The article focussed on freshwater sponge bacterial communities in different river basins across western North Carolina, U.S.A and compared these to water samples. The authors took samples across different months which also led them analyse the effect of time / sponge development stage on the sponge bacterial communities. The dataset contains valuable data and the authors explore this data in interesting ways, adding valuable information on our current knowledge of freshwater sponge bacterial communities.

In general, The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 78, 87, 135, 276 – 313, the current phrasing makes comprehension difficult. The discussion needs a revision to restructure sentences which are too long and therefor hard to understand, for example line487 – 492, 503 – 508. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.

Please see below my comments per section:

Introduction

Line 78: language – correct to led to THE discovery

Line 87: language – correct to additional sponges HAVE been analyzed

Line 135: language - correct "freshwaters" to "freshwater"

102 – 103 ,It will thus be interesting to see if a parallel phenomenon of a range of high and low microbial abundances (Hentschel, Usher & Taylor, 2006; Freeman et al., 2021) as in marine sponges has also developed in freshwater sponges.' – Indeed interesting and understudied, but were you able to conclude anything about that with this dataset?

Methods

Can the authors report the replicates of the water samples per site, maybe add them also to table 1? There is no complete overview of the samples analysed in the data.

168 – Why did you choose 80% ethanol? And not a higher concentration (> 96%) that is more usual to preserve bacterial communities? And why did you use two different methods, DNA buffer and ethanol? Can you please explain.

276 – 313 Data analysis paragraph – please correct spelling and the overall sentence structure. It is hard to read as there are several words missing or spelling mistakes.

271 – language – correct "to compared again" to "to compare against"

323 – change "the raw data of the environmental data" to "the raw environmental data"

Please report the replicates

Sponge identification – I do not have experience with sponge identification using specules so can not accurately review this part

Results

265-275 Can you report on the number of replicates in each group? I would suggest adding to table one also an overview of the water samples. From the sponge species I see the design is

unbalanced and there are only two replicates from the Watauga river. Did you leave this location out in the post-hoc testing?

281 – remove comma after included

384 – change ,,There was generally higher proportion...'' to ,,There was generally a higher proportion''

395 – 397 – was the PERMANOVA test where you included both explanatory variables (sample type and month) in the model and tested for their interaction done on the Bray-Curtis or Unifrac data?

397 – 400 – I think this is redundant? As the test where you found the interaction also already showed that month independently, significantly explained some variation in the data. Another thing, I don't understand here why month only has two levels here (F1,48) whereas before it had 4 (F3,48)? There are four months where samples were taken right?

401 – 407 – I think it is redundant to first separately report the effect of species and then the combined model. Just leave that first test out and report on the model where you included species, month and site. That shows the complete picture.

407 – 409 – Why not look at the variation in the water community over the months? I would expect the water community also varies per month.

448 – 451 – here the author states temperature did not very over the sampling months, as such, I would leave the statement made before (line 398) that month was a proxy for temperature, out.

Discussion

In general, the authors have written an interesting discussion on their findings. The readability could be improved by shortening sentences. Sometimes it is hard to understand the point when sentences are to long, for example line 487 – 492, 503 – 508.

574 -575 – Do you mean you also found *Sphingobacterium* and *Sediminibacterium* members in your data? Please rewrie because that is not clear.

583 – change 'both marine and freshwater ' to 'both marine and freshwater sponges'.

592 – remove 'the' before *E. muelleri*.

Figures and table

Table 1: Can you also add the water samples here so there is a complete overview of the number of samples. If you only have two replicates of a group the post-hoc testing is not reliable.. I understand in the field it is difficult to acquire a balanced sample design, but you should be careful claiming a statistical difference between two groups if one or both of the groups had less then 3 replicates.

Fig. 1. The map does not look very clear to me.. can you please improve, especially the overview maps on the left.

Fig. 2 To me it would be clearer if the ASVs do not occur in a particular sample don't get an annotation, just leave this spot in the graph blanc instead of the smallest circle. Then you can clearer see which ASVs are for instance sponge specific.