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COMMENT

Accounting for year effects and sampling error in temporal analyses of invertebrate population and biodiversity change: a comment on Seibold *et al.* 2019

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Abstract. 1. An accumulating number of studies are reporting severe insect declines. These studies aim to quantify temporal changes in invertebrate populations and community composition and attribute them to anthropogenic drivers.

2. Seibold *et al.* 2019 (Nature, 574, 671–674) analysed arthropod biomass, abundance and species richness from forest and grassland plots in a region of Germany and reported declines of up to 78% between 2008 and 2018. However, their analysis did not account for the confounding effects of temporal pseudoreplication.

3. We show that simply by including a year random effect in the statistical models and thereby accounting for the common conditions experienced by proximal sites in the same years, four of the five reported declines become non-significant out of six tests overall.

4. To place recent estimates of insect trends in a broader context, we analysed invertebrate biomass, abundance and richness from 640 time series from 1167 sites around the world. We found that the average trends across the terrestrial and freshwater realms were not significantly distinguishable from no net change. Shorter time series that are likely most affected by sampling error variance – such as those in Seibold *et al.* 2019 (Nature, 574, 671–674) – yielded the most extreme decline and increase estimates.

5. We suggest that the media uptake of negative trends from short time series may be serving to exaggerate the ‘insect Armageddon’ and could undermine public confidence in research. We advocate that future research uses appropriate model structures to build a more robust understanding of biodiversity change.

Key words. biodiversity loss, global change, insect, invertebrate, model structure, population declines, sampling variance, temporal analyses, year effects.

Appropriate spatial and temporal structure in time series analysis

Field studies that yield multiple observations per year from sites within a region are likely to be influenced by many shared uncontrolled variables, creating a ‘year effect’ because observations from the same year will often be more similar (Werner *et al.*, 2020). If this year-based pseudoreplication is ignored, confidence in trends estimated across years and probability of type I errors can be greatly inflated (Knape, 2016). A simple remedy for year effects is to include a year-intercept random term in statistical models (Knape, 2016; Werner *et al.*, 2020). Seibold

et al. (2019) presented an analysis of linear arthropod diversity trends across 140 (30 in some analyses) forest plots and 150 grassland plots over a 10-year period (a 9-year period in some cases). Their statistical analysis considered the spatial structure of the data in relation to consistent differences among plots and included plot ID as a random intercept nested within region. While Seibold *et al.* (2019) noted that there were ‘high numbers of arthropods in 2008’, and among year heterogeneity is clearly visible in Fig. 1 of the Seibold *et al.* (2019) paper, their analysis did not include a year-intercept random term. Instead, they assessed the sensitivity of their findings to exclusion of different years, which revealed that the significance of their evidence for declines was dependent on inclusion of data from 2008 (Seibold *et al.*, 2019; Supporting Information Fig. S2 of that study). They also included multiple environmental correlates, which suggest that they were concerned about year effects. However, even well-chosen covariates will

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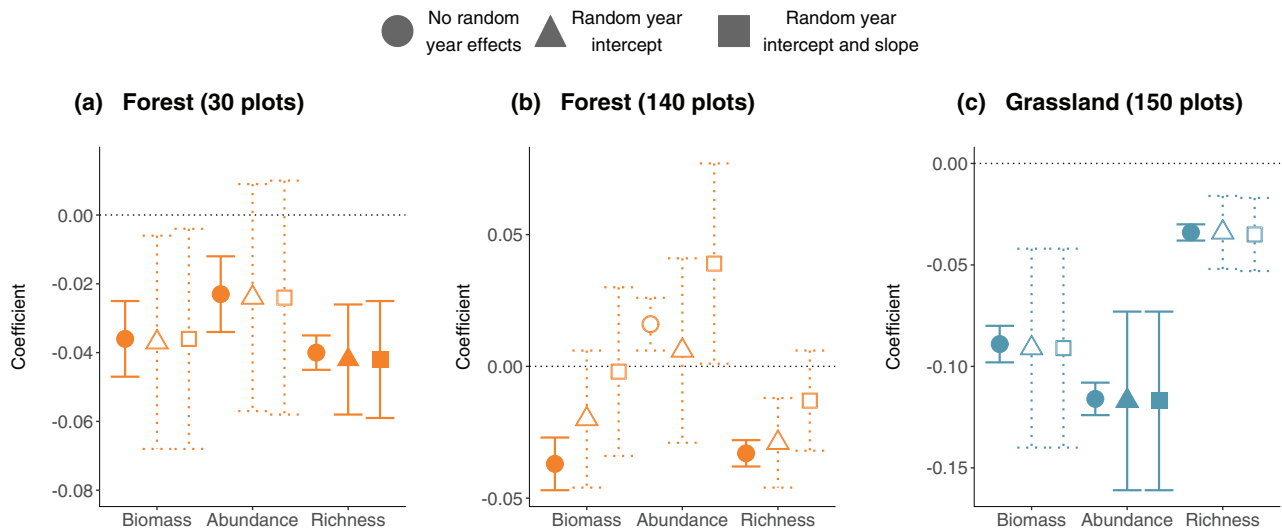


