Dear Editor Dr. Richard, and two anonymous referees,

We appreciate all your comments greatly on our previous draft entitled “Flower litters of alpine plants affect soil nitrogen and phosphorus rapidly in the eastern Tibetan Plateau” (bg-2016-68). The revised version has been completed based on your favorable suggestions item by item. Besides, all text after revision have been yellow highlighted in the new version for your consideration, except the introduction part since it has been totally re-organized according to Referee #2’s recommendation.

Reply to referee #1

Major comments about the methods,

“**However, employed methods are not clear, especially litter collection and chemical analyses of litters. Litter collection method is very important to assess the production of litter per unit area and researchers employ litter trap in general. However, authors did not give any specific methods, but said only “collect”. Also, there is no method to analyse chemical properties of litter. For example, I could not find how authors measured total nitrogen and phosphorous in litter**”.

Thanks. Yes, these details should have been added for better interpreting the exact processes and following results in the context. We have supplied the method of litter collection and analysis methods of chemical properties. Besides, total phosphorus (TP) and available phosphorous (A-P) have been clarified.

See Line 155-168. **Litter collection**, “In the study, 4 litter traps were placed under the crown of each individual shrub in different communities (5–8 individuals were chosen for the placement of litter traps), which were processed and modified based on the litterfall monitoring protocol (Muller-Landau and Wright, 2010). The litter trap was composed of 1 cloth bag and 4 support legs. Window screen (with a mesh size of 0.8 mm) was used to seize the cloth bag. Its size was about 50 cm deep and 25 cm long. Four legs (made with 80 cm PVC pipe) were tied with a cloth bag and frame. The frame of the opening was made of iron wire with 3 mm diameter. After inserting it into the soil under the shrub’s crown, the plant litter was collected twice per week, which was later sorted as flower litter and other types during the blooming period. Given the small size of herbaceous individuals, flowers were plucked at the end of the flowering phase, and their mass ratios to aboveground biomass were calculated. Freshly fallen leaves of different species were collected from the floor of the alpine meadow (i.e., mixed leaf litters, ca. 3950 m a.s.l.).

See Line 250-258. **Definition of TP and A-P**: Total phosphorus (TP) consists of phosphorus mineral and organic phosphorous compound in the soil, which can be converted into the dissolved orthophosphate. Available phosphorous (A-P) is the fragments in soil that can be absorbed by plants, which consist of water-soluble phosphorus, some adsorbed phosphorus, organic phosphorus, and precipitated phosphorus in certain soil types. Chemically, A-P is defined as the phosphorus and phosphate in soil solution that can be isotope exchanged with ^{32}P or can be easily extracted by some chemical reagents. TP and A-P in soils were estimated by extraction with 0.5 M sodium hydroxide sodium carbonate solution (Dalal, 1973).

See Line 262-268. **Chemical analyses**, "For plant samples, the contents of C and N were determined by dry combustion with a CHNS auto-analyzer system (Elementar Analysen Systeme, Hanau, Germany) (Brodowski et al., 2006). The content of P was obtained colorimetrically by the chloro molybdophosphoric blue color method after wet digestion in a mixture of HNO_3, H_2SO_4, and $HClO_4$ solution (Institute of Soil Academia Sinica, 1978). Lignin and cellulose were estimated by the method described by Melillo et al. (1989).

References:

Muller-Landau, H.C. and Wright, S.J., 2010. Litterfall Monitoring Protocol.

Melillo, J.M., Aber, J.D., Linkins, A.E., Ricca, A., Fry, B. and Nadelhoffer, K.J., 1989. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. Plant and Soil 115, 189-198.

Other minor comments

1. Line 68: mineralization of soil organic matter and decomposition of plant residues have the same meaning. It is meaningless to divide these two.

Yes, we agree that there was no need to distinguish two terms since plant residues can be included in soil organic matter. In the revised version, we addressed the sentence to be "the plant residue is one principal component of soil organic matter, whose decomposition can supply available N to plants and microorganisms." **See Line 89-91**.

