Biogeosciences Discuss., 12, C1394–C1411, 2015
www.biogeosciences-discuss.net/12/C1394/2015/

Biogeosciences Discussions
Open Access

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.

Q.-Y. Tian et al.

whzhang@ibcas.ac.cn

Received and published: 18 April 2015

We greatly appreciate the constructive comments and suggestions on our submitted manuscript (bg-2014-599) made by the reviewer. We thoroughly revised the manuscript by addressing the concerns and comments. The point-to-point responses to the reviewers' comments are given below.

Reviewer #1

(1) 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 multi-collinearity 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 vari-able 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 speciïňĄcally de-signed 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.

Answer: We thank the reviewer's constructive suggestions on the statistical analysis. We re-analyzed the relation between diversity/biomass and soil variables, using a mul-

BGD
12, C1394–C1411, 2015

tiple regression model instead of RDA as suggested by the reviewer. We also used the different PCA axes instead of the raw soil variables to determine the predominant soil variable that drives changes in diversity and biomass. The analyses of the data revealed that reduction in forb richness was primarily driven by changes in soil Mn2+ concentrations (Fig 6, Table 2 and Table A3).

(2) 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 speciïňĄc comments. I would nevertheless still strongly suggest that the authors have the manuscript proof-read by a native English speaker.

Answer: The manuscript has been carefully revised and edited.

(3) 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.

Answer: We deleted the repetitive sentences in the revised manuscript.

(4) 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.

BGD
12, C1394–C1411, 2015

I would suggest that the authors remove this statement from the manuscript, or try to provide appropriate references.

Answer: We acknowledge the reviewer's comments on the references which we referred to. We agree with the review's comment on the effect of P availability on species richness. We reworded these sentences and deleted the two references as suggested by the reviewer in the revised manuscript. In the present study, we found that N addition significantly increased P availability in soils, which mainly due to soil acidification induced by N addition, because P is mainly precipitated as calcium phosphate in calcareous and alkalinous soil, its solubility is dependent on soil pH.

(5) 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 (ïňĄeld experiment) and clearly state their research aims and hypotheses.

Answer: We deleted the sentences about the results in the introduction and added a short paragraph about our research aims and hypotheses as suggested by the reviewer in the revised manuscript.

(6) Maybe the authors could add a small description of the speciïňĄc 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.

Answer: We included a brief description about the community of the study area and a list of the different species was given in Table A4 as suggested by the reviewer in the revised manuscript.

(7) 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 ïňĄgures containing symbols to indicate signiïňĄcances. I

BGD
12, C1394–C1411, 2015

ïňĄnd it difïňĄcult 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.

Answer: We included the results of statistics analyses in the results section as suggested by the reviewer.

(8) 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'.

Answer: In our study, the grass group does not exclusively include species of the Poaceae family, and it also includes the speceis of Cyperaceae family. The detailed species characteristics of the vegetation were listed in the revised manuscript.

(9) P3, L. 5: Replace 'has increased drastically due to: : :fossil fuels globally' by 'has worldwide increased drastically due to: : : fossil fuels'.

Answer: We reworded these sentences accordingly as suggested by the reviewer.

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

Answer: We changed "ecosystem structure" to "community composition of ecosystem".

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

Answer: We have revised these sentences accordingly.

(12) 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.

BGD
12, C1394–C1411, 2015

Answer: For the N limited ecosystems, elevated atmospheric N deposition generally has positive effects on productivity. However, with increase of N deposition or N addition, especially for N-saturation ecosystems, species richness declines and consequently results in reduction in ecosystem productivity (Isbell et al., 2013).

(13) P4, L3: Replace 'which is thought to' by 'which are thought to'.

Answer: We changed 'which is thought to' into 'which are thought to'.

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

Answer: We have deleted the herbaceous species.

(15) P4, L23-24: Confusing sentence, please rewrite.

Answer: We rewrote these sentences in the revised manuscript.

(16) 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.

Answer: The 'acid grasslands' refer to those grasslands with acidic soils. We acknowledge the reviewer's comments on the references which we referred to. We deleted the reference 'Van der Putten et al. 2013' in the revised mansucript.

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

Answer: We deleted this sentence.

(18) P5, L26: Replace 'fallen' by 'falling

Answer: We made the change accordingly.

(19) P6, L1: Is the China's soil classiïňĄcation system internationally known? If not,

BGD
12, C1394–C1411, 2015

please shortly specify what a ‘chestnut type’ implicates.

Answer: We added an interpretation about chestnut type soil in the revised manuscript.

