Addendum: Agrochemicals interact synergistically to increase bee mortality

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In our original article, we assessed whether interactions between stressors that impact bee health were antagonistic, additive or synergistic. To do this, we followed the methods developed by Jackson et al.[1] and determined the overall interaction effect as the standardized mean difference (SMD) (Hedges' *d*) between the predicted additive effect and observed effect of both stressors. Since then, it has been brought to our attention that (i) there were errors in our uploaded dataset, (ii) the formula for the sampling variance of the interaction effects in Gurevitch et al.[2] was more appropriate for our dataset than the one we had previously used (see ref. 1) (with thanks to Y. Yefeng and S. Nagakawa for this personal communication), and (iii) it is possible to use proportion difference approaches for this kind of interaction analysis, rather than the SMD approach we used. We have corrected our analyses with respect to these points, and the details of this are outlined below.

Dataset: an error in the dataset initially uploaded disassociated a subset of means from their standard deviations. We have corrected this and, in the process, reassessed each row of data against the original paper, removing or updating any instances where errors had been made in either data extraction or study interpretation ($n = 75$). This resulted in the n values of each analysis changing from the values seen in the original analysis (Fig. 1). The correct datasets have now been uploaded to the original repository. However, this final dataset is not our complete dataset, as 63 unpublished effect sizes were confidentially shared with us by the authors of these studies.

Sampling variance calculation: we recalculated, where appropriate (see below), the variance for interaction effects using the formula reported in Gurevitch et al.[2].

SMD: mortality data are proportion data and, as such, the appropriate meta-analytical approach is to use the proportion difference rather than the SMD[3]. We originally used SMD in the absence of a method for calculating variance that could be applied to interaction effects derived from proportion data[1]. In this correction, we now use proportion difference with an appropriate variance calculation (with thanks to S. Nagakawa for deriving this). In addition, because our dataset is a mixture of studies where standard deviations could be extracted at the cage level or the individual level, we calculated the standard deviation differently for each type of data. For cage-level data, we used the extracted standard deviations from the paper to calculate the sampling variance, as reported in our original manuscript. For individual-level data, we calculated standard deviation (s) using the formula

$$s = \sqrt{\frac{pq}{n}}$$

where p is the number of surviving individuals, q is $(1 - p)$ and n is the total number of individuals.

Finally, to improve the robustness of our analysis, in the updated analysis we (i) included 'stressor type' as a moderator in the main meta-analytic model as opposed to sub-setting the data, and (ii) used cage-level data wherever possible (e.g., the data provided in the Supplementary Material 19 data points were re-extracted at cage level). The results of analyses using the above approach, but with data extracted at the individual level when available (as reported in the paper initially published), were

