Dear Professor Yakov Kuzyakov, Handling Associate Editor,

Thank you very much for kindly considering our manuscript (bg-2016-483), “Alteration of soil carbon and nitrogen pools and enzyme activities as affected by increased soil coarseness”. We have carefully considered the thoughtful and valuable comments and suggestions from you and the reviewers and revised our manuscript accordingly. To improve the language, we also invited Dr. Feike A. Dijkstra for linguistic improvement and thus included him as a co-author. We believe that we have addressed and answered the major and minor comments and questions. We hope the manuscript now meets the level of Biogeosciences.

Below we addressed all the comments point by point. Reference to line numbers is for the revised manuscript with marked changes.

Response to Reviewer #1:

General comments:

1. There is no control of original soil without any (excessive) manipulations. The latter included withdrawal of soil down to 60 cm, thorough mixing with sand (strong altering of the soil structure per se), removal of the topsoil with the heating up to 105 ℃ and the subsequent return, transplanting of vegetation. It remained unclear to which extend the used control (C0) with no sand addition was subjected to the listed manipulations.

Response: We fully agree with the reviewer’s comments. This is a limitation of our experiment that without having a control treatment with undisturbed soil. We cannot assess the effect of the disturbance caused by the san addition. Still, we can isolate soil coarseness effects because all the soils from C0 to C70 plots were subjected to the same manipulations of excavation and sterilization. By knowing this, we have planned to enclose a new adjacent plot to serve as control of original soil in this field experiment. The absolute control would be good for our future research conducted in this experiment. We have clarified in the methods section that the current control soil was subjected to the same manipulation as in the sand addition treatments (Page 7 Line 17).

2. A key parameter for a desertification model study and a semi-arid grassland as such, the soil moisture dynamics is not shown or even mentioned. With all the reported soil characteristics, a basic one—the water content, WHC—is not shown. However, availability of water may strongly vary among soil coarseness gradient and, obviously, affect the majority parameters of interest as microbial biomass and activity, pH, nutrients mobilization etc.

Response: Thanks for the great observation. Indeed, soil moisture and water holding capacity are essential parameters for semi-arid ecosystem. Thus, we have added data on soil moisture and WHC in the results section (Page 12 Line 7-11) and discussed their potential effects on soil enzyme activities (Page 21 Line 4-9). For soil moisture, the data were only available for the time of soil sampling (October 2015). Considering the advice provided, we have decided to measure soil moisture dynamics during the whole growing season in our future work.

3. The effect of sand addition. On a mass basis, relatively low contents of C, N and especially P (not reported in the study) could in fact substantially influence the soil elements stoichiometry, and, critically important, the microbial community structure. This was not clarified in the methods and results, or in discussion.

Response: We agree with this observation. The river sand contains 1.29 g kg^{-1} C and 0.15 g kg^{-1} N which are 31.9% of SOC and 31.6% of TN of the original untreated soil. Due to the fact that the C and N contents in the sand are not negligible, we recalculated the theoretical values by considering C and N from the sand. This has been mentioned in Page 11 Line 4-9 in the methods section and Page 14 Line 20 in the result section.

4. Secondary but still important methodological issue: some soil samples were frozen for storage purpose and the enzyme activity was measured upon freezing. In the draft file, I mention several papers (but based on my own experience), the freezing/unfreezing could strongly affect enzyme activities and the direction of change is difficult to predict. So, in fact, authors had an additional treatment for the enzyme activities distribution among coarseness gradient. Most important, the frozen soil samples were not identified as the fresh soil was also used for the analysis.

Response: Thanks so much for this observation and we fully agree with this. Unfortunately, we did not compare the difference in enzyme activities between fresh soils and frozen soils. In the reference that was provided by reviewer, β-glucosidase and acid phosphomonoesterase activities were also determined from different soils, but the activities showed no response to freezing-thawing cycles in the laboratory experiment (Daou et al., 2016, Soil Biology & Biochemistry 93, 142-149). This was explained in detail in the following specific comments (comment on Page 9 Line 16). But we are not sure if the activities still remain stable in wider soil and ecosystem types. Thus, we will follow the reviewer's advice on determining enzyme activities in fresh soils or clarify the effect of the freezing-thawing process during storage on enzyme activities in our future research.

5. Finally, the "theoretical dilution approach" should probably be reconsidered. According to authors, the "dilution" occurred for the very initial soil properties, which existed at the moment of excavation and mixing with sand in respective treatments. However, after replanting of sites, additional C was introduced which was not accounted in the "dilution". Thus, depending on an amount of "new C", the theoretical dilution values would be higher as currently presented and probably approach some of the "actual" measured parameters. From this point of view, comparison of "theoretical" and "actual" values could be erroneous. Authors could estimate how much C, N, and especially microbial biomass was introduced with the replanting and correct the dilution (C0 at the same time will not change).

Response: Before plant transplantation, the roots of plants were washed to remove soil associated with transplantation. This information has been added in Page 8 Line 1-4. Therefore, replanting would not introduce C, N and microbial biomass to native soils. However, we have realized that the river sand contains non-negligible C and N, which could comprise 31.9% of SOC and 31.6% of TN. We recalculated theoretical values of SOC and TN by considering the C and N contents in the river sand. Please see the details in the specific comments (comment on Page 11 Line 7-11).

6. There are other shortcomings, such as too detailed results section (all the

observations are excessively described) as well as speculative and controversial statements in the discussion.

Response: Thanks so much for pointing out these. We have shorted the results section to avoid excessive description. Please see our responses in the specific comments (comment on Page 11 Line 8, Page 11 Line 10, Page 11 Line 7-22, and Page 12 Line 1-12). The speculative and controversial statements that were pointed in the specific comments were also addressed (see comments on Page 14 Line 21, Page 15 Line 2, Page 15 Line 10, Page 16 Line 2-3, Page 16 Line 7, Page 17 Line 18, etc.).

Specific comments:

Page 1 Line 1: The title is somewhat misleading: the elements' stoichiometry was actually not measured in the study. Next, "their related enzymes" may be understood as sets or groups of enzyme catalyzing soil C, N and P turnover; however, just three enzymes were tested. I recommend to adjust the title to better reflect the outcome.

Response: We agree with the reviewer's comment. Thus, the title has been modified to "Alteration of soil carbon and nitrogen pools and enzyme activities as affected by increased soil coarseness".

Page 2 Line 2: added "subjected to desertification" after "grasslands".

Response: These words have been added. It now reads "Soil coarseness decreases ecosystem productivity, ecosystem carbon and nitrogen stocks, and soil nutrient contents in sandy grasslands subjected to desertification" (Page 2 Line 2).

Page 2 Line 6: % of what? Natural (initial) sand content? Please, specify.

Response: It is a percentage of soil mass by keeping total mass of the soil-sand mixture constant. To be specific, this has been rephrased into "a field experiment was conducted by mixing native soil with river sand in different mass proportions: 0%, 10%, 30%, 50%, and 70% sand addition" (Page 2 Line 5).

Page 2 Line 7: Over which period of time? Please, add here briefly.

Response: We have added the period of time. It's "Four years after establishing plots and two years after transplanting" (Page 2 Line 7).

Page 2 Line 10: change "by up to" into "down to".

Response: This has been changed in Page 2 Line 10.

Page 2 Line 12: Not clear why exactly these enzymes were chosen?

Response: These three enzymes are commonly measured to represent microbial acquisition of assimilable C, N and P from recalcitrant organic matter (Waring et al., 2014). Related information has been added in Page 2 Line 14 to clarify the reason.

Page 2 Line 15: The enzyme catalyzes decomposition of chitin in soil, which is also a source of C, not just N, for microorganisms. How C from chitin was accounted in stoichiometry to N and P?

Response: The reviewer makes a great observation. The enzyme of N-acetyl-glucosaminidase (NAG) plays a role in the degradation of chitin and other β-1,4-linked glucosamine polymers (Sinsabaugh et al., 2008). It was selected to represent N-acquiring enzyme according to the studies from Sinsabaugh et al. (2008) (Ecology Letters, 11: 1252-1264) and Sinsabaugh et al. (2009) (Nature, 462: 795-798).

Page 2 Line 20: Authors stated they conducted field study. So, they should be aware of the effect of sand additions on plants. Next, microbial immobilization is a time-dependent process. Upon re-mobilization and MB turnover, the MBP should also be plant available. So, in fact, P immobilization in the medium- and long-term should be positive for plant supply with P. Please, explain better the main message here.

Response: Thank you for this observation. We have corrected the main message here into ‘Enhanced microbial recycling of P might alleviate plant P limitation in nutrient-poor grassland ecosystems that are affected by soil coarseness’ (see Page 2, Line 20-22). And we also corrected this in the text into ‘In this case, plant P limitation might be alleviated due to microbial P immobilization, because microbial biomass turnover and P re-mobilization from MBP would increase P availability to plants in the medium and long term’ (see Page 23 Line 11-14).

Page 2 Line 21: The conclusion is too simplistic and elusive. Authors should specifically highlight the key finding, relate it to a mechanism and/or explain its ecological relevance.

Response: Thank you for pointing out this issue. The conclusion has been stated as ‘Soil coarseness is a critical parameter affecting soil C and N storage and increases in soil coarseness can enhance microbial C and N limitation relative to P, potentially posing a threat to plant productivity in sandy grasslands suffering from desertification’ (see Page 2 Line 22).

Page 4 Line 19: Does it mean it can not be higher? If not, please rephrase.

Response: Thank you for this observation. It has been rephrased to read ‘Microbial biomass generally comprises 1-4% of soil organic C’ (see Page 4 Line 17).

Page 5 Line 5: ‘environments’ is not necessary.

Response: This sentence has been rephrased in Page 5 Line 3-5.

Page 5 Line 6: ‘this kind of essential microbial function’ is not clear, please rephrase.

Response: This has been rephrased to ‘microbial mineralization of SOM’ see Page 5 Line 3.

Page 5 Line 9: unnecessary words.

Response: These words have been deleted (Page 5 Line 6-7).

Page 5 Line 10: In which soil/ecosystem? Why exactly these enzymes? For instance, peptidases also reflect the N turnover, several key enzyme are catalyzing decomposition of cellulose and hemicellulose (main components of plant residues), i.e. cellulose, xylosidase, alfa-glucosidase, etc.

Response: The authors appreciate and fully agree with this observation. Indeed, peptidases (e.g. aminopeptidase), cellulase and hemicellulase are also key enzymes for N and C turnover. However, the selected enzymes of β-glucosidase (BG), NAG and acid phosphomonoesterase (AP) are commonly measured enzymes (Moorhead et al., 2012; Sinsabaugh et al., 2008, 2009, 2011; Waring et al., 2014). Also, these three enzymes catalyze rate-limiting steps of C, N and P turnover which supply assailable substrates of β-glucose, amino sugars and phosphate to microorganisms (Waring et al., 2014). Thus, we chose to determine the activities of BG, NAG and AP at the beginning of our study. For sure, we will determine the activities of peptidases, cellulase, hemicellulase and other essential enzymes in our future work. Also, we have added the soils/ecosystems in Page 5 Line 7.

Page 5 Line 16: replace ‘researches concerning’ by ‘studies on’

Response: This has been replaced; please see Page 5 Line 14.

Page 6 Line 22: The initial properties of the soil must be included, especially texture, bulk density, C-org, nutrients.

Response: Thanks so much for the observation. The information has been added in Page 7 Line 3-4.

Page 7 Line 4: change ‘simulated’ into ‘simulate’

Response: This has been corrected in Page 7 Line 8.

Page 7 Line 6: How did the bulk density change? Texture? Aggregates structure and stability? Seemingly, some protected (occluded) OM became available due to destructive manipulations. Was this accounted for at later stages? Another important and lacking here information, how the soil moisture changed with the coarseness?

Response: The bulk density significantly increased in the C10 treatment as compared to control. For soil texture and aggregate structure, the soil became sandier after sand addition, because the fraction of soil particles smaller than 0.25 mm (< 0.25 mm, or so-called soil microaggregates) decreased with increasing coarseness (please see Lü et al., 2016, Solid Earth 7: 549-556). This important information has also been mentioned in Page 18 Line 1-2. Indeed, destructive manipulation would make occluded SOM more available to microbial degradation, and this has been mentioned in the text (see Page 18 Line 12-14). Also, the information of soil moisture and water holding capacity has been added (Page 12 Line 7-10) and discussed in Page 21 Line 4-9, Page 24 Line 2-7.

Page 7 Line 8: So, this should mean the total mass (and volume) increase, correct?

Response: Thanks for this observation. But this is not the case. We kept the total mass constant and added different mass proportions of sand. This has been clarified in Page 7 Line 11.

Page 7 Line 8: -ed (Past Tense).

Response: This has been corrected in Page 7 Line 14.

Page 7 Line 10: ‘soils of 0-5 cm depth were taken out form all plots…’. Was this done in the control? How much of SOM was lost due to such a manipulation?

Response: Yes, this manipulation was also done in the control soil to keep all the conditions constant across treatments, except for sand mass proportion (Page 7 Line 17-18). However, we did not quantify the loss of SOM as compared to untreated soil right after we did the sterilization (mentioned in Page 7 Line 18-21). Due to the fact that all soils with and without sand addition were autoclaved at 105 ℃, this would not influence our interpretation of the data. Thanks so much for this observation.

Page 7 Line 15-16: replace ‘quadrat’ by ‘area’, and replace ‘at August’ by ‘annually (in August)’.

Response: These have been corrected in Page 8 Lines 4-5.

Page 7 Line 20: What was the moisture level between different plots?

Response: From C0 to C70, soil moisture decreased from 10.6% to 6.8%. Please see Page 12 Line 7-9.

Page 8 Line 1: I guess ‘in soils’

Response: This has been corrected in Page 8 Line 14.

Page 9 Line 3: replace ‘extracted’ by ‘amended’

Response: Thanks for the observation. This detailed description has been deleted as suggested by Reviewer 2 to omit details where a reference was cited for a technique (Page 9 Line 14-20).

