How deep do we dig for surface soil? A comparison of patterns of microbial C:N:P stoichiometry between topsoil and subsoil along an aridity gradient

General comments

This paper focus on the sampling depth for analysing microbial C : N : P stoichiometry at 0-10 cm or 10-20 cm soil depth. It is an interesting study made in permanent grassland with an aridity index, but the interpretation and presentation of the data should be improved.

The paper will profit from clearer hypothesis that can be tested, and more clear wording and presenting of the results. I do also suggest putting the correlation analyses given in 3.1 into a table, which would make it more accessible for the reader. Your question: "How deep do we dig for surface soil?" Should be clearly answered in the conclusion.

Response: Thanks for your positive comments. We perfer to demonstrate the correlation analyses by figure, which would be more direct in our view. Then, limited by sampling soil depths, we tended to remove "How deep do we dig for surface soil?" in title. Additionally, we have carefully revised our manuscript according to your suggestions. Please see more details in our reply to your specific comments.

Specific comments

Normally subsoil is used for the soil under the surface soil/ topsoil that are less affected by plant roots and tillage operations. However, I assume there were no tillage at the sites referred to in the present paper. The root distribution and rooting depth for the different sites are not given, but in permanent grassland most of the rooting and microbial activity is in the upper soil layer. I would still be reluctant to use the "subsoil" as a term for the soil layer at 10-20 cm depth as the roots would likely go deeper than 10 cm. Surface soil and topsoil are in many cases used as synonyms and the heading is therefore confusing. I suggest in stead: How deep do we dig for surface soil? A comparison of patterns of microbial C : N : P stoichiometry between an upper and lower soil layer along an aridity gradient.

Response: Thanks for your suggestions. This study is based on previous research conducted in China's grassland which found the largest proportion of roots near the soil surface (0-30cm). The title has been modified as follows "A comparison of patterns of microbial C : N : P stoichiometry between topsoil and subsoil along an aridity gradient."

When you present hypotheses, it should be possible to test them and to either confirm or reject them and the result of the testing of the hypotheses should be clearly presented in the conclusion.

(i) microbial C:N and C:P ratios increase and the microbial N:P ratio decreases across an aridity gradient because of differences in nutrient-use efficiency.

The first part of this hypotheses "microbial C:N and C:P ratios increase and the microbial N:P ratio decreases across an aridity gradient », you have actually tested in the present paper, but the result is not clearly written in the conclusions. In Figure 2, C:N, C:P and N:P ratios are given along an aridity Index (Gradient). Because of the very low relationships between the ratios and the aridity index, this part of the hyphothesis cannot be confirmed. R^2=0.1 is very low. In discussion you write: "microbial C:N and C:P ratios increase and the microbial N:P ratio decreases across an aridity gradient » I do not agree with this statement. Because of the low R^2, a P<0.05 does not say much. If you look at figure 2, you see that the variation in within C:N and C:P sites at the same aridity is much larger than the impact of Aridity index. I would rather call it a trend, then to state a significantly impact.

Response: Response: Thanks for your suggestions. We assume that R2 is good enough to exhibit the change trend. Firstly. The variations in microbial C:N and C:P ratios were partly induced by the measurement method. At the small scale, correlations between fumigation-incubation and fumigation-extraction were variable, which might cause variations in microbial biomass C:N:P stoichiometry (Wardle & Ghani, 1995). Therefore, we assume the variations in microbial biomass C:N:P stoichiometry are inevitable systematic errors.

Second, in a previous study, low R2 also was found along environmental

gradients (precipitation, temperature, soil pH, soil content percentage, etc.) at regional scale (Chen et.al 2016). Finally, several global researches only showed the trend but not even R2(Xu et al., 2013; Li et al., 2016). This study offered the regional evidence through measurements across a 2100-km climatic transect in the Inner Mongolian grasslands.

All in all, we do believe the R2 is good enough to exhibit the trend of microbial C:N along the aridity index gradient. We appreciated that you could accept our explanations.

The second part of the first hypothesis "because of differences in nutrient-use efficiency. », you do only discuss and do not test. I would leave that out from the hypothesis.

Response: thanks for your helpful suggestion. We have removed the speculative statement.

(ii) Due to variations in resource supply among different soil depths, the effects of driving factors on microbial C:N, C:P and N:P ratios might decrease with soil depth.

This hypothesis you have not tested and cannot do, as you do not know if variations in resource supply among different soil depths actually do effect driving factors on microbial C:N, C:P and N:P ratios. What you can test is: "Microbial C:N, C:P and N:P ratios do vary with soil depth." In the results 3.1 lines 222 to 223 you write: "Moreover, the microbial C:N ratio in the subsoil was significantly higher than that in the topsoil (Fig. 2b)." I assume you must mean table 2? If this is the case, such a hypothesis could be confirmed for C:N ratio, and rejected for C:P and N:P ratios. Obs,. You write in the abstract (line 32-34) :" We found that the microbial C:N , C:P and N:P ratios varied with soil depth. »

According to table 2, they do not.

