

This manuscript (soil-2015-87) aims to provide a better understanding of the role of extracellular biological materials (EPS), esp found in biofilms, in soil aggregation stabilisation. It uses a series of additions of hydrolytic degradative enzyme to test the hypothesis that EPS materials contribute to the stability of soil aggregation, while also affecting SOM availability.

I have major concerns regarding this manuscript:

1. Poor study design and poor description of the methodologies used:
 - a. Section 2.2: confusingly written maths section
 - b. Section 2.2: poor justification of numbers used:
Eg the supposed soil bulk density number seems odd, as this can be measured for field core samples and be recreated to field soil density. Otherwise explain the assumption for this particular experiment as normal dried and sieved soil without repacking does not get to this density.
 - c. Section 2.2/2.3: poor justification of numbers used:
The 'scenarios' have been explained (though could be improved in clarity) but do not actually contain any information regarding the technical set up. How much enzyme activity units were applied? What was the level of purity of the enzyme preparations? How where the enzymes added? Was there mixing involved? There is a severe lack of information, especially as the whole manuscript depends on contact of these enzymes with EPS materials. How have the authors assured that these enzymes have reached the materials processed further?
 - d. Section 2.2/2.3: the E4 scenario seems to suggest a large excess of enzymes was applied. How have the authors ensured that such a large excess is not damaging to resident live microbial cells? E.g. a large excess of lipase may affect the membrane integrity of cells. This may in turn impact on DNA quantification without actually directly affecting soil aggregate stability.
 - e. Section 2.3: information/studies on basal respiration at 30C/37C, the temperature of the actual experiments performed, are missing.
 - f. Section 2.4: this experiment was performed on a separate soil incubation experiment within kit tubes. The experiment should however have been performed on subsamples taken from the experiment in 2.2/2.3 as the conditions in (closed?) kit tubes are very different from regular soil incubations. The authors attempt to link the results from both experiments, which in my opinion is not warranted as the experiments have been performed under different conditions.
 - g. Section 2.4: for especially scenario E4, with an apparent excess of enzymes including DNase, I am surprised to see the authors report successful DNA purification. How have the authors achieved DNA purification in the presence of excess DNase? Idem for the scenarios with lower amount(s) of DNase added?
2. Most results not significantly different from control experiments or have missing statistical analyses.
 - a. The results of soil stability/SOM measurements indicate that none of the 'scenarios' are significantly different from the control experiment. The only significant difference the authors report concerns between-treatment results, which leaves me wondering about the relevance of the whole study.

- b. The results shown in Figure 2 have been reported without statistical analyses on significant difference. Please include statistical analyses on significant difference between control and treatments. The figure's error bars of the control and the experimental treatments could suggest that differences between control and treatment scenarios are unlikely to be significant, leaving doubt about the experiment's relevance and study design.
 - c. Figure 3 is missing a control on DNA present in the added enzyme mixtures. Can the authors ensure that the DNA extracted and amplified is not derived from the enzyme preparations added? Especially scenario E4 might lead to addition of a lot of DNA.
 - d. Figure 3: In contrast to the above, DNase is added in the scenarios, which should then lead to degradation of DNA present in the samples. Can the authors therefore please clarify the puzzling details of this experiment?
 - e. Figure 3: Can the authors please provide (control) data on (expected) cell lysis from treatments, esp E4? This will enable untangling of results due to lysis and any EPS-biofilm effect on soil aggregation.
3. Discussion of results
- a. The significant in-between-treatment results are given too much focus and attention, especially in the knowledge that none of the treatments were significantly different to controls. The majority of the conclusions drawn are not supported by the actual data provided.
 - b. Line 390 '... our results give a qualitative evidence for the influence of biofilms on aggregate stability...' This conclusion is not supported by the data provided.
 - c. Figure 4: this diagram can be omitted.
4. I am a bit puzzled by the section 'Data Availability', shouldn't these references be included in the References section? If this is provided according to the journal's instructions, then fine.

In conclusion, I have severe reservations regarding the study design and technical approach used in combination with the actual results (mostly not significantly different from controls). This in turn leads me to believe this manuscript cannot be improved substantially through major revision as a severe overhaul of study design and methodology is needed. My recommendation is therefore to reject the current manuscript for publication.