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Interactive comment on "Limited protection of macro-aggregate occluded organic carbon in Siberian steppe soils" *by* Norbert Bischoff et al.

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Dear Reviewer,

thank you for commenting on our manuscript and giving helpful advices to improve our manuscript. All of your comments from the first revision (before the ms was published online) were intensively discussed among the authors and a point-by-pont reply to your comments was submitted to the journal. In this reply, we indicate what we have changed in the ms according to your comments. We also show where the ms remained unchanged and argue why we would like to keep with the presentation style. However, it appears you have not been informed about this point-by-point reply. Therefore, we copy paste the complete reply to your first comments on the ms below (we also attach a reader-friendly PDF-version as a supplement to this comment –> see the hyperlink

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below!). This reply covers all of your "new" comments from 18 January 2017. For example, we changed already the terms "aliquot" to "subsample" and referred to the MRT as being "shorter/longer". We also explained more clearly the novelty of the study and made respective changes in the ms. The reply was adressed to the Associate Editor (Y. Kuzyakov) but includes all of your comments.

Best regards,

Norbert Bischoff, on behalf of all co-authors

Reply to your first comments on the ms:

"Dear Prof. Dr. Yakov Kuzyakov, thank you very much for considering our manuscript for publication after minor revision. We intensively discussed the concerns raised by Reviewer#1 among co-authors and are pleased to present you a revised version of the manuscript. In this version, we better clarify the novelty of the study and point more precisely to the implications of our results (see for example P12 L6–17). We further improved the presentation of the data to facilitate the understanding for the reader (see for example Fig. 3–6 and Table 3). Moreover, we deleted unnecessary information from the main text (for example the results section "Soil organic carbon mineralization along the chronosequences" was completely rewritten) and focused on significant results and relevant trends to draw scientific conclusions (see for example P13 L24–27 or P14 L2–4). Thus, the revised manuscript is more concise. In the following, we respond step by step to each point raised by Reviewer#1. Best regards, Norbert Bischoff, on behalf of all co-authors

Reviewer#1 comments R#1: ms has several shortcomings, namely: i) the novelty of the study should be provided more clearly, because making a study similar to other 10 studies, but with different objects, is not the novelty, ii) figures need to be redrawn, because it is not clear what exactly they show (mean, median?), iii) result and discussion parts need to be rewritten with presentation only significant results. Please, see detailed comments below. For this stage, I advise major revision for the ms and sug-

gest to strongly improve it. A: We thank Reviewer#1 for this critical comments. To the first (i) point: Research on the effect of land-use change from grassland to cropland on soil OM in steppe soils has, up to now, focused on the prairie soils (i.e. steppe soils) of the North American Great Plains. Very little is known about soil OC dynamics in the Siberian steppe soils, though this area belongs to the greatest grassland conversion areas of the world. Thus, studies about soil OC stabilization in the Siberian steppe biomes are quantitatively severely underrepresented. This is a critical imbalance and our study presented here is the first attempt to correct this (we summarized this problem already in the original manuscript P4 L6–9). Thereby, our study showed that the tillage-induced break-down of macro-aggregates and the subsequent release of OM is not the key factor driving OC losses due to land-use change in the Siberian soils. In contrast, most OC in steppe soils of Siberia appears protected by occlusion within micro-aggregates and/or association with minerals. This, in part, contrasts with previous research of prairie soils from the North American Great Plains. The here presented study is the first one documenting these differences between Siberian and American steppe soils and gives possible explanations to these findings. In our opinion, these are crucial novelties of our study. We more precisely point to that novelty in the revised manuscript and improved amongst others, the discussion section (P12 L6–17, P13 L24–27). Moreover, we indicated already in the original manuscript that a serious shortcoming of previous studies is the short incubation time. This complicates an accurate determination of the size of the macro-aggregate protected OC fraction and its turnover time. Our study is the first conducting a long-term incubation of intact and crushed macro-aggregates. By that, we could show that the fraction of macro-aggregate protected OC was negligibly small and that it was mineralized within 100–400 days. This approach, in fact, is a novelty and now pronounced more precisely in the revised manuscript (P4 L2–5). To the second (ii) point: It might be that some of our figures were not clear enough. To make clear what exactly they show, we defined the drawn boxplots in the statistics section of the revised manuscript (P8 L23–25). We went through the reviewer's suggestions regarding the figures (see below)

