Dear Dr Burdon,

Here you will find the new version of the manuscript entitled “The return of the trophic chain: fundamental vs. realized interactions in a simple arthropod food web”, which addresses all the suggestions raised by the referees. We would like to thank the referees as they have certainly helped to improve the manuscript. We also provide a document with a detailed answer to each of the comments provided by the referees. We hope that the manuscript is now ready for recommendation from PCI Ecology.
All the best,
Marta Montserrat, on behalf of all authors

Reviewers Comments

Reviewer 1
In this experimental study of effects of community structure on interactions among predatory and herbivorous mites, there was strong and frequent evidence that interactions that occurred when only pairs of species were present did not occur when other species were also present.
Evidence was from factorial experimental manipulations of species presence/absence showing frequent strong two-way interactions in the effect of species presence/absence on predation/mortality rates.
As the Discussion points out, these results, if generalizable to other and more complex food webs, would imply that the food web structure predicted from knowledge of interactions from pairwise trials would/could predict a more connected food web than would be realised. Furthermore, and again as pointed out in the Discussion, observations of interactions in the field will be more important than previously thought, and models that can predict the community context-dependence of pairwise interaction strengths might be preferable.
The information in the manuscript was clearly presented, conclusions were supported by the results, and the methods were appropriate to produce the results. The Introduction and Discussion were appropriate.

R: Thank you!

Reviewer 2
The authors conducted a series of arena-based tests to determine which feeding links occur when various combinations of 5 species are placed together. They found that, in general, intraguild predation links between two mite species are weak to nonexistent. The major dynamics of this system therefore tend to resolve into one or more food chains rather than omnivory motifs.

1 - The testing seems to have been well done and comprehensive; my only major comment for the methods and results sections is that it can be difficult to keep track of *which results are being described (in particular, which mite is serving as the IG predator and prey). The authors can likely resolve this by adding subheadings or something along that line.*

R: The text has now been improved following the indication of this referee (and that of referee # 3). Throughout the text, the reader can now keep track on when species are acting as IG-predator or as IG-prey. To do so, we have either added the subscripts ES or NC when applicable (see L204-206), or we have specifically indicated the role of each species in the text. For example, if the IG-predator is *E. stipulatus*, this is indicated in the text as follows: "IG-predators$_{ES}$ attacked IG-prey$_{NC}$ (…)". We hope that in this way it is much easy to follow the role each predator is playing at each moment.

2 - One minor comment about tables 1-2: there seems to be a typo of Generalized Lineal Models. This should almost certainly be Generalized Linear Models.

R: This has been corrected. See Tables 1 and 2.

3 - The simple and intuitive hypothesis tested (i.e., predators with the choice of two food items will tend to consume the preferred prey rather than both prey) and the thoroughly documented results are, unfortunately, rather weakened by the introduction and discussion. The introduction dismisses some very early work on food webs (e.g., Elton, 1927) to suggest an exclusive focus on linear chains, and then builds up quite a straw man by saying that intraguild predation/omnivory are expected to destabilize food webs. This was indeed the early expectation, but more recent tests have shown that weak links in omnivory/intraguild predation modules can increase stability (e.g., McCann et. al, 1998). Omitting this research makes the introduction read as overly dismissive of prior work and, to me, risks biasing readers against this manuscript. I strongly suggest that the authors carefully revise the introduction to include more recent theoretical tests of omnivory/intraguild predation effects on stability and to make clear that, despite this theoretical work, it is often assumed that all links in empirical webs are equally strong. c) This then leads naturally to the idea that a species' fundamental niche (all of its potential interactions) are unlikely to be realised at a particular place/time. Lines 56-63 give many excellent examples of how potential interactions may not always occur despite forming part of the food web at large.