Response: Thanks for your reminder. Firstly, according to the Table.2, the result showed that the microbial C:N ratio in the lower soil was significantly higher than that in the upper soil. We have revised the error in the manuscript.

Second, Our hypothesis is based on the homeostasis theory and previous

literature. Under the framework of homeostasis theory, microorganisms are constrained by basic metabolic needs, which results in microorganisms having fixed C:N:P ratios (Sterner and Elser, 2002). To adapt to the resource imbalances and limitations caused by substrate heterogeneity, microbes exhibit stoichiometric non-homeostasis by regulating their ecological processes such as mineralization and immobilization (Fanin et al., 2013; Mooshammer et al.,2014). As the depth of the soil changes, a shift in resource supply might lead to a variation in microbial stoichiometry. Published studies also show the variation in microbial C:N, C:P and N:P ratios between soil depths.

Therefore, we hypothesised that microbial C:N, C:P and N:P ratios do vary with soil depth. We have revised this sentence as "From the topsoil to the subsoil, the microbial C:N, C:P and N:P ratios varied from 6.59 to 6.83, from 60.2 to 60.5 and from 9.29 to 8.91, respectively. Only that the microbial C:N ratio significantly increased with soil depth. "

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, which could influence the research on the vertical patterns of microbial stoichiometry.

I do not understand what you mean by this hypothesis. You should convert it to a hypothesis that can be tested and clearly present the result of the hypothesis. Do you mean "Microbial C:N, C:P and N:P ratios do vary with soil depth. At 0-10 cm depth the ratios are more influenced of an aridity gradient and other ecological factors than at 10-20 cm soil depths"?

Response: Thanks for your suggestion. Here we mean that soil microbes may shift their C:N:P stoichiometry to adapt to the imbalance of resource between soil depths. We have revised this sentence accordingly.

In 3.1 you refer to "environmental gradient» in the title, but you do not refer to what you mean with «environmental gradient. »

Response: We majorly foucused on the Aridity gradient as our environmental gradient, which combined the effects of temperature and precipitaition.

You do focus on the impact of Latitude, but I do not understand for which purpose. And again the degree of explanation is low (R^2= 0.14) and the variation is large.

Response: Thanks for your suggestion. Until now, there are controversial latitudinal pattern of microbial C:N, C:P and N:P ratios. This study offered the regional evidence through measured data across a 2100-km climatic transect in the Inner Mongolian grasslands. we have added analysis related to the average annual temperature, to make our conclusions more robust. As we mentioned above, we do believe the R2 is good enough to exhibit the trend of microbial C:N along environment gradient.

Because this study is done on three grassland types (meadow steppe, typical steppe and desert steppe) with corresponding soil types, I do miss the discussion on impact of grassland types, plant roots and rooting pattern on the microbial stoichiometry.

Response: Thanks for your suggestion. First, This study is based on previous research conducted in China's grassland which found the largest proportion of roots near the soil surface (0-30cm). From our previous survey, above ground biomass was nearly proportional to below ground biomass with a scaling exponent across various grassland types in China's grassland. Therefore, above-ground biomass was chosen as important indicator in the SEM.

Second, it was ture that this study was conducted on three grassland types and it also was done along natural environment gradient (e.g. temperature, precipitation, aridity index) in this grassland transect. Owing to our uneven samping, we conducted the correlation analysis to see the change trend along environment gradient.

Because aridity gradient (index) is central in this study it should be given how it was calculated (Line 171-172).

Response: Thanks for your suggestion. We are sorry that we missed this information. We have included more details on the methods of data extracting and data acquiring in the revised manuscript. 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:

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

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

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.

Figure A3 need some introduction. How did you develop this?

Response: Thanks for your suggestions. We showed the direct pathway and related introduction in Figure. A3. We have revised the table as follows:

Pathway	Interpretation	Reference
SOC → Microbial C:N:P	Influence of SOC on microbial C:N:P stoichiometry	(Hartman et al., 2013; Maria et al., 2014; Mooshammer et al.,2014)
AGB → Microbial C:N:P	Plant necromass represents the fundamental resource for microbes to maintain element balance	(Cleveland et al., 2007; Aponte et al., 2010; Manzoni et al., 2010; Li et al., 2012; Zechmeister-Boltenstern et al., 2016)
AI→Microbial C:N:P	Influence of increasing temperature on microbial C and N cycle	(Wang et al., 2014; Zechmeister-Boltenstern et al., 2016; Chen et al., 2016)

Sand percentage → Microbial C:N:P	Influence of soil texture associated water-holding capacity and nutrient availability on microbial C:N:P ratios	(Cleveland et al., 2007; Xu et al., 2013; Maria et al., 2014; Li et al., 2015; Zechmeister-Boltenstern et al., 2016)
F:B ratio→ Microbial C:N:P	Influence of shift in the composition of microbial community on microbial C:N:P ratios	(Ross et al.,1993; Cleveland et al.,2007; Aponte et al., 2010; Tischer et al., 2014; Zechmeister-Boltenstern et al., 2016; Chen et al., 2016)

Technical corrections

Line 181 and line 189, You must explain what a universal conversion factor is, what the units are and give a reference to where you got it from.

