

Article

Associations Between Landscape Quality, Pesticide Occurrence, and Adult-to-Brood Ratio in Honey Bee Colonies

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Abstract

Honey bee (*Apis mellifera*) colonies are exposed to multiple environmental stressors, including pesticide contamination and landscape changes, which may affect colony dynamics and colony condition. This study evaluated pesticide residue occurrence, landscape quality through the Agroecosystem Apibotanical Interest Index (AABI), and colony demographic structure across five contrasting agricultural systems in Uruguay using a comparative case-study approach. Pesticide residues were analyzed in multiple hive matrices, and residue occurrence was assessed using cumulative hazard quotients (HQ). Colony dynamics were characterized using population variables and the adult-to-brood ratio, associations were evaluated with generalized linear models. Pesticide diversity and cumulative HQ values were higher in intensively managed landscapes, particularly in horticultural and soybean systems, whereas forestry systems showed consistently low values. Residues were detected in all matrices, with greater diversity in wax and bee bread. Colony dynamics varied among landscapes, with more stable patterns in native forest systems. Demographic states based on the adult-to-brood ratio were consistently associated with colony demographic structure. We identified a significant negative statistical association between AABI and the demographic state defined by the adult-to-brood ratio, whereas cumulative HQ showed only marginal effects. Given the observational nature of the study and the multifactorial determinants of colony dynamics, this relationship should be interpreted as an observed pattern within the studied dataset rather than evidence of a direct biological effect of landscape quality on colony adult-to-brood ratio. Overall, the results suggest associations between environmental conditions and colony adult-to-brood ratio.

Keywords: agroecosystems; pollinator health; pesticide mixtures; honey bee; adult-to-brood ratio; landscape context; colony dynamics; environmental stressors



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1. Introduction

Pollinators play a key role in ecosystem functioning through their contribution to plant reproduction and pollination services [1,2]. The honey bee (*Apis mellifera*) is widely used in managed pollination systems and represents a model organism for studying colony-level responses to environmental stressors due to its ecological and economic importance. Honey bee colonies function as highly organized superorganisms whose

population dynamics emerge from the interaction between internal demographic processes, such as brood production and worker survival, and external environmental conditions that regulate resource availability and colony growth [3–5].

Multiple stressors, including pesticide exposure, habitat loss, and climate variability, have been associated with declines in colony stability [6,7]. Recent studies have emphasized that interactions among multiple stressors, including nutritional limitation, pesticide mixtures, and climatic variability, are critical drivers of colony-level responses in honey bees [7,8]. Among these, pesticide exposure represents an important component of a broader set of interacting drivers, as colonies are exposed to multiple compounds through contaminated floral resources and hive matrices such as wax, stored pollen, and honey [9,10]. The effects of pesticides depend not only on toxicity but also on environmental context and exposure pathways. At the same time, landscape structure influences the availability, diversity, and temporal continuity of floral resources, which are key factors regulating colony growth and stability [11,12]. Simplified agricultural systems may co-occur with higher pesticide exposure and reduced resource continuity, potentially affecting colony demographic responses [13–15].

Colony performance can be described using demographic indicators such as the adult-to-brood ratio, which reflects the balance between brood production and the adult worker population and provides information on colony demographic status [4,5]. This balance has been associated with the capacity of colonies to buffer environmental perturbations and maintain functional stability [15,16]. However, the relative importance of internal colony structure compared to external environmental drivers remains insufficiently understood under real agricultural conditions.

In Uruguay, agricultural expansion under no-tillage systems has increased agrochemical use [17] and altered floral resource availability, generating landscapes that differ in both chemical pressure and resource continuity. Previous studies have demonstrated that honey bee colonies can function as biomonitors of pesticide residue occurrence, as hive matrices integrate chemical residues and reflect landscape-level contamination patterns [18]. Building on this approach, integrative indicators combining pesticide residues and colony demographic parameters have been developed to characterize environmental conditions in agroecosystems [18–20]. The Agroecosystem Apibotanical Interest Index (AABI) has been proposed to evaluate landscape capacity to support colony development through the availability and continuity of floral resources [21].

Despite these advances, most approaches have addressed pesticide residue occurrence, landscape quality, and colony dynamics separately. As a result, the relative importance of internal colony structure versus external environmental drivers remains insufficiently understood under real agricultural conditions.

In this context, the present study integrates pesticide residue occurrence, landscape context, and climatic variables to evaluate their association with colony population dynamics. By combining cumulative pesticide indicators (HQ), landscape quality (AABI), and demographic measures of colony structure, this work provides a comprehensive framework to assess how multiple environmental factors are associated with colony dynamics under field conditions.

We hypothesized that (H1) pesticide residue occurrence varies across landscape contexts and is higher in intensively managed systems; (H2) pesticide distribution differs among hive matrices; and (H3) adult-to-brood ratio balance states differ among landscape contexts and levels of pesticide exposure.

2. Materials and Methods

2.1. Study Area and Landscape Selection

This study was conducted from spring 2014 to summer 2017 across five contrasting agricultural landscapes in Uruguay: native forest (NF), eucalyptus forestry plantations (F), dairy systems (D), soybean systems (S), and horticultural–fruit systems (HF) [22].

Each landscape represents a single case system within a gradient of agricultural intensification and was not spatially replicated. Therefore, landscape categories were treated as individual systems rather than independent experimental units. This design supports a comparative case study approach and does not allow formal statistical inference at the landscape category level.

Landscape quality was assessed using the Agroecosystem Apibotanical Interest Index (AABI), calculated based on weighted land-cover classes within the foraging range of each colony, integrating resource availability, diversity, and temporal continuity [21]. For analytical purposes, AABI values were categorized into three levels: low (<3), intermediate (3–4), and high (≥ 4). The geographic locations of the study sites and monitored apiaries are provided in Table S1 and in Appendix A, Table A1.

2.2. Colony Monitoring and Pesticide Residue Occurrence Assessment

2.2.1. Colony Monitoring

In each landscape, one apiary, managed under standardised beekeeping conditions, was selected, with at least 20 Langstroth hives. A total of 25 colonies (five per landscape) were monitored, resulting in 166 sampling events across the study period.

Colony assessments were conducted seasonally from spring 2014 to summer 2017. During each visit, demographic variables were recorded, including adult bee population, brood area, and honey reserves. The adult bee population was estimated using a standardized visual frame-counting method, while brood area was quantified using a reference grid placed over brood frames. In the present analysis, brood was used as a demographic variable based on brood area measurements. Direct pesticide residue analyses in brood samples were not performed. Honey reserves were estimated using a frame-based visual assessment [23]. All measurements were performed by two trained observers to ensure consistency.

The present study is based on a retrospective analysis of a previously generated dataset obtained within the INIA-FPTA monitoring program [22]. The five study landscapes were not selected randomly but were chosen because they included apiaries managed by experienced beekeepers participating in a sanitary monitoring program under the technical supervision of the Uruguayan Ministry of Livestock, Agriculture and Fisheries (MGAP). Throughout the monitoring period, trained technical personnel conducted sanitary inspections, colony population assessments, brood measurements, and sample collection. Colonies were routinely monitored for *Varroa destructor* and *Nosema ceranae*, sanitary management practices were supervised, and colonies exhibiting severe sanitary problems were not incorporated into the monitored dataset. Consequently, the analyzed dataset represents colonies maintained under relatively controlled sanitary conditions, allowing the present study to focus on associations between demographic variables, landscape context, and pesticide residue occurrence.

However, potential biological effects associated with colony health status, disease pressure, acaricide treatment history, and sanitary management practices cannot be completely excluded. The acaricides commonly used for *Varroa* control (e.g., coumaphos, fluvalinate, and amitraz) were excluded from the data treatment and cumulative HQ analyses when detected in the samples in order to focus the analyses on environmental pesticide residue occurrence associated with landscape conditions.

2.2.2. Hive Matrice Collection

At each sampling event, five hive matrices were collected per colony: adult bees, honey, corbicular pollen, bee bread, and wax.

Samples were collected following standardized procedures and stored in clean containers. During transport, samples were kept refrigerated and subsequently stored at -20°C until laboratory analysis.

Colony population dynamics were monitored until summer 2016, whereas pesticide residues were additionally analysed in summer 2017. Therefore, peak cumulative HQ values reported for 2017 represent chemical residue occurrence conditions that are not directly paired with demographic measurements, and are interpreted as indicators of maximum residue occurrence rather than concurrent colony responses.

Honey samples consisted of capped comb sections collected from honey supers. Corbicular pollen was obtained using pollen traps installed at hive entrances. Bee bread and wax samples were collected from brood frames, while forager bees were captured at hive entrances during early morning hours. When necessary, composite samples from multiple frames were combined to ensure representativeness.

2.2.3. Chemical Analysis of Pesticides

Pesticide analyses were performed using QuEChERS-based methodologies validated according to the European Commission guidance document. Insecticides, fungicides, herbicides, and acaricides were determined using liquid chromatography–tandem mass spectrometry (LC–MS/MS) and gas chromatography–mass spectrometry (GC–MS), following previously validated methods adapted for beehive matrices [19,20].

For each compound, concentration (mg kg^{-1}) and physicochemical properties were recorded, including water solubility, octanol–water partition coefficient ($\log K_{ow}$), Henry's constant, and environmental half-life (DT_{50}). Seasonal pesticide application calendars were constructed using technical reports, agronomic records, and expert knowledge. These calendars represent estimated application patterns and not direct measurements of pesticide use. Pesticide residue occurrence in the present study was inferred from residues detected in hive matrices and therefore reflects integrated environmental contamination rather than direct measurements of pesticide applications at the study sites.

2.2.4. HQ-Based Screening Indicator of Potential Toxic Pressure

Toxicological risk was assessed using the Hazard Quotient (HQ), defined as

$$\text{HQ} = C / \text{LD}_{50}$$

where C is the pesticide residue concentration ($\mu\text{g kg}^{-1}$), and LD_{50} is the acute oral median lethal dose ($\mu\text{g bee}^{-1}$) [24,25]. HQ values were calculated for each pesticide, hive matrix, and sampling event.

Cumulative HQ was calculated as the sum of individual HQ values across all detected pesticides and matrices within each sampling event, assuming additive effects as a first-order approximation of cumulative residue-associated chemical pressure. HQ values were log-transformed ($\log_{10}(\text{HQ})$) for statistical analyses.

HQ should be interpreted as a comparative screening-level indicator of potential toxic pressure based on detected pesticide residues rather than as a direct measure of toxicological risk, actual environmental residue occurrence conditions, or biological damage at the colony level.

