April 28th, 2016

Editor-in-Chief

Biogeosciences

Dear Editor,

Thank you very much for your willingness of considering a revised version of our manuscript '*Variations of leaf N, P concentrations in shrubland biomes across Northern China: phylogeny, climate and soil*' (bg-2015-414). We appreciate very much this opportunity and the comments from the referees. We carefully considered every comment when revising the manuscript. As we repeatedly find, the review process causes the creation of a stronger and more accurate manuscript. We think that the manuscript is now greatly improved.

The main revision we conducted is that we included soil pH in our analyses. Both reviewers suggested that our work is limited by only look at temperature, precipitation, soil total nitrogen and soil total phosphorus concentrations as environmental factors. Soil pH is an integrated index of soil nutrient availability, and is correlated with various processes such as soil enzymatic and microbial activities. The inclusion of soil pH improved our understanding on the influence of soil nutrient on leaf N concentration, and did not change our major conclusions.

In addition, we revised the manuscript according to the other comments from the reviewers (Please see below for our point-to-point response). We hope you will find the revision to be satisfactory, but we will be happy to make any additional changes that you think are necessary.

I look forward to your replying in due time.

Best regards

Zhiyao Tang

PhD, Associate Professor of Ecology

Dept. of Ecology, College of Urban and Environmental Sciences

Peking University

Beijing 100871, China

Tel/Fax: +86-10-6275-4039

Response to referee's comments:

Referee: 1

General comments:

The authors use a series of shrubland sites in northern China and investigate the relationship of leaf N and P concentrations to several environmental factors. In essence, the study attempts to assess the leaf "economics spectrum" (sensu Wright et al. 2004), but over a much smaller latitudinal gradient (i.e within northern China). I think the paper has potential but needs reworking and additional analyses (it is perhaps an editorial decision as to whether that constitutes a minor or major revision, given the amount of additional work finally recommended). The key difference between the Wright et al. (2004) paper and this study is the large range of sites in the former. Wright et al. (2004) attempted to explore worldwide patterns; however, when the scale is considerably smaller (albeit large) other factors may confound the results.

Overall, I think the paper is limited by only looking at leaf N and P and only temperature and precipitation; other (leaf) traits are not touched upon, yet recent syntheses (e.g. Reich 2014, J Ecol) highlight the importance of other traits and factors, not just leaf N and P. I think it would be far better to take other traits into account in some way, not only because it brings it up to date with broader hypotheses (see Reich 2014) but also because with such varying habitats (desert through to alpine shrublands) these traits could affect the results. For example, plants at the "slow" end of the economics spectrum may have lower absolute concentrations of leaf N and P even if the ratios (N:P) are roughly similar. At the very least, these varying habitats should be accounted for. I suspect that a linear mixed model, treating the different type of shrubland as a random effect, would likely indicate that within each region there was limited, or no, effect and that the significant regressions observed were due to the changes from one region to the next. All of which is fine, but other factors certainly play a part in that. Some of those factors might be correlated with (co-linear to) the traits assessed (e.g. annual precipitation) but only additional analyses would reveal that. Considering additional factors/traits I think would place the study better in the journal's stated scope. Furthermore, I would be surprised if the authors did not have additional data available to them to extend the analyses.

Response: Thank you for your comments. Your major concern is that we only considered limited leaf traits and environmental factors. We agree that other plant functional traits, especially other essential nutrient element concentrations, are very important. However, due to the large area our extensive survey covered and limited labors we had, measurements of functional traits that need to be conducted on site using fresh plant tissue were minimized. Instead, we kept dried plant samples for potential future measurement on other nutrient elements. We have discussed the importance of include other nutrient elements in future studies in Discussion in our revised manuscript. **Page 16, Line 4-9** for detail.

