

Point-to-Point Response

Reply to Reviewer #2

1. Overall comments: This manuscript presents plant functional traits at species, plant functional groups and community level measured in paired grazed areas (60 years or longer of free grazing) and enclosures (between 18 and 28 years fenced plots), which are located in six representative vegetation communities of the Xilin River Basin, Inner Mongolia. All six sites are said to have similar climatic and soil conditions and a gradient of standing aboveground biomass (Zheng et al., 2010), soil nitrogen and organic carbon contents and field holding capacity (present manuscript). Altitude is variable. While the title refers to linkages to ecosystem functioning, the abstract say “We test functional trait-based mechanisms underlying the responses of different life forms to grazing and linkages to ecosystem functioning along a soil moisture gradient in the Inner Mongolia grassland”. Such study of plant functional traits along a moisture gradient and contrasting land use seems to be useful investigation.

I missed the moisture gradient in the title and further wonder (still) what authors refer to with “trait-based mechanisms” and “ecosystem functioning”. Reading the manuscript I have missed firstly, a concrete mention of the ecosystem functioning the title refers to – I need to assume this has to do with plant strategies and vegetation functioning, although effects on functions such as nutrient cycling and specifically, ANPP are not mentioned.

Reply: We appreciate the constructive comments made by the reviewer. We agree with the reviewer that the current title is inexact, and we have revised it as “Functional trait responses to grazing are mediated by soil moisture, plant functional group identity and composition”.

2. Comments: Secondly, I also miss a clear definition of the gradient under study, soil moisture and other associated soil properties. As reader, it is very difficult to understand why a moisture gradient would exist along sites that receive equal precipitations and are located in

similar soils; in a way, I certainly missed precipitation data from these six different sites and certainly, “Field holding capacity (%)” does not clearly represents the moisture gradient or explain why this is such (Is it an acceptable proxy of moisture gradient in the Inner Mongolian grasslands?).

Reply: In this study, the six plant communities are subjected to similar climatic conditions (i.e. patterns of temperature and precipitation), but they differ in soil moisture and other soil properties (e.g. soil organic carbon and nitrogen contents). This is mainly caused by topography-controlled wind and water erosion and deposition processes (Hoffmann et al. 2008; Kolbl et al. 2011). We have revised the text accordingly.

We agree with the reviewer that field holding capacity may not be an appropriate proxy of soil moisture in the Inner Mongolian grasslands. Thus, we directly used the soil moisture instead of field holding capacity as a continuous variable, and the results in Figures 2, 3, 4, 5 were presented along the soil moisture gradient in the revised manuscript. The *Carex appendiculata* meadow had the highest soil moisture (33.32%), followed by the *Stipa baicalensis* meadow steppe (15.55%), and the four typical steppe communities have lower soil moisture (11.40%, 9.49%, 7.75%, 7.14%, respectively) (see Table 1).

Hoffmann, C., Funk, R., Wieland, R., Li, Y., and Sommer, M.: Effects of grazing and topography on dust flux and deposition in the Xilingele grassland, Inner Mongolia, J. Arid Environ., 72, 792-807, 2008.

Kölbl, A., Steffens, M., Wiesmeier, M., Hoffmann, C., Funk, R., Krümmelbein, J., Reszkowska, A., Zhao, Y., Peth, S., Horn, R., Giese, M., and Kögel-Knabner, I.: Grazing changes topography-controlled topsoil properties and their interaction on different spatial scales in a semi-arid grassland of Inner Mongolia, P.R. China, Plant Soil, 340, 35-58, doi: 10.1007/s11104-010-0473-4, 2011.

3. Comments: Results are not always presented following the moisture gradient (see Figs. 5 and 6) but along vegetation communities as in previous publications. This inconsistency created confusion and wonder whether discussion and conclusions can actually talk about vegetation responses to grazing along a (soil nutrient, soil water availability or ANPP?)

gradient.

Reply: We have totally revised the Results section and Figures as suggested.

4. Comments: To my mind, the moisture gradient needs to be better defined at the very beginning of the manuscript and both,

Reply: We have defined the moisture gradient in the revised Introduction and Methods sections.

5. Comments: Figures and Tables in results section adapted accordingly so this manuscript discuss strongly a gradient. The feeling is that otherwise, there has not been a significant progress from the published article Zheng et al., 2010. “Effects of grazing on leaf traits and ecosystem functioning”.

Reply: We have revised the entire manuscript by focusing on how plant responses to grazing are mediated by soil moisture, plant functional group identity and composition.

