nature portfolio

Peer Review File

Context-dependent indirect effects mediate ecological transitions between parasitism and mutualism

Corresponding Author: Professor Syuan-Jyun Sun

This manuscript has been previously submitted at another journal. This document only contains information relating to versions considered at Communications Biology.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In this study, Lee and co-authors examine a multitrophic system involving a beetle that feeds on animal carcasses, and its associated mite and nematodes. Both the mite and the nematode are phoretic on the insect and colonise carcasses once adult beetles lay their eggs. By doing so, these phoretic animals can be transmitted to the next generation. The authors demonstrate, with a suite of elegant experiments, that the pathogenic nematode incurs lower fitness costs in the presence of the mite. The mite plays a crucial role in this system. This species feeds on the nematode and becomes a defensive symbiont of the beetle, but in the absence of the nematode the mite can incur costs, as it can feed on the beetle too. This is a classic system where the benefits conferred by the mutualistic symbiont (the mite) are context-dependent. Such context dependency classically explains variable prevalences of the symbiont in nature. By performing field sampling, the authors demonstrate such variable prevalence in the field. I do not have much to say about this part: the experiments are well performed, with good controls, the topic is timely, and it will for sure attract the audience of the journal.

I am a bit less enthusiastic about the second part of the study, where the authors characterise the microbiota of these carcasses. They report that carcass microbiota depends on the presence of the beetle, the mite, and the nematode (particularly the mite and the beetle). In an independent experiment, they also demonstrate that both the mite and the nematode can feed on some bacteria from these carcasses. However, it is not entirely clear to me whether the changes in microbiota (and the feeding assays) imply (i) a direct effect of mite activity on bacteria that then feeds back to beetle fitness (in this situation the mite is ensuring the mutualism), or (ii) whether microbiota changes are simply a consequence of beetle fitness (that the mite and the nemathode affect) that cascades down to carcass microbiota. Ideally, a manipulative experiment would be needed to demonstrate that beetle fitness decreases in certain symbiotic scenarios in carcasses that are either infested or not with particular bacterial communities. I do not think these experiments are required for publication, but a clear identification of this limitation is needed.

Enric Frago
CBGP - Centre for Biology and Management of Populations - Montpellier - France
(I sign all my reviews)

Reviewer #2

(Remarks to the Author)
The manuscript “Context-dependent indirect effects mediate ecological transitions between parasitism and mutualism” examines interactions between phoretic mites and nematodes and their beetle hosts, and how fitness outcomes of the players are shaped under different contexts. Overall, the study was interesting in that it examined interactions within a multi-species symbiosis, and the results were supportive of the overall message of the paper. I thought the manuscript was fairly clearly written as well. I have a few comments below on some conceptual clarifications:

Phoresy and parasitism:
1. Most of the measurements taken in this study were done before or after the transportation stage (e.g., on carcass), so it is

reasonable that the interactions were harmful or beneficial toward the host in the traditional sense. If the phoronts were harming the host during transportation, then I would see it more as support for the mites/nematodes being parasites instead of phoronts. That being said, I think the questions and results were still interesting and highlight the complex interactions between these players pre/post dispersal, and how hosts can transport organisms to breeding grounds that can ultimately affect their fitness.

2. Some of the language in the Introduction was focused on the host (beetle in this case), which was unclear at certain points. For example, in line 56, "fitness effects are neutral", but symbiont would be benefiting in some way?

3. Lines 56 – 57. I appreciate that the authors cited recent papers, but context-dependency has been an interest at least in the last decade (e.g., Chamberlain et al. Ecol. Lett. 2014).

4. Line 90. "Nutritional dependence" is somewhat vague and also not the only reason for non-phoretic interactions. I would define it more generally, such as without harming the host during the transportation phase following a reference that the authors cited (White et al. Curr. Biol. 2017).

5. Lines 94 – 96. "neutrality" by itself is vague because the phoront is benefiting.

6. Lines 280 – 281. Interactions on carrion can still be considered harmful/helpful toward the host without re-defining what happens during the transportation stage.

Relevance of bacteria:

7. It was not clear to me what the bacteria were doing in this symbiosis and why the authors were measuring them (lines 132 – 133, 268 – 269), at least until toward the end of the manuscript.