2. Line 152: Add "genus" before *Kobresia* and *Carex* and add spp. after *Festuca*, *Gentiana*, and *Leontopodium* to deliver exact meaning.

Thanks, we added. **See Line 144.**

3. Line 164: Table 1 is not necessary. Delete it.

Sorry, after discussed with each other and also referred to the comments from referee #2, we would like to keep table 1 for better presenting results. Besides, we revised description of relevant text.

4. Line 164: Mixed litter- What is this litter composed of? It might be composed of flower and leaf litters. This is not clear.

Thanks for your kind reminder. It is mixed leaf litter, which was composed of dominant species' leaf litters in the alpine meadows.

5. Lines 184-187: It is not clear that collected samples were mixed through sieving or each sample was mixed through sieving.

Thanks, it has been revised-"After 50 days, each soil sample was collected from three points of each pot in the center and then mixed to avoid the boundary layer effect. Each soil sample from different PVC pots was mixed evenly by sieving through a 2 mm mesh respectively. The samples were stored and marked separately in an ice box prior to chemical determination." **See Line 207-211.**

6. Line 215: It is not necessary to use abb. of DHN and DNN for NH4+-N and NO3-N, respectively.

Thanks, NH_4^+-N and NO_3^--N are just used in the latest version.

7. Table 1, 4 and 5 should go to the place after text which were mentioned.

Yes, Table 4 and 5 have been edited to right place after combined with Table 2 and 3.

8. Table 6 and explanation should go to results part, rather than Discussion part.

Ok, please kindly notice that it is **Table 4** in revised version after pooled new figures and tables. **See Line 373-387.**

Effects of flower litter addition on soil solution N pool and soil MBC and MBN

Soil solution N pool has been improved noticeably from 31.46 mg g^{-1} to 47.35 mg g^{-1} in flower litter treatment compared with the control, particularly in fragment of NO_3^--N, which has been greatly increased (from 30.93 mg g^{-1} to 46.8 mg g^{-1}). (**Table 4**). In mixed leaf litter treatment, there were no obvious variations after litter decomposition, with 32.4 mg g^{-1} NO_3^--N and 0.45 mg g^{-1} NH_4^+-N, respectively. There were notable differences of both MBC and MBN between different treatments. Litter addition not only increased soil microbial biomass C (102.05 mg kg^{-1}, 68.08 mg kg^{-1}, and 46.25 mg kg^{-1}

for flower litter, mixed litter, and control, respectively) and MBN (73.02 mg kg^{-1}, 69.29 mg kg^{-1}, 67.13 mg kg^{-1} for flower litter, mixed litter, and control, respectively) but also their C:N ratios (1.40, 0.98, and 0.69 for flower litter, mixed litter, and control, respectively).

Table 4 Comparing median value of soil solution pool and soil microbial biomass between litter addition treated (flower litter and mixed leaf litter) and control.

Treatments	Soil solution N pool (mg g^{-1})		Soil microbial biomass (mg kg^{-1})		
	NO_3^--N	NH_4^+-N	MBC	MBN	MBC/MBN
Flower litter	46.8	0.55	102.05	73.02	1.40
Mixed leaf litter	32.4	0.45	68.08	69.29	0.98
Control	30.93	0.53	46.25	67.13	0.69

9. Line 259: delete per unit.

Yes, done.

10. Table 2 and 3: DIN and DON are not necessary because these are deliberated from TN, DNN, and DHN do not have any special meaning.

Yes, please notice the response of the item 11 above.