Our study included temperature, precipitation, and soil nutrients as the environmental factors because of their strong influence on plant growth and leaf chemical traits. Based on the suggestions from you and the other reviewer, we included soil pH in our analyses. We found soil pH was positively correlated with leaf N and leaf N:P, but explained relatively small portions of variation. We have included the analyses of soil pH in Method (**Page 6, Line 12-13**), Result (**Page 9, Line 18-21**), and Discussion sections (**Page 13, Line 19-26**). In additional to mean annual temperature (MAT) and annual precipitation (AP), we also analyzed the influence of mean temperature (GST) and precipitation (GSP) of the growing season (from May to September) as climatic factors during our preparation for the manuscript. However, the relationships of leaf chemical traits with GST and GSP are very similar to those with MAT and AP. Also, MAT and AP explained more variation than GST and GSP and they were used in some previous studies (Reich and Oleksyn 2004; Han et al. 2005; Liu et al. 2012; Chen et al. 2013).

Another concern is that whether the observed pattern holds within each type of shrubland. We didn't treat the different type of shrubland as a random effect, because our study region only covers two major types of shrubland (i.e. temperate shrubland and desert shrubland), and we did not randomly select sample types of shrubland from a larger population. Instead, to address your concerns, we analyzed the temperate shrubland and desert shrubland separately using general linear models. The result of temperate shrbland was similar as that of using all data, indicating the same pattern holds within temperate shrbland. For desert shrubland, however, rainfall is the major factor influencing leaf chemical traits. We presented these result in **Figure S3** and **Table S3**.

Specific Comments:

Specific comments relating to the following pages and line numbers are as follows:

p. 18977 line 14,15: The Allen paper was concerned about the prevalence of ECM vs AM in different habitats (e.g., mesic vs xeric) and the generalisation "the infection of mycorrhizal fungi mainly depends on environments" is neither quite right or justified by that reference.
line 15: "in contrast, N is relatively sufficient". I don't know quite what you mean here and I'm not sure how it relates to the Allen reference.
p. 18986 line 24-25: That's not a true statement and not what the Allen paper concludes.

Response: Thank you for the comments. Based on comments from you and the other reviewer, we have rewritten the third hypotheses to make it clearer. According to niche conservatism theory, traits that define species competition on limited resources are less likely to be phylogenetically conserved as they are under strong selection and more adapted to the environment. Moreover, Fyllas et al. (2009) proposed that leaf N concentration tend to be more genetically constrained, while leaf P concentration tend

to be more environmentally constrained, with higher level of plasticity. These evidences could support our third hypothesis that leaf P concentration might be less phylogenetically conserved than leaf N concentration, because P is particularly low in soils in China and leaf P concentration is environmentally constrained. **Page 5, Line 9-14** for detail.

p. 18978 line 15: It would be good to see the correlations/other data in a supplement for those depth intervals.

Response: We reported the Pearson correlations of soil total nitrogen (STN) and phosphorus (STP) concentrations between different soil intervals in **Table S1**.

p. 18980 line 22: It would be better to include the AIC values (in the supplement).

Response: We included the AIC values in **Table S2**.

p. 18984 line 18: These ratios are indicative only but further tests are needed to say for sure.

Response: We tested the N:P ratio using one sample t-test. The N:P ratio was significantly greater than 16 (p<0.001), which indicates a significant P limitation. We included this result in our revised manuscript. **Page 13, Line 3** for detail.

p. 18985 line 2: Available nutrients are not necessarily the best, as "available" measures are typically based on agricultural plants that often lack the root specialisations or root symbionts (mycorrhizal fungi, for example) that can allow access to other forms of N and P. That is, there is other literature to support the use of total elements.

Response: Thank you very much for the insightful suggestion. This is a very strong support of using total element. We included this point in Discussion in our revised manuscript, citing Aerts and Chapin (1999). **Page 13, Line 15-18** for detail.

pp. 18975, 18976: The three paragraphs starting at line 14 could be condensed a little; structurally, it might all be better as one paragraph.

p. 18976 line 14: "proved to prior to others" needs correction; there's a wrong word there. line 15: insert "been" after "have"

p. 18977 line 4: insert "the" after "have"
line 6: ", plants" does not flow. I'm guessing you meant "and plants"
line 7: Did you mean "soil P availability"? If not, you need to explain why "nutrient availability" in general would affect leaf [P].

