

Dear Editor,

Thank you for allowing us to submit a revised version of our manuscript. We greatly appreciate the detailed and constructive comments of you and two reviewers which helped us to improve the manuscript. We addressed all comments as attached below (highlighted in the manuscript in blue for reviewer 1's comments and in red for reviewer 2's comments) and hope that we adequately solved the requests.

With kind regards,

Xiaojuan Feng

(We attached a marked version of the manuscript and the supplement with changes highlighted at the end of this pdf.)

Response to Editor

Comment:

Both reviewers agree that your manuscript has improved substantially, but several issues remain. Reviewer 1 provides some very useful editorial suggestions; reviewer 2 provides important editorial suggestions as well.

In addition, reviewer 2 points out a few results that really need a closer look; for instance, I fully agree with reviewer 2 that the reported specific soil respiration rates on page 7 are not consistent with reported soil C contents and total respiration rates (compared to the other two soils, the XLHT soil has roughly 60% less organic carbon, yet the respiration rates are also about 60% lower. So, the specific respiration rate should not be substantially higher than for the other soils). Reviewer 2 also suggests to include a brief discussion on two issues, and I agree on both accounts. In your revised version, please address all comments in detail.

Response:

Thank you. Below we provide a point-to-point response to all the issues raised by the referee. We hope our replies and revisions will satisfy all the requests.

Response to Referee #1

Thank you for providing so much detailed suggestions on improving this manuscript. All the comments are very constructive and helpful, and we have revised the manuscript thoroughly. Suggestions including language correction and rearrangements of sentences have been adopted accordingly, and we supply a pdf showing the point-to-point response. Here we only demonstrate the response to comments that need to discuss in detail.

Comment 1:

“It should be mentioned that the DIC concentration may be underestimated due to CO₂ outgassing during leachate collection. However, the potential underestimation is lower than 7% owing to the low proportion of outgassed CO₂ in total DIC (Table S2) as calculated using formulas in Ran et al. (2015).”

Table S2 indicates that there is outgassing of CO₂ from solution into the atmosphere even, for *p*CO₂ in solution that are smaller than the atmospheric *p*CO₂. How can this be?

Response:

First of all, we apologize for the incorrect use of the term “outgassing”. We actually meant “exchange”. According to Table S2, the calculated partial pressure of CO₂ in the leachate ranged from 18–409, 59–1368 and 601–14029 μ atm in the XLHT, KQ, and GC soils, respectively, under a series of alkalinity conditions (300–7000 μ mol/L) using our leachate pH (7.5–9.0) and incubation temperature (23 °C) according to equations listed in Ran et al. (2015). Hence, depending on the assumed alkalinity, leachate *p*CO₂ could be higher or lower than the mean atmospheric *p*CO₂ (400–500 μ atm in our lab) and CO₂ exchanges in both directions (outgassing and absorption) could happen. However, as the leachate pH was high (7.5–9.0) in all samples, bicarbonate (HCO₃⁻) rather than dissolved CO₂ (more abundant in acidic conditions) was the predominant form in the leachates. We estimate that dissolved CO₂ represented <7% (0.2–6.8%) of total DIC (HCO₃⁻ + CO₃²⁻ + dissolved CO₂) in the range of pH we measured. Therefore, regardless of outgassing or absorption, exchange-induced alterations in DIC concentrations were relatively small. We have revised this sentence and Table S2 as follows:

“It should be mentioned that the DIC concentration may vary due to exchanges between dissolved and atmospheric CO₂ during leachate collection. However, potential contribution from this process was < 7% owing to the low proportions of dissolved CO₂ in total DIC of our samples (Table S2) as calculated using formula in Ran et al. (2015).”

Table S2: Proportion of dissolved CO₂ in total dissolved inorganic carbon under the given pH, temperature and alkalinity conditions.

pH ^a	T ^b	Alkalinity ^c (μ mol/L)	Proportion of dissolved ^d CO ₂ (%)	Partial Pressure of CO ₂ ^d (μ atm)
9.0	23	300	0.2	18
8.5	23	300	0.7	59
7.5	23	300	6.8	601
9.0	23	500	0.2	29
8.5	23	500	0.7	98
7.5	23	500	6.8	1002
9.0	23	1000	0.2	58
8.5	23	1000	0.7	195
7.5	23	1000	6.8	2004
9.0	23	2000	0.2	117
8.5	23	2000	0.7	391
7.5	23	2000	6.8	4008
9.0	23	3000	0.2	175
8.5	23	3000	0.7	586

7.5	23	3000	6.8	6013
9.0	23	7000	0.2	409
8.5	23	7000	0.7	1368
7.5	23	7000	6.8	14029

^a: pH of the soil leachates in this study, pH_{XLHT} ~ 9, pH_{KQ} ~ 7.6, pH_{GC} ~ 8.6;

^b: incubation temperature;

^c: alkalinity range of natural and soil waters.

^d: calculation of partial pressure and proportion of dissolved CO₂ was based on equations in Ran et al. (2015).

Comment 2:

“According to Hendy (1971) and Doctor et al. (2008), isotopic fractionation of leached DIC due to CO₂ loss in an open system is insignificant when the partial pressure of CO₂ ($p\text{CO}_2$) in the solution is lower than twice that of the surrounding atmosphere. Therefore, due to the much lower $p\text{CO}_2$ in the XLHT leachates (~ 200 μatm ; Table S2) compared to that in the ambient atmosphere (> 400 μatm), the influence of CO₂ outgassing on the $\delta^{13}\text{C}$ of leached DIC was not considered in the present study.”

How can the $p\text{CO}_2$ that was established in soil water in contact with soil air be smaller than the $p\text{CO}_2$ of atmospheric air? The $p\text{CO}_2$ in soil air is typically higher than the atmospheric $p\text{CO}_2$.

Response:

Again, according to Table S2, we calculated $p\text{CO}_2$ in the soil leachates under a series of alkalinity conditions (300–7000 $\mu\text{mol/L}$) using the measured leachate pH and incubation temperature (23 °C). Leachates from the XLHT soil had a lower dissolved CO₂ concentration and hence lower $p\text{CO}_2$ (18–409 μatm) compared to other two soils (59–1368 and 601–14029 μatm in the KQ, and GC soils, respectively) due to its higher soil pH (9.0). There are three main reasons for the lower $p\text{CO}_2$ relative to the atmosphere in the XLHT leachates: (1) due to the high pH of this soil, bicarbonate (HCO_3^-) rather than dissolved CO₂ (more abundant in acidic conditions) was the predominant form in soil leachates; (2) the high flux of precipitation (1 L in 3-4 h per EPE) largely diluted dissolved CO₂ in soil solutions rather than allowed respired CO₂ to accumulate in solution; (3) (maybe less importantly) respiration rate was rather low in the XLHT soils due to its lower SOC content (5.1–15.6 mg C m⁻² h⁻¹ relative to 16–49 mg C m⁻² h⁻¹ in the KQ and GC soils; Fig. S3).

To clarify, the paragraph is revised:

“Isotopic fractionation of leached DIC due to CO₂ loss in an open system is insignificant when the partial pressure of CO₂ ($p\text{CO}_2$) in the solution is lower than twice that of the surrounding atmosphere (Hendy, 1971; and Doctor et al., 2008). In the present study, $p\text{CO}_2$ in the XLHT leachates was low (~ 400 μatm assuming alkalinity equals to DIC concentration; Table S2) due to its high pH, low soil respiration and dilution of dissolved CO₂ under EPE, the influence of CO₂ outgassing on the $\delta^{13}\text{C}$ of leached DIC was thus considered not important.”

Response to Referee #2

General Comments:

Comment 1:

The manuscript was significantly improved by the authors, they responded to all comments properly. There are two points, which should be more discussed, first: the transferability of the results to field conditions, especially the low water content in arid and semi-arid climates and what does it mean for soil carbon leaching.

Response:

Soil water content is indeed low in the temperate grasslands of Inner-Mongolia for the majority of the year. However, as we mentioned in the Materials and Methods, extreme precipitation events tend to occur in the summer with 70% of the annual precipitation falling within June-Aug (Fig. S2), which leads to oversaturation and leaching of soil carbon. There are normally 2-4 heavy precipitation events per summer (Fig. S2) in the study area, and our experiment design was consistent with the field precipitation frequency and strength. Furthermore, as we showed later, neither DOC nor DIC fluxes showed significant relationships with leachate volume during EPEs (Figs. 6e-f), indicating that we used sufficient amount of precipitation and these fluxes represent soil carbon's leaching potential under EPEs. These being clarified, we agree with the reviewer and add discussions on the implication of our findings for field conditions:

“Interestingly, neither DOC nor DIC fluxes showed any significant relationships with the volume of leachates during EPEs (Figs. 6e-f). This indicates that we used sufficient amount of precipitation in this study to “scavenge” dissolved carbon from soils and hence these fluxes represent soil carbon’s leaching potential under EPEs.”

“Also, soil water content was set to be ~60% of max WHC initially in our experiment, higher than that in the field of temperate grasslands (XLHT and KQ). Thus, our measured DOC and DIC fluxes are likely to be higher than carbon leaching in the field due to greater water retention in drier soils. Hence, our estimate may represent an upper limit of soil carbon leaching potential under EPEs.”

Comment 2:

Second the comparison of soil respiration from bare vs. litter-covered soil.

Response:

Thank you for this suggestion. Comparison of soil respiration between litter-amended and non-amended soils is now added into Section 3.2:

“Total respired CO₂ was higher in the litter-amended than non-amended soils before and after EPEs (Fig. S5). The cumulative respired CO₂ in the litter-amended XLHT, KQ, and GC soils were 16.7, 54.8, and 44.6 g C m⁻² during three EPEs, 20%, 22%, and 15% higher than that of the non-amended soils, respectively. Due to the wide presence of litter coverage in our studied soils, litter effect on soil respiration should be considered when estimating carbon budgets for these grassland soils.”

Specific Comments:

Additionally there are some points which are confusing, and for a final publication these parts should be

rewritten.

Comment 3:

Page 7, line 29: The authors present the soil respiration normalized for different SOC contents, with 2.2, 2.6 and 7.6 [$\mu\text{g C g}^{-1} \text{SOC h}^{-1}$], it is not clear which values are presented, maximum or average values – please specify.

Furthermore, I think the specific respiration rate of the XLHT site is too high with 7.6 [$\mu\text{g C g}^{-1} \text{SOC h}^{-1}$], given the maximum respiration rate of 13.7 [$\text{mg C m}^{-2} \text{h}^{-1}$] (same page line 27) and SOC stocks of 7.4 [kg C m^{-2}] (based on the numbers given in table 1). There must be a mistake in the calculation or in the presented numbers. In consequence, SOC of the XLHT site is not easier to degrade.

Response:

We reported maximum respiration rates herein and we indeed made a mistake in presenting the numbers. Specific soil respiration rates should be 2.2, 2.6, and 2.0 $\mu\text{g C g}^{-1} \text{SOC h}^{-1}$ in the GC, KQ, and XLHT soils, respectively. Detailed parameters used for the calculation of specific respiration rate were shown in the following table:

Station	Maximum soil respiration rate ($\text{mg C m}^{-2} \text{h}^{-1}$)	SOC (g)	Bottom area of column (m^2)	Maximum specific respiration rate ($\mu\text{g C g}^{-1} \text{SOC h}^{-1}$)
GC	37.30	132	0.00785	2.21822
KQ	40.60	123	0.00785	2.59114
XLHT	13.70	55	0.00785	1.95536

Accordingly, we have revised this sentence as follows:

“The maximum specific soil respiration rates normalized to SOC were 2.2, 2.6, and 2.0 $\mu\text{g C g}^{-1} \text{SOC h}^{-1}$ in the non-amended GC, KQ, and XLHT soils, respectively. Therefore, SOC degradability was quite similar in the alpine and temperate grassland soils.”

Comment 4:

Page 8, line 3:

“Total respired CO_2 was higher in the litter-amended than non-amended soils before and after EPEs (Fig. S3), likely caused by one or two following reasons: (1) the degradation of labile components in the fresh litter; (2) increased degradation of SOC primed by litter additions (Fröberg et al., 2005; Ahmad et al., 2013). These two reasons might affect CO_2 release in litter-amended soils in two ways: (1) both of them had positive effects; (2) one of them had positive effects and the other one had negative ones, but the total net effects was positive.”

The second sentence is confusing and makes no sense, I'd suggest to delete it and rewrite the first a little bit.

Suggestion: “... (1) the degradation of labile compounds in the fresh litter; (2) induced priming effects due to the addition of an easily available energy source.”

Response:

Thank you. Revised accordingly.

Comment 5:

Page 8, line 3-18:

In that paragraph, I really miss the comparison between soil carbon loss from bare soil versus litter covered and the resulting discussion. What are the field conditions for the studied area is the soil normally covered by litter or not? What does it mean for SOC losses due to respiration and leaching? - As mentioned in the introduction the authors want to compare soil carbon loss from bare vs. litter-covered soil.

Response:

The following is added to this section:

“Respired CO₂ was higher in the litter-amended than non-amended soils before and after EPEs (Figs. S3 and S5). The cumulative respired CO₂ in the litter-amended XLHT, KQ, and GC soils were 16.7, 54.8, and 44.6 g C m⁻² during three EPEs, 20%, 22%, and 15% higher than that of the non-amended soils, respectively. Due to the wide presence of litter coverage in our studied soils, litter effect on soil respiration should be considered when estimating carbon budgets for these grassland soils.”

Comment 6:

Page 8, line 25: “The specific EPE-induced CO₂ was significantly different for the litter-amended soils among sites ($p < 0.05$), showing a pattern of KQ > XLHT > GC.”

In my opinion you can not compare the specific EPE-induced CO₂ release of the litter amended soils, because you have no information about the source of CO₂ especially on the XLHT and KQ site. Due to the coarser soil texture of these two sites, it might be also possible that labile C is dissolved from the litter, transported down column and led to positive priming effects in the deeper parts of the column. However, since you have no information about the contribution of SOC mineralization to total CO₂ this statement is highly speculative. You can only compare the non-amended sites. It would be more interesting if you could show the reduction of SOC mineralization of the GC site, after EPE. Here you have the isotopic data.

Response:

This is a good point. As the respired CO₂ from litter-amended soils originated from both SOC and litter mineralization, it is not appropriate to normalize respiration to SOC. We have therefore deleted relevant results and discussion.

SOC-derived CO₂ flux in the litter-amended GC soils ranged from 6.2 to 15.7 mg C m⁻²h⁻¹ after the first EPE, with specific SOC-derived CO₂ flux of 0.4-0.9 μg C h⁻¹ g⁻¹ SOC. By comparison, CO₂ flux in the non-amended GC soils ranged from 17.2 to 27.1 mg C m⁻²h⁻¹, with specific CO₂ flux of 1.0-1.6 μg C h⁻¹ g⁻¹ SOC. The specific SOC-derived CO₂ flux was hence lower in the litter-amended than non-amended GC soils, indicating that litter addition induced a negative priming effect on SOC mineralization.

The above results and discussion are added:

“Similarly, the specific CO₂ flux derived from SOC was lower in the litter-amended GC soils (0.4–0.9 μg C h⁻¹ g⁻¹ SOC) than in the non-amended GC soils (1.0–1.6 μg C h⁻¹ g⁻¹ SOC), further proving the negative priming effect.”

Comment 7:

Page 8, line 32:

“These results suggest that SOC contents and SOC:N ratios are important factors influencing the total EPE induced CO₂ release”

I disagree; figure 3b shows that there is no significant difference in specific respiration of the non-amended sites. Therefore, differences between sites might be less important.

Response:

We have removed this sentence.

Comment 8:

Page 9, line 2

For me it is not clear what the authors mean with “ ... a total of 0.57–0.71, 0.56–0.94, and 0.73–0.89 L of leachates were collected for the XLHT, KQ, and GC soils, respectively.”

Does it mean e.g.

- that after 3 EPE in total 0.57 L of leachates were collected for the XLHT site?
- or does it mean a range, that after the first EPE 0.57 L were collected for the XLHT site and after the third EPE 0.71 L

Is there any explanation why the XLHT soil retain more water than the GC soil, due to the lower sand content in GC I would expect the highest retention on this site.

Response:

First, to clarify, there were 0.57, 0.71 and 0.69 L of leachates after the addition of 1 L of precipitation during the first, second, and third EPEs for the XLHT soils, respectively. Similarly, leachate volumes were 0.56, 0.94 and 0.83 L for the KQ soils and 0.73, 0.87 and 0.89 L for the GC soils, respectively. Such information is shown in Figure 4.

Second, although water holding capacity (WHC) is primarily controlled by soil texture, structure, and organic matter, water movement in soils is also affected by preferential flow (an uneven and rapid movement of water in soils; Soares et al., 2015). Higher clay contents tend to facilitate the formation of preferential flow in soils (Karup et al., 2016), which can also be triggered by heavy rainfall events (McGrath et al., 2009). The GC soils have a higher clay content than the other two, presumably causing potential preferential flows and thus a higher leaching volume. In addition, although we added the same amount of water (10 mL) to the surface of all soil columns daily, it is difficult to ensure a constant water content for such large soil columns. Hence, water evaporation may have also differed for soils with different textures (low water loss from clay soils; Harris and Robinson, 1916) and the GC soils, with the highest clay and silt contents, needed less precipitation to compensate for the evaporated water loss. Regardless of the cause for varied leachate volumes from different soils, neither DOC nor DIC fluxes showed significant relationships with leachate volume during EPEs (Figs. 6e-f), indicating that we used sufficient amount of precipitation in our experiment and these fluxes represent soil carbon's leaching potential under EPEs.

