Interactive comment on "Accumulation of physically protected organic carbon promoted biological activity in macro-aggregates of rice soils under long term rice cultivation" *by* Yalong Liu et al.

Anonymous Referee #2

The manuscript addresses interactions between aggregate turnover, SOM sequestration and microbial activity in rice paddy soils. This is a relevant and timely topic within SOM research which fits well to the scope of the journal. The manuscript presents a comprehensive data set that has the potential to advance process understanding in this line of research. However, I unfortunately can not recommend publication because the manuscript lacks of clarity. In my opinion, the main problem is that it does not present a clear and understandable conceptual framework that can be used to develop testable research questions and to guide the discussion. The authors address many different concepts and keywords of current SOM research without properly explaining

them. The links between the concepts are oftentimes not clear. Furthermore, I did not understand how the conclusions were deduced from the data. Below, I will provide individual examples of sentences/paragraphs taken from the introduction, discussion and conclusion sections, in which a much clearer explanation of concepts and conclusions is needed (it is a selection; several other paragraphs also lack of clarity):

Introduction:

Line 106 ff.: "The distribution of soil microbial biomass and activity in particle size fractions could be important in determining how agro-ecosystems accumulated and stabilized SOC." - This is an important sentence, because it introduces the motivation of the work. However, it is not at all clarified by which mechanisms microbial biomass and activity in particle size fractions might determine SOC accumulation and stabilization. Does microbial activity cause the formation of aggregates? And if so, by which mechanism? Are there also other factors of aggregate formation besides microbial activity? Are there differences in formation of macro-aggregates and micro-aggregates? And: what is the difference between accumulation of SOM and stabilization of SOM? Both terms need to be explained.

Lines 112 ff.: "interactions of organic matter, microbial and enzyme activities in aggregate size fractions of long term cultivated soils and their dynamics with soil development had been not yet fully understood." - This sentence also presents the motivation of the work. In part, it repeats what has been outlined in the sentence above. Here, the authors argue that enzyme activity might play a role in turnover of SOM stored in aggregates. This needs clarification: what might be the role of enzymes? And: what is meant by 'dynamics of the interactions with soil development'?

Lines 117 ff.: "In early studies, greater persistence of OC in rice paddies than in dry croplands had been often attributed to enhanced aggregation and thus the aggregate stability (Lu et al., 1998; Yang et al., 2005), and to increased humification of SOC (Olk et al., 2000). " -In my opinion, this is also an important sentence of the introduction,

as it sums literature evidence that aggregate turnover and SOC turnover are related. However, what is meant by humification? In the earlier paragraphs, the terms accumulation and/or stabilization of SOC were used? Is humification the same as stabilization, or is it another mechanism which causes long-term storage of C in soil?

Lines 134 ff.: "SOC accumulation had been shown driving enhancement of microbial biomass and evolution of microbial community in long-term cultivated paddy soils (Bannert et al., 2011; Jiang et al., 2013; Liu et al., 137 2015). Nevertheless, the dynamics of SOM and bio-activity in size fractions of soil aggregates had not yet been characterized for understanding carbon sequestration in relation to soil microbial structure and functioning of rice paddy soils." - How can SOC accumulation cause an increase in microbial biomass? Organic matter that accumulates is not degraded; so it is not used as energy source by microbes, and thus accumulation should not enhance biomass production. Furthermore, what exactly is meant by dynamics of SOM and bioactivity in aggregates? Is it temporal changes of different parameters during soil development? Here I think one should provide presumptions about which parameters may change over time (and why they should change). The research questions can then be developed on basis of these presumptions. What exactly is meant by functioning of rice paddy soil?

Lines 140 ff.: "Taking a rice soil chronosequence as a case, we looked into the changes in organic matter (SOM) stabilization and microbial activity in different size fractions across the sequence and to infer how SOM accumulation and stabilization relate to soil bio-activities and to their dynamics along long term rice cultivation up to 700 years. We aimed to address if organic carbon stabilization could confront soil bioactivity in rice soils." - The research aims are vague. First, why using a chronosequence? What might be learned about the turnover of aggregates and the stabilization of SOM by using this research approach. Why studying paddy soils? I assume that aggregate turnover in paddy soil is strongly affected by the "puddling" activites of the farmers. This aspect is not discussed in the manuscript. Why should organic carbon stabilization confront soil

bioactivity? This has not been explained in the introduction.

Discussion:

Lines 562 ff.: "All these information above could suggest that organic carbon had been stabilized rather via physical protection in coarse sand fraction of macro-aggregates than via chemical recalcitrance due to mineralogical binding in clay." - I did not understand how this conclusion can be drawn from the data. Figure 3 clearly suggests that the resistance of SOM depends on recalcitrance. Maybe the problem here is the definition of recalcitrance in the manuscript. Conventionally, recalcitrance means that a compound is resistance against being degraded due to its intrinsic chemical properties (such as a high content of stable aromatic rings, see e.g., the discussion on bio-char decomposition). It should not be confused with stabilization of organic compounds by adsorption onto mineral surfaces.

Conclusions:

Lines 807 ff.: "This study further supported our previous finding for bulk soils that long term rice cultivation led to accumulation of SOC and promoted soil biological activities through physical protection of labile carbon in line with enhanced soil aggregation. And labile organic carbons accumulated in macro-aggregates helped enhancing microbial C use efficiency and improving potentially ecosystem functioning." - This is the major conclusion, and it is not clear to me. How can protected organic compounds promote biological activity? Furthermore, why does the protection and accumulation of labile organic compounds improve ecosystem functioning? I would assume that it slows down turnover processes, because the energy-rich compounds are not available for microorganisms.

In conclusion, I recommend a comprehensive revision of the introduction and of the development of the conclusions. I assume that this will require extensive and time-consuming work on the conceptual framework of the manuscript. Hence, I recommend rejection with the possibility for re-submission at a later point in time.