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Interactive comment on "Adsorption to soils and biochemical characterization of purified phytases" *by* Maria Marta Caffaro et al.

Anonymous Referee #2

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The paper adsorption to soils and biochemical characterization of purified phytases, by Caffaro et al, uses conventional techniques for the evaluation of known commercial phytases. They have some success trying to prove that phytases have the potential to be used as complement for soil fertilizers. There are many issues that need to be clarified before publication: The title itself is ambiguous and misleading Recently it has been a discussion about the term phytase. Certainly one definition is that all enzymes which area use phytate as substrate are phytases. However, several authors i.e Greiner have pointed out that many of those are actually phytate degrading enzymes particularly the ones in E coli. Therefore it might be that those are not true phytases, The main reason is that their function is not related to processing phytate, different from some other's "real" phytases in plants i.e. PAPphy. The authors refer to the work

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Discussion paper

of Misset 2003 as a reference of E coli phytases and their relevance in the industry. There are a couple of issues here. First I'm not really sure of the relevance of all strains of E coli phytases for the industry. If any which ones?. Many strains of E coli possess an active phosphatase A gene witch can provide a certain level of phytate degradation but a real level of commercial degradation, I'm not sure about it.

Only until the lines 60 to 70, the really important point of the work was revealed. The main point in my perspective is the usage of phytases as biological fertilizers to release inorganic P from organic P sources. But so far the whole history sounded more focused on something else. The major problem of the paper starts with the first hypothesis: Phytases have the ability to release P from different organic P sources, with a preference for phytic acid. In that way is redacted that is not a hypothesis that contributes at all with new knowledge in the field. It is already known that some phytases are highly specific and others are not but have preferences for phytate. Similar to the other two. Many references for that just two examples: doi:10.1128/AEM.01384-15 doi: 10.1128/mBio.01966-18 Is the norm of the journal to include only some of the line numbers? That makes it more difficult for review. The abstract is very misleading because implies that the authors isolated the phytases from the fungi by themselves. That is not the case. Line 18-19: The proportion of phytases found in the solid phase of the soil 60 minutes after addition was lower than that found in the liquid phase (23-34% vs. 66-77%). This result is not well connected in the abstract, is coming out of nowhere.

Lines 38-39: There are different forms of inositol-phosphates and the most abundant from phytate (refers only to the salt form). But what exactly is the meaning of phytates in these lines and in the subsequent text in general?.

Line 48. E coli and the rest of the text please italicize where required

The hypotheses are not real hypotheses in the way their current state. It is already known that phytases can use different substrates. The number two was proved by a paper that the authors cite https://doi.org/10.1002/jpln.201600421. Finally, the hypothesis number 3 is way too basic for being a good work hypothesis

The biochemical characterization needs to include the catalytic efficiency of the reactions. It has been demonstrated recently by the works of Tan et al (doi: 10.1007/s00253-015-7097-9) and others in 2019 using metagenomes that phytases are also present in metagenomes of soils. In fact, their presence is underestimated. Where is the experiment which proves that the used soils have low phytase activity?

The control reactions of the initial experiments are missing.

Line 134 I don't think is a good idea to use a demo or student versions of any software for statistical analysis in a publication.

The characterization of the enzymes are very poor. Soils are complex matrices with multiple variants i.e ions. That was not evaluated here.

Were the buffers set at the optimal temperature ?

The authors refer at the begging of the manuscript to the type of enzymes as 3-phytases. But they do not mention what type of enzymes are from the structural point of view. Are they acid phytases? Maybe that's is why need pH relatively low to act. But nothing of this is mentioned in the text.

Is the optimal pH was determined before that the temperature is obvious that they did not set the buffer for the pH test at the right temperature. Therefore the pH characterization is not trustworthy.

It seems that the authors did not review any literature about phytases in 2019.

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