Fig. 1. Accounting for temporal pseudoreplication shifts four of the five detected declines out of six tests in the study by Seibold *et al.* (2019) from significant to non-significant declines. The six Seibold *et al.* (2019) tests refer to their six models of biomass, abundance and richness over time in forests and grasslands (three metrics, two habitats). Points represent the model coefficient for the trend over time and error bars show standard error, as derived from the three different model structures (see legend). We focused on the key results in the study by Seibold *et al.* (2019) (a, c) and did not include separate models for different trophic groups, but we anticipate that the significance of other findings reported in this study would be similarly affected by inclusion of a year random effect. We found a statistically significant relationship for abundance in the forest habitat without inclusion of a year random effect (a). We also analysed the three-year in addition to annual interval data from the forest plots (b) and found no net declines once random effects are included for the full dataset (b). See Supporting Information Figure S1 for model prediction fits, Supporting Information Table S1 for full model outputs and Supporting Information Table S2 for summary of analyses including environmental covariates. [Color figure can be viewed at wileyonlinelibrary.com]

generally be unable to capture all aspects of the environment that affect diversity in a year, thus year effects are likely to remain and inflate type I errors.

Methods

Data sources

We used the data published in Seibold *et al.* (2019) to test the influence of temporal pseudoreplication and model structure. We used 640 time series of biomass, abundance and species richness from the publicly available data from Dornelas *et al.* (2018) and van Klink *et al.* (2020) to demonstrate the larger distribution of the varying trends of invertebrate biodiversity over time as well as our concerns about model structure (Supporting Information Fig. S2). The time series had a duration of at least five years and together represented 1169 locations. The BioTIME data were rarefied based on sample size and study area as per Blowes *et al.* (2019). The van Klink *et al.* (2020) data were used in their original form.

Statistical methods to reanalyse the data in the study by Seibold *et al.* 2019

We reanalysed the data provided by Seibold *et al.* (2019) by applying three alternative model structures to data on biomass (log transformed), abundance and species richness (both using

Poisson error structure) separately for forest and grassland plots. Model 1 was intended to be similar to the structure used by the authors, and included year as a continuous predictor, region as a fixed effect (rather than random term, as there are only three levels) and plot within region as a random term. Model 2 included year as a factor as an additional random intercept term and model 3 included a random year slope term across sites. The motivation for including model 3 was to estimate whether diversity trends vary across sites. To account for overdispersion, when modelling count data using a Poisson error structure, abundance and richness models also included a random intercept for a `PlotIDYear` variable (concatenation to specify each plot in each year) for models 2 and 3.

We replicated the model structure used in the study by Seibold *et al.* (2019) which included environmental covariates and compared the detected temporal trends among models with and without a year random effect. We included the same environmental covariates as Seibold *et al.* (2019) – mean winter temperature, precipitation over the growing season, their interaction, land-use intensity, grassland cover, arable land cover and the interactions between year and land-use intensity, grassland cover and arable land cover. We advocate that the random effect model structure should be determined *a priori* rather than *post hoc* based on retaining only terms that are statistically significant. Models were fitted using the same lme4 package as the authors, via the `lmer` and `glmer` functions (Bates *et al.*, 2014). Significance of the temporal trends was estimated using `lmer` via the `lmerTest` package (Kuznetsova *et al.*, 2017). We inferred the

