

# Declines in insectivorous birds are associated with high neonicotinoid concentrations

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**Recent studies have shown that neonicotinoid insecticides have adverse effects on non-target invertebrate species<sup>1–6</sup>. Invertebrates constitute a substantial part of the diet of many bird species during the breeding season and are indispensable for raising offspring<sup>7</sup>. We investigated the hypothesis that the most widely used neonicotinoid insecticide, imidacloprid, has a negative impact on insectivorous bird populations. Here we show that, in the Netherlands, local population trends were significantly more negative in areas with higher surface-water concentrations of imidacloprid. At imidacloprid concentrations of more than 20 nanograms per litre, bird populations tended to decline by 3.5 per cent on average annually. Additional analyses revealed that this spatial pattern of decline appeared only after the introduction of imidacloprid to the Netherlands, in the mid-1990s. We further show that the recent negative relationship remains after correcting for spatial differences in land-use changes that are known to affect bird populations in farmland. Our results suggest that the impact of neonicotinoids on the natural environment is even more substantial than has recently been reported and is reminiscent of the effects of persistent insecticides in the past. Future legislation should take into account the potential cascading effects of neonicotinoids on ecosystems.**

Although concerns have been raised about the direct effects of neonicotinoids on non-target vertebrate species<sup>8</sup>, neonicotinoids are in general thought to be less harmful to mammals and birds than to insects. The main mode of action of neonicotinoids occurs through binding nicotinic acetylcholine receptors in the central nervous system of invertebrates<sup>9</sup>, and neonicotinoids bind with substantially less affinity to these receptors in vertebrates<sup>10</sup>. This property has made neonicotinoids highly favoured agrochemicals worldwide over the past two decades<sup>11</sup>. In the Netherlands, imidacloprid was first administered by the Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) in August 1994. Annual use increased rapidly from 668 kg in 1995 to 5,473 kg in 2000 and 6,332 kg in 2004 (ref. 12). Since 2003, imidacloprid has ranked consistently in the top three pesticides that exceed the environmental concentrations permitted by quality standards in the Netherlands<sup>4,13</sup>.

As neonicotinoids have relatively long half-lives in soil and are water soluble, they have the potential to accumulate in soils and to leach into surface water and ground water. Their systemic property (that is, their ability to spread through all of the tissues of the plants under treatment), together with their widespread use, indicates that many organisms in agricultural environments are likely to become exposed<sup>8</sup>. Indeed, studies have shown, both in experimental and in field conditions, that neonicotinoids may affect non-target invertebrate species across terrestrial and aquatic ecosystems<sup>4–6</sup>. The question remains, however, whether the effects are sufficiently severe to affect ecosystems through trophic interactions: that is, beyond the direct lethal and sublethal effects on individual species. In the past, the introduction of insecticides has caused prey-base collapses, which in turn affected avian populations<sup>14–16</sup>, showing that pesticide-induced declines in invertebrate densities can cause food deprivation for birds. Thus, if natural insect communities are indeed affected by neonicotinoids to the extent of causing disruptions in the food chain, we may expect insectivorous bird species to be affected as well.

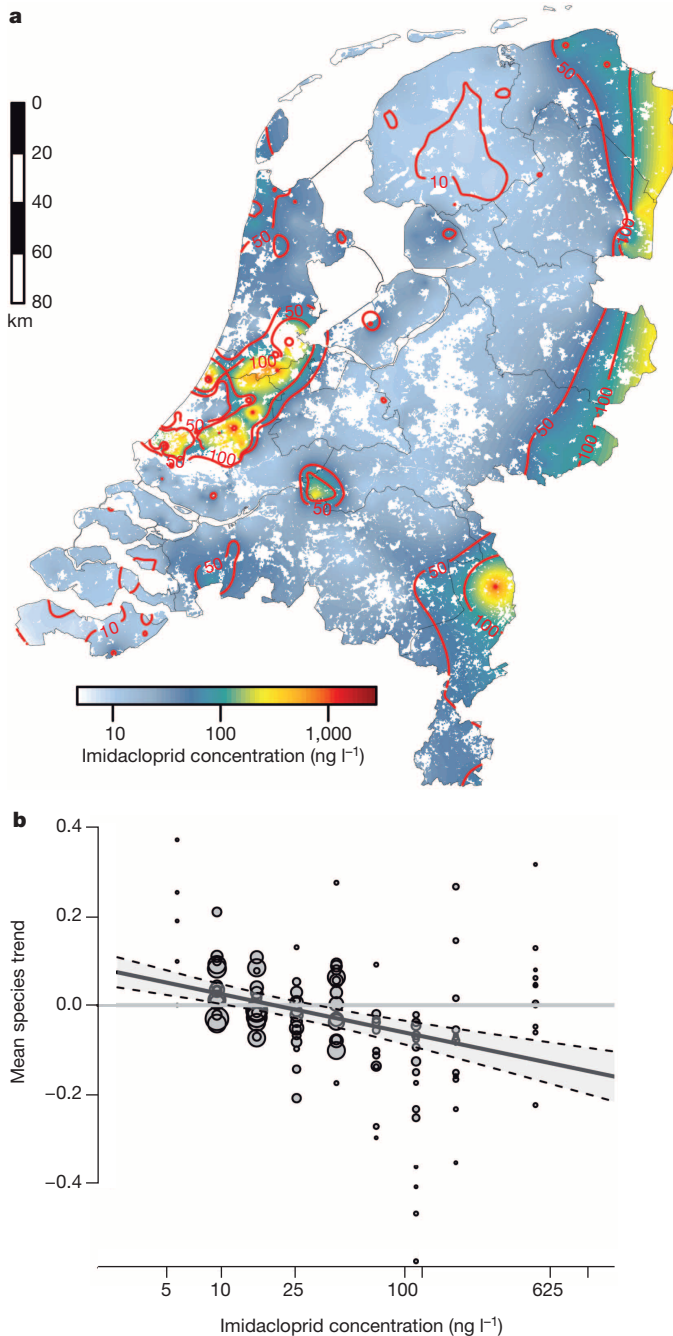
The present study takes advantage of two standardized, long-term, country-wide monitoring schemes in the Netherlands (see Methods)—the Dutch Common Breeding Bird Monitoring Scheme<sup>17</sup> and surface-water quality measurements<sup>4</sup>—to investigate the extent to which average concentrations of imidacloprid residues in the period 2003–2009 spatially correlate with bird population trends in the period 2003–2010. We selected 15 passerine species that are common in farmlands and depend on invertebrates during the breeding season (Extended Data Table 1 and Supplementary Methods). We interpolated concentrations of imidacloprid in surface water to bird monitoring plots (Extended Data Figs 1–3, Supplementary Data and Supplementary Methods) and examined how local bird trends correlate with these concentrations (Fig. 1).

