

*Answer to comments from the reviewers.*

*Comments from the reviewer were left intentionally in this document and written in roman font. Our answers are written in italics.*

### **Anonymous Referee #1**

General comments:

The manuscript entitled "Strong stoichiometric resilience after litter manipulation experiments; a case study in a Chinese grassland" focuses on the evolution of C, N and P pools and ratio among several grassland ecosystems compartments (aboveground biomass, belowground biomass, litter and microbial biomass). This study is well designed and the statistical analyses well conducted. Even if it is limited to one particular type of grassland and therefore needs to be completed with forthcoming studies, my recommendation for this manuscript is accept with minor revisions.

Specific comments:

Introduction: Page 3 line 17: Previous studies also considered the microbial biomass in soils under litter addition experiments (e.g. Chemidlin Prévost-Bouré et al., Applied Soil Ecology, 2010). This should be indicated in the introduction.

*This reference is now added in the resubmitted version.*

Methods: Litter addition was done with sieved fragmented litter. Could the authors explain why they chose to do like that. I understand that this was to mimic the activity of soil fauna. However, according to the relatively high sand content and low C content (for a grassland), one might expect a relatively low faunal activity. Was it the case or not?

*Actually, we used fragmented and sieved litter mainly to standardize the litter area available to microbes and therefore reduce the variance among plots and treatments. We have no data on soil faunal activity, even though we acknowledge that this is likely the most important control on nutrient mineralization.*

P7 line 22: Why did the authors perform a 24h incubation step at 60% of water holding capacity before the fumigation-extraction procedure. This is not usual for that kind of measurement which can be done directly on fresh soil samples.

*Fresh soil samples were immediately transported to the laboratory with a portable ice box and stored at 4 °C before analysis. Therefore, to reactivate soil microbes, we performed a pre-treatment incubation at 60% of water holding capacity before starting the fumigation-extraction. This procedure is indeed not commonly done, but does follow the original procedure published by Vance et al. (Soil Biol. Biochem., 19, 703–707, 1987). To avoid any misunderstanding, we rewrote the sentence in the revised manuscript.*

Minor comments

*All the minor comments listed below were taken into account in the resubmitted version.*

Figures are not very reader friendly because of the numerous barplots they are constituted of but I do not ask to change this.

P6 line 6: "15, 30,..." should be "0, 15, 30,..." since the control did not experiment any litter addition.

P7 line 24: Please rephrase. It is unclear whether this is only the non-fumigated sample or both fumigated and non-fumigated samples that were extracted with K<sub>2</sub>SO<sub>4</sub>.

P9 Title 3.2 is unclear. Please rephrase

P9 line 16: delete "for" in "year for on aboveground"

P13 line 9: "was N limited" in "community N limited"

## **Anonymous Referee #2**

The manuscript "strong stoichiometric resilience after litter manipulation experiments; a case study in a Chinese grassland" explores effect of increasing litter input on soil nutrients, plant growth and ecological Carbon (C) : nitrogen (N) : phosphorus (P). The manuscript is well written and interesting. A better insight on the relationship between litter inputs and soil processes are indeed important for future climate change projections. However, before the manuscript can be published, I have some points which should be clarified and improved.

### General comments

Introduction. I agree it is much likely that with CC (climate change) we may increase biomass production in some regions. In this case, regions will receive have more rain, temperature and CO<sub>2</sub> and thus presumably higher litter inputs. However, in other cases this might be the opposite...!!! What I missed here, is a paragraph on litter quality (which is likely to change with CC and increase in CO<sub>2</sub> level). As we do not quite know how grassland will reaction in the future, So I recommend authors to add some lines on what we know from other CC experiments with grassland, litter quality, etc...