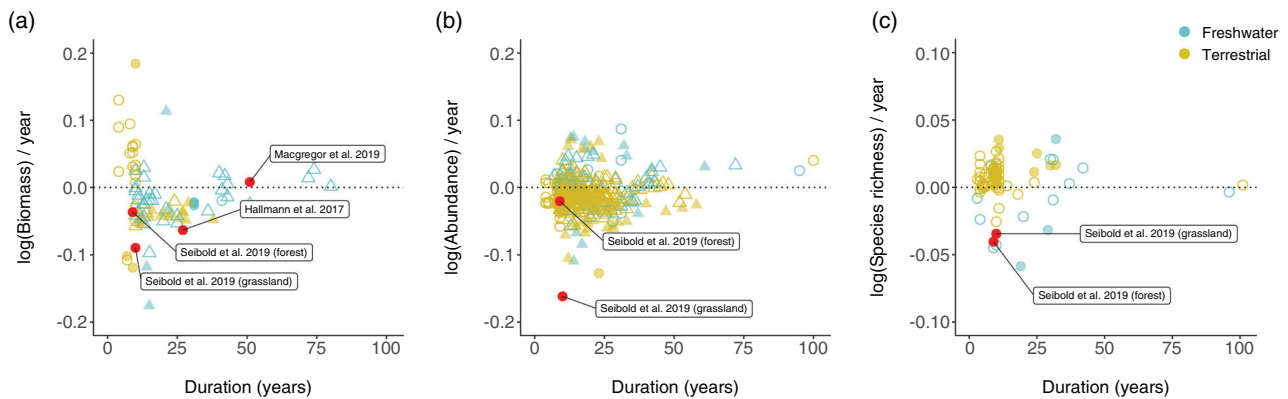


Fig. 2. Invertebrate trends over time span a spectrum of decreases, increases and no net change in biomass (a), abundance (b) and species richness (c). Points show effect sizes from time series from terrestrial and freshwater taxa, as well as effect sizes from published studies (Hallmann *et al.*, 2017; Macgregor *et al.*, 2019; Seibold *et al.*, 2019; red points, statistical significance of the literature-reported effect sizes not presented). Circles show time series from the BioTIME database (Dornelas *et al.*, 2018) and triangles show time series from the compilation of van Klink *et al.* (2020). Note that for visualisation purposes, we did not include the effect size (slope = -0.86) for abundance change in arthropods in a hurricane-dominated system from (Lister & Garcia, 2018), because this slope value was an extreme outlier in the distribution of trends (Blowes *et al.*, 2019). See Supporting Information Table S3 for full model outputs. [Color figure can be viewed at wileyonlinelibrary.com]

significance of the year random intercept term via a likelihood ratio test comparing models 1 and 2. For the forest plots, we ran two sets of analyses, one restricted to the 30 plots with annual data and a second analysis using all 140 plots. We found less pronounced diversity declines for the second analysis.

To test whether year order matters, Seibold *et al.* (2019) conducted 100 randomisations of the order of years and found that the observed insect declines were generally significantly steeper than the null hypothesis (Seibold *et al.*, 2019; Supporting Information Fig. S2 of that study). We revisited the year permutation test of Seibold *et al.* (2019), with site effects included but year effects excluded, and the order of years permuted 1000 times under the null hypothesis that year order is exchangeable. We found that with the exception of grass abundance ($P = 0.036$), the observed coefficients were not significantly steeper than expected under the null hypothesis (two-tailed test $P > 0.05$ in all cases, Supporting Information Fig. S3).

Statistical methods to demonstrate the wider distribution of invertebrate trends

We used a Bayesian modelling framework through the package MCMCglmm (Hadfield, 2010) to fit a model with a Gaussian error structure to analyse invertebrate biomass (logged) as a function of year (centred with a median of zero) and a random intercept term for year of observation, as well as year of observation grouped by ecoregion, and random slopes for the relationship between biomass and year at each plot. We allowed for covariation between the random intercepts and slopes across plots. The models we used for invertebrate abundance and richness followed a similar structure except they assumed a Poisson error distribution, since those data represent count integer data. We extracted the random slope values for each time series from

the BioTIME and van Klink *et al.* (2020) data. We presented the effect sizes, together with the effect sizes of published papers, in Fig. 2.

