

Reply to Reviewer #2

General comments: This is an interesting paper on the characteristics of leaf traits in 8 grassland communities distributed along a soil moisture gradient. The impacts of grazing on three leaf traits (leaf area, leaf mass, specific leaf area) and on community characteristics (leaf area index, leaf biomass and standing aboveground biomass) were assessed by the comparison of grazed and ungrazed sites for 6 of the communities.

In the result's part, trait's responses are analysed per species for the common ones in the paired ungrazed and grazed sites and per plant functional groups (PFGs), testing seven different classifications. It is shown among communities, different proportions of increased, decreased or unchanged trait's values in response to grazing, and also that trait's values and trait's responses differ among PFGs. Then, LAI, leaf biomass and standing aboveground biomass are compared for the first dominant species of each community, and are also calculated at the whole community level among grazed or ungrazed sites. These variables are used by the authors to describe the ecosystem functioning. Results indicate different responses at the dominant species level but more uniform response at the community level, i.e., a decreased leaf biomass and standing aboveground biomass under grazing.

In this paper, a large amount of observations and measurements have been collected and many results are presented, but the way used to collect the data and the way they are analysed need to be clarified in order to improve the manuscript, the understanding of the results and their interpretations. Thus major modifications are needed before publication.

Main comments are:

1. Comments: It is necessary to indicate if the grazed sites have been protected from defoliation before the period of measurement. If not, it is possible that shorter plants and lower level of standing biomass and LAI reflect the effect of partial defoliation. Were the quadrats protected from defoliation? If not, which were the rules to select the quadrats and the plants for leaf trait measurements in the grazed sites? Same

questions for plant trait's measurements: which procedures were used to select plants not subjected to recent defoliation?

Reply: We appreciate this important point made by the reviewer. Actually, we did not use the enclosures to protect the grazed sites from grazing in this study. This is partly because a rest-grazing practice has been proposed in our study area by local government through a period of 45-60 days each year (early May to late June) since 2005. This practice is partially compensated by the distribution of hay and maize. After this period, grasslands outside the fenced permanent research sites were grazed continuously by domestic animals (mainly sheep) as before. During the time of our field sampling, there was still about 40-50% of the areas that had not been subjected to grazing, particularly in the area near our permanent ungrazed sites. Thus, at each grazed site, 10 quadrats were randomly located in the areas that were not subjected to grazing during the current season for measuring species composition, height, density, coverage, leaf biomass, stem biomass, stem: leaf ratio, and total standing aboveground biomass. Similarly, for each species 30-50 individuals with mature and fully expanded leaves that not subjected to grazing during the current season were randomly collected at each grazed site for leaf trait measurements. We have added this information in the Methods section.

2. Comments: The utilisation of standing aboveground biomass as a proxy of ANPP needs to be justified and clarified.

Reply: Great point. In the Inner Mongolia grassland, both monthly mean temperature and precipitation reach their annual peak concurrently in July. Based on the long-term observations of vegetation dynamics, the standing aboveground biomass in our study area usually reaches its annual peak in August, which has been commonly used to approximate the aboveground net primary productivity (ANPP) in many previous studies in the Inner Mongolia grassland (see Bai et al. 2004. *Nature* 431:181-184; Bai et al. 2008. *Ecology* 89: 2140-2153) and as well as in the North

America short grass steppe (see Lauenroth and Sala. 1992. *Ecological Applications* 2: 397-403).

In this study, the vegetation and soil sampling were conducted during July 28 to August 14, 2007, when the standing aboveground biomass reached its annual peak. Thus, we used the peak standing aboveground biomass to approximate ANPP at the ungrazed sites, but it was only used for standing aboveground biomass at the grazed sites. We have added this information in the Methods section and revised the text accordingly in the current version of our manuscript.

3. Comments: Were dead and green material considered all together or only green material? This could have implication for assessment of standing aboveground biomass, as dead material could be less important in grazed than in ungrazed sites. This could also have an impact on leaf traits if mature and fully expanded leaves used for traits measurements contain or not senescent tissue. This point needs to be explained and clarified.

Reply: During the aboveground biomass measurements, for each species the green and current year dead materials were collected, and litter biomass within each quadrats were also collected. Both the green and current year dead materials were used for calculating stem biomass, leaf biomass, stem: leaf ration, and total community standing aboveground biomass, with litter being not included.

To facilitate our analysis and data interpretations, for each species only mature and fully expanded leaves were used for measuring leaf area, leaf dry mass, and specific leaf area (SLA) at leaf level. However, at species, functional group, and community levels, both the green and current year dead materials were included in calculating the leaf area, leaf biomass, and SLA.

