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Title page

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Title. Adequate statistical modelling and data selection are essential when analysing abundance and diversity trends

Matters arising. Arising from Crossley *et al.*, No net insect abundance and diversity declines across US Long Term Ecological Research sites, *Nature Ecology & Evolution* (2020)
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Author contributions

M.D. and P-A.C. performed both the detailed and overall analysis of the article and wrote the original draft. P-A.C. examined and re-programmed the R code. L.G. contributed to the argumentation and extensively edited the manuscript. J-M.B. contributed to the analysis of data selection in the article. All authors contributed to the general comment and reviewed the manuscript.

1 **Title.** Adequate statistical modelling and data selection are essential when analysing abundance and
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6

7 **Abstract**

8 In an analysis of a large number of time series on arthropod abundances in natural and managed
9 areas of the United States, Crossley et al. reported no evidence of an overall decline in insect
10 abundance and diversity¹. We identified major concerns in the statistical analysis and
11 inconsistencies in the selection of data, which, we argue, invalidate their conclusions. We call for a
12 rigorous methodology in analyses of biodiversity trends because relevant information is crucial for
13 stakeholders and policy makers.

14

15 **Matters arising**

16 The extent of the decline of insect populations worldwide is much debated²⁻⁵, with major
17 implications for public policy investment in biodiversity protection. Crossley et al. conducted a
18 statistical analysis of 5,375 geographically and taxonomically varied time series on arthropod
19 abundance during 4 to 36 years across the United States¹. They concluded that there was no
20 significant change in insect populations. However, we argue that issues in the statistical analysis and
21 inconsistencies in data selection invalidate their conclusions.

22 The modelling proposed by Crossley et al. relied on the following steps: i) collecting data, ii)
23 separating each species of each locale of each LTER (in the R script, a locale could be an arthropod
24 group, a location or a collection method), iii) pre-processing data (Box 1), iv) running a different
25 autoregressive linear model for each species of each locale of each LTER (hereafter, LLS),

26 v) combining all estimated slopes into a “sample”, vi) analysing this “sample” using violin plots, T
27 tests and confidence intervals (Fig. 1). The statistical analysis carried out in this last step relied on
28 the assumption that the observations in the sample were independent and identically distributed
29 (iid). This assumption was violated for two reasons. First, the pre-processing step included a time
30 scaling to change the minimum year of each LLS time series to 0 and its maximum year to 1. As the
31 time length varied from 4 to 36 years depending on LLS, the scaled time x varied across LLS time
32 series. Therefore, the estimated slopes did not represent abundance trends per year, but per time
33 units x varying over time series and without a clear meaning. Second, according to the linear
34 regression theory, the expectation and variance of the estimated slopes depend on the number of
35 measures of the x variable (i.e. the length of the time series) and the distances of y measures to the
36 model (i.e. the quality of the model approximation). Among LLS time series, there are different
37 time lengths, and different qualities of approximation. Therefore, the slopes cannot be iid, and
38 estimations and tests used in step vi) are not reliable. To circumvent this problem, it would be more
39 appropriate to use a hierarchical model to analyse the whole dataset.

40 Insert Box 1 and Figure 1.

41 Other problems are as follows. First, most individual time series were too short to provide reliable
42 estimations of the four unknown parameters specific to each LLS (Box 1). Indeed, 44% of LLS time
43 series only had 4 to 9 years of data. While no simple threshold exists, we do not see how to reliably
44 estimate four parameters with less than ten data points, which will only provide a very imprecise
45 estimation. Some limited sensitivity analysis was provided with a stricter data subset involving a
46 minimum of 15 years of data, but this strict dataset only included less than 6% of the time series. It
47 represented a much more limited variety of situations than the total sample and was therefore much
48 less representative. This is another argument in favour of a global modelling approach, which would
49 improve the precision of the trend estimate of any given LLS by using data from other LLS.

50 Second, the analysis was performed at a very fine taxonomic level, implying that a high proportion
51 of abundance counts was equal to zero (the full dataset contained 49% of zeros and the strict

52 dataset, 30.5%; moreover, 71% of the series in the full dataset, and 84% in the strict dataset,
53 contained at least a zero). As the logarithm of 0 is undefined, all zero abundance values were shifted
54 upwards before being log-transformed by adding an arbitrary value. Such rudimentary log-
55 transformation of count data is to be avoided because results depend on the chosen value and
56 coefficient estimates are inaccurate^{6,7}. Zero-inflated models would have dealt appropriately with the
57 problem of high occurrence of zeros in the dataset⁸.

