



Neonicotinoids and decline in bird biodiversity in the United States

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Neonicotinoid insecticides are being widely used and have raised concerns about negative impacts on non-target organisms. However, there has been no large-scale, generalizable study on their impact on biodiversity of avian species in the United States. Here we show, using a rich dataset on breeding birds and pesticide use in the United States, that the increase in neonicotinoid use led to statistically significant reductions in bird biodiversity between 2008 and 2014 relative to a counterfactual without neonicotinoid use, particularly for grassland and insectivorous birds, with average annual rates of reduction of 4% and 3%, respectively. The corresponding rates are even higher (12% and 5%, respectively) when the dynamic effects of bird population declines on future population growth are considered. The effects of neonicotinoids on non-grassland and non-insectivorous birds are also statistically significant but smaller, with an average annual rate of reduction of 2% over this period.

Bird biodiversity is declining at alarming rates, and it is estimated that the bird population in the United States has decreased by 29% since 1970 (refs. 1–3). Among avian species, grassland birds have declined faster than any other guild in recent decades, with a 53% decrease since 1970 (refs. 3,4). These changes have been attributed to various factors, including the intensification of agricultural production with increasing use of pesticides⁵, land-use changes at the extensive margin due to the continued conversion of grassland acres^{3,6} and climate change⁷.

Although overall pesticide use has been declining in the United States, the types of synthetic insecticides applied to agricultural land have shifted over the past two decades from predominantly organophosphorus and *N*-methyl carbamate pesticides to a mix dominated by neonicotinoids⁵. Figure 1a,b shows that the use of neonicotinoids has grown exponentially, while that of non-neonicotinoids has declined relative to the peak level in 1996. Although neonicotinoids are applied at lower rates per acre than non-neonicotinoids, they are considerably more toxic to insects and generally persist longer in the environment⁵.

Studies have raised concerns about the environmental risks of neonicotinoids for non-target species exposed to them^{5,8,9}. Numerous laboratory and field studies have confirmed substantial negative impacts of neonicotinoid insecticides on honeybees, wild bees and butterflies^{8,10–14}. Many studies have noted that the toxicity of neonicotinoids can affect other non-target species as well, including birds, aquatic invertebrates and mammals^{15–19}. Birds that feed on crop seeds as well as aquatic and emergent insects are widely exposed to neonicotinoid application. The consumption of even one or two neonicotinoid-treated seeds by birds and mammals is likely to cause chronic risks for their reproduction and development^{20–23}. In addition to directly affecting the birds that have direct exposure to them, these pesticides can have long-term effects on bird population and diversity by reducing the number of birds left to reproduce. These studies provide some support for a causal relationship between neonicotinoid use and the decrease in bird biodiversity according to the Bradford Hill criteria²⁴, as discussed further in the Methods. However, there has been no large-scale, generalizable study quantifying the causal

impact of neonicotinoids and their toxicity on bird biodiversity at the national scale in the United States.

The purpose of this Article is to examine the causal effects of neonicotinoid use and compare them with the effects of other pesticide use on four bird species groups: grassland birds, non-grassland birds, insectivorous birds and non-insectivorous birds in the United States. We analyse the static effect of neonicotinoids on bird populations as well as the dynamic and persistent effect caused by neonicotinoids reducing the sizes of bird populations and thus their reproductive capacity. Since the effects of exposure to pesticides are likely to differ across avian species¹⁷, we analyse the effects on three measures of biodiversity: bird population, species richness and species diversity. These metrics have been widely used to study the factors that affect bird biodiversity^{25,26}; however, no previous study has used the same statistical framework to examine the effects of pesticides on all three measures. We compile a panel dataset from the North American Breeding Bird Survey (BBS) for 29 species of grassland birds, 637 species of non-grassland birds, 36 species of insectivorous birds and 631 species of non-insectivorous birds from 2,953 routes for the contiguous United States from 2008 to 2014 (see Supplementary Table 1 for the list of grassland birds and insectivorous birds).

Results

The three measures of bird biodiversity used in this study are (1) bird population, measured by the number of birds observed; (2) species richness, measured by the number of bird species observed; and (3) species evenness, measured by the Shannon index of diversity, which takes the relative abundances of different species into account (see ‘Data’). We examine the static and dynamic effects of neonicotinoid use while controlling for a number of other factors that are likely to influence bird species, including land use (cropland and urban land), use of non-neonicotinoid pesticides, breeding season and past winter monthly mean temperature and precipitation, and human population density^{27,28} (see ‘Data’ for details).

We undertake our analysis at the county level by aggregating route-level data over 2,448 counties in the United States to match the available data on pesticide use at the county level from the US

