Title page

Title. Inconsistencies in data selection and issues in statistical modelling invalidate the results of Crossley et al., 2020.

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) doi:10.1038/s41559-020-1269-4.

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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. J.M.B. contributed to the analysis of data selection in the article. L.G. extensively edited the manuscript. All authors contributed to the general comment and reviewed the manuscript.

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7 Abstract

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

14

15 Matters arising

The extent of the decline of insect populations worldwide is much debated²⁻⁴, with major 16 17 implications for public policy investment for the protection of biodiversity. Crossley et al. 18 conducted a meta-analysis of 5,375 geographically and taxonomically varied time series on arthropod abundance during 4 to 36 years across the United States¹. They suggested that the 19 20 broad representation of taxa, habitats, feeding guilds and sampling methods made their data 21 well suited to detect any overall decline in insect biodiversity. They concluded that there was 22 no significant change in insect populations, a result that was consistent across insect feeding 23 groups and between heavily disturbed and relatively natural sites. However, we argue that 24 serious inconsistencies in the selection of data and issues in the statistical analysis invalidate

their conclusions.

26 Regarding data selection, the article is intended to be a meta-analysis of insect abundance 27 trends in US Long Term Ecosystem Research (LTER) sites, but it departs from this description in three ways. First, 39.5% of time series are from the Suction Trap Network 28 29 (STN). One suction trap of the STN is located in the Kellogg LTER site, and all STN data, encompassing ten US states, were incorporated into the Kellogg LTER dataset up to 2014⁵. 30 31 But the dataset used in the meta-analysis, spanning up to 2019, is not linked to a LTER. The 32 inclusion of this dataset may bias results because the STN exclusively provides data on 33 aphids, and was primarily aimed at documenting pest aphids⁵, which benefit from intensive 34 agriculture. Second, 9% of the time series were of non-insect arthropods or included both 35 insects and other arthropods, and therefore should have been excluded (Table 1). Third, several LTER datasets were not included in the analysis, without justification (e.g. refs⁶⁻⁹). 36 These inconsistencies add to other criticisms of this article¹⁰ regarding unaccounted-for 37 38 changes in sampling location and sampling effort at LTER sites and the unaccounted-for 39 impact of experimental conditions on insect populations in experimental datasets. 40 The statistical approach in Crossley et al. reveals further problems. These authors modelled 41 separately abundance trends of each species in each considered 'locale' of each LTER (in the 42 R script, a locale could be an arthropod group, a location or a collection method)¹¹. For each species of each locale of each LTER (hereafter, LLS), changes in abundance were estimated 43 44 by performing a univariate regression of the scaled logarithm of abundance on scaled time, 45 with a Gaussian auto-regressive error of order 1 (box 1). This statistical modelling raises four 46 issues.

47 Insert box 1.

48 First, most individual time series were too short to provide reliable estimations of the four 49 unknown parameters for each LLS: the intercept and the slope of the linear regression, the 50 autocorrelation coefficient and the variance of the error term. Indeed, 75% of LLS time series 51 had 4 to 14 years of data. While no simple threshold number of years exists, 16 years of continuous monitoring are often necessary to identify long-term changes in abundance¹². 52 53 Although some limited sensitivity analysis was provided with a better sub-dataset involving a 54 minimum of 15 years of data, this strict dataset only included less than 6% of time series. 55 Second, the analysis was performed at a very fine taxonomic level, implying that a high 56 proportion of abundance counts was equal to zero (the full dataset contained 49% of zeros and the strict dataset, 30.5%; moreover, 71% of the series in the full dataset, and 84% in the strict 57 58 dataset, contained at least a zero). In this context, the log-transformation of the data carried 59 out in the article was inadequate. As the logarithm of 0 is undefined, all zero abundance 60 values were shifted upwards before being log-transformed by adding an arbitrary value. Such 61 rudimentary log-transformation is to be proscribed because results depend on the chosen 62 value¹³. Given the high occurrence of zero counts in the dataset, zero-inflated models, 63 classically used for a long time now, would have allowed dealing appropriately with this problem¹⁴. 64

65 Third, the model corrected for scale differences between abundance series without accounting for imperfect detection, which can be of particular concern for rare species. This problem may 66 67 be illustrated by the case of *Aphis asclepiadis*, NEPAC locale, Midwest STN LTER site (external database S1 in ref¹¹). In the ten years of record included in the meta-analysis, its 68 69 abundance was 0 for the nine first years, and 1 for the last year. This time series (like the 70 others in the dataset) was not composed of abundance levels, but estimations of abundance. 71 Due to imperfect detection, a shift from an estimated abundance from 0 to 1 provides poor 72 information on the real abundance trend. After scaling log-abundances (see Box 1), this

uninformative *A. asclepiadis* data series was erroneously modelled as having the highest
abundance increase of all the time series (external database S2 in ref¹¹), while it could just
reflect the rarity of the species or its poor detection. The same slope could have been obtained
with a time series actually reflecting a significant abundance change, with for example a
hundred insects in all years except one year with a thousand insects. Simple models of
occurrence and abundance have already been developed to cope with this problem.¹⁵ Not
using them is a serious shortcoming of the article by Crossley et al.

