nature research

Peer Review Information

Journal: Nature Ecology & Evolution
Manuscript Title: No net insect abundance and diversity declines across US Long Term Ecological Research sites
Corresponding author name(s): Michael S. Crossley

Editorial Notes:

Reviewer Comments & Decisions:

Decision Letter, initial version:

30th April 2020

*Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors.

Dear Dr. Crossley,

Your manuscript entitled "No Evidence of an “Insect Apocalypse” Across U.S. Long-Term Ecological Research Sites" has now been seen by 3 reviewers, whose comments are attached. The reviewers have raised a number of concerns which will need to be addressed before we can offer publication in Nature Ecology & Evolution. We will therefore need to see your responses to the criticisms raised and to some editorial concerns, along with a revised manuscript, before we can reach a final decision regarding publication.

In particular, Referee #1 feels that the results need to be presented in a more quantitative manner, while Referee #3 feels that additional sensitivity analysis is needed to account for aphid migrant data.

We also agree with this referee that the title should be amended. Titles should be brief and not include punctuation. We suggest something like: "No net insect abundance and diversity change across US Long Term Ecological Research sites"

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file [OPTIONAL: in Microsoft Word format].

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or

unlikely to yield a meaningful outcome.

When revising your manuscript:

* Include a "Response to reviewers" document detailing, point-by-point, how you addressed each reviewer comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the reviewers along with the revised manuscript.

* If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions at http://www.nature.com/natecolevol/info/final-submission. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

Please use the link below to submit your revised manuscript and related files:

[REDDACTED]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Ecology & Evolution or published elsewhere.

Nature Ecology & Evolution is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

[REDACTED]

Reviewer expertise:

Reviewer #1: Biodiversity change

Reviewer #2: Biodiversity change, insect ecology

Reviewer #3: Biodiversity change, insect ecology

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

I previously reviewed this for another journal: I was very positive about the potential for this ms to be published in a high-profile journal, and I have not changed my opinion. The new version is broadly the same but improved in a number of areas, most notably in placing the results into a broader context. Most of the rest of this review is recycled from the old version, although I have checked for issues that no longer apply.

The authors present an impressive dataset of 5300 insect time-series, and report no evidence for the prevailing narrative of precipitous declines. They explore change in multiple dimensions of biodiversity, including mean abundance, species richness, species evenness and assemblage composition. They also report aggregated trends at the level of study site, realm (terrestrial vs aquatic) and by feeding guild (although curiously not by taxon/species). The results are broadly consistent in all cases: there are some decreasing and some increasing trends, but no consistent tendency in either direction. These results are an important counter-balance to startling headlines about "insectageddon".

The authors present a number of sensitivity analyses to explain why the results differ from widely reported declines in Europe. The key issue they attempt to address is the fact that most LTER sites are well-protected and in areas of low human disturbance. The results are not wholly convincing (e.g. it's likely that the range of variation in human footprint index across LTER sites is rather small, so the lack of correlation is not surprising), and it's clear that the LTER sites are not really representative of USA as a whole. Although the authors say that most of the sites are in undisturbed areas, they also have 45 sites in agricultural landscape and one in urban area. Yet these sites are apparently dominated by non-native species (line 193 and Fig. 4), so it's not clear whether these are representative either. However, this would not be the first high-profile study to publish trends that may not represent the entire landscape. Moreover, the sensitivity analyses are probably the best that can be done (in the absence of time machine).

The discussion is primarily focussed on a comparison with the declining trends reported for Europe, but the authors have also placed their results into the context of debate about site-level biodiversity trends (e.g. several recent papers by Dornelas and colleagues).

The case for publication rests on two questions. First: is there about the analysis that would undermine the conclusions? One could quibble with any number of details about how the data have been analysed (e.g. the criteria for pooling sub-sites is opaque and could potentially be better-handled using random effects). One could also argue that it would be more interesting to have reported which species are doing well and which species are doing poorly. However, the bottom line is that I don't believe that any unmodelled bias would lead to a failure to detect the existence of substantive declines

(at least on these sites). Second, are the results sufficiently important? I think they are, given the prevailing narrative that insect Armageddon is imminent.

My only substantive criticism is that the results are presented in a rather unquantitative manner. The key results (from line 99) are described only vaguely (e.g. "change was modest" and "broadly overlapped zero"). There are two issues here. First, the authors give no indication of what magnitude of decline would be sufficient for them to refer to it as such (i.e. what is the null model?). Second, the results are presented in units of standard deviations, which makes it impossible to compare with the 'insectageddon' literature. Most existing studies reporting change as a percentage of the starting value: this is fine when the majority of the time-series show declines, but problematic if a large proportion also increase (because percentages are not symmetrically distributed). On the previous version I recommended that the authors report some statistics on the quantiles (or rather, the CDF) of the distribution(s) of trends (e.g., what is the proportion of trends that are declining (vs increasing?). Some new material has been presented with this in mind (line 148-154), but I think the authors could do more to draw out how these results contrast with those previously reported. For example, what is the proportion of trends that exhibit a net loss of 10%, or 30% (either over the full series, or per decade)?

SPECIFIC COMMENTS
Line 52-53: Having pointed out that the survey methods differ among taxa and LTERs, it would be worth asserting they have been consistent across time. The word 'systematically' implies consistency, but it would be worth being explicit in order to clarify that methodological differences among taxa and LTERs are irrelevant to the question at hand.
Line 146: This would be an appropriate place to mention the prevalence cases where loss in one biodiversity metric is compensated by increases in another (results in figure 3). Examples include Arctic, Cedar Creek sweep nets and Sevilleta. The relevant point here is that most insect decline studies report trends in only one metric: the fact that different metrics show contrasting patterns further undermines the case that insect declines are omnipresent and pervasive.

Reviewer #2 (Remarks to the Author):

This interesting and timely study uses publicly available data from the USA's long-term ecological research network (LTER) to investigate time series trends for insects and other arthropods. The authors use a range of statistical methods to explore variation in the time series trends, and to identify potential factors that may produce false positives/negatives. They also consider trends for birds and fish at some locations for which there are also arthropod data, to investigate whether arthropod-feeding vertebrates may be influenced by loss of food resources. In general, the study reads well and data appear to be analysed appropriately. I particularly like the discussion of broader implications and caveats and the concluding paragraph. I think it will be a valuable contribution to the literature. I just have a few comments and suggestions to improve clarity and some questions about methodological details.