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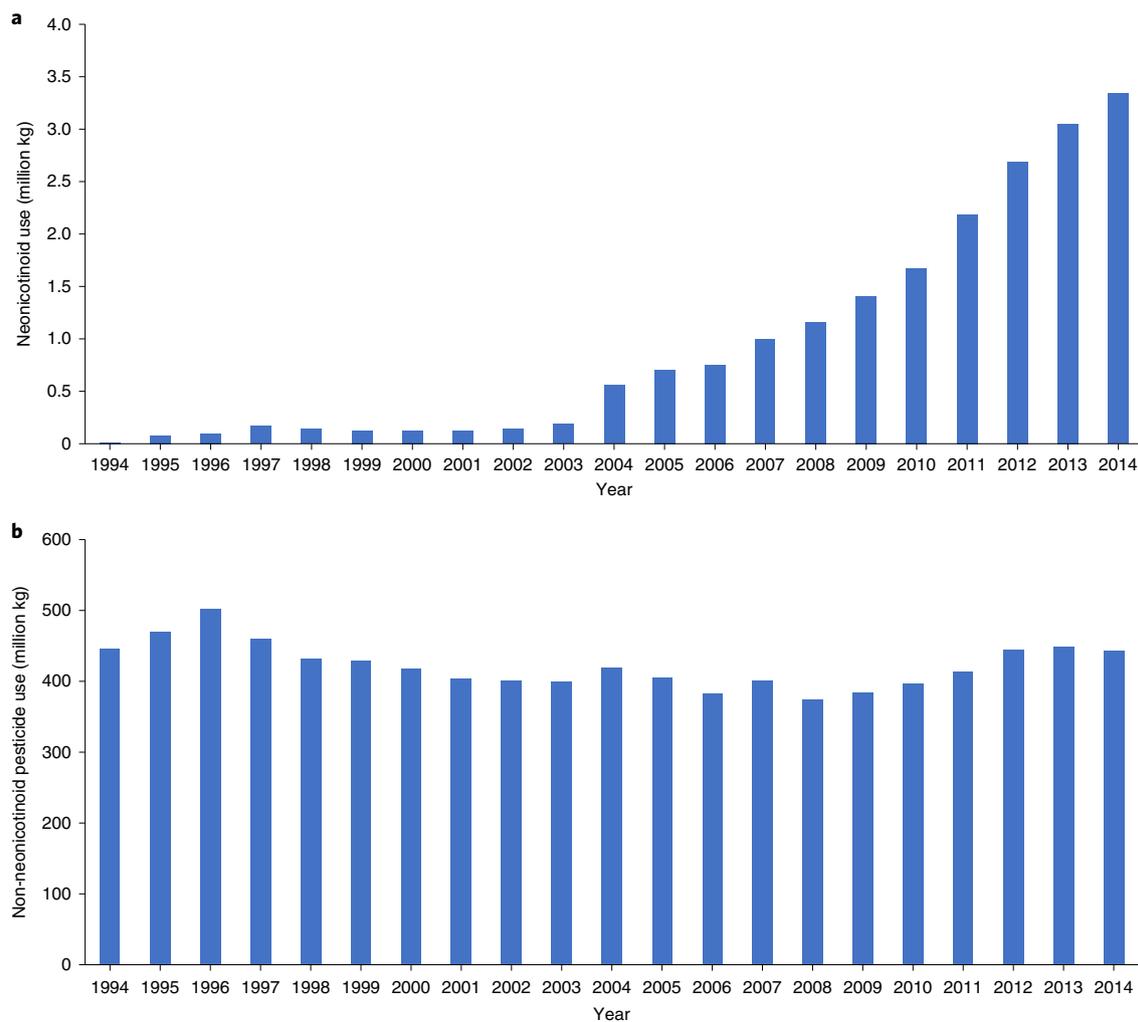


Fig. 1 | Trends in total pesticide use in the United States. a, Total neonicotinoid use in the United States from 1994 to 2014. **b**, Total non-neonicotinoid pesticide use in the United States from 1994 to 2014.

Geological Survey (USGS) over the 2008–2014 period (see summary statistics of the data at the county level in Supplementary Table 2; and see Supplementary Table 3 for toxicity range of neonicotinoid pesticides). Our analysis is restricted to this period because land-use data from the Cropland Data Layer (CDL) provided by the National Agricultural Statistics Service (NASS) of the US Department of Agriculture (USDA) are available at the national scale from 2008 onwards, and data on neonicotinoid use from the USGS were discontinued after 2014 (ref. ²⁹).

The causal effects of pesticide use and land use on bird biodiversity could be confounded by unobserved factors that may also affect bird biodiversity metrics, such as types of vegetation or land management practices. We conduct the Durbin–Wu–Hausman test for endogeneity of pesticide use, cropland acreage and developed land acreage. We find that we can reject the null hypothesis of exogeneity of pesticide use and cropland acreage but not of developed land acreage (Supplementary Tables 4 and 5). We therefore analyse the effect of neonicotinoid use on bird biodiversity by estimating a fixed-effects panel data model with instrumental variables for pesticide use and cropland use (see Methods for details). We estimate Moran’s *I* statistic to test for spatial autocorrelation in the error term and reject the null hypothesis of no spatial autocorrelation (see Supplementary Tables 4 and 5 for the details). We therefore apply Conley spatial standard error models

to control for spatial autocorrelation³⁰; these standard errors are reported together with the unadjusted standard errors in the regression results tables in the Supplementary Information. Our results on the significance of parameter estimates are robust to the type of standard error used.

We test the robustness of our findings by estimating alternative specifications and regression models. Specifically, we consider different toxicities of the various neonicotinoid insecticides and analyse the effects of a toxicity-weighted measure of neonicotinoid use on bird biodiversity (Supplementary Tables 6 and 7). To do so, we construct a toxicity-weighted sum of neonicotinoid use as the new explanatory variable, where the weights are based on the toxicity of each type of neonicotinoid ingredient relative to a benchmark toxicity (see Supplementary Table 3 and ‘Data’ for details). The benchmark is set to be the toxicity of the most toxic ingredient, which is given a weight of 1; ingredients with lower toxicities have weights less than 1 (see the last column in Supplementary Table 3). Thus, one unit of toxicity-weighted neonicotinoid use is equivalent to one unit of the most toxic neonicotinoid.

Since pesticide data at the county level can be noisy³¹, we also undertake our analysis by aggregating the data to the crop reporting district level (see summary statistics of the data at the crop reporting district level in Supplementary Table 8 and regression results in Supplementary Tables 9–16). To account for the small-integer,

Table 1 | Impact of pesticide use on grassland bird and non-grassland bird biodiversity

% change in grassland bird diversity			% change in non-grassland bird diversity		
Population	Species richness	Shannon index	Population	Species richness	Shannon index
Using county-level data					
Due to 100 kg increase per county in:					
Aggregate neonicotinoid use					
-2.2 ± 1.1%	-0.5 ± 0.3%	-0.4 ± 0.2%	-1.4 ± 0.6%	-0.5 ± 0.3%	-0.05 ± 0.07%
Aggregate non-neonicotinoid use					
-0.05 ± 0.03%	-0.01 ± 0.01%	-0.01 ± 0.01%	-0.03 ± 0.01%	-0.01 ± 0.01%	<0.01%
Aggregate pesticide use					
-0.05 ± 0.03%	-0.01 ± 0.00%	-0.01 ± 0.01%	-0.03 ± 0.01%	-0.01 ± 0.01%	<0.01%
Toxicity-weighted neonicotinoid use (normalized to the most toxic chemical)					
-5.1 ± 2.7%	-1.2 ± 0.8%	-1.0 ± 0.9%	-3.3 ± 1.4%	-1.1 ± 0.6%	-0.1 ± 0.2%
Using crop-reporting-district-level data					
Due to 100 kg increase per county in:					
Aggregate neonicotinoid use					
-2.4 ± 1.4%	-0.03 ± 0.11%	-0.08 ± 0.07%	-2.0 ± 0.8%	-0.03 ± 0.05%	<0.01%
Aggregate non-neonicotinoid use					
-0.06 ± 0.05%	<0.01%	<0.01%	-0.04 ± 0.02%	<0.01%	<0.01%
Aggregate pesticide use					
-0.06 ± 0.04%	<0.01%	<0.01%	-0.04 ± 0.02%	<0.01%	<0.01%
Toxicity-weighted neonicotinoid use (normalized to the most toxic chemical)					
-5.9 ± 3.6%	-0.06 ± 0.27%	-0.2 ± 0.2%	-4.8 ± 1.9%	-0.1 ± 0.1%	<0.01%

Numbers after ± specify the 95% confidence intervals. For the crop reporting district results, we report the percentage changes using the county-level mean values of biodiversity measures for the convenience of comparison.

non-negative nature of the data for species richness, we also estimate a fixed-effect instrumental variable Poisson model to test the robustness of our results³² (Supplementary Table 17).