We have added the above information in the Methods section of the revised manuscript.

4. Comments: The analyses are presented by the authors at three different levels,

species, population and community. Nevertheless, it is not clear why the authors use the population level, because the data which are presented (p9956, § 3.4) are in fact data of the first dominant species in each of the grazed or ungrazed sites of the 6 communities. This needs to be clarified. What are the objectives of the presentation of the dominant species of each community?

Reply: Great point. In the revised manuscript, we organised data into a nested hierarchy of four levels: leaf, species, plant functional group, and community.

At species level, the effects of grazing on leaf trait of dominant and common species (relative biomass >1%) were examined in each of the six communities. For *C. appendiculata* meadow, the subdominant species, *Poa subfastigiata*, which accounted for 26% of the community standing biomass, was excluded from the leaf trait analysis; because only stems were available during early August. Thus, a total number of 13 species was selected, which together accounted for more than 65% of the community standing biomass in both ungrazed and grazed sites. For the other five steppe communities, 7-22 dominant and common species were selected in each community, which accounted for more than 90 % of the community standing biomass. The SLA, stem: leaf ratio, and standing aboveground biomass (green and current year dead) of each species were used for calculating species-level leaf area and leaf biomass for each quadrat in the paired ungrazed and grazed sites across six communities.

At plant functional group level, leaf area and leaf biomass of species that belong to a specific life form or water ecotype were summed for each quadrat in each ungrazed and grazed site, and SLA for each functional group was determined as the ratio of leaf area to leaf biomass. At community level, total leaf biomass was calculated by using plant biomass and stem: leaf ratio of each species at each quadrat in the ungrazed and grazed sites across six communities. Leaf area index (the area of leaves per soil surface area, $\text{m}^2 \text{m}^{-2}$) was determined by leaf biomass and specific leaf area of each species at each quadrat. The following formulae were used:

$$\text{Leaf biomass (g m}^{-2}\text{)} = \sum_{i=1}^n \frac{B_i}{R_i + 1} \quad (1)$$

$$\text{Leaf area index (m}^2 \text{ m}^{-2}\text{)} = \sum_{i=1}^n B_{Li} \times SLA_i \quad (2)$$

$$\text{Specific leaf area (cm}^2 \text{ g}^{-1}\text{)} = \frac{\sum_{i=1}^n B_{Li} \times SLA_i}{\sum_{i=1}^n \frac{B_i}{R_i + 1}} \quad (3)$$

where B_i , R_i , B_{Li} and SLA_i are the aboveground biomass, stem: leaf ratio, leaf biomass and specific leaf area of the species i in a community, respectively, and n is the number of species in a community.

We have added the above information in the Methods section and revised the Introduction, Results, and Discussion sections accordingly.

5. Comments: Trends in the results for population and community are not the same (Table5, Fig. 4). This needs to be discussed.

Reply: We very much appreciate this valuable comment by the reviewer. We have revised the Discussion section substantially by eliminating the redundant parts and improving the clarity of seemingly confusing places.

Our findings demonstrate that the effects of grazing on leaf traits are scale dependant and may change with vegetation type or site conditions. Several mechanisms are likely to be responsible for the observed responses of leaf traits to grazing at different levels of organization and among vegetation types. First, soil properties, particularly soil water and nutrient availability, are two major factors driving the differential responses of leaf traits to grazing between the meadow and typical steppe communities. Second, the observed responses of leaf traits to grazing at different levels of organization are largely governed by functional trade-offs between plant traits. Third, the differentiation in avoidance (escape from grazers) and tolerance (regrowth capacity after defoliation) strategies among coexisting

species is likely to be responsible for the different responses among life forms and water ecotypes.

In addition, our study showed that the magnitude of grazing impacts on leaf traits increased at higher levels, suggesting that the drivers may also change across the nested hierarchy of organizational levels. At leaf level, the response patterns of leaf traits to grazing are mainly governed by the direct effects of grazing and site conditions. At species level, the leaf traits responses are mediated primarily by the number of leaves per individual, population density of each species, and their interactions with the direct effects of grazing on leaf-level traits. At functional group and community levels, the grazing impacts on leaf attributes are caused mainly by changes in dominant species and functional groups.

These points are now clearly stated in the revised text.

6. Comments: Trait's responses are presented per PFG, but thereafter, the composition of communities in PFGs or the effects of grazing on PFG composition are not presented, thus, the utility of the presentation of trait's values per PFGs do not appeared as very useful to understand the results at the communities level. It could be suggested to present the distribution of different PFGs in grazed and ungrazed sites?