and incorporated many of these good advices. Part of the graphical design remained unchanged and we argue below in detail why we would like to keep with the presentation style. However, the helpful advices of the reviewer significantly improved the quality of the figures and the understanding for the reader. To the third (iii) point: We revised the discussion section and pointed towards the significant results. However, we keep some of the results which rather indicated trends than significant results. This concerns particularly the results relating to the MRT. First, Reviewer#1 argued that this is the most interesting part of the study, thus, we kept it in the manuscript. Second, differences of the MRT are mainly present between plots (and not between fractions). Owing to the experimental design, detecting significant differences between the plots is quite "difficult" as we have only three real field replicates per plot. Thus, all parameters which are compared between plots should be viewed as indicating trends rather than significant results. We satisfied this fact by removing statistical tests regarding differences between plots from the manuscript (see Fig. 3, 4, and 6 and Table 2) to not mislead the reader, and changed the statistical method description accordingly (P8 L2–8). This makes the manuscript more concise and the information presented in the figures and tables are easier to understand. Furthermore, in the revised manuscript we solely refer to the relevant trends.

General comments R#1: Please, make the sequential line numbering in all ms next time, and not in each page from line 1. A: This was done according to the template provided by the journal "Biogeosciences" for manuscript preparation. This can be seen also in articles of "Biogeosciences Discussions". Therefore, we have not changed this in the manuscript.

R#1: Instead of ”aliquot” please use ”subsample”. A: Corrected.

R#1: How did you fit the model to the experimental data for the CO2 emission: for the each replication separately or for all replications together? If you have fitted the exponential model to all replication together, how did you then compare MRT of C pools between the plots (in this case you have only mean and st. error)? A: We have

fitted the model to the experimental CO2 emission data for each replicate separately. This was already described in the original version of the manuscript (P7 L13–16). To better clarify this, we added "i.e. analytical replicate" in P7 L14.

R#1: How was the moisture controlled during the experiment? A: The moisture was controlled during the experiment by periodically weighing the incubation jars and adding the necessary amount of ultrapure water. This procedure is also described, for example, in Creamer et al. (2013). We added this information to the revised manuscript (P6 L29–30).

R#1: How can you be sure that aggregates were not formed again from crushed ones during incubation? A: As we mixed all samples (also those of crushed aggregates) with quartz powder, the probability of aggregate formation is very small. We added a respective note to the revised manuscript (P6 L27–28).

R#1: For whole ms - be consistent with the terms - if you wrote arable, crop and pasture - use only them and not grasslands, croplands and so on. Use only one term - SOC or SOM. If you did not investigate N, use SOC only. A: We changed the terms according to the comment. In the revised manuscript we use solely the terms “pasture” and “arable” land with respect to our studied plots. However, if we refer to results from other studies, we use the terms used in the cited study. For example, “grassland” refers in many studies to virgin grasslands and pastures. Moreover, we changed OM for OC in most cases throughout the manuscript. Also the title of the manuscript changed accordingly.

R#1: For MRT - it was longer/faster. A: We changed it accordingly to "the MRT is longer/shorter".

R#1: For whole ms - write only about significant results, go through all ms and delete all sentences where ”non-significant trends” and results are written. If you write only about significant results, please delete ”($p<0.05$)” everywhere in the text. A: All of the "non-significant trends" referred to differences between the plots. As described above

(response to the third (iii) point), we revised our statistical approach and concluded that our experimental design did actually not allow for the detection of significant differences between plots, as we had only three field replicates in each plot. Our study was designed to detect statistical differences between soil fractions (bulk soil, intact, crushed macro-aggregates), as this was the main purpose of the study. Thus, we deleted all statistical tests relating to differences between plots. In the revised manuscript, we point towards the relevant trends of parameters (e.g. MRT, size of fast OC pool) across the plots of the chronosequences, and draw respective conclusions. Thus, we improved the understanding of the manuscript. When comparing parameters between soil fractions, we consider statistical significance and add "p<0.05" to clarify at which significance level we detect differences. This is common when reporting statistical results and this was also done in many recent studies in the journal "Biogeosciences" (e.g. Gentsch et al., 2015; Hall et al., 2015; Schrumpf et al., 2013).

R#1: For whole ms - do not repeat the numbers which are on the tables and on the figures, write about trends and make conclusions. A: We deleted those parts of the manuscript where numbers on the tables and figures were solely repeated. For example, we completely revised the results sections "Soil organic carbon contents along the chronosequences" and "Soil organic carbon mineralization along the chronosequences".