*We thank the referee for this constructive comment. We modified the introduction to better present these aspects in the revised manuscript as follows: "The anticipated doubling of the atmospheric CO<sub>2</sub> concentration within the next 100 years (Houghton et al., 2001) due to continued anthropogenic carbon emissions is generally predicted to increase net primary production of most terrestrial ecosystems. Nevertheless such effects do not scale linearly with increases in atmospheric CO<sub>2</sub> because productivity is also partially controlled by climate. Regions with drying climates are therefore likely to present reduced net primary production in the next decades (IPCC, 2013). Although uncertainties exists in the magnitude of the changes (e.g. Campbell et al., 1991; Arnone and Körner, 1995; Gill et al., 2002), increases in net primary production are probably predominant and will simultaneously increase litter inputs to soils. Modification of atmospheric CO<sub>2</sub>, of climate and of nutrient cycling may also modify the chemical composition of the litter (Cotrufo et al., 1999). Elevated CO<sub>2</sub> generally increases the lignin content and reduces the N concentration of plant tissues, although Norby et al., (2001) showed that this response depends on the experimental system used (open top chamber, free air CO<sub>2</sub> enrichment, etc.)."*

In objectives (P10490), authors highlight "priming effect" ( P10490L21) this was not mention before and is neither introduced nor discussed. : : . So up to authors to either take this subject better into account or to reword.

*Priming is now defined in the new version: "We assumed that litter additions could increase nutrient release through a priming effect on decomposition rate, thereby provoking an increase in plant biomass. Priming is defined here as a modification of the soil organic matter decomposition rate induced by an input of litter and mediated by the altered activity of the microbial community. Priming effects can be negative (reduction of the decomposition rate), but are typically positive (increase of the*

decomposition rate) (Blagodatskaya and Kuzyakov, 2008).”

Along the same lines, priming effect, this effect depends on litter quality, soil conditions and microbial pools. Authors do not mention at all changes in soil conditions with respect to CC. I also miss some lines on how grassland store C: : . this is mostly through root biomass than aboveground litter.

*The introduction was modified following the reviewer comment as follows: “Moreover, climate change and increased atmospheric CO<sub>2</sub> would not only affect NPP but also environmental conditions for decomposers in soils (soil moisture, temperature), inducing modification of microbial community structure and activity (Singh et al., 2010). Associated to litter additions, these effects would disturb the C cycle in soil, affecting the net ecosystem exchange particularly in grasslands where the vast majority of the C stock is stored belowground, due to their high root:shoot biomass and productivity ratios (Mokany et al., 2006).”*

According to objectives authors like to asses the Relationship between litter additions and the plant biomass response (P10490L24). However, data analyses in this direction were made and reader can not determine if the relation is linear or not (see comments to possible graphs). A long the same lines I miss some correlation analyses between litter quantity (e.g. amount of nutrients added) and biomass, soil, microbes ect: : . From Bar graphs reader can not conclude on regressions, bar seem to increase with quantity added!!!

*We thank the referee for this constructive comment. To check this point, we performed an ANCOVA on the response variables, using the absolute litter additions as independent variable and not as factor. This ANCOVA yielded very similar results than the ones we presented in the paper with ANOVA. Therefore, we now presented the results of the ANCOVA, which are more coherent with the scatter plots. The significant covariance also implies that there was a correlation between the observed variables and the amount of litter added. Nevertheless the correlations were only significant when including the highest litter input treatments. No effects were observed under a certain threshold; only when litter additions exceeded this threshold, plant stoichiometry was affected. Nevertheless, we modified the figures and now all the results are presented as dot plots.*

M&M needs several points to get clarified as reader get no information : : .what about climatic conditions out of growing season, and how this might affect litter decomposition.

*Means of temperature and precipitation are now described in the M&M section with some details on the intra-annual variability: “The long-term mean annual temperature at the site is 2.1°C, with monthly mean temperatures ranging from -17.5°C in January to 18.9°C in July. Mean annual precipitation is about 380mm, with 90% of the precipitation falling in the growing season between May and October.” Moreover, fig 1 was added showing the daily temperature and precipitation for the entire experimental period.*

Is there an agricultural management on that site? 1000g/m<sup>2</sup> biomass production => 10t DM/ha this is quite high for the low fertility of the soil

*No, the site is not managed. We agree that 1000g/m<sup>2</sup> biomass production is very high. But the site receives the necessary precipitation during the growing season and these grasses have adapted to nutrient-poor conditions, by very high nutrient retranslocation at the end of the growing season. The low C/N ratio of the soil organic matter (10) also suggests that N cycling rates are probably high enough to sustain such high productivity.*

Results:

Effects of litter input seem to be related to amount of input. I wonder how would look a dot-graph having the nutrient ( or DM) input on the X-axis and le results Fig 2a,c on the Y. Idem plotting inorganic soil N (x-axis) with results of 3a,b and so on y-axis to see if there is any relation between quantity and effect.