8. Lines 308 – 309. Is this a known phenomenon? Is this why the authors tested bacterial composition in carrion? I would include some of this context in the Introduction (maybe lines 323 – 327 as well if pertinent).

Clarification:

9. The authors were consistent with wording throughout when they were referring to the mites, nematodes, or beetles. In some places, however, I feel it would be clearer to indicate which organism the authors were referring to:

Line 36. Perhaps adding that "offspring" will refer to the beetle host going forward unless indicated.

Lines 103. Which offspring?

199 – 200. Nematodes on beetle offspring?

Lines 298 – 299. Is "larval" referring to nematodes or beetles?

Line 355. "arthropod" is confusing because it could include beetles and mites

Other comments:

Figure 2D. Because these are proportion data, it may be clearer to use bar graphs?

Line 212. Reducing CFUs compared to?

Line 306. Should be "feed".

Lines 314. what are mites feeding on in the absence of nematodes?

Line 320. Paragraph is too long.

Lines 348 – 351. This sentence is claiming too much without direct support. E.g., "Their [Wohlfahrtiimonadaceae members] preferential association with mites" suggests the enrichment of these bacteria are due to bacterial traits, when it could be due to the host or other environmental conditions not tested. Similarly, there was no evidence for coevolution (at least in this study) between the bacteria and their hosts. It is not specificity if the bacteria have not been tested on other kinds of hosts.

Line 365. Low densities of which organism?

Line 377. "refining models of symbiosis" is vague.

Line 378. "nematodes and microbial communities"—would it be microbial or mites?

Line 532. Extra period.

Line 670. "along" should be "alone".

Reviewer #3

(Remarks to the Author)
In this paper, Lee et al. demonstrate fitness effects, interactions during host development, and changes to environmental/dietary microbial community composition using the burying beetle and its symbiotic mite and nematode. The biological system is very interesting and ecologically relevant to add to our growing understanding of symbiotic interactions. The empirical data from the study alone is rigorous and fits within the confines of the journal. The results importantly shed light on inter-symbiont interactions as a condition tilting host fitness outcomes on the mutualism-parasitism spectrum. However, I have some concerns with how the paper is presented to the scholarly community and would strongly encourage it to be revised to avoid making broad conclusions:

- Multi-symbiont interactions especially in the context of phoresy where no overt host fitness benefit is evident is an interesting system to demonstrate what truly is commensalism. The authors could better explain the misconceptions and intractability of commensalism - Is everything in nature a cost-benefit tradeoff? Diverse systems such as the one in this study help advancing our view of commensalism as just a grey area or the continuity across the mutualist-parasite spectrum, and not as a categorical phenomenon.

- The results section starts off strongly with field surveys where beetles were found to carry either one of the symbionts. This is followed by lab experiments which show that mite-nematode interactions actually tilt the host fitness costs. This is a logical flow that shows what is ecologically selected for in the field, and how we are able to experimentally test these patterns. Could the authors explain these results better in the larger context? For example, is it surprising given the later results on inter-symbiont interactions, that beetle hosts are often found to host one symbiont at a time? Perhaps some info on the environment where some of these singly-infested beetles were collected, and in relation to the type of carrion they feed on could explain these patterns?

- For the nematode transmission results, it would be interesting to note mite presence or absence during nematode transmissions across beetle development. Given the result that nematodes may become extracellular at pupal stage and re-enter the adult host, would presence of mites affect this cycling? In natural populations, is this known? Fig 1 seems to imply that mites are present across all host developmental stages. Could nematode transmission assays be discussed better within the tripartite interaction?

- It is interesting that carrion presence enhanced predation – could this indicate that mites have a diverse diet? Do they just not prefer to target nematodes in the absence of carrion or does the carrion actually affect nematodes somehow that causes this increase in predation? Could this indicate ecological context is relevant for nematode parasitism of the beetle host? When beetles feed on carrion that already has a substantially altered nematode composition, are the mite symbionts influencing priority effects of which parasitic nematodes are able to interact with the beetle host?