2.3. Statistical Analyses

Principal component analysis (PCA) was performed using physicochemical properties (water solubility, logKow, Henry's constant, and DT₅₀). Variables were log-transformed when necessary and standardized before analysis. PCA was conducted using the `prcomp` function in R. Hierarchical clustering was performed using Euclidean distance and Ward's method.

The relationship between pesticide application and detection was evaluated using Spearman's rank correlation based on four pesticide categories.

Colony adult-to-brood ratio was assessed using a binomial generalized linear model (GLM) with a logit link, where the response variable (demographic state) was defined based on the adult-to-brood ratio (≥ 2). The adult-to-brood ratio was used as an indicator of colony adult-to-brood ratio because it reflects the relationship between brood demand and the adult worker population responsible for brood care, thermoregulation, and resource acquisition. The selected threshold was used as an operational criterion to distinguish contrasting demographic states within the studied colonies and should not be interpreted as a universal biological threshold for colony resilience.

Explanatory variables included cumulative pesticide residue occurrence (HQ_log), landscape quality (AABI), and their interaction. Predictors were standardized before analysis.

The GLM framework was selected to evaluate general associations between demographic state and environmental predictors at the sampling-event level rather than to model colony-specific temporal trajectories. Because repeated measurements were obtained from the same colonies over time, potential temporal autocorrelation may occur and should be considered a limitation of the study.

Mixed-effects models including colony identity as a random effect were used for analyses of colony population size, fitted using the `lme4` package. Additionally, exploratory GLMM structures including colony identity as a random effect were evaluated for resilience analyses. However, due to the limited number of colonies within landscape categories and convergence limitations associated with the dataset structure, the simpler GLM framework was retained to maintain model stability and interpretative consistency.

To address potential separation issues, penalized likelihood logistic regression (Firth's correction) was applied.

Model selection was based on Akaike's Information Criterion (AIC), and significance was assessed at $\alpha = 0.05$. All R scripts used for statistical analyses are available from the corresponding author upon reasonable request. Statistical analyses were conducted using R version 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria).

To further evaluate the potential influence of colony demographic structure on the interpretation of pesticide-associated chemical pressure, additional exploratory analyses were conducted. These analyses included cumulative HQ calculations incorporating coumaphos residues, demographic-adjusted HQ indicators derived from the adult-to-brood ratio, and an integrated indicator combining landscape quality (AABI), adult-to-brood ratio, and cumulative HQ. The results of these exploratory analyses are presented in Supplementary Tables S7 and S8. The integrated indicator was calculated as $(AABI \times \text{adult-to-brood ratio}) / (\text{cumulative HQ} + 1)$. A constant value of one was added to cumulative HQ to avoid division-by-zero problems in sampling events where HQ values were equal to zero and to maintain numerical stability across all observations.

2.4. Conceptual Framework for Colony Demographic Trajectories

Colony demographic structure was quantified using the adult-to-brood ratio. Demographic trajectories were described using key temporal points (t_0 , t_d , t_r , t_c) and associated metrics (population decline, recovery time, and recovery magnitude). A detailed description of the identification of temporal points and the calculation of resilience metrics

is provided in Appendix B. The resilience framework proposed by Middleton [16] was used as a conceptual and interpretative tool to describe observed colony demographic trajectories. Therefore, the framework should be viewed as a heuristic approach for understanding demographic responses under field conditions rather than as a direct assessment of colony resilience.

2.5. Ethical Considerations and Data Availability

No experimental manipulation of colonies was performed beyond standard apicultural management practices. Therefore, specific ethical approval was not required.

All data supporting the findings of this study are available within the article and its Supplementary Materials. Additional datasets and R scripts used for statistical analyses are available from the corresponding author upon reasonable request.

3. Results

3.1. Pesticide Residue Occurrence Across Landscapes

Pesticide residues were detected across all evaluated landscapes, allowing for the characterization of chemical residue occurrence in relation to land-use intensity, compound diversity, and concentration patterns. Detected compounds were classified into four functional groups: acaricides, fungicides, insecticides, and herbicides (Table S1). Pesticide diversity varied among landscapes, being higher in horticultural (17 compounds) and soybean systems (15) and lower in forestry systems (7).

Intensively managed landscapes (horticulture, soybean, and dairy) showed the presence of all pesticide categories. In contrast, forestry landscapes were dominated by herbicides, with limited detection of other pesticide groups. Pesticide residues were also detected in native forest landscapes, including herbicides, fungicides, and insecticides, generally at very low concentrations.

Across all landscapes, several compounds were consistently detected, including azoxystrobin, carbendazim, pyraclostrobin, tebuconazole, chlorpyrifos, chlorpyrifos-methyl, and atrazine. Concentration ranges varied among compounds and landscapes (Table S1), with higher values observed in horticultural and soybean systems, particularly for insecticides and herbicides.

Overall, pesticide residue occurrence varied among landscapes in terms of compound diversity and concentration ranges.

3.2. Seasonal Patterns of Pesticide Detection in Relation to Agricultural Calendars and Climatic Variability

Seasonal variation in pesticide detection was evaluated in relation to agronomic application calendars (Table S4) and climatic conditions. A positive but non-significant association was observed between application frequency and detection frequency (Table S2) across pesticide categories ($\rho = 0.80$, $p = 0.33$; $n = 4$).

Seasonal patterns differed among landscapes. In soybean systems, detections of fungicides, insecticides, and acaricides were concentrated during spring and summer. In horticultural systems, pesticide detections were more evenly distributed throughout the year. In dairy systems, insecticides and fungicides were more frequently detected during spring and summer, whereas herbicides were more common in autumn and winter. Forestry landscapes showed sporadic detections, mainly associated with herbicides. In native forest landscapes, pesticide residues were also detected despite the absence of direct applications.

Climatic variables showed clear seasonal variation (Figure S2). Mean temperature ranged from approximately 10–12 °C in winter to 23–24 °C in summer, while precipitation exhibited temporal variability, with higher rainfall during spring 2014 and summer 2015.

3.3. Multivariate Grouping of Pesticides Based on Physicochemical Properties

Principal component analysis (PCA) based on physicochemical properties showed general differentiation among pesticides along gradients of solubility, lipophilicity, and persistence (Figure S3). The first two principal components explained 69% of the total variance (PC1 = 43%, PC2 = 26%).

Overall, the PCA provided a descriptive overview of physicochemical variation among compounds and supported the interpretation of heterogeneous residue distribution across hive matrices.

3.4. Distribution of Pesticide Residues Across Hive Matrices and Multivariate Differentiation of Contamination Profiles

Pesticide residues were mainly detected as mixtures, dominated by combinations of atrazine, azoxystrobin, pyraclostrobin, and tebuconazole, with additional carbendazim in bee bread. Wax and bee bread showed the highest diversity and concentration of pesticide residues (Figure 1). At the landscape level, the frequency of pesticide mixtures was consistently high in wax and bee bread across all environments analyzed, whereas in honey and adult bees, it was lower and more variable.

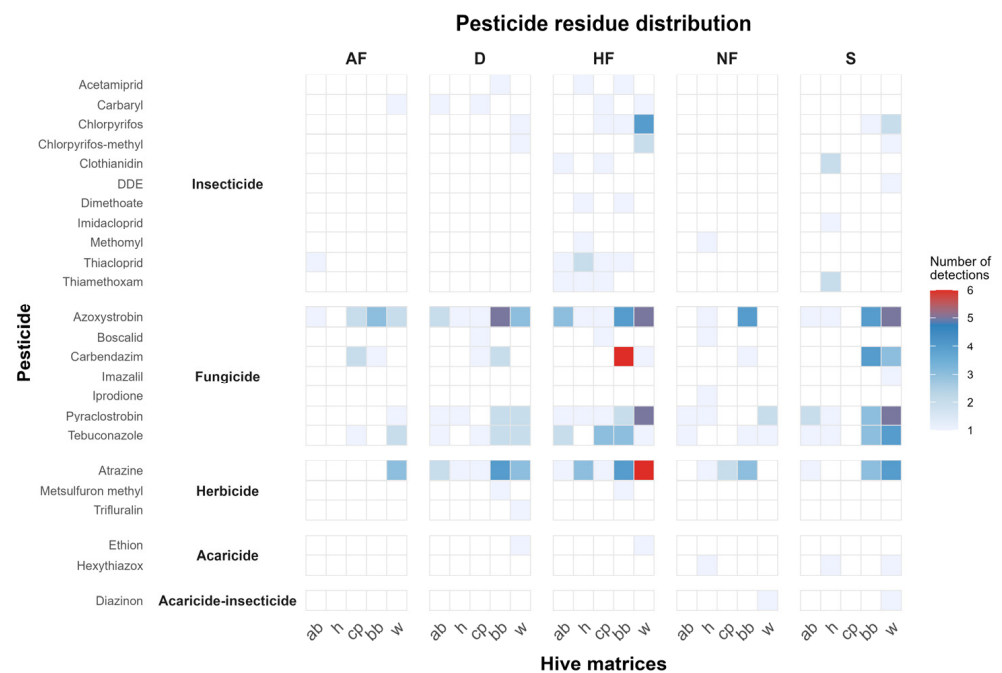


Figure 1. Heatmap of pesticide residue occurrence across hive matrices in the evaluated landscapes. Columns represent hive matrices: adult bees (ab), honey (h), corbicular pollen (cp), bee bread (bb), and wax (w), while rows correspond to individual pesticide compounds grouped by functional category (acaricides, fungicides, insecticides, and herbicides). The colour gradient represents the number of detections recorded for each pesticide, with lighter colours indicating fewer detections, whereas blank cells indicate non-detection.

A heatmap of pesticide residue occurrence across hive matrices highlights these differences, showing clear contrasts in both residue diversity and accumulation patterns among matrices (Figure 1). In addition, the relative composition of pesticide categories among matrices is presented in the Supplementary Material (Figure S3), illustrating differences in the proportional representation of fungicides, insecticides, herbicides, and acaricides.

The patterns observed in the heatmap are consistent with differences in detection frequency among pesticide categories (Table S3), where fungicides showed the highest frequency of occurrence, followed by herbicides and insecticides, while acaricides were

less frequently detected. These patterns are consistent with detection frequency among pesticide categories. Compounds with higher lipophilicity were more frequently associated with wax, whereas more soluble compounds were more frequently detected in pollen and bee bread. However, overlap of compounds across matrices was observed.