The average intrinsic rate of increase in local farmland bird populations was negatively affected by the concentration of imidacloprid (Fig. 1b, linear mixed effects regression (LMER): d.f. = 1,443,  $t = -5.64$ ,  $P < 0.0001$ ). At the separately tested individual species level, 14 out of 15 of the tested species had a negative response to interpolated imidacloprid concentrations, and 6 out of 15 had a significant negative response at the 95% confidence level after Bonferroni correction (Table 1 and Extended Data Fig. 4). Thus, higher concentrations of imidacloprid in surface water in the Netherlands are consistently associated with lower or negative population growth rates of passerine insectivorous bird populations. From our analysis, the imidacloprid concentration above which bird populations were in decline was  $19.43 \pm 0.03 \text{ ng l}^{-1}$  (mean  $\pm$  s.e.m.) (Fig. 1b). In areas with imidacloprid measurements above this concentration, bird populations declined by 3.5% on average annually.

We checked whether two alternative explanations could have caused spurious correlations between imidacloprid concentrations and bird population trends over the period 2003–2010. First, it is possible that our results could simply reflect a spatial pattern of local farmland bird declines that started before the introduction of imidacloprid<sup>18</sup>. Therefore, we tested whether declines were present before the introduction of imidacloprid, in 1994. In contrast to the strongly negative relationship between imidacloprid concentration and bird population trends in 2003–2010 (Fig. 1b), the 2003–2009 imidacloprid concentrations were not significantly associated with bird trends in the period 1984–1995 ( $t = -1.43$ ,  $P = 0.15$  for LMER<sub><1995</sub>;  $t = -2.16$ ,  $P = 0.031$  for LMER<sub>>2003</sub>; using plots only with trend data for both periods, d.f. = 365; see Extended Data Fig. 6 and Supplementary Methods). Overall, bird population trends in these two periods, paired by plot and species, were uncorrelated ( $r = -0.028$ , Pearson product moment test;  $t = -0.5455$ , d.f. = 379,  $P = 0.56$ ). We can thus conclude that the spatial pattern observed does not reflect long-term ongoing local declines caused by other factors. This finding suggests that imidacloprid is likely to have contributed to the declining population trend of the local birds.

Second, we tested whether spatial differences in land-use changes related to agricultural intensification confounded the effects of imidacloprid in our analyses. We performed multiple mixed effects regression analyses in which we included the local changes in land area use (urban area, natural area, and the production areas of maize, winter cereals and fallow land) and the amount of fertilizer applied (nitrogen in  $\text{kg ha}^{-1}$ ) as fixed

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**Figure 1 | Effect of imidacloprid on bird trends in the Netherlands.**

**a**, Interpolated (universal kriging) mean logarithmic concentrations of imidacloprid in the Netherlands (2003–2009). **b**, Relationship between the average annual intrinsic rate of population increase over 15 passerine bird species and imidacloprid concentrations in Dutch surface water. Each point represents the average intrinsic rate of increase of a species over all plots in the same concentration class, whereas the size of the point is scaled proportionally to the number of species–plot combinations on which the calculated mean is based. Binning into classes was performed to reduce scatter noise and aid in visual interpretation. Actual analysis, and the depicted regression line, was performed on raw data ( $n = 1,459$ ). The regression line is given by  $0.1110 - 0.0374 \text{ (s.e.m.} = 0.0066) \times \log[\text{imidacloprid}]$  ( $P < 0.0001$ ). Dashed lines delineate the 95% confidence interval.