- Lee et al. do a good job in being thorough in their literature survey of previous experiments and how their study adds new knowledge to this interaction. But, the discussion section is too broad in speculations of results from the current study. For example, the authors describe mites as bacterivorous, which to a non-expert seems unsubstantiated. Do we know that these mites are actually eating the bacteria or simply altering their abundances (directly or indirectly – eg: through antibacterial secretions?). I think the discussion could be used to more critically point at interesting and surprising trends from the results. This will set up the system to dissect the mechanisms favoring host mutualism v parasitism. The overarching message of the paper is diluted by pinning mechanisms on microbial community shifts, with actual little evidence provided for it in the results. Instead of predicting co-evolutionary relationships level without more evidence (Eg lines 348-356), these results are better discussed in an ecological rather than a mechanistic or evolutionary context. It would benefit researchers both immediate and broadly in the symbiosis community to understand how a tripartite intimate interaction among these invertebrates both shapes their environment (carrion microbial communities in this case) and how this feeds back into shaping the symbiosis itself (Eg host or symbiont fitness as a result of feeding on restructured environments). Overall, I would motivate the authors to limit hypothesizing to the scope of their results and encourage them to focus on the very interesting results (ex: shifts in microbial communities due to symbionts alone, even in the absence of the host beetle).

- Minor comments: Microbial taxa where appropriate were not italicized in the document. Please proofread for spelling errors.

- Figures: Particularly for fig 5, it would benefit a reader to see Fig S6 -c relative bacterial abundance at genus level instead of panels a and c which instead could be supplemental.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Communications Biology initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I have read this manuscript for the second time and noticed that the other reviewers were also somewhat critical of the microbiological analyses. I think the authors have done a good job addressing these concerns and clearly stating that, regarding microbial communities, the direct link to insect fitness, and thus to the maintenance of the symbiotic system, is not fully demonstrated. As written now, the conclusions are much less speculative, while at the same time opening novel avenues for future research.

Enric Frago
CBGP - Centre for Biology and Management of Populations - Montpellier - France
(I sign all my reviews)

Reviewer #2

(Remarks to the Author)
Thank you to the authors for thoughtfully addressing all of my concerns. I think the manuscript is much improved.

Reviewer #3

(Remarks to the Author)
Lee et al have provided an adequate rebuttal and revision for the comments provided. I am satisfied with the new shape this interesting manuscript has taken, and I'm excited to see it in press soon.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Communications Biology initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewers' comments:

Reviewer #1 (Remarks to the Author)

Comments to the Author(s)

Reviewers' comment 1:

In this study, Lee and co-authors examine a multitrophic system involving a beetle that feeds on animal carcasses, and its associated mite and nematodes. Both the mite and the nematode are phoretic on the insect and colonise carcasses once adult beetles lay their eggs. By doing so, these phoretic animals can be transmitted to the next generation. The authors demonstrate, with a suite of elegant experiments, that the pathogenic nematode incurs lower fitness costs in the presence of the mite. The mite plays a crucial role in this system. This species feeds on the nematode and becomes a defensive symbiont of the beetle, but in the absence of the nematode the mite can incur costs, as it can feed on the beetle too. This is a classic system where the benefits conferred by the mutualistic symbiont (the mite) are context-dependent. Such context dependency classically explains variable prevalences of the symbiont in nature. By performing field sampling, the authors demonstrate such variable prevalence in the field. I do not have much to say about this part: the experiments are well performed, with good controls, the topic is timely, and it will for sure attract the audience of the journal.

I am a bit less enthusiastic about the second part of the study, where the authors characterise the microbiota of these carcasses. They report that carcass microbiota depends on the presence of the beetle, the mite, and the nematode (particularly the mite and the beetle). In an independent experiment, they also demonstrate that both the mite and the nematode can feed on some bacteria from these carcasses. However, it is not entirely clear to me whether the changes in microbiota (and the feeding assays) imply (i) a direct effect of mite activity on bacteria that then feeds back to beetle fitness (in this situation the mite is ensuring the mutualism), or (ii) whether microbiota changes are simply a consequence of beetle fitness (that the mite and the nemathode affect) that cascades down to carcass microbiota. Ideally, a manipulative experiment would be needed to demonstrate that beetle fitness decreases in certain symbiotic scenarios in carcasses that are either infested or not with particular bacterial communities. I do not think these experiments are required for publication, but a clear identification of this limitation is needed.

Enric Frago

CBGP - Centre for Biology and Management of Populations - Montpellier - France

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Author response 1:

We agree with this viewpoint. Although our feeding experiments showed that mites could inhibit bacteria, our experimental design could not confirm a direct causal relationship. We also cannot rule out the possibility that changes in the cadaver microbiota were influenced by improved beetle larval health and their secretions. Therefore, we have added a new section (Line 356-359) in the discussion section, explicitly stating that the inability to clarify this effect is both a limitation of this study and a direction for future research.