3.5. Toxicological Risk (HQ) Across Landscapes, Hive Matrices, and Pesticide Groups

The distribution of hazard quotient (HQ) values varied markedly across landscapes, pesticides, and hive matrices (Figure 2), spanning several orders of magnitude. This variability indicates that HQ values are heterogeneous both within and among agricultural systems.

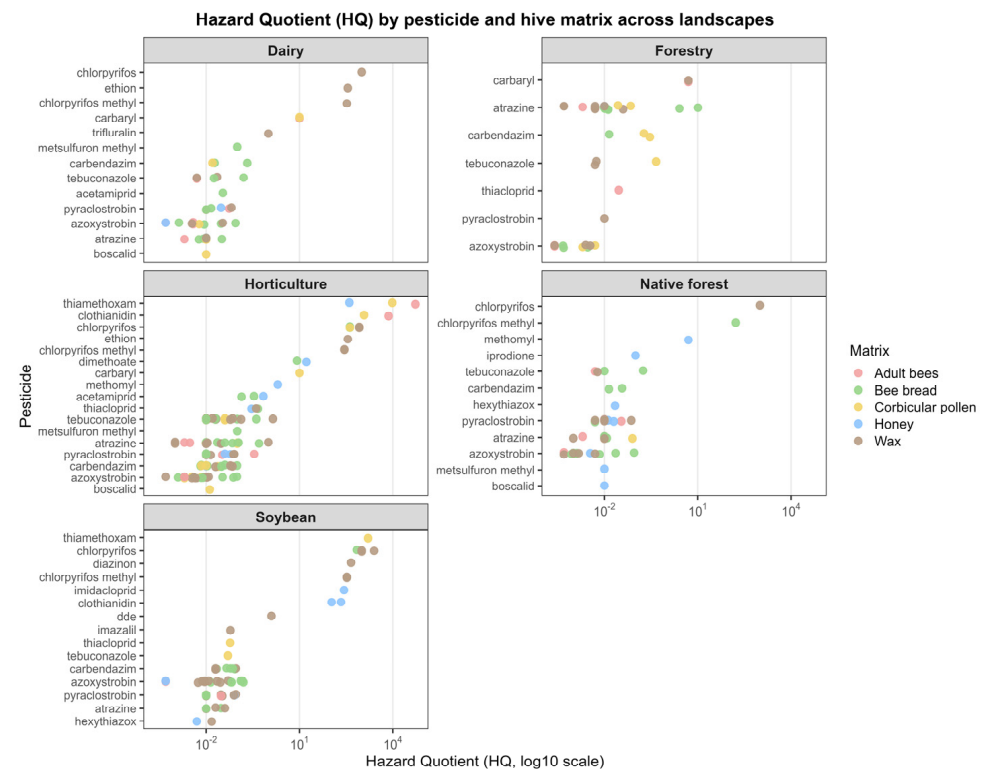


Figure 2. Hazard Quotient ($HQ = C/LD_{50}$ oral) values by pesticide, sampling event, and hive matrix (adult bees, corbicular pollen, bee bread, honey, and wax) across five landscape types. Each point represents an individual observation, allowing visualization of variability in residue-associated HQ values across matrices and sampling events. HQ values are presented on a logarithmic ($\log_{10}$) scale to facilitate comparison across several orders of magnitude.

Across landscapes, horticultural systems exhibited the highest magnitude and variability of HQ values (Figure 2), a pattern consistent with cumulative metrics (Table 1). Horticulture showed the highest mean (8673) and maximum cumulative HQ (72,120), as well as the highest total cumulative HQ (78,061). Soybean systems also showed elevated HQ values, although at lower magnitudes (mean = 831.43; maximum = 3287), while dairy and native forest landscapes presented intermediate values. Forestry systems exhibited the lowest cumulative HQ values (mean = 4.24; maximum = 10.81).

At the matrix level, higher HQ values were more frequently observed in adult bees, corbicular pollen, and bee bread, whereas honey and wax exhibited lower and less variable HQ values (Figure 2). These results indicate differences in HQ distribution among hive matrices. Differences among pesticide groups were also evident. Insecticides such as thiamethoxam, chlorpyrifos, and methomyl were associated with the highest HQ values across multiple landscapes (Figure 2) and contributed substantially to cumulative HQ

(Table 1). In contrast, fungicides and herbicides generally showed lower HQ values, although they were frequently detected.

Table 1. Summary of cumulative Hazard Quotient (HQ) by landscape.

Landscape	Sampling Event	Mean HQ	Median HQ	Maximum HQ	Total HQ	Mean No. Pesticides
Horticulture	9/9	8673.49	834.25	72,120	78,061	5.89
Soybean	8/9	831.43	162.48	3287	6651	4.75
Dairy	5/9	342.52	0.30	1201	1712	5.20
Native forest	9/9	130.29	0.09	1000	1172	3.56
Forestry	5/9	4.24	0.28	10.81	21	3.20

Note: Cumulative HQ values were calculated as the sum of individual HQ values across pesticides and hive matrices within each sampling event. Mean, median, and maximum values describe the distribution of cumulative HQ across sampling events within each landscape. Total cumulative HQ represents the overall chemical pressure, while the mean number of pesticides indicates the average diversity of compounds detected per sampling event.

The analysis of peak residue occurrence events (Table 2) further highlights the uneven distribution of HQ values. The highest cumulative HQ was recorded in a horticultural sampling event (2017Sm; HQ = 72,120), due primarily to thiamethoxam residues, followed by several high values in horticultural and soybean systems.

Table 2. Sampling events with the highest cumulative Hazard Quotient (HQ) across landscapes.

Landscape	Sampling Event	Cumulative HQ	Number of Pesticides	Number of Records
Horticulture	2017Sm	72,120	7	19
Soybean	2015Au	3287	5	6
Dairy	2015Sm	1701	9	12
Soybean	2015Sp	1701	6	10
Horticulture	2015Sp	1676	7	14
Soybean	2016Sm	1333	7	10
Horticulture	2016Sp	1244	10	28
Horticulture	2015Au	1111	4	5
Native forest	2015Sp	1000	3	4
Horticulture	2016W	834	6	25

Note: Cumulative HQ was calculated as the sum of individual HQ values across pesticides and hive matrices within each sampling event. The table highlights peak residue occurrence events associated with elevated chemical pressure. These peaks reflect the combined occurrence of multiple pesticides and higher detection frequencies. Sm = Summer; Au = Autumn; W = Winter; Sp = Spring.

3.6. Colony Demographic Structure and Population Dynamics Across Landscapes

3.6.1. Population Dynamics and Demographic Structure

Colony population dynamics varied across landscapes and seasons during the study period (2014–2016). Both brood area and adult bee populations exhibited seasonal patterns, increasing during spring and summer and declining during autumn and winter. The magnitude and temporal variability of these fluctuations differed among landscape types (Figure 3).

The native forest system showed higher and more stable population levels, whereas colonies in horticultural systems showed lower brood and adult bee populations. Soybean and dairy landscapes exhibited greater temporal variability, including marked seasonal

declines, while forestry systems showed intermediate patterns. The adult-to-brood ratio was used to describe colony demographic structure. Higher and more stable adult-to-brood ratio values were observed in the native forest and forestry systems landscapes, whereas horticultural and soybean landscapes showed lower ratios and greater variability. Across all observations ($n = 166$), 120 observations were classified within lower adult-to-brood demographic states.

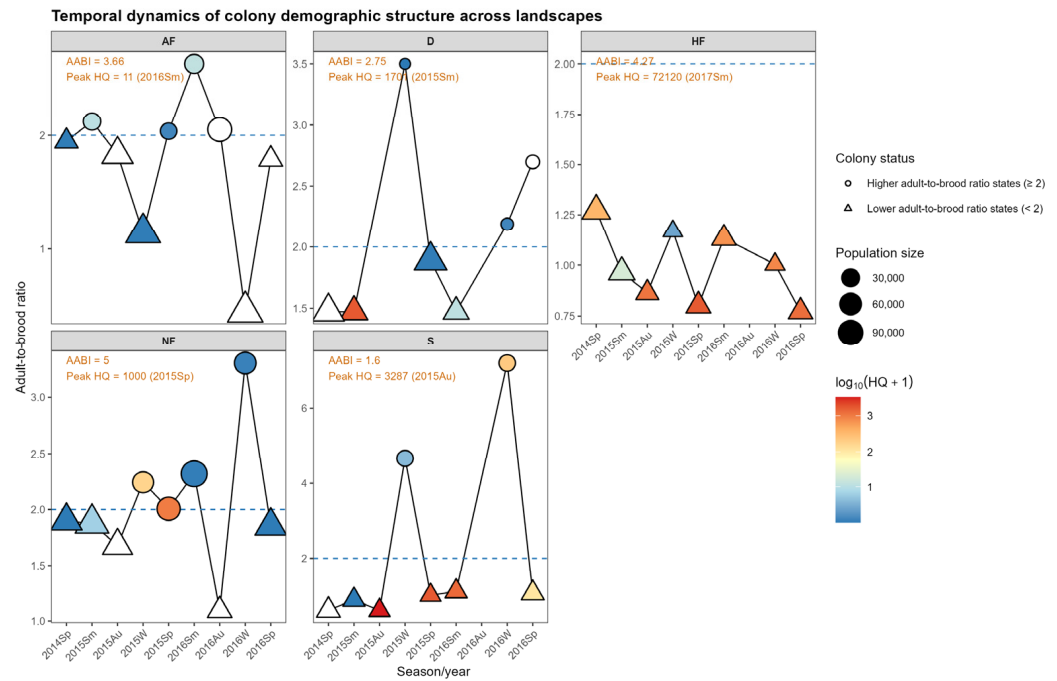


Figure 3. Temporal dynamics of colony demographic structure across landscapes based on the adult-to-brood ratio. Each panel represents a landscape type. The solid line shows temporal variation in the adult-to-brood ratio, while the dashed horizontal line indicates the operational threshold (ratio = 2) used to distinguish contrasting demographic states. Points represent seasonal observations, with circle symbols indicating higher adult-to-brood ratio states (≥ 2) and triangle symbols indicating lower adult-to-brood ratio states (< 2). Point colour represents $\log_{10}$ -transformed cumulative HQ values for each sampling event, and point size reflects colony population size. Mean AABl values and peak cumulative HQ values are shown in each panel for each landscape. The dashed blue line indicates the adult-to-brood ratio threshold (ratio = 2) used to classify demographic states. Points represent individual colony observations. Black dots represent observed mean colony population size at each sampling event.

