Interactive comment on "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" by Yuqing Liu et al.

Anonymous Referee #3

Received and published: 7 December 2019

The manuscript requires further clarification on methods, resolution of data and a more realistic presentation of the data analysis. The study design cannot answer the title of the paper, the 'gradient' variables are poorly described, and the methods are lacking with respect to the most important 'variables'. To some extent, the required revisions are minor. However, the focus on 'depth' in the manuscript title suggests that the authors need a major revision with respect to their study hypothesis. Please see the specific comments for further direction on the required revisions.

Response: Thanks for your helpful comments. Then, limited by sampling soil depths, we tended to remove "How deep do we dig for surface soil?" in title. Finally, we have carefully revised our manuscript according to your suggestions. Please see more details in our reply to your specific comments.

Title: you cannot answer the question 'How deep do we dig for surface soil'? Because you did not dig very deep / or a high dig with high incremental accuracy. Two 10 cm samples do not answer the question.

Response: Thanks for your constructive comments. We have deleted "How

deep do we dig for surface soil?" and revised the title as "A comparison of the patterns of microbial C:N:P stoichiometry between topsoil and subsoil along an aridity gradient".

L42 is influenced the correct term? What was the relationship?

Response: Thanks a lot. This sentence has been modified as "The results also revealed that the aridity index (AI) and plant aboveground biomass (AGB) exerted NEGATIVE impacts on the microbial C:N ratio at both soil depths, and the effects of AI decreased in the subsoil."

L87 why 'might be'?

Response: We have removed the speculative statements. We revised the sentence as "Moreover, edaphic variables, such as SOC (Maria et al., 2014; Chen et al., 2016) and soil texture (Li et al., 2015), could be associated with nutrient mineralization and availability, thus influencing the C:N:P stoichiometry in microbial biomass (Griffiths et al., 2012). "

L109 revise wording 'climate change background'. This study does not truly address deeper soils.

Response: Thanks for your suggestions. We revised the sentence as "Such knowledge of the nature of soil microbial stoichiometry is fundamental to understanding ecosystem function, especially within the soil depth of 10-

20cm, which remains uncertain in the published researches."

L132 it this truly an 'ideal' platform. The resolution of the resolution of the aridity index is less than ideal.

Response: Thanks. The sampling sites of this experiment covered meadow steppe, typical steppe and desert steppe, which is a natural environmental gradient. Aridity index ranges from 0.16 to 0.54 along the grassland transect, which offers an ideal experiment platform.

L135 at two depth: why especially in the surface. This is common?

Response: Thanks for your comment. Most studies of soil microbiology have focused exclusively on the soil surface limited to 20 cm in depth, where the densities of microorganisms are highest.

L150 what is the proportion of snow?

Response: Thanks for your suggestion. This sentence has been modified as follows: "The mean annual precipitation (MAP) ranges from 104 to 412 mm, about 80 % of which falls in the growing season from May to August (Chen et al., 2013). "

L158 define slightly? Agricultural? Heavy grazing? Infrequent grazing?

Response: Many thanks for your comments. We defined the slightly

disturbed as the condition that occasional animal bite marks have been observed in our plots， but without agricultural activity or grazing.

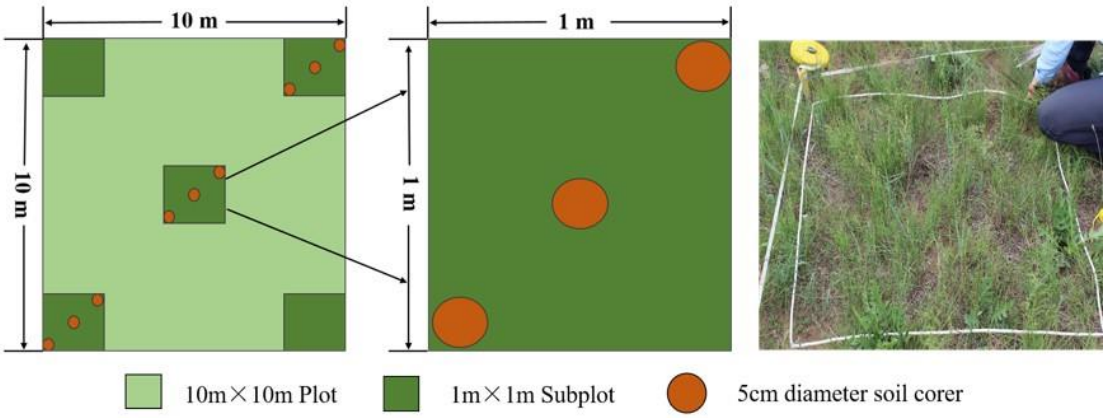
L159 why the uneven sample numbers per grassland type? Was this weighted by area?