R#1: Discussion section - change the structure of the sentences - put the references in the end. Try to avoid sentences like: ”The authors of the mentioned study suggested’...’. Actually, the MRT is the most interesting part of the results, so you need to put more effort to discuss these results. A: In the revised manuscript, we list the references at the end of the sentences in most cases. Only in some cases we kept the references within a sentence, as they are placed behind the statements they represent. In the revised manuscript we changed sentences like ”The authors of the mentioned study suggested’...’ to better English, as we agree with the reviewer that these expressions were not optimal. The results about MRT are intensively discussed in our manuscript

in the discussion section "Effect of land management and soil characteristics on the mineralization potential of soil organic carbon" (in detail see P12 L23–32 and P13 L1–2). Conclusions about the MRT results and their implications are drawn in the conclusions section (P13 L29–32, P14 L1–4). Specific comments R#1: P3 L 15-24 Actually this is already well known. Accordingly, Al-Kaisi et al. (2014) showed that macro-aggregates were faster disintegrated upon disturbance than micro-aggregates - This statement was already shown by Six et al., 2000. A: We are well aware that this is already known and recapitulate the gained knowledge from previous studies in order to show that macro-aggregates are thought to play a crucial role for OC stabilization in the course of land-use change. This is important as it highlights the motivation of our study. We better clarified this in the revised manuscript and added three more references (P3 L23–25). The reference of Six et al. (2000) was a good advice and we changed the citation accordingly (see P3 L21–24 of the revised manuscript).

R#1: P5 L5-10 - Put information about the MAP and MAT into the table 1 and delete from the text. A: We now included MAP and MAT into Table1. However, we think it improves reading of the Material & Methods section if we remain the data about temperature and precipitation also in the text.

R#1: P5 L12-13, L24-25 The soil types are written in table 1, delete them from the text. A: As with the comment above, we believe that mentioning the soil types also in the text improves fluent reading and understanding of the experimental setup. Thus, we kept the soil types in the text.

R#1: P5 L7 Add abbreviation for TS here. A: The abbreviation was added as suggested.

R#1: P6 L6 Actually HCl fumigation is not the best method to remove carbonates from the soil, especially if you have high CaCO3 content. Is it possible to check the initial CaCO3 content and total C, to ensure that you have removed all CaCO3? A: The CaCO3 contents of A horizons of the investigated profiles were very small, typically

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<0.1% of dry soil mass. Therefore, HCl fumigation was carried out to remove traces of CaCO3. Complete removal of CaCO3 was controlled via measurements of δ13C of various subsamples. δ13C ranged from -23.9 $\pm$ 0.2‰ to -26.3 $\pm$ 0.2‰ hence, indicating that carbonates were removed. If carbonates were present in the samples, δ13C would show values closer to zero (Walthert et al., 2010). We added a sentence concerning this issue to the revised manuscript (P6 L6).

R#1: P6 L18 It is not clear, did you incubate real field replications or analytical? A: We agree that we have not sufficiently clarified the experimental design, which has now been corrected. We investigated 8 plots and took 3 field replicates per plot. All of these field replicates were divided into 3 fractions (bulk soil, intact, crushed). The 3 fractions, in turn, were divided into 3 analytical replicates. This gives a total of 216 samples (8 x 3 x 3 x 3). For clarification we added the information to the revised manuscript (P6 L17–19). Moreover, in the original version of the manuscript we already stated that we used three field replicates and remained this information in the revised manuscript (P5 L28–30).

R#1: P6 L28 What was the reason to add SiO2 ? You wrote to increase the volume, but maybe it was to prevent the formation of aggregates? A: Our primary intention of adding quartz to the samples was to increase the sample volume. Another aspect was, as the reviewer mentioned, that the formation of aggregates is prevented. We have added this to the revised manuscript (P6 L27–28).

R#1: P9 L3-12 Completely delete. This information is presented in table 1, and you do not need to repeat it in the result section. Please write about trends. A: We have not deleted this section completely, as it contains some important information (e.g., that increasing duration of agricultural land-use caused no further decrease of soil OC contents in arable soils of FS). However, we revised that section considerably and focused on the relevant trends presented in Table 1. Thus, the section became clearly shorter (P9 L3–8).