To make this paragraph clear, it is now revised as follows:

“During the first EPE, a total of 0.57, 0.56 and 0.73 L of leachates were collected from the XLHT, KQ, and GC soils, respectively. The leachate increased to 0.71, 0.94 and 0.87 L during the second EPE and was 0.69, 0.83 and 0.89 L during the third EPE, respectively (Fig. 4). Soil water content was set to be ~60% of max WHC before the first EPE, and leaching did not occur until soil water reached saturation. Therefore, the leachate volume was lowest during the first EPE and similar for the second and third EPEs. There were some variations in the volume of leachates from different soils, possibly related to preferential flows created during EPEs in the soil columns (McGrath et al., 2009) and water evaporation between EPEs.”

References:

- Harris, F., and Robinson, J.: Factors affecting the evaporation of moisture from the soil, *J. Agr. Res.*, 7, 439-462, 1916.
- Karup, D., Moldrup, P., Paradelo, M., Katuwal, S., Norgaard, T., Greve, M. H., and de Jonge, L. W.: Water and solute transport in agricultural soils predicted by volumetric clay and silt contents, *J. Contam. Hydrol.*, 192, 194-202, 10.1016/j.jconhyd.2016.08.001, 2016.
- McGrath, G. S., Hinz, C., and Sivapalan, M.: A preferential flow leaching index, *Water Resour. Res.*, 45, W11405, 10.1029/2008wr007265, 2009.
- Soares, A., Moldrup, P., Vendelboe, A. L., Katuwal, S., Norgaard, T., Delerue-Matos, C., Tuller, M., and de Jonge, L. W.: Effects of Soil Compaction and Organic Carbon Content on Preferential Flow in Loamy Field Soils, *Soil Sci.*, 180, 10-20, 10.1097/ss.000000000000105, 2015.

Comment 9:

Page 9, line 16:

”However, for the KQ soil having a relatively low SIC content which was similar to the added litter-OC (0.7 g per column; Table 1), the influence of litter addition on the DIC flux was quite obvious. Therefore, although the contribution of dissolved CO₂ to DIC fluxes should be more important in high-pH soils, the relative effect of litter amendment on DIC fluxes under EPEs seemed more significant in soils with a low SIC content.”

I do not see the “quite obvious influence” of litter addition to DIC flux for the KQ site in figure 4b. Therefore I would recommend to give a number on the relative effect of litter amendment on DIC fluxes. Furthermore, comparing the GC with KQ site, the absolute / relative effect of litter addition on DIC fluxes was higher/equal on the GC site. Which contradicts the statement “the relative effect of litter amendment on DIC fluxes under EPEs seemed more significant in soils with a low SIC content.”

Response:

To better clarify, this section is revised:

“However, for the KQ soil having a relatively low SIC content similar to the added litter-OC (0.7 g per column; Table 1), litter amendment had a significant effect on the DIC flux ($p < 0.05$), increasing by $21 \pm 13\%$ and $15 \pm 7\%$ relative to the non-amended KQ soils during the second and third EPEs, respectively. There was also a $30 \pm 19\%$ increase in the DIC flux from the litter-amended GC soils relative to its non-amended counterpart during the second EPE. Therefore, litter amendment had a significant influence on DIC fluxes from soils with a relatively low SIC content (KQ and GC) under EPEs compared with the high-SIC XLHT soil.”

Comment 10:

Page 10, line 1:

“... despite their higher SOC contents and CO₂ release rates (fig S5).”

Figure S5 does not show a CO₂ release rate, See comment to Figure S5.

Response:

Sorry, there was a mistake—we referred to Figure S3. This is corrected and we have re-checked all other figures as well.

Comment 11:

Page 10, line 12 ff.:

“In addition, DIC loss exclusively resulting from SIC dissolution or weathering was also a significant fraction of soil carbon loss, equivalent to 219% SOC loss in the form of CO₂ during EPEs (Fig. 5). These results collectively corroborate that inorganic carbon loss is the main form of soil carbon loss in alkaline soils during EPEs.”

In my opinion this paragraph is a little bit confusing and needs more information especially the meaning of CO₂ loss. I guess the authors mean here EPE induced CO₂ release, however in the current form I would understand “SOC loss in form of CO₂” as total respiration. I’d suggest to stay with the term “EPE induced CO₂ loss”.

But if the authors mean total CO₂ release during EPE the presented numbers are wrong.

E.g. In figure 5 the CO₂ loss during the first EPE was ca. 1 g C m⁻², however given the numbers from figure S3 for the first EPE for the XLHT site, basal respiration with ca. 7.8 mg C m⁻² h⁻¹. Calculating a CO₂ loss for the first 20 days, would lead to 3.7 g C m⁻² for the site.

Furthermore, it is not clear which treatment is shown in figure 5 – non-amended or amended?

Response:

Thank you. We meant “EPE induced CO₂ loss” and consistently use the term here.

The treatment shown in figure 5 was the non-amended XLHT soils.

To make this sentence and Figure 5 clearer, we have revised these parts as follows:

“In addition, DIC loss exclusively resulting from SIC dissolution or weathering was also a significant fraction of soil carbon loss, equivalent to 219% SOC loss in the form of EPE-induced CO₂ during EPEs (Fig. 5). These results collectively corroborate that inorganic carbon loss is the main form of soil carbon loss in alkaline soils during EPEs.”

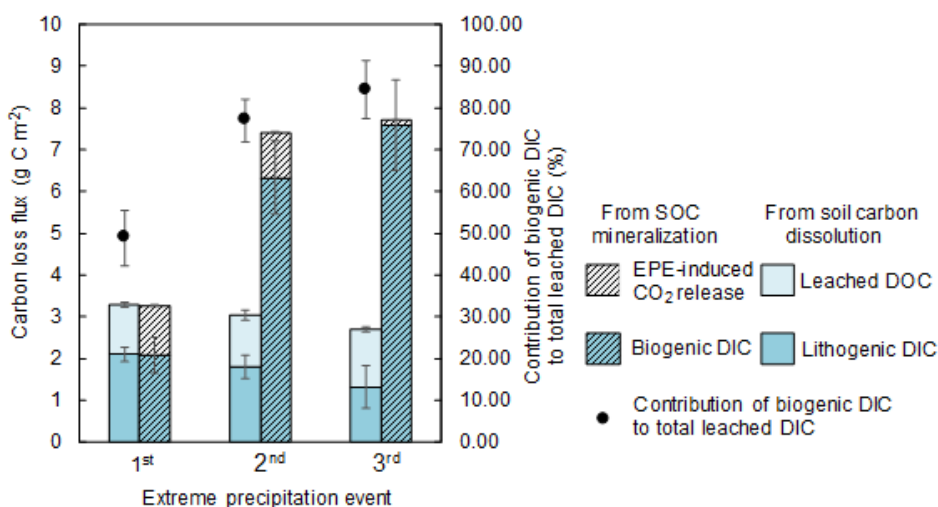


Figure 5: Carbon loss fluxes from soil organic carbon (SOC) mineralization in the non-amended XLHT soils. Fluxes include extreme precipitation event (EPE)-induced CO₂ release and leaching of biogenic dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and lithogenic DIC. Mean values are shown with standard error (n = 3).”

Comment 12:

Page 12, line 7:

Here I miss the discussion about the adjusted WHC of the soil columns, which might be important to estimate the losses of soil carbon in these arid and semi arid climates. In fact, under field conditions I’d expect a much lower DIC loss because under field conditions the water content in soils might be lower <

60% of WHC and soils can retain more water.

On page 9 line 2, the author mentioned that the sum of the collected leachates during 3 EPE was between 0.57 - 0.94 L, after the addition of in total 3 L precipitation. Therefore, this indicates a strong retention of rain water in the investigated soils, which of course depends on soil texture and water content before an EPE. It would be interesting to know how much water was added to adjust the WHC of the different soils. This would allow to give a more realistic estimation about soil carbon leaching losses under field conditions.

Further, the time between two EPE might be also an important factor for DIC losses, because the longer the time between two EPEs the lower the soil water content. In consequence DIC losses might be smaller. Therefore, leaching of soil carbon might be smaller under field condition. In addition, after an EPE under field conditions the moisture content is altered and may provide better conditions for SOC mineralization which would increase CO₂ losses.

Response:

Our original statements about leachate volume were a little confusing. In fact, there was 0.57 L of leachate after the addition of 1 L of precipitation during the first EPE for the XLHT soils. We have revised this paragraph completely to make it clearer—please see details in our reply to Comment 8.

As mentioned in our reply to Comment 1, soil water content is indeed very low in the field in the dry season but can be high in the summer due to the occurrence of heavy precipitation in this monsoon affected region. We designed the precipitation frequency and strength as close to the summer field condition as possible. However, the effect of low water content on DIC loss is important, especially in the dry season. Hence, we have added the following discussion to remind the readers of the limitations of our study:

“Also, soil water content was set to be ~60% of max WHC initially in our experiment, higher than that in the field of temperate grasslands (XLHT and KQ). Thus, our measured DOC and DIC fluxes are likely to be higher than carbon leaching in the field due to greater water retention in drier soils. Hence, our estimate may represent an upper limit of soil carbon leaching potential under EPEs.”

Comment 13:

Figure S4: Which bulk density was used for the GC site? There is no bulk density given in table 1. How did you calculated the numbers in figure S4.

Response:

Bulk density at the GC site was not determined. However, we measured the weight of wet soil in each soil depth. Combined with the field water content (FWC), soil inorganic carbon (SIC) and soil organic carbon (SOC) contents shown in Table 1, we can calculate soil carbon in each column. We have revised Figure S4 by adding the data of wet soil weight at each site.

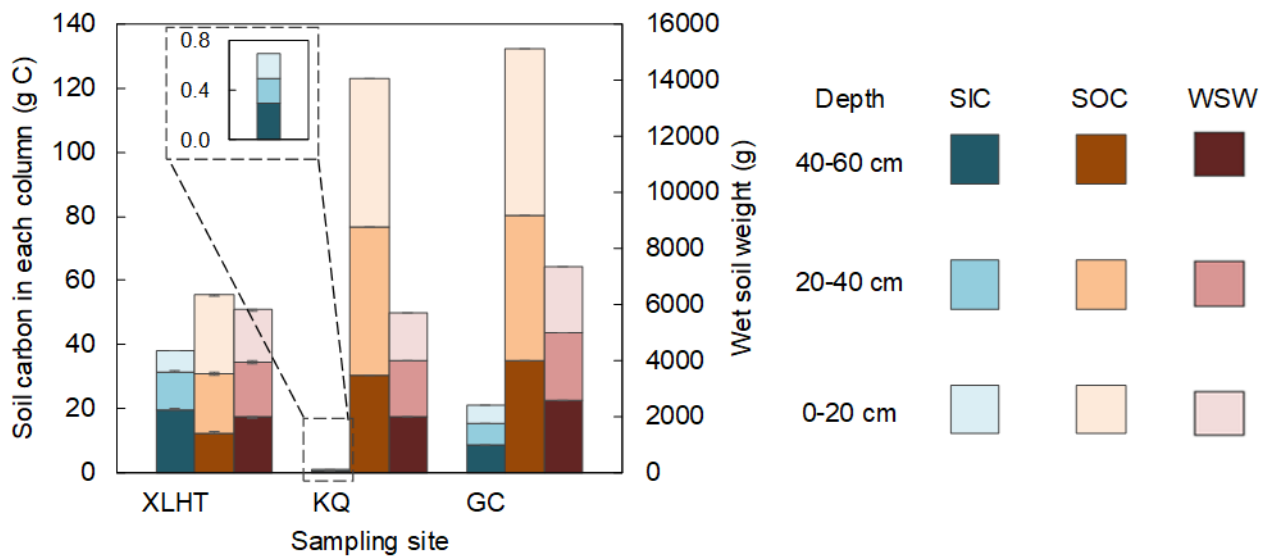


Figure S4: Total amount of soil organic carbon (SOC), soil inorganic carbon (SIC), and wet soil weight (WSW) in different layers of the artificial soil columns.

Comment 14:

Figure S5: The y axis label and figure caption is wrong. The figure does not show a rate rather it show a sum of total CO₂ produced during the three EPE.

Response:

We cited the wrong figure previously (see our reply to Comment 10; we referred to Figure S3 for rates). The axis label and figure caption in Figure S5 were actually consistent with our data, which showed the cumulative respiration during the three EPE.

Comparing soil carbon loss through respiration and leaching under extreme precipitation events in arid and semi-arid grasslands

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Abstract.

Respiration and leaching are two main processes responsible for soil carbon loss. While the former has received considerable research attention, studies examining leaching processes are limited especially in semiarid grasslands due to low precipitation. Climate change may increase the extreme precipitation event (EPE) frequency in arid and semiarid regions, potentially enhancing soil carbon loss through leaching and respiration. Here we incubated soil columns of three typical grassland soils from Inner Mongolia and Qinghai-Tibetan Plateau and examined the effect of simulated EPEs on soil carbon loss through respiration and leaching. EPEs induced transient increase of CO₂ release through soil respiration, equivalent to 32% and 72% of the net ecosystem productivity (NEP) in the temperate grasslands (Xilinhot and Keqi) and 7% the alpine grasslands (Gangcha). By comparison, leaching loss of soil carbon accounted for 290%, 120% and 15% of NEP at the corresponding sites, respectively, with dissolved inorganic carbon (DIC) as the main form of carbon loss in the alkaline soils regardless of DIC sources. Moreover, DIC loss increased with re-occurring EPEs in the soil with the highest pH due to elevated contribution of dissolved CO₂ from organic carbon degradation (indicated by DIC-δ¹³C). These results highlight that leaching loss of soil carbon (particularly in the form of DIC) is important in the regional carbon budget of arid and semiarid grasslands, and also imply that SOC mineralization measured by CO₂ fluxes might be drastically under-estimated in alkaline soils. With a projected increase of EPEs under climate change, soil carbon leaching processes and its influencing factors warrant better understanding and should be incorporated into soil carbon models when estimating carbon balance in grassland ecosystems.

1 Introduction

Soils store approximately 2500 Pg of carbon (including organic and inorganic carbon) globally, equivalent to 3.3 and 4.5 times the carbon in the atmosphere (760 Pg) and terrestrial plants (560 Pg), respectively (Lal, 2004). Slight variations of the soil carbon pool will hence severely influence atmospheric CO₂ concentrations and have important implications for climate change (Davidson and Janssens, 2006; Trumbore and Czimczik, 2008). Respiration and leaching are two main processes responsible for soil carbon loss. While respiration has received considerable research attention (Raich and Schlesinger, 1992; Raich and Potter, 1995; Hoover et al., 2016; Burri et al., 2015; Escolar et al., 2015), leaching is relatively poorly constrained despite its importance in certain ecosystems (Cole et al., 2007; Battin et al., 2008). For instance, soil carbon leached from forests, grasslands, and croplands is estimated to be 15.1, 32.4, and 20.5 g C m⁻² yr⁻¹ across Europe, representing 4%, 14%, and 8% of net ecosystem exchange (NEE), respectively (Kindler et al., 2011). Additionally, leaching of carbon previously preserved in surface litter and soil layers is believed to be a main source of dissolved organic and inorganic matter in inland waters (Spencer et al., 2008). In particular, soil inorganic carbon (SIC) that occurs widely in the arid and semiarid regions is more prone to leaching during sporadic high precipitation events (Lal and Kimble, 2000). Despite the importance of leaching loss in regional soil carbon budget, very few detailed data exist to investigate and compare the relative contribution of respiration and leaching processes to soil carbon loss.

Climate change is reported to increase the frequency as well as intensity of extreme precipitation events (EPEs; Knapp et al., 2002; Goswami et al., 2006; Parry et al., 2007; Min et al., 2011; Reichstein et al., 2013), especially in arid regions (Donat et al., 2017). In northwestern China, the frequency and intensity of EPEs have showed an increasing trend in the recent 50 years, constituting a much higher proportion of total precipitation than light precipitation events (Liu et al., 2005; Chen et al., 2012; Wang et al., 2012; Fu et al., 2013; Wang et al., 2014). Increasing EPEs will not only enhance soil carbon leaching but also affect soil respiration processes through increasing soluble substrates for microbial decomposition and potentially inducing hypoxic conditions (Knapp et al., 2002; Harper et al., 2005; Morel et al., 2009; Unger et al., 2010). Hence, it is critical to evaluate the effects of EPEs on soil respiration and leaching processes in order to better understand the impact of climate change on terrestrial carbon cycling, especially in the arid and semiarid regions.

Grasslands, containing 20% of global soil carbon pool, are the most widespread ecosystems in arid and semiarid regions globally (Jobbagy and Jackson, 2000). The deposition rate of carbonate is relatively high in the grassland soils with a high alkalinity and aridity (Lal, 2008; Yang et al., 2012), and hence SIC is the major form of soil carbon in many grasslands (Mi et al., 2008). SIC storage in China is approximately 53.3–77.9 Pg (Li et al., 2007; Mi et al., 2008), 54% of which is mainly distributed in the temperate and alpine grasslands located in Inner Mongolia and Qinghai-Tibetan Plateau (Mi et al., 2008). From 1980s to 2000s, SIC in the topsoil of Chinese grasslands was estimated to decrease by 26.8 g C m⁻² yr⁻¹, mainly attributed to soil acidification (Yang et al., 2012). Alternatively, precipitation is one of the main factors influencing the distribution and storage of SIC in arid and semiarid regions (Batjes, 1998; Lal and Kimble, 2000). Mi et al. (2008) found that

84% of SIC in China was distributed in areas with a mean annual precipitation (MAP) of < 500 mm and that SIC content decreased significantly with the increase of MAP. Given the high leaching potential of SIC in grassland soils under altered precipitation patterns in the future, we hypothesize that EPEs may significantly enhance SIC loss through leaching processes and further reduce SIC storage in grasslands.

