Dear Prof. Dr. Yakov Kuzyakov,

thank you very much for considering our manuscript for publication after minor revision.

We discussed the comments given by Reviewer#2 among co-authors and are pleased to present you a revised version of the manuscript. In this version, we better present the state of current research on that topic and deduce the hypotheses more clearly. Moreover, the experimental design is explained more concisely. Many of the helpful advices of Reviewer#2 were incorporated into the manuscript. However, in some aspects we are still arguing against his/her major concerns. We were pleased about the many suggestions of Reviewer#2 and thank him/her for the critical discussion on our manuscript.

We would like to note, that Reviewer#2 commented on our originally submitted manuscript. After a first revision of the manuscript, according to comments of Reviewer#1, our manuscript was published in Biogeosciences Discussions. In that manuscript, many of the concerns of Reviewer#2 were already revised. That particularly concerns the discussion section and the abstract of the manuscript.

In the following, we respond step by step to each point raised by Reviewer#2. Those parts of the manuscript which were changed during the revision are indicated in parentheses (page, line) and refer to the marked-up version of the revised manuscript.

Best regards,

Norbert Bischoff, on behalf of all co-authors

Reviewer#2 comments

R#2: Page 2 Abstract. As a matter of fact, an abstract gives the paper (highly concentrated) consequently, all comments and recommendations given below apply also for the abstract.

A: Done as suggested.

R#2: Page 3. Line 7: please add a reference to the statement “As the stabilization. . ...for maintaining soil fertility".

A: Done as suggested.

R#2: Line 9-11: in my understanding Lützow et al. do not point out that mineral-ass. and physical disconnection are the main ones, they rather want to strengthen that recalcitrance is not that important than thought.

A: We changed the reference to Lehmann & Kleber (2015) who pointed towards the importance of mineral-associations and aggregates in their soil continuum model.

R#2: Line 11 13: delete this sentence, as your investigation is not done in the dry steppe, rather substitute by own data (Bischoff 2016). (And to your knowledge, Kalinina et al. (2014) found comparable C rations in aggregate and clay fractions for dry steppe soils)

A: We have not meant the "dry steppe" but wanted to pronounce that steppe ecosystems are "dry" and this lack of water might inhibit the formation of mineral-organic associations. Because of your comment we realized that this verbalization is confusing and therefore deleted "dry" from the sentence. We did not add our own data (Bischoff et al. 2016) in this sentence since this study has not explicitly detected the importance of aggregates for OC stabilization in steppe soils. In Bischoff et al. (2016) we rather found a relation between the soil OC decline and aggregate stability to which we refer on page 4 line 12.

R#2: Line 16: is “primary particle” the right term? What about “detached” or “isolated”?

A: We considered using “detached” instead of “primary”, but came to the conclusion that “primary” is the better term. In the literature “primary particle” is used to describe particles which assemble aggregates. However, we deleted “free” as this term is not necessary here.

R#2: Line 22-23: I would add as explanation for the importance of aggregate C the very pronounced crumble structure (at least in Chernozems the best I have ever seen).

A: We did add the advice of Reviewer#2. We inserted this sentence on page 3 line 14-15 as we think it fits better there.

R#2: Page 4 Line 1: what is meant by “complicate reliable assessment”? Please explain more detailed.

A: The expression "complicate reliable assessment" was changed to: "which results in an unreliable assessment of the size of the macro-aggregate protected OC fraction and its turnover time". (page 4 line 5-6).

R#2: Line 5: please explain why Siberian steppe soils need special attention? Are results of the same soils but different regions not transferable? They must!

A: From a theoretical point of view results of the same soils but different regions should be transferable. But results of our previous study (Bischoff et al. 2016) showed that soils of the Kulunda steppe had different characteristics with respect to their OM quality, e.g. the partitioning between particulate OM and OM in mineral-organic associations, than soils of the North American prairies. Hence, despite soils of both regions are classified as Chernozems or

Kastanozems at level of soil groupings, differences in soil quality criteria might be expected. This suggests that soils in the Siberian steppes respond different to disturbances like land-use change than soils of the same soil groupings in the North American prairies. In fact, it is open whether results of soils of the same soil grouping are transferable between North America and Siberia. As to now, very little is known about the soil OC dynamics in the Siberian steppes, which is evident from the lack of studies in the literature.

R#2: Line 15: delete "agricultural"

A: We would not like to delete "agricultural" as this term specifies the fact that we were investigating chronosequences on agricultural land. The term "agricultural chronosequence" was e.g. also used by Insam and Domsch (1988) and Panettieri et al. (2014).

R#2: Line 18-20: first hypothesis is not consistently deduced from the literature! The authors state themselves that increases and decreases were found (page 3)

A: It is right that we state that increases and decreases were found. However, beforehand we deduce from the literature that OC is stabilized within aggregates. Moreover, we state that the disruption of aggregates was shown to be the reason for a decline of OC along agricultural chronosequences. These results/conclusions from the literature suggest that the disruption of macro-aggregates leads to an increased OC mineralization, as a previously occluded OC fraction becomes available to microbial decomposition. Thus, we made this to our first hypothesis. The question on why other studies have not found this increase is not part of our hypothesis, but is rather discussed in the Discussion part of our manuscript.

R#2: Line 20-21: also inconsistent: the authors refer to the opposite (page 3, line 29). The second part "land-use duration" and "intensity" (what is exactly meant by this term?) is not derived from knowledge from the literature (missing state of the art)

A: In the revised version of the manuscript we referred to "*bulk soil* OC mineralization rates (...) are higher in pasture than in arable soils" (page 4 line 22-23). This is not the opposite of what was written on page 3 line 29, as the statement in line 29 refers to the response of OC mineralization rates after aggregate crushing and not to OC mineralization rates of bulk soil. We agree with Reviewer#2 that we not precisely derived the second hypothesis ("land-use duration and intensity") from the literature. Therefore, we added a sentence clarifying the state of the current knowledge to the manuscript (page 3 line 27-29). The term "land use intensity" refers to the comparison of the pasture soil (low land use intensity) and the forage crop soil (relatively higher land use intensity) in the forest steppe. This is explained in the Material & Methods section (page 5 line 9-10). The term "land-use duration" refers to the "time since land-use change from pasture to arable land". To better clarify that, we changed the term and used it throughout the revised manuscript (page 4 line 20).

R#2: Line 24-25: is the approach of getting pools from fitting decay models an appropriate one? Please explain to those who are not familiar with it, add references

A: The use of double exponential decay models with two distinct carbon pools and associated mineralization rates constants is standard in describing the decomposition pattern of soil organic matter (e.g., Kalbitz et al., 2005). Therefore, in our opinion, it is not necessary to incorporate an explicit note on that topic into the manuscript. It is important that the time of incubation is sufficiently long (in most cases at minimum 1 year) that two C pools can be fitted accurately. Hence, we used an incubation time of >1 year.

R#2: Page 6 Line1-2: As stated, the Kulanda steppe is semi-arid. How can FS be part of this steppe (forest steppe in semi-arid steppe?)?

A: We thank Reviewer#2 for this attentive note and agree that a forest steppe cannot be part of a semi-arid steppe. Therefore, we deleted the term "semi-arid" from the manuscript.

R#2: Line 4: hopefully with comparable grain sizes within each chronosequence, please confirm.

A: Of course, the grain size of the soils was similar within one chronosequence. To avoid misunderstandings we added a respective sentence to the manuscript (page 5 line 4-5).

R#2: Line 5 (Tab. 1): I. missing data on grain size distribution, please add, so that any grain size effect on analyzed process can be excluded. II. The term "soil type" is not used in WRB, please correct. III Replace the term "Typical Steppe" by "Semi-arid Steppe" and introduce abbreviation (throughout the text). IV here 30yr in line 12 ten years, what is correct?

A: I. We added the necessary information on grain size distribution to Table 1. II. We deleted the term "type" and referred to "soil classification". III. We would not like to replace the term "Typical steppe" by "Semi-arid steppe" as (i) it is a characteristic term for the steppes of south-western Siberia and was already used in previous studies (e.g. Bischoff et al. (2016); Frühauf et al., 2004; Lebedeva (Verba) et al., 2008), and (ii) the term "semi-arid steppe" is broader and could also include the "dry steppe", which is located further south. IV. We thank the Reviewer for this attentive note. By mistake we wrote "ten years" in the main text, but "thirty years" is correct. This has been corrected.

R#2: Line 7: use one term throughout the text for "more arid typical steppe, you introduced before "semi-arid" (much more consistent) and be stay thereafter

A: In the previous comment we explained why we would not like to introduce the term "semi-arid" instead of "typical". Therefore, we keep the term "typical" and use it consistently throughout the manuscript. We agree that the expression "more arid typical steppe" can be confusing and changed the sentence in the revised manuscript (see page 5 line 8-9).

R#2: Line 8: please clarify, how you identified sites

A: We identified the two sites by interviewing farmers and land owners about land-use history and management. The plots within a site were checked for comparable pedological conditions by inspecting the soils with a hand-auger. We added this information to the manuscript to clarify how we identified sites (page 5 line 9-13).

R#2: Line 9: I. Why did you resign to include a natural plot? All plots of second chronosequence have a management history, hence, no discussion on land use change can be done. Additionally, can you prove that the first plot (FS) has no cropping history at all, although cropped sites are that near and land use change seems to be distributed all over the investigation area , is crucial point (see discussion). II. The forage plot also has a time of cultivation, please add, otherwise infeasible. III What exactly makes the forage site to an intensively used one?

A: I. We could not include a natural plot in our study, as we could not identify natural grasslands nearby our chronosequences. All grasslands in the region are normally used as extensive pastures. Nevertheless, it is possible to discuss effects of land-use change as the conversion of pasture to arable land, in fact, represents a change in land-use. Based on local farmers and land owners, we are very sure that the pasture plot in FS has no cropping history at all (at least not for the last ~100 years). This is underpinned by the fact, that there exists no relict/former plough horizon (Ap) which usually stays for decades once a soil was ploughed. II. The time of cultivation of the forage plot is 10 years, but we resigned to include this information into the manuscript, as this plot is not part of the chronosequence in FS, but rather used for the comparison of land-use intensity (pasture vs. forage crop). Therefore, the time of cultivation of the forage plot is not important for the interpretation of our results and we would not like to refer to it explicitly, to not create misunderstandings during reading of the manuscript. III. The forage crop plot is more intensively used than the pasture plot, as the cultivation of forage crops includes occasional soil management while soil management is absent on the pasture. The comparison of land-use intensity includes only the comparison between the pasture and forage crop in FS. This is already mentioned in the Material & Methods section of the manuscript (page 5 line 13-14).

R#2: Line 13: abbreviation “TS” not introduced, should be exchanged by SAS (see above)

A: In the version of the paper published in Biogeosciences Discussions the abbreviation "TS" was already introduced on page 5 line 7. As mentioned in a previous comment we would like to keep the term "typical steppe (TS)" in the manuscript and not replace it by "semi-arid steppe (SAS)".

R#2: Line 19: ”meanwhile”?, I guess since 1983

A: “Meanwhile” is the correct term. The plot was left as fallow since 1983 and is now used as pasture. Unfortunately, we do not know the exact year since it was used as pasture. Thus, it is “meanwhile” used as pasture. With respect to our experimental design it is not important since when it was used as pasture. The important fact is that it was not cropped and tilled since 1983.

R#2: Line 16: 30 yr fallow means sampling year was 2013, correct? Please add more information on sampling design

A: This is correct, sampling year was 2013. We added more information on sampling design to the manuscript (page 5 line 9-11).

R#2: Line 22: “which are attributed to erosion” is a speculation, delete. E.g. position top hill vs. slope toe might also be feasible

A: We would not like to delete the expression "which we attributed to erosion", to give the reader an idea why we did not measure a decline of soil OC along the chronosequence in TS, as is typical for chronosequence studies. Thereby, we point to the fact that it is very likely that another process superimposes the effect of soil management along the chronosequence in TS. It is important to note (and we included this sentence in the manuscript --> page 5 line 26-28), that the possible effect of macro-aggregate crushing on soil OC mineralization, if existent, will be also evident on slightly eroded plots. Therefore we decided to include the chronosequence in our study.

R#2: Line 24: and if erosion is the case you have mixed material at plot 19 yr (autochthonous and from above) which makes plot 10 yr unfeasible. Strictly argued: Plot 10 yr has to be deleted, but what is left?

A: We were discussing among co-authors to exclude the plot arable 10yr as it probably accumulated soil material from above (fallow 30 yr). Nevertheless, we decided to keep the plot in the study as it supports the general result of our study: "macro-aggregate protected OC is not stabilized against decomposition in the studied soils". Nevertheless, we agree that based on the chronosequence in TS there are no conclusions possible about the effect of land use duration. However, these effects are discussed based on the chronosequence in FS.

R#2: Line 25: How you prove that key profiles are representative?

A: We proved that by inspecting the soil with a hand-auger down to 1m depth.

R#2: Line 26: I. delete “genetic” II. arable 30yr plot not introduced before

A: We deleted "generic". II. We thank Reviewer#2 for this advice. As the reviewer correctly mentioned in a previous comment, we denoted the arable 30 yr erroneously as "10 yr" before, thus "arable 30 yr" was not introduced. We corrected that accordingly.

R#2: Line28: despite not all sites were investigated by a key profile, all plots have to be analyzed in respect of grain size distribution (see above). II. Why EC was measured? Delete, if you do not refer to somewhere. III: what means “composite”? Mixed samples?

A: Of course we checked all plots with respect of grain size distribution. On those plots where we did not establish a key profile we determined grain sizes by hand analysis and confirmed that all plots within a site had comparable grain size distribution. We added this information to the manuscript (page 6 line 1-4). II. Some of our colleagues argued that in steppe soils the electrical conductivity can vary considerably and hence affect microbial activity and in consequence OC mineralization. Therefore, we measured EC on those plots which were not located directly adjacent to each other. Our measurements show that in all plots EC was in a comparable range and confounding effects of EC on our results are unlikely. We agree with the Reviewer, that we did not refer to it elsewhere in the text. Thus, we decided to add a note in the results section of the main text (page 9 line 3-4). III. The term "composite" is not part of our manuscript, since it was deleted beforehand after a comment of Reviewer#1.

R#2: Line 30: Tab S1 not required, coordinates can be integrated in Tab 1

A: We agree with the Reviewer and included the coordinates in Table 1.

Page 6

R#2: Line 2: which samples are “all samples”? Those from the profiles? And if so, why is always only one data set per soil is given, and not those per horizon? And from what horizon were the given data?

A: "All samples" refers to all incubated samples. We added the information to the manuscript to avoid misunderstandings (page 7 line 9).

R#2: Line 3: why was the residual water measured? Nowhere appearing again

A: This is a standard procedure in our laboratory and necessary to calculate the soil mass as basis for subsequent calculations of elemental contents. For example, if we measure the OC and TN contents (mg g^{-1} soil) on air-dry samples we need to subtract the residual soil water content, otherwise we would underestimate the OC and TN contents. We added this information to the manuscript (page 6 line 9-10).