Line 53: states 13 LTER sites here, but the map shows 12

The term sub-sites is a bit confusing (Table 1 & line 271). Perhaps call these 'sampling points'?

The ticks/person/hour dataset seems an odd inclusion, and I wonder how it compares to the other sampling methods. The sampling methods for other datasets described (lines 55-61) would be more representative of what's in the area at the time, whereas counting ticks on humans would be quite a specific measure of host density/availability/attractiveness, rather than tick abundance? There are no details on how these ticks were sampled, so I'm not really clear on the relevance here.

The aphid data (Midwest farmland site) appear to be from 46 sampling points pooled into one site, if I've interpreted correctly? On the map, the sampling points cover 4 different ecoregions. Would this be an issue to pool them into one site, to analyse with each of the other LTER sites that represent one ecoregion?

Line 299-305: I understand how difficult it can be to classify insects into feeding guilds, and I don't think there is a major problem with the author's approach here. However, based on the brief information given, I did wonder about potential mismatches between feeding guilds and location found. The authors classify species as terrestrial vs. aquatic based on the location found, but does the feeding guild also represent this location? For species with short-lived adults, the authors may classify them as terrestrial because they were found at a terrestrial site, but if they only live a few days/don't feed in that development stage does that match the feeding guild assigned to them? And species that have different feeding guilds at different development stages? E.g. flies that have aquatic larvae that are decomposers, and adults are nectar-feeders. Should the feeding guild be matched to the development stage they were collected as? This could perhaps be explained a bit more clearly in the methods.

Line 307: it says n=50 here for bird species, but above it says 775 species (line 294)?

Line 334: should be classify, not classifying

Reviewer #3 (Remarks to the Author):

This is a high-level analysis of change over time for herbivores, carnivores, omnivores, detritivores, parasites, parasitoids, birds and fish at US LTER sites using various model responses, including abundance and diversity, amongst others. The main conclusion is that there was no evidence of widespread insect abundance declines in North America and therefore no insect apocalypse. The paper is extremely well written and the figures and tables are of high quality, and are for the most part complete bar a few minor changes. I am impressed with the overall quality of the presentation. However, I think the authors should be more cautious, particularly given that their dataset is dominated by aphid migrants and the locations studied are drawn from sites that are generally healthy and in good order.

Herein, I have some comments that the authors should address

1. The paper must draw on comparisons with van Klink's work which has just been published and summarises 166 studies, 1676 sites across 41 countries, including those in North America and particularly some of the LTER sites.

Roel van Klink, Diana E. Bowler, Konstantin B. Gongalsky, Ann B. Swengel, Alessandro Gentile, Jonathan M. Chase. Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. Science, 2020; 368 (6489): 417-420 DOI: 10.1126/science.aax9931

For example, why are the patterns different between US Midwest and US West that indicate a decline in insect abundance for van Klink but this study the authors concludes that these 'showed unchanged or slightly increasing overall insect densities, species richness, and/or evenness broadly consistent with the less-disturbed sites (Fig. 2, 3)'? I believe that this result is most likely weighted by the Midwest aphid data set used in this study which is strictly not from any habitat as such but individuals are collected whilst migrating at 6 m (Lagos-Kutz et al. 2020). Table 1 shows that these aphid data number over 2000 time series, far greater than any other dataset used. There's no problem using these data, but what do those data say about whether these sites are from heavily disturbed versus relatively natural sites, a classification which dominates this study? I would argue that it is difficult to know and further, it is difficult to conclude about the health of the habitat if indeed the migrants aren't in it but migrating over it.
Doris Lagos-Kutz, David J Voegtlin, David Onstad, David Hogg, David Ragsdale, Kelley Tilmon, Erin Hodgson, Christina Difonzo, Russell Groves, Christian Krupke, Joseph Laforest, Nicholas J Seiter, Emily Duerr, Benjamin Bradford, Glen L Hartman, The Soybean Aphid Suction Trap Network: Sampling the Aerobiological "Soup", American Entomologist, Volume 66, Issue 1, Spring 2020, Pages 48–55,

I believe there is a need to do a sensitivity analysis with and without these data included and this would dispel any doubt. Importantly, Van Klink appear not to have used these aphid data but an old UK dataset. There is now an update on this which has also just been published

Bell, J.R., Blumgart, D. and Shortall, C.R. (2020), Are insects declining and at what rate? An analysis of standardised, systematic catches of aphid and moth abundances across Great Britain. Insect Conserv Divers, 13: 115-126. doi:10.1111/icad.12412

This paper is part of a special issue and one other study on the Poweshiek skipperling [Oarisma poweshiek (Parker, 1870; Lepidoptera: Hesperiidae)] shows that there are major habitat issues here which the authors of this paper seem to gloss over.

Belitz, M.W., Monfils, M.J., Cuthrell, D.L. and Monfils, A.K. (2020), Landscape-level environmental stressors contributing to the decline of Poweshiek skipperling (Oarisma poweshiek). Insect Conserv Divers, 13: 187-200. doi:10.1111/icad.12399

Most notable is that 99% of tallgrass prairie in North America has been destroyed since 1830. How can insects numbers be stable when the scale of habitat destruction is so great? Not one sentence is dedicated to this and I find this unsettling.

2. I would like to see some further direct geographical comparisons with van Klink et al's work. van Klink write that 'By contrast, the overall negative trends for terrestrial insects in North America have tempered and were no longer negative since 2000.' which might be consistent with this study but I think there is a need to compare van Klink et al.'s Fig 3, S1, S7 and draw direct parallels. Herein, could the authors also explain why van Klink seems to suggest an increase in freshwater inverts and compare with the current study's finding of which I am uncertain? It is clear that arctic stream inverts have been used in this paper, but I struggle to find any discussion of them.