Reviewer #2 (Remarks to the Author):

The manuscript "Context-dependent indirect effects mediate ecological transitions between parasitism and mutualism" examines interactions between phoretic mites and nematodes and their beetle hosts, and how fitness outcomes of the players are shaped under different contexts. Overall, the study was interesting in that it examined interactions within a multi-species symbiosis, and the results were supportive of the overall message of the paper. I thought the manuscript was fairly clearly written as well. I have a few comments below on some conceptual clarifications:

Phoresy and parasitism:

Reviewers' comment 2:

1. Most of the measurements taken in this study were done before or after the transportation stage (e.g., on carcass), so it is reasonable that the interactions were harmful or beneficial toward the host in the traditional sense. If the phoronts were

harming the host during transportation, then I would see it more as support for the mites/nematodes being parasites instead of phoronts. That being said, I think the questions and results were still interesting and highlight the complex interactions between these players pre/post dispersal, and how hosts can transport organisms to breeding grounds that can ultimately affect their fitness.

Author response 2:

Thank you for this comment. We have refined the definition of 'phoresy' in the Introduction to emphasize the lack of harm to the host, specifically during the transportation phase by referring to White et al. (2017).

Reviewers' comment 3:

2. Some of the language in the Introduction was focused on the host (beetle in this case), which was unclear at certain points. For example, in line 56, "fitness effects are neutral", but symbiont would be benefiting in some way?

Author response 3:

Thank you for pointing this out. By 'fitness effects are neutral' we indeed mean the effect of symbionts on hosts. We have explained this in detail.

Reviewers' comment 4:

3. Lines 56 – 57. I appreciate that the authors cited recent papers, but context-dependency has been an interest at least in the last decade (e.g., Chamberlain et al. Ecol. Lett. 2014).

Author response 4:

We totally agree that Chamberlain et al. 2014 is a key reference. We have cited it now.

Reviewers' comment 5:

4. Line 90. "Nutritional dependence" is somewhat vague and also not the only reason for non-phoretic interactions. I would define it more generally, such as without harming the host during the transportation phase following a reference that the authors cited (White et al. Curr. Biol. 2017).

Author response 5:

We have rephrased this sentence to ensure it explicitly refers to host fitness outcomes during the dispersal stage.

Reviewers' comment 6:

5. Lines 94 – 96. "neutrality" by itself is vague because the phoront is benefiting.

Author response 6:

We have rephrased it.

Reviewers' comment 7:

6. Lines 280 – 281. Interactions on carrion can still be considered harmful/helpful toward the host without re-defining what happens during the transportation stage.

Author response 7:

We agree with the reviewer's distinction. Strictly speaking, phoresy refers to the transport phase, whereas the fitness impacts we observed manifest primarily during the subsequent interactions on the carrion resource. It was not our intention to redefine the mechanism of phoresy, but rather to characterize the net fitness outcome of the symbionts' presence. We have revised the text to refer to 'phoretic associations' rather than 'phoresy' to encompass the broader relationship without conflating the transport phase with the interaction phase.

Reviewers' comment 8:

Relevance of bacteria:

7. It was not clear to me what the bacteria were doing in this symbiosis and why the authors were measuring them (lines 132 – 133, 268 – 269), at least until toward the end of the manuscript.

Author response 8:

We thank the reviewer for identifying this omission. We agree that the motivation for the bacterial assays should be established earlier to frame the study correctly. We have now added a paragraph to the Introduction (Lines 117) explicitly stating that the carrion resource is an intense arena for microbial

competition. We clarify that both mites and nematodes are bacterivores that may actively regulate the carcass microbiome to suppress putrefaction, which provides the rationale for our bacterial quantification and microbiome profiling.

Reviewers' comment 9:

8. Lines 308 – 309. Is this a known phenomenon? Is this why the authors tested bacterial composition in carrion? I would include some of this context in the Introduction (maybe lines 323 – 327 as well if pertinent).