Page 9 Line 16: This is not good for comparing the enzyme activities. To my own experience, as well as here (Sorensen et al., 2016. Biogeochemistry, 128, 141-154) and here (Daou et al., 2016. Soil Biology & Biochemistry, 93, 142-149) the freezing affects the enzyme activities and should be considered as a treatment. Therefore, authors should clearly define frozen samples and distinguish them from the fresh (unfrozen) soil. All the observed effects should also be considered through the prism of an additional freezing treatment.

Response: Thanks so much for this constructive suggestion. When we determined the enzyme activities, we actually did not compare activities from frozen soil samples with the fresh ones. Because we froze all sampled soils in the laboratory for later enzyme assays according to Allison et al. (2009, Soil Biology & Biochemistry, 41, 293-302) who determined β-glucosidase, N-acetyl-glucosaminidase and acid phosphatase, and Creamer et al. (2013, Biogeochemistry, 113, 307-321) who determined N-acetyl-glucosaminidase. According to the paper by Razavi et al. (2016), the pre-treatment of freezing-thawing can be assumed to correspond to snow thaw in the spring. Indeed, freezing-thawing cycles would affect activities of some of the enzymes. However, according to Daou et al. (2016, Soil Biology & Biochemistry, 93, 142-149), freezing-thawing cycles had no or a marginal significant effect on activities of β-glucosidase and acid phosphomonoesterase of different soils, who simulated freezing-thawing cycles in the laboratory. Thanks for the reviewer's observation and we will definitely consider the effects of laboratory storage (e.g. freezing) on soil enzyme activities in our future work. We have rephrased "fresh soil" into "frozen and field moist soil" (Page 10 Line 8) and added more information in the text (Page 10 Line 9-10) to be more accurate.

Page 10 Line 6: -ed (Past Tense)

Response: The 'express' has been replaced by 'expressed' in Page 10 Line 22.

Page 10 Line 10: This is actually not fully correct. The thing is, the "dilution" occurred for the very initial soil properties, which existed at the moment of excavation and mixing with sand in respective treatments. However, after replanting of sites, additional C was introduced (how much?) which was not accounted in the "dilution". Depending on an amount of "new C", the theoretical dilution values could be higher as the current and probably approach some of the "actual" measured parameters. From this point of view, comparison of "theoretical" and "actual" values could be erroneous. Authors could estimate how much C, N, and especially microbial biomass was introduced with the replanting and correct the dilution (C0 at the same time will not change).

Response: Thanks so much for the observation. Before replanting, we washed the plant roots to remove the soil associated with transplantation (descried in Page 8 Line 1-4). In this case, there would be no additional C, N and microbial biomass was not introduced with replanting. Due to the fact that C and N contents in the sand was not negligible, we have corrected the theoretical value based on mass proportions of sand and native soil by considering total C and N concentrations in river sand (please see Page 11 Line 4-9, Page 14 Line 20, and Figure 3a,b).

Page 11 Line 6: delete ‘decreased’

Response: This has been deleted in Page 12 Line 4.

Page 11 Line 8: Please, describe only the significant differences (nonsignificant – only those which are really key findings). In such a case, there is no need to use the word “significant” (provide respective references to statistical data, where necessary).

Response: Thanks so much for the suggestion. We checked throughout the manuscript to make sure to describe only the significant differences and only nonsignificant results when they are really key findings. Also, we deleted the word “significant” and “significantly” and provided respective references to statistical data in the Result section.

Page 11 Line 10: Not necessary, if the above mentioned prerequisite is met.

Response: This has been deleted (Page 12 Line 13). And the above mentioned prerequisite has been met throughout the manuscript.

Page 11 Line 7-22: The whole paragraph should be substantially shorted: there is no need to describe every singly difference and number. Leave only key message (result).

Response: This paragraph has been shortened and some of the numbers have been deleted (Page 12 Line 12-22).

Page 12 Line 1-12: Same as above: too detailed description and too much text/numbers. Enough to say that addition of sand decreased SOC content and stocks as well as TN by these many percent from the control (initial?).

Response: Thanks for the suggestion. We have shortened the text and deleted some of the numbers (Page 12 Line 12-22).

Page 13 Line 14: Many studied parameters could depend on a soil moisture dynamics in sites with increasing sand concentrations. Is such information available? Without such a key background, all other described parameters look secondary (or may directly result from a change in moisture). The same is true for the temperature regime if it changed due to the treatments.

Response: We measured soil moisture when sampling the soils (presented in Page 12 Line 7-9). Also, we have added the information of soil water holding capacity (Page 12 Line 9-11). The information has also been discussed in Page 21 Line 4-9.

Page 14 Line 9: Please, refer to my comment in the section “Statistical analyses”. The values reported here could vary after the respective correction for the “new C” (from replanting).

Response: We added a description of the new calculation in “Statistical analyses” (Page 11 Line 4-9). As mentioned above, there is no new C from replanting.

Page 14 Line 21: “faster decrease”—the statement is misleading: there were no measurements conducted in dynamics. Please, rephrase.

Response: We have rephrased the statement. And it now reads “For microbial biomass C, N, and P, measured values decreased less with increased soil coarseness than expected when accounting for theoretical dilution” (Page 15 Line 12-13).

Page 15 Line 2: “decreased faster”—same as above: rephrase.

Response: This has been corrected and it now reads “However, the acid PME activity decreased more strongly than the theoretical activity in C50 and C70 treatments” (Page 15 Line 15-16).

Page 15 Line 6: delete “in values” and replace “laboratory measurements” by “measure parameters”.

Response: These have been corrected, and it now reads “The difference between measured and theoretical soil parameters accounting for dilution effects” (Page 16 Line 4).

Page 15 Line 9: Actually, authors should know this. As the sand admixture was done on a weight basis and the C, N contents were measured, so the calculation is rather simple. Please, do this and state more exactly.

Response: We have done the calculation again by considering the total C and N contents in river sand (Page 11 Line 4-9, Page 14 Line 20), and interpreted the new data in Page 16 Line 6-11.

Page 15 Line 10: “might”—This means a very low probability of the observation/event. However, indeed, litter inputs and decomposition increase SOC and TN. Authors, should trust their findings!

Response: Thanks for this advice. After calculation of the theoretical C and N concentrations, we have rewritten this paragraph. Please see Page 16 Line 6-11.

Page 15 Line 11-13: Replace “plan” into “plant”, and “was” into “is”. Add “as compared with the theoretical dilution”. Delete “as their mobility”.

Response: We have replaced “plan” into “plant” and changed “was” into “is” in Page 16 Line 8. And “compared with theoretical accounting for dilution” has been added in Page 16 Line 12. “as their mobility” has been deleted in Page 16 Line 13.

Page 15 Line 15: Actually, authors should be able to prove this, as they collected soil samples from three soil depths. However, it is not clear, why DOC and other parameters were not measured in deeper sampled layers…

Response: Thanks so much for the reviewer’s observation. We did not measure enzyme activities in deeper soil layers because microbial activities in surface soil are more important in nutrient cycling than that in subsoils and that we did not have the means to measure all soil layers. Correspondingly, we did not measure available C, N and P concentrations in subsoils which are essential for microbial secretion of extracellular enzymes. However, we would like to consider this suggestion in our future work when doing researches on C and nutrient transportations across soil profile.

Page 15 Line 16: delete “which resulted in higher measured values than theoretical dilution”

Response: These words have been deleted in Page 16 Line 17.

Page 15 Line 18: Change “was not the case for” into “was not accounted for the”.

Response: This has been changed in Page 16 Line 19.

Page 15 Line 20: “soil physiochemical properties”— Exactly! Such as moisture regime…

Response: Thanks for the observation. And the information of soil moisture has been added in Page 12 Line 7-9, Page 21 Line 4.

Page 16 Line 2-3: Well, it is not that straightforward, especially based on the mentioned complexity of the field conditions. Another point, sand as such contained native microbial populations. As the sand was not sterile, they obviously were combined with the native soil microorganism. It is difficult to predict their fate, but under the conditions of higher nutrients supply and plant-derived deposits, they could proliferate and contribute to the less pronounced decrease with the coarseness (Fig. 2d) as compared with the theoretical dilution.

Response: Thanks so much for the suggestion. Even though the sand was not sterilized, the mixture of sand and native soil was sterilized in August 2012 (Fig. 1b). According to your advice, we have rephrased the sentence to make it straightforward (Page 16 Line 22; Page 17 Line 1-5).

Page 16 Line 6: I doubt they were conducted on the same site, so in the studied soil, number of other related parameters could change the picture.

Response: Yes, they were not conducted on the same site. And we agree with the reviewer that number of other related parameters could change the picture. Thus, we deleted this sentence (Page 17 Line 8-9).

Page 16 Line 7: There is no need to speculate: it could be other way round. If not measured, then omit as speculation.

Response: Soil bacteria were actually not measured in this study and this sentence was speculation. In this case, we followed the reviewer's advice to omit the speculation. This statement has been deleted in Page 17 Line 9-11.

Page 16 Line 18: "the decline of soil fine particles"—Was this really decline or dilution?

Response: It was a decrease mainly caused by dilution. And this has been rephrased in Page 18 Line 1-2 to read as "We previously found that the decrease of soil fine particles was mainly a result of sand dilution in this field experiment".

Page 16 Line 20: "decline"—Better to use deterioration, degradation…

Response: It has been replaced by "deterioration" (Page 18 Line 3).

Page 17 Line 1: Repetition of the statement 4 lines above. Omit or combine both sentences.

Response: Thanks. The repetition part has been deleted, and it now reads "Moreover, loss of SOC could result from limited stabilizing effects of mineral associations with increased soil coarseness" (Page 18 Line 7-9).

Page 17 Line 4: Again, this is just a repetition. Omit.

Response: This has been deleted (Page 18 Line 9-12).

Page 17 Line 8: as compared to what?

Response: As compared to later desertification stages (Page 18 Line 16), the loss of C and N was greater in light and moderate stages.

Page 17 Line: This is not exactly true! Of course, manipulations allow to distinguish between different effects, but probably authors were so far very lucky that they could "precisely control" and usually got "clear trends". I am afraid, authors confusing field and lab experiments. However, it is indeed worth to compare natural gradient studies and the current manipulation experiment in terms of potential (and actual) drawbacks of the latter. For instance, the level of manipulation such as digging the whole soil out, mixing (destroying of the natural structure), sterilization with heat etc., all these are not common for the natural desertification. Still, we need strongly manipulated experiments to better understand factors/mechanisms. So, I would like to encourage authors to look onto their results with criticism and caution when relating to natural pristine ecosystems.

Response: Thanks so much for this suggestion and it's really constructive. According to reviewer's comment, we deleted the original statement and described the difference between field manipulations and natural pristine ecosystems. Also, we emphasized the necessity to conduct field manipulation experiments and to caution when relating them to investigations from natural pristine ecosystems (Page 18 Line 19-22, Page 19 Line 1-2).

Page 17 Line 18: This is misleading: reduction of C stocks due to dilution with sand does not necessarily mean there should be an increased transfer to the atmosphere. Why? The opposite could be hypothesized: with the increasing mineral component (sand) the total surface of minerals should increase and absorb (stabilize) available C. So, in the long-term the C storage capacity should increase.

Response: We agree with the reviewer's point that the decrease in C stocks due to dilution does not mean there should be an increased transfer to the atmosphere. In this case, we deleted the original statement in Page 19 Line 5.

Page 17 Line 22: "decrease faster"—This is misleading: authors did not measure parameters in dynamics. Rewrite or omit as speculation.

Response: We have reworded this, and it now reads “Our results indicated that N stocks in the surface soil decreased more than that of soil C” (Page 19 Line 8-9).

Page 18 Line 1: replace “with” by “the”

Response: This has been corrected in Page 19 Line 9.

Page 18 Line 4: The sentence is confusing: “this” means the mentioned study of Zhou et al., or the current study? Next, the explanation of the difference between C and N stocks decrease is not clear at all. Clarify!

Response: Thanks for the observation. “this” means the current study and it has been corrected in Page 19 Line 13. The difference between C and N stocks decrease was clarified in Page 12 Line 17-18 where “Across all soil coarseness levels, soil C and N stocks decreased by as much as 31.8% and 54.0%, respectively”. This information has also been added in Page 19 Line 8-9.

Page 18 Line 7: “might”—this means such a probability is very low, so maybe no need to pay attention to this problem?

Response: As suggested by our plant productivity measurements, soil coarseness indeed decreased plant biomass (but data were not shown in this manuscript). Thus, we rephrased “might” into “would” (Page 19 Line 16).

Page 18 Line 14: Please, add here “discussed below (section 4.4)”. Otherwise, there is an impression authors “forgot” about P while discussing C and N in microbial biomass.

Response: Thanks for mentioning this and related information has been added. It now reads “while the increase in MBP under soil coarseness (discussed below, section 4.4) was not expected” (Page 20 Line 1).

Page 18 Line 19: Flawy statement: it is not possible to inhibit MBC (MBN). Inhibition occurs for the living organisms. If they die then MBC (MBN) may decrease.

Response: This has been rephrased to “decreasing MBC and MBN” (Page 20 Line 5).

Page 18 Line 19: Do authors mean the “synthesis” or properties of enzymes as chemical compounds/molecules? This difference is important.

Response: It is “synthesis”. This sentence has been rewritten as “factors directly or indirectly decreasing MBC and MBN would also suppress the synthesis of extracellular enzymes of BG, NAG and PME” (Page 20 Line 5).

Page 18 Line 21: delete “element”, and replace “microbial biomass production” by “microorganisms”.

Response: These have been corrected and it now reads “Soil C is essential for microorganisms and a vital source of growth” (Page 20 Line 8).

Page 19 Line 17: The reference is inappropriate: in the referred study, i.e. Wang et al. (2015), term “desiccation” is mentioned just once and with the reference to the study of Zhang et al. (2013). However, there are number of studies about the drought/drying effects on soil microbial populations. So, authors should refer to proper studies over the topic.

Response: Thanks for the observation. We have changed the reference into Alster et al. (2013) which studies drought effect on soil enzyme activities in a grassland ecosystem (Page 21 Line 4).

Page 20 Line 7: “salinity from sand”—This is not clear: the sand was a river sand, so why the salinity should increase? Did authors measure concentrations of salts? Please, explain.

