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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.

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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 often times 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): Response: Considered. Thank for your review and consideration for the scientific quality of the paper. Your opinion is considered in this revision. For a better clarity, the manuscript has been rewritten and revised, especially those you indicated.

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. Response: Considered. The sentence is now rephrased as "The distribution of soil microbial biomass and activity among different particle size fractions of soil aggregates had been shown in active response to the accumulation and stabilization of soil organic matter in agro-ecosystems". Sorry, here we did not intend to address the formation of aggregates by microbial activities. Also, accumulation refers to the quantity change but stabilization to the change in the degree by which the accumulated SOM could be decomposed due to a shift in environmental conditions. For example, SOM can be quickly increased with crop residues input in short term, which may be decomposed shortly after a cease of residue input. SOM stabilization involves a variety of physical, chemical, faunal, and microbial processes. In the processes, SOM increases with recalcitrance and decreases with accessibility. It means a decrease in the potential for SOM loss by respiration, erosion or leaching. SOC accumulation means an increase in the quantity of SOM which include labile and stable SOM.

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'? Response: Considered. The sentence is revised as "inter-link between organic matter, microbial community and enzyme activities in aggregates of different size fractions and the evolution with soil development due to continuing hydroagric pedogenesis had not yet been quantitatively characterized". Throughout the manuscript, the authors intended to address if OM (OC) accumulation/stabilization could promote soil biological activity including both the microbial abundance and their functioning activity, such as soil enzyme activity. The role of soil enzyme could be another issue for soil aggregation and the stability, which could deserve further study. The dynamics of the interactions with soil development refers to the potential changes in the quantitative relationship between SOM, microbial abundance and soil enzyme activity, with the rice soil development due to continuing hydroagric pedogenesis under prolonged rice cultivation. Sorry for the misleading sentence. Bioactivity here refers basically to microbial activity including soil enzymatic activity and basal respiration as well as microbial biomass. Soil enzyme activities are closely associated with SOM level and distribution in soils (Leinweber et al., 2008). And, a cascade system of heterogeneous extracellular and intracellular enzymes controls the decomposition of organic C (Stemmer et al., 1998). Therefore, measurements of the activity of soil enzymes can be used to evaluate the decomposition mechanisms, spatial patterns of microbial succession and C storage

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in different size aggregates (Fansler et al., 2005). Some relevant references below: Leinweber P, Jandl G, Baum C, et al. Stability and composition of soil organic matter control respiration and soil enzyme activities[J]. Soil Biology and Biochemistry, 2008, 40(6): 1496-1505. Stemmer M, Gerzabek M H, Kandeler E. Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication[J]. Soil Biology and Biochemistry, 1998, 30(1): 9-17. Fansler S J, Smith J L, Bolton Jr H, et al. Distribution of two C cycle enzymes in soil aggregates of a prairie chronosequence[J]. Biology and fertility of soils, 2005, 42(1): 17-23.

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? Resposne: Considered. Sorry for the unclear expression. The sentence now rephrased as "Great persistence of OC in rice paddies, compared to dry croplands, had been often attributed to enhanced aggregation and thus the aggregate stability in the studies by Lu et al., (1998) and by Yang et al., (2005), and to increased humification of SOC in the study by Olk et al., (2000)". Here we addressed that the earlier understanding of SOM persistence in rice paddies was already pointed to enhanced aggregation and aggregate stability, apart from to humification. There have been some good developments about SOM persistence in soils, including a new contribution by Lehmann and Kleber (2015), and Schmidt et al (2011) , published in Nature, Six and Paustian (2014) in SBB and Kleber et al. 2009 published in GCB. SOM persistence in soil has been increasingly accepted as mediated by soil and ecosystem property rather than chemical recalcitrance. Humification, as a traditional concept for long term persistence of stabilized OC, could be directly relevant to chemical stabilization. More recently, SOM stabilization is concerned of a variety of physical, chemical, microbial, edaphic and ecosystem processes. However, stabilization of SOM is indicated generally by a decrease in accessibility apart from an increases in chemical recalcitrance. This paper does not intend to deal with the stabilization mechanisms but the direct or indirect bearing on biological activity.

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? Response: considered. Indeed, the question “How can SOC accumulation cause an increase in microbial biomass” has been an important issue in current study of soil carbon sequestration and ecosystem functioning. And this is just the focus of this study on the link between SOM stabilization (SOM accumulated and stabilized) and bioogica activity in rice soils. Stabilization refers to an increased inaccessibility of carbon substrates to microbes, limiting microbial use of SOM as their energy source. However, microbial growth is an overall result of adaptation to increased available substrate supply and improved habitat conditions, involving changes in community structure and their energy use efficiency. Consequently, overall microbial biomass change in response to SOM accumulation and stabilization could be explained either by changes in SOM fractions (labile or particulate carbon, for example, could represent the available substrates) or by changes in community structure or diversity (increases in low energy demand species). In a study by Wang et al., (2015), increase in labile organic carbon (LOC) and particle organic carbon (POC), and an increase both in microbial biomass and enzyme activity was found in line with the total organic carbon accumulation in a rice soil chronosequence under hundreds years rice cultivation. On other hand, microbial biomass and diversity was seen increased with amendment of biochar, a generally recognized stable carbon material, in rice soils (Chen et al. 2013, 2015). The dynamics in this study refers to the changes with increasing rice cultivation, which need some key parameters to trace. For this, OC normalized gene abundance, microbial gene abundance scaled enzyme activity or OC normalized respiration, are examples of these potential parameters in our analysis. In addition, soil functioning is also a general term for a soil performing its functions of biogeochemical cycling and biological preservation and so on. Particularly, preservation of a carbon storage, supporting a high microbial biomass and diversity as well as bearing a high overall enzyme activity, are among the aspects of soil functioning for the rice soils in our study.