explanatory variables (see Supplementary Data), in addition to imidacloprid concentrations. These variables have been put forward frequently as causal factors related to farmland bird declines<sup>19–21</sup>, although their major effect may have already occurred earlier in the twentieth century. As imidacloprid usage is likely to be related to horticulture and greenhouses<sup>4</sup>, spatial changes in these variables may confound the effects of imidacloprid on bird trends. We therefore also incorporated changes in the area of greenhouses and the area of flower bulb production in our analysis. The results indicate that the concentration of imidacloprid and the changes in urban and natural areas were negatively correlated with local population trends, whereas the changes in the bulb and fallow land were positively correlated (Fig. 2). However, only imidacloprid and bulb area were significantly correlated with local trends (Extended Data Table 2).

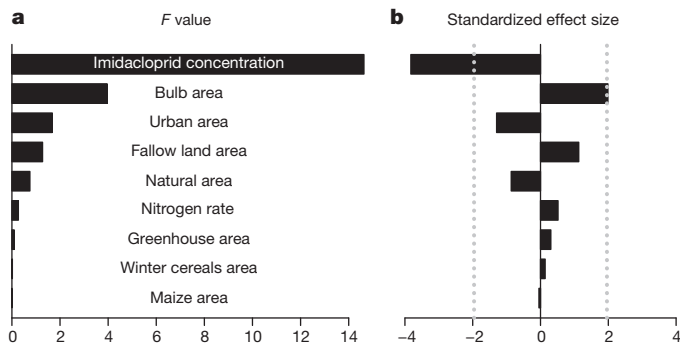
So far, the suggested potential risks of neonicotinoids for birds have focused on the acute toxic effects caused by direct consumption<sup>8</sup>. Our results suggest another possibility: that is, that the depletion of insect food resources has caused the observed relationships. Two lines of evidence seem to support this. First, 9 out of 15 species tested in the present study are exclusively insectivorous. All 15 species feed their young (almost) exclusively with invertebrates, and food demand is the highest in this period. Adult skylarks, tree sparrows, common starlings, yellowhammers, meadow pipits and mistle thrushes are also granivorous to some extent and may thus directly consume coated seed. However, meadow pipits and mistle thrushes forage on seeds only outside the breeding season, and for all 15 species the bulk of the diet during the breeding season consists of invertebrates<sup>7</sup>. Second, recent *in situ* research involving the same areas as the present study revealed strong declines in insect macrofauna, including species that have a larval stage in water, where imidacloprid concentrations were elevated<sup>4</sup>. These insects (particularly Diptera, Ephemeroptera, Odonata, Coleoptera and Hemiptera) are an important food source in the breeding season for the bird species that we investigated<sup>7</sup>. However, as our results are correlative, we cannot exclude other trophic or direct ways in which imidacloprid may have an effect on the bird population trends. Food resource depletion may not be the only or even the most important cause of decline. Other possible causes of decline include trophic accumulation of this neonicotinoid through

**Table 1 | Effect of imidacloprid on insectivorous bird species population trends**

Species	Effect (mean)	Error (s.e.m.)	t value	P	n
Marsh warbler ( <i>Acrocephalus palustris</i> )	0.0110	0.0187	0.5871	0.5584	105
Sedge warbler ( <i>Acrocephalus schoenobaenus</i> )	-0.0229	0.0152	-1.5070	0.1351	99
Reed warbler ( <i>Acrocephalus scirpaceus</i> )	-0.0348	0.0145	-2.3949	0.0180	138
Eurasian skylark ( <i>Alauda arvensis</i> )	-0.0684	0.0189	-3.6164	0.0004*	125
Meadow pipit ( <i>Anthus pratensis</i> )	-0.0299	0.0184	-1.6273	0.1053	200
Yellowhammer ( <i>Emberiza citrinella</i> )	-0.0385	0.0179	-2.1578	0.0367	44
Icterine warbler ( <i>Hippolais icterina</i> )	-0.0705	0.0313	-2.2501	0.0285	57
Barn swallow ( <i>Hirundo rustica</i> )	-0.2313	0.0544	-4.2540	0.0007*	17
Yellow wagtail ( <i>Motacilla flava</i> )	-0.1255	0.0272	-4.6145	0.0000*	124
Tree sparrow ( <i>Passer montanus</i> )	-0.1301	0.0815	-1.5971	0.1211	31
Willow warbler ( <i>Phylloscopus trochilus</i> )	-0.0036	0.0094	-0.3827	0.7025	154
Stonechat ( <i>Saxicola rubicola</i> )	-0.0279	0.0211	-1.3241	0.1891	85
Common starling ( <i>Sturnus vulgaris</i> )	-0.1070	0.0315	-3.3991	0.0013*	57
Common whitethroat ( <i>Sylvia communis</i> )	-0.0408	0.0125	-3.2751	0.0013*	179
Mistle thrush ( <i>Turdus viscivorus</i> )	-0.1093	0.0277	-3.9480	0.0003*	44

Effect of imidacloprid concentration on annual intrinsic rate of increase in individual insectivorous bird species populations in the Netherlands.