*All the figures are now dot-graphs.*

P10490L24 non-linear relationship between litter additions and the plant biomass response.

*We were not really sure what this comment meant, but because the sentence was deleted there was no more need to address it.*

Discussion -I miss some discussion on the effects of litter quality (and soil climate) on decomposition (turnover time of litter ect). These topics needs to be mentioned somewhere (P10497L20ff??):...

*To accommodate the referee request, we added a few sentences in the revised version to discuss the effect of litter quality on decomposition. The sentence stating the implications for our study is rather speculative, but this was made clear by explicitly stating the last sentence as an assumptio : “It must be noted that the litter used here was harvested under natural conditions. Thus, the modifications of the litter chemical composition expected under climate change are not taken into account here. Litter C:N ratio may increase in the future (Norby et al., 2001) inducing a higher microbial N demand. Microorganisms may still decrease their carbon use efficiency as assumed here or may increase the N uptake. Since the microbial N uptake in our experiment only increased for very high and unrealistic litter inputs, we assume that, at our site, microorganisms will likely modify their carbon use efficiency in response to the modification of litter C:N ratio instead of competing with plants for N.”*

It's not that because more is available more is decomposed... Thus a real litterbag experiment would have been nice.

*We fully agree with this comment; litter bag decomposition estimates would have been a nice addition. However, because the objective of the study was not to estimate the effect on litter decomposition but to better understand how modifications of litter inputs impact on the plant C:N:P ratios, the lack of a litter bag experiment is not problematic for the paper.*

-As litter was inserted to 10-20cm litter incorporation is an issue as well.

*We agree that it makes our experimental systems a bit different from natural, but we inserted the litter in the soil to avoid export of added litter during storms or high rainfall events, thereby better controlling the inputs. We now justified this procedure in the revised manuscript by adding the following statement: “We did not add the litter at the soil surface, but inserted it in the soil to reduce export due to wind or rain and thereby better control the amount of litter added.”*

-I wonder if authors ca add un paragraph on some mechanisms, future processes: : : some thoughts here: Are the applied litter amendments realistic in the future? What is the future climate in this region? (more rain, T??) How do authors expect the vegetation will change. Future variation of litter input?? What about soil C stock?

*We modified the discussion following the reviewer comment: “The NPP in our study sites is expected to increase between 10 and 60% (Arora and Boer, 2014; Todd-Brown et al., 2014). Moreover, temperature is also expected to increase in China during the next decades (Piao et al., 2010), likely accelerating litter and soil organic matter decomposition and nutrient release. Future predictions about the evolution of precipitation are still highly uncertain (Piao et al., 2010).”*

## Conclusion

Conclusion mention topics which were not tackled before neither intro nor discussion: such as increase in biomass production (eg quantity) , changes in soil climatic conditions and CO<sub>2</sub>: : : with future climate (see comments to intro). So as said before these I suggest to add some lines at the mentioned places. As also said befor, reader ca not conclude on in terms of stoichiometry and this resilience as no relation between litter inputs and plant growth etc were shown.

*We thank the referee for this suggestion. These topics are now being touched upon in the revised discussion.*

#### Specific comments

P10488L7 what kind of litter, quality/N? Amounts? How can we scale them? Is this twice, 4, 5 times as much as expected for this steppe? Replace with “ correspond to litter input increases of 15, 30, 60 and 120 % respectively” here

*We modified the abstract to clarify these issue. Please see the resubmitted version.*

L13 future prediction? This is strange, not measured?

