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Discussions

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Interactive comment on "Disruption of metal ion homeostasis in soils is associated with nitrogen deposition-induced species loss in an Inner Mongolia steppe" *by* Q.-Y. Tian et al.

Anonymous Referee #1

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General:

- My most important concern with this paper involves the statistical analysis of the dataset. Although the research questions that are asked in this paper are very interesting, I believe that the current statistical approaches do not allow a clear understand and discussion of the processes shaping the biomass and diversity patterns. Since a high number of soil variables were measured in this study, which are all expected to be strongly affected by the N addition treatments, we can expect very high multicollinearity to occur in this dataset. This is indeed strongly suggested by the results of the 'stepwise regression' between soil pH and several soil variables, with very high $R2$ for only one soil variable, combined with very low $R2$ for the remaining soil variables (P10, L27;

P11, L2-4). The PCA analysis also clearly indicates a strong correlation among most of the measured soil variables. I am furthermore skeptical of how the reported RDA models were performed. According to Table 2, 94.5% of the variation in forb richness is explained through the model. To me, this seems an unrealistically high percentage, which is furthermore not supported by the clearly high variability of forb richness in Figure 5. I suspect that the RDA results given in Table 2 refer to three individual RDA analyses for forb richness, one for the macro-element group, one for the heavy metal group and one for the base cation group. If this is indeed the case, then the high levels of explained variation is a direct result of the high multicollinearity of the dataset, and should not be interpreted as is presently done in the manuscript. To resolve these issues, the relation between diversity/biomass and soil variables should be analyzed using a multiple regression model (GLM) rather than RDA. Instead of using the raw soil variables, the authors should use the different PCA axes (maybe use three instead of two) as dependent variables to disentangle the patterns and evaluate what soil variable is most strongly driving changes in diversity and biomass. I furthermore believe GLM is more appropriate for this analysis than RDA, since only one response variable is evaluated per model (forb/grass Richness/biomass), while RDA was specifically designed for the analysis of multiple-response variable datasets. Only after the reanalysis of this data can we be sure if patterns in biomass/diversity are indeed primarily driven by changes in metal ion availability, rather than by changes in acidity or N availability.

- The overall quality of the English writing is somewhat poor. Throughout the manuscript I encountered several strange and unclear sentences. I have suggested a few changes concerning the English writing in the specific comments. I would nevertheless still strongly suggest that the authors have the manuscript proof-read by a native English speaker.

- The manuscript contains several repetitions of information, that hamper its readability and clarity. I would suggest that the authors try to reduce the redundancy throughout the manuscript. For example: repetition of the hypotheses (P3, L21-25, P4 L11-15),

the repetition of rationale behind the use of certain techniques in the results section, which were previously discussed in the methods section (e.g. P9, L21-23), and the repetition of the results in the discussion section.

- The authors state that the loss of species during nitrogen enrichment can be caused by reduced levels of phosphorous availability (e.g. P3, L23, P14, L15). However, I am very skeptical that this has been shown in nature. N addition usually does not affect P availability in the soil. Limited levels of soil P are furthermore known to enhance species richness rather than reduce it in many ecosystems. For this statement the authors refer to the papers of Phoenix et al. (2003) and Carroll et al. (2003). Both papers do, however, not discuss the authors statement, or imply that this process might explain species loss following N enrichment. Both papers look at how P addition in P limited grassland systems affects patterns of N uptake during N enrichment treatment. I would suggest that the authors remove this statement from the manuscript, or try to provide appropriate references.

- I miss the rationale of the study at the end of the Introduction. Instead of discussing the results of the study at the end of the introduction, the authors should add a short paragraph that explains their study design (field experiment) and clearly state their research aims and hypotheses.

-Maybe the authors could add a small description of the specific grassland community that occurs at the studied site to the materials and methods section (P6). It might also be useful to add a species list of the different species observed in this study to an appendix.

- The authors fail to report test statistics and p-values throughout the results section. For example, none of the performed ANOVA analyses results are reported in the manuscripts, beside in figures containing symbols to indicate significances. I find it difficult to evaluate the strength of certain statistically tested patterns without this information. I would suggest that the authors provide all test statistics, p-values and, where

relevant, R2-values and degrees of freedom, either directly in the results section, or in a table.

- The authors explain that they split up species composition in 'grasses' and 'forbs'. Does the 'grasses' group exclusively include species of the Poaceae family? If it also contains species of other, related, families such as the Cyperaceae or the Juncaceae, it might be better to refer to this group as 'graminoids' rather than 'grasses'.

Specific:

Introduction

- P3, L. 5: Replace 'has increased drastically due to…fossil fuels globally' by 'has worldwide increased drastically due to… fossil fuels'.

- P3, L. 7: It is not clear to me what the authors mean by 'ecosystem structure'. Please clarify.

- P3, L. 17/19: Similar to L. 5, do not end sentences with the words 'globally' or 'consequently', this is not correct English.

- P3, L 19: Here the authors state that N deposition can result in a reduction of ecosystem productivity. However in L. 8 they stated that N deposition usually results in an increase of ecosystem productivity.

- P4, L3: Replace 'which is thought to' by 'which are thought to'.

- P4, L4: I think 'herbaceous species' and 'forbs' would refer to the same species in this context.

- P4, L23-24: Confusing sentence, please rewrite.

- P5, L6: This seems somewhat general. To what grasslands do the authors refer when they talk about 'acid grasslands'. Do they refer to steppes with more acidic soils, or does this refer to European semi-natural acidic grasslands? The reference 'Van der

Putten et al. 2013' does furthermore not seem to support this statement.

- P5, L19-20: The results of this study should not be discussed in the introduction section of the paper. Remove this sentence.

Materials and Methods

- P5, L26: Replace 'fallen' by 'falling'.

- P6, L1: Is the China's soil classification system internationally known? If not, please shortly specify what a 'chestnut type' implicates.

- P6, L16: Please specify in what year biomass/diversity measures were obtained, and thus over how many years N addition was performed.

- P6, L16: What 'samples' were collected?

-P6, L19: Replace 'Aboveground biomass (AGM) classified artificially as forbs and grasses' by 'aboveground biomass (AGM) of forbs and grasses separately'.

- P6, L22: What do the authors mean by 'clipping'?

- P6, L23: What do the authors mean by 'biomass was measured 'separately'?

- P8, L 18-18: Replace 'the difference between species richness and AGB under six levels of N addition' by 'the difference in species richness and AGB among six levels of N addition'.

- P8, L 20-21: Awkward sentence, please rewrite.

Results

- P9, L 8: Was it AGB or N addition that was 4 g/(m2 yr) at peak values? It might furthermore be informative to give the mean values of AGB and species richness over the treatments.

- P10, L 2: Why was soil EC compared among N treatments using linear regression,

when all other soil variables were compared among N treatments using ANOVA analyses?

-P10, L26-29: If the results of the stepwise regression are retained in the manuscript, then please provide a summarizing table for these results containing test-statistics, R2-values, p-values and degrees of freedom.

-P12, L5: I would argue that Zn2+ is strongly correlated with the second PCA axis.

Discussion

- P13, L15-16: Please specify by what organisms the authors assume urea to be converted in ammonium and nitrate.

- P13, L17: Add 'show'.

- P14, L2: Replace 'in Europe grasslands' by 'in semi-natural European grasslands'.

- P15, L18-19: Please specify that the study of Chytry et al. (2007) refers specifically to Siberian vegetations (tundra, grassland & forest).

Tables & Figures

- Figures 4 and 5: Please indicate which regression curves/point symbols refer to grasses and which refer to forbs.

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