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

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Final response to #Referee2

Dear Referee2.
Thank you for your ideas for the improvement of this article and for your X-ray vision to find unreferenced details. In the following I want to answer your questions.

1) The title did not completely meet the content of the paper: Most of the discussion focusses on the effects of inoculation on soil microbial diversity and its possible effects on net and cumulative SOC release (may be used as a measure for aggregate stability, however a reference is missing).
The method of ultrasonication/density fractionation invented by *Golchin et al. (1994)* is used as a blue-print for diverse surveys to analyze functional carbon Pools, e.g. in *Cerli et al. (2012)*. As the dispersion cut-off (applied J/ml to release all occluded light fraction without destructive effects on mineral matrix and redistribution of SOM between fractions) strongly depends on soil properties, this method is not applicable to compare aggregate stability of *different* soils via SOC release. On the other hand, *similar* soils with similar mineralogy, SOC content, SOC distribution in fractions and binding patterns can be compared. Therefore discussion about the relation between SOC occlusion and aggregate stability will be included and the title is replaced by “Two different microbial communities did not cause differences in occlusion of POM and soil aggregate stability”.

2) Additionally the manuscript focused on a mixture of soil plus 5vol. May be the statement at L137 will reflect to a larger extent the content of the paper: “Testing the influence of two different microbial communities on aggregate stability in a sandy soil”?? Here the biochar effects should also be considered. Explain why not using pure soil but a mixture of soil plus biochar.
In the beginning, this experiment was part of a more extensive trial also including charcoal-free samples and further methods. Therefore, biochar is a relict. Lines 137-139 are replaced by “The aim of this work is to do a first step in this field by testing the influence of two fundamentally different microbial populations on the aggregate stability of a sandy agricultural soil containing 5 vol% biochar.” Bacteria are colonizing biochar, and microbial community structure is affected by biochar *(Jin, 2010).* However, it is not the aim of this work to consider biochar effects and this is also not possible because of no biochar-free control.

3) Further I would like to ask the authors to consider the aspect that gamma radiation to free organic matter because of cell damage.
Gamma radiation is surely damaging cells and therefore enhancing soil DOC. However, the biochar soil was mixed before irradiation. It is distributed to the parallels of both incubated variants and SP_{pure} afterwards. Therefore, we do not expect difference in DOC

between variants in the beginning of the experiment.

4) Abstract L43. Please correct into "Therefore samples of a were."
Thank you. Done.

5) L46 A none-incubated subsamples is used as a control. The abstract should also contain a statement reflecting the conclusion of your study.
Thank you. Extension of the last sentence: "..., whereas comparison of incubated variants with a non-incubated sub-sample indicates strong stabilization during the incubation."

6) Introduction L148 please add an explanation why your studied the effects of microbial communities of aggregate formation by using a soil + biochar mixture instead of a soil itself? Please note within the conclusion how this statement is tested: true or false?
See 2). Extension of the last sentence in line 148: "... because of their influence on aggregate stability."

7) Material and Methods Please add references on the methods used for -homogenization of biochar + soil mixture, - gamma radiation, -filtration and -autoclave procedure etc.
We will add the following text to the manuscript: "Homogenization took place by over-head-shaking for approximately 1 minute. Gamma-irradiation was performed following *McNamara et al. (2003)* using additional 20 kGy. We used a 1.5 µm pore size glass fibre filter for adequate separation of POM, although 0.45 µm are required to retain single bacterial cells. And the autoclave program is typical for sterilization of liquid and solid media *(Atlas, 2010)*."

8) Note where the "R2A broth" ("mixture" may be a more common synonym for broth, or is "R2A broth" a trade name?, in this case please mark it accordingly), add a reference.
To our knowledge "R2A broth" is a very common term. The reference for the composition of R2A is *Atlas (2010)*.

9) L191 Are there any references on these procedures please add them or add missing information (e.g. testing incubation conditions to be constant)
No references. In a pre-trial we add 100 ml of tap water to 300 g of soil sample. Impounded water was rejected within 15 minutes. The adjustment to 37 vol% soil water content at pF=2 took place within 4 days. This information will be added to the text.

10) L202 Please explain why "soil extract could exceed the adjusted water content " (at line 194 you stated a "constant matrix potential.."). Please explain the reason to discard these exceeds.
"The soil has a bulk density of 1.36 g/cm^3. A water content of 35 vol% equates to 77 ml. Giving 100 ml soil extract to the sample, 23 ml are subsequently removed by the hydrostatic head when water capacity is adjusted to pF=2.1." This will be added to the manuscript.

11) L204ff please add references or explain the reasons for choosing the mentioned gradient in temperature, the disconnection of the hanging water columns etc.
As drought stress is one factor inducing biofilm buildup (see introduction), decrease of soil water content is mentioned to raise EPS production. Therefore the water columns were disconnected to facilitate drying.
Although there are only a few studies about the influence of temperature on biofilm production, e.g. *Di Bonaventura et al. (2008)* points to increasing biofilm production with increasing temperature. Reduction of summer time incubation temperature to 8°C was mentioned to simulate soil temperatures in the early spring and autumn. Low temperatures give an advantage of fungal compared to bacterial growth *(Borowik and Wyszkowska, 2015)* and therefore have influence mainly on the domain composition of SP_{soil} samples.
This information will be included to the manuscript.