11. After delete DIN and DON, I suggest to combine Tables 2 and 4, Tables 3 and 5, and Figures 4 and 5.

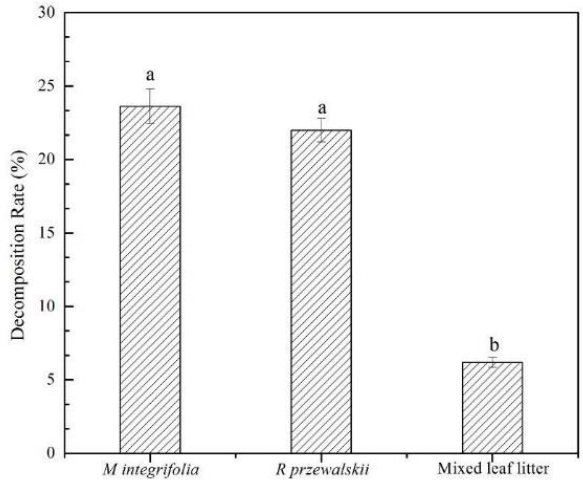
Thanks for your professional recommendation, which greatly improved the manuscript's whole structure. Tables and figures have been combined for your further review. **See Line 325-371, and Line 699-702.**

12. Fig 4: add explanation of (a) and (b) in the Figure caption.

Yes, we have added more detail explanation of (a) and (b) in Fig 4. **See Line 703-707.**

13. Fig 6: Where did ab of lower case letters come? To use ab, there should be b but there is no b. Check the statistical analysis.

Sorry for this unnecessary mistake. It has been corrected in the revised version (kindly refer to the below, which is Fig. 5 in the revised version).



Reply to referee #2

Major comments

"**However, I would also suggest the authors seek assistance with English grammar and translation where appropriate, as the impact of this paper is currently obscured**."

Ok, we agree that it is necessary to seek assistance from native English speakers. After revision of the whole structure in details, this paper has been sending to professional polish company with attached certificate for your consideration.

"**The introduction of this paper is scattered and somewhat confusing. I think spending time re-organizing/framing this section will provide clarity for the results and discussion sections. Perhaps the authors could introduce the topic of flowering bodies and their higher litter quality (N content), then discuss how aboveground litter quality influences belowground biogeochemical cycling through microbial subsidies, and conclude with a section discussing alpine ecosystems and evolved traits**."

Thanks, very good recommendation. We revised introduction part according to your kind suggestions for better addressing scientific questions, results, and discussion sections. Please see **Line 66-133**.

Other comments

"**Lines 137-142 provide specific research questions that will be addressed by the authors. Currently they are a little unclear and seem to set up questions that are not directly tested. I would suggest refocusing on the major comparisons being made- is flower litter of higher quality than leaf litter? do**

these traits facilitate faster decomposition? does the time of litter fall influence ecosystem productivity?"

Yes, the draft will be definitely improved if we focus on the major comparison. Thus, after supplying method in details of litter collection, we revised three questions as follows: 1) Should flower litter be considered in the alpine ecosystem's biogeochemical cycles for their relatively innegligible biomass production and/or allocation? 2) Does flower litter of higher quality and with unique traits have faster decomposition than leaf litter? 3) Does the time of litter fall influence soil available nutrients and soil microbial productivity of alpine meadow ecosystem? See **Line 128-133**.

"**The methods/materials section is generally clear. However, the authors do not provide details regarding their litter collection method (makes question 1 difficult to assess). The number of replicates and control treatments are sound. In my personal opinion I think it is important the field moves beyond litter bag experiments and mass loss. Litter bags exclude fauna and litter fragmentation, which contribute greatly to litter decomposition. While the authors used mesh bags with two layers of differing mesh size, the smaller mesh actually surrounding the litter still excludes faunal decomposers and minimizes biophysical perturbation. Since the study is focused on nutrient cycling more than soil organic matter mass loss/formation I think the litter bag approach is okay, but in the future it would be good for us to move beyond these techniques**."