R: Indeed, the whole body of work dealing with the strength of trophic interactions is relevant for our data. In an earlier version, we had two whole paragraphs about that in the introduction, but decided to eliminate them from the submitted version as (a) our data is not about interactions in general, but more specifically about two interaction modules and (b) our data does not test stability, which is the question posed by McCann and followers. However, after reading the manuscript again in light of this comment, we realize that it does sound weird not to mention it. We have thus followed the referees' suggestion and placed back a paragraph dealing with this research. We have done it in a way that is different from our original inclusion, which

allows being briefer and putting the emphasis on the assumptions of the models, rather than on their outcomes, which is the focus of our paper.
Concerning the reference to Elton, we would prefer to avoid it, as it would open the door to all the body of literature on niche theory that, although relevant, is somewhat lateral to the general topic of the paper.
Changes can be found in L55-70, 91-94, 100-115.

4 - The discussion has similar problems with over-simplification and loses the "fundamental vs. realised" thread almost completely. The authors seem to assume that, because predators tend to take only the preferred prey when both are offered together, predators in a field system will only ever take the preferred prey. This disregards the likely case that prey availability will vary such that, over its lifetime, an intraguild predator will indeed act as an omnivore. There is an interesting discussion to be had about how best to consider the different strengths of interaction between omnivory motifs (particularly stage-structured omnivory, as in this system), but this is largely skipped in the present manuscript. I particularly missed a discussion of weak interactions given the weight the authors place upon stability in the introduction. The author's discussion of different biocontrol strategies might offer a good place to include this: if occasional intraguild predation among mites occurs when aphids are scarce, might this allow both mite species to persist and provide better biocontrol? The authors could also incorporate theoretical work showing that weak interactions tend to stabilize food webs in order to tie their findings of weak IGP back to the stability theme in the introduction.

R: We have modified the Discussion to incorporate the points raised by the referee. We believe these changes (mostly in L463-486) have considerably improved the Discussion.

5 - Finally, both sections vastly over-state the "complexity" of the study system (5 species is certainly more complex than a pairwise system but is unlikely to accurately represent the hundreds-thousands of species in a real food web). Testing all combinations of species will therefore be impossible in many systems, and the authors' findings from a small and tightly-controlled system may not generalize to larger systems with varying abundances and densities of potential prey. I would therefore suggest that the authors soften their general claims and try to add more nuance to their discussion.

R: We only found one sentence in which we state that our system is relatively complex and have now changed that. Mostly, we have stated that our system is “relatively simple”, as it mimics simple community configurations refereed to as “community modules” (sensu, Holt 1997). We have now clarified this in L48-49, and in L109, for example. We also believe that in the discussion we have softened our general claims.

To sum up, I think the authors have conducted a well-done and important test of the strength of different feeding links in a system where intraguild predation is possible. The introduction and discussion are a little over-blown and tend to over-simplify the results, and this risks reducing the potential uptake of these results.
I suggest the authors carefully revise the introduction and discussion before submitting the manuscript for publication, being careful to look for recent theoretical tests of different intraguild predation strengths. My impression is that this work is generally in agreement with the authors' results (i.e., stable systems tend to have weak IGP), so including it would help to reduce the somewhat combative tone of the manuscript.
Speaking as a theoretical ecologist: we are aware that not all interactions occur with equal frequency and that occurrence of many interactions is contingent upon the sets of species present. Strong data on interaction frequencies (especially with respect to contingencies) is, however, almost always lacking. This is the root of the simplifying assumption of constant interaction frequencies. Studies like this one are an important step towards providing this information and so I hope that the authors will adjust the manuscript for a broader audience.

R: We are very grateful to this referee who has provided very nice insights and inputs which, with no doubt, have improved the quality of the manuscript.

Reviewer 3

The ms 'The return of the trophic chain: fundamental vs realized interactions in a simple arthropod food web' proposes an interesting idea of how increasing the number of species in a community might not necessarily increase the number of interactions.

1 - Unfortunately, I don't think this can be tested with the experiment presented here, as the actual occurrence or frequency of inter-specific interactions was not measured, but rather the authors present results on whether the presence of one species affects the mortality and oviposition of another species.