3. I do like the novel use of the Human Footprint Index. The Fig S4B states that The average HFI value for locations within the US is 7; LTER sites ranged from 1 to 38. This indicates that across all datasets, the habitats were in relatively good condition and some almost pristine. In what way has this affected the conclusions? Afterall, I assume that declines would have been observed if the sites chosen has HFI >50? I think the authors should caveat their findings by this limitation.

Minor issues
Fig 2. What are the clear violin plots representing? Not urban, not freshwater but what?
Supplementary file
T test – is LC t test
Figs S1/S2 – what is the significance of blue text and blue bars in the supplementary graphs? The legend is not clear and should indicate that the plots show the species average slope from the regression models, indicating the overall rate of change in abundance at each of the LTERs.
Fig S1 right panel. Right panels depict average change in diversity metrics and 95% confidence intervals among LTERs. Please could you explain why parasitoids have a wide CI relative to the plots presented for Cedar Creek, Phoenix and Coweeta?
Fig S3 Abundance trends – of what? Across all taxa?

**********END**********

Author Rebuttal to Initial comments

Below we provide line-by-line responses to reviewer comments. Reviewer comments are provided in black font. Our responses follow in indented blue font.

Reviewer #1

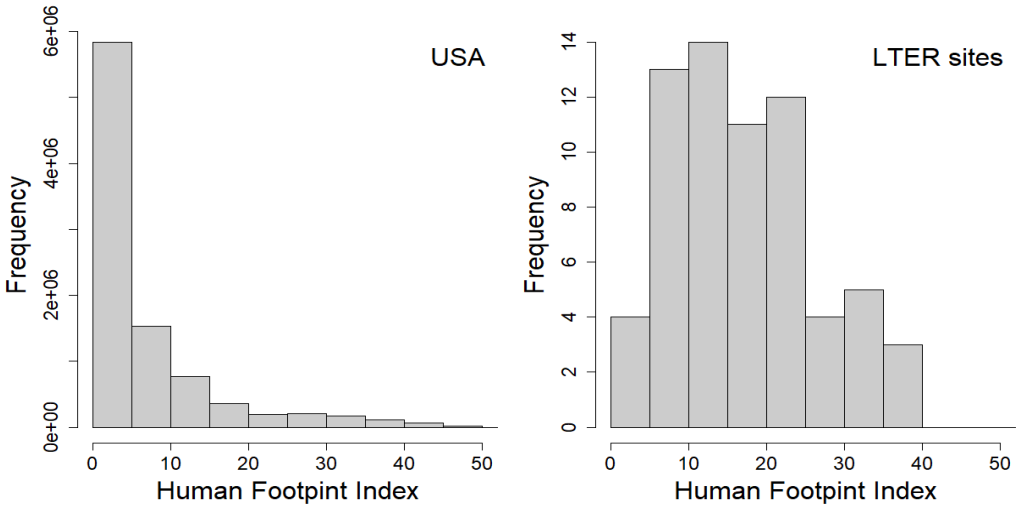
I previously reviewed this for another journal: I was very positive about the potential for this ms to be published in a high-profile journal, and I have not changed my opinion. The new version is broadly the same but improved in a number of areas, most notably in placing the results into a broader context. Most of the rest of this review is recycled from the old version, although I have checked for issues that no longer apply.

The authors present an impressive dataset of 5300 insect time-series, and report no evidence for the prevailing narrative of precipitous declines. They explore change in multiple dimensions of biodiversity, including mean abundance, species richness, species evenness and assemblage composition. They also report aggregated trends at the level of study site, realm (terrestrial vs aquatic) and by feeding guild (although curiously not by taxon/species). The results are broadly consistent in all cases: there are some decreasing and some increasing trends, but no consistent tendency in either direction. These results are an important counter-balance to startling headlines about "insectageddon".

We thank the reviewer for these positive remarks.

The authors present a number of sensitivity analyses to explain why the results differ from widely reported declines in Europe. The key issue they attempt to address is the fact that most LTER sites are well-protected and in areas of low human disturbance. The results are not wholly convincing (e.g. it's likely that the range of variation in human footprint index across LTER sites is rather small, so the lack of correlation is not surprising), and it's clear that the LTER sites are not really representative of USA as a whole. Although the authors say that most of the sites are in undisturbed areas, they also have 45 sites in agricultural landscape and one in urban area. Yet these sites are apparently dominated by non-native species (line 193 and Fig. 4), so it's not clear whether these are representative either. However, this would not be the first high-profile study to publish trends that may not represent the entire landscape. Moreover, the sensitivity analyses are probably the best that can be done (in the absence of time machine).

We have created a new supplementary figure (Fig. S8) to address this concern (please see page 13/lines 33-35):



Importantly, the Human Footprint Index (HFI) ranges between 0 and 50 by design, so by this measure, the majority of our LTER sites are in moderately disturbed areas, though our data includes sites on either extreme as well.

While we acknowledge that the Baltimore mosquito data set is now dominated by non-native mosquitoes, the dominant species in the Midwest Farmland sites is a native generalist aphid, *Rhopalosiphum padi*.

The discussion is primarily focussed on a comparison with the declining trends reported for Europe, but the authors have also placed their results into the context of debate about site-level biodiversity trends (e.g. several recent papers by Dornelas and colleagues).

The case for publication rests on two questions. First: is there about the analysis that would undermine the conclusions? One could quibble with any number of details about how the data have been analysed (e.g. the criteria for pooling sub-sites is opaque and could potentially be better-handled using random effects). One could also argue that it would be more interesting to have reported which species are doing well and which species are doing poorly. However, the bottom line is that I don't believe that any unmodelled bias would lead to a failure to detect the existence of substantive declines (at least on these sites). Second, are the results sufficiently important? I think they are, given the prevailing narrative that insect Armageddon is imminent.

We thank the reviewer for their positive remarks.

We also note that we have added some discussion of which species are doing well or poorly in a new paragraph (page 7/line 24 – page 8/line 6) and in page 9/lines 1-7.

