Dear Dr. Farwig,

Thank for the opportunity to revise this manuscript. We very much appreciate the careful review and comments left by the two reviewers and feel that their suggestions have greatly improved the quality of this manuscript.

Below, please find our responses to the reviewer comments, shown in red. We have also uploaded a revised version of the manuscript through the author portal.

Thank you again, and we look forward to further comments and suggestions from reviewers.

Sincerely,

Micah Freedman

Reviewer #1: This manuscript reports that a bird-free island has a much higher prevalence of ants and honeydew-producing insects, compared to islands with abundant insectivorous birds. Thus, as the authors stress, the manuscript extends previous findings from small scale exclosure experiments to large spatial scales and indicates that strong top-down control by insectivorous birds is not restricted to small scales. Considering the previous research (e.g. on spiders) from the same system, the results are, in my opinion, expected but nevertheless interesting.  
The manuscript is well-written, easy to follow, and has no major flaws except the limited spatial replication. Please let me extend on this: The authors use an unique system, where birds have largely been extirpated by an invasive snake, and compare this to two islands where native bird communities thrive. Thus, the study necessarily suffers from pseudoreplication and spatial autocorrelation in a statistical sense, as all bird-free sampling localities are in close spatial proximity (the same for all locations with birds). While I would usually not encourage publishing studies with severe pseudoreplication/autocorrelation issues, the matter is different here and I think the study should be published (once revised). A statistically perfect design is for many reasons simply not possible at larger spatial scales. Nevertheless, you must openly address these issues in the discussion, which is currently not the case. Other than this, my comments are mostly pointing towards  
clarifications (see below) and how to adjust/extend the analyses.

We have updated our discussion and added a paragraph highlighting the shortcomings of our study design. In particular, we emphasize the spatial pseudoreplication inherent in our design and the limits that this imposes when inferring the mechanisms behind changes in ants and honeydew producing insects.  
  
Further general points:  
The referencing is sometimes a bit old and you mostly ignore the literature from the last 5 years or so. Please update.

Where possible, we have updated our referencing to including more contemporary literature. Below are the articles that we have added that were published within the last five years:

1. Staab *et al.* (2015), *Oikos*
2. Schuldt and Staab (2015), *Biotropica*
3. Schuldt et al. (2017), *Proc. Roy. Soc. B.*
4. Züst and Agrawal (2017), *Ecology*
5. Vidal and Murphy (2018), *Ecology Letters*

I suggest providing clear expectations/hypotheses at the end of the introduction. The questions currently used are a bit vague and cannot necessarily be deduced from the previous introduction text. Also, question (2) is only very superficially introduced. Please devote some text on this, as there are some potentially interesting findings and implications related to it (see below).

We added a section to the end of the introduction that provides specific predictions for each of the three hypotheses that we outline. Likewise, we have added a full paragraph to the introduction in an attempt to better contextualize question 2.

In one of their previous papers (Rogers et al. 2012 PLoS ONE) some of the authors report very high spider abundances on Guam (as opposed to the other islands also investigated here). Could your results also be related to the very higher spider abundances on Guam? Web-building spiders might reduce the numbers of predators and parasitoids (e.g. various taxa of wasps) that attack HPIs? From my own experience, a large share of HPIs can be attacked by parasitoids, which could have also been reduced by spiders (as a more indirect consequence of the absence of birds). Add on this in the discussion.

We have added this possibility to the same paragraph that highlights the limitations of our study design in the discussion.

Why did you not also calculate a community composition analysis for HPI that is equivalent to the composition analysis for ants? Depending on the outcome, this might help to infer if the effect of birds is stronger on ants or on HPI.

Unfortunately, due to the relatively small number of sampled branches that had HPIs present (n=59 total), we have limited power to conduct analyses on HPI communities. While we do have enough data to conduct multivariate analyses of the overall community of herbivorous arthropods, this includes many taxa that are not HPIs, and this would seem to be too much of a departure from the rest of the manuscript to discuss at length. The other shortcoming of our HPI data is that identifications are very coarse, as individuals were never sent to taxonomic experts for positive identification and are not present in local insect collections on Guam. Thus, in contrast to our ant data, the HPI data only includes identifications to the level of family or in some cases superfamily. We have added text to the methods section explaining our reasoning for omitting HPI community level analyses, and we have also added a supplemental figure showing an NMDS plot for the entire herbivore community, although we do not discuss this in the main text. We have also added a bipartite network depicting associations between HPIs and tree species, as per your request below.

