Anonymous Referee #1

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General comments: This manuscript (MS) examined soil microbial C:N:P stoichiometry along a large aridity gradient across different temperate grassland biomes, using 58 plots sampled from a 2100-km transect in Inner Mongolian, China. The dataset is good not only in that studies of soil microbial stoichiometry along great aridity gradient is still limited, and also because they examined difference in patterns and potential drivers between top and subsoil. The MS is generally well written, though there were some clear typewriting flaws and some sentences not easy to understand. I suggest a minor revision, mainly on the improving of the statistical analyses, and clarity and readability of the MS.

Response: Thanks for your positive comments. We have carefully revised our manuscript according to your suggestions. Please see more details in our reply to your specific comments.

Specific comments: The title: from the title I have first thought that you have sampled much deeper than 10 cm. However, I then realized that you have sampled to a depth of 20 cm. I would suggest to revise the title and delete “How deep do we dig for surface soil?”. The rest part of the title is good enough.

Response： Thanks for your constructive comments. Following your suggestion, we have revised title as "A comparison of patterns of microbial C : N : P stoichiometry between topsoil and subsoil along an aridity gradient".

Methods: In 2.2, how the above-ground biomass data was obtained was not clarified, but this data was used in statistical analyses. In addition, the method to calculate aridity index need to be introduced. Though the data was extracted from database, the Equation needs to be introduced for readers to better understand the biological meanings. Further, there were several different indices for aridity.

Response: Thanks for your suggestions. We are sorry that we missed this information. We have included more details on the methods of data extracting in the revised manuscript. We measured the aboveground biomass by harvesting the aboveground part of the plants in the sampling plot. We have revised as" Thanks for your suggestions. We are sorry that we missed this information. We have included more details on the data extraction and data acquisition methods in the revised manuscript. We have revised it as "Aridity index was extracted them from the Global Aridity Index (Global-Aridity) dataset, which provide high-resolution (30 arc-seconds or ~ 1km at equator) global raster climate data for the 1950-2000 period (http://www.cgiarcsi.org) (Zomer, Trabucco, Bossio, & Verchot, 2008). The specific calculation formula is as follows:

$$Aridity\ Index\ (AI) = MAP / MAE$$

$$PET=0.0023 \cdot RA \cdot (Tmean+17.8) \cdot TD^{0.5} \text{(mm/month)}$$

where MAP represents mean annual precipitation, obtained from the WorldClim Global Climate Data (Hijmans et al. 2005); MAE represents mean annual potential evapo-transpiration (PET); Tmean represents monthly mean temperature, TD is calculated as the difference between monthly maximum and minimum temperatures; RA represents the extra-terrestrial radiation on top of atmosphere.

Statistical analyses: The plots were sampled from a northeast to southwest transect, which include variations in both temperature and aridity. It remains unclear how temperature contribute to the geographic patterns reported here. Considering the large difference in aridity from typical steppe to desert steppe, personally I agree with your results on the role of aridity on microbial stoichiometry. However, you may consider to include temperature as a predictor, to make your conclusions more robust.

Response: thank you for your helpful suggestions. We have added related figure, result and discussion in the manuscript.