Thanks, we suppose this is similar as the referee #1 raised. Litter collection procedure has been added in the revised version. Yes, you are quite right. The smaller mesh affected faunal decomposers and minimizes biophysical perturbation of litters to some extent. We did similar kind of comparison with quantification method between different meth sizes. If chose smaller size, tiny size litter can be hold in the litter bag better, but it may exclude some soil fauna. If chose bigger size, tiny litters might be easily dropped in the procedure. We do agree reviewer's constructive recommendation that in the future it would be great to move beyond these techniques to closely simulate real statues. For example, dual-labelled litter has been applied in recent decomposition studies.

Litter collection method: In the study, 4 litter traps were placed under the crown of each individual shrub in different communities (5–8 individuals were chosen for the placement of litter traps), which were processed and modified based on the litterfall monitoring protocol (Muller-Landau and Wright, 2010). The litter trap was composed of 1 cloth bag and 4 support legs. Window screen (with a mesh size of 0.8 mm) was used to seize the cloth bag. Its size was about 50 cm deep and 25 cm long. Four legs

(made with 80 cm PVC pipe) were tied with a cloth bag and frame. The frame of the opening was made of iron wire with 3 mm diameter. After inserting it into the soil under the shrub's crown, the plant litter was collected twice per week, which was later sorted as flower litter and other types during the blooming period. Given the small size of herbaceous individuals, flowers were plucked at the end of the flowering phase, and their mass ratios to aboveground biomass were calculated. Freshly fallen leaves of different species were collected from the floor of the alpine meadow (i.e., mixed leaf litters, ca. 3950 m a.s.l.).

Determine the weight of litter after decomposition: Firstly, the debris or mud was remove outside the litter bags carefully, then litter was taken outside and sank into small water basin for short period of time, which would go through 0.5 mm mesh filter to sort out clay and litter. Lastly, litters were dried at 60℃ in an oven for 48 hours and measured the weight on the balance (accuracy 0.001 g). See **Line 230-235**.

"**A-P is never defined in the manuscript. Correction factors for microbial biomass C and N are commonly employed, but are highly specific to soil mineraology/sorption. Direct testing of recovery efficiency at a particular site should be assessed before a correction factor is applied**."

Sorry for missing definition of A-P. It has been supplied in the methods section. Total phosphorus (TP) consists of phosphorus mineral and organic phosphorous compound in the soil which can be converted into the dissolved orthophosphate. Available phosphorous (A-P) is the fragments in soil can be absorbed by plants, which consist of water solvable phosphorus, some adsorbed phosphorus, and organic phosphorus, even including precipitated phosphorus in certain soil types. Chemically, A-P is defined that phosphorus and phosphate in soil solution can be isotope exchanged with ^{32}P or can be easily extracted by some chemical reagents. see **Line 250-258**.

We agree with your professional point that correction factors for microbial biomass C and N are highly specific at different soil sampling sites or soil types. This issue has been discussed with the lab staff in our institute, who also referred the same opinion about methodology of MBC and MBN given it was processed by general international method and relevant correction factor. However, as referee also mentioned that this paper is not specially focused on the microorganism scope, this flaw will not make a decisive change to the results and conclusions, for the same soil type and also fully mixed through sieve before litter addition. Surely, we will pay more attention to site-specific correction factors in the future's research. Many thanks for your helpful suggestions.

"**Line 357: clarify which species were used to compare decomposition rates between flower and mixed litters**."

Yes, we have clarified. *R. przewalskii* and *M. integrifolia* are two typical plant species widely distributed and easily collected. Both species were assessed to compare their decomposition rates of flower litter and mixed leave litter.

"**Line 397: lignin/N and C/N ratios are commonly accepted as good indicators of decomposition rates under short time frames, but there is little evidence lignin is preferentially preserved in soils, compared with bulk soil, over long-time periods (Cotrufo et al., 2015, Mikutta et al., 2005, Kleber et al., 2007)**."

