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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 by Liu et al., deals with the link between organic C distribution among different aggregate-size fractions and microbial activity in paddy soils under long term cultivation. Our knowledge on C stabilization in soil subjected to alternating redox conditions, and the role of physical protection (as well as other mechanisms of OM stabilization) in C accumulation in these soils is rather limited. This manuscript can therefore represent an important and novel contribution to our understanding of these processes in paddy soils. The concepts proposed are very interesting, the scientific

approach adequate, and the data set impressive. However, the manuscript lacks focus, is rather long and often repetitive, and requires a significant work on the English language. Moreover the interpretation and discussion of data is often unfounded. This does not do justice to an otherwise valid contribution. In my opinion, the manuscript should be reconsidered for publication in BG after major revisions. Response: Considered. We thank for the encouragement for such a work on a potential link of OC to microbial activity among aggregate classes of rice soil. We also acknowledge the constructive comments on the data interpretation, discussion and text writing of the manuscript. We are sorry that the previous version of the manuscript was not well organized and written in good English. Herewith we submit our revised manuscript by carefully addressing all the reviewer's comments and revising the manuscript editing, by joint efforts of all the coauthors. The manuscript overall had been rewritten and organized, particularly the introduction and discussion sections. Hope both the scientific and language quality have been improved.

Specific comments 1) The introduction is rather general, long and tends to be repetitive. The authors should rewrite this part providing a more focused outlook on the interaction between C stabilization, aggregate stability and biological activity in rice paddies. They should also provide one or more hypotheses which the manuscript lacks. It is not clear from the introduction alone, why the authors choose a 700 y chronosequence to test their hypothesis. This time is longer than the expected residence time of physically protected organic C that is the subject of the manuscript. Response: Accepted. Manuscript has been largely rewritten and phrased, and the length much reduced, with focusing on the issue of C stability and bioactivity of rice soils. We proposed two hypothesizes. Firstly, 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 mineral-bound or chemically stabilized organic carbon; And secondly, the C bioactivity in macro-aggregates C sequestration could be enhanced through prolonged rice paddy management. This is tested by taking advantage of soil chronosequence up to 700 years of rice cultivation,

which was previously investigated for bulk soils. Yes, labile OC or POC particularly, had been considered as a short term C pool (Cambardella and Elliott, 1992), and subject to fast change in rice soils shifted to dry cropland (Li et al 2007). We provided explanation for the ever increasing of physically protected carbon in sand sized fractions of macro-aggregates, mainly attributed to prolonging submergence and pudding activities with long term continuing paddy management.

2) The authors utilized a fractionation procedure that provides a number of aggregate size fractions. The functional distinction between these fractions, and consequently the interpretation of all the results obtained, strongly depends on the sonication energy applied. The authors suggest that they adopted a "low energy sonication procedure" with applying 170 J/g for all soils irrespective of the pedogenetic processes that characterize their formation (known to have a direct bearing on aggregate stability). It is essential that the authors justify the fractionation procedure, provide further details on how they determined this energy input, and what the size-fractions represent. ResponseïijŽConsidered. A certain level of energy is required to destruct soil clods into soil aggregates. Indeed, the functional distinction between the aggregate fractions, and consequently the interpretation of all the results obtained strongly depends on the sonication energy applied. A sonification dispersion assay with the use of low-energy (170J/g) was developed by Stemmer et al., (1998) and were later on followed by Sessitsch et al., (2001) and others (Zheng et al., 2007; Chen et al., 2014). The method offers a gentle destruction of soil clods and results in separation of different size fractions of water stable soil aggregates, thus allowing characterization of SOM, microbes and enzyme activities within the aggregate fraction obtained. The size class was set up basically following the soil particle size composition, in an attempt to observe the aggregation of single mineral particles over the particle size composition. Unlike for the dry croplands (for example, Lobe et al 2011 in Geoderma), aggregates larger than 2000μm was not accounted for rice soils mostly have smaller sized aggregates due to long term pudding activities under mostly submergence condition. Provided references for the rational in MM section. The method had been employed for the last decade in our rice

soil research at the corresponding authors group (Li et al., 2007b, Pan et al., 2008). With the use of this method, we could address the changes in OC pools after land sue change (Li et al., 2007a), in microbial abundance and activities with different fertilization practices (Zheng et al., 2007) and in C and respiration under metal contamination (Chen et al., 2014) of rice paddies. We also noted there was alternative separation methods, including wet sieving with capillary water pretreatment by Six et al., 1998 and with gentle shaking by Kristiansen et al (2006). In the future, dispersion and separation incorporating these new developments will be tested for rice soil aggregates.