R#2: Line 18: statement on amount of samples is redundant

A: Since Reviewer#1 did not understand exactly the amount of samples, we clarified the quantity precisely in this sentence. We cannot find where this statement is redundant in the manuscript, as the sentence with the number "giving a total of 216 samples" is only given there.

R#2: Line 20-25: it is not clear how the quantification was done. II. Fig "1 is not required, because 1) it does not help to understand the quantification and 2) does not appear again

A: I. The quantification was done as following: the fraction of crushed macro-aggregates was sieved through a 63µm-sieve and the percentage remaining on the sieve and that passing the sieve was calculated by mass balance calculations. We added this information to the manuscript (page 6 line 28-29). II. In our opinion "Fig S1" is required as it highlights the condition of the crushed aggregates. Only by that figure we can conclude that the fraction <63µm consisted mainly of small micro-aggregates and only few primary particles (see page 6 line 31-32). Without "Fig S1" we could not rule out that the fraction <63µm is only composed of primary particles.

R#2: Line 27: how was WHC determined?

A: WHC was determined according to Schlichting et al. (1995). We added the information to the manuscript (page 7 line 2).

R#2: Line 31: replace "sampling" by "filling"

A: "Filling" is not right in this sentence as gas was sampled from the headspace of each jar and not filled.

R#2: Fig. 1: not introduced. II. site photos not meaningful (delete). III. profile too small. IV. Map not meaningful (medium scale is missing) + scale not stated + missing north arrow

A: I. We introduced "Fig. 1" on page 5 line 5. II. In our opinion the site photos are indeed meaningful and we would like to keep them in. In the site photos it is clearly visible that the fallow 30 yr (pasture) in TS is degraded and has a sparse vegetation cover, to what we refer on page 5 line 22-23. This is in contrast to the "good" condition of the extensive pasture in FS, which is clearly visible in the site photos. III. We increased the size of the profile pictures. IV. In our opinion the map is meaningful as it quickly gives an overview to the reader where the study took place (without explicitly looking for the given geographical coordinates in Table 1 on a map). As we denote latitude and longitude in the figure a separate scale is not necessary. We added a north arrow to the figure.

Page 9

R#2: Line7: what is meant by “increasing duration”? A reached equilibrium after 5 yr cropping?

A: We do not mean that an equilibrium was reached after 5 years of cropping. This could be a possible interpretation but we do not know about the soil OC content after >30 yrs cropping. Thus, we kept the expression "neutral" and solely stated that our data showed that the increasing duration of land use (pasture --> 5 yr arable --> 30 yr arable) has not led to a further decrease of soil OC contents.

R#2: Line 11: see comment on this issue above. In addition: at the top plot you might have include sub soil material as top soil was eroded. However, any erosion process includes addition from elsewhere or losses from top and addition from the sub soil, processes completely destroying investigation approach and hence have to be completely avoided (make sure). II. In addition, erosion statement was only given for 10 yr above, but not for 1 yr, as firstly described here.

A: I. No subsoil material was included at the top plot. As was denoted in the Material & Methods section (page 5 line 29) we took samples from 0-10 cm. In the profile picture of Figure 1 it is visible that the A horizon of the top plot (fallow 30 yr) was >20 cm, hence, subsoil material cannot be present when taking samples from 0-10 cm. In a previous comment we argued that we are aware that the possible erosion process along the chronosequence in TS will affect any interpretation regarding the effect of land use duration on bulk soil OC mineralization. Therefore, we interpret the effect of land use duration on bulk soil OC mineralization rates and sizes of the fast OC pool (hypothesis 2) solely based on results of the chronosequence in FS. However, the possible erosion process has no influence on the effect of macro-aggregate crushing on soil OC mineralization (hypothesis 1), as confirmed by the similar results of soil OC mineralization upon macro-aggregate crushing on the arable 10 yr plot. Thus, the general result of our study is not altered because of the possible erosion process. II. In this sentence we did not state that erosion took place on arable 1yr. We solely stated that "soil OC contents (...) did not follow the gradient over time since cultivation, as the site was affected by erosion". This means, that the arable 10 yr has not the smallest soil OC contents as we would have expected.

R#2: Line 7 + 12: it might be interesting to point out the differences in C/N of both chronosequences?

A: We pointed out the difference of C : N ratios between both chronosequences and included that topic in the discussion section (page 9 line 9, page 13 line 13-16).

R#2: Line 14 and following: it is not clear when the measurements were done, after incubation? It hampers a reviewing with regard to content

A: The measurement of OC and TN were done before the incubation experiment. We added this information to the Material & Methods section (page 6 line 13-14).

R#2: Line 16 (Fig S2): if the figure shall only show which is stated in the sentence beginning in line 14, the figure is not required (delete)

A: Fig. S2 indicates the respiration rates of the samples during the incubation. This figure is not necessary for the understanding of the manuscript. Therefore, we placed it in the supplements. In our opinion the figure should be kept in the supplements as it is a standard figure in incubation studies, giving an overview about the measured data (respiration/CO_2 emission of the studied samples), which otherwise cannot be given to the reader. We think that it is good style to show also primary data.

R#2: Line 17-19: I do not understand the relationship between the two sentences given here

A: We changed the formulation of the two sentences.

R#2: Line 17 (Fig. 2): Figure does not show different scales, as written in the heading

A: The figure does show different scales. Please note the different y-scale for the fallow 30 yr (pasture). We added "y-scale" to the manuscript to clarify that it is the y-scale and not x-scale.

R#2: Line 19-21 please interpret Fig 2 more correctly, what about TS?

A: Line 19-21 refer to Fig. 3 and not Fig. 2, as is mentioned in the text. We clarified this part of the text and indicated that the amount of soil OC mineralized was larger than that in the intact and crushed macro-aggregates in all plots along the two chronosequences (including TS), but significant differences were only detected in FS (page 9 line 21).

R#2: Line 14-21: these few lines are supported by four figures/tables. I propose just to keep Fig. 3.

A: We think that the four figures/tables are, in fact, not redundant and would like to keep them all in. Table S2 (in the revised manuscript Table S1) shows the absolute OC and TN content of the samples in mg g^{-1} soil. Figure S2 indicates the original/measured incubation data and is in our opinion a standard graph in incubation studies which should be kept in the manuscript, though it is sufficient to place it in the supplements. Figure 2 highlights the fitted models. Based on these models we calculated the size of the fast and slow OC pool and the respective MRT´s. It is thus important that the reader can see how the models fitted the data and therefore it is necessary to keep Fig. 2 in the manuscript. Figure 3 summarizes statistics on data which is also present in Fig. 2, but could not have been integrated into Fig. 2 as that figure would become overloaded. Therefore, in agreement with Reviewer#2, Fig. 3 is also necessary to present the data to the reader in an appropriate way. Because of these reasons, we would like to keep all of the four figures/tables in the manuscript.

R#2: Line 23: advice to Fig. 3 needed but not to Fig 2 (delete)

A: See our comment above. However, in the revised manuscript we only refer to Fig. 3 and not Fig. 2, as Fig. 2 gives no information about the statistical significance of the results (page 9 line 24).

Page 10

R#2: Line 10: what is meant by "the sites", please indicate more precisely

A: In the revised manuscript we do not refer to the sites anymore and indicated the results more precisely (page 10 line 11-12).

R#2: Line 30 (Fig S3): not meaningful, delete

A: In our opinion it is important to check for the relation between the percentage of OC mineralized and the quantity of soil microbial biomass, since soil microbes are the ones who respire and thus mineralize soil OC. We placed the figure to the supplements, as it does not indicate any of the main results. Nevertheless, it illustrates the reader the relation between the two parameters.

R#2: Line 32 (Fig S4): not meaningful, delete

A: In our opinion it is also important to check for the relation between the percentage of OC mineralized and the share of the microbial biomass C in the total soil OC. The share of the microbial biomass C in the total soil OC was used by several authors as an indicator for soil OM quality (Allison et al., 2007; Hurisso et al., 2014), with larger values indicating a substrate with high OM quality. We would expect that a substrate with a high OM quality leads to larger OC mineralization rates, but this was not the case. As the figure did not show any of the main results we placed the figure in the supplements.

Page 11 and 12 Discussion on limited protection macro-aggregate C

R#2: As already noted above I recommend a more careful discussion on this aspect. All plots of the second sequence have a cropping history. Thus, it might by possible that macro-aggregate occluded C was lost than, never built up again (Kalinina et al., 2011, found during self-restoration of post-agrogenic Chernozems an increase in C, however in relations to other fractions increase of aggregate C was less existing) and thus, you find no differences. A cropping history can in all probability also not be excluded for the first plot of the first chronosequence. This means your chronosequences lack of proven uncropped former stage. So, once again this aspect has to be included into the discussion.

A: According to Kalinina et al. (2011) the increase of aggregate C (oPOM) after self-restoration of post-agronomic Chernozems was less pronounced than for other C fractions. But, particularly in 0-10 cm (the same sample depth as used in our study), Kalinina et al. (2011) observed an increase of oPOM to slightly >20% of total soil OC within 8 years, thus proving a build-up of aggregate C in the topsoil. Moreover, in our reply to a previous comment we clarified that we can be very sure that the extensive pasture in FS was never ploughed/cropped before. Hence, we can exclude that macro-aggregate occluded OC was already lost upon cultivation and never build up again on the extensive pasture in FS. The Reviewer is certainly right that we cannot prove this for the other plots as all of them were ploughed in former times. However, since the effect of macro-aggregate crushing on OC mineralization was not evident in the extensive pasture in FS, it is very unlikely that a missing build-up of previously lost macro-aggregate occluded OC is the reason that we found no differences in the other FS plots. In TS, the last ploughing of the fallow/pasture plot was 30 years ago and according to Kalinina et al. (2011) (Fig. 5 in their paper) aggregate-occluded OC increased by about 25–30% after 8 years of cultivation. Thus, we could expect a larger OC mineralization in crushed than in intact macro-aggregates at least in the fallow/pasture plot in TS, if a protection of macro-aggregate occluded OC was present. Since we did not find this increased OC mineralization, it is in our opinion correct to conclude, that macro-aggregate protected OC was not present in the studied soils. As explained in that comment, these conclusions remain even given the results from Kalinina et al. (2011). Apart from that, we changed many parts of the discussion on the effect of macro-aggregate crushing based on comments of Reviewer#1, and discussed the topic more carefully. Please refer to this formerly revised manuscript to see that changes.

Page 12 and 13 Discussion on effect of management, soil and sites on mineralization

R#2: The discussion has to be done more tentatively, as differences in fast soil pools of grassland and cropland was not significant (see Fig. 4 and page 10, line 11). In this respect, statement beginning in line 28 is too offensive (just trends), the same is true for line 31 (or was this a literature statement, then add references). On the other hand the statement “our results support. . .. .” (page 13, line 5) is too general (what results are explicitly meant?). In addition be again be careful in discussion LUC from grass to crop (see comments for page 11, 12). At least, it is nice to see homogenous effects upon ploughing by your data, however, this is an old story.

A: In the revised manuscript we deleted all statistical tests on parameters which were compared between plots, since our experimental design did actually not allow for powerful statistics on differences between plots (see our comments to Reviewer#1 which are published in the Biogeosciences Discussions forum). The experimental setup of our study was designed to test for significant differences between fractions (intact vs. crushed aggregates) to which we refer in the discussion section on the effect of macro-aggregate crushing. Since we removed statistical tests on differences of fast soil OC pools (see Fig. 4 and page 10 line 6-8) we had to discuss the results more tentatively. In our opinion, the statement in line 28 is not too offensive as it just expresses what we measured. In line 31 we denote that MRTs "tend" to become shorter along the chronosequence in FS. The statement "our results support..." was clarified in the revised manuscript (page 12 line 33). The effect of LUC from grassland to cropland is only discussed once in this section (page 12 line 25-26). There we state that the fast OC pool is highly vulnerable to LUC as it became diminished within 1-5 yrs after LUC. This is based on the results of the chronosequence in FS. As mentioned in a previous comment, the chronosequence in FS is valid and conclusions on the effect of LUC are therefore feasible.

Page 13-14 Conclusion

R#2: I again recommend a more careful writing many statement are not underlined by significant data, and again see comments for page 11, 12.

A: In the revised manuscript we changed many parts of the conclusion section in order to be more careful with the significance of the results. As we responded already on the comments for page 11 and 12, we would like to keep with our conclusion on the effect of macro-aggregate crushing, since in our opinion the results of Kalinina et al. (2011) do not question our results.

References:

Allison, V. J., Yermakov, Z., Miller, R. M., Jastrow, J. D. and Matamala, R.: Using landscape and depth gradients to decouple the impact of correlated environmental variables on soil microbial community composition, Soil Biol. Biochem., 39(2), 505–516, doi:10.1016/j.soilbio.2006.08.021, 2007.

Bischoff, N., Mikutta, R., Shibistova, O., Puzanov, A., Reichert, E., Silanteva, M., Grebennikova, A., Schaarschmidt, F., Heinicke, S. and Guggenberger, G.: Land-use change under different climatic conditions: Consequences for organic matter and microbial communities in Siberian steppe soils, Agric. Ecosyst. Environ., 235, 253–264, doi:10.1016/j.agee.2016.10.022, 2016.

Frühauf, M., Meinel, T. and Belaev, V.: Ecological consequences of the conversion of steppe to arable land in Western Siberia, Eur. Reg., 12(1), 13–21, 2004.

Hurisso, T. T., Norton, J. B. and Norton, U.: Labile soil organic carbon and nitrogen within a gradient of dryland agricultural land-use intensity in Wyoming, USA, Geoderma, 226–227, 1–7, doi:10.1016/j.geoderma.2014.02.025, 2014.

Insam, H. and Domsch, K. H.: Relationship between soil organic carbon and microbial biomass on chronosequences of reclamation sites, Microb. Ecol., 15(2), 177–188, doi:10.1007/BF02011711, 1988.

Kalbitz, K., Schwesig, D., Rethemeyer, J., Matzner, E.: Stabilization of dissolved organic matter by sorption to the mineral soil, Soil Biology & Biochemistry, 37, 1319-1331, 2005.

Kalinina, O., Krause, S.-E., Goryachkin, S. V., Karavaeva, N. A., Lyuri, D. I. and Giani, L.: Self-restoration of post-agrogenic chernozems of Russia: Soil development, carbon stocks, and dynamics of carbon pools, Geoderma, 162(1–2), 196–206, doi:10.1016/j.geoderma.2011.02.005, 2011.

Lebedeva (Verba), M. P., Lopukhina, O. V. and Kalinina, N. V.: Specificity of the chemical and mineralogical composition of salts in solonchak playas and lakes of the Kulunda steppe, Eurasian Soil Sci., 41(4), 416–428, doi:10.1134/S106422930804008X, 2008.

Panettieri, M., Knicker, H., Murillo, J. M., Madejón, E. and Hatcher, P. G.: Soil organic matter degradation in an agricultural chronosequence under different tillage regimes evaluated by organic matter pools, enzymatic activities and CPMAS 13C NMR, Soil Biol. Biochem., 78, 170–181, doi:10.1016/j.soilbio.2014.07.021, 2014.