Response: Thanks for your suggestions. 0.45 is the conversion factor (k_{EC}), no units (Jenkinson et al., 1976). The specific calculation formula is as follows:

$$k_{EC} = EC/ FC$$

where EC represents the difference between organic C extracted by 0.5 M K_2SO_4 from fumigated and non-fumigated soil, fumigation-extraction method. FC represents the flush of CO_2-C caused by fumigation during a 10 day incubation, fumigation-incubation method .(Jenkinson et al., 1976; Vance et al., 1987; Wu and Joergensen et al., 1990; Joergensen et al., 1996).

Line 185 Which principal method is used? Cloroform fumigation? Hedley and Stewart (1982) is not given in the reference list.

Response: Both methods were calculated by the difference in total microbial-P content before and after $CHCl_3$ fumigation. For microbial biomass P, calculation was based on the difference between P removed by $NaHCO_3$ extraction of $CHCl_3$ fumigated and nonfumigated samples. We have added the source of the method as

follows: Hedley, M. J., & Stewart, J. W. B. (1982). Method to measure microbial phosphate in soils. Soil Biology and Biochemistry, 14(4), 377-385.

Line 201 Was the log10 transformed ratios normally distributed?

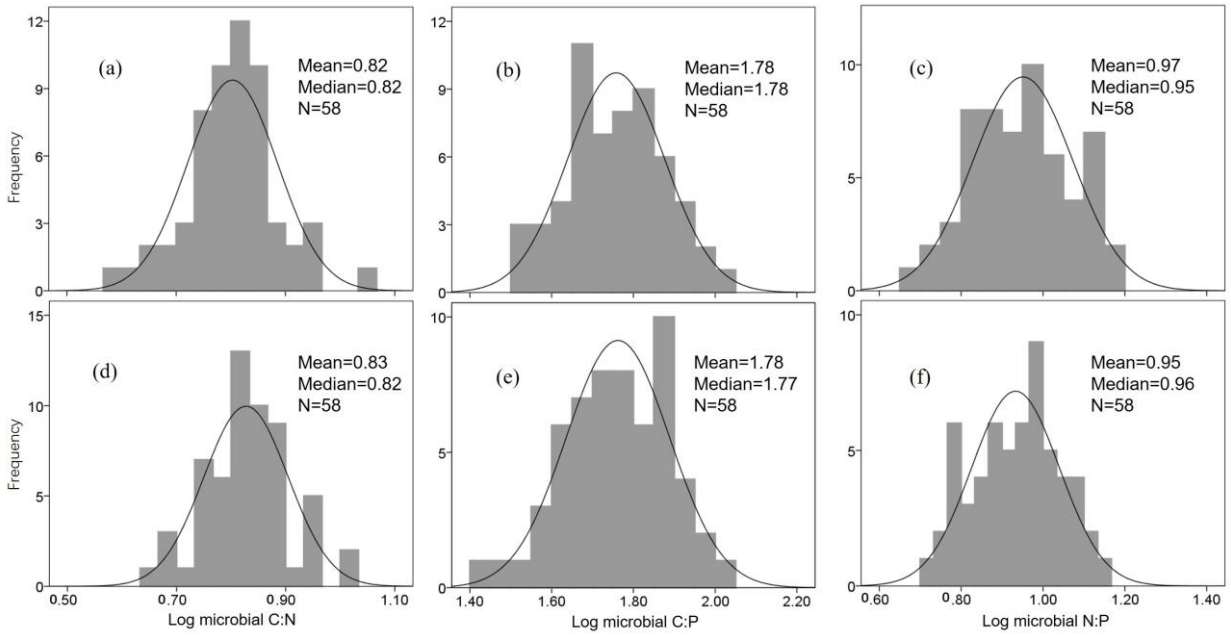

Figure A4. Histograms showing the frequency distributions of the soil microbial C:N, C:P and N:P ratios in the topsoil (a-c) and the subsoil (d-f).

Response: Thanks for your suggestion. The log10 transformed microbial C:N, C:P and N:P ratios in both soil depths demonstrated normal distribute. We have added the Figure A4 in the manuscipt.

Line 223 , Should it be table 2, not Fig. 2b?

Response: Thanks for your suggestion. Here should be Table.2. We have revised in the manuscript.

Reference:

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