Yes, we have revised previous statement as follows: "Generally, tissues with high lignin, polyphenol, and wax contents and higher lignin/N and C/N ratios exhibit slow decomposition. Lignin/N and C/N ratios are commonly accepted as good indicators of decomposition rates under short time frames, but there is little conclusive evidence that lignin is preferentially preserved in soils, compared with bulk soil, over long-time periods (Melillo et al., 1982; Mikutta et al., 2005; Kleber et al., 2007; Cotrufo et al., 2015). Moreover, lignin plays dual role in plant litter decomposition if taken photochemical mineralization these abiotic decomposition into account (Austin and Ballaré, 2010)". According to the literature from Austin and Ballaré (PNAS, 2010), it was said that biotic decomposition in mesic ecosystems is generally negatively correlated with the concentration of lignin, which is a typically recalcitrant material that is resistant to microbial decomposition. However, for its dual role in plant litter decomposition, lignin is quite complicated if we take photochemical mineralization of carbon into account. See **Line 429-437**.

"**Line 467: microbial community composition was not directly tested (no sequencing/PLFA analysis etc) so it cannot be concluded that flowering litter increases nutrient status and therefore changes microbial assembly. There is support that MBC and MBN pools increase, but that could be due to faster turnover or growth, not necessarily to a change in species composition**."

Yes, this not quite convincing deduction has been revised since there was no direct evidence. We have been processing another study this year, which is aiming to compare the microbial community composition (sequencing) in soil after flower litter addition (two dominate shrubs- *Rhododendron capitatum* and *Rhododendron przewalskii*), which has been conducted both in the field and incubator.

We have revised the relevant statement: "Flower litter contains more than twice MBC (increased from 46.25 to 102.05), and both MBC and MBN pools increased potentially after flower litter addition.

Therefore, microbial functional groups might be changed for nutrient supplement from litters, or could also be due to their faster turnover or growth, which need more evidences in the further study by directly testing of soil microbial community composition. See **Line 501-506**.

"**Line 499: the impact of this paper is significant and should be re-stated clearly in the conclusion**."

Thanks for your compliment. We have re-stated the significance of this paper in the last paragraph. See **Line 535-546**.

Comments regarding tables

"**Make sure to clearly define variables tested in each caption**."

Table 1: it is not clear how species dominance is assessed (Y/N).

We have added in the method part about how to assess dominance of species for both shrub and herbaceous species in the study sites. Target species were firstly decided by visual observation. For herbaceous species, their dominances were determined using quadrat methods. Each quadrat (1 m × 1 m) was spaced at least 2 m apart from each other along the transect for recording community composition (totaling 10 quadrats along one transect, and three transects at each site). Weighted means of frequency and biomass of target species were sorted and used to assess their dominances. For shrubs, line-point intercept method was conducted to calculate targeted species' frequency, height, and cover, which represented by "hit" (3 transects at each site, use 20 m rope with ca. 1 cm diameter or measuring tape), whose weighted means of were sorted to determine dominant species (Herrick et al., 2005). We also consulted expert who already has prior knowledge or researches about the dominant species at the selected sites. See **Line 170-181**.

Reference: Herrick, J.E., Van Zee, J.W., Havstad, K.M., Burkett, L.M. and Whitford, W.G., 2005. Monitoring manual for grassland, shrubland and savanna ecosystems. Volume I: Quick Start. Volume II: Design, supplementary methods and interpretation. USDA-ARS Jornada Experimental Range.

Line 303, 324, 329 etc.: the authors are assessing N pools, not fragments.

Thanks for this precise comment. We combined Fig. 4 and Fig. 5 together and deleted DIN and DON. Besides, the relevant content has been revised regarding the modified figures, in particular, focused more on N pools.

Table 3: DNN/DHN are not necessary; although defined as such in the text, NO3- and NH4+ are clearer.

Yes, revised and just used NO_3^- and NH_4^+.