Response: This study was conducted along natural environment gradient (precipitation, temperature etc.) which shapes the grassland types in this grassland transect. The experiment was designed for comparing the difference between the upper soil and lower soil, not the difference among grassland types.

L161 what stop at 20 cm? L161 where were the three plots sampled? Corners and centre?

Response: Most studies have focused exclusively on the surface 20 cm soil where the densities of microorganisms are highest. However, most studies used 0-10 cm as the surface soil to facilitate sampling and comparative research (Cleveland and Liptzin, 2007; Li and Chen, 2004; Chen et al., 2016). To identify the soil depth that is appropriate for sampling and to improve the understanding of surface soil research at a global scale, we designed a study that divided the surface soil into 0-10 cm and 10-20 cm depths to compare the differences in microbial stoichiometry at the regional scale.

As shown below, there are five 1×1 m^2 subplots established at each corner and the center of a 10×10 m^2 plot.



L164 sentence is incomplete.

Response: Thanks. We have modified this sentence: “After gentle homogenization and removal of roots, the soil was sieved through a 2-mm mesh and then stored for further experiments. ”

L168 what elemental contents? Carbon only? What are the other elements? If other elements, how were they measured?

Response: These is no other element discussed in this paper. The SOC is obtained by subtracting the soil inorganic carbon from the total carbon in this paper.

L170 how was organic matter and carbonates removed from the soil? Carbonates should not be removed before texture is estimated. They are

part of the mineral soil texture.

Response: Carbonate is removed by hydrochloric acid water wash. It is true that carbonates are part of the mineral soil texture. However, the microbial C:N:P stoichiometry was not affected by carbonate. Thanks for your understanding.

L172 what was the resolution of the AI database? Is this adequate to evaluate against site specific measurements? If the metric is important why not calculate at each site?

Response: 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.

L172 what about bulk density? How was it measured? Reported? Why not use loss-on-ignition?

Response: Bulk density with soil volume measured by coating natural clods in cutting ring then weighing the oven-dried clod in drying oven at 105℃ for 24h. Bulk density is calculated by dividing the weight of the oven dried clod by this volume ($g \cdot cm^{-3}$).

How was AGB biomass measured. This is not explained but is an important measure (as indicated by the abstract)

Response: Thanks. We have revised in manuscript as “We measured the aboveground biomass by harvesting the aboveground part of the plants.”

L180 is that ration based on mass or volume?

Response: Thanks for your suggestion. We have revised as follow: The

fumigated and nonfumigated samples were extracted using 0.5 M K2SO4 with a soil:solution mass ratio of 1:4.

L193 what different phases?

Response: Thanks for your comment. Here we mean that there are different phases in the process. Phospholipids were separated from neutral and glycolipids on solid-phase extraction columns by eluting with $CHCl_3$, acetone and methanol, respectively. We have revised as follows: "The resultant fatty acid methyl esters were separated, quantified, and identified using capillary gas chromatography."

L201 why t-test? Maybe an ANOVA should be used to account for the different grassland types? Or was a t-test applied to each type? If the latter, was the p value corrected for multiple tests?

Response: It was ture that this study was conducted on three grassland types, and it was also done along the 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 the environment gradient.

L206 AGB is not defined. L206 provide more details on the source of AI and AGB. What is there resolution? Is there a gradient in the data?

Demonstrate that they are gradients. How are they estimated / measured? Provide a description of the data in the results (if they are important variables).

Response: 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. The plant community in subplots was identified, and the above-ground biomass (AGB) was harvested. As to the calculation of AI, we mentioned in the previous reply.