line 11: "remarkably", I presume you meant "markedly".

line 21: "expanding", I suppose you meant "encompassing".

p. 18978 line 2: insert "then" after "were"

line 4: "sites" should be singular.

line 10: "leaves under 950C for combustion" needs rewording, e.g., "were combusted at 950..."

p. 18980 line 10: "and explain them" needs rewording. I'd suggest something like: "with climatic and soil nutrient factors as explanatory variables".

line 13 and in other parts of the paragraph: tense should be the past tense, e.g. "We can then extract the SS" should be in the past tense.

p. 18981 line 3, 4 and 5: This sentence is not really necessary.
line 7: You don't need to mention the base package because it's loaded by default. line 10 and the Results section in general: Appropriate numbers of significant figures

should be used – there are too many in some places.

line 22, 23 : insert "a" before "significant"

p. 18982 line 13: "Come to the" doesn't make sense here.

line 20: "focused" should be "focus" presumably.

p. 18983 line 7: shrub should be "shrubs".

line 24: P does not diffuse well at all in most soils, but rather is usually quickly and tightly bound to soil particles.

p. 18984 line 17: "is" should be "are"

p. 18985 line 20: Better to change the tense here, e.g. "Climate influenced..." line 24: insert "a" after "exhibited"

p. 18986 line 1, 2: That's a big statement and as other factors weren't included I don't think it's justified.

line 14, 15: A somewhat obvious statement.

line 18: nutrient should be "nutrients". Additionally, given the results it would be better to suggest/surmise, e.g. "we surmise that the influence...".

Paragraph starting with line 15: The sentences in this paragraph to line 22 seem to be a bit of a rehash of the paragraph starting at line 7.

p. 18987 line 10: "along climate" should be "along the climatic"

p. 18993: Table 1. Appropriate numbers of sig. figures should be used (i.e. less in some places). It would be better to have only one header column, giving a taller table. "non-significance" should be "not significant".

p. 18994: Table 2. Again, appropriate numbers of sig. figures should be used. "non-significance" should be "not significant".

p. 18995: Figure 1. "Dessert" should be "Desert".

p. 18996: Figure 2. "Dessert" should be "Desert". A dash between "community level" would be better. "logarithm transferred" should be "log-transformed".

p. 18997: Figure 3. "Dessert" should be "Desert". A dash between "community level" would be better.

In one of these figures (e.g. Fig 1), or all, the acronyms (AP etc) should be spelt out in the caption.

Response: Thank you for your corrections. We have corrected accordingly.

Reference:

Aerts, R. and Chapin, F. S.: The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns, Adv. Ecol. Res., 30, 1–67, 1999.

Chen, Y., Han, W., Tang, L., Tang, Z. and Fang, J.: Leaf nitrogen and phosphorus concentrations of woody plants differ in responses to climate, soil and plant growth form, Ecography, 36, 178–184, doi:10.1111/j.1600-0587.2011.06833.x, 2013.

Fyllas, N. M., Patiño, S., Baker, T. R., Bielefeld Nardoto, G., Martinelli, L. A., Quesada, C. A., Paiva, R., Schwarz, M., Horna, V., Mercado, L. M., Santos, A., Arroyo, L., Jiménez, E. M., Luizão, F. J., Neill, D. A., Silva, N., Prieto, A., Rudas, A., Silviera, M., Vieira, I. C. G., Lopez-Gonzalez, G., Malhi, Y., Phillips, O. L. and Lloyd, J.: Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate, Biogeosciences, 6, 2677–2708, doi:10.5194/bg-6-2677-2009, 2009.

Han, W., Fang, J., Guo, D. and Zhang, Y.: Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China, New Phytolist, 168, 377–385, doi:10.1111/j.1469-8137.2005.01530.x, 2005.

Liu, C., Wang, X., Wu, X., Dai, S., He, J.-S. and Yin, W.: Relative effects of phylogeny, biological characters and environments on leaf traits in shrub biomes across central Inner Mongolia, China, J. Plant Ecol., 6, 220–231, doi:10.1093/jpe/rts028, 2012.