My only substantive criticism is that the results are presented in a rather unquantitative manner. The key results (from line 99) are described only vaguely (e.g. "change was modest" and "broadly overlapped zero"). There are two issues here. First, the authors give no indication of what magnitude of decline would be sufficient for them to refer to it as such (i.e. what is the null model?). Second, the results are presented in units of standard deviations, which makes it impossible to compare with the 'insectageddon' literature. Most existing studies reporting change as a percentage of the starting value: this is fine when the majority of the time-series show declines, but problematic if a large proportion also increase (because percentages are not symmetrically distributed). On the previous version I recommended that the authors report some statistics on the quantiles (or rather, the CDF) of the distribution(s) of trends (e.g., what is the proportion of trends that are declining (vs increasing?). Some new material has been presented with this in mind (line 148-154), but I think the authors could do more to draw out how these results contrast with those previously reported. For example, what is the proportion of trends that exhibit a net loss of 10%, or 30% (either over the full series, or per decade)?

We appreciate this suggestion, and understand that trends measured in terms of percent increase or decrease seem more interpretable. In response, we have added a sentence to page 4 / lines 25-28: "In terms of net percent change per year, 2,319 (43%) and 1,665 (~31%) of trends exhibited decreases and increases greater than 1%, respectively; while 1,047 (19%) and 619 (12%) of trends exhibited decreases and increases greater than 5%, respectively. We also added a sentence to the methods describing how we calculated net percent change (please see page 13 / lines 20-24).

SPECIFIC COMMENTS

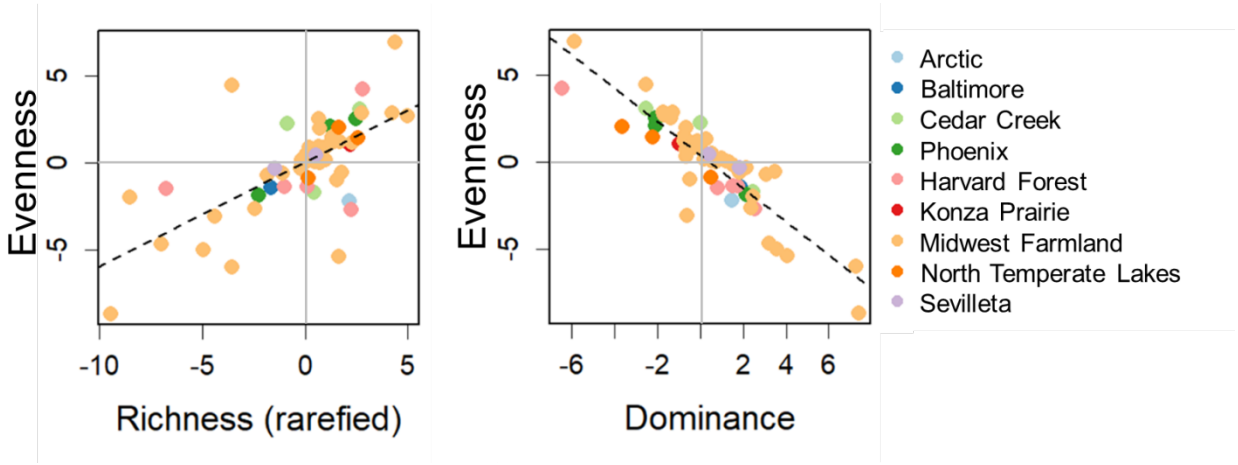
Line 52-53: Having pointed out that the survey methods differ among taxa and LTERs, it would be worth asserting they have been consistent across time. The word

'systematically' implies consistency, but it would be worth being explicit in order to clarify that methodological differences among taxa and LTERs are irrelevant to the question at hand.

> Because Table S1 contains the necessary detail to demonstrate how LTER sites have been sampled in a way that is consistent over time within each data set, we have modified this sentence to say (page 2 / lines 9-11): "Arthropod data have been systematically collected from at least 13 different LTERs (Fig. 1) using a variety of approaches (but in a consistent way over time within each data set; Table S1), with some reporting multiple, separate datasets based on taxa considered and/or method used for sampling (see Methods)."

Line 146: This would be an appropriate place to mention the prevalence cases where loss in one biodiversity metric is compensated by increases in another (results in figure 3). Examples include Arctic, Cedar Creek sweep nets and Sevilleta. The relevant point here is that most insect decline studies report trends in only one metric: the fact that different metrics show contrasting patterns further undermines the case that insect declines are omnipresent and pervasive.

> Great point. In response, we have updated Fig. S7 to help visualize cases where biodiversity metrics compensate each other, and have added a sentence in the Discussion (page 7/line 24 – page 8/line 6): "Similarly, species richness loss was sometimes accompanied by gains in evenness (Fig. S7; one Cedar Creek sweep net and two Midwest Farmland sampling points), or vice versa (Fig. S7; Arctic stream insects, Cedar Creek grasshoppers, Harvard Forest ants, and three Midwest Farmland sampling points), indicating that degradation in one aspect of biodiversity does not necessarily mean a wholesale decline."
>
> Including Fig. S7 here for convenience:



Reviewer #2

This interesting and timely study uses publicly available data from the USA's long-term ecological research network (LTER) to investigate time series trends for insects and other arthropods. The authors use a range of statistical methods to explore variation in the time series trends, and to identify potential factors that may produce false positives/negatives. They also consider trends for birds and fish at some locations for which there are also arthropod data, to investigate whether arthropod-feeding vertebrates may be influenced by loss of food resources. In general, the study reads well and data appear to be analysed appropriately. I particularly like the discussion of broader implications and caveats and the concluding paragraph. I think it will be a valuable contribution to the literature. I just have a few comments and suggestions to improve clarity and some questions about methodological details.

We thank the reviewer for these positive remarks.

Line 53: states 13 LTER sites here, but the map shows 12

The 13th site in the map is "Midwest Farmland", depicted as black diamonds. We have fixed the Fig. 1 legend to say N=13.

The term sub-sites is a bit confusing (Table 1 & line 271). Perhaps call these 'sampling points'?