5 In this study, soils were collected from varied depths of three typical temperate and alpine grasslands in Inner Mongolia and Qinghai-Tibetan Plateau to construct soil columns for a laboratory incubation study. Using simulated EPEs, we examined soil carbon loss through respiration and leaching processes and compared their fluxes after EPEs. In addition, leaf litter of a C4 grass was added to the surface of one set of soil columns to compare soil carbon loss from bare versus litter-covered soils and to estimate the contribution of litter-derived carbon to soil respiration after EPEs. Our research objectives were: (1) to
10 investigate the influence of EPEs on soil respiration; (2) to quantify the loss of SIC and soil organic carbon (SOC) through leaching during EPEs; and (3) to compare the relative importance of respiration and leaching in EPE-induced soil carbon loss from grassland soils.

2 Materials and Methods

2.1 Study area

15 For the incubation experiment, soils were collected from three different sites of temperate and alpine grasslands of China with varied environmental characteristics. Temperate grasslands were sampled near Xilinhot (XLHT, 116°22' E, 44°8' N, mean elevation of 1170 m) and Keqi (KQ, 117°15' E, 43°18' N, mean elevation of 1250 m) within the arid and semiarid regions of Inner Mongolia (Fig. S1) with MAP of 299 and 402 mm and mean annual temperature (MAT) of 1.2 and 0.4°C, respectively. Soil in this region is mainly chestnut soil, classified as Calcic Chernozems according to the World Reference
20 Base for Soil Resources (Steffens et al., 2008; IUSS working group WRB, 2015), with *Stipa klemenzii*, *Stipa Goboca*, *Stipa breviflora*, and *Stipa glareosa* as the dominating species (Sui and Zhou, 2013). The alpine grassland was sampled in Gangcha (GC, 100°7' E, 37°19' N, mean elevation of 3500 m) located north of the Qinghai Lake on the northeastern edge of the Qinghai-Tibetan Plateau. The GC site has an MAT of 0.4°C, an MAP of 370 mm and a mean annual evaporation (MAE) of 607 mm. Soils at this site are mainly Gelic Cambisol (IUSS working group WRB, 2015), with *Potentilla ansrina*
25 *Rosaceae*, *Elymus nutans Griseb*, and *Deyeuxia arundinacea* as the dominant species.

Soils were collected by digging soil pits of 25 cm × 25 cm × 70 cm from the temperate (XLHT and KQ) and alpine (GC) sites in October, 2014 and August, 2015, respectively. At each site, three plots (200 m × 200 m) were selected (> 200 m in between) with three random soil pits (distance of ~ 5 m in between) sampled within each plot. Soils from the same depth (0–20, 20–40, and 40–60 cm) of the three soil pits were mixed *in situ* for each plot, shipped back to the laboratory immediately,
30 and stored at 4°C before the experiment started within one month. As a result, each sampling site had three “true” replicates from the field for the soil column experiment.

2.2 Soil column experiment and simulated EPEs

For the laboratory experiment, we reconstructed soil columns of similar structures and texture under controlled conditions and used gravity to collect soil leachates. This approach is commonly used in process-related research (Hendry et al., 2001; Thaysen et al., 2014; Ahmad and Walworth, 2009; Aslam et al., 2015) as it minimizes experimental errors and bias caused by unknown factors including soil heterogeneity and microbial community variations. It is also more favourable in terms of quantifying soil carbon leaching loss as it circumvents pore-water contamination by vacuum suction in the field. In particular, leachate sampling by gravity from soil columns prevents alterations to DIC concentrations, which may be caused by CO₂ outgassing using vacuum suction in field studies. Artificial soil columns were constructed in the laboratory with polymethyl methacrylate frames (diameter: 10 cm; height: 70 cm; Fig. 1). The bottom of each column had an aperture (inner diameter: 0.6 cm; height: 3 cm) for the collection of soil leachates, and the column top was fitted with an airtight lid connected to two tubes for gas exchange and collection. Empty columns were soaked in 0.1 mM hydrochloric acid (HCl) solutions for 12 h and rinsed with distilled water before use. Column bottoms were packed with pre-cleaned quartz sand (5-cm thick; soaked in 0.1 mM HCl and combusted at 450°C for 6 h before use) with a layer of nylon net (pore size: 150 µm; diameter: 10 cm) on both sides to prevent the movement of soil particles. Subsequently, soils were passed through 2-mm sieves with roots removed and packed into each column at the corresponding depths (in the sequence of 40–60, 20–40, and 0–20 cm). Soils were compacted gently to maintain a similar bulk density as in the field (Table 1). Water content of each soil layer was separately adjusted to 60% of the maximum water holding capacity (Table 1) to provide an ideal moisture condition for microbial growth (Howard and Howard, 1993; Rey et al., 2005). There was a 10-cm headspace unfilled with soil for each column.

Six soil columns (one litter-amended and one non-amended column for each of the three sampling plots) were set up for each site as described above, and pre-incubated for two weeks in the laboratory to allow the recovery of microbial communities after disturbance. Subsequently, leaf litter of a C4 grass, *Cleistogenes squarrosa*, a dominant species in the grasslands of northern China (Tian et al., 2015), was added to the surface of three columns in an amount equivalent to the aboveground biomass in the field (1.26 g for the XLHT and KQ sites and 1.59 g for the GC site; Bai et al., 2008). The isotopic signal of the leaf litter ($\delta^{13}\text{C}$ of -16.2‰) would allow us to estimate the contribution of litter-derived CO₂ to total soil respiration. The columns were pre-incubated again for seven days. Basal respiration rate was measured by collecting CO₂ gas in the column headspace after 4 h of incubation. Temperature was recorded every day during the whole incubation period ($23 \pm 1^\circ\text{C}$).

According to historical precipitation records (Fig. S2), more than 70% of the annual precipitation occurs from June to August in the study area, mainly in the form of 2–4 heavy precipitation events. Therefore, a total of three EPEs were simulated over a period of 2 months for each soil using artificial rainwater prepared according to rainwater's composition at the corresponding sites (pH of 7.3; Table S1; Tang et al., 2014; Zhang et al., 2013). A maximum rainfall intensity of ~100 mm per precipitation event has been recorded in the past two decades in the study area (Fig. S2) and is predicted to increase by 18.1% in the late 21st century in north China (Chen et al., 2012). Hence, approximately 1 L of rainwater (rainfall of ~127

mm), comparable to 30% of the MAP of the investigated sites, was added to the surface of each soil column over 3–4 h at rates of one drop per second using syringes and allowed to leach through the column to be collected with a clean beaker within 12–14 h. The leachates were weighed, filtered through a 0.45- μ m PTFE syringe filter and analyzed for dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) concentrations immediately. To monitor soil respiration every 1–2 days following each EPE, soil columns were first aerated for 1 h using CO₂-depleted air that had been passed through saturated sodium hydroxide (NaOH) solutions (twice; Fig. 1) and then incubated for 4 h with lids closed. CO₂ gas in the column headspace was collected by gas-tight syringes for the subsequent measurement. After collection of CO₂ gas, the lids were open to allow the exchange with the ambient air. Soil respiration was monitored for 30 days after the first EPE and observed to stabilize approximately on the 20th day (Fig. S3). Hence, the first, second, and third EPEs were conducted on the 1st, 31st, and 51st day of incubation, and the CO₂ measurement was conducted for approximately 30, 20, and 20 days after the first, second, and third EPEs, respectively. Basal respiration was considered to be represented by the stabilized respiration rate at the end of each EPE cycle.

2.3 Sample analyses

Soil pH was measured at a soil:water ratio of 1:2.5 (w:v) using a pH meter (Sartorius PB-10). Soil texture was examined by laser diffraction using Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK) after removal of organic matter and calcium carbonates. Soil field water content was determined by difference between moist and dried soils (dried at 105°C for 8 h). Maximum water holding capacity was estimated by weighing soils before and after removal of redundant water from fully soaked soils (in water for 8 h). For SOC analysis, dried soils were decarbonated by exposure to concentrated HCl vapor for 72 h, followed by saturated NaOH solutions for 48 h to neutralize extra HCl, and then dried at 45°C. Total soil carbon, SOC (after decarbonation) and nitrogen (N) contents were measured by combustion using an elemental analyser (Vario EL III, Elementar, Hanau, Germany). SIC was calculated as the difference between total carbon and SOC contents. Small aliquots of the soil leachates were analyzed immediately on a Multi N/C 3100-TOC/TN Analyzer (Analytik Jena, Germany) for DIC and DOC concentrations (with the latter acidified to pH < 2 with concentrated HCl before analysis). It should be mentioned that the DIC concentration may be underestimated due to CO₂ outgassing during leachate collection. However, the potential underestimation is lower than 7% owing to the low proportion of outgassed CO₂ in total DIC (Table S2) as calculated using formulas in Ran et al. (2015). CO₂ concentration in the soil column headspace was determined by gas chromatograph (Agilent 7890A, USA) coupled with a flame ionization detector (FID).

To examine the contribution of SOC- and litter-derived carbon to soil respiration, the $\delta^{13}\text{C}$ values of SOC and CO₂ gas were determined on an isotope ratio mass spectrometer (Delta plus xp, Thermo, Germany) with a precision of $\pm 0.2\%$. To estimate the contribution of SOC degradation to leached DIC, the $\delta^{13}\text{C}$ values of DIC were determined on a Picarro isotopic CO₂ analyzer equipped with an automated DIC sample preparation system (AutoMate) based on wavelength scanned cavity ring down spectroscopy technique (Picarro AM-CRDS, USA). The precision for the DIC- $\delta^{13}\text{C}$ measurement was $\pm 0.3\%$. Due to

budget constraints and logistic reasons, we only measured the $\delta^{13}\text{C}$ of the respired CO_2 in the GC soils during the first EPE and the leached DIC in the XLHT soils.

2.4 Data analysis and statistics

The relative contribution of litter- and SOC-derived CO_2 to total respired CO_2 in the litter-amended soils was estimated using the following mass balance model:

$$f_{\text{litter-derived}} + f_{\text{SOC-derived}} = 1 \quad (1)$$

$$f_{\text{litter-derived}} \times \delta^{13}\text{C}_{\text{litter-derived}} + f_{\text{SOC-derived}} \times \delta^{13}\text{C}_{\text{SOC-derived}} = \delta^{13}\text{C}_{\text{respired-CO}_2} \quad (2)$$

where $f_{\text{litter-derived}}$ and $f_{\text{SOC-derived}}$ are the proportion of litter- and SOC-derived CO_2 in the total respired CO_2 ; $\delta^{13}\text{C}_{\text{litter-derived}}$ is the $\delta^{13}\text{C}$ value of litter-derived CO_2 , equivalent to -16.25% ; $\delta^{13}\text{C}_{\text{SOC-derived}}$ is the $\delta^{13}\text{C}$ value of SOC-derived CO_2 , which assumes the same value as that in the non-amended soils at the beginning of incubation (-23.1%) according to Cerling et al. (1991); $\delta^{13}\text{C}_{\text{respired-CO}_2}$ is the measured $\delta^{13}\text{C}$ of respired CO_2 .

Similarly, the relative contribution of lithogenic carbonate and biogenic DIC derived from SOC degradation to leached DIC was assessed according to the following isotopic mass balance model:

$$f_{\text{carbonate}} + f_{\text{biogenic-DIC}} = 1 \quad (3)$$

$$f_{\text{carbonate}} \times \delta^{13}\text{C}_{\text{carbonate}} + f_{\text{biogenic-DIC}} \times \delta^{13}\text{C}_{\text{biogenic-DIC}} = \delta^{13}\text{C}_{\text{DIC}} \quad (4)$$

where $f_{\text{carbonate}}$ and $f_{\text{biogenic-DIC}}$ are proportion of carbonate- and biogenic DIC in total DIC; $\delta^{13}\text{C}_{\text{carbonate}}$ is the $\delta^{13}\text{C}$ value of soil carbonate, equivalent to 0% (Edwards and Saltzman, 2016); and $\delta^{13}\text{C}_{\text{biogenic-DIC}}$ is the $\delta^{13}\text{C}$ value of biogenic carbonate/bicarbonate derived from the dissolution of CO_2 produced by SOC degradation, which is estimated to shift by approximately 8% compared with the $\delta^{13}\text{C}$ value of soil-respired CO_2 (-24% here) due to isotope fractionation during CO_2 dissolution (Zhang et al., 1995). Hence, $\delta^{13}\text{C}_{\text{biogenic-DIC}}$ is estimated to be -16% . $\delta^{13}\text{C}_{\text{DIC}}$ is the measured $\delta^{13}\text{C}$ signature of leached DIC. According to Hendy (1971) and Doctor et al. (2008), isotopic fractionation of leached DIC due to CO_2 loss in an open system is insignificant when the partial pressure of CO_2 ($p\text{CO}_2$) in the solution is lower than twice that of the surrounding atmosphere. Therefore, due to the much lower $p\text{CO}_2$ in the XLHT leachates ($\sim 200 \mu\text{atm}$; Table S2) compared to that in the ambient atmosphere ($> 400 \mu\text{atm}$), the influence of CO_2 outgassing on the $\delta^{13}\text{C}$ of leached DIC was not considered in the present study.

EPE-induced CO_2 release via respiration was assessed following two steps. First, cumulative respiration during the first 20 days after each EPE (until respiration rate stabilized) was calculated. Second, difference between the measured cumulative respiration and that estimated using the stabilized basal respiration rate after each EPE was calculated as the EPE-induced CO_2 release.

Independent samples T test (group size = 2) and One-way ANOVA analysis (group size > 2) was used to compare the dissolved carbon concentrations and fluxes among different columns. Linear regression analysis was used to assess correlations between leachate carbon flux and influencing factors (carbon content, soil pH, soil texture, etc.). All these

analyses were performed using IBM SPSS Statistics 22. Differences and correlations are considered to be significant at a level of $p < 0.05$.

3 Results and Discussion

3.1 Bulk properties of grassland soil samples

5 In the investigated grassland soils, SOC represented 59–99% of soil carbon and exhibited $\delta^{13}\text{C}$ values typical of C3 plant inputs (ranging from -24.1‰ to -26.3‰ ; Table 1). The XLHT soil had a much lower SOC and nitrogen (N) contents than the KQ and GC soils despite a similar soil texture ($p < 0.05$; Table 1). The SOC:N ratio was also lowest in XLHT (7.09–8.03), indicating a more decomposed state of soil organic matter (Weiss et al., 2016). Conversely, the SIC content was highest in XLHT and lowest in KQ, mainly due to pH variations at these sites, i.e., lowest pH in KQ and highest in
10 XLHT. This dependence of SIC content on soil pH is consistent with the results of Shi et al. (2012), showing that pH is the most important factor controlling SIC variation across the Mongolian and Tibetan grasslands. In terms of depth variations, soils became coarser with depth in XLHT and GC but became finer in KQ. The SOC and N contents decreased with depth in all soils due to declining plant inputs ($p < 0.05$; Table 1), while the SOC:N ratio remained relatively similar (except a small decrease with depth in XLHT). By contrast, XLHT and GC soils showed an increasing SIC content with depth ($p < 0.05$;
15 Table 1), because SIC, with a good solubility, is prone to leaching from the topsoil and subsequently gets deposited in the deeper soil via salt formation (Mi et al., 2008; Tan et al., 2014). The KQ soil, showing an almost neutral pH, had an invariant SIC content and pH with depths. Overall, the varied properties (including SOC, SIC, pH, etc.) of these soils allowed us to compare the effects of EPEs on soil respiration and leaching processes in different grassland soils.

3.2 EPE-induced changes to soil respiration

20 Shortly after each simulated EPE, soil respiration was similar to or lower than basal respiration (Fig. S3). The latter case may be attributed to hypoxic conditions induced by water saturation during EPEs (Hartnett and Devol, 2003; Jessen et al., 2017). Subsequently, soil respiration increased and peaked after approximately one week due to the recovery of microbial activity with improved soil aeration (Borken and Matzner, 2009). It then decreased to a constant level approximately 20 days after each EPE (Fig. S3). The transient increase of respiration was consistent with the “Birch Effect” proposed by (Birch, 1964),
25 i.e., a pulse of soil respiration after rewetting events due to resuscitation of microorganisms and improved diffusive transport of substrate and extracellular enzymes (Borken and Matzner, 2009; Navarro-García et al., 2012; Placella et al., 2012). The maximum soil respiration rates were 40.6 and $37.3 \text{ mg C m}^{-2} \text{ h}^{-1}$ after EPEs in the non-amended KQ and GC soils, respectively. These rates were significantly higher than that in the XLHT soil ($13.7 \text{ mg C m}^{-2} \text{ h}^{-1}$), likely related to the higher SOC content in the former soils. The specific soil respiration rates normalized to SOC were 2.2 , 2.6 , and $7.9 \text{ } \mu\text{g C g}^{-1} \text{ SOC}$

h^{-1} in the GC, KQ, and XLHT soils, respectively. This indicated that SOC in the XLHT soils was easier to degrade despite its low content.

Total respired CO_2 was higher in the litter-amended than non-amended soils before and after EPEs (Fig. S3), likely caused by one or two following reasons: (1) the degradation of labile components in the fresh litter; (2) increased degradation of SOC primed by litter additions (Fröberg et al., 2005; Ahmad et al., 2013). These two reasons might affect CO_2 release in litter-amended soils in two ways: (1) both of them had positive effects; (2) one of them had positive effects and the other one had negative ones, but the total net effects was positive. To distinguish the influences of above two reasons on total respired CO_2 and further differentiate the contribution of litter (C4) and SOC (C3) to the respired CO_2 , we examined the $\delta^{13}\text{C}$ values of CO_2 evolved from the GC soils after the first EPE. On the first day after EPE, CO_2 from the non-amended and litter-amended GC soils had a $\delta^{13}\text{C}$ value of -23.1‰ and -18.7‰ , respectively. The latter was close to the $\delta^{13}\text{C}$ signature of the added litter (-16.25‰). Using the two-endmember mixing model of Eq. (1) and (2), we calculated that litter contributed $\sim 64\%$ of the respired CO_2 in the litter-amended GC soils. However, along with the consumption of labile carbon in litter, the $\delta^{13}\text{C}$ signature of CO_2 decreased from -18.7‰ on Day 1 to -21.8‰ on Day 25 after EPE in the litter-amended soils (Fig. 2). Accordingly, the proportion of litter-derived CO_2 decreased from 64% to 20%. The litter-derived CO_2 flux in litter-amended GC soils was estimated to range from 7.0 to 17.5 $\text{mg C m}^{-2} \text{h}^{-1}$, while the SOC-derived CO_2 flux increased from 6.2 to 15.7 $\text{mg C m}^{-2} \text{h}^{-1}$ after the first EPE (Fig. S3). Compared with the SOC-derived CO_2 flux in non-amended GC soils (ranging from 17.2 to 27.1 $\text{mg C m}^{-2} \text{h}^{-1}$), litter addition had a negative priming effect on the degradation of native SOC while increasing total respiration through labile litter degradation.