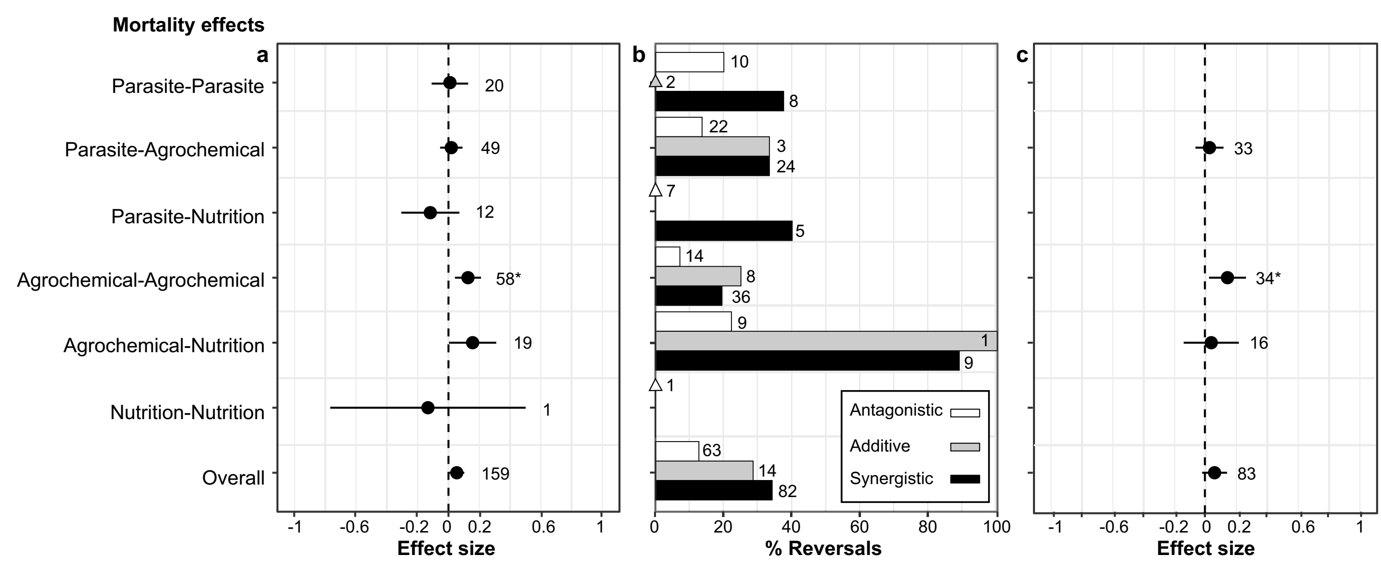

Fig. 1 | Revised – The interaction effects of parasites, agrochemicals, and nutritional stressors on bee mortality. a, The effect sizes for the indicated interaction effects. Data are PD values ± 95% CI. Interactions are synergistic when the effect size is positive and the 95% CI does not include zero, antagonistic when the effect size is negative and the 95% CI does not include zero, and additive when the 95% CI includes zero. **b**, The percentage of additive, antagonistic and synergistic interactions between stressors that were reversal interactions (Methods). **c**, Effect sizes (PD values ± 95% CI) for analyses in which bees are exposed to field-realistic concentrations of agrochemicals.