3) Linked to the previous comment, the interpretation of the results and the discussion is somewhat confusing. The fractionation procedure does not allow to separate physically-protected organic matter from organic matter stabilized by interaction with mineral surfaces. I would assume (but the authors should confirm in the manuscript) that with the low energy sonication applied, macro-aggregates have been broken releasing micro-aggregates, mineral particles and inter-aggregate particulate OM. This would mean that all fractions except the clay-sized fraction, could have different amounts of OM stabilized by different mechanisms. This has to be taken into account during the discussion. Response: Accepted that the fractionation procedure does not allow to separate physically-protected organic matter from organic matter stabilized by interaction with mineral surfaces. As indicated in the study by Stemmer et al (1998), the low ultrasonic energy input of 170 J g-1 dry soil does not disrupt aggregates completely but disrupt soil clods into macro-aggregates ($\geq$200 μm in size), micro-aggregates and fine aggregates (finer than 2μm in size) particles such as clay sized organo/mineral complexes, with a recovery of all size fractions of aggregates up to 98-100%. We are in agreement with the reviewer that all fractions except the clay-sized fraction, could have different amounts of OM stabilized by different mechanisms. The OM in the small sized clay fraction, however, could be dominantly mineral bound or chemically stabilized. In other words, macro-aggregates obtained after the sonification dispersion may contain micro-aggregates and fine aggregates, and inter-aggregate POM within the macro aggregates. Thus, either OM stabilized by mineral

binding is embedded in, or POM is protected by, the macro-aggregates. In this sense, we refer the C in the macro-aggregates to physically protected carbon, either primarily for POM or secondarily for mineral-bound OM.

4) FTIR spectroscopy analysis: I am not aware how the authors obtained a quantitative distribution of OM functional group constituents (Table 3) from specific peak bands in the IR spectrum, considering that each functional group vibration has a different molar absorptivity. In fact, Table 3 suggests that there is more phenolic than aromatic C and this is not possible since all phenols are also aromatic. I suggest using the ratio between specific peaks within each spectrum to obtain comparative results on OM composition. Response: Accepted and followed. Yes, all phenols are aromatic. Now we use total aromatic to calculate the OC recalcitrance, with the proportions estimated from the peak intensity of different groups. . 5) The objective of carrying out an incubation of soils with maize biomass is not totally clear to me and must be justified. I do not understand how results from an incubation under oxic conditions may contribute to understanding the role of physical protection in paddy soils. It seems that most of the maize-OM added was mineralized over the incubation period. The authors do not provide information on how maize application influenced the distribution of aggregate-size fractions. Moreover, they associate the C gain in the sand sized fractions to physical protection in macro-aggregates (L661-665), however stating that this is the predominant mechanisms of OM stabilization in these soils is incorrect considering (1) the fractionation procedure does not distinguish between free particulate OM and that occluded within macro-aggregates, and (2) the relatively short incubation period does not allow to take into consideration other stabilization mechanisms with longer turnover times. Response: Accepted. The experiment was intended to look at the carbon sequestration potential for exotic OC input. Considering (1) maize was used and (2) anaerobic incubation conducted, we now give up presenting the data of this experiment. And we agree that the relatively short incubation period does not allow to take into consideration other stabilization mechanisms with longer turnover times. All contents related to this experiment now deleted. However, this exclusion of data does

not confront the main finding of the study.

6) Soil respiration: This approach involved measuring the emission of CO2 over a 37 d anaerobic incubation period. However, I would expect CH4 and dissolved CO2 to contribute to the total anaerobic OM mineralization. These were not taken into account. Response: Considered. We measured soil respiration for addressing the potential microbial use of organic carbon and also for soil microbial activity in different aggregate size fractions of the soils. For rice soils have been conventionally under submerged conditions, an anaerobic incubation was performed and gas samples from the headspace of the incubation jar were collected and analyzed for CO2 evolution. Methane was produced but not measured in this study. According to a previous study (Zheng et al. 2007 AGEE) methane contributed to a minor quantity to total carbon release. CO2 could be dissolved in soil water (pH 6.4-6.5 for soils cultivated for 100-500 years but alkaline and neutral for uncultivated and cultivated for 50 years, Wang et al., 2015 SREP) but not quantified for no discharge of the soil water in the incubation. We had not taken into account of these components from the total CO2 release as this study is not a field study of carbon balance or greenhouse gas emission from a rice soils system. In addition, to help interpret the results, a new Table of basic soil properties added.

7) The discussion requires rewriting considering all the previous comments. Moreover, it need to be more concise and focused. The authors often cite other works to support their interpretations that are based on soil processes in upland soils where oxic conditions predominate. In my opinion this is not always correct especially when referring to microbial biomass composition and activity, gene abundance and their influence on soil processes. Response: Accepted. We rewrite the discussion part and tried our best to be concise and focusing on the key issues. Also, the subtitle are meanwhile revised. Now in the discussion was followed the logic as variation of OC accumulation and stabilization among aggregate fractions, OC stabilization versus bio-activity between macro aggregates and fine aggregates and the trend of bioactivity with OC

accumulation and stabilization across soils with increasing length of rice management. We are sorry that most of the citations were from the upland soils generally in oxic condition. In this revision, we added some more references from relevant soils.

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