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

Answer: We added the information about the year in which biomass/diversity measures were obtained and the N addition was performed.

(21) P6, L16: What ‘samples’ were collected?

Answer: The samples were soil and plant samples.

(22) P6, L19: Replace ‘Aboveground biomass (AGM) classiïňĄed artiïňĄcially as forbs and grasses’ by ‘aboveground biomass (AGM) of forbs and grasses separately’.

Answer: We have changed this sentence as suggested by reviewer.

(23) P6, L22: What do the authors mean by ‘clipping’?

Answer: The clipping means cutting plant shoots with scissors close to the ground.

(24) P6, L23: What do the authors mean by ‘biomass was measured ‘separately”?

Answer: ‘Biomass was measured separately’ means the biomass of each species was measured individually.

(25) 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’.

Answer: We reworded this sentence in the revised manuscript.

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

Answer: We rewrote the sentence in the revised manuscript.

BGD
12, C1394–C1411, 2015

(27) 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.

Answer: We verified that AGB peaked 425.8 g m-2 at N addition rate of 2 g m-2 yr-1, and the mean values of AGB were also presented in the manuscript.

(28) 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?

Answer: We compared soil EC among N treatments using ANOVA analyses and included the result in Fig. A1.

(29) 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.

Answer: We provide a summarizing table for the results of the stepwise regression as suggested by the reviewer in Table A3.

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

Answer: We re-analyzed the results using the statistical methods as suggested by the reviewer. The results show that the six metal cations (Ca2+, Mg2+, Mn2+, Fe3+, Cu2+, Al3+) were significantly correlated with soil pH, and that they were used for the PCA analysis. Because Zn2+ was neither correlated with soil pH nor correlated with N addition rate (Table A1), Zn2+ was not included in the PCA analysis.

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

Answer: The organisms converting urea into ammonium and nitrate were added.

(32) P13, L17: Add ‘show’.

BGD

12, C1394–C1411, 2015

Answer: We added ‘show’ as suggested by the reviewer.

(33) P14, L2: Replace ‘in Europe grasslands’ by ‘in semi-natural European grasslands.

Answer: We modified this sentence accordingly.

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

Answer: The sentence was revised accordingly.

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

Answer: We added the indication of the regression curves/point symbols referring to grasses and forbs, respectively.

Reviewer #2

(1) The manuscript from Tian et al. addressed the effects of increasing N availability on species loss based on a 9-yr and multi-level N addition experiment in a temperate steppe in Inner Mongolia. The effects of increasing N deposition on biodiversity have been received more and more attention. While many underlying mechanisms have been reported for the negative effects of N on biodiversity, this study showed that the changes of soil ions would account for such a negative effect. The experiment is well designed and performed, and the manuscript is well written. In the third paragraph of the introduction, authors mentioned that the soil acidiïňĄcation-mediated processes in species loss have been evaluated in acidic grasslands. I am wondering why we need to know whether such cases are occurring in other grasslands or not. What is the potential difference of acidic grassland and other types of grassland? And then, are these differences would lead to different changes of ions in soils? I think the explanations are important scientiïňĄc basis for carrying out this study. Actually, they may be more important than what have been shown in the introduction (different mechanisms from light limitation to ammonia toxicity). We have known much of those mechanisms

BGD
12, C1394–C1411, 2015

and case studies. I suggest authors to address those questions in the introduction. I also suggest authors to focus more on the ion toxicity to make the introduction more concrete.

Answer: We greatly appreciate the positive comments made by the reviewer on our work. In contrast to the acidic grasslands, little is known of the involvement of soil acidification-mediated processes in species loss under elevated N deposition in other type's grasslands, including the temperate steppe. A major difference between acidic grasslands and temperate steppe used in the present study is the basic properties of soils, such as soil pH, ion contents and acid buffering systems. In acidic grasslands, soil pH is usually <5.0, and availbilities of metal ions, such as Al3+, Fe3+, Mn2+ are high compared to those in the alkaline soils, and acid buffering is mainly dependent on aluminium, leading to lower acid buffering capacity (Bowman et al., 2008). However, soils in neutral or alkaline grasslands have more base cations, higher acid buffering capacity and low availbilities of metal ions (Al3+, Fe3+, Mn2+). In addition to the differences in soil traits, plants grown in acidic and alkaline grasslands may also have evolved adaptative strategies to their edaphic conditions. Plants in the alkaline temperate steppe would be imposed to high levels of metal concentrations due to N deposition-driven soil acidification, rendering them metal toxicity. Therefore, plants in the alkaline grasslands and acid grasslands may differ in their sensitivity to N deposition-induced changes in soil traits. We included this hypothesis in the revised manuscript.