3.6.2. Associations Between Demographic States, Cumulative HQ, and Landscape Quality

Demographic states defined by the adult-to-brood ratio were evaluated using generalized linear models. The generalized linear model showed that landscape quality (AABl) had a significant negative association with the demographic state defined by the adult-to-brood ratio ($p = 0.012$), while cumulative HQ values (HQlog) and their interaction with AABl showed marginal associations (Table S2).

At the landscape level, in the horticultural system (HF), colonies were associated with relatively high landscape quality (AABl = 4.27) and high cumulative HQ values (peak HQ = 72,120). Most sampling events were classified within low demographic-balance states (adult-to-brood ratio < 2) (Figure 3). Comparison of population size between demographic states revealed a significant difference, with a higher mean population size in colonies classified within lower demographic-balance states (Welch's t -test: $t = 7.65$, $df = 103.1$, $p < 0.001$; Table S6).

3.6.3. Discussion of Demographic-Adjusted HQ Indicators

Model comparisons among the original and exploratory approaches are summarized in Table S8. Demographic-adjusted HQ indicators based on cumulative HQ and adult-to-brood ratio produced only minor differences in model performance relative to the original analytical framework. Likewise, the inclusion of coumaphos residues did not substantially alter model fit.

The integrated indicator combining AABI, adult-to-brood ratio, and cumulative HQ showed the lowest AIC among the evaluated models (Table S8). The association between the integrated indicator and demographic state was marginally significant ($p = 0.060$).

3.6.4. Colony Demographic Trajectories Interpreted Through a Conceptual Framework

Colony demographic trajectories were interpreted using a conceptual resilience framework based on resistance, reorganization, and recovery phases [16]. Differences in demographic trajectories were observed among landscapes (Figure 3). Colonies in native forest landscapes showed more stable trajectories, with moderate population decline and relatively consistent dynamics over time. In contrast, horticultural systems showed more pronounced population declines and irregular trajectories. Forestry landscapes showed intermediate responses, with partial recovery and moderate variability.

Soybean and dairy systems exhibited highly variable trajectories (Figure 3). In soybean landscapes, some colonies showed temporary population increases following perturbation, whereas dairy systems showed no consistent disturbance–response pattern. The timing of key trajectory phases, including perturbation onset (td), minimum population (tr), and recovery (tc), is illustrated in the conceptual framework (Figure 4). Across landscapes, the onset of perturbation (td), defined as the first occurrence of an adult-to-brood ratio < 2 , did not consistently coincide with peak cumulative HQ values.

3.6.5. Honey Production, Landscape Quality, and Colony Size

Honey production varied across landscapes and was associated with both colony size and landscape quality (AABI) (Figure 5). Colonies located in native forest landscapes exhibited the highest honey production values, which coincided with higher AABI values and larger adult bee populations. In agricultural landscapes, both colony size and honey production showed greater variability (Figure 5). Horticultural systems presented relatively high AABI values but lower honey production compared to native forest landscapes. Soybean systems showed lower AABI values and intermediate to low levels of honey production.

Across all landscapes, higher honey production values were observed in colonies with larger adult bee populations, whereas colonies with smaller populations exhibited lower production levels (Figure 5). This pattern was consistent across landscape types, although the magnitude of honey production differed among them. Overall, the distribution of honey production reflects variation among landscapes in relation to colony size and AABI values, as illustrated in Figure 5.

Pesticide residue occurrence increased with agricultural intensification and varied across landscapes.

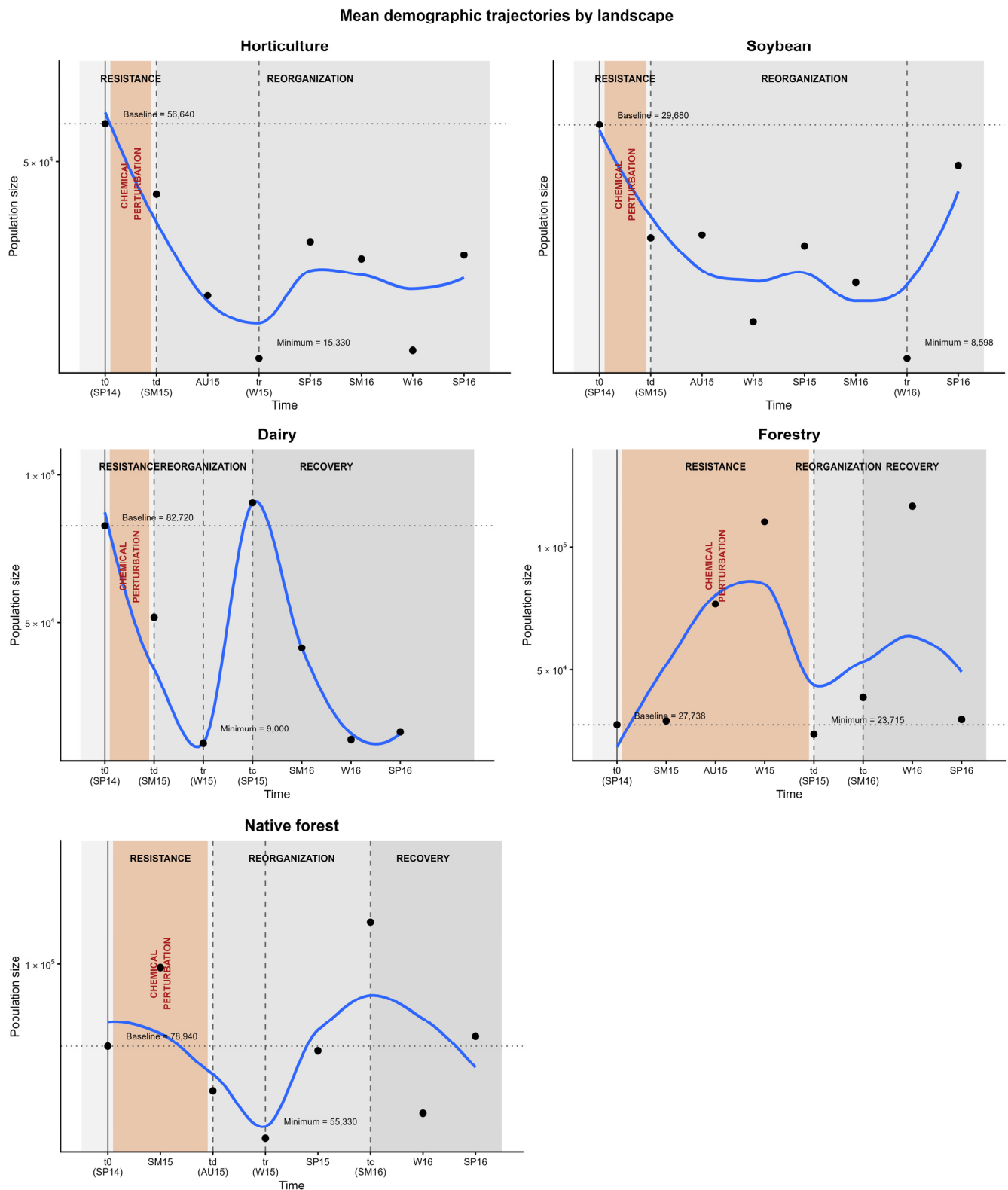


Figure 4. Conceptual demographic trajectories of honey bee colonies adapted from Middleton [16]. The figure illustrates the resistance, reorganization, and recovery phases following a demographic perturbation, including the key demographic points: baseline (t_0), perturbation onset (t_d), minimum population (t_r), and recovery (t_c) (see Appendix B). The trajectories depict mean colony population size over time for each landscape, with smoothed curves shown for visualization purposes. Black dots indicate the observed mean colony population size at each sampling event, whereas the blue curve represents the smoothed demographic trajectory used for visualization purposes. Vertical dashed lines mark the transition points (t_d , t_r , and t_c), and shaded areas indicate the resistance,

reorganization, and recovery phases. The orange shaded area denotes the period of chemical perturbation. In this study, the framework is used exclusively as a conceptual representation of colony demographic responses under field conditions and should not be interpreted as a direct measurement of resilience.

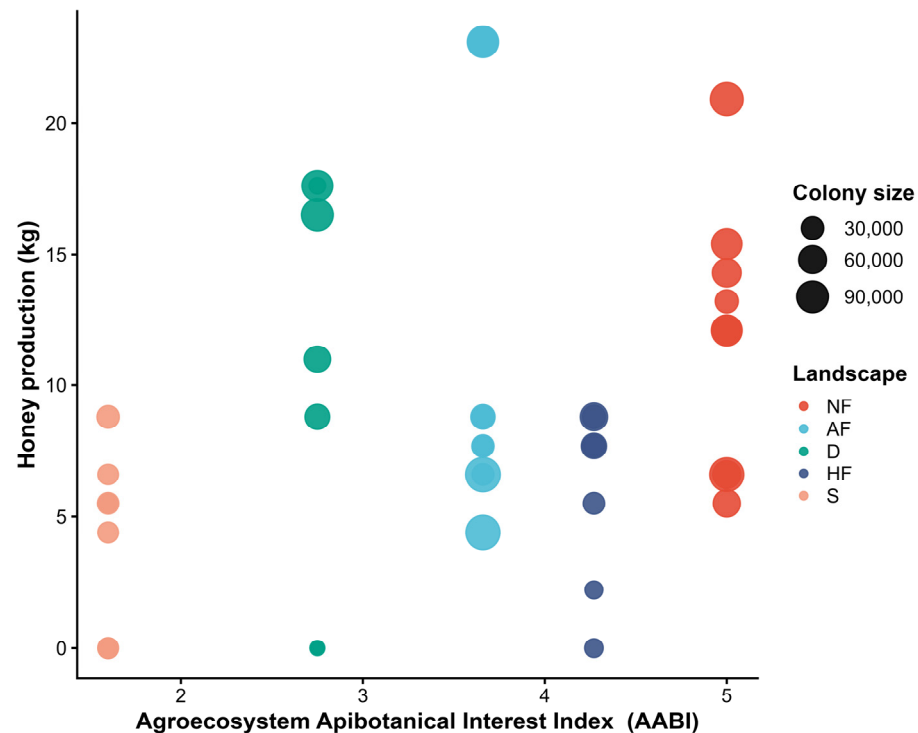


Figure 5. Relationship between the Agroecosystem Apibotanical Interest Index (AABI) and honey production (kg). Point size represents colony size (apicultural population), and color indicates landscape type (NF, AF, D, HF, S). Honey production was estimated by counting honey frames and multiplying by a conversion factor of 1.1, following standard beekeeping methodologies [23].