R: The scope of our manuscript was not to measure frequency at which interactions occurred, but to measure the impact of the presence/absence of species on the predation rate. We have now clarified this in the introduction and M&M sections. Clearly, the way we explained it caused confusion to the referee. In lines L108-115 it is now stated: "Specifically, we explore how pairwise trophic interactions between species are modified by the inclusion of other species in a simple community. The interaction we focus on is predation rate as it is an excellent proxy for trophic interaction strength, used both in modelling (e.g. the equivalent to the "catching efficiencies" in Kuijper et al 2003) and in empirical work (Wootton and Emmerson 2005; Novak and Wootton 2010; Novak 2013). Measurements of other relevant non-trophic interactions, such as competition, would require experiments at the population and community level that are beyond the scope of this manuscript."

2 - I also wonder if testing for apparent competition is legitimate when the authors only have 1 individual of the top predator and such a short time scale. As Holt and Bonsall mentioned in their Apparent Competition paper (Annual Review of Ecology, Evolution, and Systematics 2017), 'the qualitative assumptions leading to apparent competition are that an increase in the abundance of each prey benefits the predator, boosting its numbers, and that an increase in predator numbers then harms each prey'.

R: The communities we tested here were named following the configurations described in Holt (1997). We have now stated this explicitly in L48-49, L118, L208-209, and L207-208. Indeed, there is a lingering ambiguity concerning this term, which refers to either the community module or the impact of sharing a predator on population dynamics. We focus on the former, and hope that this is now clearer.

I do think that the experiment was well designed for testing changes in mortality and oviposition rates under different combination of species, and I really appreciate all the different control treatments performed.
There are several points within the manuscript that need to be clarified:

R: Thank you!

<u>Introduction</u>
3 - It would be useful to clearly define here what the authors mean by modules, as this term can have many interpretations in ecology.

R: This is now explained in L48-49

4- Similarly, stability can have multiple definitions, so the authors should clarify what they mean exactly by this term. Also, do all those studies mentioned (L55-67) use the same 'stability' measure? It would be useful to clarify this point.

R: We have now provided an explicit definition of this term (L55-56).

5 - In L95 the idea of a ´more complex´ community is introduced, but the author's haven´t explained well what they mean by this.

R: Following the changes made in reply to referee 2 and 3, we believe that now readers will understand better that by referring to "more complex" communities we mean communities with more species and more potential interaction occurring among them. See, for instance L106-109.

6 - L102: What do the authors mean by interactions are stronger? More frequent?

R: This has been clarified in L123-126.

Methods

7 - L214-216: I interpreted the results as the number of dead herbivores was estimated based on the number of herbivores that remained alive at the end of the experiment. The authors should clarify this.

R: Actually no, mortality was assessed counting the number of dead individuals. As for the cases when death was caused by predation, predation events are very easy to detect in our system as predators suck the contents of their prey and leave empty exoskeletons in the arenas. This is now more clearly stated in L234-238: "Twenty-four hours later, the number of dead herbivores/IG-prey (predation/mortality rate), and the number of eggs laid by predators/IG-predators (oviposition rate), were recorded. Predation events are very easy to detect in our system: predators suck the contents of their prey and leave empty exoskeletons in the arenas."

8 - In the Data analyses section, it would be useful to clearly explain what each interaction term means, i.e. why were they used? What would the interaction terms tell me if they were significantly positive or negative? What would a three interaction term mean?

R: We believe that the strength of our manuscript is the fact that we have included all possible controls. However, this is also its weakness, as the conclusions are based upon interpretations from comparing several treatments. In the first version of the manuscript, we opted for an over-simplified Results section and providing the interpretations in the Discussion only. However, we do agree with the referee in that this represents a considerable leap of faith for the readers. However, explaining the interpretation of all interactions would be extremely tedious and probably not very effective. Instead, we opted for an intermediate solution, which was to explain the meaning of the interaction terms not in the Data Analyses section of the M&M but in the Results section, only when interactions were statistically significant. We hope that this will help the future reader.
We also would like to thank the referee because his/her remark helped us to realize that in part of the table 2 (Table 2a) the values were wrongly transcribed. They have now been corrected.

9 -In this subsection the authors also mention 'rates of oviposition'. What exactly do they mean by this? Abundance of eggs laid per female?