Schlichting, E., Blume, H.-P. and Stahr, K.: Bodenkundliches Praktikum – Eine Einführung in pedologisches Arbeiten für Ökologen, insbesondere Land- und Forstwirte, und für Geowissenschaftler, 2nd ed., Blackwell Wissenschafts-Verlag Berlin, Wien, Austria., 1995

Limited protection of macro-aggregate occluded organic carbon in Siberian steppe soils

Norbert Bischoff[1], Robert Mikutta[2], Olga Shibistova[1,3], Alexander Puzanov[4], Marina Silanteva[5], Anna Grebennikova[5], Roland Fuß[6], Georg Guggenberger[1,3]

[1]Institute of Soil Science, Leibniz Universität Hannover, Hannover, 30419, Germany

[2]Soil Science and Soil Protection, Martin-Luther University Halle-Wittenberg, Halle, 06120, Germany

[3]VN Sukachev Institute of Forest, Siberian Branch of the Russian Academy of Sciences, Krasnoyarsk, 660036, Russian Federation

[4]Institute for Water and Environmental Problems, Siberian Branch of the Russian Academy of Sciences, Barnaul, 656038, Russian Federation

[5]Faculty of Biology, Altai State University, Barnaul, 656049, Russian Federation

[6]Institute of Climate-Smart Agriculture, Johann Heinrich von Thünen Institute, Braunschweig, 38116, Germany

Correspondence to: Norbert Bischoff (bischoff@ifbk.uni-hannover.de)

Abstract.

Macro-aggregates especially in agricultural steppe soils are supposed to play a vital role for soil organic carbon (OC) stabilization at a decadal time scale. While most research on soil OC stabilization in steppes focused on North American prairie soils of the Great Plains with information mainly provided by short-term incubation experiments, little is known about the agricultural steppes in south-western Siberia, though they belong to the greatest conversion areas in the world and occupy an area larger than that in the Great Plains. To quantify the proportion of macro-aggregate protected OC under different land-use ~~and~~ as function of land-use ~~duration and~~ intensity and time since land-use change (LUC) from pasture to arable land in Siberian steppe soils, we determined OC mineralization rates of intact (250–2000 µm) and crushed (<250 µm) macro-aggregates in long-term incubations over 401 days (20°C; 60% water holding capacity) along two agricultural chronosequences in the Siberian Kulunda steppe. Additionally we incubated bulk soil (<2000 µm) to determine the effect of ~~land-use change (~~LUC~~)~~ and subsequent agricultural use on a fast and a slow soil OC pool (labile *vs.* more stable OC), as derived from fitting exponential decay models to incubation data. We hypothesized that (i) macro-aggregate crushing leads to increased OC mineralization due to an increasing microbial accessibility of a previously occluded labile macro-aggregate OC fraction, and (ii) bulk soil OC mineralization rates and the size of the fast OC pool are higher in pasture than in arable soils with decreasing bulk soil OC mineralization rates and size of the fast OC pool as land-use ~~duration and~~ intensity and time since LUC increase. Against our hypothesis, OC mineralization rates of crushed macro-aggregates were similar to those of intact macro-aggregates under all land-use regimes. Macro-aggregate protected OC was almost absent and accounted for <1% of the total macro-aggregate OC content and to maximally 8 ± 4% of mineralized OC. In accordance to our second hypothesis, highest bulk soil OC mineralization rates and sizes of the fast OC pool were determined under pasture, but mineralization rates and pool sizes were unaffected by ~~the duration and intensity of~~ land-use intensity and time since LUC. However, at one chronosequence mean residence times of the fast and slow OC pool tended to ~~become shorter along one chronosequence~~decrease with increasing time since establishment of arable use. We conclude, that the tillage-induced break-down of macro-aggregates has not reduced the OC contents in the soils under study. The decline of OC after LUC is probably attributed to the faster soil OC turnover under arable land as compared to pasture at a reduced plant residue input.

Introduction

Steppe soils comprise about 7% of the terrestrial soil organic carbon (OC) storage down to 1m (Calculation see supplementary material) and cover about 885 million ha worldwide (FAO, 2001). As they are rich in organic matter (OM) and well-suited for agriculture they encompass about 14% of agricultural land globally (FAO, 2013). Intensive management of steppe soils reduced their OC stocks significantly, with estimated OC losses between 24 and 40% associated with conversion of grassland to cropland (Beniston et al., 2014; Mikhailova et al., 2000; Rodionov et al., 1998; VandenBygaart et al., 2003). As the stabilization of OC in agricultural ~~steppe~~ soils is crucial for maintaining soil fertility and to reduce the emission of CO_2 to the atmosphere (Lal, 2004), further insights into the processes that govern OC stabilization in agricultural steppe soils are needed. Next to temperature and moisture, chemical stabilization by formation of mineral-organic associations and physical disconnection of OM from microorganisms by occlusion of OM in aggregates, were identified as main factors stabilizing soil OC ~~(von Lützow et al., 2006)~~(Lehmann and Kleber, 2015). For ~~dry~~ steppe ecosystems the role of aggregation might be more decisive for OC stabilization than the one of mineral-organic associations, as the latter requires sufficient water for the formation of pedogenic minerals and the interaction of OM with mineral surfaces (Kleber et al., 2015). The potential relevance of aggregate-occluded OC is also suggested by the markedly crumbled soil structure usually found in steppe soils.

The mean residence time of aggregate-occluded OC ranges from decades to several hundreds of years (Six et al., 2002). Tisdall and Oades (1982) proposed a concept in which aggregates are structured hierarchically with respect to their size and binding agents. According to this aggregate hierarchy concept, ~~free~~ primary particles or silt-sized aggregates (<20 µm) are bound together to micro-aggregates (<250 µm) by persistent binding agents, e.g. humified OM, polyvalent metal cations or oxides. The micro-aggregates, in turn, are linked together to form larger macro-aggregates (>250 µm) by temporary (e.g. fungal hyphae, roots) or transient binding agents (e.g. microbial and plant-derived polysaccharides). Due to the hierarchical order of aggregate structure and the different persistence of the involved binding agents, macro-aggregates are less stable and more vulnerable to soil management than micro-aggregates (Tisdall and Oades, 1982). Accordingly, Six et al. (2000b) showed that macro-aggregates disintegrated more readily upon disturbance than micro-aggregates, particularly in soils with increasing cultivation intensity. By that, macro-aggregate occluded OC becomes available to microbial decomposition, hence, this fraction is supposed to play an important role for the decline of soil OC in intensively managed steppe soils (Cambardella and Elliott, 1993, 1994; Elliott, 1986). Furthermore, previous work indicated decreasing amounts of labile OM and OC mineralization rates with increasing duration and intensity of agricultural management (Cambardella and Elliot, 1992; Grandy and Robertson, 2007; Hurisso et al., 2014).

One way to quantify the proportion of macro-aggregate protected soil OC is to compare mineralization rates from intact and crushed macro-aggregates. Previous studies found an increase of soil OC mineralization after macro-aggregate crushing (Beare et al., 1994; Bossuyt et al., 2002; Elliott, 1986; Gupta and Germida, 1988; Pulleman and Marinissen, 2004), though not all studies revealed consistent results (Garcia-Oliva et al., 2004; Goebel et al., 2009; Plante et al., 2009; Tian et

Formatiert: Schriftartfarbe: Rot

al., 2015). Moreover, OC mineralization after macro-aggregate crushing differed also with respect to land-use. Pulleman and Marinissen (2004) found larger mineralization after crushing of macro-aggregates in croplands than in grasslands and ascribed this to the physicogenic nature of macro-aggregates in arable soils, which have smaller pore sizes than biogenic macro-aggregates in grasslands, and therefore larger protection capacity. Also Elliott (1986) observed the increase of OC mineralization with macro-aggregate crushing to be more pronounced in arable than in grassland soils, while Gupta and Germida (1988) observed the opposite effect. A shortcoming of previous studies is the short incubation period of only few weeks under largely ~~in~~ non-equilibrium conditions~~mineralization rates~~, which results in an unreliable ~~makes an accurate~~ assessment of the size of the macro-aggregate protected OC fraction and its turnover time. This fact, therefore, asks for long-term incubation experiments to address the vulnerability of macro-aggregate protected OC.

The majority of research on OC protection in aggregates of steppe soils focused on prairie soils of the Great Plains, while little is known for Siberian steppe soils. This is surprising as the ~~semi-arid~~ steppe ecosystems in Siberia belong to the greatest agricultural production areas in the world with an area greater than that of the Great Plains and cover some of the most intensively managed soils globally (Frühauf, 2011). In the West Siberian Plain 420,000 km² natural steppe was converted into cropland between 1954 and 1963 in the frame of the so-called "Virgin Lands Campaign" (Russian: *Zelina*). Conversion from grassland to cropland reduced soil OC stocks by about 31% in 0-25 cm, of which most occurred within the first years after land conversion and was associated with a decline in aggregate stability (Bischoff et al., 2016). This indicated an interrelation between aggregate stability and OC storage also in these soils. Moreover, Bischoff et al. (2016) found about 10% of OC in the studied soils was existent in particulate OM of which some is probably occluded within aggregates. In the present study we aimed to quantify the proportion of macro-aggregate protected OC under different land-use ~~and~~ as function of land-use ~~duration and~~ intensity and time since land-use change (LUC) from pasture to arable land in Siberian steppe soils. This was done by comparing OC mineralization rates of intact (250–2000 µm) and crushed (<250 µm) macro-aggregates in long-term incubations over 401 days along two agricultural chronosequences of the south-western Siberian Kulunda steppe. In addition, bulk soil samples (<2000 µm) were incubated to determine the effect of ~~land-use change (~~LUC~~)~~ from pasture to arable land on a fast and a slow soil OC pool (labile *vs.* more stable OC), as derived from fitting exponential decay models to incubation data. We hypothesized that (i) crushing of macro-aggregates leads to increased OC mineralization due to an increasing microbial accessibility of a previously occluded labile macro-aggregate OC fraction, and (ii) bulk soil OC mineralization rates and the size of the fast soil OC pool are higher in pasture than in arable soils with decreasing bulk soil OC mineralization rates and size of the fast OC pool as land-use ~~duration and~~ intensity and time since LUC increase. In this study, we refer to fractions as physically separated soil OC components (macro-aggregate occluded soil OC), while pools refer to mathematically derived OC constituents from fitting exponential decay models to incubation data (fast and slow soil OC pool).

Material & Methods

Study sites and soil sampling

The Kulunda steppe is part of the Russian Federation (Altayskiy Kray) and located within the ~~semi-arid~~ steppes of south-western Siberia. We selected two sites in two different steppe types ~~under~~ which were characterized by different climate ~~with soils of different textures~~and distinct soil texture ~~-~~(Fig. 1). Within a site the texture of the soils was comparable. The first site is located in the forest steppe (FS) near Pankrushikha (53°44'19.53"N, 80°41'2.88"E) with a mean annual precipitation (MAP) of 368 mm and a mean annual temperature (MAT) of 1.1°C (Table 1). The second site is situated near Sidorovka (52°30'1.43"N, 80°44'41.68"E) and part of the ~~more arid~~ typical steppe (TS) with a MAP of 339 mm and a MAT of 2.0°C and which is more arid than FS (climate data from "WorldClim" data base; Hijmans *et al.*, 2005). We identified the two sites by interviewing farmers and land owners about land-use history and management. The plots within a site were checked for comparable pedological conditions by inspecting the soils with a hand-auger. Soil sampling took place in 2013. At each site we identified a land-use chronosequence with four plots. At FS, we also included two plots with varying land-use intensity (extensive pasture *vs.* arable land with forage crops). The FS site comprised an extensive pasture (vegetation: *Festuca valesiaca - Fillipendula vulgaris - Bromopsis inermis*), an arable land with ~~with~~ forage crops and arable land after five and ~~ten~~ thirty years of cultivation (arable 5 yr, arable ~~10~~30 yr). Crop rotations on the arable 5 yr and arable ~~10~~ 30 yr included summer wheat, summer barley and peas. The soils were classified as Protocalcic Chernozems (Siltic) according to IUSS Working Group WRB (2014). The TS site consisted of four plots which were all cultivated since the 1950s (*Zelina*) but left as fallow since 1983 because of low agricultural productivity. After 1983 all plots were used extensively as pasture but three of the four plots were recultivated at different points in time, allowing for a chronosequence with a 30-year old fallow (meanwhile used as pasture) and plots with one, three, and ten years arable land-use (arable 1 yr, arable 3 yr , arable 10 yr). The 30-year old fallow (pasture) is characterized by *Agropyron pectinatum*, *Bromopsis inermis* and *Artemisia glauca*. The absence of some typical steppe species like *Stipa sp.* or *Festuca sp.* and the sparse vegetation cover (Fig. 1) pointed to the fact that the vegetation of this plot was degraded from grazing. The site was located on a small hillslope with <2° inclination, where the fallow 30 yr was located at the highest point and the arable 10 yr at the base level. Though the inclination was very small, we measured larger soil OC contents in the arable 10 yr plot than in the upslope arable 1 yr and arable 3 yr plots, which we attributed to erosion. Nevertheless, we decided to include this site in our study, as chronosequences are very sparse in the study area and the possible effect of macro-aggregate crushing on soil OC mineralization, if existent, will be also evident on slightly eroded plots. Soils at the TS site were classified as Protocalcic Kastanozem (Loamic). At both sites ~~one~~ characteristic key profiles w~~as~~ere identified ~~by scanning the soil with a hand-auger down to 1m depth. Key profiles were~~and established from 0-150 cm on the pasture plots for soil description and sampled in ~~generic~~ horizons. As the arable 5 yr and arable 30 yr at the FS site were >500 m distant from the other two plots, we additionally established a key profile on each of these two plots. All other plots were located directly adjacent to the pasture plot of the respective site, and checking the plots with a hand-auger showed that key profiles from pasture plots were also representative for these plots. Hence no additional key profiles were established on these plots. Key profile samples were analyzed for pH, soil texture, and electrical conductivity (EC). For those plots where no key profile was excavated a comparable soil texture between plots within a site was verified by hand analysis. Further, on all plots three additional soil samples (field replicates) were randomly collected in 0-10 cm depth for determination of soil OC and total nitrogen (TN) content and for use in the soil incubation experiment. Geographical coordinates of all plots are summarized in Table S1.

Sample preparation and basic soil analyses

Soil samples were air-dried and sieved to <2 mm. Big clods were gently broken apart to pass the 2 mm sieve and all visible plant residues were removed. A subsample was dried at 105°C for 24 h to determine the residual soil water content which was subtracted from air-dry samples for calculation of elemental contents. Another subsample was homogenized with a ball mill (Retsch MM200, Haan, Germany) and measured for OC and TN via dry combustion with an Elementar vario MICRO cube C/N Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Traces of inorganic carbon ($CaCO_3$-content <0.1%) were previously removed by HCl fumigation (Walthert et al., 2010). Organic C and TN measurements were done prior the incubation experiment. Soil pH was measured at a 1:2.5 (w:v) soil-to-water$_{deion}$ ratio after leaving the suspensions for one day to reach equilibrium, and soil EC was measured at a soil-to-water$_{deion}$ ratio of 1:5 (w:v). The texture of the soils was determined according to the standard sieve-pipette method (DIN ISO 11277, 2002).