4. Discussion

The results of this study provide a comparative evaluation of pesticide residue occurrence, landscape context, and colony demographic structure across representative agricultural systems. Because each landscape type was represented by a single case system, the observed differences among landscapes should be interpreted as comparative case-study patterns rather than replicated landscape-category effects. Therefore, these associations should not be interpreted as causal evidence, but rather as context-dependent patterns useful for hypothesis generation.

Pesticide diversity and cumulative HQ values were higher in more intensively managed landscapes, while statistical models indicated that associations between environmental variables and demographic states defined by the adult-to-brood ratio were weak or context-dependent. In contrast, colony demographic structure, particularly the adult-to-brood ratio, showed consistent variation across landscape types and provided a useful basis for interpreting demographic patterns.

4.1. Discussion of Pesticide Residue Occurrence Across Landscapes

Before interpreting the observed associations, it is important to clarify that the HQ metric should be interpreted as a comparative screening-level indicator of potential residue-associated chemical pressure based on analytically detected pesticide residues in hive matrices. It does not represent a direct measure of colony-level toxicological risk, brood-level residue occurrence, actual environmental residue occurrence, or biological damage

to the colony. Consequently, associations involving cumulative HQ values should be interpreted as comparative patterns of residue occurrence rather than evidence of direct toxicological effects.

The higher diversity of pesticide residues detected in horticultural and soybean systems is consistent with previous studies showing that agricultural intensification increases both the number of compounds and the complexity of residue-occurrence scenarios in honey bee colonies. It has been reported that landscape composition strongly influences pesticide residue profiles, with intensively managed systems showing higher residue diversity and frequency [18,26–28]. The detection of pesticide residues in native forest landscapes, despite the absence of direct applications, suggests the influence of landscape-scale processes such as drift and other environmental transport mechanisms. Similar patterns have been reported in studies showing that pesticide contamination can extend beyond treated areas, affecting the occurrence of pesticide residues across heterogeneous landscapes [29,30].

These findings suggest that pesticide residue occurrence is not solely associated with local management practices, but rather reflects broader spatial processes, reinforcing the importance of landscape-scale approaches in pollinator potential assessment [5].

4.2. Seasonal Patterns of Pesticide Detection in Relation to Agricultural Calendars and Climatic Conditions

The seasonal patterns observed in pesticide detection indicate that colony residue occurrence is partially structured by agricultural management practices, although this relationship is not strictly linear. While agronomic calendars provide a useful approximation of pesticide application timing, they do not fully explain the observed detection patterns, which are also influenced by landscape-scale processes such as environmental transport, persistence, and landscape connectivity. Similar patterns have been reported in previous studies, where pesticide residue occurrence in hive matrices reflects not only application intensity but also broader environmental and spatial processes [18,31]. These findings reinforce that pesticide residue occurrence in honey bee colonies cannot be fully predicted from agronomic practices alone, but must be understood within a broader ecological context.

The clear seasonal signal across landscapes highlights the role of crop phenology and management timing in shaping residue profile patterns. In soybean and dairy systems, higher detection frequencies during spring and summer coincide with periods of active crop management, increasing the likelihood of pesticide presence in the environment. In horticultural systems, the more continuous detection of residues throughout the year reflects sustained application regimes typical of intensive production systems, resulting in more persistent residue occurrence conditions.

The predominance of herbicide detections during autumn and winter in dairy systems further illustrates how crop-specific management practices influence temporal residue occurrence patterns. Although herbicides generally present lower acute toxicity for bees, their widespread and seasonal use contributes to the overall chemical background in the environment [5,8].

Climatic conditions also provide an important context for interpreting pesticide residue occurrence. Temperature and precipitation influence pesticide behaviour by affecting degradation rates, mobility, and environmental persistence. Higher temperatures during spring and summer may enhance degradation processes, but also coincide with periods of increased application, while precipitation can facilitate redistribution through runoff or leaching. The variability in rainfall observed during the study period may therefore have contributed to differences in the occurrence of pesticide residues across seasons and landscapes.

This indicates that the occurrence of pesticide residues cannot be fully explained by local management alone, but must be interpreted within a broader spatial and environmen-

tal context. Overall, these findings indicate that seasonal pesticide detection emerges from the combined influence of agricultural practices and climatic variability, both of which modulate the timing, distribution, and persistence of residues in agroecosystems. This is consistent with the lack of statistical significance observed in the correlation analysis (Table S2).

Climatic variables available in the monitoring dataset, including temperature and precipitation, were evaluated in the statistical analyses. However, neither variable showed significant associations with the demographic states considered in this study, and therefore, they were not retained in the final explanatory models. These findings reinforce the multifactorial nature of honey bee colony dynamics, where demographic patterns may emerge from the combined influence of landscape characteristics, floral-resource availability, pesticide residue occurrence, climatic variability, colony health status, and management practices. Nevertheless, climatic conditions remain important contextual factors influencing pesticide fate and colony dynamics under field conditions.

4.3. Discussion of Pesticide Residue Distribution Across Hive Matrices

4.3.1. Differential Distribution of Residues Across Hive Matrices

The occurrence and composition of pesticide residues varied markedly among hive matrices, indicating that pesticide residue occurrence within colonies is not homogeneous but structured by matrix function and compound properties. Wax and bee bread showed the highest residue diversity and concentrations, acting as the main reservoirs of contaminants within the colony. This pattern is consistent with previous studies identifying wax as a preferential accumulation matrix for lipophilic and persistent compounds [31]. Due to its composition and structural function, wax can retain pesticide residues over extended periods, potentially acting as an internal source of chronic residue occurrence for both adult bees and brood.

Bee bread also emerged as a critical matrix, not only because of its accumulation capacity, but also due to its functional role as the primary protein source for larvae. This suggests that contamination in bee bread may represent a relevant residue occurrence pathway for brood development and colony health, even in the absence of acute toxicity. Previous studies have shown that pesticide residues in bee bread are more closely associated with sublethal effects and developmental alterations than those detected in honey [32–34]. The distribution of residues among matrices was consistent with the physicochemical characteristics of the detected compounds. Lipophilic and persistent compounds tended to accumulate preferentially in wax and bee bread, whereas more polar compounds were more frequently associated with pollen and, to a lesser extent, honey. Accordingly, each matrix reflects a different dimension of colony residue occurrence and provides complementary information on contamination pathways.

In contrast, corbicular pollen showed lower relative accumulation, indicating that it primarily functions as an entry pathway for pesticides rather than a long-term storage matrix. Adult bees reflected more recent residue occurrence events, while honey occupied an intermediate position, particularly for compounds with higher solubility and mobility. Across all landscapes, pesticide residues were predominantly detected as mixtures, with a recurrent core of compounds (including atrazine, azoxystrobin, pyraclostrobin, tebuconazole, and carbendazim) consistently co-occurring. This pattern indicates that residue occurrence within colonies occurs mainly through complex mixtures, with greater intensity in wax and bee bread. These findings reinforce that pesticide residue occurrence within honey bee colonies is compartmentalized rather than uniform and highlight the importance of multi-matrix approaches for accurately assessing residue occurrence pathways and potential biological effects.

4.3.2. Physicochemical Drivers and Multivariate Differentiation of Residue Profiles

The PCA results indicate that pesticide physicochemical properties vary along gradients of solubility, lipophilicity, and persistence, which may contribute to differences in residue occurrence across hive matrices. However, these patterns should be interpreted descriptively, as multiple environmental and colony-level processes likely influence pesticide distribution within colonies.

4.4. Toxicological Pressure (HQ) Across Landscapes, Hive Matrices, and Pesticide Groups

The distribution of HQ values across landscapes, matrices, and pesticide groups (Figure 2) highlights the heterogeneous nature of pesticide residue occurrence in honey bee colonies. This variability is consistent with previous studies showing that colonies are exposed to complex mixtures of pesticides that vary spatially and temporally across agricultural systems [9,18,28,31].

Higher HQ values observed in horticultural and soybean systems (Figure 2; Table 1) align with findings from intensively managed agroecosystems, where increased pesticide use leads to greater residue diversity and higher cumulative HQ values [27,31]. In contrast, lower HQ values in forestry and native systems are consistent with reduced chemical inputs and simpler pesticide profiles reported in less intensive landscapes. The occurrence of peak residue occurrence events (Table 2), particularly in horticultural systems, indicates that although higher HQ values were observed in these environments, estimated residue occurrence levels based on detected residues did not exceed acute LD₅₀ reference values. Therefore, the results should be interpreted as comparative indicators of potential chemical pressure rather than evidence of direct toxicological effects at the colony level.

This finding further supports the idea that residue-associated chemical pressure in honey bee colonies is primarily driven by the co-occurrence of multiple compounds rather than by isolated pesticides. Similar patterns have been reported by [31], who found that honey bee colonies are chronically exposed to pesticide mixtures, with potential implications for colony health even when individual compounds are present at low concentrations.

Cumulative HQ provided a more integrative representation of chemical residue occurrence than individual HQ values, as it accounts for the simultaneous presence of multiple residues within sampling events. This is consistent with previous work emphasizing that cumulative and mixture-based approaches better reflect real residue occurrence conditions in honey bee colonies than single-compound assessments [31]. The contribution of fungicides to cumulative HQ, despite their low individual HQ values, is also in agreement with studies showing that fungicides are frequently detected in hive matrices and may interact with other pesticides in co-residue occurrence scenarios [32–35]. Although fungicides are often considered less toxic to bees in isolation, their widespread occurrence suggests a potential role in shaping overall residue occurrence conditions.

Despite higher cumulative HQ values in intensively managed landscapes, the weak or non-significant association between HQ and colony demographic states observed in this study suggests that chemical residue occurrence alone does not fully explain differences in colony outcomes. This is consistent with studies indicating that colony-level responses depend on multiple interacting factors, including nutrition, environmental conditions, and internal colony dynamics [5,36,37].

Overall, our results suggest that honey bee colonies are exposed to mixtures of different pesticides that change depending on the environment. Measures like HQ help us to summarize this complex chemical residue occurrence, but they are only a simplified estimate. HQ shows the possible level of toxic stress, but it does not account for subtle (sublethal) effects or interactions between chemicals (synergistic effects).

4.5. Colony Demographic States, Population Dynamics, and Comparative Differences

4.5.1. Discussion of Population Dynamics and Demographic Structure

Before interpreting the observed associations between environmental variables and colony demographic states, it is important to acknowledge several limitations associated with the adult-to-brood ratio used in the present study. Although this ratio provides useful information on colony demographic structure, it should be considered a context-dependent indicator rather than a direct or universal measure of colony resilience. Adult-to-brood ratios may vary in response to seasonal brood cycles, floral-resource availability, queen performance, colony health status, disease pressure, *Varroa* infestation, climatic variability, and management practices. Consequently, the demographic states identified in this study should be interpreted as comparative indicators of colony demographic organization under field conditions rather than as definitive measures of colony condition.

