

# Relation of aggregate stability and microbial diversity in an incubated sandy soil

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## Final response to #Referee3

Dear Referee3.

Thank you very much for your suggestions to improve the quality of this article.

**1) The introduction to the discussion paper focusses too greatly on biofilms, EPS composition and formation and bacterial composition with little or no discussion of aggregate stability (the aim of the paper being to relate the former to the latter). Aggregate stability is determined by both biotic and abiotic factors and this should be commented upon.**

In line 103 we add as follows: "... : EPS is one of manifold factors of soil aggregate stabilization. Permanent and variable charges of silicates, (hydr)oxides of Fe, Al and Mn, phosphates, carbonates as well as POM interact to each other mediated by multivalent cations with small hydrate shells (e.g. Ca<sup>2+</sup>, Fe<sup>3+</sup> and Al<sup>3+</sup>). Also dissolved organic matter (DOM) like humins, plant exudates and diverse decomposing products builds physico-chemical links between soil particles and covers mineral surfaces. In addition, fungal mycelia and fine roots form a stabilizing network in and around soil aggregates. (*Jastrow and Miller, 1997; Bronick and Lal, 2005*)". Also "However, the contribution of biofilms to this stabilization was not yet quantified." was added to line 429.

**2) The authors use sonication to disperse aggregates and then measure the release of organic carbon (OC) as a measure of aggregate stability. I am not familiar with any studies which state that aggregate stability can be measured by the quantity of OC released. The authors refer to Kaiser & Berhe as the basis for their method, but in this paper Kaiser & Berhe do not state their approach is a means to measure aggregate stability. Aggregate stability is typically measured by successive reduction in particle size (typically mean weight diameter) of aggregates, not by reference to the quantities of OC released. If it were possible to show a strong linear relationship between aggregate size and OC released then it might be possible to infer aggregate stability, but I do not consider the current approach in the discussion paper to be a measure of aggregate stability. The authors need to justify their approach in the context of the published literature on aggregate stability.**

We will include the following statement in line 420 before discussion of SOC release. "Generally, aggregate stability is characterized by determining the reduction in aggregate size after application of mechanical force. The commonly used methods are dry and wet sieving. However, the destruction of soil aggregates by ultrasonication has an advantage over these methods, which is the quantification of the applied energy (*North, 1976*). It is used for studying reduction of aggregate size (*Imeson and Vis, 1984*) as well as detachment of occluded POM carbon (*Golchin et al., 1994*). *Kaiser and Berhe (2014)* reviewed 15 studies using ultrasonication of soil aggregates in consideration of its destructiveness to the soil mineral matrix and occluded POM. They found destruction of POM at applied energy levels >60 J/ml, destruction of sand-sized primary particles at >710 J/ml and of smaller mineral particles at higher energy levels. We used this method of gentle POM detachment from soil aggregates to measure the oLF carbon release as a

result of mechanical force and linked it to aggregate stability. Since *Cerli et al. (2012)* showed that the release of free and occluded light fractions strongly depends on soil properties like mineralogy, POM content and composition, this method is restricted to comparison of soils differing in none of these properties.”

**3) The language and grammar used in the paper requires a considerable amount of revision before the paper could be accepted for publication. I have suggested several amendments in the technical corrections but there are many more than this.**

Thank you very much. We will do our best and consult a native speaker.

1. **Correct spellings are: therefore, proteins:** Done
2. **use mineral, not inanimate:** Done.
3. **line 177; create, not receive:** Done.
4. **line 206; addition, not add-on:** Done.
5. **line 217; it is not clear what soil parallels are - please clarify: “soil samples”**
6. **line 264; statistical analysis:** Done.
7. **line 340-341; it is not clear what is meant by ‘but between the two and SP pure: “... whereas both differ significantly to SP<sub>pure</sub>.”**
8. **line 480; Our hypothesis was not supported by the data:** Done.

**4) I was not convinced by the evidence that biofilms are formed as a reaction to ecological stress - the citation referred does not relate to this. Please provide clear evidence/citation to this association. *Flemming and Wingender (2010)* only refers to “..., but also act as genetic cross-over hotspot and collective digestive system for diverse soil nutrients.”** References for the evidence of reaction on ecological stress are given in lines 73-74 (*Roberson and Firestone, 1992; Mah and O'Toole, 2001; Weitere et al., 2005; Chang et al., 2007; Ozturk and Aslim, 2010*) and will be included in this paragraph in line 119 ff. to avoid misunderstanding.

**5) What statistical significance can we place on results with only three replicates?**

A more quantitative analysis would require more replicate samples. We will add the following to the end of the discussion: “Our results give a first insight to the relation of microbial community composition, SOC release and aggregate stability. A more quantitative analysis would require more replicate samples, probably inclusion of soils from different land use and different microbial communities. However, this was beyond the scope of the present study.”

**6) Line 219 - ‘were separated’ - how were the aggregates separated?**

Thank you. I will delete “... separated and ...”

## References

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(*Chang et al., 2007*) Chang, Woo-Suk, et al. "Alginate production by *Pseudomonas putida* creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions." *Journal of bacteriology* 189.22 (2007): 8290-8299.

(Flemming and Wingender, 2010) Flemming, Hans-Curt, and Jost Wingender. "The biofilm matrix." *Nature Reviews Microbiology* 8.9 (2010): 623-633.

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(Jastrow and Miller, 1997) Jastrow, J. D., and R. M. Miller. "Soil aggregate stabilization and carbon sequestration: feedbacks through organomineral associations." *Soil processes and the carbon cycle* (1997): 207-223.

(Kaiser and Berhe, 2014) Kaiser, Michael, and Asmeret Asefaw Berhe. "How does sonication affect the mineral and organic constituents of soil aggregates?—A review." *Journal of Plant Nutrition and Soil Science* 177.4 (2014): 479-495.

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(North, 1976) North, P. F. "Towards an absolute measurement of soil structural stability using ultrasound." *Journal of Soil Science* 27.4 (1976): 451-459.

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(Weitere et al., 2005) Weitere, Markus, et al. "Grazing resistance of *Pseudomonas aeruginosa* biofilms depends on type of protective mechanism, developmental stage and protozoan feeding mode." *Environmental Microbiology* 7.10 (2005): 1593-1601.

Best regards,  
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