6. Comments: Finally, any moisture gradient in a arid/semi-arid grassland will result in a standing biomass gradient and this is the case on this study – there is a linear association (linear regression with $R^2=0.77$) between standing biomass and field holding capacity of these communities (combining data from Zheng et al., 2010 and the present manuscript).

Reply: Previous studies have proposed that water availability is the key limiting factor controlling the spatial and temporal patterns of primary production in arid and semiarid grasslands on the Mongolian plateau, which along explained more than 76% of the variation in primary production at regional scale (Bai et al., 2008, 2012).

Bai, Y. F., Wu, J. G., Xing, Q., Pan, Q. M., Huang, J. H., Yang, D. L., and Han, X. G.:
Primary production and rain use efficiency across a precipitation gradient on the

Mongolia plateau, *Ecology*, 89, 2140-2153, 2008.

Bai, Y. F., Wu, J. G., Clark, C. M., Pan, Q. M., Zhang, L. X., Chen, S. P., Wang, Q. B., and Han, X. G.: Grazing alters ecosystem functioning and C:N:P stoichiometry of grasslands along a regional precipitation gradient, *J. Appl. Ecol.*, 49, 1204-1215, doi: 10.1111/j.1365-2664.2012.02205.x, 2012.

7. Comments: I found the manuscript readability fair. I got distracted with many questions and unclear statements. Several of these queries were clarified after reading Zheng et al., 2010. Abstract: Suggest that the study will refer to life forms results.

Reply: We agree with the reviewer that the original version of our manuscript was not well organized. Thus, we revised the entire manuscript by eliminating the redundant parts and improving the clarity of seemingly confusing places as suggested by the reviewer.

8. Comments: Introduction: On the one hand, this section introduces the reader to very fundamental functional concepts such as the leaf economics spectrum, leaf traits and growth rate associations, plant functional groups as well as the stress-gradient hypothesis and resource availability hypothesis. On the other hand, it presents models of plant strategies, e.g. conservative vs. acquisitive, grazing tolerant vs avoidance. It also mentioned “linkages to ecosystem functioning”. All these concepts are intimately related but not necessarily well connected in this manuscript.

Reply: We agree with reviewer that these interrelated concepts were not well connected in the previous version of our manuscript. We have reorganized the Introduction section by removing the seemingly confusing parts, such as “mechanisms”, “biotic factors “leaf economics spectrum”, and “linkages to ecosystem functioning”. We also added the definition of “avoidance and tolerance strategies” and their expected association with traits and the interpretation of “the mixed strategies” as suggested by the second and third reviewers.

Specifically, we focused on presenting the resource availability hypothesis and context-dependency hypothesis. The context-dependency hypothesis proposes that plant

responses to grazing are not only determined by site productivity or resource availability (Pérez-Harguindeguy et al., 2003; Daleo and Iribarne, 2009; Eldridge et al., 2013), but also by plant species or functional group identity (Wardle et al., 2008; McLaren and Turkington, 2010). Although extensive studies have shown that plant responses to grazing are mediated by resource availability (e.g., soil moisture) (Adler et al., 2004; Pakeman, 2004; Díaz et al., 2007; Laliberte et al., 2012), few studies, however, have examined the context-dependent effects of plant functional group identity and composition. In this study, we examine the effects of grazing on plant functional traits and shift in functional group composition along a soil moisture gradient, and test the context-dependency hypothesis of grazing effects on plant functional traits mediated by both soil moisture and plant functional group identity and composition.

In addition, the view of mixed strategies of plant defense against herbivores has been paid more attention in recent years. However, it remains largely unexplored (Carmona and Fornoni, 2013; Turley et al., 2013). For example, Carmona and Fornoni (2013) found that the complexity of herbivore communities selects for mixed resistance–tolerance strategies of plants, which is contrary to a long-standing prediction that resistance and tolerance are functionally redundant (Vandermeijden et al., 1988). Several key functional traits (e.g. SLA) may provide important insights into the mixed strategies of plants to grazing and environmental fluctuations. In this study, we explore whether the dominant perennial bunchgrasses in the arid and semiarid grasslands have developed the mixed tolerance–resistance strategies to grazing and mixed acquisitive–conservative strategies in resource utilization in the process of evolutionary adaptation to arid environments and co-evolution with herbivores. Many studies have proposed that SLA is positively linked to potential growth rate (Reich et al., 1999; Wright et al., 2004), resource acquisitive strategies (Díaz et al., 2004; Tecco et al., 2010), and plant tolerance to grazing (Díaz et al., 2001; Zheng et al., 2011). Low SLA is tightly related to physical toughness (Villar and Merino, 2001; Wright et al., 2004), resource conservative strategies (Díaz et al., 2004; Tecco et al., 2010), and plant resistance to grazing (Hanley et al., 2007). Thus, we test the hypothesis of mixed strategies of plant anti-herbivore defense and resource utilization, by using SLA of perennial bunchgrasses. Although the variation in SLA could have arisen