Using data shown in Fig. S3-4, we calculated that total EPE-induced CO_2 release during three EPEs was higher in the KQ and GC soils than in the XLHT soil ($p < 0.05$; Fig. 3a) with a lower SOC content and a lower SOC:N ratio (Table 1). However, the specific EPE-induced CO_2 release normalized to SOC content showed no significant difference in the non-amended soils among three sites (Fig. 3b), indicating that a similar proportion of SOC ($\sim 4\%$) was subject to EPE-induced CO_2 release in the alpine and temperate grassland soils. The total EPE-induced CO_2 release (including CO_2 from both litter and SOC mineralization) was significantly higher in the litter-amended KQ soils than the non-amended ones, similar to the specific EPE-induced CO_2 in the KQ and XLHT soils. The specific EPE-induced CO_2 was significantly different for the litter-amended soils among sites ($p < 0.05$), showing a pattern of $\text{KQ} > \text{XLHT} > \text{GC}$. This pattern was consistent with the mean sand content in the order of $\text{KQ} (46.9\%) > \text{XLHT} (39.1\%) > \text{GC} (27.2\%)$. In addition, the higher total and specific EPE-induced CO_2 release in the litter-amended KQ soils may be related to its relatively lower soil pH (~ 7.7), which facilitates the release rather than the dissolution of respired CO_2 in soil solution. We therefore conclude that the KQ soil, with a coarser texture and a lower pH (Table 1), may have provided less sorptive protection for the labile DOC components after EPEs (Kell et al., 1994; Nelson et al., 1994) and allowed less dissolution of the respired CO_2 , and hence showed a more responsive respiration to the precipitation events. These results suggest that SOC contents and SOC:N ratios are important factors influencing the total EPE-induced CO_2 release, while the availability of labile organic carbon, soil texture and pH are key factors affecting the specific EPE-induced CO_2 release in these grassland soils.

3.3 EPE-induced leaching of soil carbon

During three EPEs, a total of 0.57–0.71, 0.56–0.94, and 0.73–0.89 L of leachates were collected for the XLHT, KQ, and GC soils, respectively. DIC was the main form of carbon in the leachates from the alkaline soils with a high SIC content (XLHT and GC) but low from the KQ soil with a neutral pH and low SIC content (Fig. 4). The resulting DIC flux was much higher for the XLHT soils ($\sim 21.3 \text{ g C m}^{-2}$) than the other two (2.9 g C m^{-2} for KQ and 7.4 g C m^{-2} for GC soils) during three EPEs, equivalent to five times of its DOC flux ($3.8\text{--}4.2 \text{ g C m}^{-2}$, Fig. 4). In contrast, DIC flux in the KQ soils was only one third of its DOC flux during EPEs. The form of leached carbon was mainly linked to the amount of SOC and SIC in the columns (shown in Fig. S5).

Litter amendment did not increase DOC fluxes in any of the investigated soils but increased DIC fluxes leached from the KQ soil during the second and third EPEs and from the GC soil during the second EPE ($p < 0.05$, Fig. 4b-c). We postulate that, while litter contribution to DOC was minor, CO_2 derived from litter degradation contributed to dissolved CO_2 in soils and hence increased DIC in the leachates (Monger et al., 2015). This effect was not evident during the first EPE when litter decomposition just started and was not significant for the third EPE in the GC soil due to a high sample variability associated with the litter-amended soil (Fig. 4c). Due to the high SIC content in the XLHT soils (38.15 g per column) and the low litter-OC amendment (0.7 g per column), there was no significant difference of DIC fluxes between the non-amended and litter-amended XLHT soils (Fig. 4a). However, for the KQ soil having a relatively low SIC content which was similar to the added litter-OC (0.7 g per column; Table 1), the influence of litter addition on the DIC flux was quite obvious. Therefore, although the contribution of dissolved CO_2 to DIC fluxes should be more important in high-pH soils, the relative effect of litter amendment on DIC fluxes under EPEs seemed more significant in soils with a low SIC content.

Between different EPEs, leachate DOC fluxes did not vary in any of the investigated soils. By comparison, DIC fluxes increased in the XLHT soil from 4.5 g C m^{-2} after the first EPE to 9.0 g C m^{-2} after the third EPE ($p < 0.01$, Fig. 4). This increase may be caused by (i) an increased contribution of SOC degradation to soil DIC and/or (ii) an elevated dissolution of soil carbonates induced by higher soil CO_2 concentrations with repeated EPEs (Gulley et al., 2014; Ren et al., 2015). To evaluate these contributions, the $\delta^{13}\text{C}$ values of DIC were measured for the non-amended XLHT soil. The $\delta^{13}\text{C}$ of leached DIC ranged from -10.0‰ to -6.6‰ during the first EPE. Based on the isotopic mass balance of Eq. (3) and (4), lithogenic carbonate (with a $\delta^{13}\text{C}$ value of 0‰) contributed 51.2% to the leached DIC while biogenic DIC produced by SOC degradation contributed 48.4% (Fig. 5). The $\delta^{13}\text{C}$ value of leached DIC decreased to -12.3‰ and -13.5‰ during the second and third EPEs, corresponding to a contribution of 77.0% and 84.4% by biogenic sources in the total DIC, respectively (Fig. 5). These results confirm our previous hypothesis that SOC decomposition contributed significantly to soil DIC fluxes. Combined with the total flux rate, we calculated that both lithogenic and biogenic DIC fluxes were $\sim 2.1 \text{ g C m}^{-2}$ in the first EPE. Subsequently, lithogenic DIC flux decreased to $\sim 1.3 \text{ g C m}^{-2}$ while biogenic DIC flux increased to 7.6 g C m^{-2} in the third EPE. This demonstrates that the increased DIC flux with repeated EPEs was mainly derived from increased contribution of SOC mineralization. Interestingly, increasing DIC fluxes with repeated EPEs were not observed in the KQ

and GC soils (Fig. 4) despite their higher SOC contents and CO₂ release rates (Fig. S5). Given that the XLHT soil had the highest soil pH, the high alkalinity may have favored the retention of respired CO₂ in the soil solution compared with the other soils (Parsons et al., 2004; Yates et al., 2013; Liu et al., 2015), leading to its high contribution to DIC fluxes.

Regardless of its source, the EPE-induced leaching loss of inorganic carbon was 31.5 and 10.6 μg DIC g⁻¹ soil from the alkaline XLHT and GC soils, respectively, approximately three and five times higher than the corresponding DOC leaching loss (5.9 and 3.9 μg DOC g⁻¹ soil, respectively). However, the KQ soil had a relatively lower EPE-induced DIC loss (4.4 μg DIC g⁻¹ soil) than the DOC leaching loss (11.6 μg DOC g⁻¹ soil) mainly due to its lower initial SIC content and relatively neutral soil pH value. Hence, DIC was the main form of soil carbon loss in alkaline soils during EPEs regardless of its source. When the source of the leached DIC is taken into account, dissolution of CO₂ produced by SOC mineralization (biogenic DIC) constituted more than half of the leached DIC (at least from the XLHT soils; Fig. 5), whose contribution increased with re-occurring EPEs (Fig. 5). This implies that SOC mineralization measured by CO₂ fluxes was underestimated by approximately 8 times in the XLHT soils during the three EPEs (Fig. 5). In addition, DIC loss exclusively resulting from SIC dissolution or weathering was also a significant fraction of soil carbon loss, equivalent to 219% SOC loss in the form of CO₂ during EPEs (Fig. 5). These results collectively corroborate that inorganic carbon loss is the main form of soil carbon loss in alkaline soils during EPEs.

As for the influencing factors on soil carbon leaching loss, the DIC flux was positively correlated to the amount of SIC in the soil columns and soil pH ($p < 0.05$; Fig. 6a-b). These two relationships may be self-correlated due to a positive relationship between soil pH and SIC (Liu et al., 2016). By comparison, DOC flux was linked with the amount of SOC in the soil columns, but decreased with an increasing content of silt and clay ($p < 0.05$; Fig. 6c). This may be explained by the stronger retention of SOC on small-sized particles with more sorption sites (Barré et al., 2014; Mayer, 1994). Overall, total soil carbon loss through leaching under EPEs was positively related to soil pH values ($p < 0.05$; Fig. 6d), suggesting that soil pH is a critical factor determining the magnitude of soil carbon loss under EPEs.

3.4 Main pathways of grassland soil carbon loss under EPEs

In this study, EPE-induced soil carbon loss was composed of three parts: leachate DIC including lithogenic and biogenic DIC, leached DOC and EPE-induced CO₂ release through respiration. Regardless of the carbon sources, DIC and DOC fluxes accounted for 90%, 62%, and 68% of EPE-induced total loss at XLHT, KQ, and GC, respectively, representing the major pathway of soil carbon loss in these grassland soils under EPEs. Soil carbon leaching fluxes were 25.3, 10.4, and 10.1 g C m⁻² yr⁻¹ in XLHT, KQ, and GC soils during three EPEs, respectively, with DIC as the dominant form in XLHT and GC soils. While DIC fluxes in this study generally fell within the range reported for grassland soils (1.3–47.8 g C m⁻² yr⁻¹; Parfitt et al., 1997; Brye et al., 2001; Kindler et al., 2011), the XLHT soil had a DIC flux higher than the majority (> 50%) of the reported values (Fig. 7). This may be attributed to the higher SIC content and stronger dissolution of respired CO₂ in the XLHT soils due to its higher soil pH (9.1 ± 0.1) relative to other grassland soils (pH: 5.4–7.5; Kindler et al., 2011), and the high intensity of our simulated EPEs (precipitation: 40 mm h⁻¹). Nonetheless, DIC fluxes in grassland soils reported in this

study and elsewhere (Brye *et al.*, 2001; Kindler *et al.*, 2011) were significantly higher than in forest and cropland ecosystems ($p < 0.05$; Rieckh *et al.*, 2014; Lentz and Lehrs, 2014; Gerke *et al.*, 2016; Herbrich *et al.*, 2017; Siemens *et al.*, 2012; Walmsley *et al.*, 2011; Wang and Alva, 1999; Kindler *et al.*, 2011), highlighting the importance of leaching as a major pathway of soil carbon loss in grasslands. By contrast, DOC fluxes in this study ($4.8 \pm 2.5 \text{ g C m}^{-2}$) were lower than most of the reported values in forest and grassland ecosystems due to the low SOC contents in our soils (Fig. 7).

Net ecosystem production (NEP) in the temperate steppe of Inner Mongolia (XLHT and KQ) is $8.7 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Sui and Zhou, 2013). While the EPE-induced CO_2 release (2.8 ± 0.6 and $6.3 \pm 3.0 \text{ g C m}^{-2}$) accounted for 32% and 72% of the NEP at XLHT and KQ, respectively; soil carbon leached during three EPEs was equivalent to 290% and 120% of NEP, with total DIC loss accounting for 244% and 33%, respectively. It is worth mentioning that biogenic DIC loss ($16.0 \pm 3.4 \text{ g C m}^{-2}$) caused by SOC degradation accounted for 184% of NEP at XLHT, indicating the importance of biogenic DIC to leached inorganic carbon loss during EPEs. By comparison, NEP in the studied alpine grassland ($68.5 \text{ g C m}^{-2} \text{ yr}^{-1}$; Fu *et al.*, 2009) is much higher than in typical temperate steppe. Hence, soil carbon loss through leaching and respired CO_2 release accounted for 15% (DIC: 11%, DOC: 4%) and 7% of the NEP at GC, respectively. Nonetheless, the EPE-induced soil carbon loss relative to NEP was higher in this study than that estimated for grassland topsoil across Europe (12% for DIC loss, 2% for DOC loss; Kindler *et al.*, 2011) where Net Ecosystem Exchange (NEE) reported by Kindler *et al.* was used as NEP according to the report of Kirschbaum *et al.* (2001). This was partially attributed to the lower NEP and higher SIC content in XLHT and KQ soils, underscoring that soil carbon leaching is more important in fragile ecosystems with low productivity.

An uncertainty related to the importance of leaching processes in the overall carbon budget along the “soil-river-ocean” continuum lies in the ultimate downstream fate of the leached carbon. If part of this carbon is retained in the surrounding soils or carried along from the river to the ocean in the form of DIC without outgassing into the air, it will not constitute a source of atmospheric CO_2 on a relatively short term (over years or decades). However, soil columns used in our study has a depth (60 cm) typical of or even deeper than the average soil depth in the alpine grasslands of Qinghai-Tibetan Plateau (Wang *et al.*, 2001). Hence, we assume that carbon leached in our experiments will have minimum retention in the soil. Furthermore, compared to DOC and DIC in the soil solution, the leached carbon is more likely to be subject to more intensified mineralization and outgassing during the land-ocean transfer, given more intensified mixing processes, oxygen exposure and photo-oxidation of terrestrial carbon upon releasing into the river (Hedges *et al.*, 1997; Battin *et al.*, 2009). Hence, we postulate that carbon leached from soils is more vulnerable to decomposition and/or release compared to that retained in the soil. That being said, it will be necessary to confirm our results and hypothesis using field-based leaching experiments to better understand the ultimate fate of leached soil carbon: whether it will be retained in the deeper soil or show a higher degradability upon leaving the soil matrix. Such information will be complementary to our study and further elucidate the importance of leaching processes in terms of ecosystem carbon budget.

In summary, this study quantified and compared soil carbon loss through respired CO_2 release and leaching in three typical grassland soils of northern China under simulated EPEs. Soil CO_2 release was stimulated shortly after each EPE, leading to an EPE-induced CO_2 release equivalent to 32% and 72% of the NEP at XLHT and KQ (temperate grasslands) and 7% at GC

(alpine grassland). By comparison, regardless of carbon sources, soil carbon leaching fluxes accounted for 290%, 120% and 15% of the NEP at XLHT, KQ, and GC, respectively, with DIC as the main form of carbon loss in the SIC-enriched XLHT and GC soils. In view of DIC sources, biogenic DIC loss derived from SOC mineralization contributed to more than half of the total leached DIC fluxes and accounted for 184% of the NEP at XLHT. Moreover, DIC loss increased with re-occurring EPEs in the XLHT soil with the highest pH due to increased dissolution of soil carbonates as well as elevated contribution of dissolved CO₂ from SOC degradation. These results also imply that SOC mineralization measured by CO₂ fluxes might be drastically under-estimated in alkaline grassland soils during EPEs. Admittedly, our results are based on artificial soil columns which destroyed natural soil structures, hence potentially increasing the contact between pore water and soil particles through eliminating macropore structures and preferential flow (Seyfried et al., 1987; Singh et al., 1991). Hence, our estimate may represent an upper limit of soil carbon leaching potential under EPEs. Nonetheless, these results highlight that leaching loss of soil carbon, especially in the form of DIC originated from biogenic and lithogenic carbonates, plays an important role in the regional carbon budget of grasslands located in arid and semiarid regions. Further research effort is needed to combine short-term laboratory experiments with long-term field measurements to fully assess the impacts of EPEs on soil carbon budget in these areas. In addition, with a projected increase of EPEs under climate change, soil carbon leaching processes and its influencing factors warrant better understanding and should be incorporated into soil carbon models when estimating carbon balance in grassland ecosystems.

Data availability. All data is available within this paper (Table 1) and in the Supplement (Dataset S1).

Competing interests. The authors declare that they have no conflict of interest.

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Table 1: Bulk properties of soil samples collected from the temperate and alpine grasslands for the soil column experiment (mean \pm standard error; n = 3).

Station	Depth (cm)	SOC (%)	SIC (%)	N (%)	SOC:N ratio	pH	$\delta^{13}\text{C}$ (‰)	FWC (%)	Max WHC (%)	BD (g cm^{-3})	Clay (%)	Silt (%)	Sand (%)
Xilinhot (XLHT)	0–20	1.48 \pm 0.02	0.41 \pm 0.01	0.18 \pm 0.00	8.03 \pm 0.18	8.98 \pm 0.03	-24.1	10.65 \pm 0.11	47.12 \pm 0.37	1.06 \pm 0.02	0.4	64.6	35.0
	20–40	1.00 \pm 0.05	0.64 \pm 0.00	0.13 \pm 0.00	7.69 \pm 0.22	9.09 \pm 0.01	-24.1	6.48 \pm 0.24	44.92 \pm 0.25	1.24 \pm 0.05	0.5	58.2	41.3
	40–60	0.67 \pm 0.03	1.05 \pm 0.01	0.09 \pm 0.00	7.09 \pm 0.22	9.09 \pm 0.04	-23.7	5.56 \pm 0.11	39.78 \pm 0.39	1.31 \pm 0.03	0.6	58.5	41.0
Keqi (KQ)	0–20	3.36 \pm 0.05	0.02 \pm 0.00	0.29 \pm 0.00	11.48 \pm 0.24	7.79 \pm 0.10	-26.0	19.59 \pm 0.22	65.57 \pm 0.82	1.14 \pm 0.03	0.4	41.0	58.6
	20–40	2.52 \pm 0.04	0.01 \pm 0.00	0.22 \pm 0.00	11.59 \pm 0.27	7.63 \pm 0.04	-25.9	8.56 \pm 0.05	53.59 \pm 1.98	1.22 \pm 0.01	0.2	55.7	44.1
	40–60	1.65 \pm 0.03	0.02 \pm 0.00	0.14 \pm 0.00	11.49 \pm 0.42	7.57 \pm 0.12	-25.5	8.00 \pm 0.27	42.92 \pm 0.57	1.19 \pm 0.01	0.2	61.6	38.1
Gangcha (GC)	0–20	3.32 \pm 0.23	0.34 \pm 0.04	0.31 \pm 0.03	10.70 \pm 1.28	8.53 \pm 0.07	-26.3	33.24 \pm 0.68	60.79 \pm 0.21	n.d.	1.3	75.9	22.8
	20–40	2.90 \pm 0.18	0.44 \pm 0.10	0.29 \pm 0.01	9.93 \pm 0.69	8.60 \pm 0.03	-24.0	36.15 \pm 0.52	62.03 \pm 0.30	n.d.	0.9	75.8	23.3
	40–60	2.12 \pm 0.22	0.52 \pm 0.06	0.20 \pm 0.02	10.55 \pm 1.50	8.76 \pm 0.10	-25.3	35.79 \pm 0.91	62.85 \pm 0.61	n.d.	0.6	64.0	35.4

SOC: soil organic carbon; SIC: soil inorganic carbon; N: nitrogen; FWC: field water content; Max WHC: maximum water holding capacity; BD: bulk density; Clay: soil particle size $<$ 0.2 μm ; Silt: 0.2 μm $<$ soil particle size $<$ 20 μm ; Sand: soil particle size $>$ 20 μm ; n.d.: not determined.