If you feel that it would appropriate, we can more prominently feature the results of our herbivore community analysis. Perhaps interestingly, we found that herbivore communities are driven much less by island (PERMANOVA F2,138 = 6.95, R2 = 0.06) and more by tree species (F12,138 = 3.61, R2 = 0.20). This is in contrast to ant communities, for which island identity (F2,114 = 241.6, R2 = 0.60) was far more important than tree species (F12,114 = 2.62, R2 = 0.04).  
  
Line-by-line comments (in order of appearance not importance):  
Title: How about shortening to 'Landscape-level bird loss increases the prevalence...'.?

The title has been updated.

Abstract: I suggest mentioning the brown tree snake and its consequences already in the abstract.

We now mention the brown tree snake in the abstract.

L 66: This is not fully correct; there are also some Hemiptera groups (e.g. Plataspididae) that produce honeydew and are tended by ants.

Updated to reflect this.

L 95ff: Like in the abstract, mention somewhere that the absence of birds is a consequence of the brown tree snake.

Updated accordingly.

L 106: Add the size for each island.

Added.

L 123ff: Are these birds mostly feeding on the ants or the HPI?

This is hard to know, and I am not aware of any studies of foraging behavior or dietary composition in the insectivorous birds of the Mariana Islands. My best guess is that they feed more extensively on HPIs, as this has been reported in a closely related white-eye on Christmas Island that feeds on invasive scale insects.

L 137: How is the situation of the lizards today? Is the 1992 study still valid, or have the snakes after all birds and Geckos were consumed now shifted to skinks and other vertebrates?

I am not aware of any more contemporary island-wide surveys of Guam’s herpetofauna. At least one recent study (Campbell et al. 2012, *Ecology*: https://www.jstor.org/stable/23213513) did include estimates of lizard densities and biomass, although the methods are not directly comparable to the 1992 paper. Their paper suggests that *Carlia* skinks are the predominant lizard taxa and also make up a substantial portion of the brown treesnake’s diet on Guam.

L 143ff: How large was your sample size per island? This is currently completely unclear, unless you one has a look in the supplement. I suggest adding table S1 to the main manuscript.

Table S1 has been added to the main manuscript as Table 1.

L 157: Please change feet to meters.

Changed.

L 159: Did you somehow analyze the herbivore data? I can't find it in the text.

As mentioned above, we previously did not include any analysis of the herbivore community data, in part because of the sparseness of the data matrix for HPIs and the coarse identifications for herbivores.

L 159: Would the number of leaves per branch not be a useful covariate in your models? I think so, as the size of a branch (number of leaves could be a proxy for that) is for sure related to how many ants + HPI one can find.

Yes, we have updated the model structure so that leaf number per branch is a covariate. This did slightly impact the estimation of our main parameters but did not change any of main findings, and as expected, detection probabilities for ants and HPIs were higher on branches with more leaves.

L 183: Which package did you use? 'lme4'? Please state.

Yes, this has been added.

L 185: Specify the data type (e.g. categories, numerical, binary...) for all variables. I know, it is somewhere in the text, but it will be easier for the reader to understand your analyses if the information is provided together.

These have been added to the methods section.

L 192: What AICc threshold did you use?

Our threshold was 2.0, which seems to be standard practice and has been added to the text. In Table S2, we show ΔAICc values and associated model weights.

L 202: It is good that you use presence/absence data here. Abundances are not very meaningful for such analyses but are still commonly used.

Noted.

L 204-205: A further argument is that many aphids and mealybugs are parthenogenetic and a single individual can very rapidly extend to a colony.

This point has been added to the text.

L 214: What are the abundances here? Individual counts?

Yes, this refers to counts of individual ants and has been clarified in the text.

L 214: Specify on the 'scree' plots.

Scree plots depict how stress values change with increasing dimensionality and provide a guide for deciding an appropriate number of dimensions to include for an NMDS analysis.

L 220ff: Which dissimilarity measure did you use in the end? How many dimensions? Provide justification for your choices.

We used the Bray-Curtis dissimilarity metric, as our ant community dataset was based on abundance and not presence/absence data. We used k=6 dimensions, as this seemed to correspond to the point where multidimensional stress values fell below the 0.1 threshold.

L 251f: I am not familiar with this type of analyses (compare L 223-228). Maybe you can briefly explain on it in the methods? Otherwise, would simply excluding T. albipes from the composition analyses and comparing the outcome with the full data set also be a way to test for this? If composition among islands is much more similar without T. albipes, this could also be convincing inference.