The seasonal population patterns observed across landscapes are consistent with the biological dynamics of honey bee colonies, in which brood production and adult population size naturally fluctuate throughout the year. Increases during spring and summer and reductions during autumn and winter were observed across all landscape types, although the magnitude and temporal variability of these changes differed among systems.

Colony population dynamics followed consistent seasonal patterns across landscapes, with increases during spring and summer and declines during autumn and winter (Section 3.6.1), a pattern widely reported in temperate systems and associated with seasonal variation in floral resource availability and colony activity [3]. However, beyond this general seasonal trend, clear differences among landscapes were observed in both population size and temporal variability (Figure 3), indicating that landscape context was associated with differences in colony dynamics.

Colonies in native forest and forestry landscapes showed higher and more stable population levels, whereas horticultural and soybean systems exhibited lower population sizes and greater variability (Figure 3). This pattern is consistent with studies showing that heterogeneous landscapes with more continuous and diverse floral resources support more stable colony development [13,14]. In contrast, intensively managed agricultural systems are often characterized by temporal discontinuities in resource availability, which can constrain colony growth and increase variability [6].

Differences in demographic structure, as reflected by the adult-to-brood ratio, were also evident across landscapes (Section 3.6.1). Lower ratios were more frequently observed in horticultural and soybean systems, whereas higher and more stable ratios were observed in native forest and forestry landscapes. Similar patterns have been reported in studies linking colony demography to environmental conditions, where imbalances between adult population and brood can affect colony functioning and stability [3,4].

In addition, statistical comparisons showed that population size differed significantly between demographic states, with a higher mean population size in colonies classified within lower demographic-balance states (Welch's *t*-test: $t = 7.65$, $df = 103.1$, $p < 0.001$; Table S6). This result contrasts with the common assumption that larger colonies are necessarily more demographically stable. Instead, it suggests that colony condition is not solely related to population size but also depends on internal demographic organization. Taken together, these findings support the view that colony dynamics are associated with both external environmental conditions and internal demographic structure.

In this context, the adult-to-brood ratio provides a useful indicator of colony state, as it captures the balance between growth and maintenance processes, which may be more informative than population size alone for understanding colony responses to environmental variability. Although colony sanitary status was controlled during the original monitoring program, including *Varroa destructor* management and *Nosema ceranae* analyses, brood

was not directly analyzed as a pesticide-residue matrix in the present study. Therefore, potential brood-level toxicological effects or specific brood-health alterations cannot be directly inferred from these results.

However, this metric should be interpreted cautiously as a context-dependent demographic indicator rather than as a direct or universal measure of colony resilience [36]. This ratio may vary in response to multiple interacting factors, including seasonal brood cycles, floral-resource availability, queen performance, colony health, disease pressure, *Varroa* infestation, climatic variability, and management practices. Consequently, the demographic states described in this study should be interpreted as comparative indicators of colony adult-to-brood ratio under field conditions rather than as definitive measurements of colony resilience capacity.

4.5.2. Influence of Environmental Conditions and Pesticide Residue Occurrence

Patterns of residue occurrence and landscape context varied across systems and were consistently reflected in both individual HQ distributions (Figure 2) and cumulative HQ metrics (Table 1). Horticultural and soybean systems showed higher cumulative HQ values and greater variability compared to forestry and native forest landscapes, indicating that both the magnitude and diversity of pesticide residue occurrence differ among land-use systems. Peak residue occurrence events (Table 2) were primarily associated with these intensively managed systems and were characterized by higher cumulative HQ values and a greater number of detected pesticides, highlighting the importance of multi-compound residue occurrence scenarios.

The distribution of HQ values across hive matrices (Figure 2) further indicates that residue occurrence is not uniform within the colony. Higher HQ values were more frequently observed in adult bees, corbicular pollen, and bee bread, whereas honey and wax showed lower values. This pattern is consistent with previous studies describing differential residue occurrence and accumulation pathways among hive matrices, related to both the physicochemical properties of compounds and colony-level processes [25,33].

Pesticide residues were also detected in native forest landscapes, supporting the idea that residue occurrence is not restricted to areas of direct application but is influenced by landscape-scale transport processes [29,38]. This widespread occurrence of residues across landscapes reinforces the need to consider residue occurrence as a spatially integrated process rather than a strictly local phenomenon. In addition to residue occurrence, landscape quality may also influence colony demographic dynamics through the availability and continuity of floral resources. Although floral resources were not quantified directly during each sampling event, their potential influence was incorporated through the Agroecosystem Apibotanical Interest Index (AABI), which integrates resource availability, diversity, and temporal continuity within the foraging range of colonies. Likewise, climatic variables available in the original monitoring dataset, including temperature and precipitation, were evaluated during model development but were not significantly associated with the demographic states evaluated, and therefore were not retained in the final explanatory models. These results should not be interpreted as evidence that climatic conditions do not influence colony development, but rather that their effects were not detectable within the variability represented by the monitored colonies and study period. Furthermore, the monitored colonies originated from a long-term sanitary surveillance program that included routine *Varroa destructor* management and *Nosema ceranae* monitoring, reducing the likelihood that major sanitary disturbances were the primary drivers of the observed demographic patterns.

Although individual HQ values were generally low, cumulative HQ revealed more complex residue occurrence scenarios, particularly in horticultural and soybean landscapes

(Table 1). This decoupling between low individual compound values and higher cumulative residue occurrence has been widely documented and highlights the importance of considering mixture and chronic residue occurrence effects. In this context, fungicides, despite their low individual HQ values, contributed substantially to cumulative HQ due to their frequent detection—a pattern consistent with studies showing that fungicides can play a relevant role in mixture toxicity and interactions with other compounds [33,37].

Statistical models (Table S2) indicated that cumulative residue-associated HQ showed only a marginal association with the demographic states defined by the adult-to-brood ratio, while AABI showed a significant negative association within the studied systems. Importantly, AABI represents the availability, diversity, and temporal continuity of floral resources of apicultural interest rather than environmental quality in terms of chemical safety.

In the evaluated landscapes, relatively high AABI values co-occurred with intensive horticultural systems characterized by elevated cumulative residue-associated HQ. Therefore, the observed negative association between AABI and the demographic states defined by the adult-to-brood ratio should be interpreted cautiously and within the specific environmental context of the studied systems, where high floral-resource availability and elevated residue-associated chemical pressure occurred simultaneously.

Accordingly, the observed negative association should not be interpreted as evidence that floral-resource availability negatively affects resilience per se, but rather as a context-dependent association observed under the environmental conditions evaluated in this study.

At the landscape level, this pattern was reflected in the horticultural system (HF), where relatively high AABI values co-occurred with high cumulative HQ (peak HQ = 72,120) and frequent lower adult-to-brood ratio states (Figure 3). In the horticultural–fruit system (HF), the detection of thiamethoxam should be interpreted within the regulatory and landscape context of the study period. At the time of sampling (2014–2017), thiamethoxam was permitted for agricultural use in Uruguay, including applications in horticulture and fruit production. Regulatory restrictions on neonicotinoid insecticides were introduced later, from 2019 onwards, by the Ministry of Livestock, Agriculture and Fisheries (MGAP). Therefore, its presence in HF is consistent with its use in intensive production systems targeting pest control in fruit crops such as apple and plum, as well as in horticultural crops. In addition, the study site is located within a mixed agricultural landscape that includes surrounding soybean fields, which may also contribute to environmental contamination through drift or landscape-scale transport processes. A detailed description of the site characteristics and the surrounding area of influence is provided in Appendix A. These observations indicate that, within the evaluated systems, relatively high landscape quality in terms of floral-resource availability co-occurred with elevated pesticide residue occurrence. Therefore, the observed associations between environmental variables and the demographic states defined by the adult-to-brood ratio should be interpreted within this specific environmental context.

One possible explanation is that resource-rich environments may increase foraging activity, thereby increasing the likelihood of residue occurrence on contaminated resources. Honey bees are primarily exposed to pesticides during foraging, when nectar and pollen are collected and transported back to the colony [30]. In addition, sublethal pesticide residue occurrence has been shown to affect foraging behaviour and colony performance [8,34,38], suggesting that residue occurrence effects may operate through behavioural and physiological pathways not captured by acute toxicity metrics alone.

No strong evidence was found for a consistent interaction between cumulative HQ values and landscape quality (AABI) (Table S2), indicating that their effects may operate independently within the studied systems. This context-dependent pattern is consistent

with recent work showing that nutritional conditions and resource continuity can modulate colony responses to environmental stress [36,39,40].

Taken together, these findings indicate that pesticide residue occurrence and landscape context define the environmental conditions under which colonies operate, but do not by themselves explain variation in demographic states defined by the adult-to-brood ratio. Instead, their effects appear to be mediated by internal colony processes, supporting a multifactorial view in which external drivers shape the environmental context while colony-level organization influences response capacity.

The negative association observed between AABI and demographic state was unexpected and should be interpreted with caution. Although AABI was developed to represent landscape quality through floral-resource availability, diversity, and temporal continuity, the observed relationship does not necessarily indicate a direct biological effect of landscape quality on colony adult-to-brood ratio. The counterintuitive direction of the association suggests that AABI may be correlated with ecological and management-related factors not explicitly represented in the present analyses. Furthermore, because the present study is based on a comparative case-study design without landscape replication, the observed relationship should be considered an exploratory statistical association rather than evidence of a causal effect. Further studies using replicated landscape designs and direct measurements of resource availability are needed to clarify the mechanisms underlying this relationship.

Additional exploratory analyses incorporating colony demographic structure and integrated landscape-toxicity indicators suggested that demographic states may be better understood when landscape quality, pesticide-associated chemical burden, and the colony adult-to-brood ratio are considered simultaneously. Although these analyses should be interpreted cautiously, they support the multifactorial interpretation proposed throughout this study (Tables S7 and S8).

4.5.3. Adult-to-Brood Ratio in Relation to Interacting Drivers

The significant negative association between landscape quality (AABI) and the demographic states defined by the adult-to-brood ratio observed in the statistical model (Table S2) should not be interpreted as a direct effect of landscape quality per se, but rather as an emergent pattern resulting from the co-occurrence of high resource availability and elevated chemical residue occurrence in specific systems. Although higher AABI values are generally expected to support colony performance, this relationship was context-dependent across landscapes.

