Author's response to comments from Associate Editor

Dear Dr. Zhongjun Jia,

We would like to thank you for your helpful comments. We changed the manuscript according to your suggestions.

(1) L7-8. abbreviation. CLSM was used referring to confocal laser scanning microscopy. Pls provide the abbreviations for Scanning electron microscopy and Fourier transform infrared spectra; state the results that are directly obtained from these technique in abstract if flexible.

Abbreviations (SEM, FTIR) were added (Page 2, Line 8) and abstract was changed as follows:

'Fe(III)-precipitates on algal cells identified as ferrihydrite and schwertmannite by FTIR were associated with microbes …' **(Page 2, Line 13)**

'SEM imaging revealed that while the green algae were fully encrusted with Fe-precipitates, …' **(Page 2, Line 14)**

(2) L17. Delete either "based on DNA and RNA" or "RNA and DNA" in Line 18

'based on DNA and RNA' **was deleted (Page 2, Line 18).**

(3) L20. Any evidence for "fully oxygen-saturated conditions" in this study, the phrase might be deleted.

We rephrased *'fully oxygen-saturated conditions'* to *'putative oxygen-saturated conditions'* since there is no conclusive evidence (Page 2, Line 20).

(4) L23. Any experimental evidence for "lower photosynthetic activities" in this study? If not, pls rephrase it.

We rephrased abstract as follows:

'A loss of chloroplasts in the brown algae could have led to lower photosynthetic activities and reduced EPS production which is known to affect predator colonization.' **(Page 2, Line 23)**

(5) L30-31. I am not sure whether this statement can be rephrased in a better manner. It seems that the co-existence of these stuffs are clearly demonstrated in this study. But it might not be very safe to suggest the Tribonema sp. provide microenvironment for neutrophilic FeOB, why not the other way around; In addition, if there were no solid evidence of oxygen concentration dynamics as the reviewer specifically pointed out, the author may put himself/herself in a dangerous position to challenge the current dogma. I guess these statements can be tuned down to some degree. For example. Collectively, our results suggest that in heavy metal-rich environment the coexistence of oxygen-generating alage Tribonema-like and strictly microaerophilic neutrophilic FeOB-like microbes (can be better rephrased)

The sentence was changed as follows, as you suggested:

'Collectively, our results suggest the coexistence of oxygen-generating algae Tribonema sp. and strictly microaerophilic neutrophilic FeOB in a heavy metal-rich environment' **(Page 3, Line 25)**

(6) P7708. I side with reviewers that one cannot rule out the possibility of chemolithotrophic lifestyle of organisms in this study. It is certainly true that Emerson et al., 2013 showed the absence of genes for organic carbon assimilation; and no heterotrophic growth was observed for their isolate. However, this may not be the case in this study because the absence of evidence is not the evidence of absence.

The sentence was changed as follows:

'Our results cannot exclude the possibility that FeOB utilize algal EPS as organic carbon source, whereas G. capsiferriformans and S. lithotrophicus were reported to be unable to grow heterotrophically (Emerson et al., 2013).' **(Page 20, Line 397)**

In addition, I am wondering what is the point mentioning "metal-polluted" in the title. I had a quick look and found that the effect of heavy metal appears to be descriptive and rarely justified. Maybe it can be briefly discussed in the materials and methods, if heavy metal here is not a key concern relating iron encrustations on algae-Gallionella aggregated communities.

This study was conducted at a stream created by upcoming metal-polluted groundwater and there was no pristine system which can be used as a control site without metal load. Therefore, we could not fully understand the effects of heavy metals on the algae-FeOB communities. Our data showed that heavy metals were accumulated on the surface of the algae, implying that the iron coatings could act as buffers to help prevent the algae from

taking up these metals. In addition, heavy metal concentration in stream water could be a factor which determines the predominant FeOB strains on the algae. Therefore we suspect that heavy metals influenced the development of algae-FeOB communities, however, these hypotheses were not proved in this work.

The following sentence was added in discussion part:

'However, since there was no pristine system without metal load around our study site, we could not asses the effects of heavy metals on development of the algae-bacteria-mineral communities.' **(Page 17, Line 345)**

We hope that these corrections will help to improve our manuscript.

Sincerely yours,

Kirsten Küsel