from plastic responses of these species to grazing and varying soil moisture, however, it has evolutionary advantage for these species to persist and dominate against grazing and water fluctuations in arid and semiarid grasslands. Results from this study provide some new insights for future studies.

9. Comments: Page 13161, line 25: “we would expect that:...” these expectations cannot be depicted from your introduction.

Reply: Revised as suggested.

10. Comments: It is actually confusing the use along this section of several entities such as (i) life forms and (ii) plant functional group identity, (iii) species, (iv) vegetation types and (v) grassland vegetation communities. So one cannot know where the focus of the manuscript is.

Reply: We agree with the reviewer that these categories are confusing. We have removed the misleading expressions throughout the text in the revised manuscript. For example, in the Methods section the original sentences were replaced with, “In this study, six pairs of parallel grazed and ungrazed plant communities, i.e. *Carex appendiculata* meadow, *Stipa baicalensis* meadow steppe, *Leymus chinensis* typical steppe, *S. grandis* typical steppe, *Caragana microphylla* typical steppe, and *Artemisia frigida* typical steppe were selected along a soil moisture gradient in the Xilin River Basin.”..... “All species were classified into plant functional groups based on their life forms, including perennial grasses (PG), perennial forbs (PF), annuals and biennials (AB), and shrubs and semi-shrubs (SS).”

11. Comments: Materials and Methods: Although additional reading helped me to understand this section, it is unclear yet the total number of species sampled, and the number of species present in both grazed and enclosure plots.

Reply: In this study, a total number of 276 species were sampled across six paired plant communities, with 149 species in the ungrazed sites and 127 species in the grazed sites.

There were 113 shared species in both ungrazed and grazed sites. We have added this information in the revised Methods section.

12. Comment: Authors used a different set of species than previous articles so I suggest including the list of species as supplementary material.

Reply: We have added a list of plant species collected in the ungrazed and grazed sites across six paired plant communities as supplementary material (Appendix A).

13. Comment: Nine plant functional traits are mentioned. How about palatability scores?

Reply: In this study, we measured nine plant functional traits, including plant height, individual biomass, stem biomass, leaf biomass, stem-leaf biomass ratio, total leaf area, leaf density, specific leaf area (SLA), and leaf N content. For each species, the palatability score was collected from the Forage Plants of Inner Mongolia (Fu, 1990). As suggested by the third reviewer, the palatability score integrating plant palatability index and browsing season index is not reasonable in this study, because the browsing season index refers to external land-use factors but not the plant functioning itself. Hence, we only used the palatability index to reflect plant palatability in the revised manuscript.

Fu, X. Q.: Forage Plants of Inner Mongolia, Inner Mongolia People's Press, Hohhot, 1990.

14. Comments: Some traits were derived to community-weighted means (CWM). Traits were measured “For each ungrazed or grazed site...”. Were CWM calculated based on traits measured on separate sites or using the mean trait values of the complete data set? Definitely, sites have different composition and traits values (intraspecific variability). We don't know if these factors were accounted.

Reply: For each grazed and ungrazed sites, the community-weighted attributes for plant height ($\text{height}_{\text{CWM}}$), stem-leaf ratio (SLR_{CWM}), specific leaf area (SLA_{CWM}), and leaf N

content (LNC_{CWM}) were calculated as trait means weighted by the relative biomass of each species within each quadrat (Violle et al., 2007). In this study, the trait mean value is calculated based on 10-20 randomly selected individuals of each species at each site. Plant species composition and aboveground biomass were sampled by using 5–10 quadrats (1×1m each) at each site. For each site, 10 quadrats were collected for meadow steppe and typical steppe, and 5 quadrats were collected for the more homogeneous meadow. Thus, the community-weighted means (CWM) are calculated based on traits measured at separate sites and plant relative biomass of each quadrat at corresponding site. We have revised the Methods section for clarity.

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E.: Let the concept of trait be functional! *Oikos*, 116, 882-892, doi: 10.1111/j.2007.0030-1299.15559.x, 2007.