Reply: Great point. We have examined the relationships between the leaf trait responses and corresponding standing aboveground biomass responses at functional group level across the six communities. Our results showed that there were significantly positive relationships between the leaf area responses and aboveground biomass responses for all life forms (e.g., perennial grasses, perennial forbs, annuals and biennials, and shrub and semi-shrubs) and water ecotypes (e.g., xerophytes, meso-xerophytes, xero-mesophytes, and mesophytes) across the six grassland communities ($P < 0.05$). For meso-xerophytes and mesophytes, the aboveground biomass responses were also positively correlated with the SLA responses across the six communities. Our results further revealed that the positive effect of grazing on leaf traits was found only for perennial grasses and shrub and semi-shrubs in the

meadow steppe, and perennial forbs in the meadow. Among the water ecotypes, the positive effect grazing on leaf traits was mostly found for xero-mesophytes in the typical steppe.

We have added these results in the Results and Discussion sections of the revised manuscript.

7. Comments: Trait's values are presented per species and plant functional groups but not at the community level. It could be interesting to present mean trait's values at the community level to better inform relationships between traits and community characteristics. Questions and hypothesis on these relationships have to be better presented.

Reply: Great point. We have sharpened the focus by clearly stating three research questions at the end of the Introduction section: First, how do plant leaf traits respond to grazing at different levels of organization (i.e., at the leaf, species, plant functional group and community level) and across different grassland communities in the Xinlin River Basin? Second, how do the relationships between leaf traits and ecosystem functioning (e.g., standing aboveground biomass) are affected by grazing and soil properties, such as soil moisture and nutrients? Third, what are the possible mechanisms underpinning the observed responses of leaf traits to grazing? The Results and Discussion sections now are presented in the order of the three questions.

8. Comments: Links between soil and communities characteristics: are soil properties significantly affected by grazing? This needs to be tested, as soil properties could directly have an effect on community characteristics.

Reply: We agree that soil properties could directly affect community characters. We have tested how the soil properties are affected by grazing as suggested by the reviewer. Our results showed that soil bulk density ($P= 0.233$), field holding

capacity ($P= 0.797$), soil porosity ($P= 0.229$), soil organic carbon ($P= 0.297$), soil total nitrogen ($P= 0.345$) and phosphorus ($P= 0.247$) at the grazed sites were largely remained unchanged, as compared to those at the ungrazed sites across the six communities.

Also, no significant relationships were found between the plant community responses and soil property responses, suggesting that effects of grazing on soil properties may exist a time-lag as compared to the strong responses of plant traits and community attributes, which is consistent with the general findings proposed by Milchunas and Lauenroth (Ecological Monographs 63: 327-366, 1993).

These findings are now incorporated into the revised text.

9. Comments: Minor points

(1) Comments: The English needs to be improved

Reply: We have gone through all the text carefully and revised the manuscript substantially as suggested by the reviewer.

(2) Comments: Redaction: too many abbreviations in the text, making it difficult to read. Also, too many symbol, for example “>” or “<”. This needs to be modified.

Reply: We have replaced the most abbreviations as suggested, particularly in the Results and Discussion sections.

(3) Comments: For a better demonstration, references need to be added. For example, p9956, after “.....indicating that species with longer leaf life-span (LL) generally have less SLA values than....”; also, p9959: ”.....in dry habitats tend to store mineral nutrients in leaves and use a majority of them to construct protective structure....”, also, p958, line 16 : reference after “.....leaves to reach higher stature”, ...

Reply: We have cited the relevant references in the Discussion section as suggested by the reviewer.

(4) Comments: 3.2 Page 9954 : are grazed and ungrazed datas pooled to assess the variations in leaf traits across PFGs?

Reply: Leaf traits of 263 species from eight ungrazed communities were used to assess the variations in leaf traits across PFGs. We have added this information in the Methods and Results sections of the revised manuscript.

(5) Comments: Table 2: Data within a column and per PFGs.....

Reply: Revised accordingly.

(6) Comments: Table 6: in the legend: Field holding capacity, not Fielding holding capacity

Reply: Revised as per suggested.

(7) Comments: Fig. 3: the size of the symbols is too small. The same in Fig. 4

Reply: We have revised the size of the symbols in Fig. 3 as suggested by the reviewer. The presentations of other Tables and Figures have also been improved in the revised manuscript.

(8) Comments: Page 9960, line 13 $p < 0.05$, not $p > 0.05$

Reply: We have corrected this error. And the statistical significance was given in the caption of Fig. 7.