We have briefly expanded on this analysis in the methods section. As you suggest, the method should produce the same effect as simply omitting *T. albipes* from the community matrix. However, when I attempt to do this, I am unable to do subsequent analysis because of the fairly high proportion of observations that included only *T. albipes* (i.e. the remaining data matrix after excluding *T. albipes* is too sparse to be analyzed by itself).

Discussion general: There is sometimes much repetition of results. I suggest pruning the unnecessary redundancy a bit.

A number of sections, including some whole paragraphs, have been removed from the discussion.

L 301: Usually, the susceptibility of single tree species to HPI is highly variable but has often also a phylogenetic component (see Staab et al. 2015 Oikos). So I am surprised that in your study almost all tree species are evenly attacked by HPI. This brings me to a potentially interesting thought: Are there native HPI on your islands? You indicate that most HPI are not native... So the high susceptibility could mean that due to the isolation on islands those tree species have lost their defenses against HPI, which is why they are now highly attractive for non-native HPI (see L 322-323). Or are all HPI species highly generalist, meaning they attack many tree species across plant families? I think a simple bipartite network analyses between trees and HPI (again, see Staab et al. 2015 Oikos for an example]) will shed light on this and should be added.

As far as we are aware, there are not native HPIs present in the Mariana Islands. We agree with the general suggestion that susceptibility to colonization by HPIs is likely to be driven, at least in part, by the evolutionary naïveté of the tree species in the Marianas. The same suggestion has been made about aphid colonization of native Hawaii plants (Messing *et al.* 2007), and indeed the endemic Marianas cycad (*Cycas mariannensis*)is currently near extinction after the recent introduction of a non-native scale insect (*Aulacaspis yasumatsui*).

As per your suggestion, we created a bipartite network showing interactions between HPIs and our sampled tree species, which is included as Supplemental Figure 1. Visual inspection of this network suggests that the numerically most abundant HPIs do indeed interact with multiple plant hosts, although we have not yet formally this idea by assessing the generality index of the network (i.e. Gqw p-H) as was done in the Staab paper. We can also present separate bipartite networks for each island, although there were small numbers of HPIs recorded from Rota and Saipan.

L 323: New research shows that this relationship is context-dependent and may also be negative for the plant (i.e. even higher damage once colonized by HPI, see Schuldt et al. 2017 Proceedings Royal Society B).

We have added this reference.

L 393: Details missing for reference Bolton 2007

Details added.

L 532: Species name should be in italics.

Updated.

Figure 2: Replace 'output' with 'results' in the legend.

Updated.

Figure 3: How did you deal with trees without ants in the NMDS? Excluded? How high is the stress of the ordination you show? On how many dimensions is it based (only the two shown or are there further dimensions not shown)? The axes including labels are too tiny. Please increase size, or they will be unreadable at print size.

Trees without any ants (i.e. rows with all 0s) were omitted, leaving us with 113 samples. The stress of the ordination is low, approximately 0.08 (using the metaMDS criterion), and the plot is based on a distance matrix generated with 6 ordination axes. The axis labels have been increased in size.

Supplement: Place the legends in the supplement next to the figures and tables. It is tedious to go back and forth.

Apologies for the confusion; we were adhering to the author instructions, although we agree that it is much easier to navigate when the figure captions are adjacent to the figures themselves. We tried to resubmit with figure captions adjacent to figures, but the submission was sent back.

Reviewer #2: This study uses the island-scale loss of birds to test for effects on ants and honeydew producing insects (HIPs).  The novelty here is the large spatial scale of the natural experiment, and this work is important for this reason.  The paper is well written and easy to follow. The downside to this approach is, of course the extreme degree of pseudo-replication; there is only one island with bird loss (Guam) that is compared to 2 islands with intact avifaunas. I think this is OK as long as the limitations are frankly acknowledged because we are unlikely to ever get a truly replicated experiment of this kind for birds.

We have updated the discussion so that the third paragraph now highlights the issue of psuedoreplication in our study design.   
  
I do have some concerns and suggestions for improvement.   
  
First, the results were somewhat equivocal as to whether bird loss seems to affect HPIs as one of the two islands with intact birds did not differ from Guam.  Given you're only comparing 3 islands, the failure to find a difference seems like pretty strong evidence for a week or no effect!  So the conclusions are really oversold.  The findings are discussed in terms of the birds being less abundant on one than the other island, but the only evidence cited for this seems to be for a couple of bird species.  Why these only? Is the abundance of insectivorous birds in total really represented by these 2 or 3 species? This non-significant result then lead to the question of the power of this study to detect island differences. Include some kind of retrospective power analysis to infer the kind of differences your design could detect with ~80% certainty would be really valuable as otherwise we're left wondering if this is a case of weak power or that birds really don't matter so  
much.