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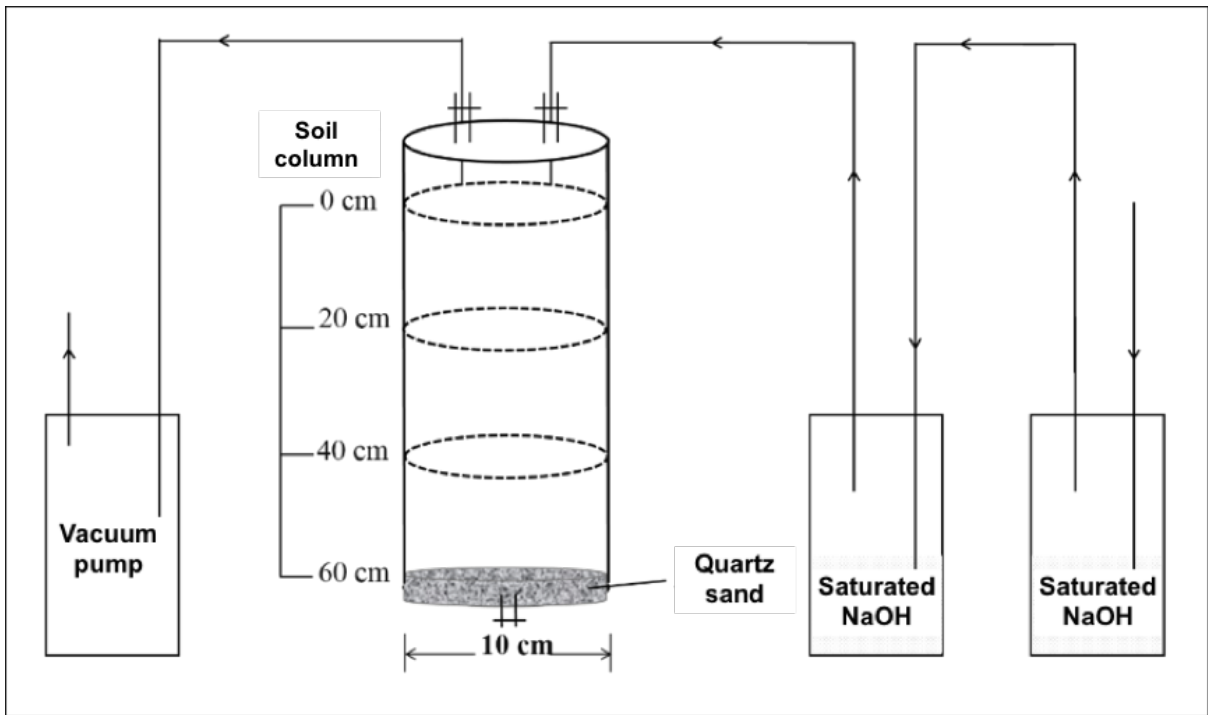


Figure 1: Design of the soil column experiment for monitoring soil respiration and leaching after simulated extreme precipitation events (EPEs).

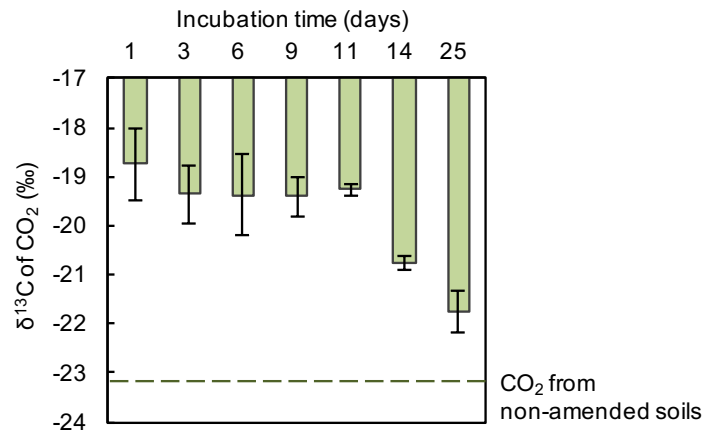
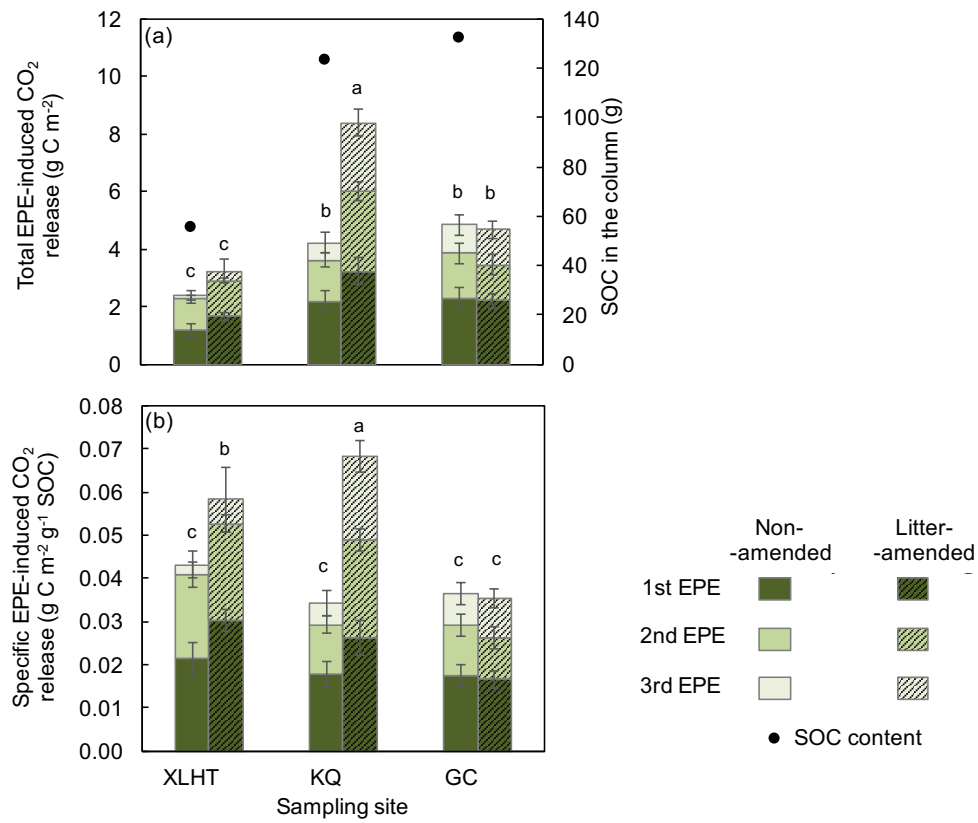


Figure 2: The $\delta^{13}\text{C}$ values of respired CO_2 in the litter-amended Gangcha (GC) soils after the first extreme precipitation event (EPE). Mean values are shown with standard error ($n = 3$).



5 **Figure 3: Total (a) and specific (b) extreme precipitation event (EPE)-induced CO₂ release in the litter-amended and non-amended grassland soils during three EPEs. Mean values are shown with standard deviation (n = 3). Lower-case letters (a, b, c) indicate significantly different levels among the litter-amended and non-amended soils determined by Duncan's multiple range test (one-way ANOVA, $p < 0.05$).**

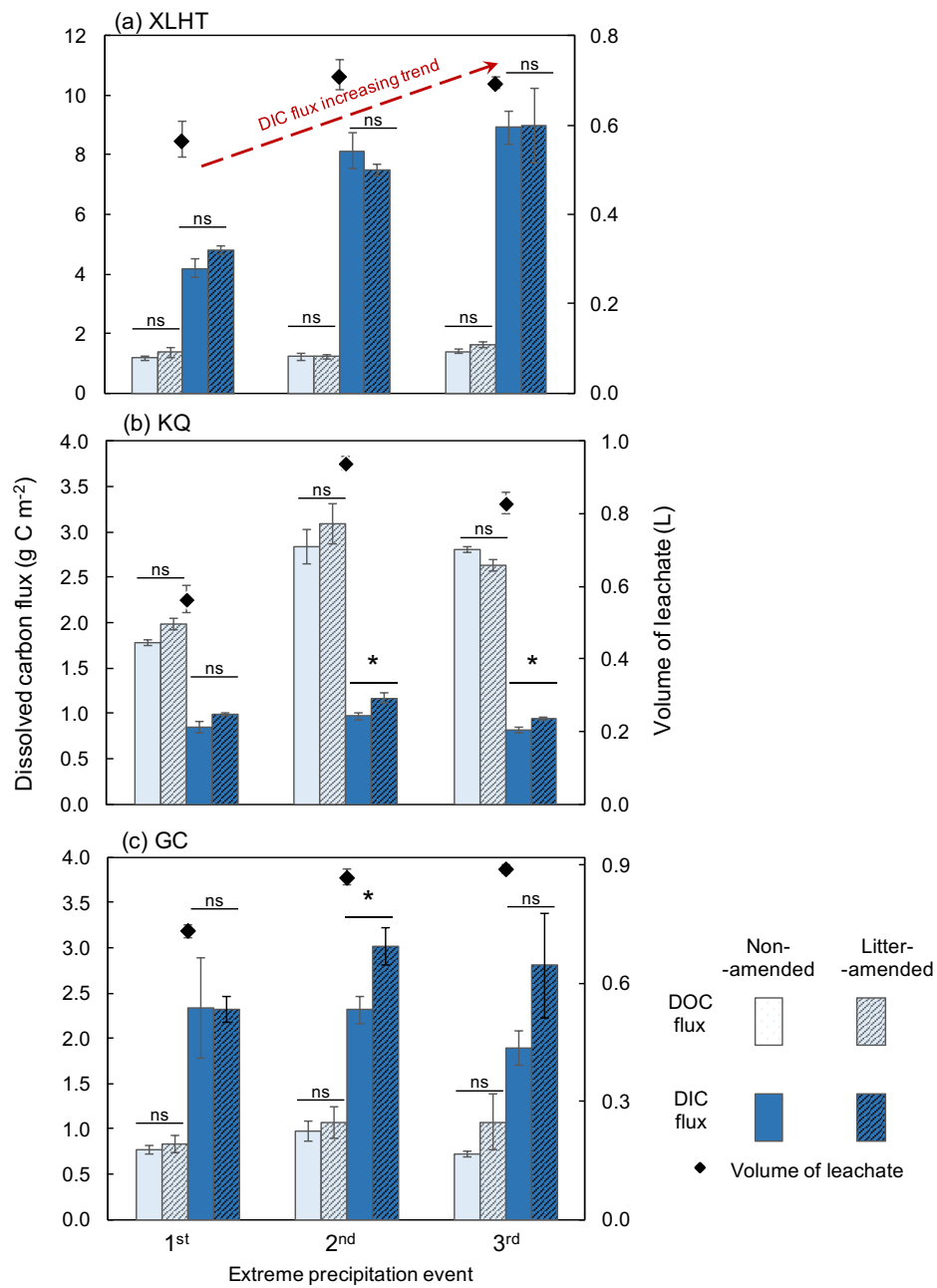


Figure 4: Fluxes of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) and volume of leachates from soil columns after extreme precipitation events (EPEs). Mean values are shown with standard error ($n = 3$). * and ns denote significant and no difference between the litter-amended and non-amended soils determined by independent samples T test, respectively ($p < 0.05$).

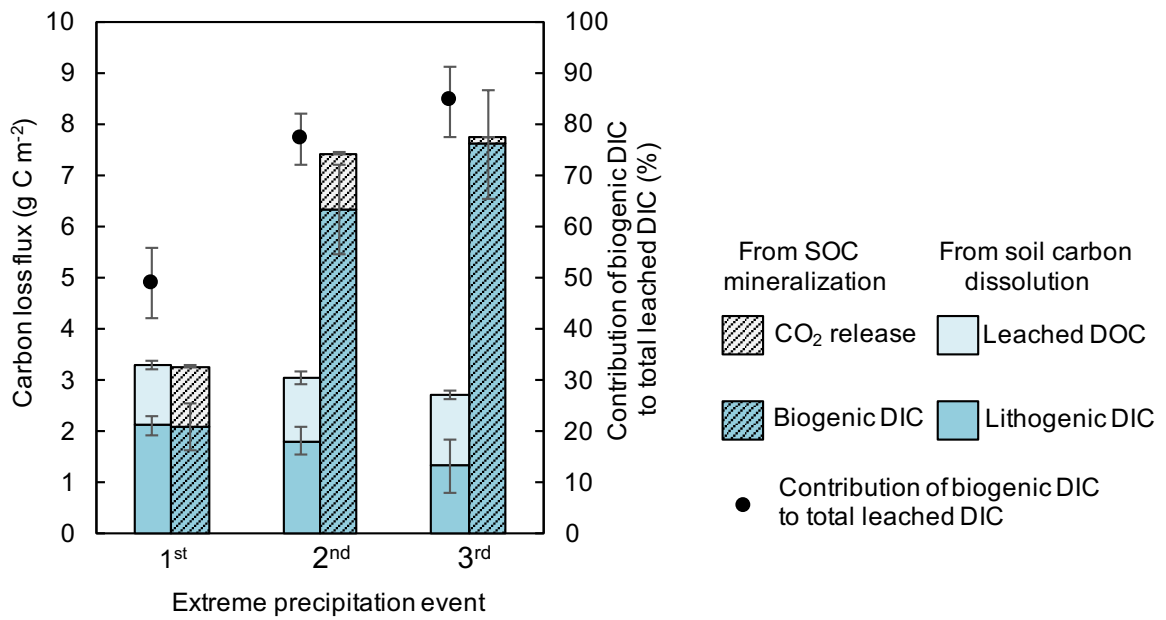


Figure 5: The flux of carbon loss from soil organic carbon (SOC) mineralization including CO₂ release and biogenic dissolved inorganic carbon (DIC), and that from soil carbon dissolution including leached dissolved organic carbon (DOC) and lithogenic DIC in the XLHT soils. Mean values are shown with standard error (n = 3).

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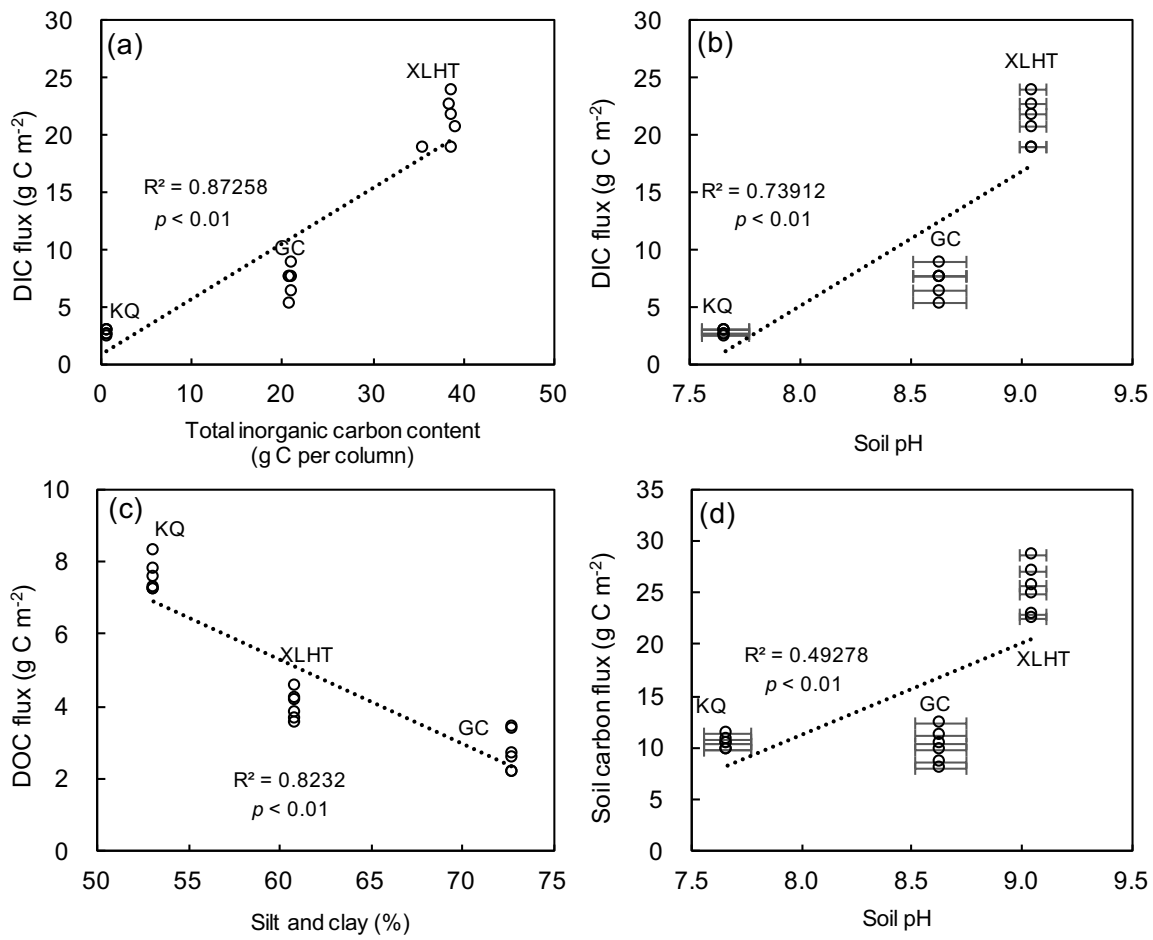


Figure 6: Relationship of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) fluxes with soil properties: (a) DIC flux with total inorganic carbon in the soil columns; (b) DIC flux with soil pH, (c) DOC flux with silt and clay content of soils, (d) total soil carbon flux with soil pH. Mean pH values are shown with standard error ($n = 3$).