\*Species whose population is significantly affected by imidacloprid, after Bonferroni correction.



**Figure 2 | Comparison of the effect of agricultural land-use changes and the effect of imidacloprid on bird population trends.** **a**, The marginal variance ratio ( $F$ ) of each effect was estimated from a mixed effects model with all species data pooled. **b**, The standardized effect size ( $t$  value) for each covariate from the mixed effects model. The vertical dotted lines represent significance thresholds at  $\alpha = 0.05$  (two-sided test). The imidacloprid concentrations and the proportional changes in bulb production areas were the only variables that had significant effects (LMER: d.f. = 1,349,  $t = -3.825$ ,  $P = 0.0001$  for imidacloprid; and  $t = 1.989$ ,  $P = 0.0468$  for bulbs).

consumption of contaminated invertebrates and, for the six partly granivorous species involved, sublethal or lethal effects through the ingestion of coated seeds<sup>8</sup>. The relative effect sizes of these pathways urgently need to be investigated.

Farmland birds have experienced tremendous population declines in Europe in the past three decades, with agricultural intensification as the primary causal factor<sup>19–22</sup>. Among aspects of intensification, pesticides are known to be a major threat to farmland birds<sup>15,23,24</sup>. Neonicotinoids have recently replaced older intensively used insecticides such as carbamates, pyrethroids and organophosphates. After neonicotinoids were introduced to the Netherlands in the mid-1990s, their application was intensified, and the concentrations found in the environment frequently exceeded environmental standards, despite these concentrations being shown to have severe detrimental effects on several insect communities. Our results on the declines in bird populations suggest that neonicotinoids pose an even greater risk than has been anticipated. Cascading trophic effects deserve more attention in research on the ecosystem effects of this class of insecticides and must be taken into account in future legislation.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** C.A.H. performed the statistical analysis. C.A.H., R.P.B.F., C.A.M.v.T., H.d.K. and E.J. wrote the manuscript.

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## METHODS

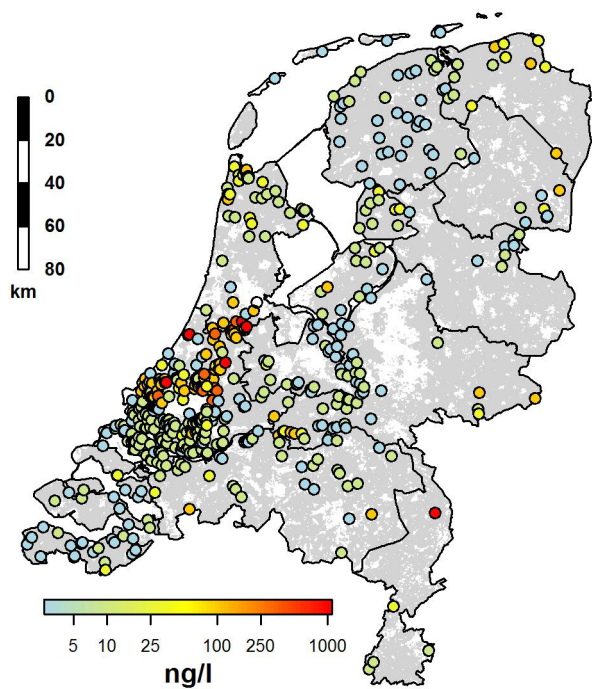
**Data.** We derived population trends for 15 insectivorous farmland passerine species (see Supplementary Data, Supplementary Methods and Extended Data Table 1 for the list of species) using long-term breeding bird data from the Dutch Common Breeding Bird Monitoring Scheme, a standardized<sup>25,26</sup> monitoring scheme maintained and coordinated by Sovon, Dutch Centre for Field Ornithology, in collaboration with Statistics Netherlands<sup>17</sup>. The scheme has been running in the Netherlands since 1984. Data originating from these monitoring plots are generally considered to be adequately representative and reliable for population trend estimation<sup>17,18,25,27,28</sup>. The monitoring plots are well scattered throughout the Netherlands and range in size between 10 ha and 1,000 ha (Extended Data Fig. 2).

We used previously described information on imidacloprid concentrations in Dutch surface water<sup>4</sup>. This data set was collected by the Dutch waterboard authorities as part of the regular monitoring of surface-water pesticide contamination<sup>13</sup> (see Supplementary Data for details). Imidacloprid concentration measurements throughout the Netherlands are available (Extended Data Fig. 1); hence, this data set is considered an adequate representation of the actual water contamination levels in the Netherlands. The geographical locations of the two monitoring programs do not generally spatially coincide. To combine the data sets, we interpolated imidacloprid concentrations from water quality measurement locations to bird monitoring plots (see Supplementary Data).