This pattern is illustrated by the contrast between horticultural (HF) and native forest (NF) systems (Figure 3). In HF, high AABI values (4.27) co-occurred with elevated cumulative pesticide residue occurrence (peak HQ = 72,120) and frequent lower adult-to-brood ratio states, indicating that resource-rich environments may act as residue occurrence amplifiers by promoting increased foraging activity and consequently greater contact with contaminated nectar and pollen [14,30]. In addition, sublethal pesticide residue occurrence can impair key processes such as foraging behaviour, navigation, and brood care [8,34,35,38], potentially reducing the capacity of colonies to reorganize following disturbance [40].

As shown in Figure 3, colonies in the horticultural system (HF) remained predominantly in a reorganisation state throughout the study period, without transitioning to the higher adult-to-brood ratio states (adult-to-brood ratio ≥ 2). The adult-to-brood ratio consistently remained below the resilience threshold, indicating a persistent imbalance between the adult workforce and brood demand. This pattern indicates that colonies were unable to restore internal demographic organisation under HF conditions.

In contrast, NF landscapes also exhibited high AABI values but were associated with substantially lower cumulative pesticide residue occurrence (Table 1) and more stable colony trajectories (Figures 3 and 5). Under these conditions, colonies were able to recover after temporary declines in the adult-to-brood ratio, indicating effective reorganization processes. These colonies also maintained relatively higher adult bee populations and a more stable demographic structure compared to more intensively managed systems, supporting their capacity to buffer perturbations.

These contrasting patterns indicate that AABI reflects resource availability rather than environmental safety, and that its association with the demographic states, defined by the adult-to-brood ratio, depends on the surrounding disturbance context. The weak interaction observed between HQ and AABI further suggests that these variables represent different dimensions of the environment rather than acting synergistically.

Together, these findings support the interpretation that pesticide residue occurrence and landscape context define the disturbance environment, but do not by themselves influence colony outcomes. Instead, colony responses appear to depend on internal demographic organization, particularly the balance between adult population and brood, consistent with the view of honey bee colonies as self-organized systems [16,40].

Within this framework, the adult-to-brood ratio can be interpreted as an operational indicator of colony organization, reflecting the balance between workforce and developmental demand. Colonies that maintained or re-established this balance were more likely to transition into reorganization and recovery phases (Figure 4), whereas colonies that remained below this threshold showed limited recovery capacity.

Overall, these results indicate that colony resilience emerges from the interaction between environmental pressures and internal demographic structure, with demographic organization playing a central role in determining colony responses to disturbance.

4.5.4. Honey Production and Landscape Context

The patterns observed in honey production (Section 3.6.4; Figure 5) indicate that colony productivity varied across landscapes in association with both colony size and landscape quality (AABI).

Colonies located in native forest landscapes showed higher honey production values, coinciding with larger adult bee populations and higher AABI values. In contrast, agricultural landscapes exhibited greater variability in both colony size and honey production, with horticultural systems showing relatively high AABI values but comparatively lower honey yields, and soybean systems showing lower AABI values and moderate to low production levels.

These patterns suggest that landscape quality alone does not fully explain variations in honey production. Instead, the results indicate that colony size is more consistently associated with honey yield across landscapes, as colonies with larger adult populations show higher production levels regardless of landscape type (Figure 5). This relationship is consistent with previous studies demonstrating that colony productivity is strongly influenced by worker population size and foraging capacity [3,41].

The mismatch observed in horticultural systems, where relatively high AABI values did not correspond to higher honey production, further indicates that the availability of floral resources at the landscape level does not necessarily translate into effective resource exploitation. Similar patterns have been reported in agricultural systems where resource availability is high but temporally variable or associated with other stressors, limiting colony performance [6,14].

In this context, the results from Sections 3.6.1 and 3.6.2 suggest that internal colony conditions, particularly demographic structure and population stability, may influence the

capacity of colonies to convert available resources into honey production. Colonies with more stable population dynamics and higher adult bee abundance are likely to sustain a more effective foraging workforce, which in turn supports higher productivity.

Together, these findings indicate that honey production emerges from the interaction between landscape context and colony-level demographic characteristics. While landscape quality defines the potential availability of resources, actual honey production appears to depend on the capacity of colonies to maintain sufficient population size and stable internal organization to exploit those resources effectively.

4.5.5. Ecological and Applied Implications

The combined results of this study suggest that pollinator pesticide residue occurrence and colony demographic responses cannot be adequately evaluated based on isolated measurements or single stressors, but instead require an integrative, system-level perspective. Across landscapes, cumulative pesticide residue occurrence (HQ) showed strong variability and reached high values in intensively managed systems (Section 3.5; Tables 1 and 2), yet its associations with the demographic states defined by the adult-to-brood ratio were weak or context-dependent in the statistical models (Table S2). This indicates that chemical residue occurrence alone is not directly associated with colony demographic outcomes, but rather contributes to a broader disturbance context.

Similarly, landscape quality (AABI) influenced resource availability and environmental conditions (Section 3.6.4; Figure 5), but did not consistently translate into improved colony performance. In particular, the mismatch observed in horticultural systems, where high AABI values co-occurred with high pesticide residue occurrence and lower demographic patterns (Figure 3), suggests that favourable resource conditions may coincide with increased environmental chemical pressure. This pattern supports previous findings showing that pollinator responses to agricultural landscapes depend not only on resource availability, but also on the interaction between multiple stressors [5,9,38,42].

In contrast, internal colony structure emerged as a key determinant of resilience across analyses. The adult-to-brood ratio was consistently associated with colony condition and trajectory dynamics (Sections 3.6.1 and 3.6.2; Figure 3; Table S3), indicating that colonies capable of maintaining a balanced demographic structure were more likely to reorganize and sustain functionality under disturbance. This finding aligns with the view of honey bee colonies as self-organized systems, in which internal regulatory mechanisms play a central role in buffering environmental variability [16,40].

From an ecological perspective, these results support a shift from single-stressor approaches toward integrative frameworks that consider both external pressures and internal colony dynamics. Recent studies have emphasized that colony-level responses to pesticides and other stressors emerge from cumulative and interacting effects rather than from acute residue occurrence alone [40,43–45]. In this context, resilience should be understood as the capacity of colonies to reorganize and maintain functionality under dynamic and interacting stressors, rather than as a simple return to a pre-disturbance state [40].

From an applied perspective, improving pollinator health in agricultural systems requires addressing both chemical and ecological dimensions. Reducing cumulative pesticide residue occurrence remains a key objective, particularly in intensively managed landscapes where repeated applications increase chemical pressure. However, this alone is unlikely to be sufficient. Management strategies should also promote conditions that support stable colony development, including continuous and diverse floral resources that sustain population size and adult-to-brood ratio. Landscapes with limited or temporally

restricted resources may constrain the ability of colonies to maintain this balance, thereby reducing their capacity to buffer environmental stress.

The widespread detection of pesticide residues across all landscapes, including semi-natural environments (Section 3.5), further underscores the importance of considering landscape-scale processes when interpreting environmental residue occurrence patterns and potential chemical pressure in honey bee colonies. Overall, this study contributes to an emerging body of evidence indicating that pollinator resilience is an emergent property arising from the interaction between environmental pressures and internal colony organization, with important implications for both ecological theory and applied pollinator conservation [40,46].

4.6. Exploratory Evaluation of Demographic-Adjusted HQ Indicators

To further address the potential role of colony demographic structure in the interpretation of pesticide-associated chemical pressure, exploratory analyses were conducted incorporating the adult-to-brood ratio into cumulative HQ calculations and evaluating alternative HQ formulations, including coumaphos residues. These analyses included demographic-adjusted HQ indicators and an exploratory integrated indicator combining landscape quality (AABI), adult-to-brood ratio, and cumulative HQ (Tables S7 and S8).

Demographic-adjusted HQ indicators based solely on cumulative HQ and adult-to-brood ratio did not substantially improve model performance relative to the original analytical framework. However, the integrated indicator showed improved model fit compared with the original models, although its association with demographic state remained marginal. Because demographic states were themselves defined using the adult-to-brood ratio, analyses directly incorporating this variable should be interpreted cautiously.

Overall, these exploratory analyses support the view that colony demographic states cannot be explained solely by cumulative pesticide occurrence and are more consistent with a multifactorial interpretation involving landscape quality, colony demographic structure, and pesticide-associated chemical pressure. Because these analyses were developed post hoc and were not part of the original study design, they should be considered exploratory and do not alter the principal conclusions of the study.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ecologies7030060/s1>, Figure S1: The geographic location of the study sites and apiaries; Figure S2: Seasonal variation in temperature and precipitation across landscapes. Seasonal variation in accumulated precipitation (PP, mm) and mean air temperature (°C) across five landscape types in Uruguay (soybean, horticulture, forestry, native forest, and dairy systems) from spring 2014 to spring 2016, corresponding to the results presented in Section 3.2; Figure S3: Principal component analysis (PCA) of pesticides based on physicochemical properties and cluster differentiation. Principal component analysis (PCA) was performed using standardized physicochemical variables, including water solubility, lipophilicity (log Kow), Henry's law constant, and environmental persistence (DT₅₀). Pesticides were grouped using k-means clustering based on PCA scores. Colors represent clusters of compounds with similar physicochemical profiles: lipophilic and persistent (red), lower lipophilicity and lower persistence (green), and higher solubility with relatively high persistence (blue). Ellipses indicate the dispersion of each cluster in the multivariate space. The first two principal components explained 69% of the total variance (PC1 = 43%, PC2 = 26%), representing major gradients in pesticide physicochemical behavior; Table S1: Pesticide residues and concentration ranges across landscapes (ng g⁻¹). Values represent minimum–maximum detected concentrations. “–” indicates not detected. “<” indicates values below the limit of quantification; Table S2: Relationship between expected pesticide application frequency and observed detection frequency. Application frequency was derived from the seasonal agronomic calendar (Table S4), and detection frequency from observed pesticide occurrences in hive matrices. The correlation

was calculated across four pesticide categories (acaricides, fungicides, herbicides, and insecticides); Table S3: Cumulative Hazard Quotient (HQ) across all sampling events and landscapes. Landscape indicates the agricultural system where samples were collected. Sampling event corresponds to the date and season of sampling. Cumulative HQ represents the sum of individual HQ values across all detected pesticides and hive matrices within each sampling event, and is used as an indicator of overall chemical pressure. Number of pesticides refers to the number of distinct pesticide compounds detected in each sampling event, while number of records indicates the total number of observations contributing to the cumulative HQ, including multiple matrices and detections per pesticide; Table S4: Estimated seasonal calendar of pesticide applications for the different agricultural systems analyzed in the study. based on agronomic management practices and regional agricultural records. Each season (spring, summer, autumn, winter) is divided into three columns representing the corresponding months. Letters indicate the type of pesticide typically applied in each period and landscape: A = acaricides; F = fungicides; I = insecticides; H = herbicides. “–” indicates periods with no expected pesticide application based on agronomic practices. This information supports the interpretation of seasonal detection patterns presented in Section 3.2; Table S5: Results of generalized linear models evaluating the effects of cumulative pesticide exposure (HQ). landscape quality (AABI). and their interaction on colony demographic state. Binomial generalized linear model: demographic state $\sim$ HQ_log $\times$ AABI; Table S6: Comparison of colony population size between demographic states using Welch’s *t*-test; Table S7: Sampling-event values of cumulative HQ, adult-to-brood ratio, and demographic-adjusted HQ indicators; Table S8: Comparison of exploratory models incorporating demographic structure and coumaphos residues.