Results and discussion

Here, we show that four of the five arthropod declines reported in the study by Seibold *et al.* (2019) that are ‘statistically significant’ without a year random term became non-significant with the inclusion of a year random term in both a simplified version of their models as well as in models that contain the environmental covariates they included (Figure 1; Supporting Information Tables S1 and S2). With a year random effect included, we estimated the among-year variances across metrics to be substantial and highly significant even when environmental covariates were included (Supporting Information Tables S1 and S2). We recognise that a more robust model in this case would also incorporate autocorrelation among sites and among years, because year and site effects tend to be more similar between neighbouring samples, but mirroring the analysis of Seibold *et al.* (2019) and for the sake of isolating the influence of the year effect, we have excluded autocorrelation terms here. However, if such autocorrelation terms were included in the model, they will likely amplify the uncertainty in estimates and increase P values. Not accounting for year pseudoreplication in time series analyses in ecology is far from an issue specific to Seibold *et al.* (2019) (e.g., see Møller, 2019). As we work toward a more comprehensive understanding of change over time across invertebrates and other taxa (Saunders *et al.*, 2019; Thomas *et al.*, 2019; Daskalova *et al.*, 2020; Leung *et al.*, 2020; van Klink *et al.*, 2020), scientists need to use statistical methods that incorporate the pronounced spatial and temporal structure of population and biodiversity data.

Incorporating baselines and year-to-year variance in time series analyses

Climate research has recognised for some time (IPCC, 2013), and population ecologists and biodiversity researchers more recently (Mihoub *et al.*, 2017; Fournier *et al.*, 2019; Didham *et al.*, 2020; Mentges *et al.*, 2020), that where there is substantial year to year variance in a metric, the start and end date of a time series can have a strong effect on the estimated effect size and significance. For this reason, it is valuable to consider the baseline conditions before monitoring began in the interpretation of the significance of trends found within data (Mihoub *et al.*, 2017). A visual inspection of Fig. 1 in Seibold *et al.* (2019) indicates that the biomass, abundance and richness were higher than average in 2008, the first year of the time series. With the start point of their analyses being 2008, the observations in subsequent years were generally lower in comparison. When 2008 is removed from the analysis, the trends between 2009 and 2017 do not exhibit large directional changes (as is also evident in Seibold *et al.* 2019; Supporting Information Fig. S2 of that study). By accounting for the fluctuations in biomass, abundance and species richness among years, our reanalysis returned estimates with much greater uncertainty and the variance in year effects was estimated to be substantial and highly significant for all metrics (Supporting Information Tables S1 and S2).

Monitored populations viewed as a sample of trends across sites globally

Concern about insect declines (Hallmann *et al.*, 2017; Sánchez-Bayo & Wyckhuys, 2019; Montgomery *et al.*, 2020), as well as the general shortage of insect data (Packer *et al.*, 2018; Thomas *et al.*, 2019), has led to a reliance on short time series, as with Seibold *et al.* (2019). To examine how Seibold *et al.*'s findings fit within a larger sample of insect biodiversity trends, we combined the trends from Seibold *et al.* (2019) with recent temporal studies of the biomass, abundance and richness of invertebrate species as compiled by the global-extent BioTIME database (Dornelas *et al.*, 2018) and data from the recent meta-analysis by van Klink *et al.* (2020) (note that both data compilations include geographic gaps, Supporting Information Fig. S2). The two compilations of time series data represent a variety of habitats and environmental conditions and were collected using different survey techniques, but the methods were always consistent within time series (for further details see original papers Dornelas *et al.*, 2018; Blowes *et al.*, 2019; van Klink *et al.*, 2020). Our analysis of 283 BioTIME freshwater and terrestrial time series from 95 sites around the world indicated a decline in freshwater invertebrate biomass (slope = -0.02 , CI = -0.04 to -0.01) and no net change in terrestrial invertebrate biomass (slope = 0.02 , CI = -0.06 to 0.11 , with units of log(biomass) per year, measured in grams), no net change in abundance (freshwater slope = 0.01 , CI = -0.08 to 0.09 , terrestrial slope = -0.01 , CI = -0.04 to 0.02 , with units of log(abundance) per year, measured in number of individuals) and no net change in richness (freshwater slope = -0.01 , CI = -0.04 to 0.02 , terrestrial slope = 0.01 , CI = -0.01 to 0.02 , with units of