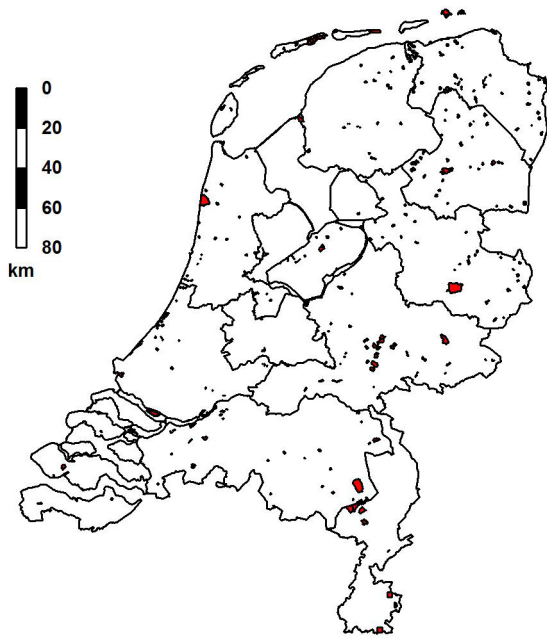
**Statistical analysis.** To assess the overall effects of expected concentrations on all species simultaneously, we used linear mixed effects models with species- and plot-specific population trends (intrinsic rates of increase or  $\log[\lambda]$ ) as the response,  $\log$ [concentration of (interpolated) imidacloprid] as the fixed explanatory variable and species as a random factor. Additionally, we performed linear regressions of the population trends against the logarithm of the imidacloprid concentrations for each species separately using weighted least squares. The trends per plot were weighted by the mean species population size of the plot, to avoid the large influence of the demographic stochasticity of small populations. Population trends were calculated

as the slope of  $\log$ [territory counts] versus year of sampling (that is, a continuous trend) (see Supplementary Data). Regressions were performed using all monitoring plots located less than 5 km between the edge of a plot and an imidacloprid measurement location. This cut-off point of 5 km balanced the preferable proximity between bird and imidacloprid measurements with the amount of data retained in the analyses. However, regardless of how we varied the cut-off value between 1 and 25 km (that is, including between 7% and 99% of the bird monitoring plots, respectively), the effect size of imidacloprid on bird population trends remained strongly significantly negative (see Supplementary Methods and Extended Data Fig. 5). We examined potential confounding of the spatial imidacloprid concentrations with several different candidate explanatory variables that have been postulated as possible causes of farmland bird declines<sup>19</sup> and that are relevant to the Netherlands<sup>17</sup>. We used eight variables<sup>12</sup> that are potentially confounded with the introduction of imidacloprid: namely, proportional change in the area of maize, proportional change in winter cereal cropping area, proportional change in flower bulb area, change in the amount of fertilizer application (nitrogen in  $\text{kg ha}^{-1}$ ), proportional change in greenhouse area, proportional change in urban area, proportional change in natural habitat area and proportional change in fallow land area (Supplementary Data). We compared the significance of all explanatory variables using a multiple mixed effects model (with species intercept as a random effect) paired with *F* tests based on single term deletions of the full model (Fig. 2a). In addition, we compared standardized effect sizes (coefficient/s.e.m.) between explanatory variables based on single species multiple linear regression models (Fig. 2b and Supplementary Methods).

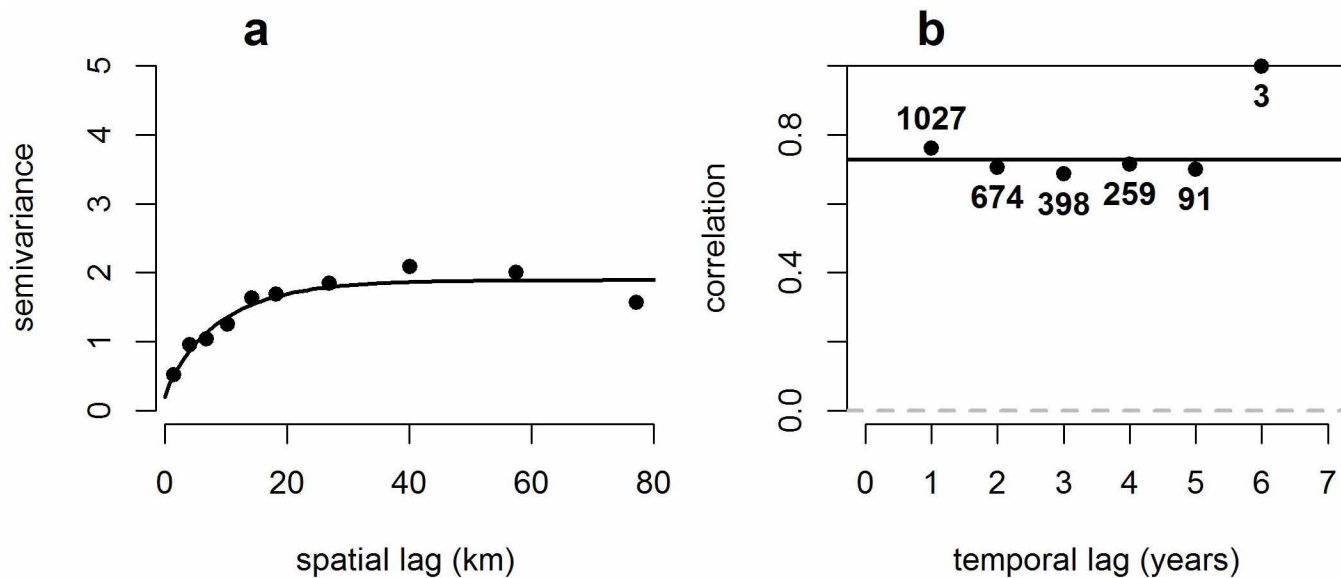
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**Extended Data Figure 1 | Distribution of the 555 imidacloprid measurement averages over the period 2003–2009, as used in the main analysis. The data are taken from refs 4 and 13.**