Response: We are sorry, it should be “alkalinity” instead of “salinity” (Page 21 Line 21). Because the pH of river sand was 7.5 (Page 7 Line 14), and sand addition increased soil pH from 6.7 (C0) to 7.3 (C70) (Page 14 Line 3).

Page 20 Line 9: This is too simplistic and misleading statement. Different soils have different microbial community structure and behind the terms “fungi” and “bacteria” are enormous number of species, which certainly have specific ecological niches. What is optimal for one group of fungi at any case similar for the other. The same is for the bacteria. But most importantly, the referred studies do not supported the statement of authors. Thus, in the study of Bååth (1998) the contrasting information is reported: “Thus, soil pH appears to have no direct effect on the number of CFUs over the pH range studied here (4 to 8)”. Therefore, I strongly recommend authors to omit such a generalization here and in their future works.

Response: Thanks so much for the detailed comment. We fully agree with the reviewer and such a generalization has been omitted (Page 22 Line 1-2). In our future work, we must be more precise and avoid the generalization like this.

Page 20 Line 10: This is speculation as the community structure was not measured in the study. Delete.

Response: We agree with this. And the speculation has been deleted (Page 22 Line 3-4).

Page 20 Line 18: The sentence is too long. Split.

Response: This sentence has been rephrased to make it short (Page 22 Line 9-11).

Page 20 Line 21: In their study, it is very likely the moisture regime changed with the coarseness. It is not clear, if the soil moisture was measured in the experiment. However, water content in a semi-arid ecosystem (and generally) is one of the basic parameter affecting ecosystem (namely, microbial in the current context) functioning. So, it is very surprising nothing is even mentioned about this key factor. Without data on soil moisture content the discussion of other important but secondary to water regime environmental parameters seems to be incorrect.

Response: Thanks so much for the reviewer pointing out this important issue. We have added the data of soil moisture content which was measured directly after the soils being sampled (Page 12 Line 7-9). Also, we added and mentioned data of water holding capacity in both result (Page 12 Line 9-11) and discussion section (Page 21 Line 5).

Page 21 Line 4: “might be”—maybe not?

Response: Thanks. We have reworded “might be” into “could be” (Page 22 Line 16).

Page 21 Line 8: “unchanged”—Flawy expression: rephrase or omit.

Response: This has been rephrased into “Olsen-P was not affected by soil coarseness” (Page 22 Line 19).

Page 21 Line 11: Does this mean there was no increase of abiotic P supply? Otherwise it is difficult to assess whether decrease of P fixation on clays could overcompensate the decrease of biotic P release. Is there any information available on the abiotic P supply due to sand increase? What was actually P content of sand used in the study?

Response: Here, “suppression of P fixation” means increase of abiotic P release (fewer fixations by clay). We did not measure P content of the river sand, but the sand should contain very low concentration of Olsen-P. Decrease of clay content would definitely decrease Olsen-P fixed on its surface which would result in increase of abiotic P supply to balance decreased biotic supply. Only in this way, it will keep Olsen-P concentration stable (Fig. 4c). To make this clear, we have rephrased this sentence (Page 22 Line 20-22).

Page 21 Line 16: add “be” after “could”.

Response: The sentence has been rephrased (Page 22 Line 16).

Page 22 Line 2: add “in” before “contrast”.

Response: The word has been added in Page 23 Line 17.

Page 22 Line 7: The sentence is too long. Split.

Response: The sentence has been split into two (Page 23 Line 17-22).

Page 22 Line 14: Again, there was no comparison with the initial state (before the experiment) provided. What is shown, relative difference between treatments after 4 years of the experiment without any intermediate data. Therefore, it is inappropriate to speak about the rates.

Response: Thanks for the observation. We fully agree with the reviewer’s advice. Thus, we have rephrased “faster” into “more” (Page 24 Line 17).

Page 22 Line 16: Maybe first of all, the soil moisture decreased with the coarseness.

Response: Thanks for the advice. We have added soil moisture data and rephrased it into “resulting from decreases of soil moisture, C pools and fraction of fine particles” (Page 24 Line 19).

Page 23 Line 3: The soil depth effects were not clearly discussed and concluded on that. So, seemingly these data could be excluded from the manuscript.

Response: Thanks so much for the comments. We have excluded these data from the manuscript.

References cited in the response

Creamer, C.A., Filley, T.R., Olk, D.C., Stott, D.E., Dooling, V., and Boutton, T.W.:

Changes to soil organic N dynamics with leguminous woody plant encroachment into grasslands. Biogeochemistry, 113, 307-321, doi: 10.1007/s10533-012-9757-5, 2013.

Daou, L., Périssol, C., Luglia, M., Calvert, V., and Criquet, S.: Effects of drying–rewetting or freezing–thawing cycles on enzymatic activities of different Mediterranean soils. Soil Biol. Biochem., 93, 142-149, doi: http://dx.doi.org/10.1016/j.soilbio.2015.11.006, 2016.

Lü, L., Wang, R., Liu, H., Yin, J., Xiao, J., Wang, Z., Zhao, Y., Yu, G., Han, X., and Jiang, Y.: Effect of soil coarseness on soil base cations and available micronutrients in a semi-arid sandy grassland. Solid Earth, 7, 549-556, doi: 10.5194/se-7-549-2016, 2016.

Moorhead, D.L., Lashermes, G., and Sinsabaugh, R.L.: A theoretical model of C-and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. Soil Biol. Biochem., 53, 133-141, doi:10.1016/j.soilbio.2012.05.011, 2012.

Razavi, B.S., Blagodatskaya, E., Kuzyakov, Y.: Temperature selects for static soil enzyme systems to maintain high catalytic efficiency. Soil Biol. Biochem., 97, 15-22, doi:http://dx.doi.org/10.1016/j.soilbio.2016.02.018, 2016.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., and Gallo, M.E.: Stoichiometry of soil enzyme activity at global scale. Ecol. Lett., 11, 1252-1264, doi:10.1111/j.1461-0248.2008.01245.x, 2008.

Sinsabaugh, R.L., Hill, B.H., and Shah, J.J.F.: Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature, 462, 795-798, doi:10.1038/nature08632, 2009.

Sinsabaugh, R.L., and Shah, J.J.F.: Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. Biogeochemistry, 102, 31-43, doi:10.1007/s10533-010-9482-x, 2011.

Waring, B.G., Weintraub, S.R., and Sinsabaugh, R.L.: Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry, 117, 101-113, doi:10.1007/s10533-013-9849-x, 2014.

Response to Reviewer #2:

General comments:

1. Additions are needed to the discussion of experimental design and assumptions, water limitation, theoretical dilutions, and implications of the results.

Response: The discussion of experimental design and assumption and implications of the results have been added in Page 24 Line 2-12. We has also added data on soil moisture and water holding capacity and discussed their potential roles in affecting soil microbial biomass and activity (Page 12 Line 7-11, Page 21 Line 4-9, and Page 24 Line 2-7). We recalculated (Page 11 Line 4-9; Page 14 Line 20-22) and discussed (Page 15 Line 19) the theoretical values by considering C and N contents in the river sand.

2. The methods should be shortened by omitting details where a reference is cited for a technique, and the results could be shortened by highlighting interesting results and their implications rather than describing every test done on every treatment.

Response: The methods have been shortened by omitting details where a reference is cited for a technique (Page 9, Line 14-20, Page 10 Line 10-16). The results have also been shortened by only highlighting the most important results, deleting unimportant numbers (as also suggested by reviewer #1).

3. A thorough editing for English grammar and usage is needed.

Response: We have did a thorough editing for English grammar and hope it reads better now.

Specific comments:

The authors should justify why an experimental approach is needed to better understand the impacts of desertification and why their design is a realistic representation of soil change observed in natural setting. What are the limitations of existing natural gradient or long-term monitoring studies? Do the amendments made represent the range of variability observed in desertified site? Is it realistic to transplant vegetation of the same composition as a native community to the treated sites, as vegetation would change along with the soil in a naturally desertifying site?

Response: We have justified the need of an experimental approach in Page 6 Line 5-10 and Page 18 Line 19. The limitations of existing natural gradient or long-term monitoring studies have been discussed in Page 6 Line 5-10. The sand amendments actually showed a larger range of variability for parameters of soil particle size distribution and SOC contents as compared to other studies based on natural desertification gradients (Zhao et al., 2006, doi:10.1016/j.still.2005.03.009; Zhou et al., 2008, doi:10.1016/j.geoderma.2008.04.003). For transplanting vegetation of the same composition as a native community, we aimed to keep the initial plant community the same among soil coarseness gradients. In this way, we could monitor the change of plant productivity and community composition at the start of the experiment which can be affected by soil coarseness (Page 8 Line 2-4).

The experiment was conducted at an arid (450 mm MAP) site, but there is no discussion of water limitation of soil processes or even the precipitation patterns observed during the study. The results of the study could have been very different if it had been conducted during a relatively dry or relatively wet period. Soil moisture data would be ideal, but a simple soil water balance model might help to form a discussion of these issues and the differences between treatments in water holding capacity. It is entirely possible that nutrient limitation is rare in those soils and difference in microbial and enzymatic activity between soil coarseness levels is driven by soil moisture differences. Additionally, it could be useful to provide data, if available, on how soils outside the treatment area changed during the study as this reflect the climate during the period.

Response: Thanks so much for the reviewer’s observation. We agree that soil moisture is an essential parameter in this water-limited ecosystem. Thus, we have added the data on precipitation patterns (Fig. 2a), soil moisture (Fig. 2b) and water holding capacity (Fig. 2c) also in the text on Page 12 Line 5-11. It turned out that the precipitation was not extremely low directly before and during the sampling year

(Page 21 Line 4). Also, we have discussed their effects on soil enzymatic activity (Page 21 Line 5-9, Page 24 Line 2-7). So far, we have not obtained data of soils outside the treatment area. We will start to collect these data from this year as suggested by the reviewer.

The methods for developing theoretical dilutions for comparison with measured values need to be explained more clearly. As I understand it, the theoretical dilution value for, as an example, SOC content in a 50% amendment plot is simply 50% of the measured SOC in the control. This seems completely wrong and oversimplified, because the added sand contains SOC (see Page 7 Line 8). The theoretical dilution should be at least a weighted average the native soil and added sand or perhaps something more detailed based on the theoretical relationships between soil texture and properties. It is unclear how theoretical dilution comparisons serve to test the hypotheses in the manuscript, so an overall better description of the objectives of this method needs to be provided.

Response: We fully agree with the reviewer’s comment on calculating theoretical values as weighted averages between the native soil and added sand. In this case, we recalculated the theoretical values by considering both C and N contents in the added sand and native soils. We have updated the information in Page 11 Line 4-9, Page 14 Line 20, and Page 16 Line 6-11. The objective of this comparison between theoretical values and measured parameters has been stated in Page 15 Line 19.

Throughout the manuscript, there needs to be a stronger connection between the analyses performed and the hypotheses tested. There is a lot of listing of results in terms of things like enzyme activity and microbial biomass carbon, and while the connection to larger issues such as nutrient limitation is explained elsewhere in the paper (perhaps five pages previously), reading and understanding the results in the context of broader implications is an onerous task.

Response: Thanks so much for the constructive advice. We have provided the implications of our results in Page 24 Line 2-9. Also, we have clarified if our hypotheses were supported or rebutted (Page 17 Line 20, Page 19 Line 22, and Page 23 Line 5).

Response to Reviewer #3:

The current work is interesting. The authors should consider few issues to improve the manuscript:

1) The authors should take into consideration about absorption/inhibition of enzymatic activity by clay particles.

Response: Thanks so much for the observation. This has been mentioned in Page 17 Line 14-17.

2) The authors focus on organic carbon and dissolved organic carbon. But, what about humic substance?

Response: We expect that humic substance would also decrease with increasing soil coarseness. This is because microbial activity decreased with soil coarseness which would slow down the humification process. In our future work, we will analyze how humic substance change with soil coarseness.

Response to Reviewer #4:

1. All the results (soil, microbe and enzyme) may be simply a mixing effect. In other words, they are not affected by soil coarseness, but caused by the dilution of native soil (much higher CNP, microbe and enzyme) with river sand (much lower CNP, microbe and enzyme). The authors should report initial results right after mixing (baseline data), and then compare these data with the data measured in 2015. An appropriate way to interpret the “effect” of soil coarseness on soil properties should account for the effect of mixing.

Response: Thanks for the observation. We agree that dilution by river sand is an important factor of decreasing soil parameters (mentioned in Page 15 Line 21). However, soil coarseness itself is a process of incorporating sand into soil to cause a dilution effect as a result of wind erosion. Besides simply dilution, we also found that there were still biogeochemical processes occurring after correcting for dilution effects (Page 16 Line 4-22). Unfortunately, we did not collect baseline data at the beginning directly after mixing. We would expect that the baseline data might be similar to the theoretical dilution values where no biogeochemical interactions happened at the very beginning.

2. It is not clear how the authors calculate the “theoretical dilution”.

Response: We have clarified the calculation of the “theoretical dilution” (Page 11 Line 4-11. It is a weighted average of native soil and river sand for C and N contents. For other soil parameters, the theoretical values were calculated based on 90% (C10 treatment), 70% (C30), 50% (C50), and 30% (C70) of the measured parameters in the control soil (C10).

3. Any soil (and microbe, enzyme) associated with the “transplant” should be accounted for in the budget.

Response: Thanks so much for the observation. There is no soil associated with the transplant which was mentioned in Page 8 Line 1-2.

4. The experimental duration is very short. The soils were sampled in 2015, only one year after plant presence (by transplant in 2014). Moreover, the authors should provide a better description of the experimental design (with a timetable and few photos for various stages of the site).

Response: We transplanted the plants in July of 2013. And soils were sampled 2 years after plant presence. We have clarified this in Materials and Methods section (Page 8 Line 10). We admit that the experimental duration is still short. We will keep monitoring the plant-soil system to compared short-term and long-term responses. As suggested, we have provided an overview with a few photos showing various stages of the site preparation (Fig. 1).

5. The novelty and uniqueness of this study should be clearly presented.

Response: This has been stated at Page 6 Line 5-10 and Page 18 Line 19. Manipulative field experiments make it easier to determine the influence caused by treatments by keeping others factors, such as climatic conditions, soil types and level of manipulations constant. Field experiments simulating desertification would help to better understand mechanisms of changes in soil properties as affected by soil coarseness.