log(species) per year). While the average trends were non-directional, we detected substantial variation including both declines and increases (Fig. 2). For example, for freshwater abundance, the model estimates are consistent with 25% of time series (i.e., 18/72 time series) exhibiting a decline of 5% or more per year. In our analysis of 357 time series from the van Klink *et al.* (2020) data compilation, and consistent with the findings of van Klink *et al.* (2020), insect abundance increased in the freshwater realm but declined in the terrestrial realm (see Supporting Information Table S3 for effect sizes and credible intervals).

In statistics, there is a general expectation that the contribution of sampling error to effect size estimation increases as sample size and precision decline, and this effect is often visualised as a funnel plot (Egger *et al.*, 1997; Gurevitch *et al.*, 2018). We found clear evidence of this effect as the most extreme biodiversity trends were for the shortest time series (Fig. 2). An increase in the severity of declines towards the present time would see the most recent – and therefore shortest – time series being the most negative. However, we observed a different pattern, in which short time series returned the most extreme positive and negative trends (Fig. 2), as expected if the effect was due to sampling variance. Additionally, longer-term studies, which should better capture the mean trend, did not present the dramatic declines reported in shorter term studies (Fig. 2, and similar to the effects found in other longer-term studies like Macgregor *et al.*, 2019; Saunders *et al.*, 2019; van Klink *et al.*, 2020 and also found in vertebrate studies like Daskalova *et al.*, 2020; Leung *et al.*, 2020). Overall, we detected considerable variation across realms and among sites, with some individual locations exhibiting both substantial increases and decreases (Supporting Information Table S3 and Fig. S1).

Our reanalysis of Seibold *et al.* (2019) returned very similar effect sizes to the linear trends reported in the original study, with estimated declines remaining substantial, yet with much higher uncertainty. When we considered the estimates from Seibold *et al.* (2019) in the broader geographic and taxonomic context of results from other temporal analyses, their effect sizes represented the negative end of the distribution but were not extreme in comparison to other time series of similarly short duration. It is striking that some of the prominent studies in the insect decline literature have been of short duration, potentially revealing a bias toward high impact journals publishing more extreme and “surprising” results and/or their subsequent amplification by the media (Fig. 2).

Conclusion

Our analysis of 640 time series from the BioTIME and van Klink *et al.* (2020) databases demonstrated no evidence for invertebrate declines on average. However, steep declines could potentially be occurring in certain parts of the world and/or for specific taxa (Macgregor *et al.*, 2019; Didham *et al.*, 2020; van Klink *et al.*, 2020). Moreover, based on the lower 95% confidence intervals, we cannot reject the possibility that the average trend may be of a shallow decline (1–6% per year) for the various biodiversity metrics and ecosystems for which data are available, which would still be very severe. Therefore, we highlight that

on the balance of evidence to date, the scientific community should prioritise data collection to monitor local and global trends in invertebrate numbers (Kunin, 2019; Saunders *et al.*, 2019; Thomas *et al.*, 2019; Didham *et al.*, 2020; Dornelas & Daskalova, 2020). Against this backdrop, we suggest that media attention that overstates the problem runs the risk of later undermining wider public confidence in biodiversity research.

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Conflict of interests

The authors declare no competing interests.

Author contributions

G.N.D., A.B.P., and I.H.M.S. conceptualised the reply. A.B.P. reanalysed the Seibold *et al.* (2019) data with input from I.H.M.S. and G.N.D. G.N.D. analysed time series data from the BioTIME database and van Klink *et al.*, (2020) with input from I.H.M.S. and A.B.P. G.N.D. created the figures. All authors contributed to writing, editing and revising of the comment.

Data availability

The data from Seibold *et al.* (2019) are available from the original paper. The raw BioTIME time-series data are available from <http://biotime.st-andrews.ac.uk/>. The rarefied version of the BioTIME time series is available from the authors upon request. All code for statistical analyses is available from the following GitHub repository https://github.com/gndaskalova/Seibold_et_al_Reply.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1: Supporting information

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