For the non-significant difference in HPIs between Guam and Rota, we have added to the discussion elaborating on why this might be the case. As per your suggestion, we conducted a retrospective power analysis, although we note that many authors question the utility of this approach after parameter estimates have already been generated. As such, we use the log response ratio reported from a separate meta-analysis (Mooney et al. 2010) for the top-down impacts of birds on Hemipterans. Based on this estimate (-0.47), we would have needed to sample 54 branches to reliably (with 80% certainty) detect a significant difference between Guam and Rota. Since we only sampled 34 branches, we suspect that the lack of a result in our data is more related to this small sample size than to a true difference between islands.

As to the second point about bird abundances, we choose to focus on these birds because they are by far the most abundant insectivores in the Marianas. Owing to the isolation of the Mariana Islands, there are actually very few bird species, and even fewer that are insectivorous. We have omitted some species such as the Saipan reed warbler (*Acrocephalus hiwae*) (only ~2,500 individuals total, and restricted to Saipan) as well as the non-native black drongo (*Dicrurus macrocercus*) because it almost never enters closed canopy forest. We also do not include some forest birds such as the Micronesian starling (*Aplonis opaca*) because while they do occasionally feed on insects, they are primarily frugivorous.

Second, the results for ants are also somewhat equivocal but not presented that way. One mechanism of assessing ants found no difference (branch presence) but the other did (trunk counts) but only the later is mentioned in the abstract and discussion. The difference between these results should be discussed. The trunk counts are likely more precise, so perhaps its not surprising this was significant whereas the branch presence was not.  It would be useful to use tree-level data to test whether your detection of HPIs on the focal branch predicts trunk counts.

We have added some discussion to the non-significant difference between islands in ant presence. As you suggest, we think that ant trunk counts are a better and more precise measure of abundance, and we highlight the reasons for this is the discussion.

As to the second question, we did include HPI presence on sampled branches as a predictor variable in our analysis of ant trunk counts, although it was only weakly positively associated with trunk counts. This may reflect the fact that foraging paths of ascending ants involved movement between trees in the canopy.  
  
Third, the study includes several components that seem outside the main hypotheses.  In particular, 13 tree species are compared, as are effects on ant community composition. Along these lines, the title states "effects on a non-native ant" but this isn't even mentioned in the abstract. And then the community / species identify of HPIs is not characterized.  To me its trivial to show community composition varies among 3 islands; I think the pseudo-replication becomes more excusable when you abstract up to trophic levels and interactions, but I don't think the community composition data really means much.  The one way I might include it is as an appendix that is cited in the methods just to show that there are relatively small differences so that the mechanism of bird effects may be likely to be driven by ant abundance, not composition. Second, tree species differences (either within or among island) also are tangential and not very meaningful.  I see why you'd want to  
include this as a random effect, or a fixed effect perhaps, but not with interactions with island.

We chose to include tree species identity as a fixed effect based on our observation that one tree species (*Macaranga*) seemed to be especially susceptible to attack by HPIs. We were interested in testing whether this was actually the case. Furthermore, because our system is comprised of primarily native trees exposed to an assemblage of almost exclusively non-native HPIs and ants, we are interested in determining whether these novel tri-partite interactions resemble those documented in other more co-evolved systems (where plant-HPI associations are only highly host-specific). Finally, we were also interested in the possibility that bird loss might change the susceptibility of certain tree species to attack by HPIs. For example, species with very low HPI prevalence in the presence of birds might become more susceptible to attack by HPIs in the absence of birds if host choice is based on selection of enemy-free space. This would be captured by an island\*species interaction, which would not be possible without including species as a fixed effect.

We agree that the ant community composition analysis is difficult to interpret because of the myriad factors that could drive differences in ant communities between islands. However, we feel that is interesting to highlight that the two islands with birds present have nearly identical ant communities, whereas Guam has a very different any community. The fact that the ant most strongly driving this difference (*T. albipes*) was also the ant species that engaged in the vast majority of observed trophobioses suggests that its abundance on Guam may in part be related to its association with HPIs. We have added this point to the discussion.  
  
I also have some detailed comments and suggestions.    
  