Reich, P. B. and Oleksyn, J.: Global patterns of plant leaf N and P in relation to temperature and latitude, Proc. Natl. Acad. Sci. U. S. A., 101, 11001–11006, doi:10.1073/pnas.0403588101, 2004.

Referee: 2

General comments:

The manuscript, entitled "Variations of leaf N, P concentrations in shrubland biomes across northern China: phylogeny, climate and soil" by X. Yang, X. Chi, C. Ji, H. Liu, W. Ma, A. Mohhammat, Z. Shi, X. Wang, S. Yu, M. Yue, and Z. Tang (BG 2015-414) proposes in a research paper to study how climate, soil and phylogeny explain the regional pattern in leaf N-P concentrations in China and the pattern components: compositional shift vs adaptive variation. With a stunning dataset where the abundance of species and their traits where measured for each of the 361 shrubland sites of the study, they highlighted that leaf N and leaf P follows different drivers: while leaf N pattern seems to be more influenced by the compositional shift of the communities than by the adaptive variation of species to climate and soil, leaf P seems to respond to the opposite pattern with a particular adaptation to soil fertility in P. To my opinion, this result is important (unless jeopardized by the issue I explain below) for the scientific community in community and ecosystem ecology and will be relevant and opportune in time for the Biogeosciences journal. It also echoes what has been founded in previous studies in another part of the world in the Amazonian basin (Fyllas et al 2009; Quesada et al 2012 in BGS).

Response: Thank you for the comments.

Specific Comments:

I see a particular major issue and some minor additional ones that I would like authors pay attention of:

1- As environmental predictors, authors used temperature, precipitation, soil total nitrogen and soil total phosphorus. At first glance, it seems very logical to use these soil variables to explain leaf nitrogen and leaf phosphorus, respectively. However, when you considered recent papers on leaf N and P over biogeographical scales (Fyllas et al 2009 in BGS; Ordoñez et al 2009; Ordonez & Olff 2013; Maire et al 2015; Simpson et al 2016 in minor revision; all in GEB), you note that leaf N is not related with soil nitrogen (available or total), for several reasons (that I won't develop here). It regresses however on soil variables related with cation availability (soil pH, soil total available bases, soil base saturation, soil CEC...). In opposite, soil phosphorus is well correlated with leaf P. Considering major soil variables to explain the variation of leaf P but not considering them for leaf N should strongly impact the results like: leaf N is less well predicted by environmental variable than leaf P, intraspecific variability of leaf N is not dependent on environmental variables... I strongly recommend to consider this point. If data has not been measured yet, a first look can be attempted by getting soil pH from a regional database that has the same resolution than worldclim (Shangguan et al 2013 Journal of advances in modelling earth systems).

Response: Thank you for your suggestion. The literature you recommended made an important contribution to improve our discussions. We included soil pH in our analyses. We found soil pH was positively correlated with leaf N and leaf N:P, but explained relatively small portions of variation. We have included the analyses of soil pH in Method (**Page 6, Line 12-13**), Result (**Page 9, Line 18-21**), and Discussion sections (**Page 13, Line 19-26**).

2- I find the introduction very interesting as well as the hypotheses that the authors want to test. However, I find hard to follow the link between hypotheses and data analyses in materials and methods – data analyses - and in the result sections. I suggest to improve the structure of MM and result sections so that they better fit the different hypotheses presented in the introduction.

Response: Changes has been made accordingly.

3- Although the manuscript is written with a fully intelligible English, I would encourage a revision by a native English speaker. Some examples: dessert of desert, L10-11 on 18978 page.

Response: Modification has been made accordingly by having a native speaker review the manuscript.

More minor comments below:

- L8-11 on page 18977: It is not clear why the plant physiology hypothesis does not consider P along an aridity gradient, while both nutrient are considered along a temperature gradients. Please make a clarification, on page 18975 for instance.