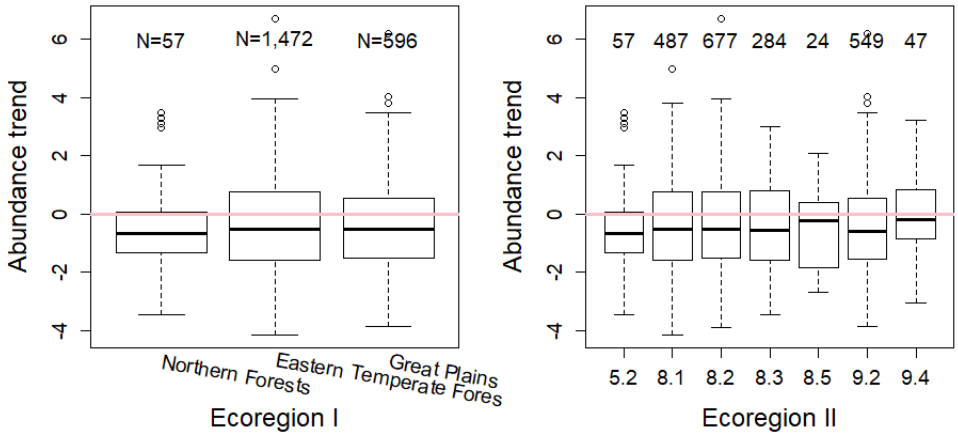
> We agree, and have made this change to Table 1 and to every occurrence throughout the methods text.

The ticks/person/hour dataset seems an odd inclusion, and I wonder how it compares to the other sampling methods. The sampling methods for other datasets described (lines 55-61) would be more representative of what's in the area at the time, whereas counting ticks on humans would be quite a specific measure of host density/availability/attractiveness, rather than tick abundance? There are no details on how these ticks were sampled, so I'm not really clear on the relevance here.

> We agree that the method used to quantify tick abundance at the Harvard Forest LTER is unique in its reliance on human attractiveness, but we chose to include data sets that had consistent within-study methods that were sampled for a long enough period of time. To reduce the chance of unintentional selection bias, we chose not to exclude any study as long as those two criteria were met.

The aphid data (Midwest farmland site) appear to be from 46 sampling points pooled into one site, if I've interpreted correctly? On the map, the sampling points cover 4 different ecoregions. Would this be an issue to pool them into one site, to analyse with each of the other LTER sites that represent one ecoregion?

> That is correct. Comparison of abundance trends broken out by Ecoregion (level I and level II) revealed no separation of trend distributions, so we have chosen to depict Midwest Farmland trends in Fig. 2 as a single violin plot:



Line 299-305: I understand how difficult it can be to classify insects into feeding guilds, and I don't think there is a major problem with the author's approach here. However, based on the brief information given, I did wonder about potential mismatches between feeding guilds and location found. The authors classify species as terrestrial vs. aquatic based on the location found, but does the feeding guild also represent this location? For species with short-lived adults, the authors may classify them as terrestrial because they were found at a terrestrial site, but if they only live a few days/don't feed in that development stage does that match the feeding guild assigned to them? And species that have different feeding guilds at different development stages? E.g. flies that have aquatic larvae that are decomposers, and adults are nectar-feeders. Should the feeding guild be matched to the development stage they were collected as? This could perhaps be explained a bit more
clearly in the methods.

> We have added some language about the complexity in assigning feeding guilds. We now recognize there can be mismatches between dominant feeding strategy and the life stage collected. We clarify that we classified the feeding guild according to the life stage collected so that it matched habitat assignment (please see page 12/lines 32-37).

Line 307: it says n=50 here for bird species, but above it says 775 species (line 294)?

We curated 775 bird abundance time series, but we only included trends for 50 arthropod-feeding birds in our analysis. Please see page 11/line 22, and page 11/line 34.

(page 12/line 22) “These data were available from four LTER sites for birds and three sites for fish, representing 775 and 171 species time series, respectively.”

(page 12/line 40): “For birds, all species that are obligate or facultative arthropodivores (insectivores and/or crustaceovore, 48) were included in the analysis (n=50).”

Line 334: should be classify, not classifying

Good catch, we have made the change.

Reviewer #3

This is a high-level analysis of change over time for herbivores, carnivores, omnivores, detritivores, parasites, parasitoids, birds and fish at US LTER sites using various model responses, including abundance and diversity, amongst others. The main conclusion is that there was no evidence of widespread insect abundance declines in North America and therefore no insect apocalypse. The paper is extremely well written and the figures and tables are of high quality, and are for the most part complete bar a few minor changes. I am impressed with the overall quality of the presentation. However, I think the authors should be more cautious, particularly given that their dataset is dominated by aphid migrants and the locations studied are drawn from sites that are generally healthy and in good order.

We thank the reviewer for their positive remarks.

In subsequent responses, we address the need to be more cautious in our interpretations of abundance trends given the nature of our data.

Herein, I have some comments that the authors should address

1. The paper must draw on comparisons with van Klink’s work which has just been

published and summarises 166 studies, 1676 sites across 41 countries, including those in North America and particularly some of the LTER sites.

Roel van Klink, Diana E. Bowler, Konstantin B. Gongalsky, Ann B. Swengel, Alessandro Gentile, Jonathan M. Chase. Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. Science, 2020; 368 (6489): 417-420 DOI: 10.1126/science.aax9931

> We agree that this study, which was published while our manuscript was in review, is an important addition to the insect decline issue. We think there are some methodological and study choice comparisons that may explain the discrepancy in findings, which we now address in detail in a new paragraph (please see page 7/line 24 – page 8/line 6).

For example, why are the patterns different between US Midwest and US West that indicate a decline in insect abundance for van Klink but this study the authors concludes that these 'showed unchanged or slightly increasing overall insect densities, species richness, and/or evenness broadly consistent with the less-disturbed sites (Fig. 2, 3)'? I believe that this result is most likely weighted by the Midwest aphid data set used in this study which is strictly not from any habitat as such but individuals are collected whilst migrating at 6 m (Lagos-Kutz et al. 2020). Table 1 shows that these aphid data number over 2000 time series, far greater than any other dataset used. There's no problem using these data, but what do those data say about whether these sites are from heavily disturbed versus relatively natural sites, a classification which dominates this study? I would argue that it is difficult to know and further, it is difficult to conclude about the health of the habitat if indeed the migrants aren't in it but migrating over it.
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Bell, J.R., Blumgart, D. and Shortall, C.R. (2020), Are insects declining and at what rate?