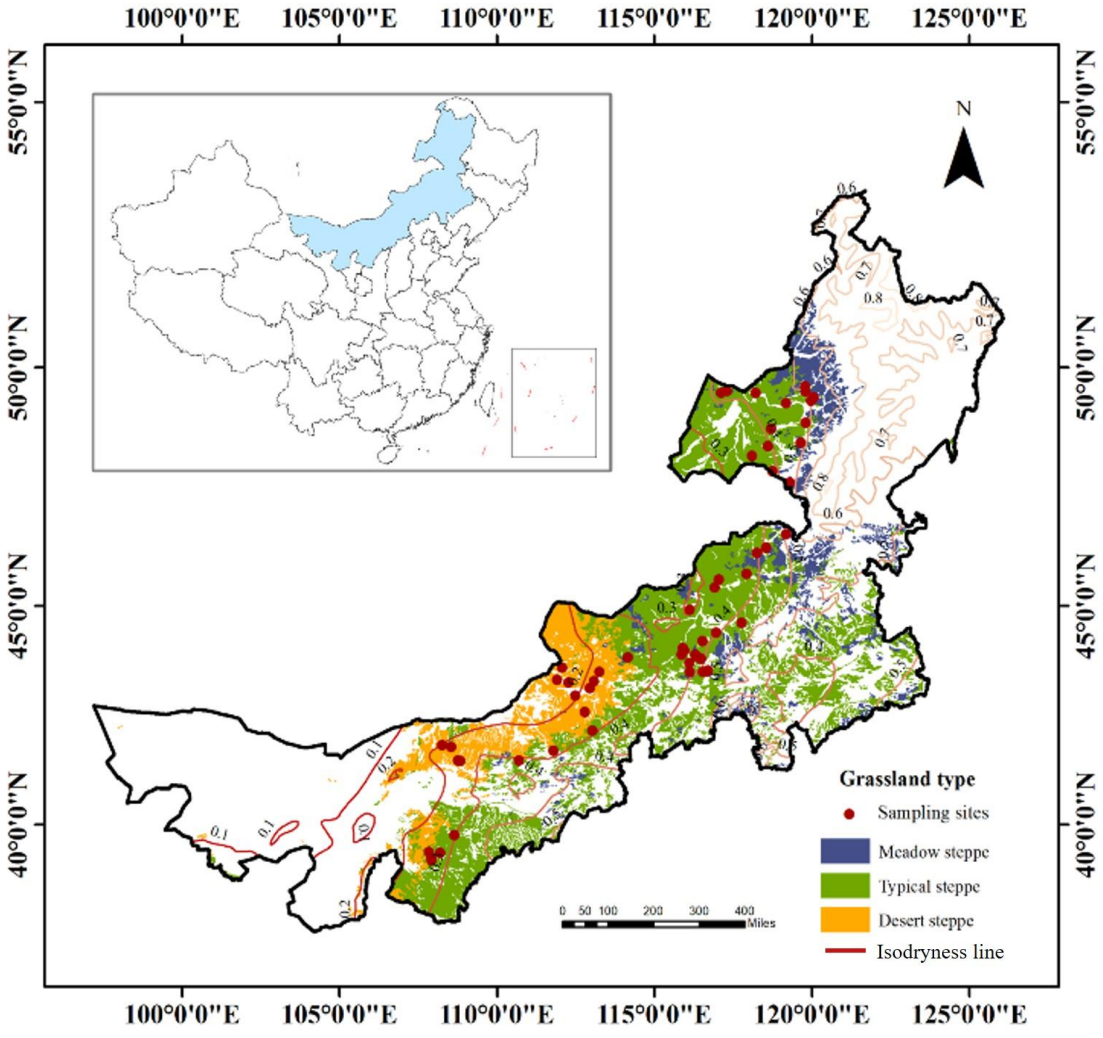


Figure A1. Geographic locations of the sampling sites in the Inner

Mongolian grassland

As Figure A1shown, our sampling sites were distributed along the aridity index gradient. In Inner Mongolia grasslands, the aridity exhibits a gradient that increases from northeast to southwest (aridity index ranges from 0.16 to 0.54). We have added the Figure A1 to the manuscript.

L214 what is the gradient?

Response: As shown in the Figure A1, the aridity exhibits a gradient that increases from northeast to southwest (aridity index ranges from 0.16 to 0.54). Besides, the study area covered both temperature (mean annual temperature ranges from -2.09 to 7.67) and precipitation (mean annual precipitation ranges from 153.9 to 401.7) gradients.

L216 does distinct mean ‘significantly different’

Response: We have revised the sentence as “Significantly different water contents, soil bulk density, sand percentages and SOC contents were found between soil depths ($P < 0.05$, Fig. 1a, 1b, 1c, 1f).”

L216 why is bulk density mentioned here: : : and only here? How was it measured? Did it differ greatly between grassland types?

Response: Bulk density is shown here to show the differences in

physicochemical properties between different soil layers. The measurement method was mentioned in the previous reply.

Why was soil microbial biomass not weighted by bulk density?

Response: In the common way, we performed the usual operation instead of weighting by bulk density. Thanks for your understanding!

L218 the concentrations were larger but was the pool larger? Use the bulk density to evaluate the pool difference.

Response: Here we mean that the concentrations of microbial biomass C, N and P, not the pool. We have revised as “The microbial biomass C, N and P concentrations in the topsoil were significantly higher than that in the subsoil ($P < 0.05$, Table. 2).” Thanks for your understanding!