Table 4/5: Define TP and A-P: α values of total phosphorus (TP) and A-P

Ok, TP and A-P are total phosphorus and available phosphorus, respectively. α values indicate natural logarithm of ratio flower litter addition to non-addition control of different soil indexes (TN, NO_3^--N, NH_4^+-N, TP, A-P). See **Line 347-351.**

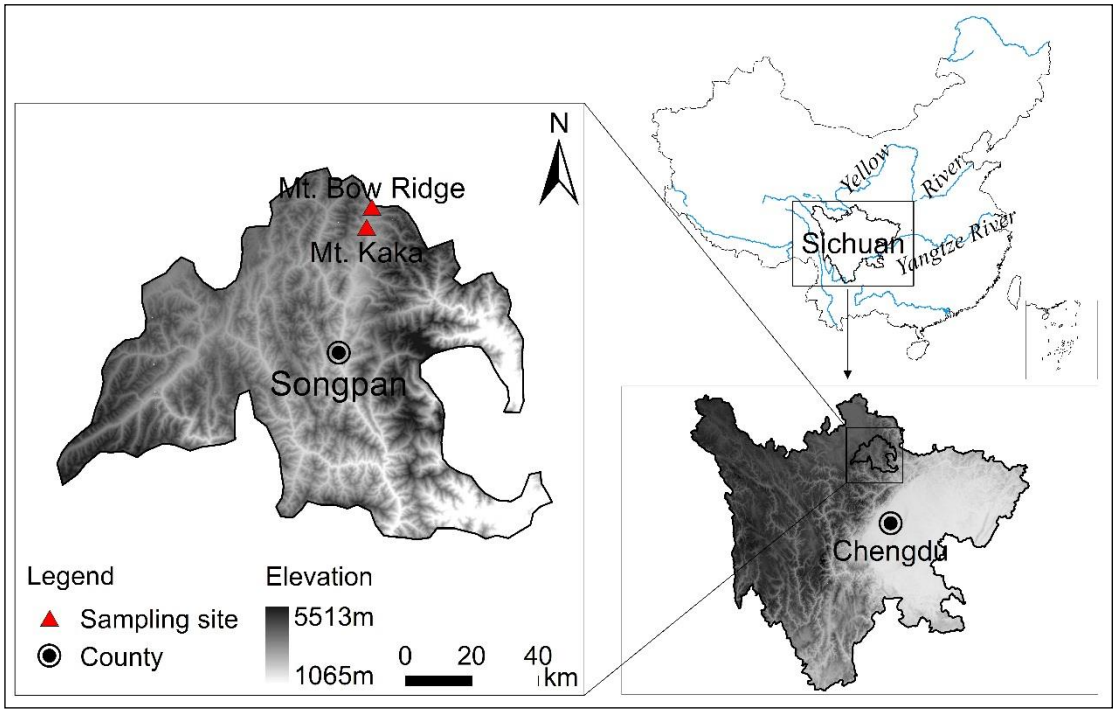
Table 6: Mean values (not comparison medium values)

Sorry for this mistake. We have corrected.