6. Plant-related data (such as above- and belowground biomass, species composition) should be presented.

Response: Thanks for the suggestion. In this manuscript, we tested 3 hypothesis: 1) soil coarseness would decrease both soil C and N contents as well as their stocks across soil depths; 2) soil coarseness would decrease microbial C, N, and P as well as the activities of C-, N-, and P-cycling enzymes because of the significant decrease in SOM; 3) soil coarseness would increase soil microbial C and N limitation relative to P as P could be supplied through abiotic processes. To test them, we believe the current data are enough. Thus, we do not present plant-related data here.

7. The three enzymes were assayed at different buffer pH (5.5, 6.0, 6.5), which is different than the soil pH (7.3). Most studies adjust the buffer pH to soil pH to make the results more reliable.

Response: The authors agree with the reviewer's comment. Buffering pH of enzyme assays to soil pH might be a way to determine enzymatic activities that is close to activities in field conditions. However, buffering the reaction system to the optimal pH (5.5, 6.0, 6.5) can obtain maximum enzymatic activities. Thus, we are measuring the maximum potential which makes it easier for researchers to compare the activities among various studies.

8. Soil pH (for all treatments and depths) should also be presented.

Response: As suggested by the Reviewer #1, all the data in subsoils have been deleted. Also, the soil pH data for all treatments and depths have been reported by Lü et al. (2016). Thus, we only cited their paper in terms of soil pH (Page 14 Line 3-4).

9. The authors should be careful in statements. For example, "our results also imply that expansion of desertified grassland ecosystems in dry regions of the world due to overgrazing and climate change might weaken the soil C sequestration potential and N retention capacity, which in turn lead to changes in grassland productivity and biodiversity in a long run." The results in this study say nothing about "soil C sequestration potential and N retention capacity", which need sophisticated studies using ^{13}C and ^{15}N isotope tracing.

Response: Thanks for the observation. We have rephrased "soil C sequestration potential and N retention capacity" into "soil C and N stocks" which can be supported by our data (Page 25 Line 5). The sentence now reads "Our results also imply that expansion of desertified grassland ecosystems in dry regions of the world due to overgrazing and climate change would decrease the soil C and N stocks, which in turn lead to changes in grassland productivity and biodiversity in a long run."

Response to Reviewer #5:

1. It is not clear how the "theoretical dilution values" were calculated. Were the river soil properties taken into account?

Response: Thanks for the observation. For C and N concentrations, the "theoretical dilution values" were calculated as weighted values of native sol and river sand based on mass proportions. However, for other soil parameters, such as DOC and microbial properties, the "theoretical values" were calculated as 90%, 70%, 50% and 30% of the measured parameters in C0 treatment for C10, C30, C50 and C70 treatments, respectively (Page 11 Line 4-12). We did this because total C and N in the sand were not negligible compared to the original soil (Page 14 Line 21), but DOC, available N and P, and microbial parameters were likely really low in the river sand.

2. After mixing and refilling, which of course destroyed the original soil structure, a new type of vegetation was planted on the plots. Soil samples were taken after one year. This is very short. I expect that the new vegetation had almost no effect of the newly created soil. Instead, the data presented in this manuscript are the result of 1) the mixing of two soils, 2) the soil-plant relations of the original soil at the plot, and 3) the properties of the river sediment. So, this has basically been a mixing of legacy effects, i.e. mixing the relicts of pre-experimental plant traits-litter quality-decomposition/mineralization process-microbial communities in a soil to get adjusted to changes in litter quality (new vegetation).

Response: The soil samples were sampled after two years of plant transplantation (from July 2013 to October 2015, clarified in Page 8 Line 10). This is indeed a short period. We agree with the point that the presented data are mainly the result of mixing of native soil and river sand (mentioned in Page 15 Line 21). However, there were still biogeochemical processes occurring after correcting for dilution effects, as suggested by the significant difference between theoretical dilution and measured parameters (Page 16 Line 4-22, Page 17 Line 1-17).

3. The experiment should have been done in a more “controlled way”. For instance, by using pure (inert) quartz sand and by analyzing the mixtures at the beginning of the experiment (new vegetation growth at time zero). Subsequently, the new vegetation should grow for at least a decade. Soil sampling at regular intervals (e.g. 3, 6, 9, … years) would yield a time series data set (C, N, P, EEA, etc.) that would reflect the adjustment of biogeochemical cycling (microbial communities, and C, N, P stocks) to the new vegetation as a function of soil texture.

Response: We agree with the reviewer’s comment that using pure quartz sand can minimize additional C, N and microbial activities inputs. Unfortunately, we did not analyze the mixtures at the beginning of the experiment. For the experimental time, we will take the reviewer’s advice to measure these parameters at least a decade to reflect the adjustment of biogeochemical cycling to the new vegetation. After getting long-term data, we can compared long-term results with short-terms ones as we did in this manuscript to better distinguish the mixing effects and interactive effects with vegetation.

With above corrections, the manuscript is hereby resubmitted to the journal. We are thankful for the reviewers’ work and glad to respond any further questions that you have. We look forward a positive response from you.

Thanking you,

Yong Jiang

Alteration of soil carbon and nitrogen pools and enzyme activities as affected by increased soil coarseness

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Abstract

Soil coarseness decreases ecosystem productivity, ecosystem carbon (C) and nitrogen (N) stocks, and soil nutrient contents in sandy grasslands subjected to desertification. To gain insight into changes in soil C and N pools, microbial biomass, and enzyme activities in response to soil coarseness, a field experiment was conducted by mixing native soil with river sand in different mass proportions: 0%, 10%, 30%, 50%, and 70% sand addition. Four years after establishing plots and two years after transplanting, soil organic C and total N concentrations decreased with increased soil coarseness down to 32.2% and 53.7% of concentrations in control plots, respectively. Soil microbial biomass C (MBC) and N (MBN) declined with soil coarseness down to 44.1% and 51.9%, respectively, while microbial biomass phosphorus (MBP) increased by as much as 73.9%. Soil coarseness significantly decreased the enzyme activities of β-glucosidase, N-acetyl-glucosaminidase, and acid phosphomonoesterase by 20.2%-57.5%, 24.5%-53.0%, and 22.2%-88.7%, used for C, N and P cycling, respectively. However, observed values of soil organic C, dissolved organic C, total dissolved N, available P, MBC, MBN, and MBP were often significantly higher than would be predicted from dilution effects caused by the sand addition. Soil coarseness enhanced microbial C and N limitation relative to P, as indicated by the ratios of β-glucosidase and N-acetyl-glucosaminidase to acid phosphomonoesterase (and MBC:MBP and MBN:MBP ratios). Enhanced microbial recycling of P might alleviate plant P limitation in nutrient-poor grassland ecosystems that are affected by soil coarseness. Soil coarseness is a critical parameter affecting soil C and N storage and increases in soil coarseness can enhance microbial C and N limitation relative to P, potentially posing a threat to plant productivity in sandy grasslands suffering from desertification.

Key words sandy grassland, grassland degradation, microbial biomass, microbial nutrient limitation, soil carbon stocks

1 Introduction

Desertification and wind erosion processes are main contributors of soil coarseness in arid and semi-arid grasslands (Su et al., 2004; Lü et al., 2016) constraining terrestrial net primary productivity (NPP) and ecosystem health (Lal, 2014; Lü et al., 2016). Currently, more than 30% of world total dryland area and 0.85 billion people are directly influenced by desertification and soil coarseness (Zhou et al., 2008; Chang et al., 2015). It has become increasingly clear that desertification and soil coarseness cause reductions in NPP (Peters et al., 2012), soil organic carbon (SOC) storage (Zhou et al., 2008), and nutrient retention (Delgado-Baquerizo et al., 2013). These effects of desertification and soil coarseness pose threats to world food security (Zhao et al., 2006), enhance the carbon-climate feedback (Lal, 2014), and cause soil deterioration and loss of soil structure (Su et al., 2004). Therefore, it is important to characterize impacts of soil coarseness on ecosystem processes in order to understand the mechanisms that cause desertification.

Microbes play a particularly important role in regulating plant nutrient availability in nutrient-poor environments (van der Heijden et al., 2008). Microbial biomass C generally comprises 1-4% of soil organic C, but it substantially contributes to stable soil C formation and nutrient supply (Brookes, 2001; Liang and Balser, 2011). For instance, microbial biomass phosphorus (MBP) has been regarded as a central feature in P cycling and plays an essential role in soil organic P mineralization (Richardson and Simpson, 2011). Soil nutrient supply is predominately controlled by microbial decomposition of soil organic matter (SOM) (although P can also be supplied through rock weathering) (Balota et al., 2014) and this process mainly relies on extracellular enzymes secreted by microorganisms and plants (Tabatabai, 1994; Wang et al., 2015). However, microbial mineralization of SOM is often constrained by C and nutrient availabilities (Cleveland et al., 2002), as well as by enzymatic stoichiometry and kinetics (Sinsabaugh et al., 2008, 2014; Wang et al., 2015). For instance, microbial P limitation decreased SOM decomposition in tropical soils~~, which could profoundly influence C cycling in tropical forests~~ (Cleveland et al., 2002). In both tropical and temperate soils, lower ratios of soil β-glucosidase (BG) to acid phosphatase (PME) and N-acetyl-glucosaminidase (NAG) to PME were observed, illustrating greater microbial P demand relative to C and N, respectively (Waring et al., 2014; Wang et al., 2015). Although a large number of studies have investigated desertification and soil coarseness effects on plant productivity (Zhao et al., 2006), soil C and N dynamics (Zhou et al., 2008), and soil nutrient availability to plants (Zhao et al., 2006; Li et al., 2013), studies on microbial biomass C, N and P, soil enzyme activities, and microbial nutrient limitations are still rare. Soil coarseness effects on stoichiometry of soil microbial biomass C:N:P and extracellular enzymes remain largely unknown (Cleveland and Liptzin, 2007; Sinsabaugh et al., 2008).

The Horqin Sandy Grassland is one of the main components of the Inner Mongolian grassland system belonging to the Eurasian steppe. The southeastern edge of the Horqin Sandy Grassland used to be a productive steppe grassland until the 1950s when overgrazing and over-cultivation occurred to support the rapidly growing human population (Li et al., 2004). After decades of over-utilization, the natural grassland has turned into an agro-pastoral zone and has undergone severe desertification and ecosystem retrogression (Yu et al., 2008). Soil coarseness is common in this area resulting from low plant cover and high annual wind speed (varying from 3.4 to 4.1 m s^{-1}) with frequent occurrence of gales (wind speed > 20 m s^{-1}) (Lü et al., 2016). Natural gradients and long-term monitoring studies have been used to examine desertification (Zhao et al., 2006; Zhou et al., 2008), but they do not control for climatic parameters, such as temperature, precipitation, and solar radiance. Therefore, controlled field experiments are necessary (*i.e.*, treatments with similar initial soil type and climatic factors) to better understand mechanisms of desertification caused by soil coarseness. In our previous work we showed that soil pH, fraction of soil fine particles (< 250 μm), soil exchangeable Ca and Mg, and soil available Fe were significantly lower with increased soil coarseness (Lü et al., 2016). In this study, we hypothesized that 1) soil coarseness would decrease both soil C and N concentrations as well as their stocks across soil depths; 2) soil coarseness would decrease microbial C, N, and P as well as the activities of C-, N-, and P-cycling enzymes because of the significant decrease in SOM; 3) soil coarseness would increase soil microbial C and N limitation relative to P as P could be supplied through abiotic processes.

2 Materials and methods

2.1 Study site and experimental design

The field experiment was located in Zhanggutai Town (42°43′N, 122°22′E, elevation 226.5 m a.s.l.) at the southeast of the Horqin Sandy Grassland of northern China. The mean annual temperature is 6.3 ℃ and the mean annual precipitation is 450 mm. The soil at this site is sandy with a bulk density of 1.66 g cm^{-3} and containing 4.04 g kg^{-1} SOC and 0.48 g kg^{-1} total N (TN). The soil is an Aeolic Eutric Arenosol in the FAO classification (IUSS Working Group WRB, 2014).

In May 2011, treatments were established in 4 m × 4 m plots arranged in a complete randomized design with five treatments and six replicates (Fig. 1a). Original plants were removed before preparing the soil. To simulate different degrees of soil coarseness, soils from three soil depths (0-20 cm, 20-40 cm, and 40-60 cm) were dug out and evenly mixed with 2 mm-sieved river sand in different mass proportions and then refilled back. By keeping the total mass constant to the control soil, we mixed different mass proportions of 0 (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70) sand with soil. Therefore, we had in total five sand addition treatments. The river sand contained 1.29±0.04 g kg^{-1} C and 0.15±0.03 g kg^{-1} N with a pH of 7.5±0.2. In August 2012, soils at 0-5 cm depth were taken out from all plots and sterilized at 105 ℃ for 3h to deactivate the seeds and prevent plant growth (Fig. 1b). The 0-5 cm soils were filled back and all plots were equilibrated for 1 year. Control plots without sand addition (C0) were subjected to the same method of sterilization. We did not include a control treatment with original soil and without sterilization, which would have allowed us to assess the effect of alteration in soil structure and physicochemical parameters caused by the sterilization method on biogeochemical processes. In July 2013, native plant species were transplanted from a local grassland in abundances similar to the native community composition (Fig. 1c). Before transplanting, roots of the plants were gently washed to remove native soil. Through soil sterilization and transplanting, we established plots that started with the same plant community similar to the nearby native grassland. Since 2014, a permanent area of 1 m × 1 m was set up within each plot to investigate plant community composition annually (in August) (Fig. 1d). Precipitation data from 2014 to 2016 were collected from the weather station located near the field site.

2.2 Soil sampling and chemical analysis

In October 2015, soil samples at 0-10 cm depth were taken by compositing three randomly placed soil cores within each plot (Fig. 1e). Fresh samples were passed through a 2 mm sieve, sealed in plastic bags, and stored at 4 ℃ until further processing.