(2) Both in the results and discussion section, authors demonstrate that loss of forbs is not caused by the competitive exclusion from increasing growth of grass after fertilization. Instead, they concluded that mobilized Mn2+ after soil acidiïňĄcation is responsible for forb loss after N addition. While this conclusion has great scientiïňĄc sense and is very interesting, authors should have more explanation (or potential reason) for the diver-gent responses of grass and forb to the mobilization of Mn2+. The explanation would be essential for this study. Authors should not simply show the phe-

BGD
12, C1394–C1411, 2015

nomenon, but they should present some potential underlying mechanisms for those different responses of grasses and forbs. In my opinion, such contents would substantially improve the quality of this manuscript.

Answer: We included the explanation for the divergent responses of grasses and forbs to the mobilization of Mn2+ as suggested by the reviewer in the revised manuscript.

(3) Page 1690 line 2-3 Enhanced N is a threat to biodiversity in almost all ecosystems instead of only in grassland.

Answer: We reworded this sentence as suggested by the reviewer.

(4) Page 1690 line 8-9 It is important to note that the changes in species composition do not mean changes of biodiversity.

Answer: We made changes accordingly in the revised manuscript.

(5) Page 1691 line 2-20 The ïňĄrst paragraph should be shorten. There many overlaps in the contents of this paragraph.

Answer: We deleted the repetitive sentences in the revised manuscript.

(6) Page 1694 line 10 Salsola collina is not a perennial species Page 1694 line 19 'aboveground biomass' Page 1697 line 7 From Figure 1a, it seems that AGB peaked at 2 g m-2 yr-1.

Answer: We corrected the mistakes, and revised them accordingly.

(7) Page 1697 line 21-23. Those two sentences can be deleted from the Results section.

Answer: We deleted the two sentences as suggested by the reviewer.

(8) Page 1698 line 17-20 This sentence should be shorten, as the ïňĄrst and second parts of this sentence are somewhat overlapped.

Answer: We removed the repetitive sentences in the revised manuscript.

BGD
12, C1394–C1411, 2015

(9) Page 1698 line 22 ‘showed’ instead of ‘were’.

Answer: We fixed the typo.

(10) Page 1698 line 25 – Page 1699 line 4 While the stepwise regression between ions and N addition makes sense, I don’t think the similar regression between ions and pH makes any sense. Authors stated that soil acidiïňĄcation is a driver for the mobilization of those ions (Page 1698 line 24), it would be self-contradictory to show that any ion change is the major explanation of variation of soil pH.

Answer: We took the the reviewer’s advice and deleted this result in the revised manuscript.

(11) Page 1701 line 27 The ïňĄnding that’ Page 1705 line 9 How did the soil nutrient depletion occur?

Answer: In neutral and alkaline grasslands, acid buffering of soils mainly depends on the base cations (Ca^{2+} and Mg^{2+}) (Bowman et al., 2008). N addition-induced production of hydrogen ion leads to release of Ca^{2+} and Mg^{2+} ions from soil particles. Because Ca^{2+} and Mg^{2+} are the moderate nutrients for plant growth, with increasing biomass of plants driven by N addition, plants more Ca^{2+} and Mg^{2+} would be taken up by plants from soils, leading to depletion of Ca^{2+} and Mg^{2+}. Compared with Ca^{2+} and Mg^{2+}, Fe^{3+}, Mn^{2+}, Al^{3+}, Cu^{2+} and Zn^{2+} are micronutrients, and the amounts of the metalsabsorbed by plants are less than those of released from soils driven by N addition-induced soil acidification. Therefore, N addition usually results in depletion of base cations and increasea in those metal ions of Fe^{3+}, Mn^{2+}, Al^{3+}, Cu^{2+} and Zn^{2+}.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C1394/2015/bgd-12-C1394-2015-supplement.pdf

Interactive comment on Biogeosciences Discuss., 12, 1689, 2015.