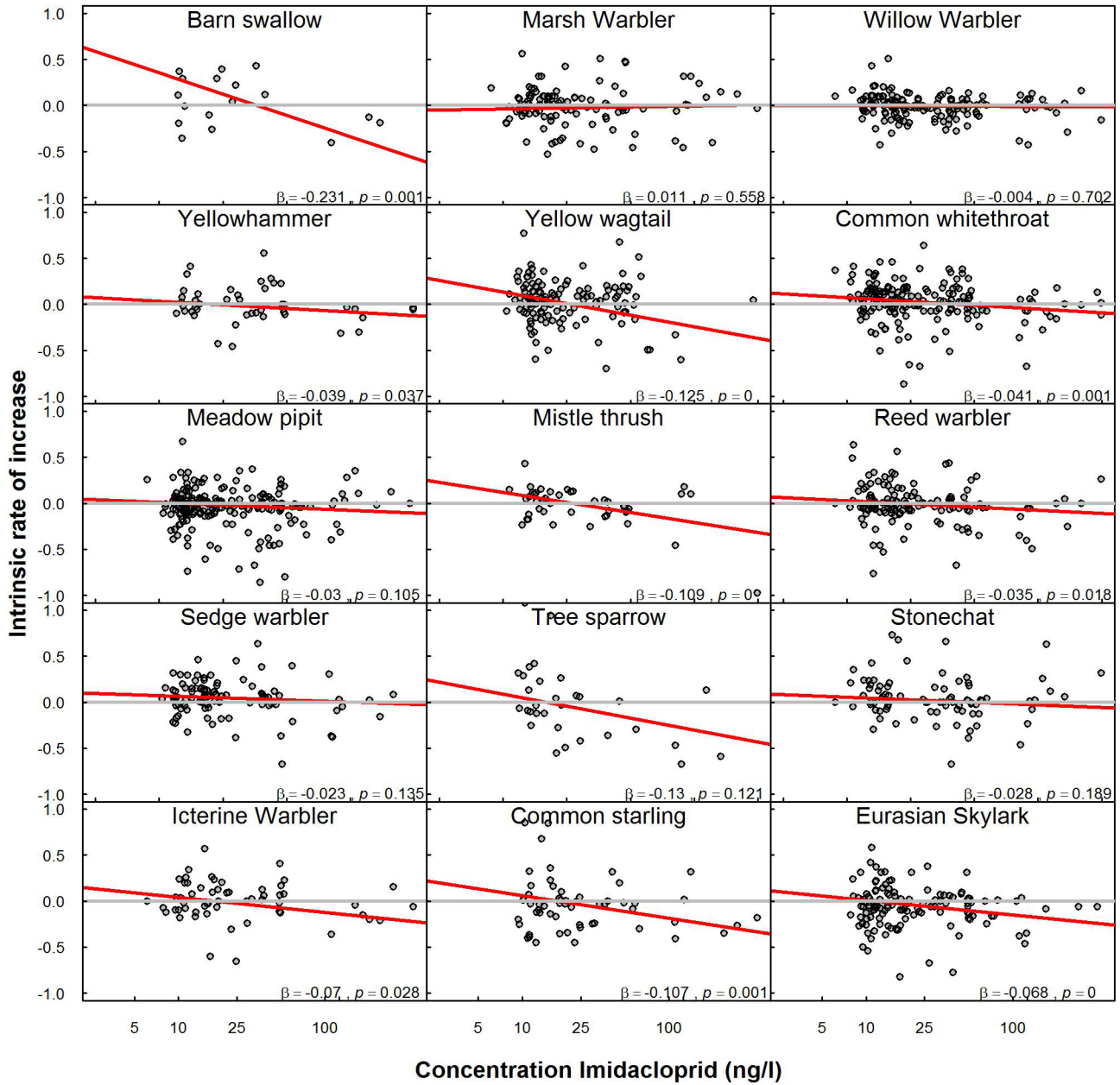


**Extended Data Figure 2 | Distribution of the 354 bird monitoring plots in the Netherlands.** The figure depicts the spatial distribution of bird monitoring plots from which local species-specific trends were calculated.