Author response 9:

We appreciate this suggestion. Yes, the suppression of specific putrefactive bacterial taxa to preserve the carcass resource is a known phenomenon in burying beetle biology, and testing whether symbionts contribute to this function was a primary motivation for our microbiome analysis. Specifically, we aimed to test whether mites participate in a cleaning mutualism. While previous evidence has been mixed regarding their ability to reduce total bacterial abundance, we hypothesized that they might functionally improve the resource by altering the bacterial community composition (e.g., suppressing *Staphylococcus* and *Proteus*) rather than simply reducing the total load. We have now incorporated this context into the Introduction (Line 119) to better frame the motivation for our microbial assays.

Clarification:

Reviewers' comment 10:

9. The authors were consistent with wording throughout when they were referring to the mites, nematodes, or beetles. In some places, however, I feel it would be clearer to indicate which organism the authors were referring to:

Line 36. Perhaps adding that "offspring" will refer to the beetle host going forward unless indicated.

Lines 103. Which offspring?

199 – 200. Nematodes on beetle offspring?

Lines 298 – 299. Is “larval” referring to nematodes or beetles?

Line 355. “arthropod” is confusing because it could include beetles and mites

Author response 10:

Thank you for the wording suggestions. We have replaced ambiguous terms such as ‘offspring’ and ‘larval’ with ‘beetle offspring’ and ‘beetle larvae’ throughout the manuscript to clearly distinguish the host from its symbionts.

Other comments:

Reviewers’ comment 11:

Figure 2D. Because these are proportion data, it may be clearer to use bar graphs?

Author response 11:

Thank you for this suggestion. We have now updated the presentation of Figure 2D using bar graphs.

Reviewers’ comment 12:

Line 212. Reducing CFUs compared to?

Author response 12:

It was the control group without any symbiont that we referred to. We have made it clearer.

Reviewers’ comment 13:

Line 306. Should be “feed”.

Author response 13:

We have rewritten this paragraph, and so this comment is no longer relevant.

Reviewers’ comment 14:

Lines 314. what are mites feeding on in the absence of nematodes?

Author response 14:

In the absence of nematodes, the mites feed primarily on the carcass fluids and the associated microbes. Our bacterial culturing assays (Fig. 4a) confirm that mites significantly reduce bacterial loads even in the absence of nematodes, supporting the conclusion that they are potentially bacterivorous (although further experimental manipulation is required; see Reviewers' comment 26). We have clarified this dietary distinction in the revised text.

Reviewers' comment 15:

Line 320. Paragraph is too long.

Author response 15:

We have restructured and split this long paragraph.

Reviewers' comment 16:

Lines 348 – 351. This sentence is claiming too much without direct support. E.g., "Their [Wohlfahrtiimonadaceae members] preferential association with mites" suggests the enrichment of these bacteria are due to bacterial traits, when it could be due to the host or other environmental conditions not tested. Similarly, there was no evidence for coevolution (at least in this study) between the bacteria and their hosts. It is not specificity if the bacteria have not been tested on other kinds of hosts.

Author response 16:

We agree that our original claims regarding co-evolution and host-specificity were speculative and extended beyond the scope of our data. We have revised the text to strictly describe our results as an observed 'enrichment' or 'elevated abundance' rather than implying an evolved preference. We have also removed the language regarding co-evolution and instead framed the potential for specificity as a hypothesis that requires future comparative sampling across arthropod taxa.

Reviewers’ comment 17:

Line 365. Low densities of which organism?

Author response 17:

This was for ‘nematode’ densities – we have clarified it.

Reviewers’ comment 18:

Line 377. “refining models of symbiosis” is vague.

Author response 18:

We have rephrased this sentence.

Reviewers’ comment 19:

Line 378. “nematodes and microbial communities”—would it be microbial or mites?

Author response 19:

We have rephrased this sentence for clarity.

Reviewers’ comment 20:

Line 532. Extra period.

Author response 20:

We have removed the duplicated period.

Reviewers’ comment 21:

Line 670. “along” should be “alone”.

Author response 21:

Thank you – we have corrected it.