R#1: P10 L16-21 Delete completely. This information is presented in table 2, you do not need to write these numbers again, write about trends and make the conclusions. A: We deleted this part completely and have written it again, thereby focusing on the relevant trends. Moreover, in the revised manuscript we present the MRT of all three fractions in Table 2. Conclusions related to MRT data are made in the discussion and conclusion section (see P12 L23–32, P13 L1–2, P13 L29–32, P14 L1–4).

R#1: P13 L20 Fig 2 is a portion of remaining C and not mineralization rate. A: We agree with the reviewer that we wrongly referred to "OC mineralization rate" in Fig. 2, which has now been corrected. However, we added "i.e. OC mineralization rates" in brackets, as the different proportion of OC mineralized in the samples translates to different OC mineralization rates.

Figures and tables R#1: Table 2 - It is not clear - is this whole soil, intact aggregates or crushed aggregates? You need to show all MRT if you have measured them. Please look at the data for all of these categories, maybe these are differences?Please add results of statistical tests for the MRT of fast OM pool between the plots. A: In the original manuscript we solely showed the MRTs as function of land-use and not depending of the fractions as there were no significant differences between fractions. However, we agree with the reviewer that we should show the MRTs also of the fractions as their investigation is the main goal of the manuscript. Thus, we included the MRTs of all fractions in Table 2. We have not added the results of statistical tests between plots, as we renounced doing statistical tests between plots in the revised manuscript (see our response to the third (iii) point from reviewer#1).

R#1: Fig. 2 - Please present the mean and st errors for each sampling point for CO2 , and not all experimental points. A: We think that showing all experimental data points gives a better representation of the variability of the data set. Therefore, we remained with showing each replicate. By just plotting means and standard errors, the variability between the plots becomes not really apparent. This can be seen in the following figure (see Fig. 1 in the supplements to this comment), which is drawn in the suggested way.

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R#1: Fig. 3 and 4 and 6 - I did not get, why you have tested the differences between the plots for all 3 fractions combined. Please provide differences for each fraction separately. Make a normal graph with mean and st. errors, what was the reason to make box plots and show the outliers? A: As stated in our previous comments, in the revised manuscript we do not present results from statistical tests between plots anymore. Thus, in Fig. 3, 4, and 6 we now solely indicate significant differences between fractions within plots. We decided to use boxplots as they give a better overview of the distribution and variability of the data than just plotting arithmetic means and standard errors. Another advantage of boxplots is that, normally, they show medians which are more robust against outliers than arithmetic means. Schädel et al. (2014), for example, used also boxplots in their analysis of incubation data. Therefore, we still use boxplots in the graphs of the revised manuscript. However, to clarify that medians are shown in the boxplots we added this information to the statistics section of the revised manuscript (P8 L23–25). R#1: Fig 4 Legend - This is not size, this is a portion of the fast mineralizable pool. Please correct. A: We corrected the figure accordingly.

R#1: Fig 5 - Figure legend - this should be the ”Mineralization rate of...” because your units are ”% of total mineralized OC d-1”, or there is a mistake in units. Make normal graphs - means (marked as dots), st. error to them and fitted exponential decay line. What does ”Mean=1.4%” (and other similar information) mean? Mean mineralization rate? But it varies during a year. A: We agree that it should correctly be termed "Mineralization rate of..." and changed it accordingly in the revised manuscript. Moreover, we deleted the text "Mean=1.4, SE=2.4%" etc. from the graph, as this information might be confusing here. This data ("Mean=1.4, SE=2.4%" etc.) refers to the share of the mineralized macro-aggregate protected OC in the totally mineralized OC during the entire incubation period. In the revised manuscript we have transferred this data to a separate table (Table 3). We also added some information to the caption of figure 5 to better understand the few negative values. However, we have not plotted means as dots, as by our statistical approach we could calculate the mineralization rate for each single day, thus, a continuous line would better represent the calculated data.

Also the standard error can be given for each single day and thus a shaded area better represents the calculated data than some error bars.

R#1: Fig. 6 You can calculate % of MBC from the total SOC, and then you can compare plots between 2 chronosequences. A:We implemented this advice in the revised manuscript.