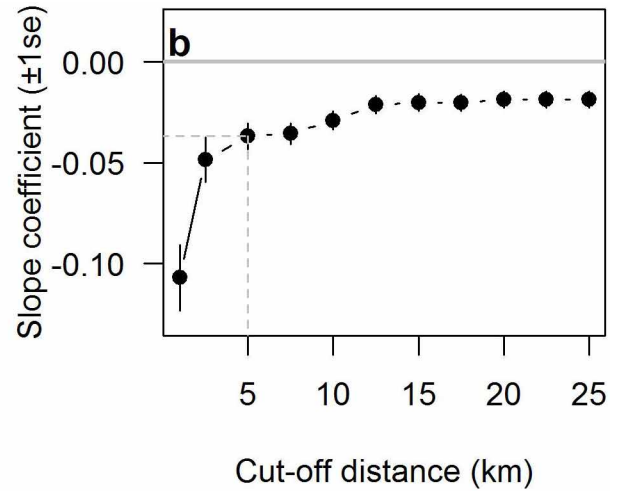
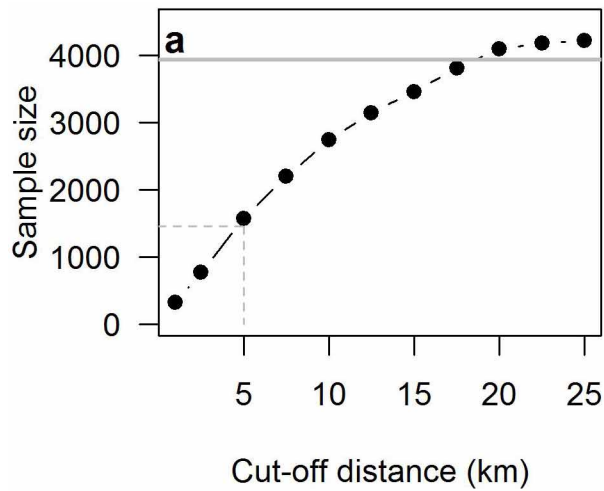
**Extended Data Figure 3 | Spatial and serial (yearly) autocorrelation of imidacloprid measurements.** **a**, Semivariance (dots) and Matern variogram model (fitted line) used in the interpolation of the concentrations (nugget = 0.1901, sill = 1.6989, range = 13.2 km). **b**, Serial correlation

(between years) of imidacloprid concentrations. Each value gives the number of pairs of measurements at each year lag that were used to calculate the coefficients. Serial correlations remain invariant with respect to temporal lag, indicating high temporal consistency in local imidacloprid concentrations.



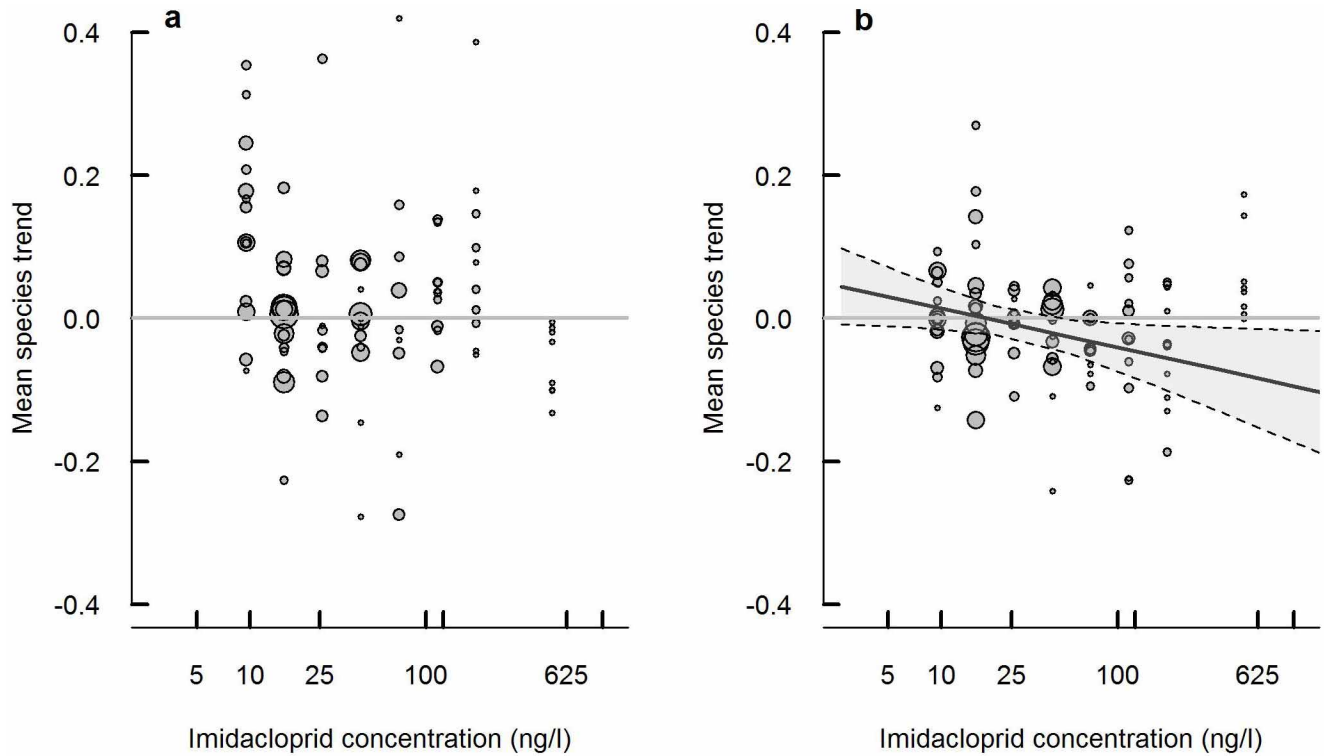
Extended Data Figure 4 | Population trends as a function of imidacloprid concentration per individual bird species. The red lines depict the weighted mean trend, also given as slope coefficients ( $\beta$ ) and with corresponding  $P$  values.