The concentrations of SOC and TN were determined in air-dried and ground soils using an elemental analyzer (Vario MACRO Cube, Elementar, Germany). Sulfanilamide (C = 41.81%, N = 16.25%) was used as the internal standard. The SOC or TN stocks were calculated by multiplying SOC or TN concentrations with soil bulk density. The soil dissolved organic C (DOC) and total dissolved N (TDN) were extracted from 15 g fresh soils with 60 ml of 0.5 M K_2SO_4 and filtered through 0.45 μm acetate filter paper after shaking at 120 rpm for 1 h (Wang et al. 2015). The concentrations of DOC and TDN in filtrate were determined by a TOC analyzer (Multi N/C 3100, Analytikjena, Germany).

Soil pH was measured in a 1:2.5 (w/v) soil-to-water slurry using a PHS-3G digital pH meter (Precision and Scientific Crop., Shanghai, China). Soil particle size distribution was measured according to Zhao et al. (2006) by the pipette method. The fraction of soil fine particles < 250 µm was calculated as the sum of fine sand, silt and clay fraction. Soil exchangeable Ca and Mg were extracted with a 1 M CH_3COONH_4 solution (Ochoa-Hueso et al. 2014). Available Fe was extracted with diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell 1978). Soil exchangeable Ca and Mg and available Fe were analyzed with an atomic absorption spectrometer (AAS, Shimazu, Japan). Soil water holding capacity was determined on air-dried soils according to Wang et al. (2017).

2.3 Microbial biomass and enzyme activities

Microbial biomass C (MBC) and N (MBN) were measured using the fumigation-extraction method (Brooks et al., 1985). ~~Soil subsamples of 15 g were fumigated with chloroform ($CHCl_3$) at 25 ℃ for 24 h and non-fumigated subsamples were kept at the same conditions. After fumigation, both fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 in a 1:4 (w/v) soil-to-extractant ratio and shaken at 150 rpm for 1 h. After filtration, the soil extracts were analyzed by a TOC analyzer (Multi N/C 3100, Analytikjena, Germany) for extractable C and N contents.~~ Microbial biomass P was determined by extracting fumigated (also by $CHCl_3$) and non-fumigated soils with 0.5 M $NaHCO_3$ (pH 8.5) (Brookes et al., 1982). Briefly, 15 g of both fumigated and non-fumigated soil was mixed with 60 ml 0.5 M $NaHCO_3$ and shaken at 150 rpm for 1 h. After filtration, the extractable P concentration in the filtrate was determined with the molybdenum blue colorimetric method (Murphy and Riley, 1962). The measured P concentration in unfumigated soil samples is referred to as Olsen-P (Wang et al., 2016). To correct for incomplete extraction, we used efficiency factors of 0.45, 0.54, and 0.40 to calculate the actual concentrations of MBC, MBN and MBP, respectively (Dijkstra et al., 2012).

Enzyme assays for β-glucosidase (BG), N-acetyl-glucosaminidase (NAG) and acid phosphomonoesterase (PME) were performed on frozen and field moist soil samples. All soil samples were frozen in the same way to minimize potential freezing effects on enzyme activities (Razavi et al., 2016). For BG activity, ~~1.0 g of soil sample was mixed with a pH 6.0 modified universal buffer (0.1 M trihydroxymethyl aminomethane + 0.067 M citric acid monohydrate compound + 0.1 M boric acid). The~~ *p*-nitrophenyl-β-D-glucopyranoside (0.05 M) was added as the indicator substrate ~~to the mixture and then incubated for 1 h. After the reaction was stopped by 0.5 M $CaCl_2$ and 0.1 M trihydroxymethyl aminomethane (pH 12),~~and the product ~~was filtered and~~from the enzyme assay was analyzed with an UV-VIS spectrophotometer (UV-1700, Shimazu) at 410 nm (Tabatabai, 1994). The measurements of NAG and PME activities were similar to the assay of BG activity but now we used *p*-nitrophenyl-N-acetyl-β-D-glucosaminidine and *p*-nitrophenyl-phosphate as the substrates, respectively. The pH values of the reaction systems for NAG and PME were adjusted to 5.5 (Wang et al., 2015) and 6.5 (Tabatabai, 1994), respectively. The activities of BG, NAG and PME were expressed as production rates of p-nitrophenol

(PNP, in mg PNP kg soil^{-1} h^{-1}).

2.4 Statistical analyses

We calculated the theoretical dilution in SOC and TN caused by the addition of river sand. Because the river sand contained small amounts of total C and N, the values of theoretical dilution were calculated based on mass proportions of sand and soil by considering the concentrations of SOC and TN in both sand and the C0 treatment (without sand addition) for the C10, C30, C50, and C70 treatments, respectively. For other soil parameters, values of theoretical dilution were calculated as 90%, 70%, 50%, and 30% of the measured parameters in the C0 treatment (without sand addition) for the C10, C30, C50, and C70 treatments, respectively. One-way ANOVA was conducted to determine the effects of soil coarseness on SOC and TN concentrations and stocks, concentrations of DOC, TDN and Olsen-P, enzyme activities, and stoichiometry of microbial biomass and enzyme activities. Multiple comparisons of Duncan’s test were conducted to compare the significant differences among treatments for SOC and TN concentrations and stocks, DOC, TDN, Olsen-P, enzyme activities, and stoichiometry of microbial biomass and enzyme activities. Pearson correlation analysis was executed to determine relationships between microbial biomass as well as enzyme activities and soil physicochemical properties. Multivariate linear regression analyses (stepwise removal) were used to determine parameters that made significant contributions to the variation of microbial biomass and enzyme activities. All statistical analyses were performed in SPSS 16.0 (SPSS, Inc., Chicago, IL, U.S.A) with $\alpha < 0.05$.

3 Results

3.1 Soil moisture, water holding capacity and soil C and N pools with soil coarseness

The annual precipitation was 383.8 mm, 419.5 mm and 615.9 mm in 2014, 2015 and 2016, respectively (Fig. 2a). Precipitation in 2014 and 2015 were below and in 2016 above the mean annual precipitation from long-term records (450 mm). Soil moisture in samples taken in October 2015 decreased with increasing soil coarseness from 10.6% down to 6.8% ($P < 0.01$, Fig. 2b). Soil coarseness also decreased soil water holding capacity in the C50 and C70 treatments compared to the C0 treatment ($P < 0.01$, Fig. 2c).

The concentrations and stocks of both SOC and TN decreased with increasing soil coarseness. The SOC concentration decreased from 4.0 to 2.7 g kg soil^{-1} from in the C0 to the C70 treatment ($P < 0.01$, Fig. 3a). The TN content ranged from 0.48 to 0.22 g kg soil^{-1} and also decreased with increased soil coarseness ($P < 0.01$, Fig. 3b). Both SOC and TN stocks declined with increased soil coarseness ($P < 0.01$, Fig. 3c,d). Across all soil coarseness levels, soil C and N stocks decreased by as much as 31.8% and 54.0 %, respectively. The ratio of SOC to TN (soil C:N) increased with increased soil coarseness ($P < 0.01$, Fig. S1a). The DOC concentration decreased with increased soil coarseness ($P < 0.01$, Fig. 4a). The TDN concentration was lower in the C50 and C70 treatment compared to the C0 treatment ($P = 0.002$, Fig. 4b). However, soil Olsen-P content was not influenced by soil coarseness ($P = 0.84$, Fig. 4c).

3.2 Changes in soil microbial biomass under soil coarseness

The MBC decreased from 97.4 (in C0) to 54.5 (in C70) mg kg soil^{-1} with increased soil coarseness ($P < 0.01$, Fig. 4d). Similarly, MBN content declined from 11.3 (in C0) to 5.4 (in C70) mg kg soil^{-1} with increased soil coarseness ($P = 0.007$, Fig. 4e). However, MBP, ranging from 5.1 to 2.9 mg kg soil^{-1}, was higher in all treatments with sand addition than in the C0 treatment ($P = 0.012$, Fig. 4f).

Soil coarseness showed no effect on the ratio of MBC to MBN (microbial C:N) ($P = 0.64$, Fig. S1b). Microbial C:P decreased with increased soil coarseness with the highest ratio of 37.8 in the C0 treatment ($P = 0.003$, Fig. S1c). The microbial N:P ratio also decreased with increased soil coarseness, ranging between 4.1 (in C0) to 1.4 (in C50) ($P < 0.01$, Fig. S1d).

3.3 Soil extracellular enzyme activities as affected by soil coarseness

The activities of BG, NAG, and PME decreased significantly with increased soil coarseness. The BG activity decreased with soil coarseness by 20.2% to 57.5% (Fig. 5a). The NAG activity varied from 6.4 to 13.6 mg PNP kg soil^{-1} and decreased with soil coarseness by 24.5% to 53.0% (Fig. 5b). The activity of acid PME decreased from 109.1 mg to 12.3 mg PNP kg soil^{-1}, or by 22.2% to 88.7% with increased soil coarseness (Fig. 5c). The BG:NAG ratio was not affected by soil coarseness ($P = 0.41$, Fig. 5d). Both BG:PME and NAG:PME ratios were highest in the C70 treatment (both $P < 0.01$, Fig. 5e,f).

3.4 Correlation between soil parameters

Soil pH significantly increased from 6.7 (C0) to 7.3 (C70) in the 0-10 cm soil layer (Lü et al. 2016). The fraction of fine particles < 250 μm significantly decreased from 83.1% to 39.1% with soil coarseness in the 0-10 cm soil layer (Lü et al. 2016). Both MBC and MBN were significantly and positively correlated with SOC, TN, fine particles (< 250 μm), and DOC, but they were negatively correlated with soil pH (Table 1). As suggested by multiple regression models, soil fine particles accounted for 57.8% of the variation in MBC, and soil pH explained 53.3% of the variation in MBN (Table 1). A significant negative correlation was detected between MBP and Olsen-P, and Olsen-P explained 16% of the variation in MBP (Table 1). The three enzyme activities (BG, NAG and PME) were positively correlated with SOC, TN, soil fine particles, DOC, and TDN (Table 1). According to multiple regression models, TN explained 64.0% of the variation in BG activity, and 51.8% of the variation in NAG activity (Table 1). For PME activity, 90.3% of its variation was explained by SOC, soil fine particles, and soil pH (Table 1). Soil pH was negatively correlated with SOC, TN, DOC, TDN, exchangeable Ca and Mg, and available Fe (Table 2).

3.5 The differences between theoretical dilution and measured parameters

We calculated what the theoretical concentrations in soil parameters were only accounting for the dilution effect of adding river sand. Because the river sand contained low concentrations of total C and N (1.29 ± 0.04 g kg^{-1} C and 0.15 ± 0.03 g kg^{-1} N), total C and N contents in the river sand were considered when calculating the theoretical concentrations of SOC and TN.

The measured SOC concentration in the C70 treatment was significantly higher than the theoretical concentration ($P < 0.01$, Fig. 3a). However, measured TN concentrations were lower than theoretical concentrations in the C50 ($P = 0.01$) and C70 ($P < 0.03$) treatments (Fig. 3b).

The DOC concentrations in the C30, C50, and C70 treatments decreased less than expected accounting for theoretical dilution (Fig. 4a). Similarly, the theoretical TDN concentrations in the C10, C30, C50, and C70 treatments were lower than measured ($P < 0.05$, Fig. 4b). The theoretical Olsen-P concentrations decreased with increased soil coarseness ($P < 0.01$), but not the measured concentrations ($P = 0.84$) (Fig. 4c). For microbial biomass C, N, and P, measured values decreased less with increased soil coarseness than expected when accounting for theoretical dilution (Fig. 4d,e,f). No difference was detected between theoretical and measured activities for both BG and NAG (Fig. 5a,b). However, the acid PME activity decreased more strongly than the theoretical activity in the C50 and C70 treatments (Fig. 5c).

4 Discussion

In this study, we added sand at different levels to plots to mimic desertification effects on soil biogeochemical processes in a semiarid grassland in northern China. Because sand addition also resulted in dilution of the soil parameters we measured, we compared observed soil parameters with theoretical values we would expect if sand addition only caused a dilution effect. Therefore, differences between measured and theoretical soil parameters are caused by effects of soil coarseness other than dilution effects.

4.1 The difference between measured and theoretical soil parameters accounting for dilution effects

Compared to theoretical values accounting for dilution effects, we observed higher measured values of SOC in the C70 treatment. This could be due to increased plant C input through litter decomposition, which is commonly recognized as one of the main controllers of SOM content (Xiao et al., 2007). Plant uptake and N leaching processes might have contributed to the lower soil TN (measured) in the C50 and C70 treatments compared to theoretical values accounting for dilution effects (Fig. 3b).

Under field conditions, higher values of DOC, TDN, and Olsen-P compared with theoretical accounting for dilution, could be influenced by various factors~~ as their mobility~~. In this dryland ecosystem, greater evaporation than precipitation (Nielsen and Ball, 2014) could bring these mobile C, N and P fractions from the subsoil to the surface soil (Luo et al., 2016), which could have been stimulated with soil coarseness~~ which resulted in higher measured values than theoretical dilution~~. Moreover, higher soil extractable C, N and P contents could be derived from plant residues in the field (Halvorson et al., 2016), which was not accounted for by the theoretical dilution. Microbial biomass and activity could be affected by plant growth (Sanaullah et al., 2011; Zhang et al., 2010) and soil physiochemical properties (Sinsabaugh et al., 2008). With plants present, soil microorganisms could benefit from rhizosphere exudates or root turnover (Sanaullah et al., 2011; Wang et al., 2010), but might also suffer from nutrient limitation caused by plant-microbe competition (Dunn et al., 2006). Although plant-derived deposits may have decreased with increased coarseness, this decrease may have been less than the dilution factor caused by sand addition, so that observed microbial parameters were larger than theoretically predicted (Fig. 4d).