R: This is explained in the M&M section, line 234-236: ""Twenty-four hours later, the number of dead herbivores/IG-prey (predation/mortality rate), and the number of eggs laid by predators/IG-predators (oviposition rate), were recorded."

10 - Also, why did the authors use a Poisson distribution? Wouldn't it be better to use a binomial distribution for assessing rates (i.e., number of dead individuals vs. number of survivors)?

R: The reason of why we use Poisson distribution is added into the text, L 243-245: "Predation rates on herbivores and on IG-prey, and rates of oviposition of IG-predators, were analysed using Generalized Lineal Models (GLM) assuming a Poisson distribution as the distribution of data is expected to be skewed towards low rather than high numbers, and a Log link function as no overdispersion of the data was detected."

11- L223-226: The authors haven't mentioned how the model is specified or that it has an interaction term, so they should move the model selection part after they describe the model.

R: We believe that this change is not really necessary, as now in lines 246-247 clearly state that all models were 3 factorials, and we indicate that the main factors in each analyses is explained in other parts of the ms.

12 - L235: I found the term 'predator juveniles' confusing. Are the authors referring to IG-prey that were juveniles?

R: We hope to have improved the track of each species' role throughout the manuscript. See answer to comment 1, from referee ·#2.

13 - L238: Please clarify if that is oviposition rates of IG-predators.

R: Done, L315

<u>Results</u>

14 - L244: Please clarify which were the 'main factors´ referred to here. I think that readers would have problems understanding from the text what is described here without looking at the table.

R: Please, see reply # 8 above.

15 - L248-259: This sentence is long and difficult to follow. Please revise.

R: The sentence is in fact composed of several sentences separated by ";".

16 -L248-251: Isn't this the same as what the glm tells us? I didn't understand why the authors used planned comparisons when they did their glm´s already, as both seem to answer the same questions.

R: We use planned comparisons because we are only interested in comparing some specific treatments, which are not necessarily reflected in the interaction term. Actually GLMs and planned comparisons are two completely independent concepts. You can apply GLM without the latter, and the latter in many other statistical models besides GLM. Planned comparisons with orthogonal or non-orthogonal contrasts are used to test specific predictions within the full model (Rosenthal and Rosnow 1985; see for instance Moya-Laraño et al. 2002), thus the answer is no, they do not answer the same questions.

References:

1 - Rosenthal, R., and R. L. Rosnow. 1985. Contrast analysis: focused comparisons in the analysis of variance. Cambridge University Press, Cambridge, UK.

2- Moya-Laraño, J., J. M. Orta-Ocaña, J. A. Barrientos, C. Bach, and D. H. Wise. 2002. Territoriality in a cannibalistic burrowing wolf spider. Ecology 83:356–361.

17 - L252-253: 'adding O. perseae did not influence mortality of the IG-prey' but the glm indicates the opposite (Table 1b, IG-predator x herbivore interaction).

R: The interaction is significant because the presence of the IG-predator does affect mortally of the IG-prey (compare, for instance, Fig 1b, columns 7 and 10 with column 8), but, indeed, such mortality is not affected by the presence of the share prey (as indicated in the text, please compare Fig 1b, column 7 with column 10).

18 - L254: 'predation of IG-predators', use the same term as used in the Figures so that it's easier to follow.

R: Cf. answer to comment 1, referee #2.

19 - L259: Since the authors reared E. stipulatus on pollen of C. edulis, could there already be a preference for this food type by E. stipulatus? Ideally these consumers would have been fed something different to the resources used in the experiment.

R: Please see answer #29, below.

20 - L267-268: Again, how is this different from the author's glm results and why do they then need both analyses?

R: Cf. answer #16.

21- L283: 'bar 5', according to the author's figure there was oviposition.

R: This rate of oviposition was not statistically different from the control (Fig 2, bar 6).

Discussion

22 - The results presented by the authors are about changes in mortality and oviposition rates when species are added, not whether an interaction occurred or not, so I would modify the discussion according to this.