Aggregate crushing and incubation of soil samples

Each of the samples from 0-10 cm was divided into three fractions: (i) bulk soil (<2000 µm), (ii) intact macro-aggregates (250–2000 µm), and (iii) crushed macro-aggregates (<250 µm). Intact macro-aggregates were isolated by gently sieving the air-dry bulk soil through a 250-µm sieve and using the fraction remaining on the sieve. A subsample from the intact macro-aggregates was crushed in a mortar and sieved again through the 250-µm sieve to obtain the fraction of crushed macro-aggregates (<250 µm). We decided to use dry-sieved aggregates for soil incubation as wet-sieving releases soluble OM, which is bioavailable and thus a critical fraction for soil OC mineralization (Sainju, 2006). Further, microbial activity is less affected by dry-sieving than by wet-sieving (Sainju, 2006). All samples of the three fractions were divided into three analytical replicates, giving a total of 216 samples for soil incubations (8 plots x 3 field replicates x 3 fractions x 3 analytical replicates).

To determine whether the crushed macro-aggregates consisted of intact micro-aggregates or free primary particles, a subsample of crushed macro-aggregates was sieved through a 63-µm sieve and obtained fractions were quantified by mass balance calculations and subsequently imaged by a JEOL JSM-6390A scanning electron microscope (JEOL Ltd., Tokyo, Japan). Our analysis revealed that 62.1 ± 3.2% of crushed macro-aggregates still existed as large micro-aggregates (>63 µm), while 37.9 ± 3.2% were found in the fraction <63 µm, which mainly consisted of small micro-aggregates and only few free primary particles (Fig. S1).

Soil laboratory incubations were carried out under aerobic conditions in the dark, at constant temperature of 20°C and 60% of maximum water holding capacity (WHC), which was determined according to Schlichting et al. (1995). An amount of 7.5 g soil sample was mixed with 12.5 g combusted (1000°C for 24 h) quartz powder (Roth, Karlsruhe, Germany; >99% pulverized, <125 µm) and filled into 120-ml glass jars. Quartz powder was used to increase the sample volume and prevent the formation of aggregates in the crushed samples. Three jars were solely filled with quartz and used as control. Soil moisture was regulated during the experiment by periodically weighing the glass jars and adding ultrapure water. All samples were pre-incubated for 14 days and respiration measurements were subsequently taken at days 1, 3, 8, 14, 21, 28, 57, 98, 127, 196, 268, and 401 by sampling the headspace of each jar using a syringe through a septum, which was installed in the jar lids prior to sampling. Gas samples were analyzed for CO_2 concentrations with a Shimadzu GC-2014 modified after Loftfield et al. (1997).

Determination of microbial biomass

After the laboratory incubations all incubated samples were analyzed for microbial biomass C using the chloroform-fumigation-extraction method (Vance et al., 1987). Briefly, 6 g soil were kept at 60% WHC and weighed in duplicate into glass jars. One sample was fumigated with ethanol-free $CHCl_3$ during 24 h while the other sample was left unfumigated. Both, fumigated and unfumigated samples, were extracted with 0.5 M K_2SO_4 at a soil-to-solution ratio of 1:10 (w:v), shaken for 30 min, and subsequently centrifuged at 2700 g. The extracts were filtered (Whatman filter paper, ashless, Grade 42) and measured for non-purgeable organic carbon (NPOC) by a LiquiTOC (Elementar Analysensysteme GmbH, Hanau, Germany). Microbial biomass C was calculated as the difference between fumigated and unfumigated soil samples and expressed as mg C g OC^{-1}.

Calculations and statistical analyses

All data analyses were carried out in R 3.1.2 (R Core Team, 2015). To calculate cumulative respiration rates, data of CO_2 measurements per day was interpolated by spline interpolation for each sample (i.e. analytical replicate) separately. Cumulative respiration rates were analyzed by fitting three different exponential-decay models to the data and choosing the model with the best fit by AIC selection (Akaike Information Criterion). The first model was a first-order exponential decay model with one pool (one-pool model; Eq. 1):

$$C_{remain} = C_1 \times e^{(-k_1 \times t)} \quad \text{(Eq. 1)}$$

The second model consisted of two pools (two-pool model; Eq. 2):

$$C_{remain} = C_1 \times e^{(-k_1 \times t)} + C_2 \times e^{(-k_2 \times t)} \quad \text{(Eq. 2)}$$

The third model was an asymptotic first-order exponential decay model with two pools (asymptotic two-pool model; Eq. 3):

$$C_{remain} = C_2 + C_1 \times e^{(-k_1 \times t)} \quad \text{(Eq. 3)}$$

where C_{remain} is the amount of OC remaining in the sample, C_1 and C_2 are the sizes of the fast and the slow pool, respectively, k_1 and k_2 the rate constant of the fast and the slow pool, respectively, and t the time. For the majority of samples the two-pool model showed the best fit. Only for the pasture plot at FS the incubation time was too short to calculate the rate constant k for the slow pool, thus the asymptotic two-pool model fitted the data best. The mean residence time (MRT) was calculated as $1/k$. The modelled parameters were used in linear mixed effects models (package lme4; Bates et al., 2012) to test for significant differences between soil fractions within plots, accounting for the nested structure of sampling by using the field replicates within each plot as random effects. Moreover, random slopes were included by allowing field replicates within each plot to have random slopes for the effect of soil fraction. Based on the linear mixed model fit, we tested whether differences of the dependent variable between soil fractions within plots were significant, including corrections for multiple comparisons (analogous to the Tukey test) with Satterthwaite degrees of freedom, using the R packages lsmeans (Lenth and Herve, 2015), lmerTest (Kuznetsova et al., 2015) and multcomp (Hothorn et al., 2008). Model assumptions were checked using residuals *vs.* fitted plots and Q-Q-plots for the residual errors and random effect estimates. The proportion of the macro-aggregate protected OC fraction to the total macro-aggregate OC content was calculated by Eq. 4:

$$C_{macro,total\ aggrC} = C_{min,crushed} - \frac{1}{n} \sum_{i=1}^{n} C_{min,intact} \quad \text{(Eq. 4)}$$

where $C_{macro,total\ aggrC}$ is the proportion of macro-aggregate protected OC to the total macro-aggregate OC (%), $C_{min,crushed}$ is the proportion of OC mineralized in the crushed macro-aggregates (%) and $C_{min,intact}$ is the proportion of OC mineralized in the intact macro-aggregates (%), while n is the number of analytical replicates per field replicate for the treatment of intact macro-aggregates and i is the ith analytical replicate per field replicate. The proportion of the macro-aggregate protected OC fraction to the total mineralized OC as function of time was calculated by Eq. 5:

$$C_{macro,mineralizableC}(t) = \frac{C_{min,crushed}\,(t) - \frac{1}{n} \sum_{i=1}^{n} C_{min,intact}\,(t)}{C_{min,crushed}\,(t)} \times 100 \quad \text{(Eq. 5)}$$

where $C_{macro,mineralizableC}$ (t) is the proportion (%) of macro-aggregate protected OC to the total mineralized OC at time t (days). Graphs were generated using ggplot2 (Wickham, 2009). Boxplots show the median, the first and the third quartile and the whiskers extend from the box to the highest or lowest value, respectively, that is within 1.5 × inter-quartile range. Individual measurements are plotted as points.

Results

Basic soil characteristics and~~Soil~~ organic carbon contents along the chronosequences

The soil pH of A horizons was characteristic for Chernozems and Kastanozems and ranged between 7.0 and 7.6, while EC was low and did not exceed 120 µS cm^{-1} at both sites (Table 1). In FS, soil OC contents decreased as a result of LUC from pasture to arable land from 55 ± 5 mg g^{-1} under extensive pasture to 39 ± 1 mg g^{-1} and 40 ± 2 mg g^{-1} under arable 5 yr and arable 30 yr, respectively (Table 1). Thus, increasing duration of agricultural land-use caused no further decrease of soil OC contents in arable soils. C : N ratios were around 12 and slightly higher for non-arable than for arable soils. Soil OC contents in TS were smaller than in FS and did not follow the gradient over time since cultivation, as the site was affected by erosion (Sect. ~~2.1~~*Study sites and soil sampling*). In TS, soil C : N ratios were around 10–11 and ~~did not vary considerably between the plots~~slightly larger for the fallow 30 yr (pasture) than for the arable soils.

Effect of macro-aggregate crushing on the mineralization of soil organic carbon

Mass balance calculations revealed~~,~~ that in both steppe types about 70% of OC was associated with macro-aggregates, indicating the importance of macro-aggregates for the OC dynamics in these soils. Organic C and TN contents did not vary considerably between intact and crushed macro-aggregates (Table S~~2~~1). As is typically for soil incubations, respiration rates were higher at the beginning and decreased with increasing incubation time (Fig. S2). The amount of OC remaining in the sample during incubation was described by either two-pool or asymptotic two-pool models (Fig. 2). The variability ~~within one plot~~ of the percentage ~~of~~ residual OC within one plot ~~remaining in the samples~~ . ~~The variability of the amount of OC remaining in the samples within one plot~~ decreased with increasing time since cultivation (Fig. 2). ~~Thus,~~This means that soil samples belonging to the plots with the longest cultivation history were more similar to each other than samples from plots in more pristine state. The amount of soil OC mineralized was slightly larger in the bulk soil fraction (<2000 µm) than in the intact and crushed macro-aggregates in ~~most of the studied plots~~all plots along the two chronosequences, though significant differences were only observed in soils of FS ($p<0.05$, Fig. 3).

There was no significant difference in soil OC mineralization between intact and crushed macro-aggregates after 401 days of incubation in all plots under study (Fig. ~~2 and~~ 3). The fraction of macro-aggregate protected OC was practically not existent and accounted for <1% of the total macro-aggregate OC content in all plots (data not shown). Furthermore, macro-aggregate crushing did not increase the size of the fast soil OC pool, which was determined by fitting exponential decay models to the incubation data (Fig. 4). Also the MRT of the fast and the slow OC pool was unaffected by macro-aggregate crushing (Table 2). However, we could determine a small contribution of the macro-aggregate protected OC fraction to the total OC mineralization during the beginning of the incubation in seven out of eight plots, where macro-aggregate protected OC contributed to about 10% to the total mineralized OC d^{-1} (Fig. 5). Cumulated over the entire incubation period, the contribution of the macro-aggregate protected OC fraction to the total OC mineralization was not existent or very small and amounted between zero and 8 ± 4% in seven out of eight plots with no clear trend with respect to the ~~land-use duration~~time since LUC (Table 3). The arable 3 yr plot in TS had clearly negative values of macro-aggregate protected OC, which resulted from a lower OC mineralization in crushed than in intact macro-aggregates. For most plots, the negligible fraction of macro-aggregate protected OC was depleted between 100 and 400 days, while the arable 30 yr in FS and the fallow 30 yr (pasture) in TS showed a constant but small (ca. 5%) mineralization rate of macro-aggregate protected OC during the complete incubation period (Fig. 5).

Soil organic carbon mineralization along the chronosequences

The bulk soil OC mineralization declined after LUC from pasture to arable land in both steppe types, but only in TS we observed also a trend of decreasing soil OC mineralization with increasing ~~duration of land-use~~time since LUC (Fig. 3). Likewise, the proportion of the fast soil OC pool decreased as a result of LUC, but it was unaffected by the ~~duration~~ intensity and time since ~~or~~establishment of ~~intensity of~~ arable land-use (Fig. 4). The MRT of the fast OC pool became shorter in the course of LUC in both steppe types, but only in FS we observed also a trend towards shorter MRTs with increasing land-use intensity and ~~duration of land-use~~time since LUC (Table 2). With respect to the slow soil OC pool, only in FS we detected shorter MRTs due to conversion of pasture to arable land and with increasing land-use intensity and ~~duration of land-use~~time since conversion to arable land-use, while no trend was apparent along the chronosequence in TS (Table 2). In general, the amount of soil OC mineralized was slightly larger in TS than in FS, while the differences were most pronounced between the pasture plots (Fig. 3). Remarkable was the pasture in TS, which had clearly the largest OC mineralization and proportion of the fast OC pool but, at the same time, also the highest variability (Fig. 3 and 4).

Microbial biomass carbon

The share of microbial biomass C in the total OC was similar in both steppe types and ranged between 1.5 and 4.0 mg C g^{-1} OC, as indicated by the first and third quartile of the boxplots (Fig. 6). Crushing of macro-aggregates caused a small decrease of microbial biomass C, which was significant when considering all plots ($p<0.05$), while bulk soil samples and intact macro-aggregates had similar amounts of microbial biomass C. There was no correlation between the amount of OC mineralized and the share of microbial biomass C in total OC (Fig. S3). Moreover, the quantity of OC mineralization was not related to the amount of microbial biomass C per gram soil (Fig. S4).

Discussion

Limited protection of macro-aggregate occluded organic carbon

Previous studies showed higher OC mineralization following macro-aggregate crushing (e.g. Beare et al., 1994; Bossuyt et al., 2002; Pulleman and Marinissen, 2004), while some studies showed no such effect (Garcia-Oliva et al., 2004; Goebel et al., 2009; Plante et al., 2009). In our study, the macro-aggregate occluded OC fraction contributed only marginally to the OC mineralization during the entire incubation (Fig. 5, Table 3). Against our hypothesis, macro-aggregate occluded OC is not protected against decomposition in the studied soils. In turn, the break-down of macro-aggregates due to soil tillage and the subsequent release of soil OC is not the reason for a decrease of soil OC contents due to soil management, as observed along the chronosequence in FS (Table 1). Plante et al. (2009) suspected that the disruption treatments used in their experiments (crushing of 2–4 mm aggregates to <0.5 mm) was insufficient to release large amounts of physically protected OC for decomposition, and that a considerable amount of OC was stabilized in micro-aggregates. Also Balesdent et al. (2000) provided some evidence that the proportion of physically protected OC is larger in micro-aggregates than in macro-aggregates. In our study, the majority of crushed macro-aggregates (62 ± 3%) consisted of micro-aggregates with 63–250 µm size (See *Material & Methods*), and as the OC mineralization of the crushed aggregate fraction was not enhanced, we suggest that most of the OC was stabilized in the micro-aggregates. However, micro-aggregates are less sensitive to soil tillage (Tisdall and Oades, 1982), therefore, in light of LUC-induced OC losses, the soil OC in macro-aggregates is generally considered to be more vulnerable for destabilization than OC in micro-aggregates. This could not be confirmed for the soils under study. Nevertheless, we cannot rule out that an increased macro-aggregate turnover due to agricultural management leads to a reduced formation of micro-aggregates within macro-aggregates and, as a result, to lower OC contents in arable as compared to pasture soils (Six et al., 2000a).