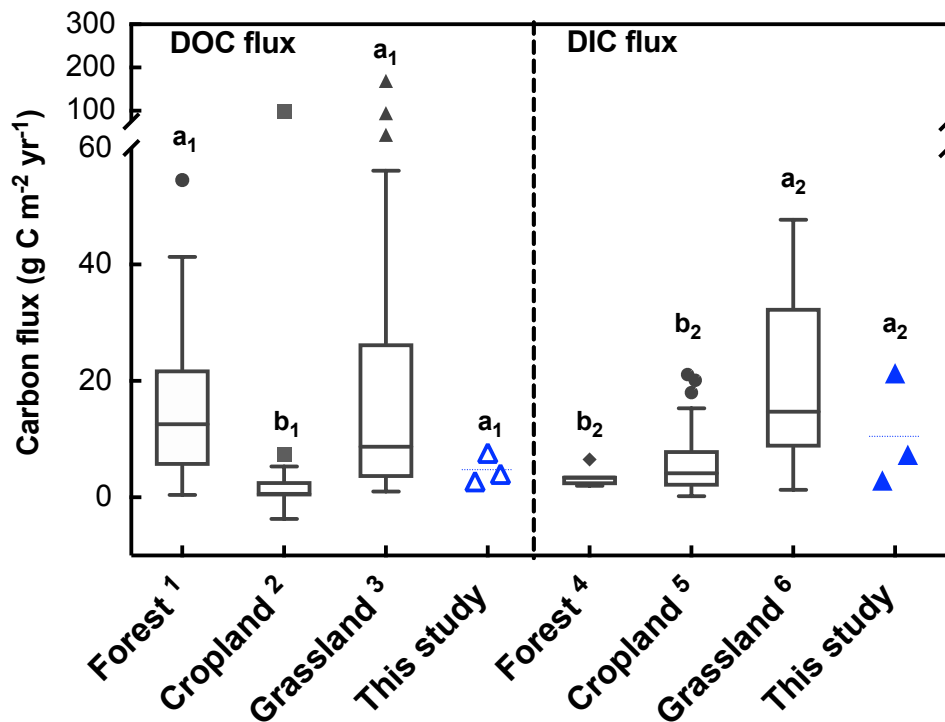


Figure 7: Leaching fluxes of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) in this study compared with that reported in the literature. ¹n = 110, data from Brooks et al., 1999; Froberg et al. 2005, 2006, 2011; Gielen et al., 2011; Kindler et al., 2011; Lu et al., 2013; Michalzik et al., 2000; Sanderman et al., 2008; ²n = 33, data from Brye et al., 2001; Kindler et al., 2011; Siemens et al., 2012; Walmsley et al., 2011; Wang and Alva, 1999; Gerke et al., 2016; Herbrich et al., 2017; Rieckh et al., 2014; Lenz, 2014; ³n = 46, data from Brooks et al., 1999; Brye et al., 2001; Ghani et al., 2010; Kindler et al., 2011; Mctiernan et al., 2001; Parfitt et al., 2009; Sanderman et al., 2008; Tipping et al., 1999; ⁴n = 8, data from Kindler et al., 2011; ⁵n = 32, data from Kindler et al., 2011; Siemens et al., 2012; Walmsley et al., 2011; Wang and Alva, 1999; Gerke et al., 2016; Herbrich et al., 2017; Rieckh et al., 2014; Lenz, 2014; ⁶n = 9, data from Brye et al., 2001; Kindler et al., 2011. Lower-case letters (a₁, b₁) and (a₂, b₂) represent significant different levels of DOC and DIC fluxes in different ecosystems determined by Duncan's multiple range test, respectively, (one-way ANOVA, *p* < 0.05). Dash lines represent mean values for the investigated soils.

Comparing soil carbon loss through respiration and leaching under extreme precipitation events in arid and semi-arid grasslands

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Abstract.

Respiration and leaching are two main processes responsible for soil carbon loss. While the former has received considerable research attention, studies examining leaching processes are limited especially in semiarid grasslands due to low precipitation. Climate change may increase the extreme precipitation event (EPE) frequency in arid and semiarid regions, potentially enhancing soil carbon loss through leaching and respiration. Here we incubated soil columns of three typical grassland soils from Inner Mongolia and Qinghai-Tibetan Plateau and examined the effect of simulated EPEs on soil carbon loss through respiration and leaching. EPEs induced transient increase of CO₂ release through soil respiration, equivalent to 32% and 72% of the net ecosystem productivity (NEP) in the temperate grasslands (Xilinhot and Keqi) and 7% of NEP in the alpine grasslands (Gangcha). By comparison, leaching loss of soil carbon accounted for 290%, 120% and 15% of NEP at the corresponding sites, respectively, with dissolved inorganic carbon (DIC, biogenic DIC + lithogenic DIC) as the main form of carbon loss in the alkaline soils. Moreover, DIC loss increased with re-occurring EPEs in the soil with the highest pH due to elevated contribution of dissolved CO₂ from organic carbon degradation (indicated by DIC- $\delta^{13}\text{C}$). These results highlight that leaching loss of soil carbon (particularly in the form of DIC) is important in the regional carbon budget of arid and semiarid grasslands, and also imply that SOC mineralization in alkaline soils might be under-estimated if only measured as CO₂ emission from soils into the atmosphere. With a projected increase of EPEs under climate change, soil carbon leaching processes and its influencing factors warrant better understanding and should be incorporated into soil carbon models when estimating carbon balance in grassland ecosystems.

1 Introduction

Soils store approximately 2500 Pg of carbon (including organic and inorganic carbon) globally, equivalent to 3.3 and 4.5 times the carbon in the atmosphere (760 Pg) and terrestrial plants (560 Pg), respectively (Lal, 2004). Slight variations of the soil carbon pool will hence severely influence atmospheric CO₂ concentrations and have important implications for climate change (Davidson and Janssens, 2006; Trumbore and Czimczik, 2008). Respiration and leaching are two main processes responsible for soil carbon loss. While respiration has received considerable research attention (Raich and Schlesinger, 1992; Raich and Potter, 1995; Hoover et al., 2016; Burri et al., 2015; Escolar et al., 2015), leaching is relatively poorly constrained despite its importance in certain ecosystems (Cole et al., 2007; Battin et al., 2008; Liu et al., 2017). For instance, soil carbon leached from forests, grasslands, and croplands is estimated to be 15.1, 32.4, and 20.5 g C m⁻² yr⁻¹ across Europe, representing 4%, 14%, and 8% of net ecosystem exchange (NEE), respectively (Kindler et al., 2011). Additionally, leaching of carbon previously preserved in surface litter and soil layers is believed to be a main source of dissolved organic and inorganic matter in inland waters (Spencer et al., 2008). In particular, soil inorganic carbon (SIC) that occurs widely in the arid and semiarid regions is [more prone to leaching than organic carbon during sporadic high precipitation events](#) (Lal and Kimble, 2000). Despite the importance of leaching loss in regional soil carbon budget, very few detailed data exist to investigate and compare the relative contribution of respiration and leaching processes to soil carbon loss.

Climate change is reported to increase the frequency as well as intensity of extreme precipitation events (EPEs; Knapp et al., 2002; Goswami et al., 2006; Parry et al., 2007; Min et al., 2011; Reichstein et al., 2013), especially in arid regions (Donat et al., 2017). In northwestern China, the frequency and intensity of EPEs have showed an increasing trend in the recent 50 years, constituting a much higher proportion of total precipitation than light precipitation events (Liu et al., 2005; Chen et al., 2012; Wang et al., 2012; Fu et al., 2013; Wang et al., 2014). Increasing EPEs will not only enhance soil carbon leaching but also affect soil respiration processes through increasing soluble substrates for microbial decomposition and potentially inducing hypoxic conditions (Knapp et al., 2002; Harper et al., 2005; Morel et al., 2009; Unger et al., 2010). Hence, it is critical to evaluate the effects of EPEs on soil respiration and leaching processes in order to better understand the impact of climate change on terrestrial carbon cycling, especially in the arid and semiarid regions.

Grasslands, containing 20% of global soil carbon pool, are the most widespread ecosystems in arid and semiarid regions globally (Jobbagy and Jackson, 2000). The deposition rate of carbonate is relatively high in the grassland soils with a high alkalinity and aridity (Lal, 2008; Yang et al., 2012), and hence SIC is the major form of soil carbon in many grasslands (Mi et al., 2008). SIC storage in China is approximately 53.3–77.9 Pg (Li et al., 2007; Mi et al., 2008), 54% of which is mainly distributed in the temperate and alpine grasslands located in Inner Mongolia and Qinghai-Tibetan Plateau (Mi et al., 2008). From 1980s to 2000s, SIC in the topsoil of Chinese grasslands was estimated to decrease by 26.8 g C m⁻² yr⁻¹, mainly attributed to soil acidification (Yang et al., 2012). Alternatively, precipitation is one of the main factors influencing the distribution and storage of SIC in arid and semiarid regions (Batjes, 1998; Lal and Kimble, 2000). Mi et al. (2008) found that 84% of SIC in

China was distributed in areas with a mean annual precipitation (MAP) of < 500 mm and that SIC content decreased significantly with the increase of MAP. Given the high leaching potential of SIC in grassland soils under altered precipitation patterns in the future, we hypothesize that EPEs may significantly enhance SIC loss through leaching processes and further reduce SIC storage in grasslands.

5 In this study, soils were collected from varied depths of three typical temperate and alpine grasslands in Inner Mongolia and Qinghai-Tibetan Plateau to construct soil columns for a laboratory incubation study. Using simulated EPEs, we examined soil carbon loss through respiration and leaching processes and compared their fluxes after EPEs. In addition, leaf litter of a C4 grass was added to the surface of one set of soil columns to compare soil carbon loss from bare versus litter-covered soils and to estimate the contribution of litter-derived carbon to soil respiration after EPEs. Our research objectives were: (1) to
10 investigate the influence of EPEs on soil respiration; (2) to quantify the loss of SIC and soil organic carbon (SOC) through leaching during EPEs; and (3) to compare the relative importance of respiration and leaching in EPE-induced soil carbon loss from grassland soils.

2 Materials and Methods

2.1 Study area

15 For the incubation experiment, soils were collected from three different sites of temperate and alpine grasslands of China with varied environmental characteristics. Temperate grasslands were sampled near Xilinhot (XLHT, 116°22' E, 44°8' N, mean elevation of 1170 m) and Keqi (KQ, 117°15' E, 43°18' N, mean elevation of 1250 m) within the arid and semiarid regions of Inner Mongolia (Fig. S1) with MAP of 299 and 402 mm and mean annual temperature (MAT) of 1.2 and 0.4°C, respectively. Soil in this region is mainly chestnut soil, classified as Calcic Chernozems according to the World Reference Base for Soil
20 Resources (Steffens et al., 2008; IUSS working group WRB, 2015), with *Stipa klemenzi*, *Stipa Goboca*, *Stipa breviflora*, and *Stipa glareosa* as the dominating species (Sui and Zhou, 2013). The alpine grassland was sampled in Gangcha (GC, 100°7' E, 37°19' N, mean elevation of 3500 m) located north of the Qinghai Lake on the northeastern edge of the Qinghai-Tibetan Plateau. The GC site has an MAT of 0.4°C, an MAP of 370 mm and a mean annual evaporation (MAE) of 607 mm. Soils at this site are mainly Gelic Cambisol (IUSS working group WRB, 2015), with *Potentilla ansrina Rosaceae*, *Elymus nutans*
25 *Griseb*, and *Deyeuxia arundinacea* as the dominant species.

Soils were collected by digging soil pits of 25 cm × 25 cm × 70 cm from the temperate (XLHT and KQ) and alpine (GC) sites in October, 2014 and August, 2015, respectively. At each site, three plots (200 m × 200 m) were selected (> 200 m in between) with three random soil pits (distance of ~ 5 m in between) sampled within each plot. Soils from the same depth (0–20, 20–40, and 40–60 cm) of the three soil pits were mixed *in situ* for each plot, shipped back to the laboratory immediately, and stored
30 at 4°C before the experiment started within one month. As a result, each sampling site had three “true” replicates from the field for the soil column experiment.

2.2 Soil column experiment and simulated EPEs

For the laboratory experiment, we reconstructed soil columns of similar structures and texture under controlled conditions and used gravity to collect soil leachates. This approach is commonly used in process-related research (Hendry et al., 2001; Thaysen et al., 2014; Ahmad and Walworth, 2009; Aslam et al., 2015) as it minimizes experimental errors and bias caused by unknown factors including soil heterogeneity and microbial community variations. It is also more favourable in terms of quantifying soil carbon leaching loss as it circumvents pore-water contamination by vacuum suction in the field. In particular, leachate sampling by gravity from soil columns prevents alterations to DIC concentrations, which may be caused by CO₂ outgassing using vacuum suction in field studies. Artificial soil columns were constructed in the laboratory with polymethyl methacrylate frames (diameter: 10 cm; height: 70 cm; Fig. 1). The bottom of each column had an aperture (inner diameter: 0.6 cm; height: 3 cm) for the collection of soil leachates, and the column top was fitted with an airtight lid connected to two tubes for gas exchange and collection. Empty columns were soaked in 0.1 mM hydrochloric acid (HCl) solutions for 12 h and rinsed with distilled water before use. Column bottoms were packed with pre-cleaned quartz sand (5-cm thick; soaked in 0.1 mM HCl and combusted at 450°C for 6 h before use) with a layer of nylon net (pore size: 150 µm; diameter: 10 cm) on both sides to prevent the movement of soil particles. Subsequently, soils were passed through 2-mm sieves with roots removed and packed into each column at the corresponding depths (in the sequence of 40–60, 20–40, and 0–20 cm). Soils were compacted gently to maintain a similar bulk density as in the field (Table 1). Water content of each soil layer was separately adjusted to 60% of the maximum water holding capacity (Table 1) to provide an ideal moisture condition for microbial growth (Howard and Howard, 1993; Rey et al., 2005). There was a 10-cm headspace unfilled with soil for each column.

Six soil columns (one litter-amended and one non-amended column for each of the three sampling plots) were set up for each site as described above, and pre-incubated for two weeks in the laboratory to allow the recovery of microbial communities after disturbance. Subsequently, leaf litter of a C4 grass, *Cleistogenes squarrosa*, a dominant species in the grasslands of northern China (Tian et al., 2015), was added to the surface of three columns in an amount equivalent to the aboveground biomass in the field (1.26 g for the XLHT and KQ sites and 1.59 g for the GC site; Bai et al., 2008). The isotopic signal of the leaf litter ($\delta^{13}\text{C}$ of -16.2%) would allow us to estimate the contribution of litter-derived CO₂ to total soil respiration. The columns were pre-incubated again for seven days. Basal respiration rate was measured by collecting CO₂ gas in the column headspace after 4 h of incubation. Temperature was recorded every day during the whole incubation period ($23 \pm 1^\circ\text{C}$).

According to historical precipitation records (Fig. S2), more than 70% of the annual precipitation occurs from June to August in the study area, mainly in the form of 2-4 heavy precipitation events. Therefore, a total of three EPEs were simulated over a period of 2 months for each soil using artificial rainwater prepared according to rainwater's composition at the corresponding sites (pH of 7.3; Table S1; Tang et al., 2014; Zhang et al., 2013). A maximum rainfall intensity of ~100 mm per precipitation event has been recorded in the past two decades in the study area (Fig. S2) and is predicted to increase by 18.1% in the late 21st century in north China (Chen et al., 2012). Hence, approximately 1 L of rainwater (rainfall of ~127 mm), comparable to 30% of the MAP of the investigated sites, was added to the surface of each soil column over 3–4 h at rates of one drop per

second using syringes and allowed to leach through the column to be collected with a clean beaker within 12–14 h. The leachates were weighed, filtered through a 0.45- μm PTFE syringe filter and analyzed for dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) concentrations immediately. To monitor soil respiration every 1–2 days following each EPE, soil columns were first aerated for 1 h using CO_2 -depleted air that had been passed through saturated sodium hydroxide (NaOH) solutions (twice; Fig. 1) and then incubated for 4 h with lids closed. CO_2 gas in the column headspace was collected by gas-tight syringes for the subsequent measurement. After collection of CO_2 gas, the lids were open to allow the exchange with the ambient air. Soil respiration was monitored for 30 days after the first EPE and observed to stabilize approximately on the 20th day (Fig. S3). Hence, the first, second, and third EPEs were conducted on the 1st, 31st, and 51st day of incubation, and the CO_2 measurement was conducted for approximately 30, 20, and 20 days after the first, second, and third EPEs, respectively.