Response: We only consider the change of N along an aridity gradient because of the important function of leaf cellular N to maintain photosynthesis rates in dry environments. Studies in arid regions proposed that plants tend to have higher leaf N concentration to exploit greater light availability while reducing stomatal conductance and transpiration rate (Cunningham et al., 1999; Wright et al., 2003). However, there is no mechanical explanation for the change of leaf P concentration along an aridity gradient. A recent study found both leaf N and P concentrations increase with aridity (Luo et al. 2015), but all the explanations of this result focused on leaf N. Biogeochemical hypothesis proposed that both leaf N and P will decrease with precipitation due to soil leaching. This hypothesis does not apply for our study region, where soil leaching is weak. Therefore, we didn't include leaf P along aridity gradient in our hypothesis due to lack of mechanical explanations.

- L15 on page 18977: '... in contrast, N is relatively sufficient'. This part of the sentence does not make sense. Do the authors mean in this particular region? In general? I strongly advise the authors to consider Mayor et al (2015 ELE) paper

showing that mycorrhiza can significantly contribute to N nutrition from mid to high latitude.

Response: Based on comments from you and the other reviewer, we have rewritten the third hypotheses to make it clearer. According to niche conservatism theory, traits that define species competition on limited resources are less likely to be phylogenetically conserved as they are under strong selection and more adapted to the environment. Moreover, Fyllas et al. (2009) proposed that leaf N concentration tend to be more genetically constrained, while leaf P concentration tend to be more environmentally constrained, with higher level of plasticity. These evidences could support our third hypothesis that leaf P concentration might be less phylogenetically conserved than leaf N concentration, because P is particularly low in soils in China and leaf P concentration is environmentally constrained. **Page 5, Line 9-14** for detail.

- L16 on page 18978: Unless I make a big mistake, I think that WorldClim does not include a correction of temperature by altitude. Could the authors check this? It may be particularly important in the context of the study. If true, the authors can use CRU database that does that. Otherwise, the authors can apply the rule of thumb: -0.6^{o}C change by 100m elevation change

Response: We have checked the methods of Worldclim database. Elevation was included.

- L21-27 on page 18983: First, it is not clear if litter (leaf or root?) is included in total nitrogen or phosphorus. Second, increase in precipitation leads to increase in litter decomposition and in phosphorus availability, only when precipitation is lower than evapotranspiration, i.e. in the context of the study. When precipitation≥evapotranspiration, it is likely the opposite. Please add that the argument concerns the climatic context of the study.

Response: For the first question, litter was not included in soil total N or P concentrations. Litter layer was removed before sampling, and all visible roots were removed at the laboratory. We have clarified this in Method in our revised manuscript. **Page 6, Line 7** for detail.

Second, we agree that litter decomposition and phosphorus availability increase with precipitation only in arid region, where precipitation is lower than evapotranspiration. We calculated aridity index (AI) defined as the ratio of total precipitation to potential evapotranspiration of all sites. AI is greater than 1 in 301 of the 361 study sites. Therefore, in our study region, precipitation is generally lower than evapotranspiration. We have added this point in revised manuscript. **Page 12, Line 2-7** for detail.

- L5-8 on page 18984: Please test this opposite effect of climate variables with multiple regression, it should give good indication on what's going on.

Response: We have tested the effects of climate and soil variables with multiple regression at both community level and individual level. The significance and relative contribution of each variable are similar at community level and individual level. Therefore, we only present the result at community level (Total variation in Table 2) to avoid redundancy. In the multiple regression model of leaf N concentration, the effect of annual precipitation is significant, while the effect of mean annual temperature is not significant. Most previous studies were conducted at regions where temperature and precipitation were positively correlated, and the effects of temperature and precipitation are often confounded. The weak negative correlation between MAT and AP in our study region (Pearson's correlation R=-0.01) allow us to test the major influencing climatic factor of leaf N concentration. We found that it is precipitation, rather than temperature, significantly influenced leaf N concentration in the study region. We have added more explanation in Discussion. **Page 12, Line 14-20** for detail.

- Paragraph 2.1: It would be important to give an estimation of the shrub contribution to the community biomass (even if community is dominated by shrub).