12) L221 Please add references on the procedures described here

It is already referenced as NucleoSpin® Soil Kit (MACHEREY-NAGEL GmbH & Co. KG, Düren/Germany

13) L224 please add information how the 260/280nm ratio is obtained (I assume an UV-vis spectral analysis. However, this needs to be mentioned within the manuscript including a reference that shows this ratio to reflect purity of DNA samples).

"260/230 nm and" added to the sentence: "DNA sample purity, represented by 260/230 nm and 260/280nm extinction ratio, was determined with a NanoDrop1000 spectrophotometer (NanoDrop Products, Wilmington, DE, USA) and assessed as free of contamination."

The desired value for the 260/280nm extinction ratio is 1.80 (our mean value is 1.83, min=1.78, max=1.88), for 260/230 nm it is 2.0 (our mean is 1.80, min=1.68, max=1.92). *(TECHNICAL BULLETIN NanoDrop)*

14) L225-240 Please add all missing references

References for Primers are listed in Table 2 following *Fierer et al. (2005)*. Devices are already referenced in the text. Melting curve analysis is implemented in the QuantStudio™ 12K Flex Real-Time PCR System (Life Technologies, Grand Island, NY/USA).

15) L257 add a reference

The Device (Elementar Vario EL III CNS Analyzer) is referenced in the text.

16) L263 please note a reference on the statistics.

Shapiro-Wilk test: *(Shapiro and Wilk, 1965)*
Welch test: *(Welch, 1947)*
Bonferroni correction: *(Bonferroni, 1936)*
one way ANOVA: *(Christensen, 1996)*

17) L275-335 PLEASE explain all abbreviations like DNAEUB (is it equal to EUB338/EUB518 primer pair?) and their meaning with respect to microbial diversity within the materials and methods section.

Sorry for the imprecise description: DNA_{EUB} = total eubacterial population amplified by Eub338/Eub518 primer pairs. All other abbreviations are already explained in the text. Archaea, eubacteria and fungi represent the 3 domains involved in soil aggregation. Fungi are known to stabilize soil aggregates, whereas influence of bacteria is shown to be minor (*Tang et al., 2011*). Acidobacteria, actinobacteria, α- and β-proteobacteria are taxa commonly used in soil ecotyping (e.g. *Jangid et al., 2011*).

18) L336 please add within materials and methods how you analyze the particle size fractions for Cfrac and Crel Please explain the meaning of "oLF500" etc.

All this is already explained in lines 253-275.

19) L379-384 seem to belong into introduction (include missing references) If you introduce an abbreviation please use it consistently throughout the whole manuscript.

All this is described in the introduction and only summarized as a liftoff at the beginning of the discussion. "EPS" is introduced in the introduction section and used consistently as the abbreviation of "extracellular polymeric substance" (the extracellular matrix of biofilms).

20) Please note within discussion and conclusion how the hypotheses (including the last statement of the introduction (L147-149) given in the introduction were tested. Add a summary of those results within the abstract.

It's all part of the abstract and the discussion. The statement in lines 147-149 is related to lines 132-133 (introduction) and taken up in lines 460-468 (discussion). However, we do not think that methods should be repeated in the conclusion section – but that may depend on the philosophy of the writer.

21) L415-420 please clarify these statements.

Thank you very much. I have really no idea what happened to this sentence :). New version: “We conclude, that both variants strongly differ in their community structure within the final period from day 49 to day 76, at which both communities are dominated by specific taxa. This development implies different EPS compositions and biofilm structures. Following the hypothesis of this work, the different composition of microbial communities should have lead to a variation of aggregate stability between SP_{soil} and SP_{air}.”

22) Please add a discussion on the effect of gamma radiation on the amount of decomposable organic matter Such organic molecules may (i) interact with mineral soil as well as the biochar components in an abiotic way and may potentially force microbial activity.

Please see 3). Measuring and discussion of this effect is not the aim of this work.

23) Figs. mention within figure 1 where the soil sample is located?

It is already mentioned in the caption of Figure 1 (“... soil sample(dark grey)...”)

24) Please explain which figure/table represents the data on aggregate stability? Are the data on “cumulative SOC release” used as a measure for aggregate stability? Explain why.

Data on “cumulative SOC release” generally show the SOC release as a function of applied mechanical force. Occluded POM, that is bond inside soil aggregates, is released after application of a certain level of mechanical force. A higher share of “weak” aggregates in soil will result in an increased release of SOC. Therefore the cumulative SOC release is used as a proxy of aggregate stability, but only if soils with similar amount and composition of SOC are compared. This relation will be presented in the discussion and is also part of the revised version of *Büks and Kaupenjohann (in revision)*. See also 1).

New caption of figure 3: “Relative SOC release of variants (SP_{soil}, SP_{air}, SP_{pure}) at different energy levels (0, 50, 500 J ml^{-1}). Highest SOC release is associated with lowest aggregate stability at the respective energy level.”

New caption of figure 4: “Cumulative data of absolute SOC release (in mg SOC per g dry soil) of SP_{soil}, SP_{air}, SP_{pure} and A_{pure} as a function of applied energy. (*) marks A_{pure} as measured at 0, 50 and 300 J ml^{-1}. Highest SOC release is associated with lowest aggregate stability.”

References

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Best regards,
Frederick Büks