Reviewer #3 (Remarks to the Author):

In this paper, Lee et al. demonstrate fitness effects, interactions during host development, and changes to environmental/dietary microbial community composition using the burying beetle and its symbiotic mite and nematode. The biological system is very interesting and ecologically relevant to add to our growing understanding of symbiotic interactions. The empirical data from the study alone is rigorous and fits within the confines of the journal. The results importantly shed light on inter-symbiont interactions as a condition tilting host fitness outcome on the mutualism-parasitism spectrum. However, I have some concerns with how the paper is presented to the scholarly community and would strongly encourage it to be revised to avoid making broad conclusions:

Reviewers' comment 22:

- Multi-symbiont interactions especially in the context of phoresy where no overt host fitness benefit is evident is an interesting system to demonstrate what truly is commensalism. The authors could better explain the misconceptions and intractability of commensalism - Is everything in nature a cost-benefit tradeoff? Diverse systems such as the one in this study help advancing our view of commensalism as just a grey area or the continuity across the mutualist-parasite spectrum, and not as a categorical phenomenon.

Author response 22:

We appreciate this insightful comment. We agree that phoresy offers a unique lens to challenge the traditional, categorical view of commensalism. In the revised Introduction, we have expanded our definition of symbiotic spectra to explicitly frame commensalism not as a static state of neutrality, but as a "dynamic grey area" defined by subtle cost-benefit tradeoffs. We emphasize that "neutrality" is often context-dependent, and that these interactions exist on a continuity that can easily shift toward parasitism or mutualism depending on the ecological context (Lines 55–58).

Reviewers' comment 23:

- The results section starts off strongly with field surveys where beetles were found to carry either one of the symbionts. This is followed by lab experiments which show that mite-nematode interactions actually tilt the host fitness costs. This is a logical flow that shows what is ecologically selected for in the field, and how we are able to experimentally test these patterns. Could the authors explain these results better in the larger context? For example, is it surprising given the later results on inter-symbiont interactions, that beetle hosts are often found to host one symbiont at a time? Perhaps some info on the environment where some of these singly-infested beetles were collected, and in relation to the type of carrion they feed on could explain these patterns?

Author response 23:

We thank the reviewer for this insightful comment connecting our field and laboratory results.

Regarding environmental context, as our field data were collected using hanging traps baited with standardized mouse carcasses (Methods, lines 188–189), we cannot retrospectively determine the "natural" carrion history of these specific beetles prior to capture.

Regarding single vs. co-occurrence, while our lab results suggest a fitness benefit to co-occurrence (particularly at high nematode densities), the observation that not all beetles carry both symbionts is not necessarily surprising for two reasons. First, dispersal to ephemeral resources is highly stochastic; beetles often arrive at carcasses where specific symbionts are absent or fail to acquire them during the brief window of dispersal. Second, our data show that the benefits of co-occurrence are density-dependent. At low nematode densities, mites do not provide a survival benefit and can even impose a fitness cost (Fig. 2d). Therefore, singly-infested beetles (e.g., those with few nematodes but no mites) are not necessarily under strong negative selection.

We have added a sentence to the Discussion to clarify that this variation likely reflects both dispersal constraints and the context-dependent nature of the benefits.

Reviewers' comment 24:

- For the nematode transmission results, it would be interesting to note mite presence or absence during nematode transmissions across beetle development. Given the result that nematodes may become extracellular at pupal stage and re-enter the adult host, would presence of mites affect this cycling? In natural populations, is this known? Fig 1 seems to imply that mites are present across all host developmental stages. Could nematode transmission assays be discussed better within the tripartite interaction?

Author response 24:

This is an excellent point. Our current data strongly support the hypothesis that mites interfere with this specific transmission window. In our system, mite deutonymphs (the phoretic stage) attach to the parents, disembark to breed on the carcass, and their offspring (new deutonymphs) attach to both the beetle adults and offspring upon emergence. Thus, mites are physically present and active on the carcass exactly when nematode dauers exit the pupae to colonize the new adults.

Our results on individual offspring loads (Fig. 3c) show that at high nematode densities (100), the presence of mites significantly reduces the number of nematodes that successfully attach to beetle offspring. Since we now know nematodes must shift externally during the pupal stage to re-colonize the adult (Fig. S4), this external exposure likely makes them vulnerable to predation by the co-occurring mites. We have expanded the Discussion (Lines 313-317) to explicitly link the external pupal window with the observed reduction in nematode transmission.

Reviewers' comment 25:

- It is interesting that carrion presence enhanced predation – could this indicate that mites have a diverse diet? Do they just not prefer to target nematodes in the absence of carrion or does the carrion actually affect nematodes somehow that causes this increase in predation? Could this indicate ecological context is relevant for nematode parasitism of the beetle host? When beetles feed on carrion that already has a substantially altered nematode composition, are the mite symbionts influencing priority effects of which parasitic nematodes are able to interact with the beetle host?