You frame your study as being important because of the large spatial scale it includes.  Some of the meta-analyses on trophic cascades (from birds and others) assess the effects of exclusion size, and you should review this.  Island scale is obviously larger than any manipulation, but this is still relevant.  You also cite the Spiller and Schoener island work; how does their island-scale manipulation compare to their manipulative studies?

This is a really great point. We have added text to the manuscript highlighting that meta-analyses that have considered exclosure size find no difference in top-down effects. As you mention, the largest of these exclosures is still relatively small (~750 m2) compared to the size of Guam (>500 km2).

We have added a section to the discussion that compares Spiller and Schoener’s island-level comparisons of top-down lizard effects to their manipulative experiments. The estimates between these two kinds of studies seem to be quite similar, further suggesting that the mechanisms involved are scale-independent.  
  
You could consider modeling the presence of ants on a branch (or abundance on the trunk) as dependent upon HPIs, island and the interaction.  The question would be whether islands differ in the HPI-ant association (vs. just the presence / absence of each), where the interaction tests this hypothesis.

This was indeed one of the candidate models that we tested and is shown in the model comparison Table S2. In all three sets of models tested, the model including a island\*ant/HPI interaction term was the second best performing model after the simpler model with no interaction terms. So, based on this, it does not seem like bird loss strengthens the association between ants and HPIs, but rather increases the abundance of both sets of interactors (at least that is how I would interpret the result). Please let us know if you would like for us to highlight this in the discussion.   
  
Fig. 1 Has detail in term of interaction strengths that are not warranted.  These are speculative and not in any way documented in this study (or substantiated by reference to other studies). In addition, for completeness it would also need to include other predators that birds impact besides ants (e.g. spiders).  The concept being tested is easy to follow and doesn't need this visual.  (It is also a bit crude, mostly because some but not all of the icons have line boxes around them.) This figure does make me think that your best approach here may be a path analysis where you include more information than is presented in this abstract.

Figure 1 was removed, since as you point out, it contains more detail than is warranted based on our data collection.   
  
Can you comment on how you lowered branches once cut and the extent to which you lost ants or HPI in the process?

We have updated the methods section to provide more detail on this. We almost certainly lost ants and HPIs in the process while branches fell from the canopy (especially some of the more mobile HPIs, like Membracids), but because the sampling effort was uniform across islands, this should not bias our estimates.  
  
L124: Why mention these birds in particular? They must be 2 of many?

See comments above.  
  
L202: It would be useful here to comment here on approximate mean proportion presence (say on each island) as this helps understand if this is a reasonable way to quantify your response.

These values have been added.  
  
L208: Counts per species? Or total? If the later, comment on what this means as different ant species will recruit at different levels to the same resource.

This was based on counts of individuals. We have added a portion to the discussion highlighting that our ant abundance data could in part be driven by ant compositional differences between islands, since as you mention, some species will recruit more abundant to a given resource.  
  
L236: The tree effect seems unrelated to your question, and perhaps species should just be included as a random effect. Your analyses include higher level interactions that would be testing whether island (bird) effects differ among tree species, and this seems unrelated to your hypotheses. So if you don't think tree species should be treated as random (i.e. perhaps because they are not selected in a random enough fashion to be representative of upsampled tree species) then I would at least exclude the interactions involving tree species.

See discussion above about the reasons for including tree species as a fixed effect. For what it is worth, we also tried including tree species as a random effect to see how this would impact the parameter estimates of our other main effects. It did not appreciably change our estimates (although the variance around some of the estimates was slightly lower), and model comparison of a model with tree species as a random effect did not drastically increase model fit (based on AICc) compared to a model with tree species as a fixed effect. Thus, we stand by our inclusion of tree species as a fixed effect.

For the interaction between tree species and island, this was included as a possible model, but it received very little support, so this is somewhat of a moot point.   
  
L159-160: How was the ant tending information used? I don't see this included.

We have added information about the ant tending data to the paper. The lone takeaways from these data are that instances of tending were far more common on Guam than the other two islands, and almost all instances of tending on Guam involved the ant *T. albipes.*  
  
L279-280: This difference in bird abundance should be described in detail in the methods.

The difference in bird abundance is described in the methods section. If you would like for us to detail the methods by which the cited papers measured these bird abundances, we can add this information as well.  
  
Instructions for accessing any attached files:

Supplemental figures should be accessible from the Editorial Manager portal. Data and scripts for analysis are available at https://github.com/EBL-Marianas/Ants\_HPIs.