Static effects of pesticide use. The upper panel of Table 1 presents a summary of results on the static impact of neonicotinoid use on grassland birds and non-grassland birds obtained from regressions estimated at the county level and shown in Supplementary Tables 4 and 6. The corresponding results for insectivorous and non-insectivorous birds are presented in the upper panel of Table 2, and the regression coefficients are shown in Supplementary Tables 5 and 7. To facilitate the narrative, we convert the estimated coefficients into semi-elasticities with respect to pesticide use (evaluated at the sample mean) at the county level and report the percentage impact of a 100 kg increase in pesticide use per county on bird diversity measures in Tables 1 and 2.

We find that neonicotinoid use has a negative and statistically significant impact on the population of each of the four groups of birds (Tables 1 and 2). We estimate that, holding everything else constant, a 100 kg increase in neonicotinoid use per county (which represents a 12% increase on average) contributes to a 2.2% (±1.1%) decrease in the grassland bird population and a 1.4% (±0.6%) decrease in the non-grassland bird population; see the first row in the upper panel in Table 1 (the numbers after ± in the parentheses specify the 95% confidence intervals). The increase in neonicotinoid use also leads to a decrease in the species richness and evenness of grassland birds. More specifically, a 100 kg increase in neonicotinoid use causes about a 0.5% (±0.3%) decrease in grassland and non-grassland species richness (roughly 0.02 species) and a 0.4% (±0.2%) decrease in grassland species evenness (measured by the Shannon index); the corresponding effect on the Shannon index for non-grassland species is not statistically significant. This negative impact on species

evenness reflects heterogeneous impacts of neonicotinoids on different types of grassland species.

Neonicotinoid use also decreases insectivorous bird populations by 1.6% (±0.9%) and non-insectivorous bird populations by 1.5% (±0.6%) (see the first row in the upper panel in Table 2). In addition, a 100 kg increase in neonicotinoid use causes a 0.5% (±0.3%) decrease in non-insectivorous bird richness. The impacts of neonicotinoid use on insectivorous species richness and evenness and on non-insectivorous species evenness are negative but statistically insignificant.

We compare the effects of neonicotinoid use on bird biodiversity with those of non-neonicotinoid pesticide use (and similarly of aggregate pesticide use) by estimating separate specifications that include each of the latter (non-neonicotinoid pesticides or aggregate pesticides) as an explanatory variable (Supplementary Tables 18–21). Supplementary Tables 18 and 19 show that the signs and statistical significance of the coefficient of non-neonicotinoid pesticide use (or, separately, of aggregate pesticide use) on the four groups of birds are similar to those for neonicotinoid use, but the overall impact per 100 kg of use is substantially smaller (Tables 1 and 2). Since non-neonicotinoid constitutes nearly 99% of total pesticide use in terms of quantity in the United States over the studied period, these effects of non-neonicotinoid use on bird populations are similar to those of aggregate pesticide use (see the third row in the upper panel in Table 1). Specifically, a 100 kg increase in non-neonicotinoid pesticide use (which represents a 0.06% increase on average) in a county contributes to a 0.05% (±0.03%) decrease in the grassland bird population and a 0.03% decrease in bird populations for the other three groups in the county, holding everything else constant (see the second row in the upper panel of Tables 1 and 2). Note that an increase by the same amount in neonicotinoid use has an effect more than 40 times larger than this (2.2% versus 0.05% and 1.4% versus 0.03%).

Table 2 | Impact of pesticide use on insectivorous bird and non-insectivorous bird biodiversity

% change in insectivorous bird diversity			% change in non-insectivorous bird diversity		
Population	Species richness	Shannon index	Population	Species richness	Shannon index
Using county-level data					
Due to 100 kg increase per county in:					
Aggregate neonicotinoid use					
-1.6 ± 0.9%	-0.08 ± 0.4%	-0.1 ± 0.2%	-1.5 ± 0.6%	-0.5 ± 0.3%	-0.07 ± 0.07%
Aggregate non-neonicotinoid use					
-0.03 ± 0.02%	<0.01%	<0.01%	-0.03 ± 0.02%	-0.01 ± 0.02%	<0.01%
Aggregate pesticide use					
-0.03 ± 0.02%	<0.01%	<0.01%	-0.03 ± 0.01%	-0.01 ± 0.01%	<0.01%
Toxicity-weighted neonicotinoid use (normalized to the most toxic chemical)					
-3.8 ± 2.2%	-0.2 ± 0.9%	-0.2 ± 0.5%	-3.5 ± 1.4%	-1.1 ± 0.6%	-0.2 ± 0.2%
Using crop-reporting-district-level data					
Due to 100 kg increase per county in:					
Aggregate neonicotinoid use					
-2.3 ± 0.9%	-0.03 ± 0.09%	-0.01 ± 0.04%	-2.0 ± 0.8%	-0.08 ± 0.07%	<0.01%
Aggregate non-neonicotinoid use					
-0.04 ± 0.02%	<0.01%	<0.01%	-0.04 ± 0.02%	<0.01%	<0.01%
Aggregate pesticide use					
-0.04 ± 0.02%	<0.01%	<0.01%	-0.04 ± 0.02%	<0.01%	<0.01%
Toxicity-weighted neonicotinoid use (normalized to the most toxic chemical)					
-5.5 ± 2.3%	-0.08 ± 0.21%	-0.02 ± 0.09%	-4.9 ± 1.9%	-0.2 ± 0.2%	<0.01%

Numbers after ± specify the 95% confidence intervals. For the crop reporting district results, we report the percentage changes using the county-level mean values of biodiversity measures for the convenience of comparison.