15. Comment: Please, consider replacing "i.e." by "e.g." in several sentences of your manuscript; for example in Page 13165-Line 21.

Reply: Revised as suggested.

16. Comments: Results: Section 3.1. Principal component analysis: Fig. 1, why PC2 is not shown? SLA and PH are in general quite important functional traits.

Reply: We have added PC2 axis in Fig. 1 as suggested by the reviewer. In PCA analysis, the three principal components reflect the syndrome of several functional traits. In this study, PC1 explained 43% of the total variance and represented an axis of plant size, reflecting by plant individual biomass, stem biomass, leaf biomass, and total leaf area. PC2, which explained 19% of the variance, was strongly associated with plant height, stem-leaf biomass ratio (SLR) and specific leaf area (SLA), representing an axis of biomass allocation and high capacity for aboveground/light competition. PC3, which explained 14% of the variance, was primarily driven by leaf N content and leaf density, representing an axis of leaf nutrient

acquisition and shoot growth. Thus, plant individual biomass loaded more scores on PC1 axis, while plant height loaded more scores on PC2 axis.

17. Comment: Unexpectedly, Fig. 1a shows low correlation of plant height and plant biomass.

Reply: Our results showed that, when relationship between two functional traits was analyzed, plant height was positively correlated with plant biomass ($r = 0.358$, $P < 0.00001$).

18. Comment: In Fig. 1b, dots representing species from grazed and enclosure plots share the same ordination space suggesting similar vegetation functioning. However, authors remark the different functioning in grazed/enclosure plots as results of Fig 1c.

Reply: Although species from grazed and ungrazed plots share the same ordination space, the magnitude of the score distribution of species differed significantly between the ungrazed and grazed plots.

19. Comment: please check here whether or not different letters should be used for PC2 where $p=0.1011$).

Reply: Done as suggested.

20. Comment: I wonder whether the PCA biplot could actually show functional difference between grazed/enclosure vegetation by displaying PC1 and PC2 instead of PC1 and PC3.

Reply: We agree with the reviewer that the effect of grazing on PC2 score was not significant, and we have rephrased the sentences as following: “The PC1 and PC3 axes distinctly separated species from the grazed and ungrazed communities (Fig. 1b). Grazing significantly decreased the loading score of plant size along PC1 axis ($P = 0.0163$), but greatly increased the loading score of leaf N content and leaf density along PC3 axis ($P =$

0.0016, Fig. 1c). However, the PC2 score of plant height, SLR and SLA was little affected by grazing ($P = 0.1011$)."

21. Comments: Finally, the study "have examined how plant responses to grazing are mediated by resources availability..." I suggest using a constrained analysis (i.e. DCA) for assessing to what extent the moisture gradient explains functional response of vegetation.

Reply: We have conducted a CCA analysis to quantify to what extent soil moisture influences plant community structure based on the relative abundance of different life forms. The results of CCA demonstrated that plant community structure in terms of life form composition was significantly influenced by environment variables. The first axis (CCA1) was mainly affected by soil moisture, explaining 89.8% of the total variance; the second axis (CCA2) was more driven by grazing, explaining 10.2% of the total variance (Fig. A1 in the Appendix C).

22. Comments: Section 3.2. In Table A2, field holding capacity (the moisture gradient under study in this manuscript) affects significantly three plant functional traits (i.e. stem-leaf biomass, specific leaf area and leaf nitrogen content, which explain functional variability of PC2. This axis is not shown and this result might be worth mentioning.

Reply: We have added PC2 axis in Fig 1 in the revised manuscript as suggested by the reviewer. We defined the moisture gradient as soil moisture instead of field holding capacity as suggested. We also revised the results of stem-leaf biomass, specific leaf area and leaf nitrogen content accordingly.

"In addition, among-site variations in plant height and individual biomass were mainly affected by grazing, while the among-site variations in SLR, SLA and leaf N content which related to plant biomass allocation and aboveground/light competitive capacity (PC2 axis) were primarily driven by soil moisture."

23. Comments: This section (3.2) includes responses of functional traits by species and by

functional groups, Figs. 2 and 3, respectively. The following section (3.3) presents results at species level and after all in section 3.5 results at community level (CWM) are given. This is difficult to follow and add confusion. Section 3.4 provides the relative biomass of functional groups, between results of plant functional traits responses. I suggest to rearrange the order of sections.

