

## Rebuttal Multiple stressor

Line 28: It is not clear to me what the control is here

With control we mean the *D. magna* that were not exposed to any stressor. We adjusted the sentence "... compared to the *D. magna* not exposed to any stressor" (Line 28).

Line 36: A bit too general. Include one or two examples

A few examples are included in the revised document: e.g., butterflies develop differently under different temperatures (Rodrigues and Beldade; 2020); many aquatic invertebrates move towards or away from light (i.e., phototaxis; negative phototaxis upon predation stress, Decaestecker et al., 2002) (Line 36-39).

Line 40-41: Both terrestrial and aquatic species?

Yes, the natural populations in this sentence refer to both terrestrial and aquatic species.

Line 58: I would incorporate here how the microbiota is affected (to avoid unclear terms) as you provide examples after that. In terms of diversity? Structure?

Thank you for this remark. We adjusted the sentence to "... differ in terms of diversity" (Line 60).

Line 63: I would incorporate this idea in the previous sentence as a third point (i.e. consequences on host fitness).

We choose to keep these sentences separately. The two points in the first sentence (less diverse microbial communities and/or less adapted host microbiomes) address differences in the microbiome diversity and composition, while the next sentence shows a link between the microbiome diversity and host fitness.

Line 67: It's a bit repetitive with the previous sentence, can you rephrase? Or this idea could come first and then you put your example with bacterial heterogeneity.

Thank you for this remark. We removed the sentence on bacterial heterogeneity in the revised document (Lines 66-69).

Line 76-92: I put this paragraph here because it's more logical to me to first say what is already known about *Daphnia* microbiota, before focussing on cyanobacteria and parasites (what you are going to test).

We agree that this order is more logic, and we accept the adjustment in the revised document (Lines 77-93).

Line 98: Put some general references on the link between parasites and global changes.

In the revised document, three references on the link between global change and parasites were added (Lines 99-100).

Line 104-106: What do they impact in particular?

The oomycetes cause high mortality in the zooplankton populations. We clarified this in the revised document: "..., by affecting survival and host population densities" (Line 107).

Line 111: What kind of trait(s)? Survival?

Thank you for this remark, we clarified this in the revised document: " ... (i.e., age at maturity, fecundity, body size and growth rate)" (Lines 112-113).

Line 140-142: It's too repetitive with the second paragraph of the introduction, I would remove this.

We agree and removed this sentence in the revised document (Lines 140-143).

Line 193: Figure 1: I would use the same abbreviation for your treatments in the figure as you presented in the text (CTL for control, C for cyanobacterium).

We thank you for this remark and adjusted the text on the figure (Line 195).

Line 218-219: Did you now receive sequencing results to identify the species/strain?

Based on microscopic pictures of the samples of the original experiment and comparison with pictures of Prowse 1954, Green 1974 and Seymour et al. 1984 (see figure SI1 in supplementary information), we estimate the infection to be an oomycete-like infection. Until now, we performed Sanger sequencing with multiple fungi specific primers such as: ITS (ITS1 (F), ITS2 (R), ITS4 (R), ITS5 (F) and ITS86 (F)), Small subunit (NSA (F) and NS4 (R)) and Large subunit (LROR (F) and LR5 (R)) primers. The sequences confirmed the presence of protists (such as *Vorticella*) and fungi (such as *Cladosporium*) but were not conclusive. As a next step, we will perform Sanger sequencing using oomycete-specific primers and will perform shotgun sequencing. This work is currently ongoing. A summary of the results so far can be found on: <https://docs.google.com/document/d/1FX9Clde-PMyejw2zK6SVPWeM76CdJo1EmAxR40qic6A/edit>.

Line 221-222: Separated or not?

The hyphae were separated. We corrected this sentence in the revised document (Line 223).

Line 228-234: Why don't you put this in the introduction when you talk about the effect of cyanobacteria alone on *Daphnia*?

Based on previous revisions, we restructured the material and methods section. We chose to specify the action of the stressor on *Daphnia* in the materials and method section and to keep the introduction more general.

Line 369: Figure 2: Could you put the legend that you included in your figures (2 + 3 + 5) bigger?

We increased the text size of the legend in figures 2, 3 and 5.

Line 437: Figure 5: Can you increase text size (including axis) in the figures 5 and 6?

We increased the text size of the legend, axis and axis title in figures 5 and 6.

Line 475: For your discussion, I would suggest to follow the order of the hypotheses that you mentioned in the introduction. For example, ideas/conclusions/hypotheses about ASV richness/recruitment of particular bacteria should be regrouped. As it is now, this info are in different paragraphs (not consecutive) and it lost me a bit. I would discuss after the different levels of interactions.

In the discussion we followed the order of the hypothesis. We hypothesized that (1) the stressor treatments would affect host fitness, and the stressors would act antagonistically, (2) *Daphnia* would have a higher fitness when they were exposed to a natural microbial inoculum than to a laboratory microbial inoculum, and (3) the effect of the stressors and microbial inoculum would be genotype dependent. In the discussion we followed the same order. A first paragraph confirmed that natural

and laboratory microbial inocula were differently structured, with the natural ones being more diverse. In the second paragraph we discussed the effect of the stressor treatment on *Daphnia* fitness without including the microbial inoculum as a factor (Hypothesis 1) In the third paragraph we discussed the antagonistic interaction between the two stressors. This antagonistic interaction is microbiome dependent (Hypothesis 2). In the fourth and fifth paragraph we elaborated on the effect of the microbial inoculum on the interaction between the stressors and its effect on *Daphnia* fitness. In the sixth paragraph we discuss that the response to stressors is genotype-dependent (Hypothesis 3).

Line 510-511: So in what way is it virulent too?

Both the yeast *Metschnikowia bicuspidata* and the bacterium *Spirobacillus cienkowskii* are highly virulent for individual *Daphnia* hosts. However, at population-level, only *Spirobacillus* epidemics were associated with significant changes in host population density and dynamics, *Metschnikowia* epidemics were not. A parameterized evolutionary epidemiological model predicted that the combination of very high selectivity of predation by bluegill on *Metschnikowia*-infected *Daphnia* and rapid evolution of host resistance in response to selection by *Metschnikowia*, may both operate to temper the virulent yeast parasite's influence on host dynamics. For more information on this topic, we like to refer to Duffy and Hall, 2008.

Line 530: This should come earlier in the discussion when you discuss about ASV richness in the second paragraph.

We thank the reviewer for this remark. In the second paragraph we confirmed that the natural microbial inocula were more diverse and differently structured than the laboratory-derived microbial inocula and that this was also the case in the gut microbiota. In the fourth paragraph we link the more diverse natural microbial community (i.e. broader pool of bacteria) with the fitness results. We chose to follow this order, to follow the order of the hypotheses that were stated in the introduction.

Line 536-538: Does it exist an example in the literature, in a freshwater model system?

We did not find any examples in literature in a freshwater model system.

Line 541-542: Put some references.

References were added in the revised document (Line 548).

Tables 2 & 3: Dots instead of commas.

We adjusted accordingly.