58 Third, the model corrected for scale differences between abundance series without accounting for
59 imperfect detection, which can be of particular concern for rare species. This problem may be
60 illustrated by the case of *Aphis asclepiadis*, NEPAC locale, Midwest STN LTER (external database
61 S1⁹). In the ten years of records, its abundance was 0 for the nine first years, and 1 for the last year.
62 This time series (like the others in the dataset) was not composed of abundance levels, but
63 estimations of abundance. Due to imperfect detection, a shift from an estimated abundance from 0
64 to 1 provides poor information on the real abundance trend. After scaling log-abundances (Box 1),
65 this uninformative *A. asclepiadis* data series was erroneously modelled as having the highest
66 abundance increase of all the time series (external database S2⁹), while it could just reflect the rarity
67 of the species or its poor detection. The same slope could have been obtained with a time series
68 reflecting a significant abundance change, with for example a hundred insects in all years except the
69 last year with a thousand insects. In total, 16% of time series included only abundance values of 0
70 and 1, and 27% of time series included only abundance values of 0, 1 and 2. Simple models of
71 occurrence and abundance have already been developed to cope with the problem of imperfect
72 detection.¹⁰

73 As all analyses of diversity (richness, evenness and β diversity) in the article relied on these
74 estimations of abundance and on the same modelling, they shared similar methodological problems.

75 We also point out that we had to re-program the R script provided by the authors using their
76 external databases S1 and S2⁹ to make it run.

77 Regarding data selection, the article is intended to analyse insect abundance trends in US Long
78 Term Ecosystem Research (LTER) sites, but it departs from this description in two ways. First,
79 39.5% of time series are from the Suction Trap Network (STN). One suction trap of the STN is
80 located in the Kellogg LTER site, and all STN data, encompassing ten US states, were incorporated
81 into the Kellogg LTER dataset up to 2014¹¹. But the dataset used in the analysis
82 (<https://suctiontrapnetwork.org>), spanning up to 2019, is not linked to a LTER. Its inclusion may
83 bias results by minimising the damages of intensive farming, because the STN exclusively provides
84 data on aphids, and primarily aims to document pest aphids¹¹, which benefit from intensive
85 agriculture^{12,13}, unlike most insects (e.g. aphid natural enemies¹³ or bees¹⁴).

86 Second, the reference to insects in the title of the article is confusing as almost 10% of time series
87 were of non-insect arthropods or included insects and other arthropods. In Fig. 2, three of the 22
88 violin plots concerned or involved crustaceans. Unlike the rest of the dataset, the violin plot from
89 the Coweeta LTER related to aquatic invertebrate communities in terms of functional feeding
90 groups, and not individual species. These inconsistencies add to other criticisms of this article¹⁵
91 regarding unaccounted-for changes in sampling location and sampling effort at LTER sites and the
92 unaccounted-for impact of experimental conditions on insect populations.

93 As a conclusion, the methodology chosen in this article is very approximate with several identified
94 problems likely to substantially bias the results. The analysis would have required an adequate
95 global model for all data, considering all our criticisms and those of ref. ¹⁵. We call for the
96 application of rigorous standards for analyses on global change, especially because results could
97 have a significant impact on policy decision-making and the fate of biodiversity.

98

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134

135 **Box 1. Model used by Crossley et al. (2020)**

136 Each time series i was composed of abundance levels A_{it} for LLS i and for years t_{i1} to t_{iT_i} .

137 The first step of data pre-processing consisted in log-transforming abundances. For LLS i in year t ,
138 the abundance value A_{it} was replaced either by its logarithm, $\log A_{it}$, or, if $A_{it} = 0$, by the logarithm
139 of a constant, $\log c_i$, where c_i was half the minimum non-zero abundance in time series i , to obtain a
140 series of log-transformed abundances a_{it} .

141 In a second step, log-abundances were scaled: the empirical mean \bar{a}_i of log-transformed abundances
142 of the series was subtracted to each a_{it} and this difference was divided by the empirical standard
143 deviation s_i of log-transformed abundances. This yielded the scaled logarithm of abundance of LLS
144 i in year t , y_{it} , defined as $y_{it} = (a_{it} - \bar{a}_i)/s_i$.