The impact of the toxicity-weighted neonicotinoid use is qualitatively similar in sign and significance to that of unweighted neonicotinoid use (Supplementary Tables 6 and 7). However, the magnitudes of the effects are different. As shown in Table 1, an increase of 100 kg of the most toxic neonicotinoid leads to a 5.1% ($\pm 2.7\%$) reduction in the population of grassland birds and a 3.8% ($\pm 2.2\%$) decrease in the population of insectivorous birds (see the fourth row in the upper panel of Tables 1 and 2). The corresponding negative effects on non-grassland and non-insectivorous birds were smaller at 3.3% ($\pm 1.4\%$) and 3.5% ($\pm 1.4\%$), respectively.

Impact of cropland expansion. We find that cropland expansion also has a negative effect on all four groups of birds (Supplementary Tables 4 and 5). On average, the effect of a 1% increase in cropland acreage is a decrease in grassland bird population ranging from 1.4% to 3.5% across the various regressions estimated when evaluated at the sample means (see the elasticities in the final row of Supplementary Tables 4, 5 and 18–21). The corresponding negative effects on insectivorous birds range from 1.7% to 3.2%. The expansion of cropland also has negative and statistically significant impacts on the species richness of both grassland birds and insectivorous birds. On average, a 1% increase in cropland acreage decreases the species richness of grassland birds by about 0.7% to 1.2% and of insectivorous birds by 0.4% across the specifications estimated. Our finding of the effects of land cover on species richness is comparable to some previous results that also show that a 1% increase in high-input but low-diversity crops (corn and soybeans) in the Midwest contributes to a decline of 0.55% to 0.77% in total avian species richness²³. We also find that the species evenness of grassland birds decreases by 0.8% to 1.1% in response to a 1% increase in cropland. However, we do not find that insectivorous bird species evenness is responsive to cropland acreage. The negative effect of a

1% increase in cropland acreage on non-grassland bird population ranges from 0.8% to 2.1% on average across the various regressions estimated when evaluated at the sample means. The corresponding negative effects on non-insectivorous birds range from 0.6% to 2.1%. We do not find that non-grassland bird and non-insectivorous bird species richness and evenness are responsive to cropland acreage.

Crop-reporting-district-level analysis. We find that the effects of neonicotinoid use on the four groups of bird species are robust to aggregation of the data to the crop reporting district level (see the lower panels of Tables 1 and 2 and Supplementary Tables 9–16). For comparability with the county-level results in the upper panels in Tables 1 and 2, we again report the percentage impact of a 100 kg increase in pesticide use in a county on bird diversity measures in the county on the basis of regression results obtained from aggregated data at the crop reporting district level. We find that the sign and significance of the effect of neonicotinoids are the same as those obtained from regressions estimated using county-level data, and that the magnitude of the effect is also generally similar.

Dynamic effects of neonicotinoid use. Next, we estimate a dynamic panel data model using the generalized method of the moments-based Arellano–Bond estimator³³ that includes a one-year lagged dependent variable as an explanatory variable. Since the inclusion of a lagged dependent variable as a regressor violates strict exogeneity, as the lagged dependent variable is necessarily correlated with the idiosyncratic error term, we use further lags of the dependent variable as instruments for the one-year lagged dependent variable. Tests for the identification of the instrumental variables are reported in Supplementary Table 22. We find that there is a positive and statistically significant coefficient of the lagged dependent variable in the specifications estimated. We also find