Formatiert: Schriftart: Kursiv

Only few studies determined the share of the macro-aggregate protected OC fraction in the total OC mineralization or the total macro-aggregate OC, respectively. Beare et al. (1994) showed that macro-aggregate protected OC accounted for about 1% of total aggregate OC and to about 8–23% of total mineralizable OC during 20 days of incubation. They detected a smaller macro-aggregate protected OC mineralization in more intensively managed soils. In our study, <1% of total macro-aggregate OC was stored as macro-aggregate protected OC, while this fraction accounted for max. 8 ± 4% of total OC mineralization (Table 3). Thus, our values are in the same order of magnitude as observed by Beare et al. (1994), who suggested that an increased macro-aggregate turnover in tilled soils is one reason for the small macro-aggregate protected OC fraction. According to Beare et al. (1994), the physically protected but relatively labile macro-aggregate occluded OC is released for microbial decomposition due to the frequent tillage-induced macro-aggregate break-down. As a result, macro-aggregates contain only little or no labile OC. This can be a reason in arable soils, but is unlikely in pasture soils where the macro-aggregate turnover is slower due to the absence of tillage (Six et al., 2002). In the untilled soils, therefore, other factors are probably responsible for the absence of labile macro-aggregate protected OC.

The mineralization of OC is driven by microorganisms and, thus, can be affected by disturbances of their physical environment (Schimel and Schaeffer, 2012). Garcia-Oliva et al. (2004) observed lower OC mineralization in crushed than in intact macro-aggregates and attributed this finding to a reduced microbial activity in crushed samples, what they explained by a disturbed soil environment with possibly anaerobic conditions. Balesdent et al. (2000) reviewed the effect of aggregate-crushing on the mineralization of soil OM and indicated a reduced microbial biomass in crushed aggregates as a possible reason for similar OC mineralization rates in intact and crushed aggregates. In our study, crushed macro-aggregates contained slightly but significantly less microbial biomass C than intact macro-aggregates (Fig. 6). This may have contributed to the missing effect of aggregate crushing on OC mineralization.

Besides stabilization of OC by physical occlusion within aggregates, formation of mineral-organic associations can be an important mechanism for OC stabilization (von Lützow et al., 2006). Bischoff et al. (2016) showed that a large OC fraction (>90% of total OC) is associated with mineral surfaces in soils of the Kulunda steppe, which is much more than generally observed in steppe soils (Kalinina et al., 2011; Plante et al., 2010). In our study, about 38 ± 3% of the crushed macro-aggregate fraction were particles <63 µm, in which the proportion of particulate OC is usually very low (Christensen, 2001). Based on the similar OC mineralization rates of intact and crushed macro-aggregates, this suggests that a considerable OC proportion is stabilized by mineral surfaces. As a result, OC in crushed aggregates became not available to microorganisms and thus did not enhance soil OC mineralization.

Summing up, our results suggest 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 LUC in the studied 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. Elliott (1986) and Cambardella and Elliott (1993, 1994) found that macro-aggregate occluded OC was rapidly lost after conversion of grassland to cropland due to the break-down of macro-aggregates and concluded that this fraction is protected from decomposition. Though, more recent research (e.g. Six et al., 2000) indicated that micro-aggregates which are formed within existing macro-aggregates are decisive for OC stabilization in agroecosystems, it is still widely accepted that the decomposition of previously occluded macro-aggregate OC is another key factor controlling the decline of OC after grassland to cropland conversion. Our results imply, that this is not the case in the Siberian steppe soils. A possible explanation for the observed differences are smaller soil OC inputs by crop residues and rhizodeposits in the Siberian soils, resulting in smaller proportions of particulate OC (Castellano et al., 2015) and, thus, less possibilities for the formation of macro-aggregate occluded OC.

Effect of land management and soil characteristics on the mineralization potential of soil organic carbon

As shown in previous studies the conversion from grassland to arable land caused a decrease of labile soil OC (Plante et al., 2011; Poeplau and Don, 2013), which corresponds to OC with fast turnover rates. In line with this, we found a larger fast OC pool under pasture than under arable land, while the proportion of the fast OC pool was unaffected by the intensity ~~and duration~~ of agricultural use and the time since land-use conversion (Fig. 4). This means, that the fast OC pool is highly vulnerable to LUC as the majority of this pool was rapidly lost within 1–5 yrs after grassland to cropland conversion. At the same time higher intensity ~~or duration~~ of agricultural land-use and longer time since land-use conversion tended to shorten MRTs in the fast and slow OC pool of the soils in FS (Table 2), thus reducing the potential to sequester soil OC. This is in line with Beare et al. (1994) and Grandy and Robertson (2007) who reported that the MRT of soil OC pools from laboratory incubations were shorter under high than under low land-use intensity. Beare et al. (1994) argued that the frequent soil disturbance in tilled soils impedes a strong association of OC with mineral surfaces, which in turn leads to a low protection of OC against microbial decomposition and thus fast turnover rates. Moreover, McLauchlan (2006) showed that the MRT of the fast OC pool was shorter in arable soils than in soils which were left as fallow, which is supported by our results. We should consider that our observations were derived from long-term laboratory incubations and that we expect the difference of MRTs between arable and pasture soils to be even more pronounced under field conditions, as soil tillage generally accelerates the turnover of soil OC. Moreover, soil OC inputs by plant residues are probably reduced in arable soils. This, together with faster soil OC turnover times would lead to a decrease of total soil OC as a result of agricultural land management.

We observed differences in the amount of mineralized soil OC between the two sites. Mineralization rates were smaller in the clayey soils of FS than in the soils of TS with larger sand content. Many studies showed smaller OC mineralization rates in clayey soils as compared to sandy soils, as OC is stabilized by clay-sized minerals and thus protected against decomposition by microorganisms (Franzluebbers, 1999; Franzluebbers and Arshad, 1997; Harrison-Kirk et al., 2013). Moreover, Bischoff et al. (2016) showed that the proportion of labile particulate OC tended to increase with aridity in the soils under study. This means, that soils in TS would have larger amounts of bioavailable and easily decomposable particulate OC than soils in FS, which in turn could leads to increased OC mineralization in the soils of TS. The differences between both sites with respect to their soil OC mineralization rates could therefore be attributed to a different contribution of mineral-organic associations, with less mineral-bound OC in TS as compared to FS. Another explanation for the larger OC mineralization rates in TS could be the slightly smaller C : N ratios in these soils, indicating a larger N availability. This is in line with the observation that increased N availability during laboratory incubation enhancesd OC mineralization rates in a laboratory incubation(Bossuyt et al., 2001).

Interestingly, we found a smaller variation of the percentage of OC mineralized (i.e. OC mineralization rates) between samples from plots with long land-use duration of arable land-use (Fig. 2). This is possibly due to the fact that tillage homogenizes the soil within plough depth and consequently minimizes the heterogeneity of soil OC at the field scale. This idea is supported by Schrumpf et al. (2011), who showed that soil OC contents are less variable under cropland as compared to grassland. We, therefore, conclude that continuous agricultural management obliterates differences of soil OC properties across a field.

Conclusion

This study set out to determine the quantity of macro-aggregate protected OC in Siberian steppe soils under different land-use and as function of land-use duration and intensity and time since LUC from pasture to arable land. This was done by crushing of dry-sieved macro-aggregates (250–2000 µm) to <250 µm and subsequent incubation of crushed and intact macro-aggregates at 20°C and 60% WHC during 401 days along two agricultural chronosequences of the Kulunda steppe. The effect of macro-aggregate crushing on OC mineralization was negligible along the two chronosequences. Macro-aggregate protected OC accounted for <1% of the total macro-aggregate OC content and for maximally 8 ± 4% of total mineralized OC. The majority of macro-aggregate protected OC was mineralized during the beginning of the incubation, showing that this represents a labile fraction with fast turnover rates. Our results imply that the tillage-induced break-down of macro-aggregates has not reduced the OC contents in the studied soils. In contrast, our data suggest that mainly OC occluded within micro-aggregates and/or associated with mineral-surfaces is decisive for OC stabilization in these soils. Long-term incubations of bulk soil samples revealed that LUC from pasture to arable land but also the cultivation with forage crops caused a rapid decrease of a fast soil OC pool within 1–5 yrs of agricultural management. At the same time the MRT tended to become shorter in the fast and slow OC pool with increasing land-use duration and intensity time since LUC at one of the investigated sites. This suggests that the potential of the soils to sequester OC is reduced under agricultural management, as OC which enters the soil from above- or belowground is released to the atmosphere within few decades. The difference of turnover times between arable and pasture soils is probably even more pronounced under field conditions, as soil tillage leads to a frequent disturbance of the soil environment which additionally accelerates soil OC mineralization. Thus, we conclude that the decrease of soil OC contents in the course of LUC is attributed to faster soil OC turnover under arable land as compared to pasture at a reduced plant residue input but not to the tillage-induced release of macro-aggregate occluded soil OC.

Acknowledgements

Financial support was provided by the German Federal Ministry of Education and Research (BMBF) in the framework of the KULUNDA project (01 LL 0905). O. Shibistova and G. Guggenberger appreciate funding from the Russian Ministry of Education and Science (No.14.B25.31.0031). We thank the entire KULUNDA team for great collaboration and good team spirit. We are thankful to all farmers of the Kulunda steppe for collaboration during sampling and Lukas Gerhard for indispensable assistance in the field. Thanks for laboratory assistance to Silke Bokeloh, Elke Eichmann-Prusch, Roger-Michael Klatt, Pieter Wiese and Fabian Kalks, while Leopold Sauheitl is appreciated for guidance in the laboratory. Andrea Hartmann is acknowledged for helpful support on the scanning electron microscope. Thanks to Norman Gentsch for valuable scientific discussions on the manuscript, while we acknowledge Frank Schaarschmidt for statistical support. We thank antwo anonymous reviewers for valuable suggestions on the manuscript. The publication of this article was funded by the Open Access Ffund of Leibniz Universität Hannover.

References

Balesdent, J., Chenu, C. and Balabane, M.: Relationship of soil organic matter dynamics to physical protection and tillage, Soil Tillage Res., 53(3–4), 215–230, doi:10.1016/S0167-1987(99)00107-5, 2000.

Bates, D., Mächler, M. and Bolker, B.: Fitting linear mixed-effects models using lme4, J. Stat. Softw., 1–51 [online] Available from: http://arxiv.org/abs/1406.5823%5Cnhttp://listengine.tuxfamily.org/lists.tuxfamily.org/eigen/2011/06/pdfKU_S0z6LjT.pdf, 2012.

Beare, M. H., Hendrix, P. F., Cabrera, M. L. and Coleman, D. C.: Aggregate-protected and unprotected organic matter pools in conventional- and no-tillage soils, Soil Sci. Soc. Am. J., 58(3), 787, doi:10.2136/sssaj1994.03615995005800030021x, 1994.

Beniston, J. W., DuPont, S. T., Glover, J. D., Lal, R. and Dungait, J. A. J.: Soil organic carbon dynamics 75 years after land-use change in perennial grassland and annual wheat agricultural systems, Biogeochemistry, 120(1–3), 37–49, doi:10.1007/s10533-014-9980-3, 2014.

Bischoff, N., Mikutta, R., Shibistova, O., Puzanov, A., Reichert, E., Silanteva, M., Grebennikova, A., Schaarschmidt, F., Heinicke, S. and Guggenberger, G.: Land-use change under different climatic conditions: Consequences for organic matter and microbial communities in Siberian steppe soils, Agric. Ecosyst. Environ., 235, 253–264, doi:10.1016/j.agee.2016.10.022, 2016.

Bossuyt, H., Denef, K., Six, J., Frey, S. D., Merckx, R. and Paustian, K.: Influence of microbial populations and residue quality on aggregate stability, Appl. Soil Ecol., 16(3), 195–208, doi:10.1016/S0929-1393(00)00116-5, 2001.

Bossuyt, H., Six, J. and Hendrix, P. F.: Aggregate-protected carbon in no-tillage and conventional tillage agroecosystems using carbon-14 labeled plant residue, Soil Sci. Soc. Am. J., 66(6), 1965, doi:10.2136/sssaj2002.1965, 2002.

Cambardella, C. A. and Elliot, E. T.: Particulate soil organic-matter changes across a grassland cultivation sequence, Soil Sci. Soc. Am. J., 56, 777–783, doi:10.2136/sssaj1992.03615995005600030017x, 1992.

Cambardella, C. A. and Elliott, E. T.: Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils, Soil Sci. Soc. Am. J., 57, 1071–1076, doi:10.2136/sssaj1993.03615995005700040032x, 1993.

Cambardella, C. A. and Elliott, E. T.: Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils, Soil Sci. Soc. Am. J., 58, 123–130, doi:10.2136/sssaj1994.03615995005800010017x, 1994.

Castellano, M. J., Mueller, K. E., Olk, D. C., Sawyer, J. E. and Six, J.: Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept, Glob. Chang. Biol., 21(9), 3200–3209, doi:10.1111/gcb.12982, 2015.

Christensen, B. T.: Physical fractionation of soil and structural and functional complexity in organic matter turnover, Eur. J.

Soil Sci., 52(3), 345–353, doi:10.1046/j.1365-2389.2001.00417.x, 2001.

DIN ISO, 11277: Soil quality – determination of particle size distribution in mineral soil material – method by sieving and sedimentation, 2002.

Elliott, E. T.: Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils, Soil Sci. Soc. Am. J., 50, 627–633, doi:10.2136/sssaj1986.03615995005000030017x, 1986.

FAO: Lecture notes on the major soils of the world, edited by P. Driessen, J. Deckers, O. Spaargaren, and F. Nachtergaele, World soil Resour. reports, 94, 336, doi:10.1136/gut.27.11.1400-b, 2001.

FAO: FAO Statistical yearbook 2013 - World food and agriculture, Rome., 2013.

Franzluebbers, A. J.: Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture, Soil Biol. Biochem., 31, 1083–1090, doi:10.1016/S0038-0717(99)00022-X, 1999.

Franzluebbers, A. J. and Arshad, M. A.: Soil microbial biomass and mineralizable carbon of water-stable aggregates, Soil Sci. Soc. Am. J., 61(4), 1090–1097, doi:10.2136/sssaj1997.03615995006100040015x, 1997.

Frühauf, M.: Landnutzungs- und Ökosystementwicklung in den südsibirischen Agrarsteppen, Geogr. Rundschau, 1, 46–53, 2011.

Garcia-Oliva, F., Oliva, M. and Sveshtarova, B.: Effect of soil macroaggregates crushing on C mineralization in a tropical deciduous forest ecosystem, Plant Soil, 259(1–2), 297–305, doi:10.1023/B:PLSO.0000020978.38282.dc, 2004.

Goebel, M.-O., Woche, S. K. and Bachmann, J.: Do soil aggregates really protect encapsulated organic matter against microbial decomposition?, Biologia (Bratisl)., 64(3), 443–448, doi:10.2478/s11756-009-0065-z, 2009.

Grandy, A. S. and Robertson, G. P.: Land-use intensity effects on soil organic carbon accumulation rates and mechanisms, Ecosystems, 10(1), 58–73, doi:10.1007/s10021-006-9010-y, 2007.

Gupta, V. V. S. R. and Germida, J. J.: Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation, Soil Biol. Biochem., 20(6), 777–786, doi:10.1016/0038-0717(88)90082-X, 1988.