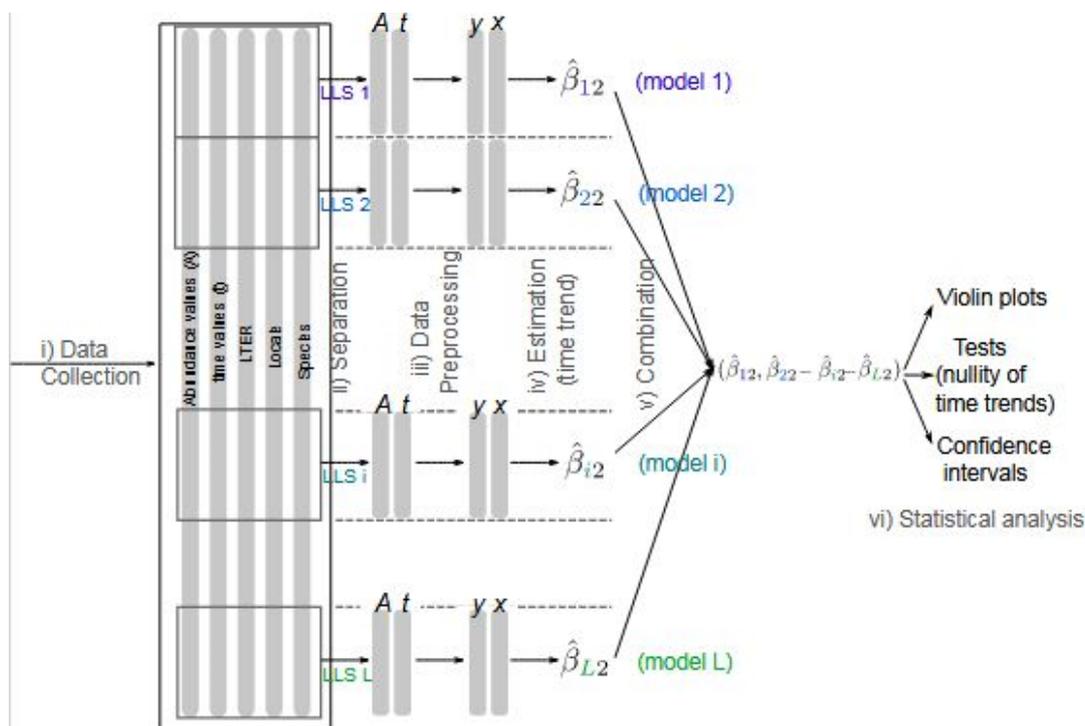
145 In a third step, the authors transformed all time units using a common scale varying between 0 (the
146 first year of the LLS abundance time series) and 1 (its last year). The scaled year x_{it} was obtained by
147 transforming the first year of time series t_{i1} to 0, and its last year t_{iT_i} to 1, and scaling all years
148 accordingly as $x_{it} = (t_{it} - t_{i1})/(t_{iT_i} - t_{i1})$.

149 The proposed modelling was a linear model with a Gaussian auto-regressive error of order 1:

150 $y_{it} = \beta_{i1} + \beta_{i2} x_{it} + \varepsilon_{it}$, where $\varepsilon_{it} = \rho_i \varepsilon_{i,t-1} + \eta_{it}$, with $\eta_{it} \sim N(0, \sigma_i^2)$.

151 For each individual LLS time series, this model implied the estimation of four parameters, β_{i1} , β_{i2} , ρ_i
 152 and σ_i , the slope β_{i2} representing the abundance trend and therefore being the parameter of interest.
 153

154 **Figure 1. Modelling steps in Crossley et al. (2020) and arising problems.** Time trends were
 155 estimated separately for each species of each locale of each LTER (LLS). The time scaling was
 156 performed on LLS of different time lengths and the quality of approximation varied across LLS.
 157 Therefore, the abundance time trends were not independent and identically distributed as assumed
 158 when calculating the average abundance trends, confidence intervals and significance tests
 159 associated with the violin plots of Fig. 2 in Crossley et al. (2020). A global hierarchical modelling
 160 would have circumvented this problem.



161

162

163

164 **Competing interests.**

165 The authors declare no competing interests.