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. Response: Considered. Sorry for the unclear and long sentence. As in the response to the similar comment by first referee. This paragraph is now revised. We have clearly expressed our two hypothesis and the research purpose as well. This paragraph is now revised as below: In this study, two hypothesizes are tested. First, the link of carbon stability to bioactivity differed among the aggregate classes, and bioactivities enhanced in macro-aggregates with physically protected carbon rather than in micro(clay sized) aggregates with largely chemically stabilized organic carbon; And second, the bioactivity in relation to physical C sequestration in macro-aggregates could be enhanced with long term continuing rice paddy management. Using a recommend sonification separation procedure, we looked into the changes in aggregate size fraction composition for aggregate stability, OC functional group composition for chemical recalcitrance, soil respiration for microbial carbon accessibility and carbon gain from amended residue for carbon sequestration potential in order to characterize the SOM accumulation and stabilization in rice soils. On other hand, changes are explored with SOM accumulation/stabilization in microbial activity for soil functioning. We analyzed total microbial gene abundance (equivalent to biomass carbon and estimated overall enzyme activity in aggregate size fractions. The potential link between carbon stabilization and bioactivity among the aggregate fractions are quantitatively assessed using the parameters of carbon - or gene abundance- scaled respiration and enzyme activity. Furthermore, the evolution of such interlink is traced by comparing the soils of sequential length of rice cultivation in a soil chronosequence up to 700 years. Finally, we aimed to address if soil bioactivity could be improved with enhanced organic carbon stabilization in rice soils under long term rice cultivation. The use of chronosequence is to trace the evolution of carbon stabilization and the link to biological activity changes over the long run of rice soil management. Turnover of soil aggregates should be an important issue deserving dedicate study, but is not dealt with here. The pudding practice with rice cultivation could certainly have impact on soil aggregation, which is a component of long term rice soil management. As raised by Janzen (2006) (Janzen H. 2006. The soil carbon dilemma: Shall we hoard it or use it? SBB, 38(3):419-424), increased carbon stabilization should not confront its bioactivity in agricultural soils. This study will help to understand that carbon stabilization would not confront but improve biological activity in soils. We have added this in the introduction. Again, yes, soil aggregation of rice soils could have been affected by the long term rice paddy management such as puddling activities, which had been well addressed in generally in an earlier study by Deng and Xiong (1965). The effects by puddling on SOM accumulation and soil development had been well addressed specifically for the similar chronosequence in studies by Kölbl, et al (2014) and by Kalbitz et al. (2013). We could not have even deeper discussion on this aspect than in these previous studies. Yet, we focus on the interlink issue of OC and bioactivities in the rice soils.

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 macroaggregates 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. Response: Considered. Figure 3 presented the correlation of either total soil OC or carbon respiratory to OC recalcitrance. Compare the data in Table 1 (a new table with basic soil properties) and Table 2 (original Table 1) and Table 3ïijĹoriginal Table2ïijĽ, over 50% of total soil OC was stored in sand and fine sand fractions but only about 15%in clay sized fraction. As indicated in Table 4 (original Table 3)ïijŇcarbon recalcitrance was estimated by the ratio of aromatic carbon to aliphatic and polysaccharide carbon. Here, OC in sand fraction was apparently recalcitrant similarly to in the clay fraction (See Table 4 (original Table 3). Thus, this recalcitrant carbon in sand and fine sand fraction was supposed again secondarily protected. For better clarity, the sentence now revised as "All these information above could suggest that organic carbon had been stabilized rather via physical protection in coarse and fine sand fraction of larger aggregates than via chemical recalcitrance due to mineralogical binding in fine aggregates of clay size".

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. Response: Considered. The quantitative assessment of the link between OC and microbial abundance and activities, as indicated by the parameters described in the text, suggested OC physically protected in larger aggregates (course and fine sand fractions) promoted biological activities, probably through persistence of labile carbon or inter aggregate particular carbon within these macro aggregates. The physically protected labile OC could slow down their turnover but still accessible to microbial community within the aggregates, which could support a diverse microbial community and the enzyme activities related to ecosystem functioning. Of course, the molecular nature and the microbial adaptation to these carbon persistence between micro-aggregates within the macro-aggregates still need further study.

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 imeconsuming work on the conceptual framework of the manuscript. Hence, I recommend rejection with the possibility for re-submission at a later point in time. Response: Thanks for your very detailed questions, which may point to our further studies on organic carbon and microbial activity in rice soils, by in depth exploring the microbial response nature to accumulated or stabilized carbon in aggregate level. We believe these issue may help improve our understanding of soil property and functions in rice soils for sustainable soil management. By addressing your questions, we have made our great efforts in revising and smoothing the manuscript, though the extensive revision is really time consuming. We enjoy the exchanges with you and this is a real part of research. Thanks again!

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