An analysis of standardised, systematic catches of aphid and moth abundances across Great Britain. Insect Conserv Divers, 13: 115-126. doi:10.1111/icad.12412

This paper is part of a special issue and one other study on the Poweshiek skipperling [Oarisma poweshiek (Parker, 1870; Lepidoptera: Hesperiidae)] shows that there are major habitat issues here which the authors of this paper seem to gloss over.

Belitz, M.W., Monfils, M.J., Cuthrell, D.L. and Monfils, A.K. (2020), Landscape-level environmental stressors contributing to the decline of Poweshiek skipperling (Oarisma poweshiek). Insect Conserv Divers, 13: 187-200. doi:10.1111/icad.12399

> We agree that suction traps measure the abundance of migrating aphids (at least ones that migrate at ~6 m). However, Rhainds at al. (2010) observed a close association between suction trap counts and field observations of soybean aphid (*Aphis glycines*), the most common aphid species in this data set, and Bahlai et al. (2014) were able to relate aphid suction trap counts to local landscape factors known to be important for soybean aphid life history. This suggests that, at least for crop-feeding aphids that account for most aphid counts in this data set, a decrease in aphid counts as measured by the Midwest Suction Trap Network likely reflects a true decrease in numbers of aphids in Midwestern farmscapes, and vice versa.
>
> Importantly, the trends among aphids in the Midwest Farmland sites were mostly negative, so excluding aphids from our analysis results in a less negative overall mean trend (-0.03 standard deviations per year, instead of -0.06 when aphids are included), and cannot account for the different conclusions between our study and van Klink et al. (2020). We discuss this in greater depth below (after comment #2).
>
> Bahlai, C. A., A. W. Schaafsma, D. Lagos, D. Voegtlin, J. L. Smith, J. Andrew Welsman, Y. Xue, C. Difonzo, and R. H. Hallett. 2014. Factors associated with winged forms of soybean aphid and an examination of North American spatial dynamics of this species in the context of migratory behaviour. Agric. For. Entomol. 16: 240–250.
>
> Rhainds, M., H. J. S. Yoo, K. L. Steffey, D. J. Voegtlin, C. S. Sadof, S. Yaninek, and R. J. O'Neil. 2010. Potential of suction traps as a monitoring tool for *Aphis*

glycines (Hemiptera: Aphididae) in soybean fields. J. Econ. Entomol. 103: 186–189.

Most notable is that 99% of tallgrass prairie in North America has been destroyed since 1830. How can insects numbers be stable when the scale of habitat destruction is so great? Not one sentence is dedicated to this and I find this unsettling.

Indeed, almost all tallgrass prairie habitat has been eliminated and converted to cropland. The LTER site in question (Konza Prairie), though, is located in the last remaining large unplowed ecoregion (Flint Hills) of tallgrass, about 25,000 km^2 of mostly contiguous habitat. So, while tallgrass prairie arthropods have indeed declined on the continental level through loss of habitat, they may not have changed that much in surviving habitat at the local level. We have added language on this issue and also included the reference (Welti et al. 2020) to a recent Konza Prairie analysis (please see page 9/lines 16-25).

2. I would like to see some further direct geographical comparisons with van Klink et al's work. van Klink write that 'By contrast, the overall negative trends for terrestrial insects in North America have tempered and were no longer negative since 2000.' which might be consistent with this study but I think there is a need to compare van Klink et al.'s Fig 3, S1, S7 and draw direct parallels. Herein, could the authors also explain why van Klink seems to suggest an increase in freshwater inverts and compare with the current study's finding of which I am uncertain? It is clear that arctic stream inverts have been used in this paper, but I struggle to find any discussion of them.

In response, we have created a new Table S3 that directly compares overall abundance trends between our study and van Klink et al. (2020). We also have added a new paragraph in the Discussion that addresses some key differences between our studies (please see page 7/line 24 – page 8/line 6).

Of the sites that were shared between our study and van Klink et al, most overall trends were in agreement (Table S3). That van Klink et al. arrived at an opposite conclusion stems mainly from differences in how study sites were chosen, namely, that van Klink et al. included four non-LTER data sets from North America that exhibited strong negative trends in total insect abundance/biomass,

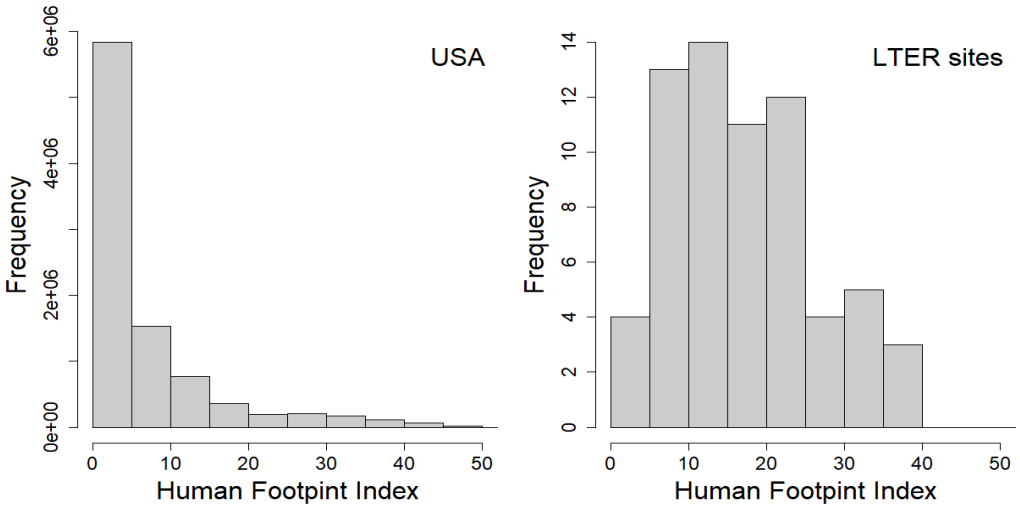
but excluded four LTER data sets that exhibited strong increasing trends (Table S3).