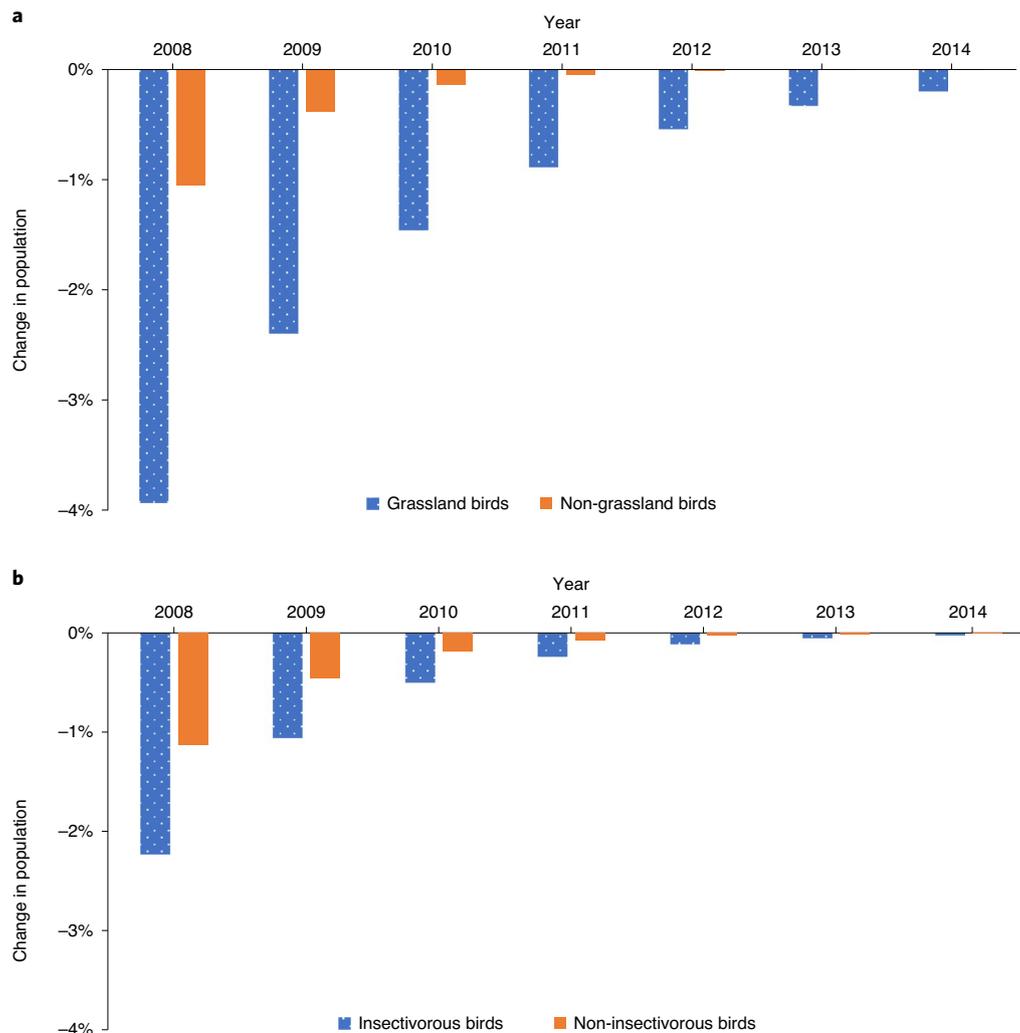


Fig. 2 | Dynamic effects on bird population due to a 100 kg increase of neonicotinoid use in 2008. a, Changes in populations of grassland birds and non-grassland birds due to a 100 kg increase in neonicotinoid use in 2008. **b,** Changes in populations of insectivorous birds and non-insectivorous birds due to a 100 kg increase in neonicotinoid use in 2008. We use dynamic model results and assume a one-time 100 kg increase in neonicotinoid use in 2008.

a negative and statistically significant effect of neonicotinoids on populations and other biodiversity measures of all four groups of bird species. The presence of the lagged dependent variable implies that the reduction in bird populations due to neonicotinoid use in one year affects the populations of birds in the following years as well (although at a diminishing rate), as shown in Fig. 2a,b. In the case of grassland birds, a 100 kg increase in neonicotinoid use per county in 2008 reduces the population of birds by almost 4% that year and cumulatively by 9.7% over the 2008–2014 period. The corresponding estimates for insectivorous birds are 2% and 4.2%, respectively. The effects on non-grassland and non-insectivorous birds are smaller, with a concurrent effect of 1% and a cumulative effect of 2%.

Effects of neonicotinoid use from 2008 to 2014 across the United States. We use the estimated parameters from the static and the dynamic regression analyses to quantify the effects of neonicotinoid use on birds both temporally and spatially across the United States from 2008 to 2014. The sample data plotted in Fig. 3 show that the populations of grassland birds, non-grassland birds, insectivorous birds and non-insectivorous birds have declined by 10%, 14%, 9% and 9%, respectively, over this period. The annual predicted values

of the four groups obtained from the static model are very close to the observed data. Although the fit of the predicted values obtained from the Arellano–Bond estimator is not as good as that of the linear fixed-effect model, the divergence between the predicted and observed values is small (by 2–6% in 2011–2013 across the various groups of bird species); an exception to this is in the case of grassland birds, where the predicted values diverge from the observed data by 12–14% in 2011–2013.

We find that on average the increase in neonicotinoid use over this period results in annual decreases of 4% and 3% in grassland bird and insectivorous bird populations, respectively, on the basis of the coefficients obtained in the static models reported in Supplementary Tables 4 and 5. The corresponding estimates from the dynamic models are 12% and 5% for grassland bird and insectivorous bird populations, respectively. The average annual reduction in non-grassland and non-insectivorous bird populations due to neonicotinoid use is much smaller at about 2% and is the same in both the static and the dynamic models.

We find considerable spatial heterogeneity in the magnitude of the decreases in bird populations due to neonicotinoid use from 2008 to 2014. The adverse impacts on bird populations are concentrated in the Midwest, Southern California and the Northern Great