10 Basal respiration was considered to be represented by the stabilized respiration rate at the end of each EPE cycle.

2.3 Sample analyses

Soil pH was measured at a soil:water ratio of 1:2.5 (w:v) using a pH meter (Sartorius PB–10). Soil texture was examined by laser diffraction using Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK) after removal of organic matter and calcium carbonates. Soil field water content was determined by difference between moist and dried soils (dried at 105°C for 8 h).

15 Maximum water holding capacity was estimated by weighing soils before and after removal of redundant water from fully soaked soils (in water for 8 h). For SOC analysis, dried soils were decarbonated by exposure to concentrated HCl vapor for 72 h, followed by saturated NaOH solutions for 48 h to neutralize extra HCl, and then dried at 45°C. Total soil carbon, SOC (after decarbonation) and nitrogen (N) contents were measured by combustion using an elemental analyser (Vario EL III, Elementar, Hanau, Germany). SIC was calculated as the difference between total carbon and SOC contents. Small aliquots of the soil

20 leachates were analyzed immediately on a Multi N/C 3100-TOC/TN Analyzer (Analytik Jena, Germany) for DIC and DOC concentrations (with the latter acidified to $\text{pH} < 2$ with concentrated HCl before analysis). **It should be mentioned that the DIC concentration may vary due to exchanges between dissolved and atmospheric CO_2 during leachate collection. However, potential contribution from this process was $< 7\%$ owing to the low proportions of dissolved CO_2 in total DIC of our samples (Table S2) as calculated using formula in Ran et al. (2015).** CO_2 concentration in the soil column headspace was determined

25 by gas chromatograph (Agilent 7890A, USA) coupled with a flame ionization detector (FID).

To examine the contribution of SOC- and litter-derived carbon to soil respiration, the $\delta^{13}\text{C}$ values of SOC and CO_2 gas were determined on an isotope ratio mass spectrometer (Delta plus xp, Thermo, Germany) with a precision of $\pm 0.2\%$. To estimate the contribution of SOC degradation to leached DIC, the $\delta^{13}\text{C}$ values of DIC were determined on a Picarro isotopic CO_2 analyzer equipped with an automated DIC sample preparation system (AutoMate) based on wavelength scanned cavity ring

30 down spectroscopy technique (Picarro AM-CRDS, USA). The precision for the DIC- $\delta^{13}\text{C}$ measurement was $\pm 0.3\%$. Due to budget constraints and logistic reasons, we only measured the $\delta^{13}\text{C}$ of the respired CO_2 in the GC soils during the first EPE and the leached DIC in the XLHT soils.

2.4 Data analysis and statistics

The relative contribution of litter- and SOC-derived CO₂ to total respired CO₂ in the litter-amended soils was estimated using the following mass balance model:

$$f_{\text{litter-derived}} + f_{\text{SOC-derived}} = 1 \quad (1)$$

$$f_{\text{litter-derived}} \times \delta^{13}\text{C}_{\text{litter-derived}} + f_{\text{SOC-derived}} \times \delta^{13}\text{C}_{\text{SOC-derived}} = \delta^{13}\text{C}_{\text{respired-CO}_2} \quad (2)$$

where $f_{\text{litter-derived}}$ and $f_{\text{SOC-derived}}$ are the proportion of litter- and SOC-derived CO₂ in the total respired CO₂; $\delta^{13}\text{C}_{\text{litter-derived}}$ is the $\delta^{13}\text{C}$ value of litter-derived CO₂, equivalent to -16.25‰; $\delta^{13}\text{C}_{\text{SOC-derived}}$ is the $\delta^{13}\text{C}$ value of SOC-derived CO₂, which assumes the same value as that in the non-amended soils at the beginning of incubation (-23.1‰) according to Cerling et al. (1991); $\delta^{13}\text{C}_{\text{respired-CO}_2}$ is the measured $\delta^{13}\text{C}$ of respired CO₂.

Similarly, the relative contribution of lithogenic carbonate and biogenic DIC derived from SOC degradation to leached DIC was assessed according to the following isotopic mass balance model:

$$f_{\text{carbonate}} + f_{\text{biogenic-DIC}} = 1 \quad (3)$$

$$f_{\text{carbonate}} \times \delta^{13}\text{C}_{\text{carbonate}} + f_{\text{biogenic-DIC}} \times \delta^{13}\text{C}_{\text{biogenic-DIC}} = \delta^{13}\text{C}_{\text{DIC}} \quad (4)$$

where $f_{\text{carbonate}}$ and $f_{\text{biogenic-DIC}}$ are proportion of carbonate- and biogenic DIC in total DIC; $\delta^{13}\text{C}_{\text{carbonate}}$ is the $\delta^{13}\text{C}$ value of soil carbonate, equivalent to 0‰ (Edwards and Saltzman, 2016); and $\delta^{13}\text{C}_{\text{biogenic-DIC}}$ is the $\delta^{13}\text{C}$ value of biogenic carbonate/bicarbonate derived from the dissolution of CO₂ produced by SOC degradation, which is estimated to shift by approximately 8‰ compared with the $\delta^{13}\text{C}$ value of soil-respired CO₂ (-24‰ here) due to isotope fractionation during CO₂ dissolution (Zhang et al., 1995). Hence, $\delta^{13}\text{C}_{\text{biogenic-DIC}}$ is estimated to be -16‰. $\delta^{13}\text{C}_{\text{DIC}}$ is the measured $\delta^{13}\text{C}$ signature of leached DIC. [Isotopic fractionation of leached DIC due to CO₂ loss in an open system is insignificant when the partial pressure of CO₂ \(\$p\text{CO}_2\$ \) in the solution is lower than twice that of the surrounding atmosphere \(Hendy, 1971; and Doctor et al., 2008\). In the present study, \$p\text{CO}_2\$ in the XLHT leachates was low \(~ 400 \$\mu\text{atm}\$ assuming alkalinity equals to DIC concentration; Table S2\) due to its high pH, low soil respiration and dilution of dissolved CO₂ under EPE, the influence of CO₂ outgassing on the \$\delta^{13}\text{C}\$ of leached DIC was thus considered not important.](#)

EPE-induced CO₂ release via respiration was assessed following two steps. First, cumulative respiration during the first 20 days after each EPE (until respiration rate stabilized) was calculated. Second, difference between the measured cumulative respiration and that estimated using the stabilized basal respiration rate after each EPE was calculated as the EPE-induced CO₂ release.

Independent samples T test (group size = 2) and One-way ANOVA analysis (group size > 2) was used to compare the dissolved carbon concentrations and fluxes among different columns. Linear regression analysis was used to assess correlations between leachate carbon flux and influencing factors (carbon content, soil pH, soil texture, etc.). All these analyses were performed using IBM SPSS Statistics 22. Differences and correlations are considered to be significant at a level of $p < 0.05$.

3 Results and Discussion

3.1 Bulk properties of grassland soil samples

In the investigated grassland soils, SOC represented 59–99% of soil carbon and exhibited $\delta^{13}\text{C}$ values typical of C3 plant inputs (ranging from -24.1‰ to -26.3‰ ; Table 1). The XLHT soil had a much lower SOC and nitrogen (N) contents than the KQ and GC soils despite a similar soil texture ($p < 0.05$; Table 1). The SOC:N ratio was also lowest in XLHT (7.09–8.03),
5 indicating a more decomposed state of soil organic matter (Weiss et al., 2016). Conversely, **the SIC content was highest in XLHT and lowest in KQ, in line with soil pH variations at these sites**, i.e., lowest pH in KQ and highest in XLHT. **This correlation of SIC with soil pH is consistent with the results of Shi et al. (2012)**, showing that pH is the most important factor controlling SIC variation across the Mongolian and Tibetan grasslands. In terms of depth variations, soils became coarser with depth in XLHT and GC, **but became finer with increasing depth in KQ**. The SOC and N contents decreased with depth in all
10 soils due to declining plant inputs ($p < 0.05$; Table 1), while the SOC:N ratio remained relatively similar (except a small decrease with depth in XLHT). By contrast, XLHT and GC soils showed an increasing SIC content with depth ($p < 0.05$; Table 1), because SIC, with a good solubility, is prone to leaching from the topsoil **and subsequently precipitates in the deeper soil** (Mi et al., 2008; Tan et al., 2014). The KQ soil, showing an almost neutral pH, had an invariant SIC content and pH with depths. Overall, the varied properties (including SOC, SIC, pH, etc.) of these soils allowed us to compare the effects of EPEs
15 on soil respiration and leaching processes in different grassland soils.

3.2 EPE-induced changes to soil respiration

Shortly after each simulated EPE, soil respiration was similar to or lower than basal respiration (Fig. S3). The latter case may be attributed to hypoxic conditions induced by water saturation during EPEs (Hartnett and Devol, 2003; Jessen et al., 2017). Subsequently, soil respiration increased and peaked after approximately one week due to the recovery of microbial activity
20 with improved soil aeration (Borken and Matzner, 2009). It then decreased to a constant level approximately 20 days after each EPE (Fig. S3). The transient increase of respiration was consistent with the “Birch Effect” proposed by (Birch, 1964), i.e., a pulse of soil respiration after rewetting events due to resuscitation of microorganisms and improved diffusive transport of substrate and extracellular enzymes (Borken and Matzner, 2009; Navarro-García et al., 2012; Placella et al., 2012). The maximum soil respiration rates were 40.6 and 37.3 $\text{mg C m}^{-2}\text{h}^{-1}$ after EPEs in the non-amended KQ and GC soils, respectively.
25 These rates were significantly higher than that in the XLHT soil ($13.7 \text{ mg C m}^{-2}\text{h}^{-1}$), likely related to the higher SOC content in the former soils. **The maximum specific soil respiration rates normalized to SOC were 2.2, 2.6, and 2.0 $\mu\text{g C g}^{-1}\text{SOC h}^{-1}$ in the non-amended GC, KQ, and XLHT soils, respectively. Therefore, SOC degradability was quite similar in the alpine and temperate grassland soils.**

Total respired CO_2 was higher in the litter-amended than non-amended soils before and after EPEs (Fig. S5). The cumulative respired CO_2 in the litter-amended XLHT, KQ, and GC soils were 16.7, 54.8, and 44.6 g C m^{-2} during three EPEs, 20%, 22%, and 15% higher than that of the non-amended soils, respectively. Due to the wide presence of litter coverage in our studied soils, litter effect on soil respiration should be considered when estimating carbon budgets for these grassland soils. The higher

total respired CO₂ in litter-amended soils might be caused by one or two following reasons: (1) the degradation of labile components in the fresh litter; (2) induced priming effects due to the addition of an easily available energy source (Fröberg et al., 2005; Ahmad et al., 2013). To distinguish the influences of above two reasons on total respired CO₂ and further differentiate the contribution of litter (C4) and SOC (C3) to the respired CO₂, we examined the δ¹³C values of CO₂ evolved from the GC soils after the first EPE. On the first day after EPE, CO₂ from the non-amended and litter-amended GC soils had a δ¹³C value of -23.1‰ and -18.7‰, respectively. The latter was close to the δ¹³C signature of the added litter (-16.25‰). Using the two-endmember mixing model of Eq. (1) and (2), we calculated that litter contributed ~64% of the respired CO₂ in the litter-amended GC soils. However, along with the consumption of labile carbon in litter, the δ¹³C signature of CO₂ decreased from -18.7‰ on Day 1 to -21.8‰ on Day 25 after EPE in the litter-amended soils (Fig. 2). Accordingly, the proportion of litter-derived CO₂ decreased from 64% to 20%. The litter-derived CO₂ flux in litter-amended GC soils was estimated to range from 7.0 to 17.5 mg C m⁻² h⁻¹, while the SOC-derived CO₂ flux increased from 6.2 to 15.7 mg C m⁻² h⁻¹ after the first EPE (Fig. S3). Compared with the SOC-derived CO₂ flux in non-amended GC soils (ranging from 17.2 to 27.1 mg C m⁻² h⁻¹), litter addition had a negative priming effect on the degradation of native SOC while increasing total respiration through labile litter degradation. Similarly, the specific CO₂ flux derived from SOC was lower in the litter-amended GC soils (0.4–0.9 μg C h⁻¹ g⁻¹ SOC) than in the non-amended GC soils (1.0–1.6 μg C h⁻¹ g⁻¹ SOC), further proving the negative priming effect.

Using data shown in Fig. S3 and S5, we calculated that total EPE-induced CO₂ release during three EPEs was higher in the KQ and GC soils than in the XLHT soil ($p < 0.05$; Fig. 3a) with a lower SOC content and a lower SOC:N ratio (Table 1). However, the specific EPE-induced CO₂ release normalized to SOC content showed no significant difference in the non-amended soils among three sites (Fig. 3b), indicating that a similar proportion of SOC (~4%) was subject to EPE-induced CO₂ release in the alpine and temperate grassland soils (Fig. 3b). The total EPE-induced CO₂ release was significantly higher in the litter-amended KQ soils than the non-amended ones. Besides the availability of labile OC provided by litter, the higher total EPE-induced CO₂ in litter-amended KQ soils might be related to its relatively lower soil pH (~7.7) that facilitates the release rather than the dissolution of respired CO₂ (from both SOC and litter mineralization) in soil solution. In addition, KQ has the highest mean sand content (46.9%) among the three soils (Table 1), i.e., the least possible mineral protection on labile OC dissolved from the litter, and this benefits the transport and mineralization of labile OC which meanwhile might play positive priming effects on the SOC mineralization. We therefore conclude that the KQ soil, with a coarser texture and a lower pH (Table 1), may have provided less sorptive protection for the labile DOC components after EPEs (Kell et al., 1994; Nelson et al., 1994) and allowed less dissolution of the respired CO₂, and hence showed a more responsive respiration to the precipitation events. Consequently, we deduced that the availability of labile organic carbon, soil texture and pH are important factors influencing the total EPE-induced CO₂ release in temperate and alpine grassland soils.

3.3 EPE-induced leaching of soil carbon

During the first EPE, a total of 0.57, 0.56 and 0.73 L of leachates were collected from the XLHT, KQ, and GC soils, respectively. The leachate increased to 0.71, 0.94 and 0.87 L during the second EPE and was 0.69, 0.83 and 0.89 L during the

third EPE, respectively (Fig. 4). Soil water content was set to be ~60% of max WHC before the first EPE, and leaching did not occur until soil water reached saturation. Therefore, the leachate volume was lowest during the first EPE and similar for the second and third EPEs. There were some variations in the volume of leachates from different soils, possibly related to preferential flows created during EPEs in the soil columns (McGrath et al., 2009) and water evaporation between EPEs. DIC was the main form of carbon in the leachates from the alkaline soils with a high SIC content (XLHT and GC) but low from the KQ soil with a neutral pH and low SIC content (Fig. 4). The resulting DIC flux was much higher for the XLHT soils (~21.3 g C m⁻²) than the other two (2.9 g C m⁻² for KQ and 7.4 g C m⁻² for GC soils) during three EPEs, equivalent to five times of its DOC flux (3.8–4.2 g C m⁻², Fig. 4). In contrast, DIC flux in the KQ soils was only one third of its DOC flux during EPEs. The form of leached carbon was mainly linked to the amount of SOC and SIC in the columns (shown in Fig. S4).

Litter amendment did not increase DOC fluxes in any of the investigated soils but increased DIC fluxes leached from the KQ soil during the second and third EPEs and from the GC soil during the second EPE ($p < 0.05$, Fig. 4b-c). We postulate that, while litter contribution to DOC was minor, CO₂ derived from litter degradation contributed to dissolved CO₂ in soils and hence increased DIC in the leachates (Monger et al., 2015). This effect was not evident during the first EPE when litter decomposition just started and was not significant for the third EPE in the GC soil due to a high sample variability associated with the litter-amended soil (Fig. 4c). Due to the high SIC content in the XLHT soils (38.15 g per column) and the low litter-OC amendment (0.7 g per column), there was no significant difference of DIC fluxes between the non-amended and litter-amended XLHT soils (Fig. 4a). However, for the KQ soil having a relatively low SIC content similar to the added litter-OC (0.7 g per column; Table 1), litter amendment had a significant effect on the DIC flux ($p < 0.05$), increasing by $21 \pm 13\%$ and $15 \pm 7\%$ relative to the non-amended KQ soils during the second and third EPEs, respectively. There was also a $30 \pm 19\%$ increase in the DIC flux from the litter-amended GC soils relative to its non-amended counterpart during the second EPE. Therefore, litter amendment had a significant influence on DIC fluxes from soils with a relatively low SIC content (KQ and GC) under EPEs compared with the high-SIC XLHT soil.

Between different EPEs, leachate DOC fluxes did not vary in any of the investigated soils. By comparison, DIC fluxes increased in the XLHT soil from 4.5 g C m⁻² after the first EPE to 9.0 g C m⁻² after the third EPE ($p < 0.01$, Fig. 4). This increase may be caused by (i) an increased contribution of SOC degradation to soil DIC and/or (ii) an elevated dissolution of soil carbonates induced by higher soil CO₂ concentrations with repeated EPEs (Gulley et al., 2014; Ren et al., 2015). To evaluate these contributions, the $\delta^{13}\text{C}$ values of DIC were measured for the non-amended XLHT soil. The $\delta^{13}\text{C}$ of leached DIC ranged from -10.0‰ to -6.6‰ during the first EPE. Based on the isotopic mass balance of Eq. (3) and (4), lithogenic carbonate (with a $\delta^{13}\text{C}$ value of 0‰) contributed 51% to the leached DIC while biogenic DIC produced by SOC degradation contributed 48% (Fig. 5). The $\delta^{13}\text{C}$ value of leached DIC decreased to -12.3‰ and -13.5‰ during the second and third EPEs, corresponding to a contribution of 77% and 84% by biogenic sources in the total DIC, respectively (Fig. 5). These results confirm our previous hypothesis that SOC decomposition contributed significantly to soil DIC fluxes. Combined with the total flux rate, we calculated that both lithogenic and biogenic DIC fluxes were ~2.1 g C m⁻² in the first EPE. Subsequently, lithogenic DIC flux decreased to ~1.3 g C m⁻² while biogenic DIC flux increased to 7.6 g C m⁻² in the third EPE. This

demonstrates that the increased DIC flux with repeated EPEs was mainly derived from increased contribution of SOC mineralization. Interestingly, increasing DIC fluxes with repeated EPEs were not observed in the KQ and GC soils (Fig. 4) despite their higher SOC contents (Table 1) and CO₂ release rates (Fig. S3). Given that the XLHT soil had the highest soil pH, the high alkalinity may have favored the retention of respired CO₂ in the soil solution compared with the other soils (Parsons et al., 2004; Yates et al., 2013; Liu et al., 2015), leading to its high contribution to DIC fluxes.