L225-235 these are very weak significant relationships. This should be acknowledged. Similarly, the relationships in Figure 2 and Figure 3 are not very convincing of a relationship(s).

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.

L225-235 you are regression carbon against a ratio that contains carbon: : : this is spurious?

Response: Soil organic matter includes the labile (rapid turnover) and stabilized (slow turnover) fractions (Parton et al, 1987). However, the clear and broad consensus is that soil microbes are primarily limited by C availability (Fierer et al. 2003). There is a clear assumption that available C limits biomass and activity (Eilers et al.2012). As soil carbon matter

changes, microbial biomass N and microbial biomass P change asymmetrically, which affects the ratios (Mooshammer et al. 2014). Therefore, we assume that the regression of SOC against microbial C:N:P stoichiometry is a reasonable analysis.

L237 clarify: : : subsoil is reported in L232, L233, and L235.

Response: Thanks for your helpful comments. We have revised this sentence as: "No or only weak association was found between the microbial C:N, C:P and N:P ratios and the AGB and F:B ratio in the subsoil (Fig. 3)."

L274 drought? Clarify.

Response: Thanks for your helpful comments. As the Table shown, decreasing aridity index means drier weather condition. We have revised the manuscript as follow: "In addition, microbial C:N ratio decreased with decreasing aridity index, which serves as a protective mechanism as microbes decrease their nitrogen use efficiency (NUE, the ratio of N invested in growth over total N uptake) and tend to be more N conservative under dry climatic conditions (Mooshammer et al., 2014; Delgado-Baquerizo et al., 2017)." Due to the weak relationship, we have mentioned in the previous reply.

Table. Generalized climate classification scheme for ***Global***-Aridity values (UNEP

1997).

Aridity Index Value	Climate Class
< 0.03	Hyper Arid
0.03 – 0.2	Arid
0.2 – 0.5	Semi-Arid
0.5 – 0.65	Dry sub-humid
> 0.65	Humid

L285 many things change across latitude. Is microbial biomass influenced by latitude or the change in grassland type / climate / etc. Will microbial biomass also change across longitude? What is the range in the aridity gradient in the current study?

Response: In general, latitude pattern was driven by temperature. Therefore, we have added results of the temperature analysis to make our conclusions more robust. It is true that microbial biomass exhibits longitudinal pattern in global study (Xu et al., 2013). As shown in Figure A1 and Table 1, aridity index ranges from 0.16 to 0.54 in this study.

L292 this is essentially stating that carbon is related to a ratio that includes carbon. This is not surprising. Is this a spurious (correlation) regression?

Response: Soil organic matter includes the labile (rapid turnover) and stabilized (slow turnover) fractions (Parton et al, 1987). However, the clear and broad consensus is that soil microbes are primarily limited by C availability (Fierer et al., 2003). There is a clear assumption that available

C limits biomass and activity (Eilers et al., 2012). As soil carbon matter changes, microbial biomass N and microbial biomass P change asymmetrically, which affects the ratios (Mooshammer et al.,2014). Therefore, we assume that the correlation regression with SOC is a reasonable analysis.

L309 how were AGB and AI measured? Are they site specific or regional indicators? They only show a weak relationship with little predicative power.

Response: Thanks a lot. The above ground biomass was site-specific while aridity index was a regional indicator. We measured the aboveground biomass by harvesting the aboveground part of the plants. As to the source of AI, more details in the previous reply.

L334 did you quantify spatial heterogeneity? How?

Thanks for your comments. We have removed the speculative statements. The highly variable N:P ratio in microbes may reflect the high variability in site-related P availability (Chen et al. 2013; Li, et al. 2015). Furthermore, the relatively high microbial N:P ratio (suggesting P limitation) are supported by direct evidence showing that low soil P availability strongly limits microbial biomass, activity, and other ecosystem processes (Cleveland et al., 2007). This sentence has been modified as “The high variability of the N:P ratio in soil and soil microbial biomass therefore

indicates that the N:P ratio could be an indicator of the ecosystem nutrient status at deeper soil depths (Cleveland et al., 2007; Chen et al. 2013; Li, et al. 2015)."

L337 you cannot answer this question.

Response: Thanks for your comment. We agree that inappropriate statement might result in uncertainty. This sentence has been modified as follows: "How deep should we dig to evaluate the surface soil the microbial stoichiometry in vertical study?"

L341 are the pools distinct?

Response: Thanks a lot. We have removed the speculative statements. We have revised as follows: The results showed significant differences in the water content and sand percentage, SOC content and F:B ratio between soil depths, suggesting that the resource supplies between topsoil and subsoil were significantly different.