References Creamer, C. A., Filley, T. R. and Boutton, T. W.: Long-term incubations of size and density separated soil fractions to inform soil organic carbon decay dynamics, Soil Biol. Biochem., 57, 496–503, doi:10.1016/j.soilbio.2012.09.007, 2013. Gentsch, N., Mikutta, R., Alves, R. J. E., Barta, J., Capek, P., Gittel, A., Hugelius, G., Kuhry, P., Lashchinskiy, N., Palmtag, J., Richter, A., Santruckova, H., Schnecker, J., Shibistova, O., Urich, T., Wild, B. and Guggenberger, G.: Storage and turnover of organic matter fractions in cryoturbated permafrost soils across the Siberian Arctic, Biogeosciences, 12, 4525–4542, doi:10.5194/bg-12-4525-2015, 2015. Hall, S. J., McNicol, G., Natake, T. and Silver, W. L.: Large fluxes and rapid turnover of mineral-associated carbon across topographic gradients in a humid tropical forest: Insights from paired 14C analysis, Biogeosciences, 12(8), 2471–2487, doi:10.5194/bg-12-2471-2015, 2015. Schädel, C., Schuur, E. A. G., Bracho, R., Elberling, B., Knoblauch, C., Lee, H., Luo, Y., Shaver, G. R. and Turetsky, M. R.: Circumpolar assessment of permafrost C quality and its vulnerability over time using long-term incubation data, Glob. Chang. Biol., 20(2), 641–652, doi:10.1111/gcb.12417, 2014. Schrumpf, M., Kaiser, K., Guggenberger, G., Persson, T., Kögel-Knabner, I. and Schulze, E.-D.: Storage and stability of organic carbon in soils as related to depth, occlusion within aggregates, and attachment to minerals, Biogeosciences, 10(3), 1675–1691, doi:10.5194/bg-10-1675-2013, 2013. Walthert, L., Graf, U., Kammer, A., Luster, J., Pezzotta, D., Zimmermann, S. and Hagedorn, F.: Determination of organic and inorganic carbon, δ13 C, and nitrogen in soils containing carbonates after acid fumigation with HCl, J. Plant Nutr. Soil Sci., 173(2), 207–216, doi:10.1002/jpln.200900158, 2010.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/bg-2016-518/bg-2016-518-AC1-supplement.pdf

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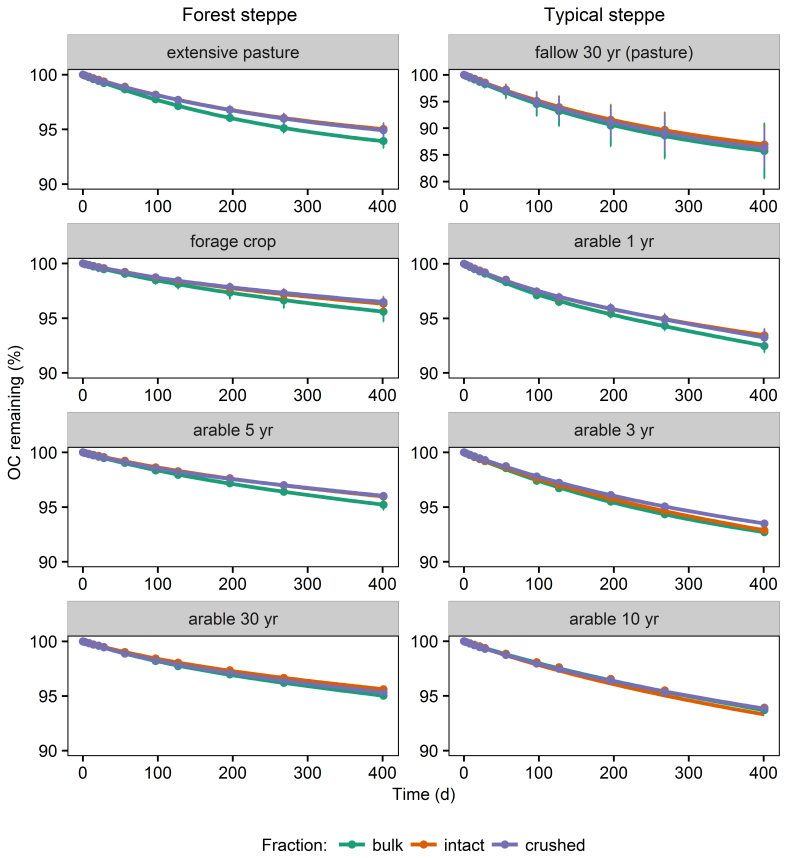

Fig. 1.

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