In our new paragraph, we specifically address the conspicuous difference in trends observed between our studies for the Konza Prairie grasshopper data set. The difference stems from how van Klink et al measured change in total abundance (aggregating across species), whereas we measured change in abundance per species. We have added a new Fig. 4 that demonstrates how strong declines in two once-dominant grasshopper species mask increases in rare species. We argue that the observed increases in richness and evenness at these sites further demonstrates how species-level analyses (like ours) may be a better indication of ecological health that measures of insect abundance in-aggregate.

We refrain from direct comparison of trends among freshwater invertebrates in the manuscript, because we included taxa from three LTERs, whereas van Klink et al. gathered 22 freshwater invertebrate data sets for the USA alone. We note here, though, that of the two data sets shared between our studies, we found the same overall trends: Artic (positive), North Temperate Lakes (trend not different from zero).

3. I do like the novel use of the Human Footprint Index. The Fig S4B states that The average HFI value for locations within the US is 7; LTER sites ranged from 1 to 38. This indicates that across all datasets, the habitats were in relatively good condition and some almost pristine. In what way has this affected the conclusions? Afterall, I assume that declines would have been observed if the sites chosen has HFI >50? I think the authors should caveat their findings by this limitation.

We have created a new supplementary figure (Fig. S8) to address this concern (please see page 13/lines 33-35):



Importantly, the Human Footprint Index (HFI) ranges between 0 and 50 by design (values > 50 are not possible), so the majority of our LTER sites are in moderately disturbed areas. We feel that for the Midwest Farmland sites, HFI is actually a conservative estimate of human impact. This is because HFI is especially sensitive to built infrastructure (roads, urban areas, e.g.), and gives less weight to agricultural land cover, which accounts for anywhere from 0% to 95% (mean=52%; median=59%) of Midwest Farmland sites.

Minor issues

Fig 2. What are the clear violin plots representing? Not urban, not freshwater but what?

We have added the following to the figure legend: “Unfilled violin plots and black font indicate LTER sites reporting terrestrial taxa in relatively less human-disturbed habitats.”

Supplementary file

T test – is LC t test

We apologize for not understanding what “LC t test” refers to, and will gladly take this comment into consideration if its meaning can be clarified.

Figs S1/S2 – what is the significance of blue text and blue bars in the supplementary graphs? The legend is not clear and should indicate that the plots show the species average slope from the regression models, indicating the overall rate of change in abundance at each of the LTERs.

> Good catch. We have added to the Fig S1 legend "Blue shading and font indicate LTER sites reporting aquatic taxa."

Fig S1 right panel. Right panels depict average change in diversity metrics and 95% confidence intervals among LTERs. Please could you explain why parasitoids have a wide CI relative to the plots presented for Cedar Creek, Phoenix and Coweeta?

> Yes, that does seem odd. But the reason is that there were only two sites (Cedar Creek and Phoenix) that reported parasitoids (the two sweep subsamples at Cedar Creek were aggregated for statistical analysis). So, in the equation for CI, the denominator was small compared to for other feeding guilds.

Fig S3 Abundance trends – of what? Across all taxa?

> We have added "of all taxa" to clarify the figure caption.

Decision Letter, first revision:

12th June 2020

*Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your co-authors.

Dear Dr. Crossley,

Your manuscript entitled "No net insect abundance and diversity declines across US Long Term Ecological Research sites" has now been seen by our reviewers, and in the light of their advice I am delighted to say that we can in principle offer to publish it. First, however, we would like you to revise your paper to address the final points made by the reviewers (especially Referee #2), and to ensure that it is as brief as possible and complies with our Guide to Authors at http://www.nature.com/natecolevol/info/final-submission.

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SPECIFIC POINTS:
In particular, while checking through the manuscript and associated files, we noticed the following specific points which we will need you to address (please provide a point-by-point response to these remaining points):

1. A brief editorial summary of the paper will appear on the journal homepage with the link to the paper. This is our proposed summary: "Analysing > 5,000 population abundance time series for insects and other arthropods from 68 sites within the US Long Term Ecological Research network, the authors find high variation but no overall trend in abundance and diversity among sites and taxa." Please let us know of any factual inaccuracies.
2. Please submit the main text, methods and figure legends as a single file (Word or LaTex), but the figures themselves as separate vector files (PDF, AI, EPS).
3. Please divide your article by subheadings. We need at least 'Results and Discussion' (we suggest at L100) but you may include other informative subheadings as well.
4. Please include citations in the main text to the Supplementary Datatsets uploaded to your files.
5. Please note that we have recently moved from having figures in the supplementary information to having them as Extended Data items, which are linked directly from the main text in the html version of the paper. Please see below for further details of how to submit supporting files.
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The main manuscript file should include the abstract, main text, methods, author contribution, data availability, code availability and competing interests statements, acknowledgements, references, and figure legends. Figures should be submitted separately as individual files. For details on other supporting material, please see below.

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All Supporting Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of two categories:

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supplied at a size that will allow both the figure and legend to be presented on a single A4 page. Each figure should be submitted as an individual .jpg, .tif or .eps file with a maximum size of 10 MB each. All Extended Data figure legends must be provided in the attached Inventory of Supporting Information, not in the figure files themselves.

2. SUPPLEMENTARY INFORMATION: Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, video files, large data sets and calculations). Each item must be detailed in the attached Inventory of Supplementary Information. Tables containing large data sets should be in Excel format, with the table number and title included within the body of the table. All textual information and any additional Supplementary Figures (which should be presented with the legends directly below each figure) should be provided as a single, combined PDF. Please note that we cannot accept resupplies of Supplementary Information after the paper has been formally accepted unless there has been a critical scientific error.

Additional Supplementary Figures and other items are not required to be referred to in your manuscript text (though they can be), but should be numbered as Supplementary Figure 1, not SI1, etc.

Methods & Notes:
Please include references for the Methods in the same list as those for the main text, following on sequentially after the main text references. Any citations in the Supplementary Information will need inclusion in a separate SI reference list.