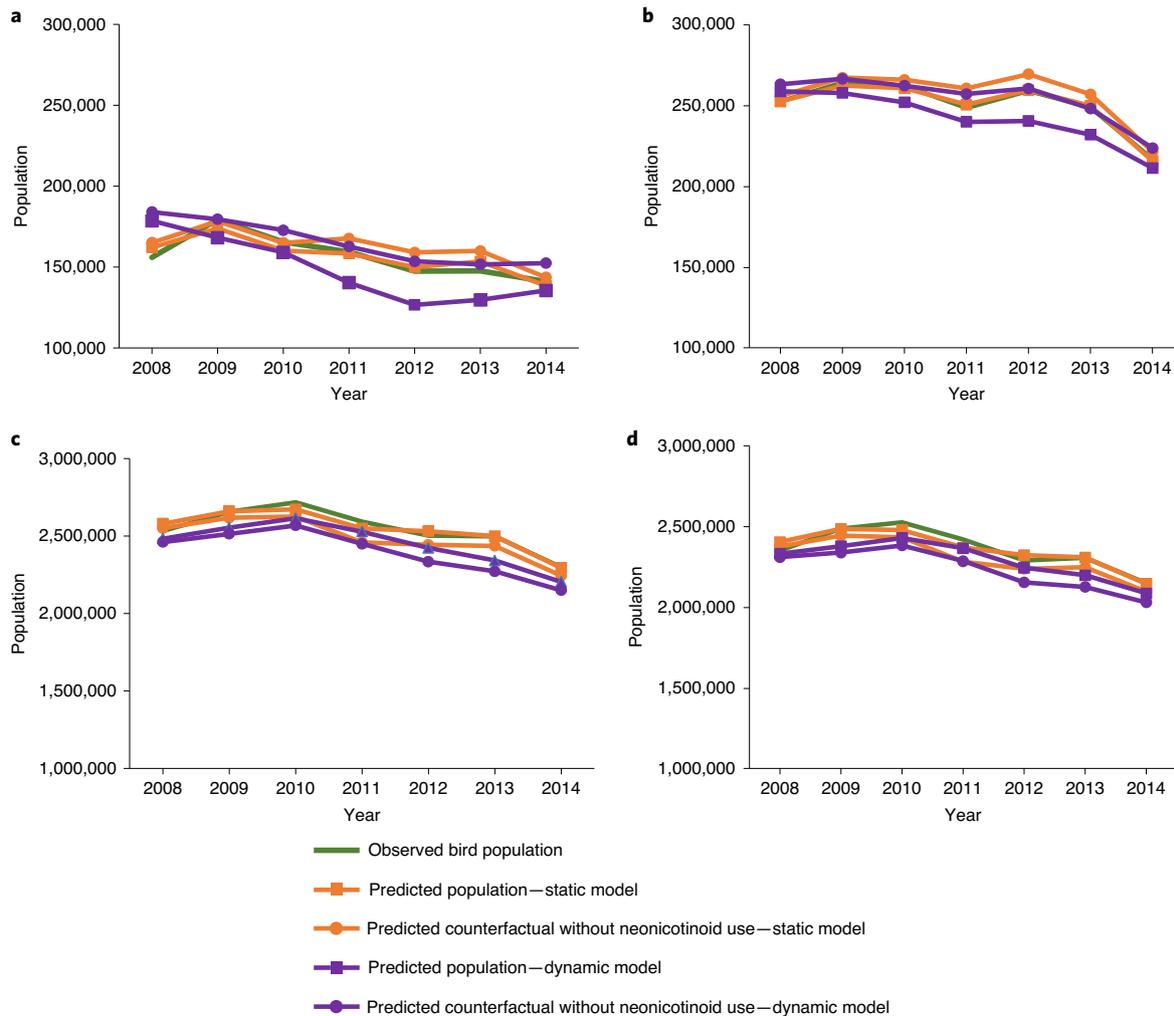


Fig. 3 | Predicted changes in bird populations due to an increase in neonicotinoid use and the observed bird populations in the United States from 2008 to 2014. a, Grassland birds. b, Insectivorous birds. c, Non-grassland birds. d, Non-insectivorous birds.

Plains (Fig. 4). Among the 1,491 counties in our grassland bird sample, 55% of them have a decrease in bird population of more than 10% due to neonicotinoid use by 2014 compared with 2008. For insectivorous birds, 35% of the 1,739 sampled counties have a decrease in bird population of more than 10%. We do find a few counties in the northeastern and western United States where the use of neonicotinoids decreased over this period, leading to a small positive impact on bird populations.

Discussion

Bird biodiversity has undergone a substantial decline in the United States over the past four decades, with land use implicated as a major factor⁴. Recent studies quantify the amount of the decline³, but empirical evidence on reasons for the decline is limited and pre-dates the expansion of neonicotinoid use. Recent experimental evidence indicates that neonicotinoids impair the biological functions of various bird species^{21–23}. Epidemiological studies have used the Bradford Hill criteria²⁴ to establish the cause–consequence relationships from experimental and observed information and make expert judgements on the verity of these relationships. While this approach can provide support for hypothesizing a cause–consequence relationship, gaps in the available experimental information can limit the extent to which these criteria can be used to

conclude a causal effect and rule out circumstantial evidence, as in the case of the application of these criteria to conclude a causal effect of neonicotinoids on honeybees³⁴. Our study relies on a rich panel dataset on neonicotinoids and bird populations to statistically isolate the effects of neonicotinoid use on bird biodiversity while controlling for the effects of other observed and unobserved confounding factors.

We find a negative and statistically significant impact of neonicotinoid use on the four groups of bird species examined here, with the effect being relatively larger for grassland bird and insectivorous bird populations. The average impact of a 100 kg increase in neonicotinoid use per county from 2008 to 2014 on grassland bird populations from the static model was 4% and on insectivorous bird populations was 3%. We also find that neonicotinoid use decreased species richness and diversity. These effects are substantially larger when the persistent effects of neonicotinoids on bird populations are considered through their reducing the number of birds left to reproduce. After incorporating this dynamic effect, we find that the average annual effect of neonicotinoids on grassland birds is 12% and on insectivorous birds is 5%.

The present study provides considerable evidence of the ecological risks of neonicotinoid insecticides to bird biodiversity, with particularly large negative effects on grassland and insectivorous bird

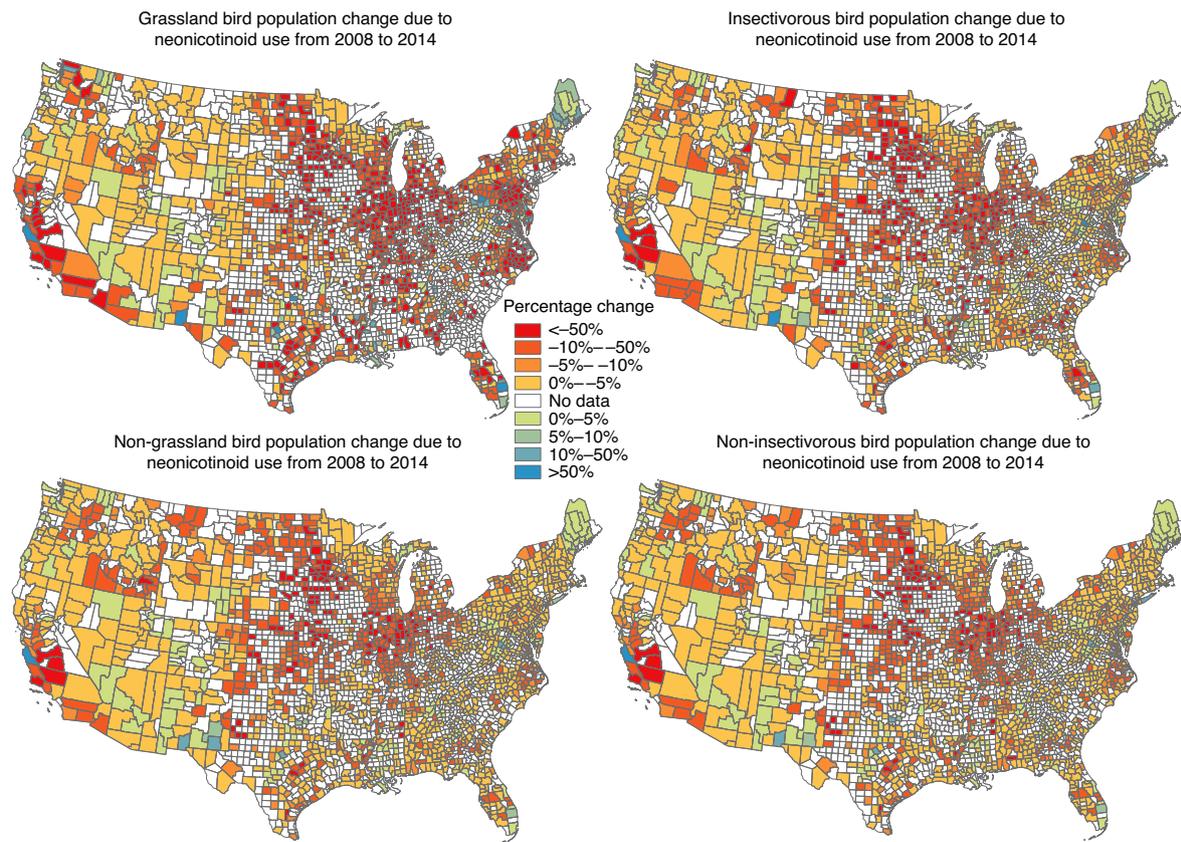


Fig. 4 | Changes in bird populations due to neonicotinoid use from 2008 to 2014. We use the following formula to calculate the change in bird population due to neonicotinoid use from 2008 to 2014 for county i : $(\text{neonicotinoid use}_{i,2014} - \text{neonicotinoid use}_{i,2008}) \times (\text{neonicotinoid use coefficient}) / (\text{bird population}_{i,2008}) \times 100\%$.