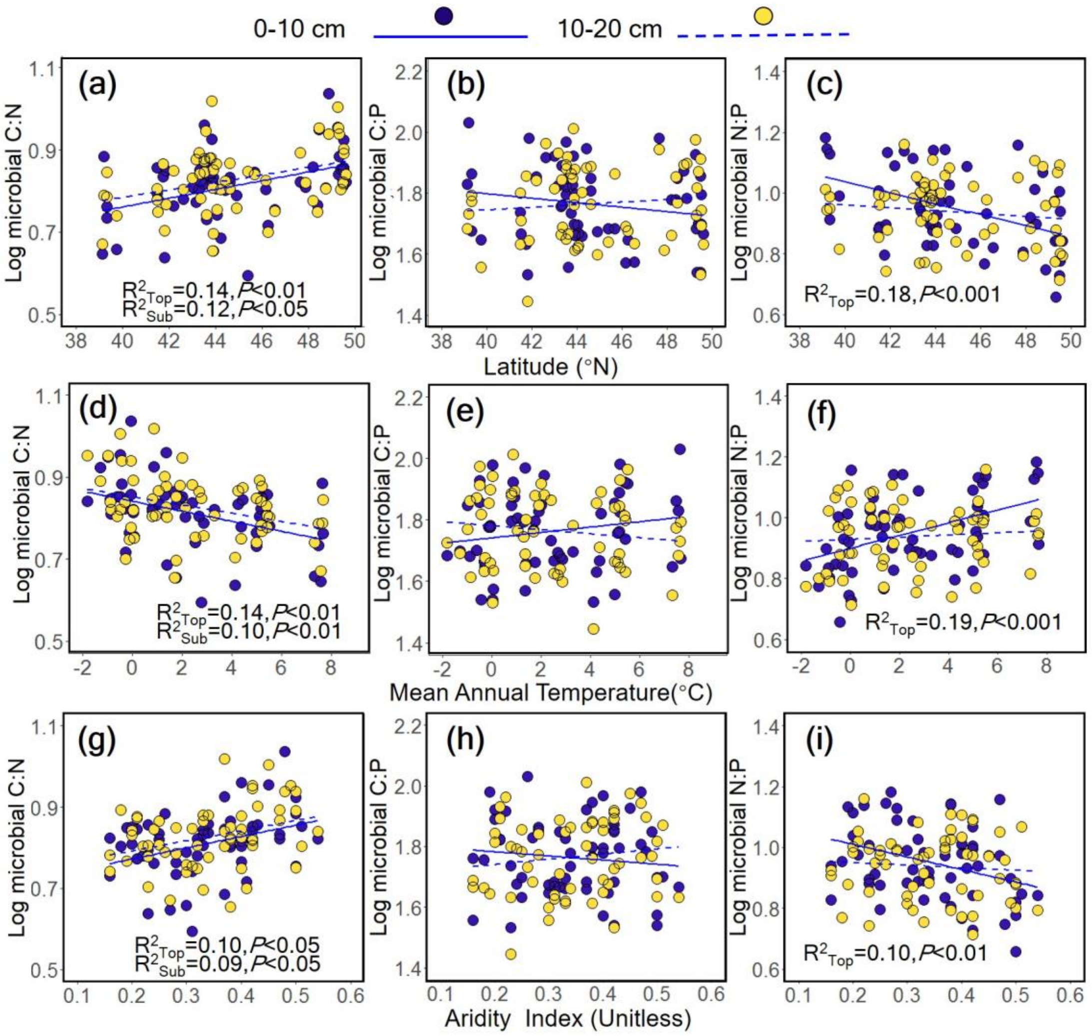

Figure 2. Relationships between the C:N, C:P and N:P ratios in soil microbial biomass and latitude (a-c), mean annual temperature (d-e) and aridity index (g-i) in the Inner Mongolian grassland.

In result: Besides, significant negative relationships were found between the microbial C:N ratio and MAT (Topsoil, R^2 =0.14, P< 0.01; Subsoil, R^2 =0.10, P< 0.01, Fig. 2d), while a negative relationship was found between the microbial N:P ratio and MAT (Topsoil, R^2 =0.19, P< 0.001; Fig. 2f).

In discussion: The increase in the microbial C:N ratio and decrease in the microbial N:P ratio that were found along a temperature gradient in this study are in accordance with the findings of Li et al. (2015) and Chen et al. (2016), who reported similar variations in microbial stoichiometry along latitudinal gradients. Temperature drives the variation in the growth of the microbial community, as high growth rates at low latitudes require high RNA contents, causing the N:P ratio to decline (Chadwick et al., 1999; Kooijman et al., 2009; Xu et al., 2013).

Results and Discussion: The SEM showed that climate have indirect effects through AGB, SOC and F:B. These are also interesting results, but was not mentioned in results. I also suggest to added some discussions of these indirect effects, though some of them were mentioned in discussion implicitly. Anyway, these indirect effects are part of the full picture how climate affects soil microbial stoichiometry.