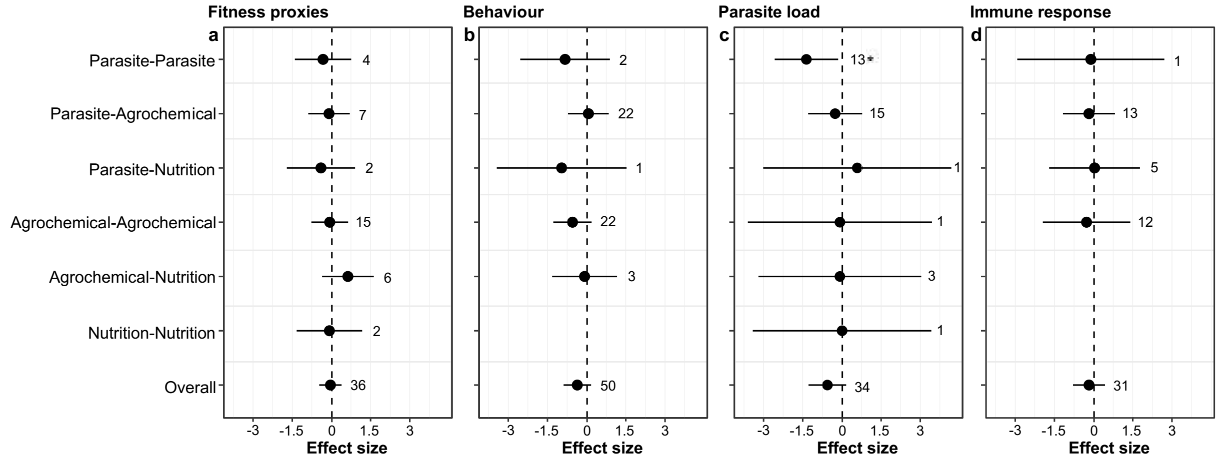

Fig. 2 | Revised – The interaction effects of parasites, agrochemicals and nutritional stressors on non-mortality response measures. a–**d**, The interaction effects of parasites, agrochemicals and nutritional stressors on (**a**) bee fitness proxies, (**b**) bee behaviour, (**c**) parasite load and (**d**) immune response. The effect sizes for the indicated interaction effects. Data are Hedges' d values ± 95% CI. Interactions are synergistic when the effect size is positive and the 95% CI does not include zero, antagonistic when the effect size is negative and the 95% CI does not include zero, and additive when the 95% CI includes zero.

qualitatively the same as our new analyses when cage-level data were used. The only exception was that the overall interaction effect remained synergistic, not additive (PD, proportion difference = 0.05; CI, 95% confidence intervals = 0.001 to 0.09 – see below for comparison with the analysis incorporating available cage-level data).

We report the reanalyses of our published results below.

Agrochemicals interact synergistically to increase bee mortality. While the overall interaction between stressors was additive in the revised analyses (PD = 0.05, CI = −0.001 to 0.09, $n = 159$), agrochemicals still showed a synergistic interaction (PD = 0.12, CI = 0.04 to 0.20, $n = 58$) (Fig. 1a). Thus, our key finding that agrochemicals interact synergistically to increase bee mortality does not change. All other interactions remained additive (parasite × parasite, PD = 0.00, CI = −0.12 to 0.12, $n = 20$; parasite × agrochemical, PD = 0.02, CI = −0.06 to 0.08, $n = 49$; parasite × nutrition, PD = −0.12, CI −0.32 to 0.06, $n = 12$; agrochemical × nutrition, PD = 0.15, CI −0.00 to 0.30, $n = 19$; nutrition × nutrition, PD = −0.14, CI = −0.78 to 0.50, $n = 1$). This pattern was mirrored in analyses where only studies using field-realistic agrochemical exposure were used (Fig. 1c).

We also found the same synergistic interactions between specific agrochemicals, as observed in the original analysis (Extended Data Fig. 1, which is a revised version of the original Extended Data Fig. 4).

Interaction effects on non-mortality end-points are largely additive. We reanalysed our non-mortality datasets using the Gurevitch et al.[2] formula for sampling variance, pooled standard deviation and j:

$$j = 1 - (3/(4 \times (\text{crossed } n + \text{stress 1 } n + \text{stress 2 } n + \text{control } n - 4) - 1)).$$

To avoid mixing dependent variables with different error distributions, only studies with Gaussian data distributions were included ($n = 28$ effect sizes removed). Again, the results were largely qualitatively similar (Fig. 2).

Fitness: the results mirrored those of the original analyses. The overall interaction between stressors was additive ($d = -0.04$, CI = −0.46 to 0.39, $n = 36$), as were the individual interactions (parasite × parasite, $d = -0.32$, CI = −1.40 to 0.76, $n = 4$; parasite × agrochemical, $d = -0.09$, CI = −0.88 to 0.70, $n = 7$; parasite × nutrition, $d = -0.40$, CI = −1.71 to 0.91, $n = 2$; agrochemical × agrochemical, $d = -0.09$, CI = −0.88 to 0.70, $n = 15$; agrochemical × nutrition, $d = 0.63$, CI = −0.36 to 1.62, $n = 6$; nutrition × nutrition, $d = -0.08$, CI = −1.33 to 1.18, $n = 2$).