biodiversity. These adverse effects of neonicotinoids could emanate both directly from the consumption of coated crop seeds and indirectly from disruptions of the food supply for birds by affecting the insect populations on which they feed and by decimating the population of birds that can reproduce in the future. These indirect effects are difficult to identify via experiments. Therefore, by using a national-level dataset, the present study complements studies that employ a laboratory or field experiment approach to examine the impact of pesticides on biodiversity. This research provides compelling support for the re-evaluation of policies permitting the use of neonicotinoids by the US Environmental Protection Agency³⁵, particularly around bird habitats.

Methods

Our empirical analysis tests the hypothesis that neonicotinoid use leads to a reduction in bird biodiversity. In epidemiological studies, causality is established using nine criteria put forth by Bradford Hill³⁶. Numerous studies have provided evidence that several of these nine criteria are met when examining the causal relationship between neonicotinoid use and decreases in bird biodiversity. For instance, studies provided experimental evidence that neonicotinoids harm the migratory ability of white-crowned sparrow^{21,22}. Results from experiments show that neonicotinoids have detrimental impacts on the thyroid of red muna and hence negatively affect their breeding²³. These studies provide direct experimental evidence regarding the toxicity of neonicotinoids to birds, satisfying the experimental evidence criterion. In these experimental studies, the results show that larger doses of neonicotinoids cause more negative impacts, which supports the biological gradient criterion. Moreover, many studies show that neonicotinoids are toxic to non-targeted species^{17,36}. Therefore, the claim that neonicotinoids are toxic to birds does not contradict any existing knowledge, which meets the coherence criterion and supports the plausibility criterion. The analogy criterion is met by the existing findings¹⁹ that show that neonicotinoid use is associated with declines in insectivorous birds. On the basis of these studies, we specify the following regression models to estimate the causal effects of neonicotinoid use on bird biodiversity.

Empirical models. We first employ a fixed-effects panel data estimator to quantify the impact of pesticide use on birds. The reduced-form regression model for each bird biodiversity measure (that is, population, species richness and species evenness) is specified as follows:

$$y_{it} = b_0 + b_1 p_{it} + b_2 k_{it} + b_3 c_{it} + s_i + u_{it} \quad (1)$$

where y represents a biodiversity measure of grassland birds or insectivorous birds, p is pesticide use, k is the land-cover vector including cropland acreage and developed land acreage, c is a vector of other controls such as human population density and climate variables (including the monthly mean temperature and monthly precipitation over the breeding season (March to June) as well as the winter before the breeding season (January and the immediate past December)), s is the county fixed effects to control for time-invariant factors such as the geographical location and soil quality of a county, and u is the error term, which does not assume any specific distributional form. The subscript i stands for the county and t for the year.

We use cropland area as a measure of land cover that can affect bird biodiversity. While disturbance in land cover threatens the habitat suitability for grassland birds and negatively affects the bird population, cropland under hay and small grains can also provide nesting habitats for grassland birds or provide food sources for insectivorous birds³⁷. Therefore, the effect of cropland expansion on bird population and species is an empirical question. We also include developed land area in the regression models, since it may be another source of neonicotinoid contamination³⁸. Apart from the land-cover and pesticide-use variables, we include the monthly mean temperature and monthly precipitation from April to June because these three months are the breeding season for most of the grassland birds and insectivorous birds. The mean temperature in the breeding season is expected to be positively correlated with the abundance of grassland birds, while an increase in precipitation might disturb the breeding of grassland birds³⁷. In addition, since harsh winter conditions are likely to affect birds in various ways, we include precipitation and mean temperature in January and in the immediate past December³⁸. We also control for human population density in the regression analysis, because we expect that human population density is negatively correlated with bird biodiversity³⁹.

Two major statistical issues need to be addressed when estimating the model. The first is the endogeneity of pesticide use, because it might be correlated with

unobserved factors (for example, plant–insect interactions) that may affect bird biodiversity. We employ the pesticide price index as an instrumental variable for pesticide use; this is a valid instrumental variable because it is correlated with pesticide use but does not directly affect bird abundance. A meta-analysis confirms negative and significant own-price elasticities of demand for pesticides⁴⁰. One might be concerned that the pesticide price index may influence birds via affecting farmer's crop choices, as crop rotation and mixing are common tools to manage insect pests⁴¹. However, since pesticide expenditure accounts for only 5–15% of the total production costs, pesticide price should not be a major driver of changes in crop mix⁴². After controlling for land use and county fixed effects, we do not anticipate any direct effect of the pesticide price index on unobserved and time-varying factors that affect bird populations. We therefore believe that the pesticide price index is a valid instrument.

The second statistical issue is that land-use changes and bird populations are likely to be affected by unobservable landscape changes and plant–insect interactions across counties, resulting in biased estimates of the regression coefficients. To address this issue, we use one-year lagged fertilizer price index as the instrumental variable to identify the effects of cropland acreage. Fertilizer is an important input for agricultural production, and fertilizer price can affect crop acreage decisions by affecting the net returns from crop production and the relative returns of various crops^{43,44}. The USDA agricultural commodity cost and return data show that fertilizer expenditure makes up about 29% of the total operating costs for all major field crops and 37% of corn operating costs in the United States⁴¹. One concern is that fertilizer prices are likely to affect bird populations through fertilizer application, which is not controlled for in the regression, because fertilizer may affect birds via affecting plants and insects. However, there is no evidence of direct effects of fertilizer use on bird populations^{45,46}; in fact, researchers have found no substantial impact of fertilizer uses on grassland birds in central Hungary⁴⁷. It is therefore reasonable to believe that the fertilizer price index affects birds only through land coverage.

