

Comments to the authors:

The manuscript titled "Using DNA metabarcoding to assess insect diversity in citrus orchards" focuses on a very timely topic, the use of metabarcoding to assess insect diversity for Agriculture.

The article is clearly written, and the background is well explained. Probably, a more focus should be put on the differences between BINs as a proxy for species, and actual species records, since based on the results and discussion, the two concept are often confused.

The figures are useful to understand the text, although figure 3 may need to be redone (see comments below). I am not sure if the raw data has been shared, since no mention of it was made in the text.

The experimental design and the methods used are fit to the purpose, with three separate sampling areas in Citrus Orchards, each subsampled (3/10) at DNA extraction stage and at the sequencing step (2x), for a total of 43 samples, and 252 reps.

However, the authors initially state they want to analyse the composition of insect communities and differentiate them between categories (i.e., pests, beneficials). While the authors indeed obtained a list of species and classified them, this doesn't provide much insight into the insect communities, since the samples were collected during a 15-month period of time. Many of these species may have never interacted with each other due to seasonality, making them hardly part of the same community.

Additional investigation should explore the community composition at a seasonal (monthly?) level.

The results obtained are valid and potentially useful, but they have not been explored sufficiently.

Furthermore, it seems the authors confuse the concepts of BINs as a proxy to species and an insect species in the strict sense. These are not the same thing and it should be made more clear in this work.

Please, see my comments below to more detailed issues.

Major points of discussion:

- At lines 222-224 there is something quite confusing. It is true that BINs can be used as a species proxy, but it is also true that the authors have used the BINs to obtain species identification. The authors cannot say they have found more than 2000 insect species just because that's the number of BINs they obtained. This is due to the fact that most of their BINs are also matching the same species. The authors managed to link a BIN to a species in 875 instances, but only 813 of these are unique instances (e.g., sometimes two BINs are linked to the same species). Even amongst these 813, there are instances of the same BIN assigned to multiple species. These are clear cases of taxonomical issues, where a species may be part of a complex or may have been recently revised.

This is one of the limitations of BINs. They can be used as a species proxy, but they are NOT the same as a species. The authors should decide if they want to stick to the BINs (in this case do not consider a BIN = a species), or if they want to go deeper in their analysis and focus on those BINs that could be identified to species.

Personally, I think that for this kind of work it would be more useful to get to a species-level. Describing how many species have been correctly identified.

- Time series of the trap could add more values. The authors have samples in the same areas for more than a year, which means that they should be able to show the seasonality of some of the insect species during this time frame. It would add a lot of value to the manuscript if this could be incorporated into the discussion. For example, can the authors record associations between pests and parasitoids/predators across time? Are the parasitoids appearing after the pests? Can they see an increase in the number of parasitoid species where/when more pests are recorded?

Minor corrections:

Line 28: change “during” to “in”. It should read “in 2018-2019”.

Line 29: change sampled to “collected”. It should read “Insects were collected using..”

Line 30: change “collections” to “samples” and remove the parenthesis. It should read “43 pooled monthly samples.”

Line 101-103: In total there should be 45 samples. The supplementary table does not explain why samplers GAN and SHI had only 14 samples and QIU had 15. If all traps had a monthly collection for 15 months, there should be 45 samples. If samples were not collected/lost/failed, the authors should specify.

Line 110: it should be “manufacturer’s protocols”.

Line 119: not sure what the reference “Prosser et al. 2016” is doing there. If it refers to the use of MID, then it should go before the full stop, at line 121.

Line 127: remove “reaction” before “volume”. It should read “All PCR reactions had a total volume”.

Line 141: the number 5 should not be in letters.

Lines 171-174: these are very true information, but they are not methods. The authors should either move these lines to the introduction (when explaining the aims of the paper) or to the discussion.

Lines 181-182: The authors should remember that the overall number of traps they analysed was 43. This doesn’t change despite the number of DNA extraction subsamples and the number of sequencing reps. The numbers reported here are not correct/misleading. The Malaise trap collections are still 14 for GAN, 14 for SHI and 15 for QIU.

Figure 2: Where did the authors find the pictures of the insect? Can the authors ensure the figure for the Psocodea is actually correct? This looks like an Hemiptera hopper.

Figure 3: I personally find the figure slightly confusing. I think this is not how a Venn diagram should report the different amounts. The numbers reported in brackets are the TOTAL BINs for each orchard, these BINs should be subdivided between the various overlapping areas. The authors should remove the percentages and stick to the number of BINs. How many BINs are shared by all the orchards? How many shared only by two? Equally important (and currently completely missing from the figure, how many BINs are present only in one orchard?