R: We now explain, "We focus on is predation rate as it is an excellent proxy for trophic interaction strength, used both in modelling (e.g. the equivalent to the "catching efficiencies" in Kuijper et al 2003) and in empirical work (Wootton and Emmerson 2005; Novak and Wootton 2010; Novak 2013). Measurements of other relevant non-trophic interactions, such as competition, would require experiments at the population and community level that are out of the scope of this manuscript" (L109-114). Therefore, the suggestion of the referee does no longer apply.

23- L299: 'But not necessarily their occurrence'. The authors haven´t observed whether an interaction occur, but rather the consequences of adding species on mortality and oviposition rates.

R: We do observe whether predation occurred or not. In our system predation is detectable. As explained above, predation events can be counted a posteriori because predators suck the contents of their prey and leave empty exoskeletons in the arenas. Furthermore, discrimination between deaths caused by predation or by natural causes in the treatments with predators could be weighed by comparing data with the control treatments without predators.

24- L299-301: If so, how do the authors explain the significant interaction terms in their glm´s?

R: We now explicitly state the interpretation of significant interactions in the Results section; we believe that this will clarify this issue.

25 - L302: 'N. californicus killed more O. perseae females per day' than… Please elaborate.

R: Done, L 339-340.

26 - L319: Please revise this sentence. Also, there were IG-prey dead in both treatments with both IG-predator species (treatment number 10).

R: We have now modified the sentence (L355-357). It is important to notice, though, that the fact that there is mortality per se does not indicate predation, there has to be a comparison with the relevant controls…

27 - L319-321: And also because E. stipulatus can feed on something else (pollen), otherwise E. stipulatus could be outcompeted.

R: Yes indeed. This is one the points stated in the Discussion

28 - L323-325: This doesn't match with treatments 7 and 8 (Fig. 1). The number of prey items dead is equal.

R: The referee detected a mistake of ours. Thank you for noticing. The text has been corrected accordingly. L359-364.

29 - L348: I wonder if this is because the authors reared them with pollen and whether this could have had an effect on the feeding preferences of these invertebrates.

R: This is explained in the sentence that follows the one referred to by the referee.

30 - L352: According to the authors treatment 11 (Fig. 2b) there were IG-prey items dead. Please explain.

R: This mortality was not different than that in the presence of the IG-predators (compare treatments 11 and 12, Fig 2b)

31- L360-361: But there were no differences between treatment 11 and 12 in Fig 1b (number of IG-prey dead)? Please explain.

R: We believe that this is now made clearer in the Results section. Overall, the fact that we now explain each interaction will hopefully clarify all the previous issues raised by the referee.

32 - L367-370: But wasn't O. perseae accessible to E. stipulatus? If it wasn't accessible, then the experimental design may be flawed, thus hindering the author's ability to invoke apparent competition here.

R: E. stipulatus cannot penetrate the nests of O. perseae, and they forage only on mobile stages that wander outside nests. This is explained now in L397-399.

33 - L377-378: This doesn't make sense to me. Why would the predator stop feeding?

R: We believe that N. californicus ceases foraging because juveniles E. stipulatus interfere with the activity of N. californicus adults. We have corrected the sentence: "These results suggest that, in presence of IG-prey (juveniles of E. stipulatus), the IG-predatorNC ceased to forage on either herbivore or IG-prey, likely because IG-preyES interferes with the foraging activities of IG-predatorsNC. " L405-408.

*34 - Tables: What does the * mean in the tables?*
R: There was a mistake in the legend. Indeed, NS* refers to the cases when double or triple interactions were removed from models because they were not significant and they yielded a lower AIC. This has been corrected in the figure legends. NS without * refer, thus, to cases in which interactions were not significant but were not removed from the model because they didn't yield a lower AIC.

35 - Figures: 1 and 2 a) sometimes the authors say prey, others herbivores, others O. perseae. It would be better to use one term throughout the entire ms. Also, what do the authors mean by 'items'? Is this individuals? Please clarify.

R: We have tried to correct this throughout the ms. to ease the reading.

Thank you!