Please include a data availability statement as a separate section after Methods but before references, under the heading "Data Availability". This section should inform readers about the availability of the data used to support the conclusions of your study. This information includes accession codes to public repositories (data banks for protein, DNA or RNA sequences, microarray, proteomics data etc…), references to source data published alongside the paper, unique identifiers such as URLs to data repository entries, or data set DOIs, and any other statement about data availability. All data that support the findings of the study must be made available. If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see:
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We hope to hear from you within two weeks; please let us know if the revision process is likely to take longer.

[REDACTED]

****

Reviewer Comments:

Reviewer #1 (Remarks to the Author):

I have read the revised manuscript and the authors' response letter. I am satisfied that all the issues I raised previously (and those of other reviewers) have been satisfactorily addressed. This article is ready for publication.

Reviewer #2 (Remarks to the Author):

Thank you for revisions addressing previous comments from all reviewers, the papers is much improved. There are some comments that I think have not been adequately addressed with relevant changes to the manuscript. If a reviewer has asked for clarification because information is lacking or confusing, then it's very likely other readers will also be just as confused when the paper is published.

Figure 1: The map caption still states "Filled black circles represent LTER sites… (n = 13)", but there are 12 filled black circles. Please improve clarity in the figure caption.

RE: my previous comment about the tick dataset. I agree with your point and I'm not suggesting you should remove the dataset. However, it seems like this dataset is different to most others, because it measures a specific interaction, rather than general abundance/diversity/occurrence, which is what your study claims to focus on. On second look, perhaps the gall insect data could also be included in this. I think it would be beneficial to acknowledge this in the methods.

Thank you for clarifying my concern about the potential ecoregion effects on Midwest sites. The figure you've provided in the response document allays this concern nicely, but I can't find mention of this anywhere in the paper or supplementary material. Please add this somewhere to help the reader interpret the results.

Line 109: the 'of' is not needed after "619 (12%).."

Reviewer #3 (Remarks to the Author):

I would like to congratulate the authors on the speed and quality of the revision. I am happy with what the authors have done to revise the MS to include the recent van Klink Science paper (Lines 192 onwards) which I found to be a fair an accurate assessment helped by Table S3, and, how they have presented a more balanced argument for the Prairies from line 246. I have two very minor recommendations that I am sure can be addressed in the proofing stage, should the MS be accepted, which are:
For Fig 3, you might like to write 'Please refer to Fig 2 legend for annotations and coloured text'
Fig 5 text in the stacked compositional species colour plot is a little hard to read.

**********END**********

Author Rebuttal, first revision:

In the following, we provide reviewer suggestions in black Times New Roman font, and our responses in blue Arial font.

Reviewer Comments:

Reviewer #1 (Remarks to the Author):

I have read the revised manuscript and the authors' response letter. I am satisfied that all the issues I raised previously (and those of other reviewers) have been satisfactorily addressed. This article is ready for publication.

Thank you!

Reviewer #2 (Remarks to the Author):

Thank you for revisions addressing previous comments from all reviewers, the papers is much improved. There are some comments that I think have not been adequately addressed with relevant changes to the manuscript. If a reviewer has asked for clarification because information is lacking or confusing, then it's very likely other readers will also be just as confused when the paper is published.

Figure 1: The map caption still states “Filled black circles represent LTER sites… (n = 13)”, but there are 12 filled black circles. Please improve clarity in the figure caption.

The reviewer is right – we are sorry to have missed this correction the first time. The caption now states n=12.

RE: my previous comment about the tick dataset. I agree with your point and I’m not suggesting you should remove the dataset. However, it seems like this dataset is different to most others, because it measures a specific interaction, rather than general abundance/diversity/occurrence, which is what your study claims to focus on. On second look, perhaps the gall insect data could also be included in this. I think it would be beneficial to acknowledge this in the methods.

We agree, and would even add the crab burrows dataset as well. In page 12/line 30 we have added “We acknowledge that some datasets, such as Harvard Forest ticks, Konza Prairie gall insects, and Georgia Coastal Ecosystems crab burrows, more directly measure arthropod activity but are nonetheless included in our abundance time series metadataset.”

Thank you for clarifying my concern about the potential ecoregion effects on Midwest sites. The figure you’ve provided in the response document allays this concern nicely, but I can’t find mention of this anywhere in the paper or supplementary material. Please add this somewhere to help the reader interpret the results.

We agree that the figure should be made available to readers, and have done so (please see new Extended Data 8).

We have added in Page 15/line 21 “Because Midwest Farmland spanned several ecoregions, we further separate aphid abundance trends from this dataset by ecoregion (Extended Data 8).”

We also added to the Fig. 2 caption: “Abundance trends for Midwest Farmland are further separated by ecoregion in Extended Data 8.”

Line 109: the ‘of’ is not needed after “619 (12%)..”

Great catch – we have made the change.

Reviewer #3 (Remarks to the Author):

I would like to congratulate the authors on the speed and quality of the revision. I am happy with what the authors have done to revise the MS to include the recent van Klink Science paper (Lines 192 onwards) which I found to be a fair an accurate assessment helped by Table S3, and, how they have presented a more balanced argument for the Prairies from line 246.

Thank you!

I have two very minor recommendations that I am sure can be addressed in the proofing stage, should the MS be accepted, which are:

For Fig 3, you might like to write ‘Please refer to Fig 2 legend for annotations and coloured text’

Great idea – we have added to the Fig. 2 legend: “Please refer to Fig. 2 legend for description of colored text.”

Fig 5 text in the stacked compositional species colour plot is a little hard to read.

We agree that species names are difficult to see. We have widened the stacked barplots and increased the font size in attempt to enhance visibility.

Final Decision Letter:

26th June 2020

Dear Dr Crossley,

We are pleased to inform you that your Article entitled "No net insect abundance and diversity declines across US Long Term Ecological Research sites", has now been accepted for publication in Nature Ecology & Evolution.

Before your manuscript is typeset, we will edit the text to ensure it is intelligible to our wide readership and conforms to house style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

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