80 A fourth major flaw lies in the drawing of the violin plots in figure 2 of the article. For each 81 LTER, or, depending on cases, each LTER locale, this figure gathered all estimated slopes 82 (one per LLS) which were used to draw a violin plot. However, a violin plot, a median or a 83 mean are statistical estimators built to estimate their counterparts in the population. These 84 estimators are based on the assumption that observations in the sample are independent and identically distributed. But in Crossley et al., each slope was estimated separately from the 85 86 others and thus had its own variability, which depended on the design of data in the series. 87 Therefore, the slopes were not identically distributed, meaning that violin plots, average 88 abundance trends and their confidence intervals were not relevant and should not have been 89 presented. Moreover, the authors transformed all time units using a common scale varying 90 between 0 (the first year of the LLS abundance time series) and 1 (its last year). Obviously, 91 since time series had different lengths, the time scale varied depending on series and thus 92 slopes cannot be compared. Figure 2 by Crossley et al. and all related discussion are thus 93 meaningless.

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

97 We also point out that we had to re-program the R script provided by the authors using their

98	external databases S1 and S2 (ref ¹¹) to make it run.

99	Overall, we argue that these methodological shortcomings invalidate the results of the meta-				
100	analysis by Crossley et al. The analysis of their dataset would have required an adequate				
101	global model for all data, considering all our criticisms and those of ref. ¹¹ . We call for the				
102	application of rigorous standards for meta-analyses on global change, especially because				
103	results could have significant impact on policy decision-making and the fate of biodiversity.				
104	Insert	Table 1.			
105					
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153		

Arthropod subphylum or class	Number of time series	Information in the "order" and/or "species" column of external database S2 ¹¹	Involved LTER sites					
Time series of only non-insect arthropods								
	423	"Amblypygida", "Anactinotrichidea", "Araneae", "Ixodida", "Mesostigmata", "Opiliones", "Oribatida", "Pseudoscorpiones", "Scorpiones", "Solifugae", "Solpugida" or "Trombidiformes" in the "order" column;	Cedar Creek, Central Arizona Phoenix, Harvard Forest, North Temperate Lakes, Sevilleta					
Arachnida		"Various" in the "order" column and "Acari" (described as "Arachnida" in the corresponding North Temperate Lakes LTER dataset) in the "species" column;						
		No "order" provided; the "species" column indicates "VAE_COA" or "VAE_RUS", for the Vaejovis coahuilae and Vaejovis russelli scorpion species						
	29	"Amphipoda", "Decapoda", "Harpacticoida" or "Isopoda" in the "order" column;	Central Arizona Phoenix, Georgia Coastal Ecosystems, North Temperate Lakes					
Crustacea		"Various" in the "order" column and "Copep", "Clado" or "Ostra" (respectively described as "Maxillopoda", "Branchiopoda" and "Ostracoda" in the corresponding North Temperate Lakes LTER dataset) in the "species" column						
Entognatha	18	"Collembola" in the "order" column	Central Arizona Phoenix, North Temperate Lakes					
Myriapoda	14	"Chilopoda", "Geophilomorpha", "Lithobiomorpha", "Scolopendromorpha", "Scutigeromorpha" or "Spirobolida" in the "order" column; No "order" provided; "Diplopoda" in the "species" column	Central Arizona Phoenix					
Time series including both insects and other arthropods								
No information provided in the dataset	10	No "order" provided; the "species" column, rather than individual species, indicates aquatic invertebrate communities in terms of the following functional feeding groups: mixed substrate filterers, gatherers, predators, scrapers or shredders; or rockface filterers, gatherers, predators, scrapers or shredders	Coweeta					

Table 1. Times series with non-insect arthropods

157 **Box 1. Model used by Crossley et al.**

Each time series *i* is composed of abundance levels A_{it} for LLS *i* and for years t_{i1} to t_{iT_i} . 158 159 The scaled logarithm of abundance of LLS i in year t, y_{it} , is obtained in two steps. First, the 160 abundance value A_{it} is replaced either by its logarithm, $\log A_{it}$, or, if $A_{it} = 0$, by the logarithm 161 of a constant, $\log c_i$, where c_i is half the minimum non-zero abundance in time series *i*, to 162 obtain a series of log-transformed abundances a_{it} . Second, the empirical mean \bar{a}_i of logtransformed abundances of the series is subtracted to each a_{it} and this difference is divided by 163 164 the empirical standard deviation s_i of log-transformed abundances, which yields 165 $y_{it} = (a_{it} - \overline{a}_i) / s_i$. 166 The scaled year x_{it} is obtained by transforming the first year of time series t_{i1} to 0, and its last year t_{iT_i} to 1, and scaling all years accordingly as $x_{it} = (t_{it} - t_{i1})/(t_{iT_i} - t_{i1})$. 167 The proposed modelling is: $y_{it} = \beta_{i1} + \beta_{i2} x_{it} + \varepsilon_{it}$, where $\varepsilon_{it} = \rho_i \varepsilon_{i,t-1} + \eta_{it}$, with $\eta_{it} \sim N(0, \sigma_i^2)$. 168 169 170 171 **Competing interests.** 172 The authors declare no competing interests.