Harrison-Kirk, T., Beare, M. H., Meenken, E. D. and Condron, L. M.: Soil organic matter and texture affect responses to dry/wet cycles: Effects on carbon dioxide and nitrous oxide emissions, Soil Biol. Biochem., 57, 43–55, doi:10.1016/j.soilbio.2012.10.008, 2013.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. and Jarvis, A.: Very high resolution interpolated climate surfaces for global land areas, Int. J. Climatol., 25(15), 1965–1978, doi:10.1002/joc.1276, 2005.

Hothorn, T., Bretz, F. and Westfall, P.: Simultaneous inference in general parametric models, Biometrical J., 50(3), 346–363, doi:10.1002/bimj.200810425, 2008.

Hurisso, T. T., Norton, J. B. and Norton, U.: Labile soil organic carbon and nitrogen within a gradient of dryland agricultural land-use intensity in Wyoming, USA, Geoderma, 226–227, 1–7, doi:10.1016/j.geoderma.2014.02.025, 2014.

IUSS Working Group WRB: World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps, World Soil Resour. Reports No. 106, 1–191, doi:10.1017/S0014479706394902, 2014.

Kalinina, O., Krause, S.-E., Goryachkin, S. V., Karavaeva, N. A., Lyuri, D. I. and Giani, L.: Self-restoration of post-agrogenic chernozems of Russia: Soil development, carbon stocks, and dynamics of carbon pools, Geoderma, 162(1–2), 196–206, doi:10.1016/j.geoderma.2011.02.005, 2011.

Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R. and Nico, P. S.: Mineral-organic associations: formation, properties, and relevance in soil environments, Adv. Agron., 130, 1–140, doi:10.1016/bs.agron.2014.10.005, 2015.

Kuznetsova, A., Brockhott, P. B. and Christensen, R. H. B.: lmerTest: Tests in linear mixed effects models. R package version 2.0-25, [online] Available from: http://cran.r-project.org/package=lmerTest, 2015.

Lal, R.: Soil carbon sequestration impacts on global climate change and food security, Science, 304(June), 1623–1627, doi:10.1126/science.1097396, 2004.

Lehmann, J. and Kleber, M.: The contentious nature of soil organic matter, Nature, 528(7580), 60–68, doi:10.1038/nature16069, 2015.

Lenth, R. V. and Herve, M.: lsmeans: Least-squares means. R package version 2.17, [online] Available from: http://cran.r-project.org/package=lsmeans, 2015.

Loftfield, N., Flessa, H., Augustin, J. and Beese, F.: Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide, and nitrous oxide, J. Environ. Qual., 26(2), 560, doi:10.2134/jeq1997.00472425002600020030x, 1997.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G., Marschner, B. and Flessa, H.: Stabilization of organic matter in temperate soils: Mechanisms and their relevance under different soil conditions - A review, Eur. J. Soil Sci., 57(4), 426–445, doi:10.1111/j.1365-2389.2006.00809.x, 2006.

McLauchlan, K. K.: Effects of soil texture on soil carbon and nitrogen dynamics after cessation of agriculture, Geoderma, 136(1–2), 289–299, doi:10.1016/j.geoderma.2006.03.053, 2006.

Mikhailova, E. A., Bryant, R. B., Vassenev, I. I., Schwager, S. J. and Post, C. J.: Cultivation effects on soil carbon and nitrogen contents at depth in the russian Chernozem, Soil Sci. Soc. Am. J., 64, 738, doi:10.2136/sssaj2000.642738x, 2000.

Plante, A. F., Six, J., Paul, E. A. and Conant, R. T.: Does physical protection of soil organic matter attenuate temperature sensitivity?, Soil Sci. Soc. Am. J., 73(4), 1168, doi:10.2136/sssaj2008.0351, 2009.

Plante, A. F., Virto, I. and Malhi, S. S.: Pedogenic, mineralogical and land-use controls on organic carbon stabilization in two contrasting soils, Can. J. Soil Sci., 90(1), 15–26, doi:10.4141/CJSS09052, 2010.

Plante, A. F., Fernández, J. M., Haddix, M. L., Steinweg, J. M. and Conant, R. T.: Biological, chemical and thermal indices of soil organic matter stability in four grassland soils, Soil Biol. Biochem., 43(5), 1051–1058, doi:10.1016/j.soilbio.2011.01.024, 2011.

Poeplau, C. and Don, A.: Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe, Geoderma, 192, 189–201, doi:10.1016/j.geoderma.2012.08.003, 2013.

Pulleman, M. M. and Marinissen, J. C. Y.: Physical protection of mineralizable C in aggregates from long-term pasture and arable soil, Geoderma, 120(3–4), 273–282, doi:10.1016/j.geoderma.2003.09.009, 2004.

R Core Team: R: A language and environment for statistical computing, [online] Available from: http://www.r-project.org/, 2015.

Rodionov, A., Amelung, W., Urusevskaja, I. and Zech, W.: Beziehungen zwischen Klimafaktoren und C-, N-Pools in Partikelgrössen-Fraktionen zonaler Steppenböden Russlands, Zeitschrift für Pflanzenernährung und Bodenkd., 161(1981), 563–569, 1998.

Sainju, U. M.: Carbon and nitrogen pools in soil aggregates separated by dry and wet sieving methods, Soil Sci., 171(12), 937–949, doi:10.1097/01.ss0000228062.30958.5a, 2006.

Schimel, J. P. and Schaeffer, S. M.: Microbial control over carbon cycling in soil, Front. Microbiol., 3(SEP), 1–11, doi:10.3389/fmicb.2012.00348, 2012.

Schlichting, E., Blume, H.-P. and Stahr, K.: Bodenkundliches Praktikum – Eine Einführung in pedologisches Arbeiten für Ökologen, insbesondere Land- und Forstwirte, und für Geowissenschaftler, 2nd ed., Blackwell Wissenschafts-Verlag Berlin, Wien, Austria., 1995.

Schrumpf, M., Schulze, E. D., Kaiser, K. and Schumacher, J.: How accurately can soil organic carbon stocks and stock changes be quantified by soil inventories?, Biogeosciences, 8(5), 1193–1212, doi:10.5194/bg-8-1193-2011, 2011.

Six, J., Elliott, E. . and Paustian, K.: Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture, Soil Biol. Biochem., 32(14), 2099–2103, doi:10.1016/S0038-0717(00)00179-6, 2000a.

Six, J., Paustian, K., Elliott, E. T. and Combrink, C.: Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon, Soil Sci. Soc. Am. J., 64, 681–689, doi:10.2136/sssaj2000.642681x, 2000b.

Six, J., Feller, C., Denef, K., Ogle, S. M. and Moraes, J. C., Albrecht, A.: Soil organic matter, biota and aggregation in temperate and tropical soils - effects of no-tillage, Agronomie, 22, 755–775, doi:10.1051/agro:2002043, 2002.

Formatiert: Abstand Vor: 0 pt, Absatzkontrolle, Abstand zwischen asiatischem und westlichem Text anpassen, Abstand zwischen asiatischem Text und Zahlen anpassen

Formatiert: Deutsch (Deutschland)

Tian, J., Pausch, J., Yu, G., Blagodatskaya, E., Gao, Y. and Kuzyakov, Y.: Aggregate size and their disruption affect 14C-labeled glucose mineralization and priming effect, Appl. Soil Ecol., 90, 1–10, doi:10.1016/j.apsoil.2015.01.014, 2015.

Tisdall, J. and Oades, J.: Organic matter and water-stable aggregates in soils, J. soil Sci., 33, 141–163, doi:10.1111/j.1365-2389.1982.tb01755.x, 1982.

Vance, E. D., Brookes, P. C. and Jenkinson, D. S.: An extraction method for measuring soil microbial biomass C, Soil Biol. Biochem., 19(6), 703–707, doi:10.1016/0038-0717(87)90052-6, 1987.

VandenBygaart, A. J., Gregorich, E. G. and Angers, D. A.: Influence of agricultural management on soil organic carbon: A compendium and assessment of Canadian studies, Can. J. Soil Sci., 83, 363–380, doi:10.4141/S03-009, 2003.

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.

Wickham, H.: ggplot2: Elegant graphics for data analysis, [online] Available from: papers2://publication/uuid/DE806F9A-5AD0-450B-AC68-5164EDBA8787, 2009.

Tables

Table 1: Land-use and soil properties in A horizons (pH, EC, sand, silt, clay) and in 0-10 cm (OC, TN, C : N) of investigated ~~sites~~ plots with mean annual temperature (MAT), ~~and~~ mean annual precipitation (MAP), and geographical coordinates. Soil ~~type~~ classification according to IUSS Working Group WRB (2014). For those plots which were situated directly adjacent to the pasture plot the soil texture (sand, silt, clay) was not explicitly measured, but the comparable soil texture was verified by hand analysis (h.a.) Abbreviation: n.d. = not determined.

Steppe	MAT	MAP	Soil ~~type~~	Land use	Coordinates		pH	EC	Sand	Silt	Clay	OC			TN			C : N
	°C	mm			Latitude	Longitude	-	µS cm^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}			mg g^{-1}			
Forest steppe	1.1	368	Protocalcic Chernozem	extensive pasture	53°44'19.53"N	80°41'2.88"E	7.6	116.6	28	609	363	54.6	±	5.4	4.5	±	0.4	12.2
				forage crop	53°44'24.92"N	80°40'58.73"E	n.d.	n.d.	~~n.d.~~ h.a.	h.a. ~~n.d.~~	h.a. ~~n.d.~~	49.3	±	1.7	4.1	±	0.2	12.1
				arable 5 yr	53°45'0.19"N	80°40'12.68"E	7.1	58.6	39	600	360	39.1	±	1.4	3.3	±	0.1	11.7
				arable 30 yr	53°45'3.32"N	80°40'2.97"E	7.0	60.4	34	598	369	40.4	±	1.6	3.4	±	0.1	11.8
Typical steppe	2.0	339	Protocalcic Kastanozem	fallow 30 yr (pasture)	52°30'1.43"N	80°44'41.68"E	7.1	34.8	292	472	236	21.6	±	2.3	2.0	±	0.2	10.9
				arable 1 yr	52°30'5.43"N	80°44'25.57"E	n.d.	n.d.	h.a. ~~n.d.~~	h.a. ~~n.d.~~	h.a. ~~n.d.~~	13.3	±	0.3	1.3	±	0.0	10.0
				arable 3 yr	52°30'10.92"N	80°44'44.44"E	n.d.	n.d.	h.a. ~~n.d.~~	h.a. ~~n.d.~~	h.a. ~~n.d.~~	14.9	±	1.6	1.5	±	0.2	9.8
				arable 10 yr	52°29'30.56"N	80°45'5.21"E	n.d.	n.d.	h.a. ~~n.d.~~	h.a. ~~n.d.~~	h.a. ~~n.d.~~	18.8	±	1.5	1.8	±	0.1	10.6

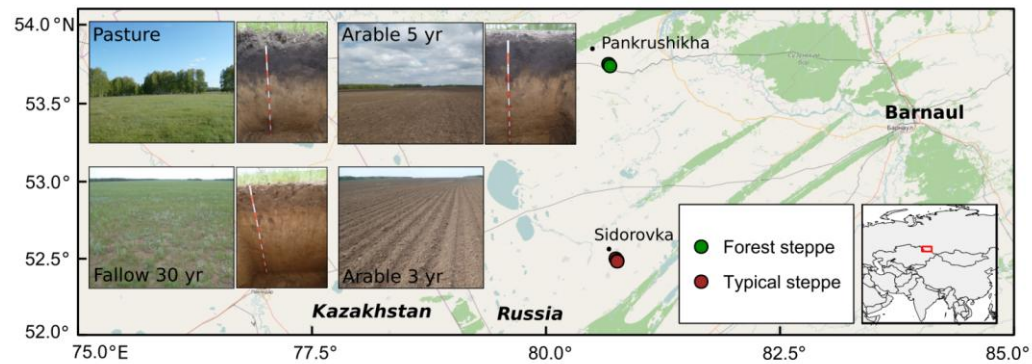
Table 2: Mean residence times of the fast and slow OC pool (years) as arithmetic mean ± SE, as derived from least-square fitting of incubation data, for two steppe types and as function of land-use and soil fraction. Significant differences ($p<0.05$) between fractions within land-use were not detected, which is indicated by same lowercase letters.

Steppe	Land-use	Fraction	Mean residence time (years)			
			Fast OC pool		Slow OC pool	
Forest steppe	extensive pasture	bulk	0.73 ± 0.07	a	62.8 ± 18.6	a
		intact	0.74 ± 0.09	a	54.5 ± 1.6	a
		crushed	0.69 ± 0.06	a	89.5 ± 19.2	a
	forage crop	bulk	0.46 ± 0.07	a	53.5 ± 7.6	a
		intact	0.68 ± 0.11	a	65.2 ± 13.6	a
		crushed	0.42 ± 0.06	a	48.3 ± 3.3	a
	arable 5 yr	bulk	0.51 ± 0.07	a	45.2 ± 8.0	a
		intact	0.51 ± 0.06	a	48.1 ± 6.4	a
		crushed	0.33 ± 0.02	a	42.4 ± 3.1	a
	arable 30 yr	bulk	0.38 ± 0.02	a	36.5 ± 1.6	a
		intact	0.41 ± 0.03	a	41.5 ± 1.9	a
		crushed	0.33 ± 0.01	a	36.9 ± 1.7	a
Typical steppe	fallow 30 yr (pasture)	bulk	0.79 ± 0.11	a	21.7 ± 7.1	a
		intact	0.79 ± 0.10	a	25.8 ± 2.4	a
		crushed	0.80 ± 0.10	a	25.9 ± 9.0	a
	arable 1 yr	bulk	0.33 ± 0.06	a	26.2 ± 4.9	a
		intact	0.43 ± 0.05	a	30.9 ± 3.2	a
		crushed	0.32 ± 0.05	a	30.3 ± 6.6	a
	arable 3 yr	bulk	0.68 ± 0.11	a	29.5 ± 4.0	a
		intact	0.64 ± 0.14	a	20.6 ± 1.3	a
		crushed	0.88 ± 0.16	a	22.7 ± 2.7	a
	arable 10 yr	bulk	0.55 ± 0.08	a	24.4 ± 1.4	a
		intact	0.86 ± 0.18	a	33.2 ± 7.2	a
		crushed	0.58 ± 0.10	a	25.9 ± 1.6	a

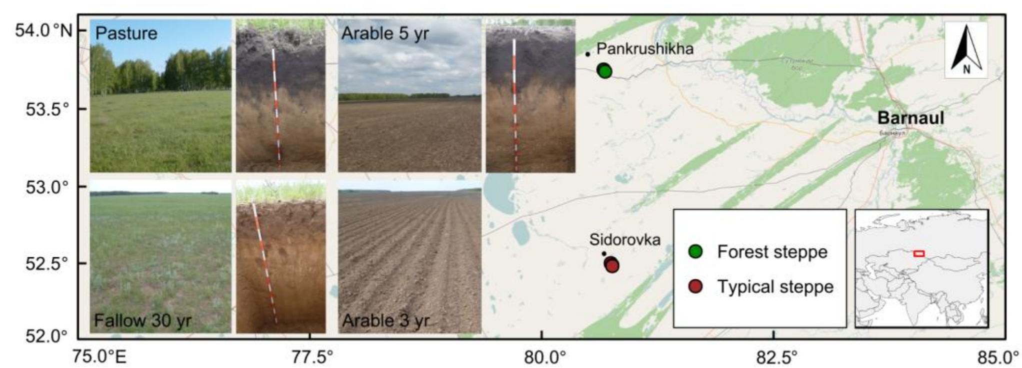
Table 3: Proportion of macro-aggregate protected OC (% of mineralized OC) for two steppe types and the respective land-use. Since the proportion of macro-aggregate protected OC was calculated by subtracting the amount of OC mineralized in intact macro-aggregates from that in crushed macro-aggregates, negative values occur when the OC mineralization was smaller in crushed than in intact macro-aggregates.