Behaviour: the overall interaction between stressors was additive ($d = -0.36$, CI = −0.88 to 0.17, $n = 50$), as opposed to the significant antagonistic interaction initially reported in the paper. However, individual interactions largely remained additive as in the original analysis (parasite × parasite, $d = -0.83$, CI = −2.54 to 0.89, $n = 2$; parasite × agrochemical, $d = 0.07$, CI = −0.71 to 0.85, $n = 22$; parasite × nutrition, $d = -0.96$, CI = −3.44 to 1.53, $n = 1$; agrochemical × agrochemical, $d = -0.54$, CI = −1.28 to 0.19, $n = 22$) with one interaction becoming additive, as opposed to antagonistic (agrochemical × nutrition, $d = -0.08$, CI = −1.32 to 1.16, $n = 3$).

Parasite load: in contrast to the original analysis published, the overall interaction between stressors was additive ($d = -0.56$, CI = −1.28 to 0.16, $n = 34$), rather than antagonistic. However, the parasite × parasite interaction remained antagonistic (parasite × parasite, $d = -1.36$, CI = −2.58 to −0.14, $n = 13$) and the remaining individual interactions remained additive (parasite × agrochemical, $d = -0.26$, CI = −1.30 to 0.78, $n = 15$; parasite × nutrition, $d = 0.59$, CI = −3.01 to 4.19, $n = 1$; agrochemical × agrochemical, $d = -0.08$, CI = −3.60 to 3.45, $n = 1$; agrochemical × nutrition, $d = -0.08$, CI = −3.19 to 3.04, $n = 3$; nutrition × nutrition, $d = 0.01$, CI = −3.41 to 3.43, $n = 1$).

Immune: the results of reanalyses mirrored the original analyses. The overall interaction between stressors ($d = -0.18$, CI = −0.79 to 0.43, $n = 31$) and the individual interactions were additive (parasite × parasite, $d = -0.11$, CI = −2.92 to 2.71, $n = 1$; agrochemical × parasite, $d = -0.18$, CI = −1.17 to 0.82, $n = 13$; parasite × nutrition, $d = 0.04$, CI = −1.70 to 1.78, $n = 5$; agrochemical × agrochemical, $d = -0.27$, CI = −1.94 to 1.40, $n = 12$).

Overall, despite the changes in dataset and in analytical methodology, our results and conclusions remain largely the same, particularly our key result regarding synergistic agrochemical interactions, and thus the original conclusions of the paper are upheld. We are extremely grateful to S. Nakagawa and Y. Yefeng for their help and support in correcting this paper.

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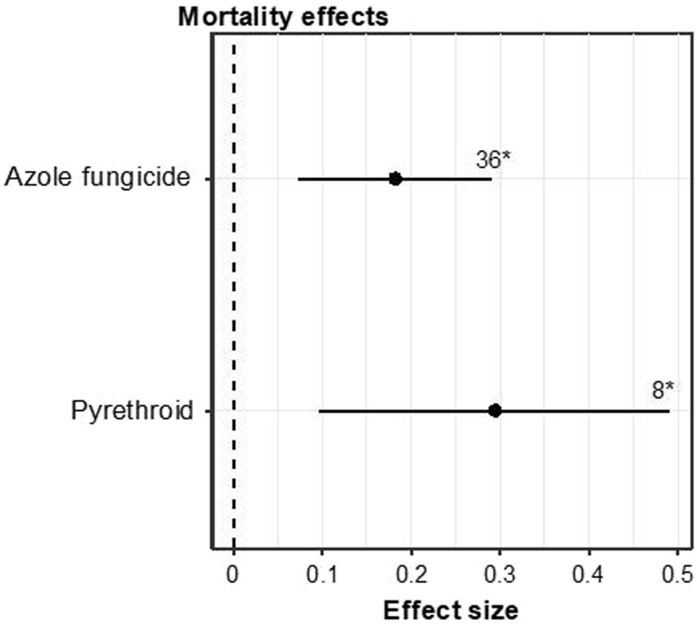

Extended Data Fig. 1 | The interaction effects of different agrochemical classes on bee mortality response measures. This is a revised version of the original Extended Data Fig. 4. PD ± 95% CI are shown. Note that effect sizes for azole fungicide × pyrethroid are included in both groups.