*No, we scaled our inputs regarding the predictions from an Earth system model for this region. We clarified this sentence.*

L14 high litter additions of what quality? I am not sure that authors will find the same with low quality+

*We agree of course that litter quality matters, but in our case the same litter was used for each treatment, so we could only focus on the quantitative modifications of the litter inputs due to climate change and not on the modification of chemical composition.*

P10490-L13ff to me objectives are quite similar and might be merged in on sentence Not sure that 3 objectives are needed here, might also be skipped

*We simplified the definition of the objectives as follows: “The primary objectives of our study were to determine whether litter addition would increase soil inorganic N and available P and thereby enhance soil nutrient availability for plant growth and if it would affect plant growth, litter, and the C, N, P pools and the C:N:P stoichiometry of plants, litter, soil and soil microbes.”*

L21 priming was not mention before and is not introduced: : . So up to authors to either take this subject better into account or to reword

*In the resubmitted version we defined the priming effect: We assumed that litter additions could increase nutrient release through a priming effect on decomposition rate, thereby provoking an increase in plant biomass. Priming is defined here as a modification of the soil organic matter decomposition rate induced by an input of litter and mediated by the altered activity of the microbial community. Priming effects can be negative (reduction of the decomposition rate), but are typically positive (increase of the decomposition rate) (Blagodatskaya and Kuzyakov, 2008).”*

P10491 -L2ff suggest to describe the climate with some words, as compared to other places it's dry and cold. What happens in out of growing season ?

*We modified the manuscript to better describe to climate of the study site: “The climate is temperate and semiarid with a dry spring and a wet summer. The long-term mean annual temperature at the site is 2.1°C, with monthly mean temperatures ranging from -17.5°C in January to 18.9°C in July. Mean annual precipitation is about 380mm, with 90% of the precipitation falling in the growing season between May and October.”*

L15ff the same for soil, BD in which soil layer, soil seems with very low C/N content, what about soil depth?

*Soil characteristics were measured in the layer 0-30cm. The soil C:N ratio is 10 (C:N:P ratio of*

57:5.7:1) which is indeed in the low range of values published (Xu et al. 2013, *Global Ecology biogeography*). We added this information in the revised manuscript.

L25-1000g/m<sup>2</sup> biomass production = > 10t DM/ha this is quite High for the low fertility of the soil Is there an agricultural management on that site?

*No, the site is not managed. We agree that 1000g/m<sup>2</sup> biomass production is very high. but the site receives the necessary precipitation during the growing season and these grasses have adapted to nutrient-poor conditions, by very high nutrient retranslocation at the end of the growing season. The low C/N ratio of the soil organic matter (10) also suggests that N cycling rates are probably high enough to sustain such high productivity.*

P10492-L3f “fresh organic matter to the soil in the 10–20 cm soil layer, at rates...” what type of litter, senescent, green, ??? move L9” fresh organic matter consisted of senescent above-ground tissues” higher equivalent to 0 (control treatment), 150, 300, 600 and 1200 g (dry mass -L7 “et primary production is assumed to increase between 10 and 60 %...” in this place?

*We reorganized this section following the reviewer comments.*

Fig 2a/b, 3a reduce Y-axis to max 3 (N) and 1 (P) to better see treatments effects Fig 4 may go to supplementary, results may be mentioned in the text say that belowground biomass was 6 times higher etc

*We modified the graphs as suggested and now mention these results in section 3.2*

P10496-L24ff suggest to reword: “ Litter amendments are substantial supply of nutrients, suggested to release nutrients during decomposition. Results show, that availability of N and P, were only modified for the two highest inputs treatment. Additionally, high litter addition also greatly increased soil microbial biomass C and N, indicating that soil microbial biomass does play an active role in nutrient transformation, conservation, and availability to plants (Wardle, 1992; Zaman et al., 1999; Tu et al., 2003). Notably, for more moderate litter additions, the observed effect on plant biomass was quite limited, suggesting that only the plant did not benefit from these inputs. Indeed, litter addition significantly increased aboveground biomass in 2009, 2010 and 2011, and belowground biomass and total biomass in 2010 and 2011 but only for the highest input level.

*We modified the text following the reviewer’s suggestion.*

P20497L19” : : efficient in using nutrient resources.”” Not clear

*In the resubmitted version we changed this sentence into : “Since plant biomass only responded to the highest litter treatments, it suggests that, in our case, microorganisms might be more efficient than plants in using nutrient resources and plant growth might be still nutrient limited.”*

L26ff suggest to reword an merge with 4.2 “, those more favourable soil moisture conditions may have caused the higher soil nutrient availability via accelerated litter decomposition. Indeed, vegetation invested and allocated more biomass toward shoots than roots biomass allocation. In our study, high 5 litter addition decreased the ratio of belowground biomass to aboveground biomass, and the decrease reached a significant level in 2010. Such an increase in photosynthates concentration is also explains the decline in C : N and C : P concentrations in aboveground biomass and litter upon high litter addition, but not for belowground biomass.