In this study site, sand addition increased soil pH from 6.7 to 7.3 (Lü et al., 2016). Soil pH is a fundamental controller on both microbial biomass and activity (Rousk et al., 2009). ~~Previous studies suggested that bacterial growth increased with higher soil pHs (Bååth, 1998; Rousk et al., 2009). Proliferation of soil bacteria with the increase of soil pH might be the reason of significantly higher microbial biomass C, N, and P in field condition than that of theoretical dilution (Fig. 2d,e,f).~~ The increase in soil pH might be the reason of a sharper decrease in acid PME activity with increased coarseness compared to the theoretical activity accounting for dilution (Fig. 5c), given that the optimal pH for acid PME activity is around 6.5 (Tabatabai, 1994). Absorption of acid PME by clay particles could also inhibit its activity (Dilly and Nannipieri, 1998), but this would not explain the greater decrease in measured acid PME activity with increased coarseness compared to the theoretical activity accounting for dilution.

4.2 Negative effect of soil coarseness on soil C and N pools

Consistent with our hypothesis, soil C and N concentrations and stocks significantly decreased with increased soil coarseness (Fig. 3). Soil fine particles (< 250 μm) are usually nutrient-rich and associated with SOM, but they are erodible during desertification (Li et al., 2004). We previously found that the decrease of soil fine particles was mainly a result of sand dilution in this field experiment (Lü et al., 2016). Removal of soil fine particles by wind erosion could result in a deterioration of soil structure and loss of SOC and nutrients (Su et al., 2004; Lal, 2014). Our results are consistent with those of Lal (2014) and Su et al. (2004), as indicated by a significant positive correlation between SOC concentration and the fraction of soil fine particles (Fig. S2). Moreover, loss of SOC could result from ~~deterioration in soil structure (or decrease in microaggregation) and~~ limited stabilizing effects of mineral associations with increased soil coarseness (Su et al., 2004). ~~Consistent with our findings, previous studies have suggested the negative responses of soil fine particles and SOM to desertification and soil coarseness (Zhao et al., 2006; Zhou et al., 2008).~~ The disturbance associated with the sand addition may further have resulted in some aggregate-protected or occluded SOC becoming more available to microbial degradation. Our findings of a linear decrease in soil C and N with increased soil coarseness are in contrast to Zhou et al. (2008) who found that declines in soil C and N concentrations were greater at light and moderate desertification stages as compared to later stages along different natural desertification gradients. The discrepancy might be due to the differences between field manipulations and field investigations along a natural gradient. Because our manipulative field experiment was conducted under somewhat artificial conditions (e.g., soil disturbance and sterilization), we caution with relating our results to natural pristine ecosystems. However, one of the advantages of our manipulative experiment is that it was done under controlled conditions, allowing for a better understanding of the mechanisms of alterations in soil C and N pools and enzyme activities caused by soil coarseness.

The soil C pool is the largest terrestrial C pool and even small changes in this pool can cause significant changes in the atmospheric CO_2 concentration (Houghton et al., 1999). ~~The reduction of soil C stocks with soil coarseness (by up to 38.2% in this study) would transfer C from soil to atmosphere which in turn would enhance the carbon-climate feedback and worsen the greenhouse effect (Duan et al., 2001; Yang et al., 2005).~~ Our results indicated that N stocks in the surface soil decreased more than that of soil C (54.0% *vs.* 31.8%, Fig. 2c,d). This is in contradiction of the findings by Zhou et al. (2008) who found a greater effect of desertification on ecosystem C storage than N storage. Greater losses of the ecosystem C stock relative to N resulted from a decrease in the soil C stock, but also from a decrease in grassland productivity in the study of Zhou et al. (2008). However, in the current study, only soil C and N stocks were determined showing a larger N decrease with increased soil coarseness relative to C. As N constrains the productivity of most terrestrial ecosystems (Vitousek et al., 1997), soil coarseness would aggravate plant N limitation in dryland ecosystems. In this case, dryland ecosystems, which cover 41% of world land area and are prone to soil coarseness, should be better protected from further degradation.

4.3 Soil coarseness decreased soil microbial biomass and enzyme activities

Significant decreases in MBC (Fig. 4d), MBN (Fig. 4e), and extracellular enzyme activities (Fig. 5a,b,c) supported our second hypothesis, while the increase in MBP under soil coarseness (discussed below, section 4.4) (Fig. 4f) was not expected. As suggested by the correlation and regression analyses, soil physicochemical properties contributed to the changes in microbial parameters (Table 1). Given the earlier findings that enzyme activities positively correlated with soil microbial biomass, factors directly or indirectly decreasing MBC and MBN would also suppress the synthesis of extracellular enzymes of BG, NAG and PME (Wang et al., 2014, 2015; Wolińska and Stępniewska, 2012).

Soil C is essential for microorganisms and a vital source of growth (Kemmitt et al., 2006). In this study, we observed positive relationships between MBC (or MBN, or enzyme activities) and SOC as well as DOC (Table 1). Based on our results, soil coarseness could possibly decrease soil microbial biomass and enzyme secretion through reduction of soil C pools (both SOC and DOC). The build-up of soil microbial biomass and secretion of enzymes (N-rich proteins) were also controlled by soil N pools (both TN and TDN), especially BG and NAG activities were mostly constrained by soil TN as suggested by multiple regression models (Table 1). These findings are consistent with large-scale surveys in grassland, agricultural and forest ecosystems (Waldrop et al., 2000; Kemmitt et al., 2006; Sinsabaugh et al., 2008).

Significant correlations between the fraction of soil fine particles and microbial parameters of MBC, MBN, BG, NAG and PME were found in our study (Table 1). The reduction in the fraction of soil fine particles with increased soil coarseness might have contributed to the decline in MBC and MBN. Soil coarseness, associated with desertification and a decrease in the fraction of soil fine particles, would provide less specific surface area where microbial cells could attach to and proliferate (Van Gestel et al., 1996). At the same time, decreases in the fraction of soil fine particles and smaller pore sizes expose microorganisms to predation by protozoa (Zhang et al., 2013) or to desiccation (Alster et al., 2013). Although it was not extremely dry during the year when soil samples were taken (2015) (Fig. 2a), a significant decrease in soil moisture (Fig. 2b) and water holding capacity (Fig. 2c) indicated that soil microorganisms were exposed to drier conditions with increased soil coarseness. Drier conditions would result in lower microbial activity and secretion of extracellular enzymes (Wang et al., 2014). With increased soil coarseness due to desertification, fewer extracellular enzyme could be stabilized by soil minerals (Dilly and Nannipieri, 1998) resulting in decreasing enzyme activities. Our results are in line with previous studies, which showed positive relationships between microbial biomass (as well as soil enzyme activities) and the size of mineral soil particles (Kanazawa and Filip, 1986; Van Gestel et al., 1996; Wang et al., 2015).

Soil pH is closely linked to biogeochemical processes in ecosystems and reflects the long-term plant-soil interactions and climatic variations (Kemmitt et al., 2006; Sinsabaugh et al., 2008; Rousk et al., 2009). Soil pH can strongly affect microbial growth, community composition, and activity through direct (*i.e.*, deformation of enzyme folding and deactivation of the enzyme active center) (Frankenberger and Johanson, 1982) and indirect pathways (affecting C and nutrient availabilities and metal solubility) (Kemmitt et al., 2006). Because the added sand was more alkaline than the soil, the pH of the surface soil increased nearly 0.6 units from the C0 to the

C70 treatment (Lü et al., 2016). ~~As previous studies suggest that the optimal pH value for fungal growth is around pH 4.5 but above pH 7 for bacteria (Bååth, 1998; Rousk et al., 2009), the decrease of MBC as affected by soil pH might mainly result from the inhibition of fungal growth instead of bacteria (Rousk et al., 2009).~~ Soil pH could decrease MBC indirectly by influencing soil C and nutrient availability (Kemmitt et al., 2005, 2006), which was also indicated by the negative correlations of soil pH with SOC, TN, DOC, TDN, exchangeable Ca and Mg, and available Fe (Table 2).

The optimal pH value for BG, NAG and PME activities are 6.0, 5.5 and 6.5, respectively (Tabatabai, 1994). Thus, the increase in soil pH from 6.7 to 7.3 with soil coarseness may have reduced the enzyme activities affecting the functional groups of amino acids and active center of enzymes (Dick et al., 2000).

4.4 Soil coarseness increased soil microbial C and N limitation relative to P

Soil coarseness increased the soil C:N ratio, which may result in decreased soil nutrient (such as N and P) availability through microbial immobilization (Marschner et al., 2015). Microbial growth or activities could be constrained by C limitation as suggested by the significant decrease in DOC with increased soil coarseness (Fig. 4a). Similarly, lower soil N availability, as partially confirmed by lower TDN in this study (Fig. 4b), might result in microbial N limitation. In contrast, Olsen-P was not affected by soil coarseness. Possibly, a decrease in SOM decomposition (and P mineralization) may have been counterbalanced by a net increase in abiotic supply of P due to suppression of P fixation associated with a lower clay content with increasing soil coarseness (Wang et al., 2016). This could alleviate microbial P limitation and even promote microbial P immobilization with lower soil C:N caused by desertification (Marschner et al., 2015). Previous studies also suggested that soil microorganisms were capable of accumulating P in biomass even under P-depleted conditions (Chapin et al., 2002; Paul, 2014). Thus, the third hypothesis was supported by our data.

In this study, significant lower ratios of microbial C:P (Fig. S1c) and N:P with increased soil coarseness (Fig. S1d), possibly due to microbial accumulation of P, suggest higher microbial P availability relative to C and N in soils (Cleveland and Liptzin, 2007). Indeed, significant increases in the BG:PME and NAG:PME ratios (Fig. 4f) suggest higher microbial C and N limitations relative to P in the C70 treatment (Wang et al., 2015). In this case, plant P limitation might be alleviated due to microbial P immobilization (Xu et al., 2013), because microbial biomass turnover and P re-mobilization from MBP would increase P availability to plants in the medium and long term. Our findings of altered microbial stoichiometry, however, suggest that microorganisms did not necessarily maintain fixed elemental ratios (or maintain stoichiometric homeostasis) like plants in response to external disturbances (Makino et al., 2003; Xu et al., 2013). These results were in contrast to findings from Cleveland et al. (2007) who suggested that C:N:P ratios of both soils and microorganisms were well-constrained at the global scale. However, our results were consistent with Sinsabaugh et al. (2008) who found that ratios of microbial C-, N-, and P-acquisition enzymes were variable and depended more on environmental parameters, such as substrate availability, soil pH and the stoichiometry of microbial nutrient demand.

Overall, by mixing soil with sand in different mass proportions to simulate various levels of soil coarseness as affected by desertification, the results implicate that desertification aggravates water limitation to plants and soil microorganisms as indicated by decreased soil moisture and water holding capacity in this semi-arid grassland. Soil moisture has proven to be the key parameter influencing soil nutrient mobilization and microbial biomass and activity in this water-limited ecosystem. Also, desertification would decrease soil C and N stocks and as well as soil C, N, and P cycling rates as suggested by lower extracellular enzyme activities. Our work sheds light on the essential role of microbial C, N, and P ratios and enzyme ratios in understanding nutrient limitation of microbial and ecosystem processes in terrestrial ecosystems subjected to desertification.

5 Conclusions

The significant decrease in both soil C and N pools was attributed to declines in the fraction of soil fine particles with increased soil coarseness. Soil TN stocks and concentrations decreased more than SOC, which might increase plant N limitation in this dryland ecosystem. Soil coarseness significantly decreased soil MBC, MBN, and activities of BG, NAG and PME resulting from the decreases in soil moisture, C pools and fraction of fine particles, and increases in soil pH. Enzymatic ratios, as well as microbial biomass C:N:P indicated higher microbial C and N limitation relative to P. This was also reflected in the decreased DOC and TDN and unchanged Olsen-P concentration with increased soil coarseness. These findings suggest that microbial biomass C, N, and P and activities of C-, N-, and P-acquiring enzymes could serve as good indicators for nutrient acquisition of microorganisms and plants. Our results also imply that expansion of desertified grassland ecosystems in dry regions of the world due to overgrazing and climate change would decrease the soil C and N stocks, which in turn lead to changes in grassland productivity and biodiversity in the long run.

Author contribution

Professor X. Han designed the experiment; and L. Lü did the field work to maintain the experiment. H. Liu and X. Feng helped with the measurements of soil analyses. R. Wang wrote the manuscript. C. Creamer and F.A. Dijkstra helped to improve the manuscript. The study was financially supported by the projects from G. Yu and Y. Jiang.

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References

Alster, C.J., German, D.P., Lu, Y., and Allison, S.D.: Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biol. Biochem., 64, 68-79, doi:http://dx.doi.org/10.1016/j.soilbio.2013.03.034, 2013.

Bååth, E.: Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques. Microb. Ecol., 36, 316-327, doi:10.1007/s002489900118, 1998.

Balota, E.L., Yada, I.F., Amaral, H., Nakatani, A.S., Dick, R.P., and Coyne, M.S.: Long-term land use influences soil microbial biomass P and S, and phosphatase and arylsulfatase activities, and S mineralization in a Brazilian oxisol. Land Degrad. Dev., 25, 397-406, doi:10.1002/ldr.2242, 2014.

Brookes, P.: The soil microbial biomass: concept, measurement and applications in soil ecosystem research. Microbes Environ., 16, 131-140, doi:http://doi.org/10.1264/jsme2.2001.131, 2001.

Brookes, P., Powlson, D., and Jenkinson, D.: Measurement of microbial biomass phosphorus in soil. Soil Biol. Biochem., 14, 319-329, doi:10.1016/0038-0717(82)90001-3, 1982.

Brookes, P.C., Landman, A., Pruden, G., and Jenkinson, D.: Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem., 17, 837-842, doi:10.1016/0038-0717(85)90144-0, 1985.

Chang, I., Prasidhi, A.K., Im, J., Shin, H.D., and Cho, G.C.: Soil treatment using microbial biopolymers for anti-desertification purposes. Geoderma, 253, 39-47, doi:10.1016/j.geoderma.2015.04.006, 2015.

Chapin III, F.S., Matson, P.A., and Vitousek, P.: Principles of Terrestrial Ecosystem Ecology. Springer Science & Business Media, 2011.

Cleveland, C.C. and Liptzin, D.: C: N: P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? Biogeochemistry, 85, 235-252, doi:10.1007/s10533-007-9132-0, 2007.

Cleveland, C.C., Townsend, A.R., and Schmidt, S.K.: Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems, 5, 0680-0691, doi:10.1007/s10021-002-0202-9, 2002.

Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., Bowker, M.A., Wallenstein, M. D., Quero, J.L., et al.: Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature, 502, 672-676, doi:10.1038/nature12670, 2013.

Dick, W., Cheng, L., and Wang, P.: Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biol. Biochem., 32, 1915-1919, doi:10.1016/S0038-0717(00)00166-8, 2000.

Dijkstra, F.A., Pendall, E., Morgan, J.A., Blumenthal, D.M., Carrillo, Y., LeCain, D.R., Follett, R.F., and Williams, D.G.: Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland. New Phytol., 196, 807-815, doi:10.1111/j.1469-8137.2012.04349.x, 2012.

Dilly, O. and Nannipieri, P.: Intracellular and extracellular enzyme activity in soil with reference to elemental cycling. J. Plant Nutri. Soil Sci., 161, 243-248, doi:10.1002/jpln.1998.3581610310, 1998.

Duan, Z., Xiao, H., Dong, Z., He, X., and Wang, G.: Estimate of total CO_2 output from desertified sandy land in China. Atmos. Environ., 35, 5915-5921, doi:10.1016/S1352-2310(01)00406-X, 2001.

Dunn, R.M., Mikola, J., Bol, R., and Bardgett, R.D.: Influence of microbial activity on plant–microbial competition for organic and inorganic nitrogen. Plant Soil, 289, 321-334, doi:10.1007/s11104-006-9142-z, 2006.

Frankenberger, W. and Johanson, J.: Effect of pH on enzyme stability in soils. Soil Biol. Biochem., 14, 433-437, doi: 10.1016/0038-0717(82)90101-8, 1982.

Halvorson, J.J., Schmidt, M.A., Hagerman, A.E., Gonzalez, J.M., and Liebig, M.A.: Reduction of soluble nitrogen and mobilization of plant nutrients in soils from US northern Great Plains agroecosystems by phenolic compounds. Soil Biol. Biochem., 94, 211-221, doi: http://dx.doi.org/10.1016/j.soilbio.2015.11.022, 2016.

Houghton, R.A., Hackler, J.L., and Lawrence, K.: The US carbon budget: contributions from land-use change. Science, 285, 574-578, doi:10.1126/science.285.5427.574, 1999.

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 Resources Reports No. 106. FAO, Rome, 2014.

Kanazawa, S. and Filip, Z.: Distribution of microorganisms, total biomass, and enzyme activities in different particles of brown soil. Microb. Ecol., 12, 205-215, doi:10.1007/BF02011205, 1986.

Kemmitt, S.J., Wright, D., Goulding, K.W., and Jones, D.L.: pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biol. Biochem., 38, 898-911, doi:10.1016/j.soilbio.2005.08.006, 2006.

Lal R. Desertification and soil erosion. In: Global Environmental Change. Springer Netherlands, 369-378, 2014.

Li, F., Zhao, L., Zhang, H., Zhang, T., and Shirato, Y.: Wind erosion and airborne dust deposition in farmland during spring in the Horqin Sandy Land of eastern Inner Mongolia, China. Soil Till. Res., 75, 121-130, doi:10.1016/j.still.2003.08.001, 2004.

Li, Y., Chen, J., Cui, J., Zhao, X., and Zhang, T.: Nutrient resorption in *Caragana microphylla* along a chronosequence of plantations: implications for desertified land restoration in North China. Ecol. Eng., 53, 299-305, doi:10.1016/j.ecoleng.2012.12.061, 2013.

Liang, C. and Balser, T.C.: Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. Nat. Rev. Microbiol., 9, 75-75, doi:10.1038/nrmicro2386-c1, 2011.

Luo, W., Sardans, J., Dijkstra, F. A., Peñuelas, J., Lü, X. T., Wu, H., Li, M.-H., Bai, E., Wang, Z., Han, X., and Jiang, Y.: Thresholds in decoupled soil-plant elements under changing climatic conditions. Plant Soil, doi:10.1007/s11104-016-2955-5, 2016.

Lü, L., Wang, R., Liu, H., Yin, J., Xiao, J., Wang, Z., Zhao, Y., Yu, G., Han, X., and Jiang, Y.: Effect of soil coarseness on soil base cations and available micronutrients in a semi-arid sandy grassland. Solid Earth, 7, 549-556, doi: 10.5194/se-7-549-2016, 2016.

Makino, W., Cotner, J., Sterner, R., and Elser, J.: Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C: N: P stoichiometry. Funct. Ecol., 17, 121-130, doi:10.1046/j.1365-2435.2003.00712.x, 2003.

Marschner, P., Hatam, Z., and Cavagnaro, T.: Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. Soil Biol. Biochem., 88, 169-177, doi:10.1016/j.soilbio.2015.05.023, 2015.

Murphy, J. and Riley, J.P.: A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, 27, 31-36, doi:10.1016/S0003-2670(00)88444-5, 1962.

Nielsen, U.N., and Ball, B.A.: Impacts of altered precipitation regimes on soil communities and biogeochemistry in arid and semi‐arid ecosystems. Glob. Change Boil., 21, 1407-1421, 10.1111/gcb.12789, 2015.

Paul, E.A.: Soil Microbiology, Ecology and Biochemistry. Academic Press, 2014.

Peters, D.P., Yao, J., Sala, O.E., and Anderson, J.P.: Directional climate change and potential reversal of desertification in arid and semiarid ecosystems. Global Change Biol., 18, 151-163, doi:10.1111/j.1365-2486.2011.02498.x, 2012.

Razavi, B.S., Blagodatskaya, E., Kuzyakov, Y.: Temperature selects for static soil enzyme systems to maintain high catalytic efficiency. Soil Biol. Biochem., 97, 15-22, doi:http://dx.doi.org/10.1016/j.soilbio.2016.02.018, 2016.

Richardson, A.E. and Simpson, R.J.: Soil microorganisms mediating phosphorus availability update on microbial phosphorus. Plant Physiol., 156, 989-996, doi:http://dx.doi.org/10.1104/pp.111.175448, 2011.

Rousk, J., Brookes, P.C., and Bååth, E.: Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl. Environ. Microb., 75, 1589-1596, doi:10.1128/AEM.02775-08, 2009.

Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., and Kuzyakov, Y.: Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. Appl. Soil Ecol., 48, 38-44, doi:10.1016/j.apsoil.2011.02.004, 2011.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., and Gallo, M.E.: Stoichiometry of soil enzyme activity at global scale. Ecol. Lett., 11, 1252-1264, doi:10.1111/j.1461-0248.2008.01245.x, 2008.

Sinsabaugh, R.L., Belnap, J., Findlay, S.G., Shah, J.J.F., Hill, B.H., Kuehn, K.A., Kuske, C.R., Litvak, M.E., Martinez, N.G., Moorhead, D.L., and Warnock, D.D.: Extracellular enzyme kinetics scale with resource availability. Biogeochemistry, 121, 287-304, doi:10.1007/s10533-014-0030-y, 2014.

Su, Y., Zhao, H., Zhao, W., and Zhang, T.: Fractal features of soil particle size distribution and the implication for indicating desertification. Geoderma, 122, 43-49, doi:10.1016/j.geoderma.2003.12.003, 2004.

Tabatabai, M.: Soil enzymes. In: Mickelson, S.H., Bifham, J.M. (Eds.), Methods of soil analysis. Part 2: Microbiological and Biochemical Properties. Soil Science Society of America, Inc., Madison, WI, pp. 775-833, 1994.

Van Gestel, M., Merckx, R., and Vlassak, K.: Spatial distribution of microbial biomass in microaggregates of a silty-loam soil and the relation with the resistance of microorganisms to soil drying. Soil Biol. Biochem., 28, 503-510, doi:10.1016/0038-0717(95)00192-1, 1996.

van der Heijden, M.G., Bardgett, R.D., and Van Straalen, N.M.: The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett., 11, 296-310, doi:10.1111/j.1461-0248.2007.01139.x, 2008.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., and Tilman, D.G.: Human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl., 7, 737-750, doi:10.1890/1051-0761(1997)007, 1997.

Waldrop, M., Balser, T., Firestone, M.: Linking microbial community composition to function in a tropical soil. Soil Biol. Biochem., 32, 1837-1846, doi:10.1016/S0038-0717(00)00157-7, 2000.

Wang, R., Creamer, C.A., Wang, X., He, P., Xu, Z., Jiang, Y.: The effects of a 9-year nitrogen and water addition on soil aggregate phosphorus and sulfur availability in a semi-arid grassland. Ecol. Indic., 61, 806-814, doi:10.1016/j.ecolind.2015.10.033, 2016.

Wang, R., Dorodnikov, M., Yang, S., Zhang, Y., Filley, T.R., Turco, R.F., Zhang, Y., Xu, Z., Li, H., and Jiang, Y.: Responses of enzymatic activities within soil aggregates to 9-year nitrogen and water addition in a semi-arid grassland. Soil

Biol. Biochem., 81, 159-167, doi:10.1016/j.soilbio.2014.11.015, 2015.

Wang, R., Filley, T.R., Xu, Z., Wang, X., Li, M.-H., Zhang, Y., Luo, W., and Jiang, Y.: Coupled response of soil carbon and nitrogen pools and enzyme activities to nitrogen and water addition in a semi-arid grassland of Inner Mongolia. Plant Soil, 381, 323-336, doi: 10.1007/s11104-014-2129-2, 2014.

Wang, R., Gibson, C.D., Berry, T.D., Jiang, Y., Bird, J.A., Filley, T.R.: Photooxidation of pyrogenic organic matter reduces its reactive, labile C pool and the apparent soil oxidative microbial enzyme response. Geoderma, 293, 10-18, doi:http://dx.doi.org/10.1016/j.geoderma.2017.01.011, 2017.

Wang, C., Long, R., Wang, Q., Liu, W., Jing, Z., and Zhang, L.: Fertilization and litter effects on the functional group biomass, species diversity of plants, microbial biomass, and enzyme activity of two alpine meadow communities. Plant Soil, 331, 377-389, doi:10.1007/s11104-009-0259-8, 2010.

Waring, B.G., Weintraub, S.R., and Sinsabaugh, R.L.: Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. Biogeochemistry, 117, 101-113, doi:10.1007/s10533-013-9849-x, 2014.

Wolińska, A. and Stępniewska, Z.: Dehydrogenase activity in the soil environment. In: Canuto (ed) Dehydrogenases, in print edn. InTech, Rijeka, pp 183-209, 2012.

Xiao, C., Janssens, I.A., Liu, P., Zhou, Z., and Sun, O.J.: Irrigation and enhanced soil carbon input effects on below‐ground carbon cycling in semiarid temperate grasslands. New Phytol., 174, 835-846, 10.1111/j.1469-8137.2007.02054.x, 2007.

Xu, X., Thornton, P.E., and Post, W.M.: A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Global Ecol. Biogeogr. 22, 737-749, doi:10.1111/geb.12029, 2013.

Yan, H., Wang, S., Wang, C., Zhang, G., and Patel, N.: Losses of soil organic carbon under wind erosion in China. Global Change Biol., 11, 828-840, doi:10.1111/j.1365-2486.2005.00950.x, 2005.

Yu, Z., Chen, F., Zeng, D, Zhao, Q., and Chen, G.: Soil inorganic nitrogen and microbial biomass carbon and nitrogen under pine plantations in Zhanggutai sandy soil. Pedosphere, 18, 775-784, doi:10.1016/S1002-0160(08)60073-9, 2008.

Zhang, S., Li, Q., Lü, Y., Zhang, X., Liang, W.: Contributions of soil biota to C sequestration varied with aggregate fractions under different tillage systems. Soil Biol. Biochem., 62, 147-156, doi:10.1016/j.soilbio.2013.03.023, 2013.

Zhang, C.B., Wang, J., Liu, W.L., Zhu, S.X., Ge, H.L., Chang, S.X., Chang, J., and Ge, Y.: Effects of plant diversity on microbial biomass and community metabolic profiles in a full-scale constructed wetland. Ecol. Eng., 36, 62-68, doi:10.1016/j.ecoleng.2009.09.010, 2010.

Zhao, H., Zhou, R., Zhang, T., and Zhao, X.: Effects of desertification on soil and crop growth properties in Horqin sandy cropland of Inner Mongolia, north China. Soil Till. Res., 87, 175-185, doi:10.1016/j.still.2005.03.009, 2006.

Zhou, R., Li, Y., Zhao, H., and Drake, S.: Desertification effects on C and N content of sandy soils under grassland in Horqin, northern China. Geoderma, 145, 370-375, doi:10.1016/j.geoderma.2008.04.003, 2008.

Tables

Table 1 Regression statistics relating soil physicochemical properties and microbial parameters.

	SOC	TN	< 250 μm	pH	DOC	TDN	Olsen-P	Multiple
MBC	0.59	0.65	0.76**	-0.67	0.52	–	–	0.76
MBN	0.50	0.56	0.58	-0.73**	0.43	–	–	-0.73
MBP	–	–	–	–	–	–	-0.40*	-0.40
BG	0.79	0.80**	0.74	-0.73	0.73	0.54	–	0.80
NAG	0.69	0.72**	0.70	-0.68	0.60	0.45	–	0.72
PME	0.86**	0.90	0.90**	-0.88**	0.86	0.60	–	0.95

When significant ($P < 0.05$), R values of linear and multiple regressions are shown. For multiple regressions (Multiple), significant contributions of soil physicochemical properties and microbial parameters are indicated with * ($P < 0.05$) and ** ($P < 0.01$), after stepwise removal of non-significant parameters.

Table 2 Relationships between soil pH and soil organic carbon (SOC), total nitrogen (TN), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), exchangeable Ca (Ca^{2+}), exchangeable Mg (Mg^{2+}) and available Fe (Fe^{2+}). ** indicates significant correlation between soil parameters at $P < 0.01$.