Response: Thanks for your comments. We agree that indirect effects are part of the full picture how climate affects soil microbial stoichiometry. However, the SEM mainly was designed to test the direct effects of potential driving factors. We have revised as follow:"In particular, drought, decreasing aridity index, could affect the growth and productivity of plant, then shape the shift in vegetation types along this grassland transect (Jaleel et al., 2009; Cherwin & Knapp, 2012). "

By the way, the MS used many abbreviations, which markedly decrease the readability. Please try to remove unnecessary ones (e.g. MS, TS, DS in Table 1)

Response： We have revised the several abbreviations such as SIC, TC and TP. We also have removed the unnecessary abbreviation like MS, TS, DS in table and figures.

Minor comments: L45: meaning not very clear.

Response: We have removed the speculative statements. We have revised the sentence as “The results of this study suggested that the flexibility of the microbial N:P ratio should be considered when establishing the minimum sampling depth in a vertical study for microbial C:N:P stoichiometry study of surface soil.”

L58: A few studies. You have listed some studies along latitudinal and environmental gradients in the subsequent text.

Response: Thanks for your suggestion. Typo corrected.

L62, 63: with higher latitude?

Response: Thank you. Typo corrected.

L64: replace values with patterns

Response: Corrected as your suggestion.

L142: and at a depth of 10 cm, what does this mean?

Response: Thanks for your suggestion. Here we mean that soil depth of 10 cm as surface soil could influence the research on the vertical patterns of microbial stoichiometry. We have revised this sentence as "(iii) to adapt to the imbalance of resources, microbial C:N, C:P and N:P ratios vary between soil depths and at a depth of 10 cm as upper soil, which could influence the research on the vertical patterns of microbial stoichiometry."

L 171: aridity index (AI)

Response: Thank you. Typo corrected.

L217: "were well constrained (Fig. A2)". It needs to be explained.

Response: The results indicate well-constrained relationships among C, N and P in soil microbial biomass (Fig. A2). Here we mean that well correlations were found among C, N and P in soil microbial biomass.

L219: larger->higher

Response: Thank you. Typo corrected.

L223: the microbial C:N ratio in the subsoil was significantly higher than that in the topsoil (Fig. 2b). This result can not be found in Fig 2b. I guess it was in Table2.

Response: We have revised Fig. 2b to Table.2

L238 (and elsewhere) Effects of potential driving factors

Response: Thank you. Typo corrected.

L303: microbial C:N:P stoichiometry impacted the microbial community structure as a result of the F:B ratio. Do you mean that C:N:P stoichiometry affects microbial community structure? This seems you be conflict with the SEM. In SEM (and the sentence in line 300-303), the logic is that C:N:P stoichiometry changes as a result of community structure.

Response: Thanks for your comments. Given that specific microorganisms (e.g. bacteria and fungi) may have unique elemental compositions, changes in soil microbial communities may lead to differences in the element ratios in biomass (Strickland & Rousk, 2010; Mouginot et al., 2014; Zimmerman et al., 2014). As we shown in SEM, here we mean that the microbial community impacted the microbial C:N:P stoichiometry. We have revised as follows:“An experiment indicated that fungi have lower resource requirements and higher C:N and C:P ratios than bacteria, and thus the microbial community structure impacted the microbial C:N:P stoichiometry as a result of the F:B ratio (Mouginot et al., 2014). "

Fig. 1 Where are the difference among the biomes here? In the caption (also in that of Table 1), you mentioned: MS, meadow steppe; TS, typical steppe; DS, desert steppe. But you did not show the results at all.

Response: Thanks for your reminder! We have removed the speculative statements. This paper mainly focused on the difference between upper soil and lower soil layer, not among the biomes.

Table 2: across 404 the Inner Mongolian grassland at ??? Biome: soil depth? You did not compared biomes here in the Table.

Response: Thanks for your reminder! We have revised the Table.2.

Fig. 3: Why the figures of F:B were different from others? It seems many plots have a same F:B value.

Response: Thanks for your suggestion. Firstly, as I mentioned in the uncertainties and perspectives, the determination of fungal and bacterial biomasses by PLFA markers, which have limited targets for fungi and bacteria.

Secondly, considering relationships to environmental factors, previous studies found that shifts in fungal:bacterial ratio dominance were not always in line with the general expectation. This is likely because the traits expected to differentiate bacteria from fungi are often not distinct (Mouginot et al., 2014).

Finally, we analyzed the data to test the reliability of F:B ratio. Compared to researches in similar study area, the result of F:B ratio demonstrated the similar pattern along precipitation gradient.

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