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Abbreviations

The following abbreviations are used in this manuscript:

AABI	Agroecosystem Apibotanical Interest Index
AIC	Akaike’s Information Criterion
PCA	Principal component analysis
HF	Horticultural-fruit system
HQ	Hazard Quotient
NF	Native forest system
D	Dairy system
AF	Artificial forest system
S	Soybean system
tc	recovery
td	perturbation onset
tr	minimum population
to	baseline

Appendix A

Table A1. Description of study sites and their surrounding areas of influence, including landscape context, land use, climate, and ecological biophysical classification.

Site/Code	Geographic Location	Landscape Context and Land Use	Climate Median Description	Ecological–Biophysical Unit
Soybean (S)	32.676506° S, 57.605665° W	The site comprises a heterogeneous agricultural matrix including pasture, soybean cultivation, and shelterbelt woodland. Pastures present a two-strata structure: a lower herbaceous layer (~100% cover) dominated by <i>Cichorium intybus</i> , <i>Lotus corniculatus</i> , <i>Lolium multiflorum</i> , and <i>Cynodon dactylon</i> , and a sparse upper layer (~10% cover) with <i>Eryngium horridum</i> and <i>Carduus acanthoides</i> . Natural grasslands are also present, with dominant species such as <i>Paspalum notatum</i> and <i>Cynodon dactylon</i> , and a secondary layer composed of <i>Acanthostyles bunnifolius</i> , <i>Baccharis</i> spp., <i>Senecio madagascariensis</i> , and <i>Nassella charruana</i> . Exotic species, particularly <i>Schinus longifolius</i> , dominate woodland patches located on elevated areas.	Temperate humid climate with warm, humid summers and cool winters. The temperature ranges from 7 °C to 32 °C. Rainfall is distributed throughout the year, with peaks in spring and summer. High relative humidity and occasional winter frosts.	Southwestern sedimentary basin—agricultural-dairy system
Horti-fruticulture (HF)	34.704082° S, 56.251371° W	Intensive production landscape dominated by greenhouse horticulture and fruit crops (apple, plum, peach, and vineyards). The matrix includes natural grasslands and small <i>Eucalyptus grandis</i> plantations, along with rural settlements and minor industrial activities. Livestock, poultry, and pig production are also present. Soils show significant erosion and degradation, partly associated with nearby quarry activities. The site is embedded in a mixed agricultural landscape where it interacts with surrounding cropping systems, particularly soybean cultivation in adjacent fields.	Mild temperate climate with annual temperatures between 6 °C and 28 °C. Mean annual precipitation ranges from 1.100 to 1.200 mm, evenly distributed with a slight autumn peak. Relative humidity remains high (>75% in winter).	Southern metropolitan sedimentary basin—horticulture, fruit-growing, and viticulture
Artificial Forest (AF)	33.11217° S, 55.48317° W	The study area and commercial forestry plantations. Anthropogenic habitats include roadside vegetation and edge environments within plantations. Native forest and natural habitats include grasslands, wetlands, and riparian forests. The arboreal layer is dominated by <i>Sebastiania commersoniana</i> , <i>Pouteria salicifolia</i> , and <i>Blepharocalyx salicifolius</i> ; the shrub layer by <i>Pavonia sepium</i> ; and the herbaceous layer by <i>Dichondra microcalyx</i> . Forestry plantations are composed mainly of <i>Eucalyptus dunii</i> and <i>Eucalyptus grandis</i> . The site interacts with surrounding cropping systems, particularly soybean cultivation in adjacent fields.	Warm temperate climate with summer maxima above 27 °C (up to ~30 °C) and winter temperatures around 6–15 °C. Annual precipitation ranges from 1.000 to 1.200 mm, with a peak in February.	Central crystalline shield—mixed livestock, agriculture, and forestry

Table A1. Cont.

Site/Code	Geographic Location	Landscape Context and Land Use	Climate Median Description	Ecological–Biophysical Unit
Dairy (D)	34.119067° S, 56.407631° W	Agricultural landscape dominated by dairy production systems. Pastures consist mainly of legume-based associations (<i>Trifolium repens</i> , <i>Trifolium pratense</i> , <i>Lotus</i> spp.). interspersed with riparian woodlands and ruderal vegetation along roadsides. <i>Shelterbelts of eucalyptus</i> are common.	Humid subtropical climate with temperatures ranging from 5 °C to 31 °C. Rainfall occurs year-round. with maxima in autumn and minima in winter.	Central crystalline shield—livestock and dairy production
Native Serrano Forest (NF)	34.3059° S, 55.2007° W	Mountainous landscape characterized by extensive native serrano forest. Vegetation structure varies along topographic gradients. with dense woody vegetation (shrubs and trees up to 3 m) in the lower. protected areas. and sparse. shrub-dominated vegetation at higher elevations. The system includes grazed natural grasslands and plant communities adapted to shallow. nutrient-poor soils (such as lichens, cacti, hardy grasses, and ferns).	Warm subtropical mountain climate with average temperatures of 25 °C in summer and 13 °C in winter. Annual precipitation ranges from 800 to 1.000 mm, with maximum rainfall in April and minimum in December.	Eastern hill systems—livestock and forestry influence

Appendix B

Conceptual framework and calculation of adult-to-brood ratio metrics in relation to interacting drivers.

This study adopted a landscape-scale comparative approach in which each production system (horticulture, soybean, dairy, artificial forest, and native forest) was treated as an ecological category rather than as a statistically replicated experimental unit. Because sampling sites were not spatially replicated within each landscape type, the analyses do not support strict causal inference but instead identify associations between landscape context and colony demographic responses.

Mean demographic trajectories were constructed for each landscape using the adult-to-brood ratio as an indicator of colony demographic structure. Demographic-response metrics were estimated following the conceptual resilience framework proposed by Middleton et al. (2016) [16]. Four key temporal points were identified along each trajectory: (i) baseline (t_0), corresponding to the initial sampling point; (ii) perturbation onset (t_d), defined as the first decline in population size relative to baseline; (iii) minimum population size (t_r), representing the maximum perturbation; and (iv) recovery (t_c), defined as the first time point after t_r at which population size equalled or exceeded the baseline value.

Based on these points, three demographic-response metrics were calculated: (1) population decline ($N(t_0) - N(t_r)$); (2) recovery time ($t_c - t_r$), expressed as the number of sampling intervals between the minimum population size and recovery; and (3) recovery magnitude ($N(t_{end})/N(t_0)$), representing the extent of recovery relative to the initial state.

Demographic trajectories were graphically represented using smoothed curves, baseline reference lines (t_0), vertical markers for t_d , t_r , and t_c , and shaded areas indicating the resistance, reorganization, and recovery phases. Smoothing was applied exclusively for visualization purposes, whereas all demographic-response metrics were calculated from observed mean values.

Chemical pressure, quantified by cumulative hazard quotients (HQ) and the number of detected pesticide compounds, was considered a potential perturbation factor. The onset of perturbation (t_d) was identified as the first decline in population size relative to baseline that

coincided with periods of increased chemical pressure. However, demographic changes may also reflect seasonal dynamics, climatic variability, resource availability, and colony health conditions.

A detailed description of the identification of temporal points (t_0 , t_d , t_r , and t_c) and the calculation of demographic-response metrics is provided in Appendix B.

Identification of temporal points and calculation of demographic-response metrics.

Demographic-response metrics were calculated from mean demographic trajectories constructed for each landscape across sampling periods. Colony demographic structure was quantified using the adult-to-brood ratio, and landscape-level mean values at each sampling time were used to identify key temporal points and calculate comparative demographic-response metrics.

Identification of temporal points

Four temporal points were identified along each landscape-level trajectory:

Baseline (t_0): initial sampling point before an observable population decline.

Perturbation onset (t_d): first sampling point at which population size declined relative to baseline ($N(t) < N(t_0)$).

Minimum population (t_r): lowest observed population value after t_d , representing the maximum demographic impact of the perturbation.

Recovery (t_c): first sampling point after t_r at which population size equalled or exceeded the baseline value ($N(t) \geq N(t_0)$). When recovery was not observed during the study period, t_c was not assigned.

Demographic-response metrics

Three demographic-response metrics were calculated:

Population decline: $N(t_0) - N(t_r)$

Recovery time: $t_c - t_r$, expressed as the number of sampling intervals between the minimum population and recovery.

Recovery magnitude: $N(t_{\text{end}})/N(t_0)$, where $N(t_{\text{end}})$ is the population size at the final sampling event.

Interpretation criteria

A demographic decline was identified when the observed mean population size at a sampling point was lower than the baseline value. Chemical pressure, represented by cumulative hazard quotients (HQ) and the number of detected pesticide compounds, was considered one potential perturbation factor. Accordingly, t_d was defined as the first demographic decline relative to baseline occurring during periods of increased chemical pressure. However, demographic changes may also reflect seasonal dynamics, climatic variability, resource availability, and colony health conditions.

No interpolation was applied to missing values. Trajectories and demographic-response metrics were based exclusively on observed mean values and were calculated only when sufficient information was available to identify the corresponding temporal points. If recovery was not observed before the end of the study, t_c remained undefined.

Landscape-level trajectories were generated by averaging colony values within each sampling period. Consequently, all demographic-response metrics represent average landscape responses rather than colony-specific estimates.

The trajectories were used to produce the conceptual demographic-response curves shown in Figure 4. Smoothed curves were included solely for visualization, whereas all temporal points and demographic-response metrics were identified and calculated from the observed mean values.

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