	SOC	TN	DOC	TDN	Ca^{2+}	Mg^{2+}	Fe^{2+}
pH	-0.77**	-0.85**	-0.76**	-0.43**	-0.67**	-0.75**	-0.87**

Figures

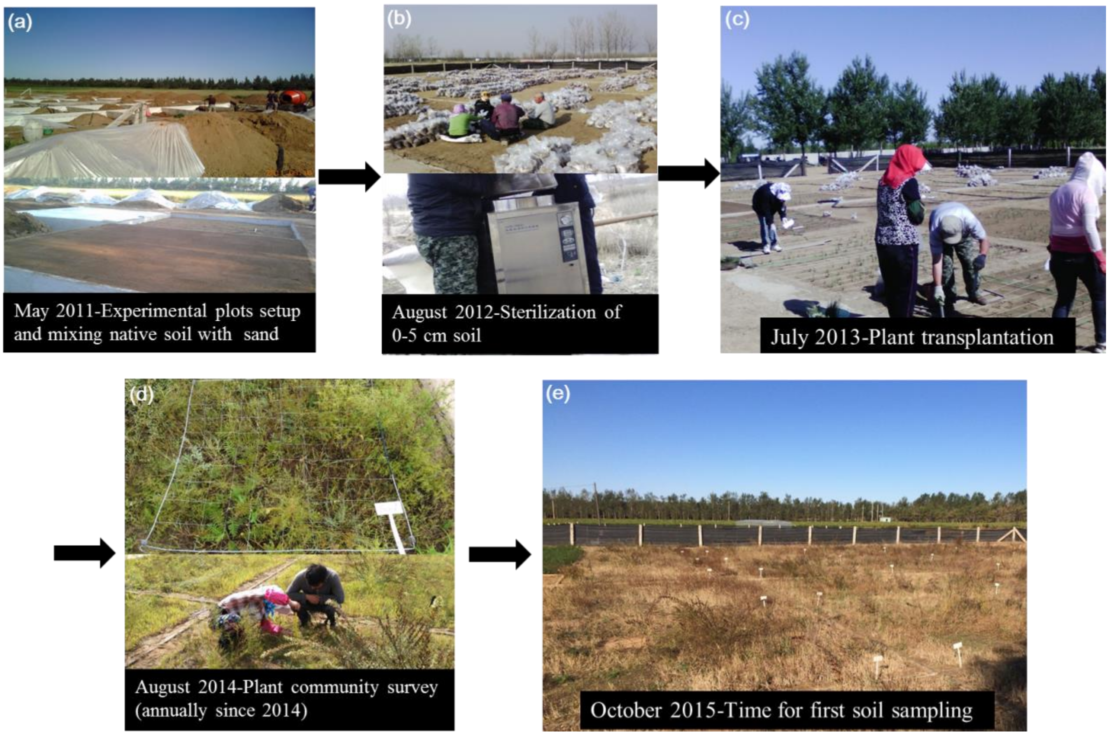

Fig. 1 Overview of field experimentation with photos taken at various stages of the experiment.

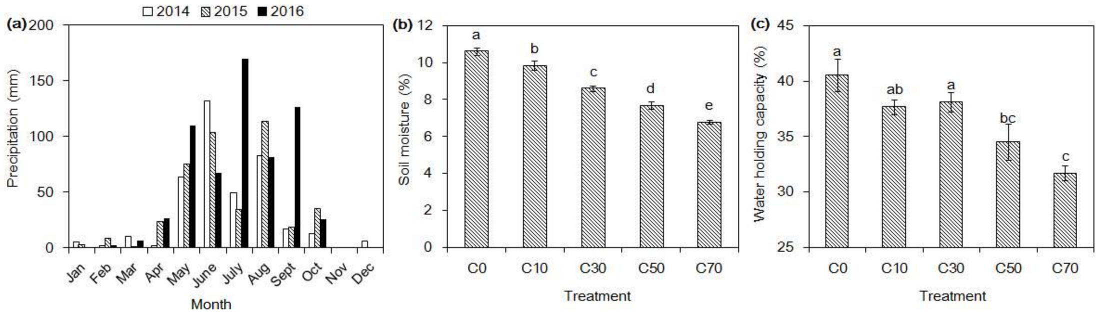

Fig. 2 Monthly precipitation from 2014 to 2016 (a) and response of moisture (b) and water holding capacity (c) in soil samples taken in October 2015 to different degrees of soil coarseness: 0% sand addition (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70). Data represent mean ± standard error (n=6). Letters indicate significant differences among treatments.

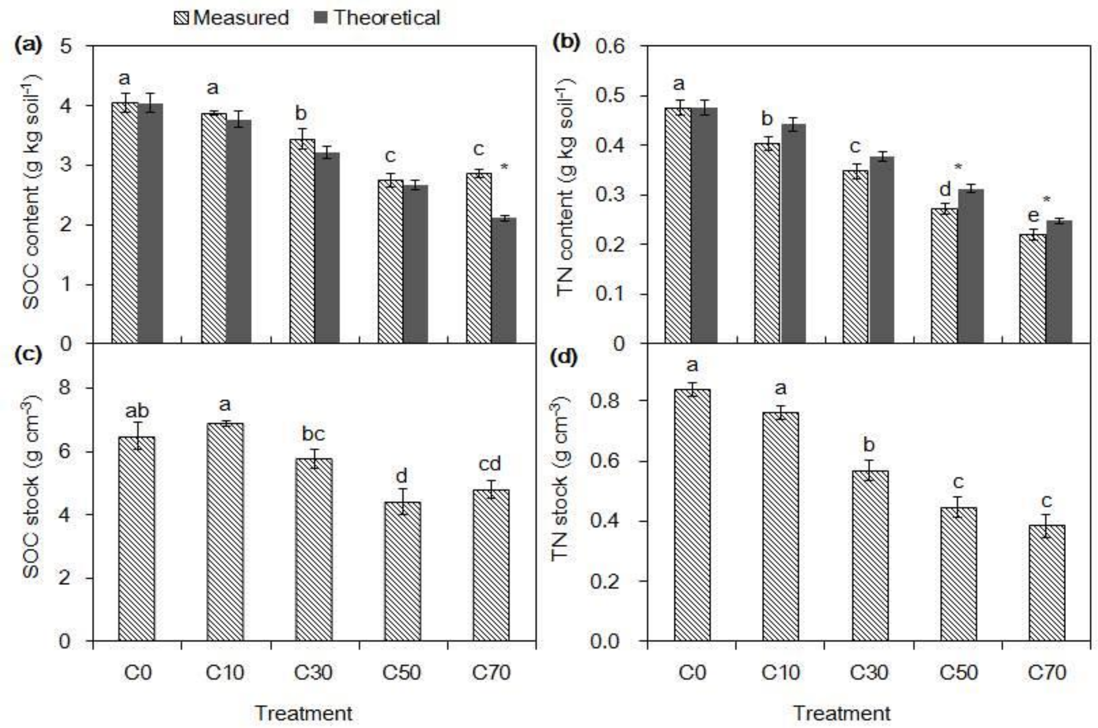

Fig. 3 Soil organic carbon (SOC) and total nitrogen (TN) concentrations (a and b, respectively) and stocks (c and d, respectively) as affected by different degrees of soil coarseness: 0% sand addition (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70). Dashed bars represent values obtained from laboratory measurements, while black bars are values calculated from theoretical dilution. Data represent mean ± standard error (n=6). Letters indicate significant differences among treatments. Asterisks indicate significance between values from laboratory measurements and theoretical values accounting for dilution within one treatment.

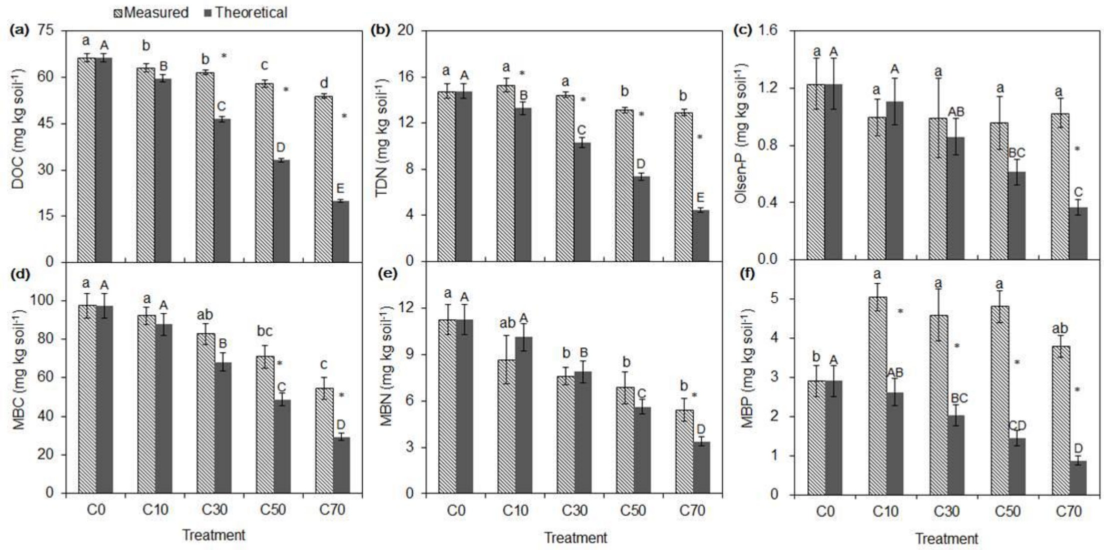

Fig. 4 Changes in soil (a) dissolved organic carbon (DOC), (b) total dissolved nitrogen (TDN), (c) Olsen phosphorus (Olsen-P), (d) microbial biomass carbon (MBC), (e) microbial biomass nitrogen (MBN), and (f) microbial biomass phosphorus (MBP) as affected by different degrees of soil coarseness: 0% sand addition (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70). Dashed bars represent values obtained from laboratory measurements, while black bars are values calculated from theoretical dilution. Data represent mean ± standard error (n=6). Letters indicate significant differences among treatments. Asterisks indicate significance between values from laboratory measurements and theoretical values accounting for dilution within one treatment.

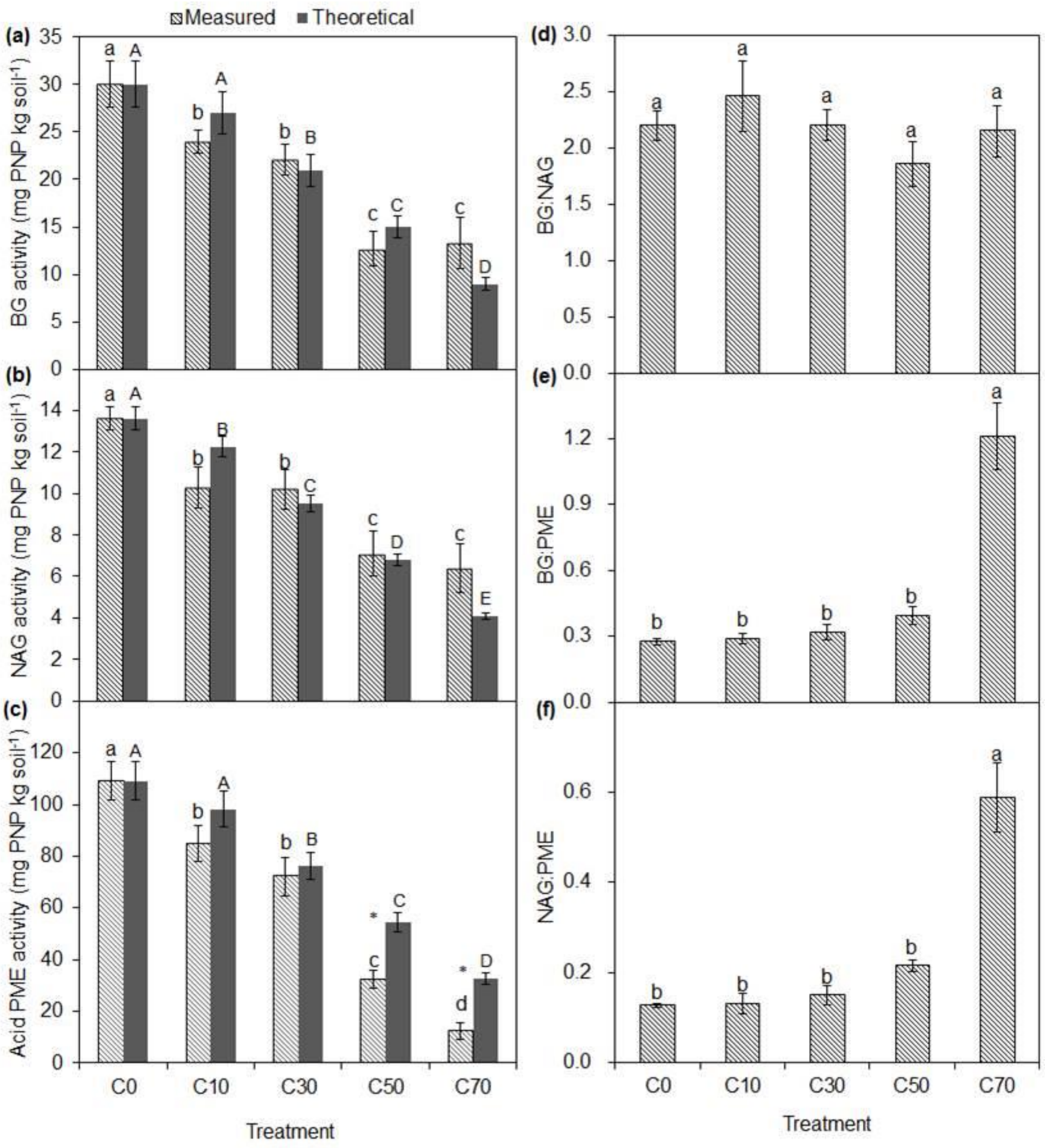

Fig. 5 Changes in (a) activities of soil β-glucosidase (BG), (b) N-acetyl-glucosaminidase (NAG), (c) acid phosphomonoesterase (PME), (d) the ratio of BG:NAG, (e) BG:PME, and (f) NAG:PME as affected by different degrees of soil coarseness: 0% sand addition (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70). Dashed bars represent values obtained from laboratory measurements, while black bars are values calculated from theoretical dilution. Data represent mean ± standard error (n=6). Letters indicate significant differences among treatments. Asterisks indicate significance between values from laboratory measurements and theoretical values accounting for dilution within one treatment.