RESPONSES TO REVIEWERS

RESPONSES TO REVIEWER 1

Comment 1 : Abstract: I find the first sentence very confusing. Why would the presence of P-accumulating bacteria invite efforts to reveal "unknown functions" if this manuscript is based on is P-accumulation as the only important function? I believe that the authors can easily rephrase this sentence to make it a bit more meaningful

Response : This is corrected in the revised manuscript (line 17, page 1).

Comment 2 : L21: what do the authors mean with multiparametric analysis here?

Response : You are correct. This is corrected in the revised manuscript (line 20, page 1).

Comment 3 :L41: I struggle to understand what the "ecological sustainability of P" could mean. The sustainable use of P in agricultural systems? Please clarify?

Response : The sentence has been reworded to make it clearer: « *In order to increase the sustainability of P-resources managements, it is crucial to significantly improve our knowledge about the detailed processes, fluxes and reservoirs involved in the geochemical cycle of P. Microorganisms have been shown to be major actors in modern and past geochemical cycles of P either as reservoirs and/or catalysts of processes exchanging P between different reservoirs (Diaz et al. 2008).* » (**lines 41-45, page 2**).

Comment 4 :L70: Should be binding of DAPI to PolyP not vice versa

Response : This is corrected in the revised manuscript (line 73, page 2).

Comment 5 :L70-72: Is there a reference that the shift and the intensity is proportional to the amount?

Response : We have added two references to the revised manuscript (line 75, page 2).

Tijssen, J. P., Beekes, H. W. and Van Steveninck, J. : Localization of polyphosphates in Saccharomyces fragilis, as revealed by 4',6-diamidino-2-phenylindole fluorescence. Biochim. Biophys. Acta 721, 394–398, doi: 10.1016/0167-4889(82)90094-5, 1982.

Aschar-Sobbi, R., Abramov, A. Y., Diao, C., Kargacin, M. E., Kargacin, G. J., French, R. J. and Pavlov, E. : High sensitivity, quantitative measurements of polyphosphate using a new DAPI-based approach. J. Fluoresc., 18, 859–866, doi: 10.1007/s10895-008-0315-4, 2008.

Comment 6 :L90: In lines 666f it is mentioned that it has recently been used. Please rephrase the sentence in the introduction accordingly

Response : The sentence has been reworded in the revised manuscript : 'This novel polyP sensor has been shown to be suitable for staining polyP in living eukaryotic cells and tissues (Angelova *et al.*, 2014), and has recently been used to target polyP in yeast extracts (Deidert *et al.*, 2024) and planktonic environmental samples (Yang *et al*., 2024).' **(page 3, lines 94-96).**

Comment 7 : L92: which can account for up to 30%...

Response : This is corrected in the revised manuscript (line 97, page 3).

Comment 8 :L130: I am not sure what a "parcel" is in this context. Maybe plots?

Response :We used the term 'parcel' because each parcel corresponds to an area of land with a specific owner and land use (e.g. organic vs. conventional).

Comment 9 :L134: incubated may not be the best term here, maybe use "agitated"?

Response : The sentence has been reworded to make it clearer : *« To separate the microbial cells from the sediment or soil particles, 10 ml of 0.01 M sodium pyrophosphate buffer (pH 7.2) was added to 1 g of soil or sediment sample in a Falcon® tube and the mixture shaken (280 rpm) at 4 °C for 30 minutes."* **(lines 139-141, page 4).**

Comment 10 :L130-135: the supernatant probably still contained many clay particles and small POM fragments. Did you verify via microscopy that your sample was consisting of mainly microbes?

Response No, this has not been done and is not generally done for this type of approach. In any case, there was no dominance of clay particles and small POM fragments in our observations of the sediments using epifluorescence microscopy.

Comment 11 :L220f: please address the fact that double labelling with DAPI is not necessary somewhere. Either here or in the discussion.

Reponse : This was noted in the revised manuscript *« It should be noted that in order to use labelling conditions similar to those used in cytometry (double labelling with polyP/DAPI and SYTO®62/DNA), we used double labelling in epifluorescence microscopy (DAPI/polyP and DAPI/DNA). However, single labelling with a high concentration of DAPI would have been sufficient."* **(lines 228-231 page 6).**

RESPONSES TO REVIEWER 2

Comment 1 : Figure 7: Change “Bioavailable inorganique P” in the legend to “Bioavailable inorganic P”.

Response : This is corrected in the revised manuscript (Figure 7, page 15).

Comment 2 : L88: “fluorescence signal” instead of “fluorescent signal”

Response : This is corrected in the revised manuscript (line 92, page 3).

Comment 3 : L271: Change “The counting of DAPI-polyP complexes in epifluorescence microscopy will then be used to validate the cytometric data.” to “The counting of DAPI-polyP complexes in epifluorescence microscopy was used to validate the cytometric data.”

Response : This is corrected in the revised manuscript (lines 278-280, page 8).

Comment 4: L449: Period after “(Table S.19)”

Response : This is corrected in the revised manuscript (line 417, page 11).

Comment 5 : L509: Show the data. There is no limit on the supplement and there are plenty of repositories for microscopy images.

Response : We would only have to show photographs of black fields because of the absence of polyP in these crops, which would be of no interest.

Comment 6 : L511: “polyP” in the title

Response : This is corrected in the revised manuscript (line 511, page 14).

Comment 7 : L557-558: “causes events with size and structure mimic nonviable cells.” should probably be “causes events which size and structure mimic nonviable cells.” If not, I do not understand this sentence.

Response : You are correct. This is corrected in the revised manuscript (line 554, page 15).

Comment 8 : L639: “dramatically lower abundance of polyP when comparing to microorganisms” to “dramatically lower abundance of polyP compared to microorganisms”

Response : This is corrected in the revised manuscript (line 636, page 17).