Response: Shrub was the dominant life form in all sites, which accounted for 87.3% aboveground biomass on average. We have clarified this point in Method. **Page 5, Line 21-22** for detail.

- Paragraph 2.2: That is a good thing to have tested for N-fixer species. Could you do the same for succulent species? Could you, please provide some explanation on K interpretation?

Response: We also conducted K statistic of leaf N after dropping succulent plants, and the K-value remained almost unchanged, indicating that the phylogenetic conservatism of leaf N concentration is not resulted from the inclusion of some clades that have higher leaf N concentrations. **Page 10, Line 19-22, Page 13, Line 2-6, and Table 1** for detail. We also added more explanation on Blomberg's K in Method. **Page 8, Line 27, Page 9, Line 1-4** for detail.

- L10 on page 18975: In Reich & Oleskyn 2004, leaf N and P increase with latitude.

Please correct it.

- L4-7 on page 18976: Meng et al 2015 could be quoted as an opposite example.

- Paragraph 2.3: Symbolism is really confusing: 'i' means two different things in the equation: value of trait for each individual and mean value of trait for each species. I strongly recommend to use different symbols for each case.

- Paragraph 4: I would not recommend to use 'cheap' and 'expensive' rationale here. It is not wrong but one would need to know westoby and wright 2006 or wright et al 2004 studies to fully understand the meaning of it. In the context of the study, it could appear a bit contradictory to have a cheap strategy with lot of nutrients in leaves. You need to understand the link between leaf nutrient and leaf mass ratio / leaf lifespan to properly understand the terminology.

- L27 on page 18983: it does not confirm the hypothesis but is in line with the hypothesis. Please change!

- L3-6 on 18985: Please correct Liu et al 2012 by Liu et al 2013. Unless I misunderstood, I do not find this result and statement in Liu et al's study. Please modify the text in consequence.

Response: Thank you for your corrections. We have corrected accordingly.

- Figure 1: Please consider using another colour than blue, which spots similar to green.

- Figures 2-3: community figures are not necessary and can be removed for at least two reasons: i) they are similar to figure showing all individuals ii) they are not related with hypotheses. Please consider using colours here to distinguish between the different ecosystem types.

- Figures 4: what is the meaning of bars in the figure? Please give more information in the figure caption

Response: Thank you for the suggestions. We have removed the community figures in **Figures 2 and 3**, changed the colors in **Figures 1, 2 and 3**, and added more information in the caption of **Figure 4**.

Reference:

Cunningham, S. A., Summerhayes, B. and Westoby, M.: Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients, Ecol. Monogr., 69, 569–588, doi:10.1890/0012-9615(1999)069[0569:EDILSA]2.0.CO;2, 1999.

Fyllas, N. M., Patiño, S., Baker, T. R., Bielefeld Nardoto, G., Martinelli, L. A., Quesada, C. A., Paiva, R., Schwarz, M., Horna, V., Mercado, L. M., Santos, A., Arroyo, L., Jiménez, E. M., Luizão, F. J., Neill, D. A., Silva, N., Prieto, A., Rudas, A.,

Silviera, M., Vieira, I. C. G., Lopez-Gonzalez, G., Malhi, Y., Phillips, O. L. and Lloyd, J.: Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate, Biogeosciences, 6, 2677–2708, doi:10.5194/bg-6-2677-2009, 2009.

Luo, W., Elser, J. J., Lü, X.-T., Wang, Z., Bai, E., Yan, C., Wang, C., Li, M.-H., Zimmermann, N. E., Han, X., Xu, Z., Li, H., Wu, Y. and Jiang, Y.: Plant nutrients do not covary with soil nutrients under changing climatic conditions, Global Biogeochem. Cycles, 29, 1298–1308, doi:10.1002/2015GB005089, 2015.

Wright, I. J., Reich, P. B. and Westoby, M.: Least-cost input mixtures of water and nitrogen for photosynthesis, Am. Nat., 161, 98–111, doi:10.1086/344920, 2003.