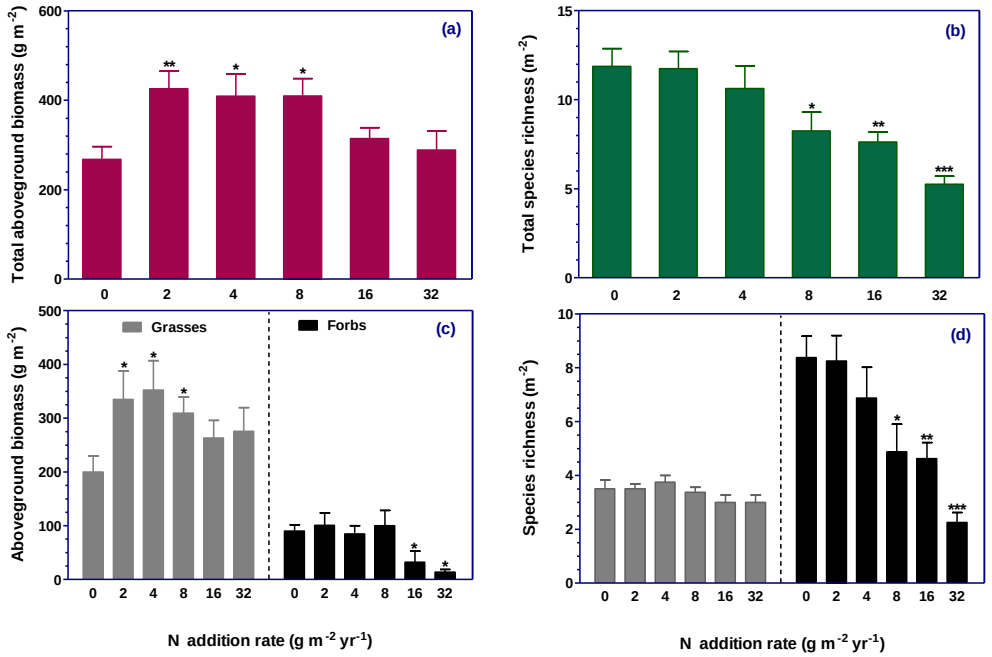


Fig. 1

ll Screen / Esc
er-friendly Version
active Discussion
Discussion Paper

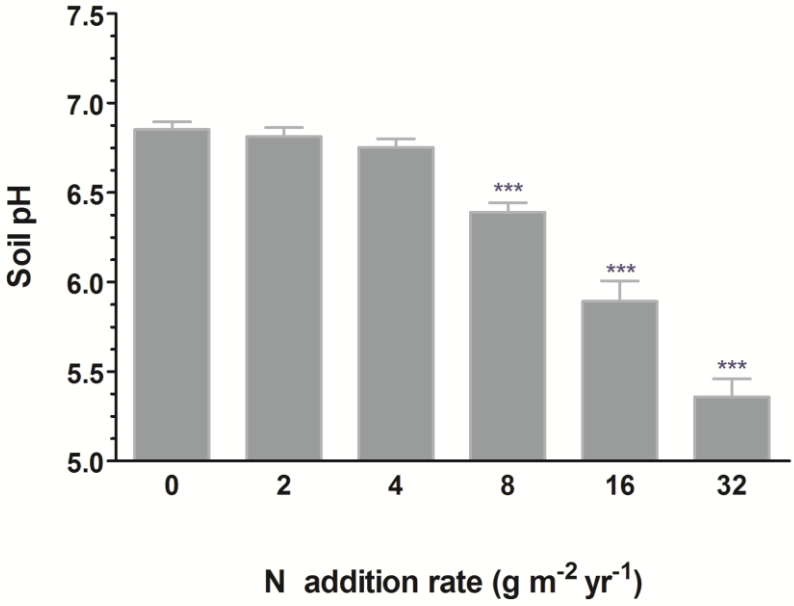
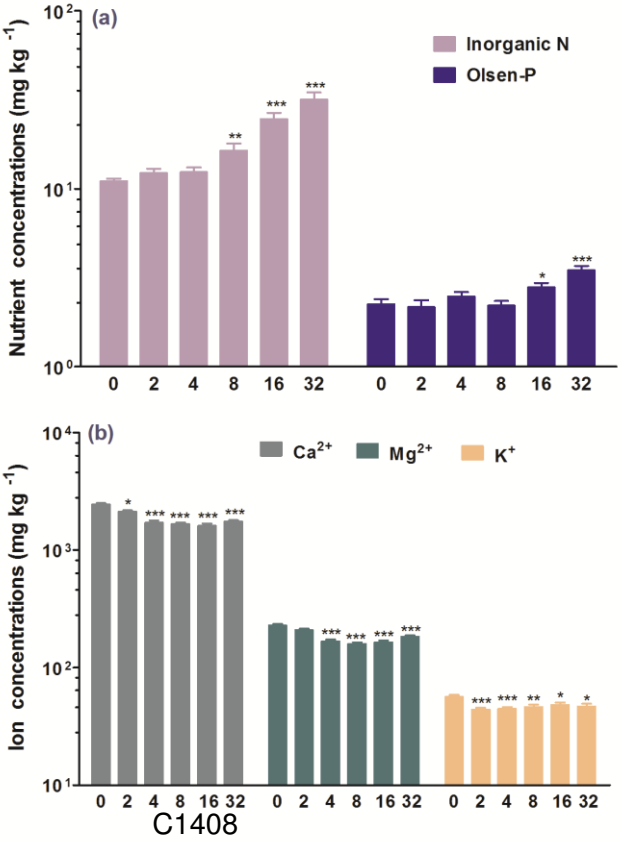