**Extended Data Figure 5 | Robustness check for the effect of the cut-off value for the distance between bird monitoring plots and water measurement locations (varied between 1 and 25 km).** The larger the cut-off distance, the more species–plot annual rates of increase are retained in the analysis subset of

the total database of 3,947 records (a) but at the cost of increased noise in the response and a decrease in the effect of imidacloprid on the bird trends (b). However, in all cases, the effect of imidacloprid was significant and negative ( $P < 0.0001$ ).



**Extended Data Figure 6 | Bird species trends before and after imidacloprid introduction.** Comparison of the relationship of bird species trends in the periods 1984–1995 (a) and 2003–2010 (b) with the imidacloprid concentrations in 2003–2009, based on all plots monitored in both time periods. Each point in the scatter plot represents the average intrinsic rate of increase of a species over all plots in the same concentration class. Binning into

classes was performed to reduce scatter noise and aid in visual interpretation. The actual analyses and the depicted significant regression line were based on raw data. The bird trends were significantly affected by the imidacloprid concentration in 2003–2010 ( $t = -2.16$ , d.f. = 365,  $P = 0.031$ ) but were not significantly affected in the period before imidacloprid administration ( $t = -1.43$ , d.f. = 365,  $P = 0.15$ ).

Extended Data Table 1 | Species information

	Species	Foraging habitat	Migratory behaviour	Trend 1990-2005
	Marsh warbler ( <i>Acrocephalus palustris</i> )	reed	long-distance	stable
	Sedge warbler ( <i>Acrocephalus schoenobaenus</i> )	reed	long-distance	strong increase
	Reed warbler ( <i>Acrocephalus scirpaceus</i> )	reed	long-distance	stable
	Eurasian skylark ( <i>Alauda arvensis</i> )	farmland/grassland	short-distance	strong decline
	Meadow pipit ( <i>Anthus pratensis</i> )	grassland	short-distance	moderate decline
	Yellowhammer ( <i>Emberiza citrinella</i> )	farmland	resident	moderate increase
	Icterine warbler ( <i>Hippolais icterina</i> )	gardens/farms	long-distance	moderate decline
	Barn swallow ( <i>Hirundo rustica</i> )	farmland/grassland	long-distance	stable
	Yellow wagtail ( <i>Motacilla flava</i> )	farmland	long-distance	moderate decline
	Tree sparrow ( <i>Passer montanus</i> )	farmland	resident	stable
	Willow warbler ( <i>Phylloscopus trochilus</i> )	shrubs	long-distance	moderate decline
	Stonechat ( <i>Saxicola rubicola</i> )	shrubs	short-distance	strong increase
	Common starling ( <i>Sturnus vulgaris</i> )	grassland	short-distance	moderate decline
	Common whitethroat ( <i>Sylvia communis</i> )	shrubs	long-distance	moderate increase
	Mistle thrush ( <i>Turdus viscivorus</i> )	grassland	short-distance	moderate decline

Extended Data Table 2 | Multiple mixed effects regression of population trends (pooled over 15 species,  $n = 1,926$ )

	Coefficient(se)	<i>t</i> -value	<i>P</i> -value
Intercept	0.0932(0.0262)	3.5500	0.0004
Imidacloprid concentration	-0.0294(0.0077)	-3.8254	0.0001
Bulb area	0.0063(0.0032)	1.9895	0.0468
Urban area	-0.2970(0.2293)	-1.2954	0.1954
Fallow land area	1.2899(1.1428)	1.1287	0.2592
Natural area	-0.1878(0.2173)	-0.8646	0.3874
Nitrogen rates	$1.15(2.22) \times 10^{-5}$	0.5174	0.6050
Greenhouse area	0.0409(0.1340)	0.3050	0.7604
Winter cereals area	0.0543(0.3950)	0.1375	0.8906
Maize area	-0.0095(0.2062)	-0.0463	0.9631

Explanatory variables include log[imidacloprid concentration] ( $\text{ng l}^{-1}$ ) and the area coverage change (difference in proportion of area, see Supplementary Data) of six land-use variables related to agricultural intensification and two variables potentially confounded with imidacloprid concentrations. For each explanatory variable, we present the slope coefficient along with the s.e.m., *t* and *P* values.