Author response 25:

We appreciate the reviewer's insightful comments regarding the mechanisms driving predation and the importance of ecological context. We agree that the presence of carrion fundamentally alters the interaction between mites and nematodes, likely through two main drivers:

1. **Encounter rates and vulnerability: While *Poecilochirus* mites are generalist predators (feeding on fly eggs and nematodes), we propose that the enhanced predation on carrion is driven by spatial aggregation. The carcass serves as a focal resource where both mites and nematodes concentrate, significantly increasing encounter rates compared to the off-carrion environment. Furthermore, nematodes transitioning from the phoretic dauer stage to the active feeding stage on carrion may become more vulnerable to mite predation.**
2. **Community assembly vs. priority effects: The reviewer raises an excellent point about priority effects. In our system, parent beetles, mites, and phoretic nematodes arrive at the carcass simultaneously, which technically precludes a classic priority effect based on differential arrival times. However, we acknowledge that mite predation during the initial carcass preparation phase (before beetle larvae hatch) could functionally reduce the nematode burden that the offspring subsequently encounter.**

We have revised our Discussion to explicitly mention this 'mite-mediated filtering' and how the ecological context (carrion presence) is a prerequisite for mites to perform this protective service effectively.

Reviewers' comment 26:

- Lee et al. do a good job in being thorough in their literature survey of previous experiments and how their study adds new knowledge to this interaction. But, the discussion section is too broad in speculations of results from the current study. For example, the authors describe mites as bacterivorous, which to a non-expert seems unsubstantiated. Do we know that these mites are actually eating the bacteria or simply altering their abundances (directly or indirectly – eg: through antibacterial secretions?). I think the discussion could be used to more critically point at interesting and surprising trends from the results. This will set up the system to dissect the mechanisms favoring host mutualism v parasitism. The overarching message of the paper is diluted by pinning mechanisms on microbial community shifts, with actual little evidence provided for it in the results. Instead of predicting co-evolutionary relationships level without more evidence (Eg lines 348-356), these results are better discussed in an ecological rather than a mechanistic or evolutionary context. It would benefit researchers both immediate and broadly in the symbiosis community to understand how a tripartite intimate interaction among these invertebrates both shapes their environment (carrion microbial communities in this case) and how this feeds back into shaping the symbiosis itself (Eg host or symbiont fitness as a result of feeding on restructured environments). Overall, I would motivate the authors to limit hypothesizing to the scope of their results and encourage them to focus on the very interesting results (ex: shifts in microbial communities due to symbionts alone, even in the absence of the host beetle).

Author response 26:

We thank the reviewer for this constructive criticism. We agree that our initial draft may have overstepped in attributing specific mechanisms (e.g., bacterivory) to the observed shifts in microbial load. We have revised the Discussion to be more conservative, clarifying that while mite presence is associated with reduced bacterial loads, our current data cannot distinguish between direct consumption, antimicrobial secretions, or indirect competitive exclusion. Furthermore, we have reframed the discussion to prioritize ecological context over co-evolutionary speculation. We now interpret the observed microbial shifts as environmental modifications arising from the tripartite interaction, rather than asserting them as definitive fitness drivers without direct evidence.

Reviewers’ comment 27:

- Minor comments: Microbial taxa where appropriate were not italicized in the document. Please proofread for spelling errors.

Author response 27:

All microbial taxa (e.g., *Koukoulia, Staphylococcus*) are now consistently italicized per taxonomic standards.

Reviewers’ comment 28:

- Figures: Particularly for fig 5, it would benefit a reader to see Fig S6 -c relative bacterial abundance at genus level instead of panels a and c which instead could be supplemental.

Author response 28:

We thank the reviewer for the suggestion. We have revised Figure 5 accordingly: The genus-level relative abundance of the top 10 bacterial genera is now shown in Figure 5b, replacing the original panels a and c. The original panels a and c (Shannon diversity index and UPGMA clustering dendrogram) have been moved to Supplementary Figure S8. The NMDS plot (panel a in the revised figure) and family-level heatmap (panel c in the revised figure) remain in the main figure to illustrate community structure and higher-level taxonomic patterns.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Communications Biology initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.