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Interactive comment on "Enzymatic biofilm detachment causes a loss of aggregate stability in a sandy soil" *by* F. Büks and M. Kaupenjohann

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Enzymatic biofilm detachment causes a loss of aggregate stability in a sandy soil.

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Final response to Referee2 (For the formatted document please see supplement)

Dear Referee2. Thank you very much for reviewing. In the following I will try to answer your important comments and to clear the objections.

1.a. Section 2.2: confusingly written maths section Pooling of equations to a single

one is a space saving way to show these manifold steps of converting concentrations of biofilm components to the final value of needed enzyme units. I will place each single step in the supplements. The paragraph beginning in line 163 will be revised to clarify the following: Literature show a wide range of enzyme-target concentrations in different soils. As we do not know target concentrations of our soil (due to a lack of extraction methods), we considered the largest published concentrations to find existing effects. Further as target molar masses vary as well, here we choose the smallest mass. Both conduce to a "worst-case" point of view with maximum enzyme targets.

1.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. Different samplings during the field experiment showed soil bulk densities of 1.4 g/cm3. These values are normal for a sandy silt (Su3) [Chaudhari et al., 2013], that is used in this experiment. For scenario E1 soil bulk density is irrelevant because ccell and therefore target maxima were estimated from cmic. For scenario E2 and the following we measured a minor soil bulk density in a sample of soil aggregates ($\sim$1.15 g/cm3). On the other hand, biofilm populations are mentioned to be mainly located in soil aggregates [Nunan et al., 2003]. Therefore – following our "worst-case"-approach – we used the bulk density of the original soil to estimate maximum target values.

1.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? Enzyme units are listed in table 3. What was the level of purity of the enzyme preparations? Enzyme purity is guaranteed by the producer (data sheets of product numbers Sigma-Aldrich: G0660, G5635, L0382 and D5025). How where the enzymes added? Was there mixing involved? Enzymes were added as described in section 2.4. Enzyme solutions were vortexed and than added to aggregate samples as described in section

2.4. 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? Contact of enzymes and EPS on the micro-scale were not demonstrated directly. Contact of enzymes to EPS can be assumed as the whole enzyme solution was absorbed by the soil aggregates. Fine pores (already filled with ARW from pre-incubation) in contrast need to be supplied by diffusion, that is probably inhibited. However, enzymes are able to diffuse into the EPS within 1 hour, as described by Böckelmann et al. (2003). Thus, observed effects are not quantitative, but qualitative. We tend to express the more cautious position "Enzymatic treatment causes an increased release of POM after sonication" to include uncertainties about enzyme contact to targets.

1.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. Cell membranes are built of phospholipids. We used purified lipase from porcine pancreas. Lipases are cutting fatty acids off e.g. glycerol, but are unable to cut fatty acids from phospholipids (as phospholipases do).

1.e. Section 2.3: information/studies on basal respiration at 30C/37C, the temperature of the actual experiments performed, are missing. Respiration data are collected at 20°C in another experiment using the same soil, where basal respiration was reached after 2 days. Therefore we concluded 3 days as sufficient to reach basal respiration at even higher temperatures.

1.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. A direct subsampling from the aggregate stability experiment to perform the DNA experiment was rejected due to its destructive capability regarding aggregates. In turn, temperature, substrate, pH and water content of the tube experiment were similar to the incubation of samples for the measurement of aggregate stability. Further differences were disregarded. As part of our hypothesis, the link between both increase in POM release and bacterial cell release can explained causally.

1.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? During incubation DNAse only digest free DNA but not DNA within bacterial cells of the biofilm. Later the pooled wash solution contains most of the DNAse. After centrifugation, this solution was discarded, whereas the bacterial pallet was resuspended in 200 μl ARW. At this stage, bacterial cells are still intact and immune to DNAse, whereas DNAse is diluted and hindered by high buffer ion concentrations. After mechanical cell lysis PPS (Protein Precipitation Solution) was added leading to e.g. precipitate DNAse. All steps of DNA extraction were conducted on ice to strongly reduce enzyme activity.

2.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. Even if there is no significant difference in aggregate stability, unfulfilled expectations (as e.g. a dramatic loss in aggregate stability after enzymatic treatment) do not minder the relevance of a study. In addition – without any attempt to prettify our results – p-values <0.05 as the limit for significance is a convention. From my point of view there is a tendency of increasing POM release (p=0.1, 5 parallels) in E4, and a tendency to decrease (p=0.06, 5 parallels) in E1 compared to the control. The first one fits to our model, the second one does not. Both

tendencies are visible and have to be explained under the restriction of being small.

2.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. Good idea. I will do this. Thereby, y-axis of figure 2 will be converted to mg POC /g dry soil.

2.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. We do not have this data. ARW for stock solutions and dilutions include ultrapure water and were autoclaved. Remaining free DNA strand amount is assumed to be far below soil DNA concentration and most probably digested by DNAse in stock and dilutet solutions. Further possible DNA additions were similar between variants and related to blind values.

2.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? Until mechanical cell lysis, extracellular DNA including eDNA from EPS is digested by DNAse. As mentioned in 2.c. small amounts of additional DNA are supposed to be irrelevant and the bulk of free DNA is rejected by washing. E4 shows the highest DNA release, although undigested biofilm in low enzyme treatments could increase DNA-concentration via centrifugation to the pallet. That could probably point to an underestimated additional DNA release in E4. It underlines the only qualitative approach of this experiment.

2.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. DOC release from bacterial cells due to enzymatic treatment and ultrasonication was not quantified. This DOC is most probably removed by repeated washing during density fractioning. Measuring the distribution of remaining bacterial DOC among fLF, oLF and HF is impossible by method. However, visibly increasing POM release in E4 (see Line 30, Final response to Marc Redmile-Gordon) points to a negligible effect of bacterial DOC sorption on measured C.

3.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. and 3.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. See point 2.a. We propose careful line of argument including a statement of insignificance and a discussion of tendencies in face of p-values nearby p=0.05.

3.b. Figure 4: this diagram can be omitted. As figure 4 illustrates the model, we prefer to retain it.

Best regards, Frederick Büks

Please also note the supplement to this comment:
http://www.soil-discuss.net/soil-2015-87/soil-2015-87-AC2-supplement.pdf

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