Steppe	Land-use	Macro-aggregate protected OC (% of mineralized OC)		
Forest steppe	extensive pasture	1.4	±	2.4
	forage crop	-0.4	±	8.4
	arable 5 yr	2.6	±	4.3
	arable 30 yr	7.7	±	4.2
Typical steppe	fallow 30 yr (pasture)	4.7	±	0.8
	arable 1 yr	0.7	±	1.8
	arable 3 yr	-8.8	±	5.7
	arable 10 yr	4.3	±	3.1

Figures



Formatiert: Schriftart: Nicht Fett, Schriftartfarbe: Automatisch



Formatiert: Schriftart: Nicht Fett, Schriftartfarbe: Automatisch

Figure 1: Map of the study area and pictures from the two sites near Pankrushikha and Sidorovka. Map modified from ©OpenStreetMap contributors, for copyright see www.openstreetmap.org/copyright.

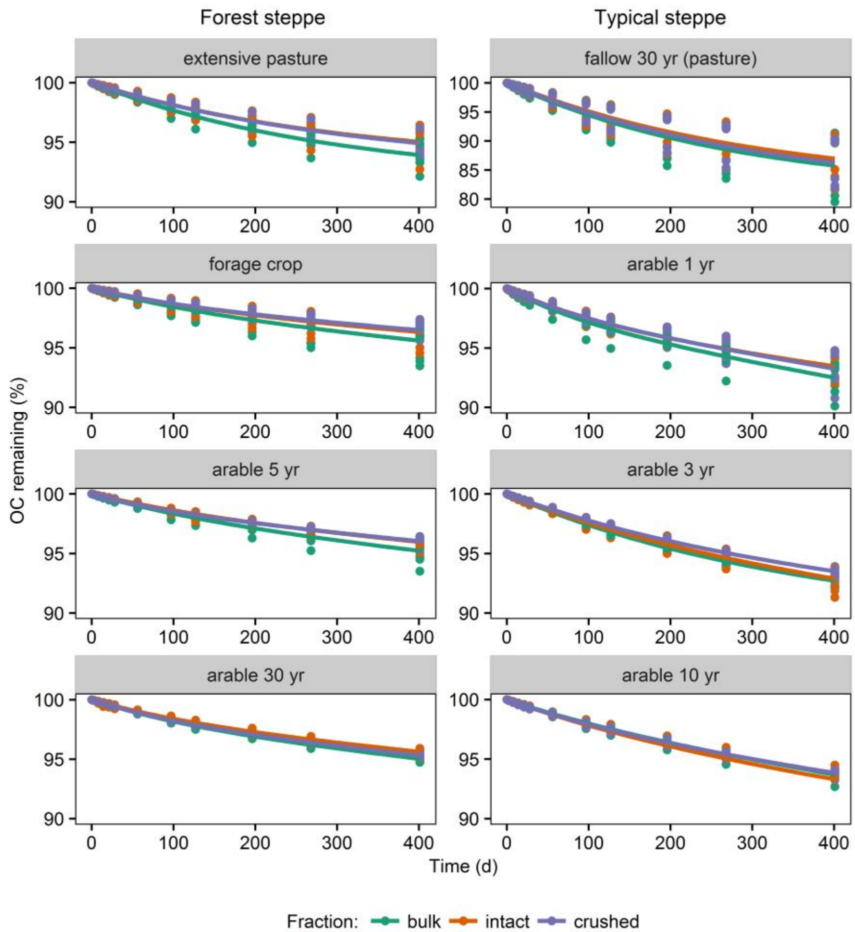


Figure 2: Percentage of soil OC remaining in the samples during 401 days of incubation for eight plots within two steppe types and for the three fractions bulk soil, intact macro-aggregates, and crushed macro-aggregates. For all plots a two-pool model (Eq. 2) was fitted, except for the extensively managed plots (extensive pasture and the fallow 30 yr (pasture)), where an asymptotic two-pool model was fitted (Eq. 3). Note the different y-scale for the fallow 30 yr (pasture).

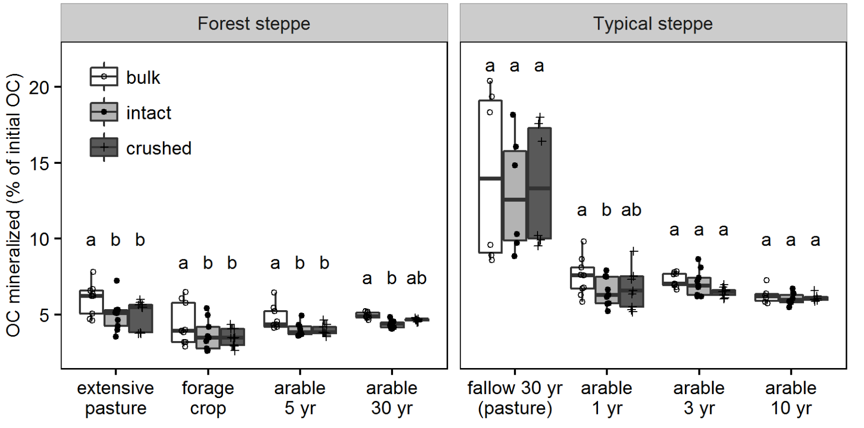


Figure 3: Percentage of soil OC mineralized during 401 days of incubation for eight plots within two steppe types and for the three fractions bulk soil, intact macro-aggregates, and crushed macro-aggregates. Different lowercase letters indicate significant differences between fractions within plots at p<0.05.

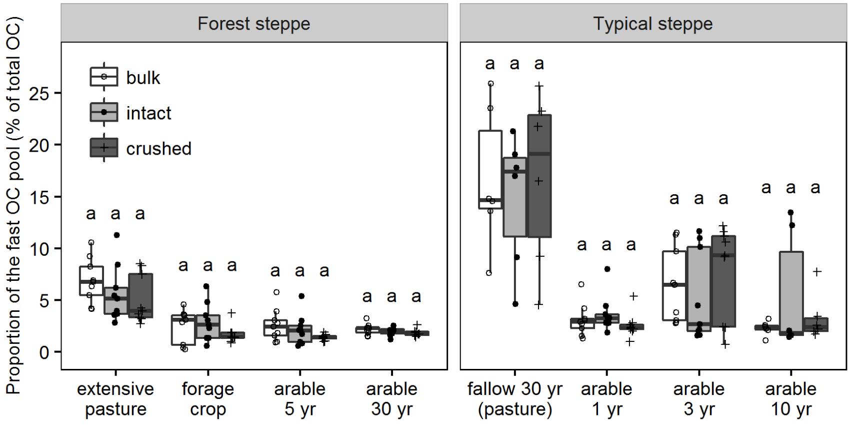


Figure 4: Proportion of the fast OC pool (% of total OC) for eight plots within two steppe types and for the three fractions bulk soil, intact macro-aggregates, and crushed macro-aggregates as derived from two-pool model fits to incubation data (Eq. 2 and 3). Significant differences (p<0.05) between fractions within plots were not detected, which is indicated by same lowercase letters.

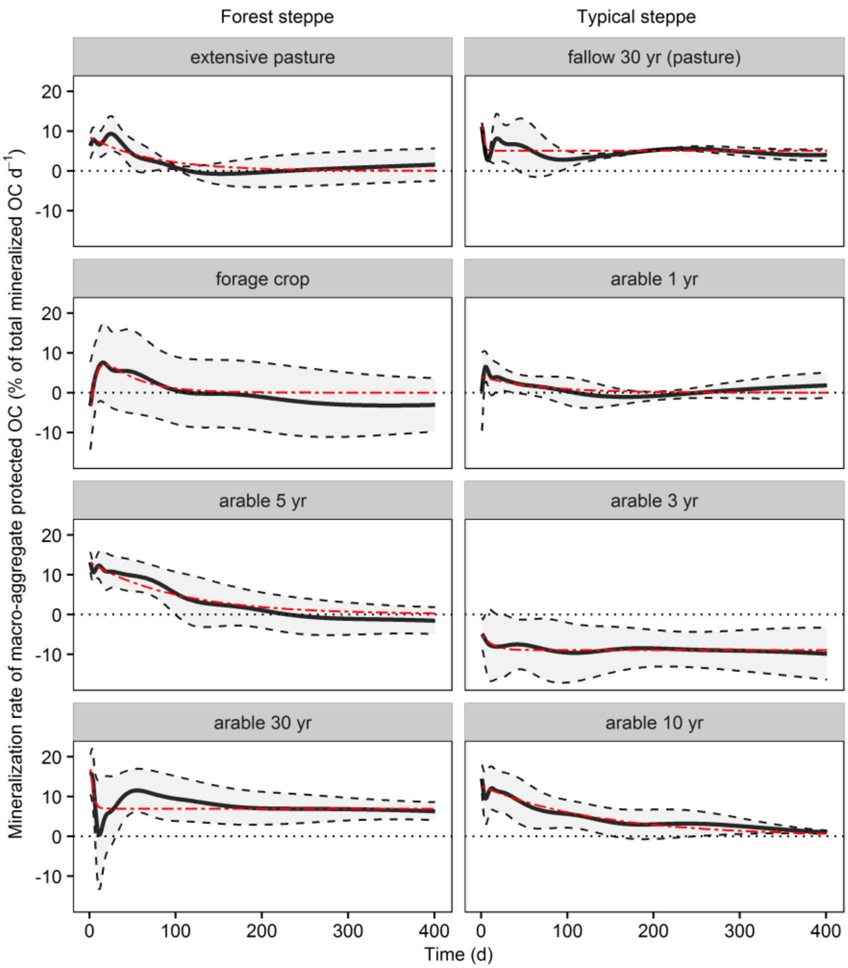


Figure 5: Mineralization rate of macro-aggregate protected OC (% of total mineralized OC d^{-1}) during 401 days of incubation for eight plots within two steppe types. The black solid line shows the mean mineralization rate per plot and the shaded grey area (confined by the black dashed lines) shows the corresponding standard error. The red dot-dashed line shows the fit of an exponential decay model (either 1-pool model, 2-pool model, or asymptotic 2-pool model according to the best fit). Since the mineralization rates were calculated by subtracting the OC mineralization rates of intact macro-aggregates from that of crushed macro-aggregates, negative values occur when the OC mineralization was smaller in crushed than in intact macro-aggregates.

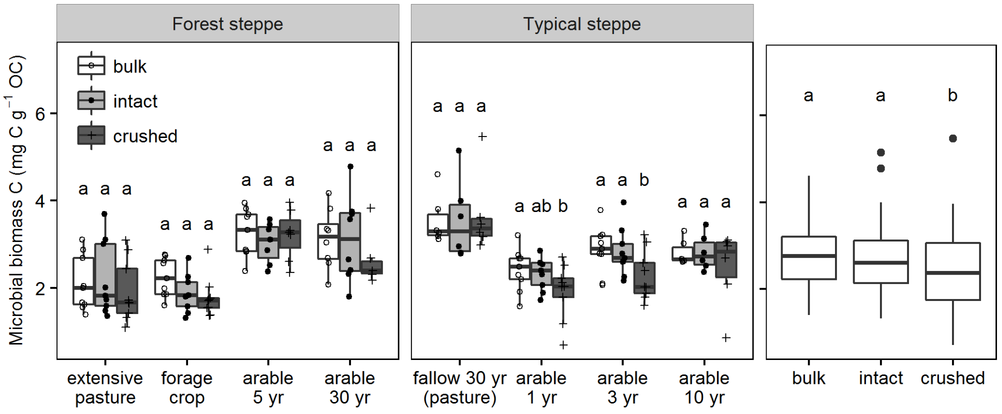


Figure 6: Microbial biomass C (mg C g^{-1} OC) for eight plots within two steppe types and the three fractions bulk soil, intact macro-aggregates, and crushed macro-aggregates. Different lowercase letters indicate significant differences between fractions within plots at $p<0.05$. The right panel shows differences between the three fractions averaged over all plots.

Supplement of

Limited protection of macro-aggregate occluded organic carbon in Siberian steppe soils

N. Bischoff et al.

Correspondence to: N. Bischoff (bischoff@ifbk.uni-hannover.de)

Supplements

Calculation of the share of steppe soils in the global soil OC stocks down to 1m depth

Steppe soils are typically the group of Kastanozems (KS), Chernozems (CH) and Phaeozems (PH; FAO, 2001). Average OC stocks of these soils are found in Batjes (1996) and their global area is estimated in the IUSS Working Group WRB (2014). By multiplying the average OC stock of these soil types by their global area and dividing the result by the global soil OC stock of about 1505 Pg OC (Batjes, 1996), we can estimate the share of steppe soils in the global soil OC stock down to 1m depth. This was done as, to our knowledge, there is no study yet, which modelled the portion of steppe soils on the global soil OC stock. The equation is as following:

$$y = \frac{\text{average OC stock (KS)} \times \text{area (KS)} + \text{average OC stock (CH)} \times \text{area (CH)} + \text{average OC stock (PH)} \times \text{area (PH)}}{\text{OC stock (worldwide)}}$$

where y is the share of steppe soils in the global soil OC stock.

Tables

Table S 1: Geographical coordinates and vegetation cover (pastures only) for the studied plots.

Steppe	Land use	Coordinates		Vegetation (pastures only)
		latitude	longitude	Dominant species (from most to least dominant)
Forest steppe	extensive pasture	53°44'19.53"N	80°41'2.88"E	*Festuca valesiaca - Fillipendula vulgaris - Bromopsis inermis*
	forage crop	53°44'24.92"N	80°40'58.73"E	
	arable 5 yr	53°45'0.19"N	80°40'12.68"E	
	arable 30 yr	53°45'3.32"N	80°40'2.97"E	
Typical steppe	fallow 30 yr (pasture)	52°30'1.43"N	80°44'41.68"E	*Agropyron pectinatum - Bromopsis inermis - Artemisia glauca*
	arable 1 yr	52°30'5.43"N	80°44'25.57"E	
	arable 3 yr	52°30'10.92"N	80°44'44.44"E	
	arable 10 yr	52°29'30.56"N	80°45'5.21"E	

Table S 112: Organic carbon (OC) and total nitrogen (TN) of the three fractions bulk soil, intact macro-aggregates, and crushed macro-aggregates for the respective land-use and steppe type.