L350 you tested limited depth, with course increments.

Response: Similar findings were reported in the top 16 cm of soil in a Mediterranean oak forest (0-8 cm and 8-16 cm), where the microbial nutrient ratios (C:N, C:P and N:P) varied between soil depth (Aponte et al., 2010).

L369 what about pools?

Response: Thanks a lot. This study focused on the C:N, C:P and N:P ratios in microbial biomass, not the pools of microbial biomass C, N, P. We don't think that's an important variable.

L375 not shown, this statement is too strongly with respect to drought. There was a weak relationship using a coarse metric.

Response: In addition, microbial C:N ratio decreased with decreasing aridity index, consistent with the perspective that microbes mediate their nitrogen use efficiency and tend to be more N conservative under drier climatic conditions. In terms of the weak relationship, we have mentioned in the previous reply.

L383 edaphic? Influence of soil on soil?

Response: Edaphic factor means any characteristic of the environment resulting from the physical, chemical or biotic components of the soil such as the microbial structure, soil texture and soil organic content. In our results, the microbial C:N, C:P and N:P ratios were influenced by SOC and F:B ratio.

L384 you need to demonstrate the gradient

Response: As the most important gradient, AI gradient is demonstrated in Figure A1. Table 1 also showed the ranges of mean annual temperature, mean annual precipitation and above ground biomass in this study.

Reference:

Chen, D., J. Cheng, P. Chu, S. Hu, Y. Xie, I. Tuvshintogtokh & Y. Bai (2015) Regional-scale patterns of soil microbes and nematodes across grasslands on the Mongolian plateau: relationships with climate, soil, and plants. Ecography, 38, 622-631.

Chen, Y., W. Han, L. Tang, Z. Tang & J. Fang (2013) Leaf nitrogen n and phosphorus concentrations of woody plants differ in responses to climate, soil and plant growth form. 36, 178-184.

Chen, Y. L., Chen, L. Y., Peng, Y. F., Ding, J. Z., Li, F., Yang, G. B., Zhang, B. B. (2016). Linking microbial C:N:P stoichiometry to microbial community and abiotic factors along a 3500-km grassland transect on the Tibetan Plateau. Global Ecology & Biogeography, 25(12), 1416-1427.

Cleveland, C. C., and Liptzin, D. 2007: C:N:P Stoichiometry in Soil: Is There a "Redfield Ratio" for the Microbial Biomass?, Biogeochemistry, 85, 235-252, 2007.

Delgado-Baquerizo, M., Powell, J.R., Hamonts, K., Reith, F., Mele, P., Brown, M.V., Dennis, P.G., Ferrari, B.C., Fitzgerald, A., Young, A., 2017. Circular linkages between soil biodiversity, fertilityand plant productivity are limited to topsoil at the continental scale. New Phytologist 215.

Eilers, K. G., Debenport, S., Anderson, S., & Fierer, N. (2012). Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. Soil Biology and Biochemistry, 50, 58-65.

Li, P., Yang, Y., Han, W., Fang, J., 2015. Global patterns of soil microbial nitrogen and phosphorus stoichiometry in forest ecosystems. Global Ecology & Biogeography 23, 979-987.

Li, X. Z., and Z. Z. Chen, 2004. Soil microbial biomass C and N along a climatic transect in the Mongolian steppe. Biology & Fertility of Soils 39(5):344-351.

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J.,Takriti, M., Watzka, M., Wild, B., 2014. Adjustment of microbial nitrogen use efficiency to carbon:nitrogen imbalances regulates soil nitrogen cycling.

5, 3694.
Wardle, D. A., & Ghani, A. (1995). Why is the strength of relationships between pairs of methods for estimating soil microbial biomass often so variable? Soil Biology and Biochemistry, 27(6), 821-828.
Trabucco, A., and Zomer, R.J. 2009. *Global Aridity Index (Global-Aridity) and Global Potential Evapo-Transpiration (Global-PET) Geospatial Database*. CGIAR Consortium for Spatial Information. Published online, available from the CGIAR-CSI GeoPortal at: http://www.csi.cgiar.org.

UNEP (1997) World atlas of desertification. United Nations Environment Programme
Parton, W. J., D. S. Schimel, C. V. Cole, and D. S. Ojima. 1987. Analysis of Factors Controlling Soil Organic Matter Levels in Great Plains Grasslands1.Soil Science Society of America Journal J. 51:1173-1179.
Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. Soil Biology & Biochemistry, 35(1), 167-176.
Xu, X., Thornton, P. E., and Post, W. M.: A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems, Global Ecology & Biogeography, 22, 737–749, 2013.