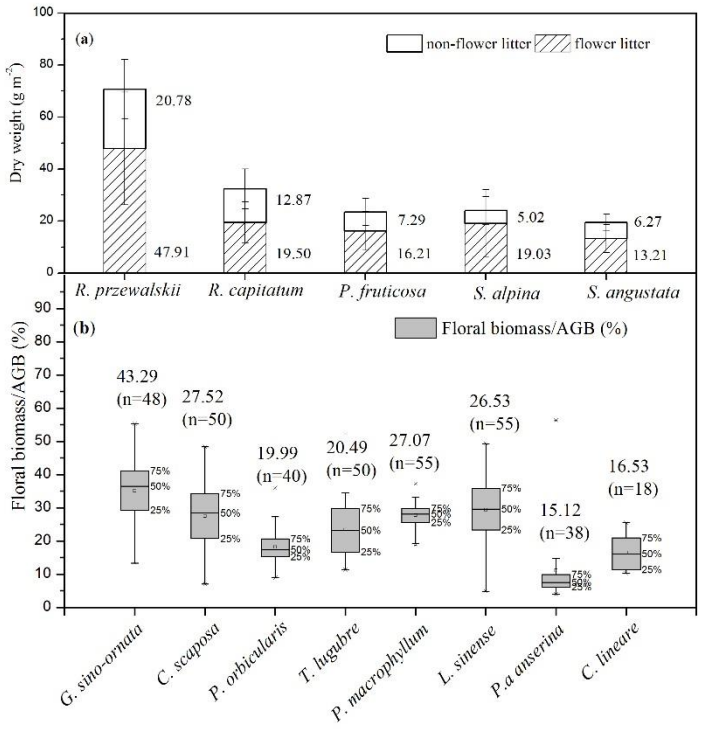
<u>Comments regarding figures:</u>

Figure 1: It is very difficult to identify where the sampling sites are on the map because the elevation shading is so dark (either increase shading transparency or make text and symbols larger)

Yes, map has been re-drawn with clearer text and symbols. Hope it is qualified.



"Figure 2: Include the mean (n=X)."



We have added the mean (n=X) for all the plots and with n value in (b). In (a), the values of sample number are the same (n=20) and we just mentioned in the figure caption. See **Line 684-687**.

Figure 3: Explicitly state the statistical analysis used (are the bars 95% confidence intervals/SE, or quantiles)? If the whiskers represent SE it seems impossible that the flower litter vs. leaf litter means are significantly different from each other. What are the values (mean, n=X)?

Ok, we supplied detailed statistical analysis in methods part. In figure 3, box-plots are used to better present the range of data distribution. Bars/whiskers refer to quantiles for comparable settings of all data distribution except extreme outliers (asterisk *). The values (mean, n=X) are also stated by One-way ANOVA. See **Line 279-282**.

Figures 4 & 5: Define the variables in the figure caption (dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), etc). Mean, n=X. What do the boxes represent? What does deviation from the 0 lines signify (significantly different at what level)?

Sorry for this unclear display. In fact, they are scatters but not boxes. However, we have to draw them a little bit bigger for the relative smaller error bar, otherwise, both will be overlapped and not well presented. These boxes (scatters) represent α mean values of different indexes in soil N and P pools

after flower litter addition (n=3). It is significantly different at *P*=0.05 level for deviation from the 0 lines. The variables in the figure caption have been defined regarding revised figure and relevant context.

Figure 6: letters indicate significant differences (at what level, p=0.05)?

Different lowercase letters indicate significant differences of decomposition rate between litter materials at *P*=0.05 level. It is Fig. 5 in revised version. See **Line 710-713**.

Comments regarding cited literature

"Overall, the authors seem well read on these topics. There are some citations that do not seem relevant to the paper the way it is currently written (for example, findings from tropical, agricultural, and Arctic sites). I think these findings are important because they show flowering litter quality influences soil nutrient status across ecotones, but this needs to be made explicitly clear. The authors also touch on soil organic matter formation/stabilization processes. There is a highly relevant body of literature that could be incorporated to strengthen these points (Kogel-Knaber, Sollins, Cotrfuo, Kleber, etc)."

Yes, after restructured introduction part, we read carefully about the literatures recommended by referee and also incorporated properly.

"**I would highly suggest a thorough language editing review is taken before the paper is published**."

Thanks, it has been done regarding your suggestion with attached certificate.

Many thanks to you time!

Jinniu Wang on behalf of all co-authors

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CERTIFICATE OF ENGLISH EDITING

To whom it may concern:

This memo is to certify that the paper titled Flower Litters of Alpine Plants Rapidly Affect Soil Nitrogen and Phosphorus in the Eastern Tibetan Plateau has been edited for language by EnPapers, a company dedicated to helping international researchers publish their findings in the best English language journals possible.

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