In the key models, we report the Cragg–Donald Wald F test statistic and the Kleibergen–Paap rk Wald F statistic for testing weak instruments for all the specifications using instrumental variables. We find that all the weak-instrument test statistics of the fixed-effect and instrumental-variable models are greater than the rule of thumb of ten, rejecting the null hypothesis that the excluded instruments are weakly correlated with the endogenous variables⁴⁸.

As a robustness check for our key model in equation (1) for species richness, we also specify a fixed-effect Poisson regression model⁴⁹:

$$y_{it} = \exp(b_0 + b_1 p_{it} + b_2 k_{it} + b_3 c_{it} + s_i) u_{it} \quad (2)$$

where y_{it} , conditional on the regressors, has a Poisson distribution. We did not employ negative binomial models because the negative binomial models cannot effectively control for fixed effects^{50,51}.

We also used the Arellano–Bond estimator for dynamic panel data models including lagged bird population, specified as follows:

$$y_{it} = b_0 + b_1 y_{i,t-1} + b_2 p_{it} + b_3 k_{it} + b_4 c_{it} + s_i + u_{it} \quad (3)$$

The estimator then uses the level or difference of further lagged dependent variables as instruments of the lagged dependent variables⁵². We use the same instrumental variables (pesticide price index and lagged fertilizer price index) for pesticide use and cropland acreage. For each bird group sample, we reject the first-order autocorrelation null hypothesis and cannot reject the second-order auto-correlation null hypothesis at 5%, indicating that the error term in equation (3) is not autocorrelated. Moreover, all four specifications passed the Hansen test of overidentification restrictions.

Data. All the data we use in the analysis are from publicly available sources, and the summary statistics are presented in Supplementary Table 2.

Bird data. We obtain the number of grassland birds by species from 2008 to 2014 from the BBS maintained by the Patuxent Wildlife Research Center of the USGS (ref. ⁵³). We identified 29 grassland bird species and 36 insectivorous bird species, following previous studies^{53,54} (Supplementary Table 1). The BBS is usually conducted in June, and participants skilled in avian identification collect bird population data along survey routes. See Supplementary Fig. 1 for the geographical distribution of these survey routes in the contiguous United States. There are over 4,100 survey routes in the United States and Canada, and each survey route is about 24.5 miles long with stops at 0.5-mile intervals. At each stop, a participant conducts a three-minute count, recording all birds heard or seen within a 400-meter radius around the stop.

Bird population abundance is calculated by aggregating the bird count of a group of birds (for example, grassland birds) observed along a survey route. Species richness is measured by the total number of species observed around a route. Species evenness is measured using the Shannon index, which is calculated by $-\sum_j a_j \ln(a_j)$, where a_j is the proportion of the j th species population over the total population of all the species within a species group. Note that the Shannon index is nonlinear in both bird population and number of species⁵⁵.

The county-level data were aggregated from all the survey routes in a county on the basis of the route geo-locations provided by the BBS (Supplementary Fig. 1). If a survey route spans two or more counties, we multiply the number of birds observed at each route by the share of the route length within a county to calculate the bird count from this route for the county. The panel is unbalanced because not all routes are surveyed each year. Note that this aggregation may cause measurement error in the county-level data because some routes might not be observed in some years. However, as long as the measurement error is independent of the bird count data, which is probably the case with the BBS data because the data collection largely relies on the (random) availability of volunteer bird observers, it will not bias the estimated coefficients.

Explanatory variables and instrumental variables. The county-level agricultural pesticide use data are obtained from the USGS Pesticide National Synthesis Project²⁹. The dataset reports the aggregate annual mass applied for each neonicotinoid active ingredient, including acetamiprid, clothianidin, imidacloprid, dinotefuran, thiacloprid and thiamethoxam. Since the USGS stopped reporting seed treatment applications of pesticide use in 2015 (ref. ²⁹), we limit our sample up to 2014 for consistency. For every county (excluding those in California), the data include values of active ingredients under EPest-low and EPest-high, which differ in their ways of treating missing values in pesticide–crop combinations. Specifically, EPest-low treats the missing values as zero, while EPest-high extrapolates the values from neighbouring crop reporting districts. We use the EPest-high values in the main results because there are fewer missing observations. For counties in California, the EPest-low and EPest-high values are the same because the county total pesticide uses are directly obtained from the California Department of Pesticide Regulation's Pesticide Use Report database⁵¹. For aggregate pesticide use and non-neonicotinoid pesticides, we aggregate all 423 and 417 types of pesticide uses, respectively, in each county in each year from 2008 to 2014.

Because not every neonicotinoid ingredient has the same toxicity, we construct the toxicity-weighted neonicotinoid use on the basis of the neonicotinoid bird toxicity range (LD₅₀ or median lethal dose) from the University of Florida Institute of Food and Agricultural Sciences website⁵⁶. The median lethal dose is defined as the ratio of the weight of the neonicotinoid ingredient to the body weight of the dosed animal (mg kg⁻¹), for a 50% mortality rate. Since the toxicity is given in a range of lethal doses, we take the middle point of the range and normalize neonicotinoid use to the most toxic neonicotinoid ingredient equivalence quantity (see Supplementary Table 3 for the detailed ranges and weights). We weight the county-level use using the inverse of the toxicity value for each neonicotinoid active ingredient.

The areas of cropland and developed land within each county from 2008 to 2014 are constructed from the CDL provided by the NASS of the USDA (ref. ⁵⁷). We aggregate CDL land areas under the categories of row crops, vegetables and tree crops to the county level. Monthly precipitation and mean temperature data between 2008 and 2014 at the county level are obtained from the Parameter-Elevation Regression on Independent Slopes Model (PRISM) provided by Oregon State University⁵⁸. County-specific human population density data from 2008 to 2014 were obtained from the Population and Housing Unit Estimates Datasets of the US Census Bureau⁵⁹. The annual national-level fertilizer price index from 2008 to 2014 is obtained from the USDA Economic Research Service data service product⁶⁰. The annual pesticide price index is from the NASS of the USDA.

Data availability

All data compiled for this study are publicly available and are available at <https://github.com/jewelli/Neonics-birds> to replicate the findings in this manuscript. Source data are provided with this paper.

Code availability

The code to replicate all the regression analyses is available at <https://github.com/jewelli/Neonics-birds>.

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Author contributions

All authors contributed to the design of the empirical methods and the writing of the manuscript. Y.L. collected the data and conducted the regression analysis.

Competing interests

The authors declare no competing interests.

Additional information

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