10498L14 “ Li and Xiao (2007) also found that the soil water content, soil organic matter, “ delete

*We modified the text as suggested by the reviewer.*

P10499LL14-17 delete this sentences has not its place here as the paper does not deals with fertilisation

*We rephrase this sentence as following: “Anthropogenic modifications of the C, N and P cycles affect the natural ecological stoichiometry and causes imbalances that will have consequences for biogeochemical cycles including C-sequestration and long term structure and function of ecosystems (Lambers et al., 2010; Vitousek et al., 2010; Peñuelas et al., 2013).”*

### **Anonymous Referee #3**

This is a very nice paper giving insights into the effects of changes in net primary production and leaf litter inputs on the stoichiometry of plant biomass, litter, microbial biomass and changes in soil nutrients. In the context of global change, the authors manipulated the litter inputs to soils and continuously monitored changes in the ecological stoichiometry for a number of years. Their results suggest that only very High additions of plant litter (twice the natural inputs) strongly affect the C:N:P stoichiometry in grassland ecosystems, which are mainly resilient to lower –more realistic changes in input additions.

I find the manuscript to be mostly well written, although some parts need clarification. At the end of the introduction section the authors state that they assumed that an increase in soil nutrients would induce an increase in plant biomass through a priming effect. The reasons for this hypothesis (in particular the priming effect) should be shortly introduced in the previous paragraphs of the introduction.

*We modified the introduction to better explain our assumption: “We assumed that litter additions could increase nutrient release through a priming effect on decomposition rate, thereby provoking an increase in plant biomass. Priming is defined here as a modification of the soil organic matter decomposition rate induced by an input of litter and mediated by the altered activity of the microbial community. Priming effects can be negative (reduction of the decomposition rate), but are typically positive (increase of the decomposition rate) (Blagodatskaya and Kuzyakov, 2008).”*

It would be also helpful to specify already in the abstract and introduction that the added litter consisted of above-ground tissues (in contrast to belowground litter).

*We modified the abstract of the resubmitted version following the reviewer’s comment*

With this, a few sentences are long and difficult to read and need to be improved (i.e. Lines 14-17 on Page 10497, and 21-23 / 24-27 on Page 10499 – see specific comments).

*All these sentences were simplified in the resubmitted version.*

Some figures are very small and their size should be increased (Figure 3, 5 and 6).

*For publication, we will provide high-resolution figures and we let the editor choose the appropriate size for the figures.*

Specific comments:

*All the specific comments are taken into account in the resubmitted version.*

(Page 10488) Line 4: Carbon should be all written in lowercase.

Line 7: “subsoil layer under a steppe community” should be changed to “subsoil layer of a steppe community”

Line 25: I think the authors mean “assessing possible changes in C, N and P cycling”. Add “changes in”

(Page 10489) Line 12: atmospheric carbon dioxide (CO<sub>2</sub>). Add (CO<sub>2</sub>).

Line 14: should read “predicted to increase the net primary production”. Add “the”. Here some references are required at the end of the sentence. Also, add “(NPP)” after the wording “net primary production”.

(Page 10493) Line 5: “the 1 m x 0.3m quadrat” should be changed to “a 1 m x 0.3m quadrat”

Title in paragraph 3.2: should be: Plant biomass, allocation and litter.

(Page 10496) Line 21: Change “Litter amendments we did” to “The litter amendments we applied

(Page 10497) Line 4-5: should read: Nevertheless. Other amounts of litter additions had no effects.

(Page 10498) Line 13-17: The work from Li and Xiao (2007) is mentioned, but it should be specified that they were working in a desert ecosystem.

Line 27: “higher than that”

(Page 10499) “when they were quite high” should be changed to “when it was quite high”

Line 24-27: This sentence is long. I suggest to add a full stop after below plant biomass and change the wording “while realistic additions: : :” to “On the other hand realistic additions: : :”.