Reply: We agree that the arrangement of Result sections is difficult to follow. We have rearranged the order of these sections. Specifically, in Section 3.1, we presents the associations among 9 functional traits of 276 plant species by base on a PCA analysis (Fig. 1). In Section 3.2, we present the responses of four key functional traits at both species (Fig. 2) and functional group levels (Fig. 3), which aims to test the hypothesis that the effects of grazing on plant functional traits are dependent on plant functional group identity and composition. In Section 3.3, we presents the variations in relative abundance of life forms (Fig. 4) and community-weighted attributes (Fig. 5) between the grazed and ungrazed communities along the soil moisture gradient, which aims to test the effects of grazing on shifts in functional group composition and community properties. In Section 3.4, we present the comparisons of functional traits among different life forms (Table A2), and responses of SLA to grazing among the four dominant perennial grasses (Fig. 6), which aims to explore the adaptive strategies of these species.

24. Comments: Section 3.5. Horizontal axes of Figs. 5 and 6 show the six plant communities instead of the moisture gradient represented with the Field holding capacity. Please, consider here to use consistently the moisture gradient along results.

Reply: We have revised the horizontal axes of Figs 5 and 6 by using soil moisture gradient as suggested by the reviewer.

25. Comments: Discussion: Section 4.1. I agree that any reference to growth rate should be included in this section because growth rate was not measured in the present study.

Unfortunately, it is not well explained how leaf traits are associated to fast/slow growth rates.

Even regrowth capacity is mentioned. I found that not sufficient explanation are provided either in the introduction or discussion.

Reply: We have added several relevant references in this section. We also revised the Introduction section accordingly.

26. Comment: Section 4.5. The third conclusion recalls to the question: Is vegetation on this study responding to grazing or to enclosure?

Reply: Based on results from this study, grazing changed plant community structure shifted plant functional group composition, which potentially alter ecosystem functioning. The perennial rhizomatous grasses (e.g., *Leymus chinensis*) and perennial bunchgrasses (e.g., *Stipa grandis*, *Agropyron cristatum*, *Cleistogenes squarrosa*,) are the dominant life forms in the Inner Mongolia grasslands. The perennial bunchgrasses, mostly xerophytes, are more resistant to grazing than perennial rhizomatous grasses (mostly mesoxerophyte) in terms of avoidance and tolerance traits, particularly under heavy grazing pressure and in dry years (Zheng et al. 2011). Moreover, the perennial bunchgrasses exhibit more conservative resource-use strategies (low leaf N content and SLA) in dry and infertile habitats. Previous studies in the same area also demonstrated that heavy grazing shifted plant species and functional group composition, reduced plant species richness, primary production, soil coverage, and increased vulnerability to soil and water erosions (Wan et al. 2011; Schönbach et al. 2011; Kölbl et al. 2011). Together, these findings suggest that destocking rate is an important management tool for mitigating the impacts of shifts in functional group composition and reduction ecosystem primary production and stability in the Inner Mongolia grassland.

Kölbl, A., Steffens, M., Wiesmeier, M., Hoffmann, C., Funk, R., Krümmelbein, J., Reszkowska, A., Zhao, Y., Peth, S., Horn, R., Giese, M., and Kögel-Knabner, I.: Grazing changes topography-controlled topsoil properties and their interaction on different spatial scales in a semi-arid grassland of Inner Mongolia, P.R. China, Plant Soil, 340, 35-58, doi: 10.1007/s11104-010-0473-4, 2011.

- Schönbach, P., Wan, H. W., Gierus, M., Bai, Y. F., Müller, K., Lin, L. J., Susenbeth, A., and Taube, F.: Grassland responses to grazing: effects of grazing intensity and management system in an Inner Mongolian steppe ecosystem, *Plant Soil*, 340, 103-115, doi: 10.1007/s11104-010-0366-6, 2011.
- Wan, H. W., Bai, Y. F., Schönbach, P., Gierus, M., and Taube, F.: Effects of grazing management system on plant community structure and functioning in a semiarid steppe: scaling from species to community, *Plant Soil*, 340, 215-226, doi: 10.1007/s11104-010-0661-2, 2011.
- Zheng, S. X., Lan, Z. C., Li, W. H., Shao, R. X., Shan, Y. M., Wan, H. W., Taube, F., and Bai, Y. F.: Differential responses of plant functional trait to grazing between two contrasting dominant C3 and C4 species in a typical steppe of Inner Mongolia, China, *Plant Soil*, 340, 141-155, doi: 10.1007/s11104-010-0369-3, 2011.