Review of the manuscript entitled '**Maternal body condition affects the response of the gut microbiome to a widespread contaminant in larval spined toads**' submitted by Tartu et al. for publication in PCI in Ecotoxicology.

General comments

The main goal of this study was to assess the effect of AMPA the main transformation product of glyphosate on the gut microbiome of spined toad tadpoles. The approach employed by the authors consist in catching tadpoles from forest or agricultural sites and keeping them in the lab until oviposition occurred. 240 tadpoles were incubated in the Lab and some were exposed to AMPA at environmental concentration, 0.4µg.L-1. Growth and development of tadpoles were monitored over time. On two subsamples of 120 tadpoles each, the feces were collected (and merged by 3, 40 samples per treatment (control and AMPA)) to study the microbial communities. Data was analysed to assess the effect of AMPA considering the body conditions of the parents (male vs female) and the site of origin of the parents (forest vs agricultural). The study demonstrates that AMPA had a significant effect on the composition of the microbial community of the feces and that this was influenced by mother body conditions. Changes in the composition of the microbial community of the feces were significant but the size of the effect was rather low: OTUs belonging to two phylum (Bacteroidetes and Actinobacteriota) were found to be responsible for the changes observed. Overall the paper is of interest but it has to be improved to better put the emphasis on the main objectives of the study: assessment of the ecotoxicological effect of AMPA on tadpoles growth, development and feces microbial community. The title has to be changed: it is not about the gut microbiome but the microbiome of feces of larval spined toads. The discussion has to be shortened and to make it less speculative.

Major comments

The origin of AMPA can be better described in the introduction by adding information on the enzyme involved in its formation: glyphosate is transformed by the enzyme GOX A (glyphosate oxidase) to AMPA.

The gut microbiome was studied by analyzing the bacterial communities found in tadpoles' feces: although the microbial communities in the feces are often used as a proxy of the gut microbiota as it has the advantage of being noninvasive, it is still debated if microbial communities of the feces are truly reflecting the ones of the gut microbiota (see https://doi.org/10.3389/fcimb.2020.00151). In addition, feces were collected after 4 to 6 days at the bottom of the aquarium. Therefore, on can hypothesize that feces have been potentially contaminated by the microbes found in the water coming from different origin. To me these two points have to be exposed in the paper: the second point in less important than the first one as we may expect that the abundance and diversity of the microbial communities in the feces are higher than those in the water. Concerning the first point it has to be mentioned that the choice has been made to use a noninvasive method to get a proxy of the gut microbiome of tadpole in order to be able to release them after the experiment done.

It is mentioned that the feces from three siblings tadpoles receiving the same treatment were pooled to increase the genetic diversity (of microbial gut microbial communities I guess?). This statement is not pretty obvious though for different reasons mainly because the environment of the siblings was the same and also because the origin of their genetic diversity (same parents) was rather limited. To

me it is better to justify this statement for technical reasons (less samples to be analyzed and higher quantity of feces obtained for DNA extraction).

The 16S rRNA amplicons were generated by PCR carried out on DNA extracted from the feces: the PCR program is a bit strange with a two-step PCR one for 10 cycles at 57°C as melting temperature and another one at 65°C as melting temperature for 25 cycles. Why this choice was made? The first part of the PCR is carried out below the theoretical melting temperature of the primers: this may led to the amplification of 16S rRNA amplicon with mismatch and create artificially diversity in the sequences amplifies latter on in the second part of the PCR.

The estimation of the microbial biomass in the feces by monitoring the spiked-in bacteria (inverse relationship) is not the classical tool used: usually as molecular-based approach (a range of other methods are available to quantify microbial biomass) 16S rDNA is quantified by qPCR and expressed in number of sequences per ng of DNA or per g of sample analyzed (here tadpole feces). As this method is not often used it is rather difficult to compare to other studies. This proxy is difficult to relate to the microbial biomass. I would suggest to remove it from the paper as it does not give much information or to present it a different way as the method used does not allow to assess properly the microbial biomass of feces.

A missing information is the fate of AMPA during the growth of spined toad Bufo spinosus tadpoles: was AMPA detected and quantified in the water of the aquarium? As it is stated in the manuscript several microbes are able to degrade AMPA. So one can hypothesize that overtime AMPA concentration decreased, changing the scenario of exposure.

Minor comments

Remove legally in line 34

Others instead of other in line 35

Fast instead of faster in line 35

Transformation products instead of breakdown products in line 38

Remove negative in line 45

Add ‘in its ecotoxicity’ after involved in line 53

Cut the sentence in line 60 and start with ‘A dysbiosis consisting in a modification in the composition and function of the gut microbiota in response to a stress’

‘Considering the widespread presence of AMPA’ instead of ‘considering the higher presence of AMPA’ in line 71

Replace predicted by hypothesized in line 93

Remove composition in line 94

Add of ‘microbial communities’ after ‘genetic diversity’ in line 144

Add information on the amount of DNA extracted from the feces (mean value ± standard error). Similarly add some information on the amount of amplicon obtained after processing them for MinION sequencing (in ng/µL of DNA) to give an idea on how much you need to reach the 150 ng loaded on the flow cell (information provided in line 179).

In the figure 5 line 313 the r2 of the regression should be added on the graphs shown in the panels A and B.

In line 340, change or remove gut microbiota biomass (see my suggestion in major comment section).

In line 446 the term agrochemicals is not appropriate: AMPA is a transformation product of glyphosate. AMPA is not an agrochemical: glyphosate is one such.

In line 448 change or remove gut microbiota biomass (see my suggestion in major comment section).

The two paragraphs from line 477 to line 495 contains elements that are too speculative at this stage. I would prefer a discussion on the ecological relevance of the observations made here: only two phyla affected by AMPA and in different ways.

Line 496 : as the effect of AMPA was not affected by the origin of the parents (forest vs agricultural sites) this last paragraph of the discussion is speculative and might be removed or shorten drastically.