Fig. 2

Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

BGD
12, C1394–C1411, 2015

Interactive Comment



Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

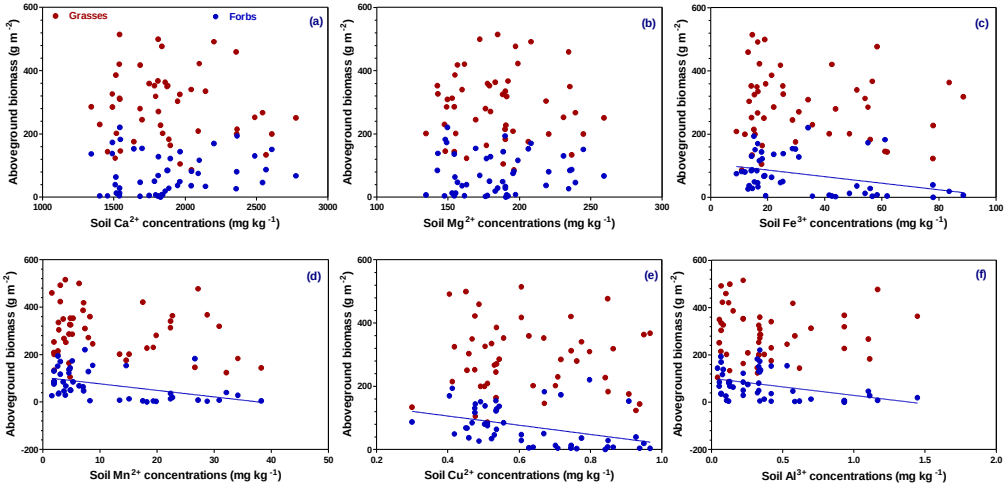


Fig. 4

Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

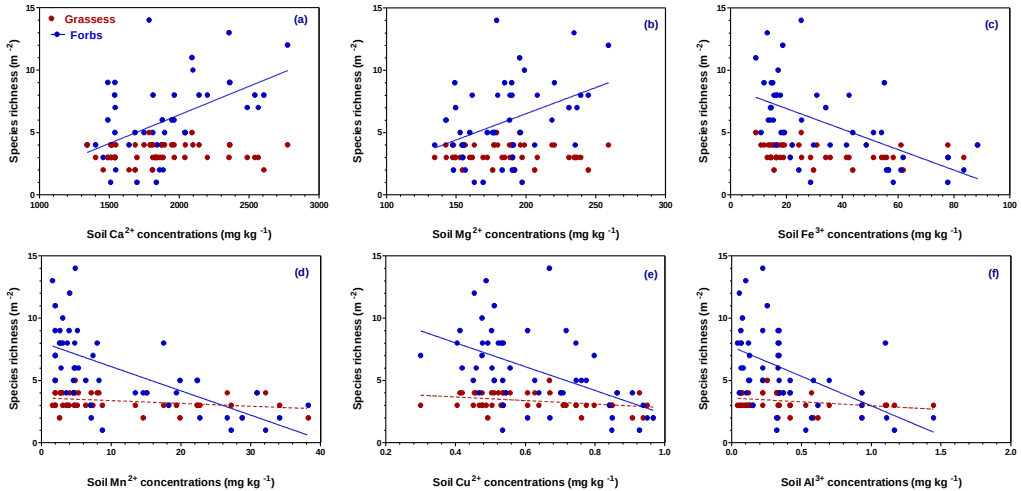


Fig. 5

Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

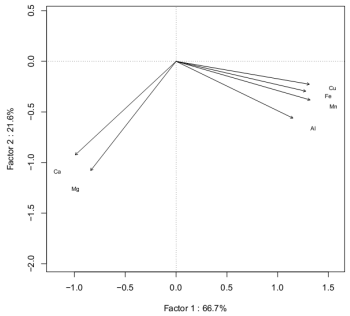


Fig. 6

Fig. 6. Fig. 6 Projection of six elemental variables for principle component analysis factors one and two.

BGD
12, C1394–C1411, 2015