Steppe	Land-use	Fraction	n	OC	TN	C : N
				mg g^{-1}	mg g^{-1}	-
Forest steppe	extensive pasture	bulk	3	54.6 ± 5.4	4.5 ± 0.4	12.2 ± 0.2
		intact	3	53.3 ± 6.5	4.4 ± 0.5	12.0 ± 0.2
		crushed	3	52.2 ± 5.7	4.4 ± 0.4	11.8 ± 0.2
	forage crop	bulk	3	49.3 ± 1.7	4.1 ± 0.2	12.1 ± 0.0
		intact	3	48.7 ± 1.2	4.1 ± 0.1	12.0 ± 0.1
		crushed	3	45.5 ± 1.1	3.9 ± 0.1	11.6 ± 0.1
	arable 5 yr	bulk	3	39.1 ± 1.4	3.3 ± 0.1	11.7 ± 0.2
		intact	3	39.2 ± 2.4	3.3 ± 0.2	12.0 ± 0.1
		crushed	3	38.4 ± 2.6	3.2 ± 0.1	11.8 ± 0.3
	arable 30 yr	bulk	3	40.4 ± 1.6	3.4 ± 0.1	11.8 ± 0.1
		intact	3	39.1 ± 2.3	3.4 ± 0.2	11.6 ± 0.0
		crushed	3	37.4 ± 1.4	3.3 ± 0.1	11.3 ± 0.0
Typical steppe	fallow 30 yr (pasture)	bulk	2	21.6 ± 2.3	2.0 ± 0.2	10.9 ± 0.1
		intact	2	20.6 ± 2.8	1.9 ± 0.2	10.6 ± 0.3
		crushed	2	20.4 ± 2.6	2.0 ± 0.2	10.3 ± 0.3
	arable 1 yr	bulk	3	13.3 ± 0.3	1.3 ± 0.0	10.0 ± 0.1
		intact	3	14.1 ± 0.9	1.4 ± 0.1	10.0 ± 0.2
		crushed	3	13.3 ± 0.5	1.3 ± 0.0	10.2 ± 0.2
	arable 3 yr	bulk	3	14.9 ± 1.6	1.5 ± 0.2	9.8 ± 0.2
		intact	3	16.6 ± 2.6	1.7 ± 0.3	9.9 ± 0.2
		crushed	3	15.6 ± 2.5	1.6 ± 0.2	9.8 ± 0.2
	arable 10 yr	bulk	2	18.8 ± 1.5	1.8 ± 0.1	10.6 ± 0.2
		intact	2	20.0 ± 0.7	1.9 ± 0.0	10.6 ± 0.3
		crushed	2	18.5 ± 0.9	1.8 ± 0.0	10.5 ± 0.2

Figures

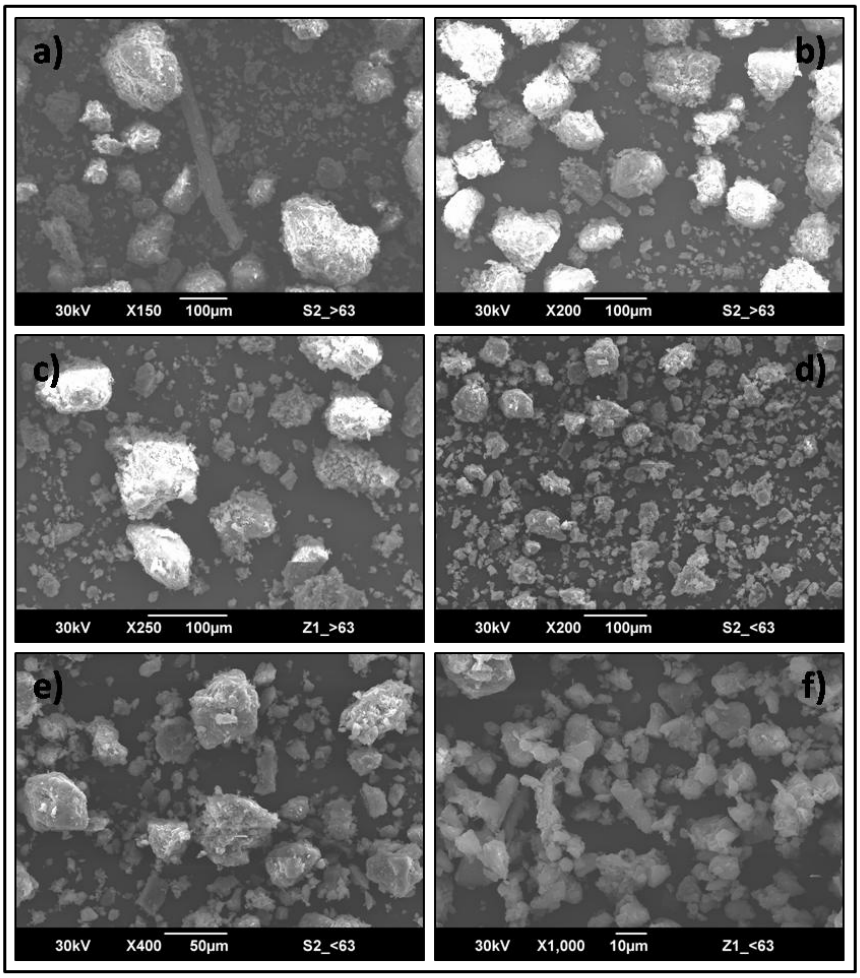


Fig. S 1: Scanning electron micrographs of crushed macro-aggregates (<250 µm). a) fallow 30 yr (pasture) 63–250 µm, b) fallow 30 yr (pasture) 63–250 µm, c) arable 5 yr 63–250 µm, d) fallow 30 yr (pasture) <63 µm, e) fallow 30 yr (pasture) <63 µm, f) arable 5 yr <63 µm.

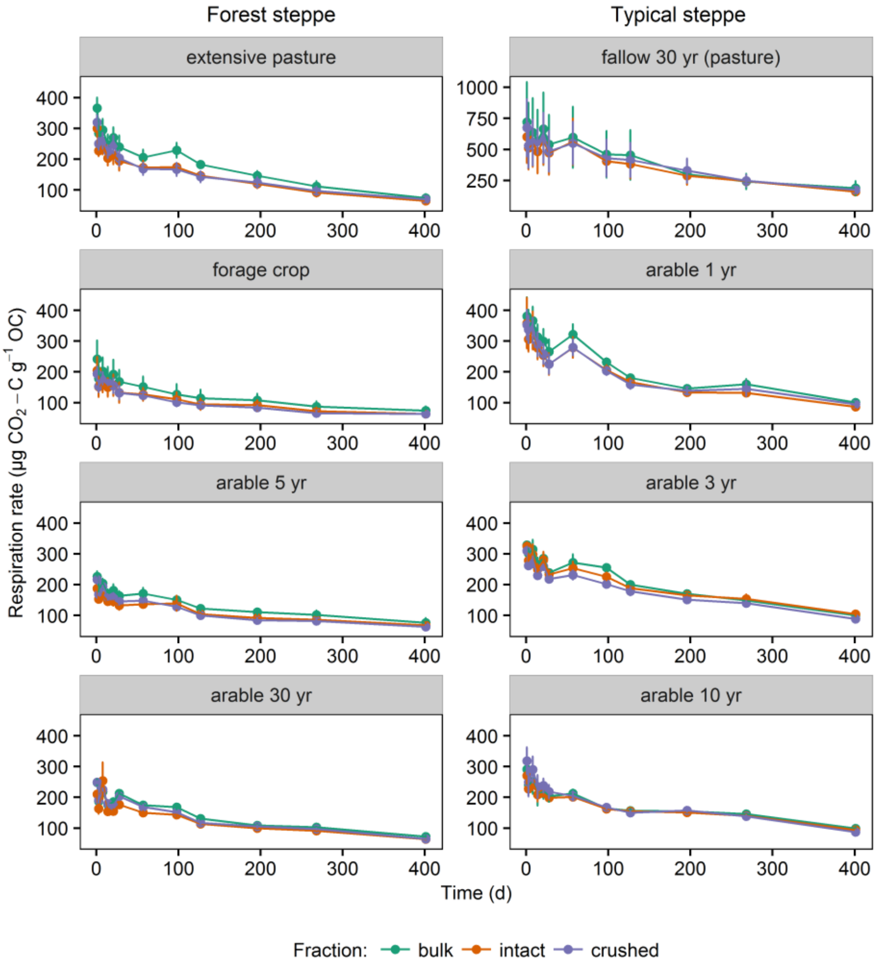


Fig. S 2: Time course of respiration rates (µg CO_2-C g^{-1} OC) for eight plots within two steppe types and for the three fractions bulk soil, intact macro-aggregates and crushed macro-aggregates. Shown are arithmetic means ± SE for all 12 time points where CO_2 gas samples were taken. Note the different y-scale for the fallow 30 yr (pasture).

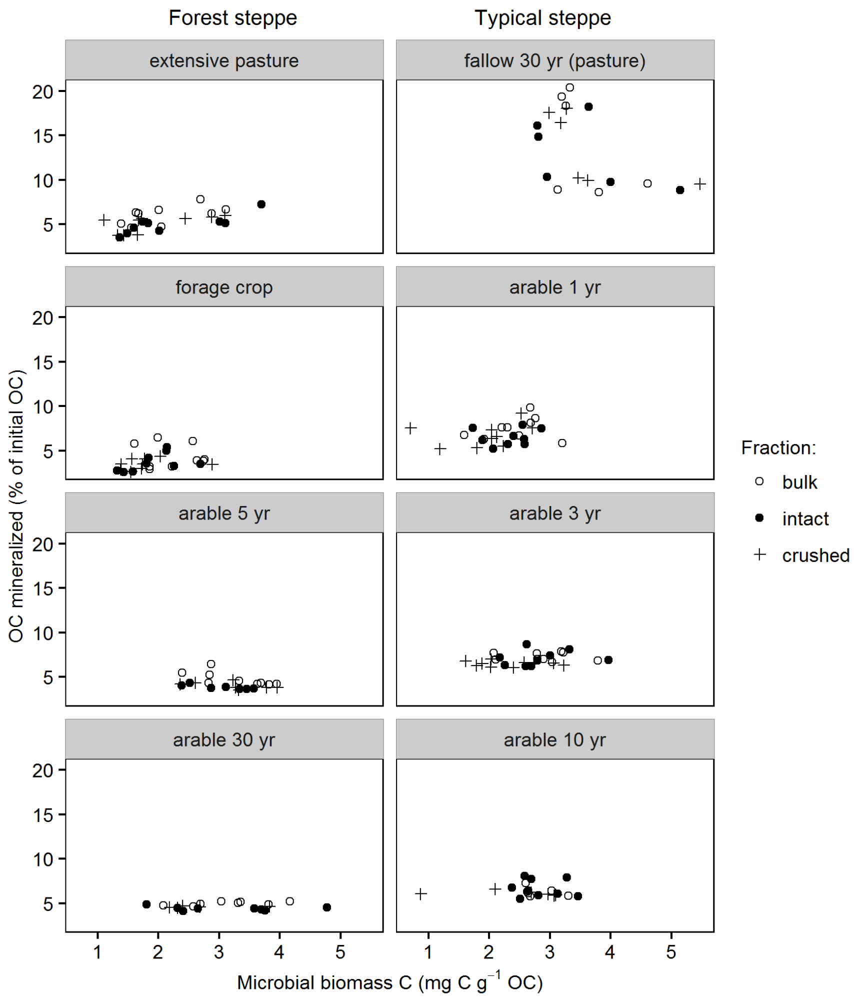


Fig. S 3: Mineralized OC (% of initial OC) plotted against the microbial biomass C normalized to total OC (mg C g^{-1} OC) for eight plots within two steppe types and the three fractions bulk soil, intact macro-aggregates, and crushed macro-aggregates.

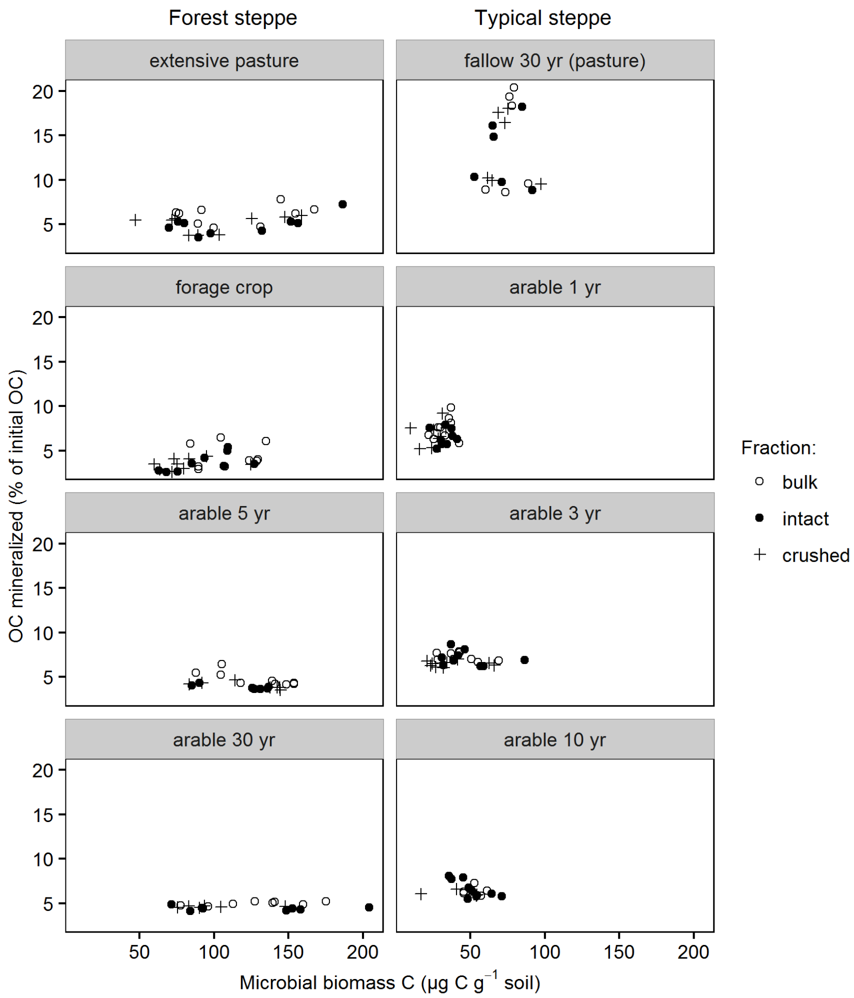


Fig. S 4: Mineralized OC (% of initial OC) plotted against the microbial biomass C per soil mass (µg C g^{-1} soil) for eight plots within two steppe types and the three fractions bulk soil, intact macro-aggregates, and crushed macro-aggregates.

References

Batjes, N. H.: Total carbon and nitrogen in the soils of the world, Eur. J. Soil Sci., 47(2), 151–163, doi:10.1111/j.1365-2389.1996.tb01386.x, 1996.

FAO: Lecture notes on the major soils of the world, edited by P. Driessen, J. Deckers, O. Spaargaren, and F. Nachtergaele, World soil Resour. reports, 94, 336, doi:10.1136/gut.27.11.1400-b, 2001.

IUSS Working Group WRB: World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps, World Soil Resour. Reports No. 106, 1–191, doi:10.1017/S0014479706394902, 2014.