Regardless of its source, the EPE-induced leaching loss of inorganic carbon was 31.5 and 10.6 μg DIC g⁻¹ soil from the alkaline XLHT and GC soils, respectively, approximately three and five times higher than the corresponding DOC leaching loss (5.9 and 3.9 μg DOC g⁻¹ soil, respectively). However, the KQ soil had a relatively lower EPE-induced DIC loss (4.4 μg DIC g⁻¹ soil) than the DOC leaching loss (11.6 μg DOC g⁻¹ soil) mainly due to its lower initial SIC content and relatively neutral soil pH value. Hence, total DIC (biogenic DIC + lithogenic DIC) was the main form of soil carbon loss in alkaline soils during EPEs. When the source of the leached DIC is taken into account, dissolution of CO₂ produced by SOC mineralization (biogenic DIC) constituted more than half of the leached DIC (at least from the XLHT soils; Fig. 5), whose contribution increased with re-occurring EPEs (Fig. 5). This implies that SOC mineralization during the three EPEs was underestimated by approximately a factor of 8 when measured as CO₂ gas flux from soil into the column headspace only (Fig. 5). In addition, DIC loss exclusively resulting from SIC dissolution or weathering was also a significant fraction of soil carbon loss, equivalent to 219% SOC loss in the form of EPE-induced CO₂ during EPEs (Fig. 5). These results collectively corroborate that inorganic carbon loss is the main form of soil carbon loss in alkaline soils during EPEs.

As for the influencing factors on soil carbon leaching loss, the DIC flux was positively correlated to the amount of SIC in the soil columns and soil pH ($p < 0.05$; Fig. 6a-b). These two relationships may be self-correlated due to a positive relationship between soil pH and SIC (Liu et al., 2016). By comparison, DOC flux was linked with the amount of SOC in the soil columns, but decreased with an increasing content of silt and clay ($p < 0.05$; Fig. 6c). This may be explained by the stronger retention of SOC on small-sized particles with more sorption sites (Barré et al., 2014; Mayer, 1994). Interestingly, neither DOC nor DIC fluxes showed any significant relationships with the volume of leachates during EPEs (Figs. 6e-f). This indicates that we used sufficient amount of precipitation in this study to “scavenge” dissolved carbon from soils and hence these fluxes represent soil carbon’s leaching potential under EPEs. Overall, total soil carbon loss through leaching under EPEs was positively related to soil pH values ($p < 0.05$; Fig. 6d), suggesting that soil pH is a critical factor determining the magnitude of soil carbon loss under EPEs.

3.4 Main pathways of grassland soil carbon loss under EPEs

In this study, EPE-induced soil carbon loss was composed of three parts: leachate DIC including lithogenic and biogenic DIC, leached DOC and EPE-induced CO₂ emission into the column headspace. Total DIC and DOC fluxes accounted for 90%, 62%, and 68% of EPE-induced total loss at XLHT, KQ, and GC, respectively, representing the major pathway of soil carbon loss in these grassland soils under EPEs. Soil carbon leaching fluxes were 25.3, 10.4, and 10.1 g C m⁻² yr⁻¹ in XLHT, KQ, and GC soils during three EPEs, respectively, with DIC as the dominant form in XLHT and GC soils. While DIC fluxes found for the

KQ and GC soils generally fell within the range reported for grassland soils ($1.3\text{--}47.8\text{ g C m}^{-2}\text{ yr}^{-1}$; Parfitt et al., 1997; Brye et al., 2001; Kindler et al., 2011), the XLHT soil had a DIC flux higher than the majority ($> 50\%$) of the reported values (Fig. 7). This may be attributed to the higher SIC content and stronger dissolution of respired CO_2 in the XLHT soils due to its higher soil pH (9.1 ± 0.1) relative to other grassland soils (pH: $5.4\text{--}7.5$; Kindler et al., 2011), and the high intensity of our simulated EPEs (precipitation: 40 mm h^{-1}). Nonetheless, DIC fluxes in grassland soils reported in this study and elsewhere (Brye et al., 2001; Kindler et al., 2011) were significantly higher than in forest and cropland ecosystems ($p < 0.05$; Rieckh et al., 2014; Lentz and Lehrs, 2014; Gerke et al., 2016; Herbrich et al., 2017; Siemens et al., 2012; Walmsley et al., 2011; Wang and Alva, 1999; Kindler et al., 2011), highlighting the importance of leaching as a major pathway of soil carbon loss in grasslands. By contrast, DOC fluxes in this study ($4.8 \pm 2.5\text{ g C m}^{-2}$) were lower than most of the reported values in forest and grassland ecosystems due to the low SOC contents in our soils (Fig. 7).

Net ecosystem production (NEP) in the temperate steppe of Inner Mongolia (XLHT and KQ) is $8.7\text{ g C m}^{-2}\text{ yr}^{-1}$ (Sui and Zhou, 2013). While the EPE-induced CO_2 release (2.8 ± 0.6 and $6.3 \pm 3.0\text{ g C m}^{-2}$) accounted for 32% and 72% of the NEP at XLHT and KQ, respectively; soil carbon leached during three EPEs was equivalent to 290% and 120% of NEP, with total DIC loss accounting for 244% and 33%, respectively. It is worth mentioning that biogenic DIC loss ($16.0 \pm 3.4\text{ g C m}^{-2}$) caused by SOC degradation accounted for 184% of NEP at XLHT, indicating the importance of biogenic DIC to leached inorganic carbon loss during EPEs. By comparison, NEP in the studied alpine grassland ($68.5\text{ g C m}^{-2}\text{ yr}^{-1}$; Fu et al., 2009) is much higher than in typical temperate steppe. Hence, soil carbon loss through leaching and respired CO_2 release accounted for 15% (DIC: 11%, DOC: 4%) and 7% of the NEP at GC, respectively. Nonetheless, the EPE-induced soil carbon loss relative to NEP was higher in this study than that estimated for grassland topsoil across Europe (12% for DIC loss, 2% for DOC loss; Kindler et al., 2011) where Net Ecosystem Exchange (NEE) reported by Kindler et al. was used as NEP according to the report of Kirschbaum et al. (2001). This was partially attributed to the lower NEP and higher SIC content in XLHT and KQ soils, underscoring that soil carbon leaching is more important in fragile ecosystems with low productivity.

An uncertainty related to the importance of leaching processes in the overall carbon budget along the “soil-river-ocean” continuum lies in the ultimate downstream fate of the leached carbon. If part of this carbon is retained in the surrounding soils or carried along from the river to the ocean in the form of DIC without outgassing into the air, it will not constitute a source of atmospheric CO_2 on a relatively short term (over years or decades). However, soil columns used in our study has a depth (60 cm) typical of or even deeper than the average soil depth in the alpine grasslands of Qinghai-Tibetan Plateau (Wang et al., 2001). Hence, we assume that carbon leached in our experiments will have minimum retention in the soil. Furthermore, compared to DOC and DIC in the soil solution, the leached carbon is more likely to be subject to more intensified mineralization and outgassing during the land-ocean transfer, given more intensified mixing processes, oxygen exposure and photo-oxidation of terrestrial carbon upon releasing into the river (Hedges et al., 1997; Battin et al., 2009). Hence, we postulate that carbon leached from soils is more vulnerable to decomposition and/or release compared to that retained in the soil. That being said, it will be necessary to confirm our results and hypothesis using field-based leaching experiments to better understand the ultimate fate of leached soil carbon: whether it will be retained in the deeper soil or show a higher degradability

upon leaving the soil matrix. Such information will be complementary to our study and further elucidate the importance of leaching processes in terms of ecosystem carbon budget.

In summary, this study quantified and compared soil carbon loss through respired CO₂ release and leaching in three typical grassland soils of northern China under simulated EPEs. Soil CO₂ release was stimulated shortly after each EPE, leading to an EPE-induced CO₂ release equivalent to 32% and 72% of the NEP at XLHT and KQ (temperate grasslands) and 7% at GC (alpine grassland). By comparison, **total soil carbon leaching fluxes** accounted for 290%, 120% and 15% of the NEP at XLHT, KQ, and GC, respectively, with DIC as the main form of carbon loss in the SIC-enriched XLHT and GC soils. In view of DIC sources, biogenic DIC loss derived from SOC mineralization contributed to more than half of the total leached DIC fluxes and accounted for 184% of the NEP at XLHT. Moreover, DIC loss increased with re-occurring EPEs in the XLHT soil with the highest pH due to increased dissolution of soil carbonates as well as elevated contribution of dissolved CO₂ from SOC degradation. **These results also imply that SOC mineralization in alkaline grassland soils during EPEs might be underestimated, if measured only as CO₂ emission from soil into the atmosphere.** Admittedly, our results are based on artificial soil columns which destroyed natural soil structures, hence potentially increasing the contact between pore water and soil particles through changing soil porosity. **Also, soil water content was set to be ~60% of max WHC initially in our experiment, higher than that in the field of temperate grasslands (XLHT and KQ). Thus, our measured DOC and DIC fluxes are likely to be higher than carbon leaching in the field due to greater water retention in drier soils. Hence, our estimate may represent an upper limit of soil carbon leaching potential under EPEs.** Hence, our estimate may represent an upper limit of soil carbon leaching potential under EPEs. Nonetheless, these results highlight that leaching loss of soil carbon, especially in the form of DIC originated from biogenic and lithogenic carbonates, plays an important role in the regional carbon budget of grasslands located in arid and semiarid regions. Further research effort is needed to combine short-term laboratory experiments with long-term field measurements to fully assess the impacts of EPEs on soil carbon budget in these areas. In addition, with a projected increase of EPEs under climate change, soil carbon leaching processes and its influencing factors warrant better understanding and should be incorporated into soil carbon models when estimating carbon balance in grassland ecosystems.

Data availability. All data is available within this paper (Table 1) and in the Supplement (Dataset S1).

25 *Competing interests.* The authors declare that they have no conflict of interest.

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Table 1: Bulk properties of soil samples collected from the temperate and alpine grasslands for the soil column experiment**(mean \pm standard error; n = 3).**

Station	Depth (cm)	SOC (%)	SIC (%)	N (%)	SOC:N ratio	pH	$\delta^{13}\text{C}$ (‰)	FWC (%)	Max WHC (%)	BD (g cm^{-3})	Clay (%)	Silt (%)	Sand (%)
Xilinhot (XLHT)	0–20	1.48 \pm 0.02	0.41 \pm 0.01	0.18 \pm 0.00	8.03 \pm 0.18	8.98 \pm 0.03	–24.1	10.65 \pm 0.11	47.12 \pm 0.37	1.06 \pm 0.02	0.4	64.6	35.0
	20–40	1.00 \pm 0.05	0.64 \pm 0.00	0.13 \pm 0.00	7.69 \pm 0.22	9.09 \pm 0.01	–24.1	6.48 \pm 0.24	44.92 \pm 0.25	1.24 \pm 0.05	0.5	58.2	41.3
	40–60	0.67 \pm 0.03	1.05 \pm 0.01	0.09 \pm 0.00	7.09 \pm 0.22	9.09 \pm 0.04	–23.7	5.56 \pm 0.11	39.78 \pm 0.39	1.31 \pm 0.03	0.6	58.5	41.0
Keqi (KQ)	0–20	3.36 \pm 0.05	0.02 \pm 0.00	0.29 \pm 0.00	11.48 \pm 0.24	7.79 \pm 0.10	–26.0	19.59 \pm 0.22	65.57 \pm 0.82	1.14 \pm 0.03	0.4	41.0	58.6
	20–40	2.52 \pm 0.04	0.01 \pm 0.00	0.22 \pm 0.00	11.59 \pm 0.27	7.63 \pm 0.04	–25.9	8.56 \pm 0.05	53.59 \pm 1.98	1.22 \pm 0.01	0.2	55.7	44.1
	40–60	1.65 \pm 0.03	0.02 \pm 0.00	0.14 \pm 0.00	11.49 \pm 0.42	7.57 \pm 0.12	–25.5	8.00 \pm 0.27	42.92 \pm 0.57	1.19 \pm 0.01	0.2	61.6	38.1
Gangcha (GC)	0–20	3.32 \pm 0.23	0.34 \pm 0.04	0.31 \pm 0.03	10.70 \pm 1.28	8.53 \pm 0.07	–26.3	33.24 \pm 0.68	60.79 \pm 0.21	n.d.	1.3	75.9	22.8
	20–40	2.90 \pm 0.18	0.44 \pm 0.10	0.29 \pm 0.01	9.93 \pm 0.69	8.60 \pm 0.03	–24.0	36.15 \pm 0.52	62.03 \pm 0.30	n.d.	0.9	75.8	23.3
	40–60	2.12 \pm 0.22	0.52 \pm 0.06	0.20 \pm 0.02	10.55 \pm 1.50	8.76 \pm 0.10	–25.3	35.79 \pm 0.91	62.85 \pm 0.61	n.d.	0.6	64.0	35.4

SOC: soil organic carbon; SIC: soil inorganic carbon; N: nitrogen; FWC: field water content; Max WHC: maximum water holding capacity; BD: bulk density; Clay: soil particle size $<$ 0.2 μm ; Silt: 0.2 μm $<$ soil particle size $<$ 20 μm ; Sand: soil particle size $>$ 20 μm ; n.d.: not determined.

5

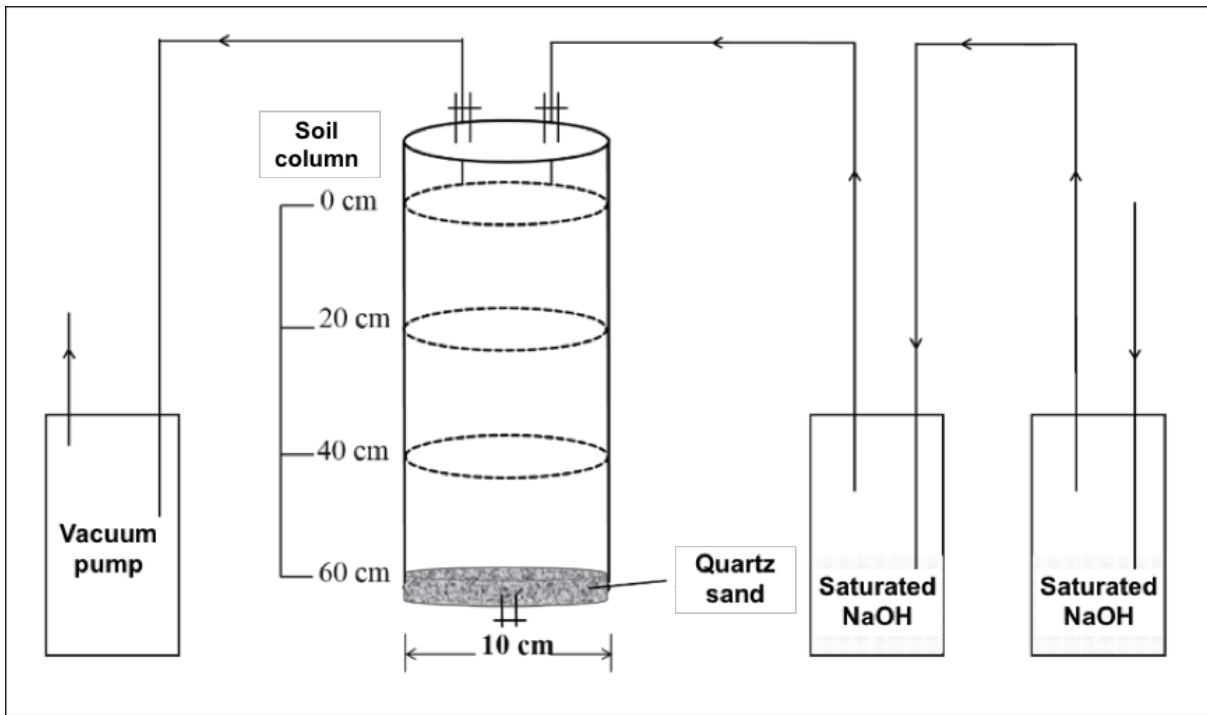


Figure 1: Design of the soil column experiment for monitoring soil respiration and leaching after simulated extreme precipitation events (EPEs).

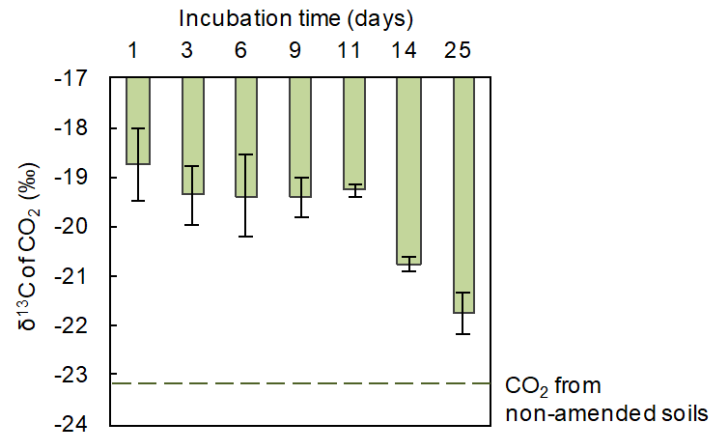
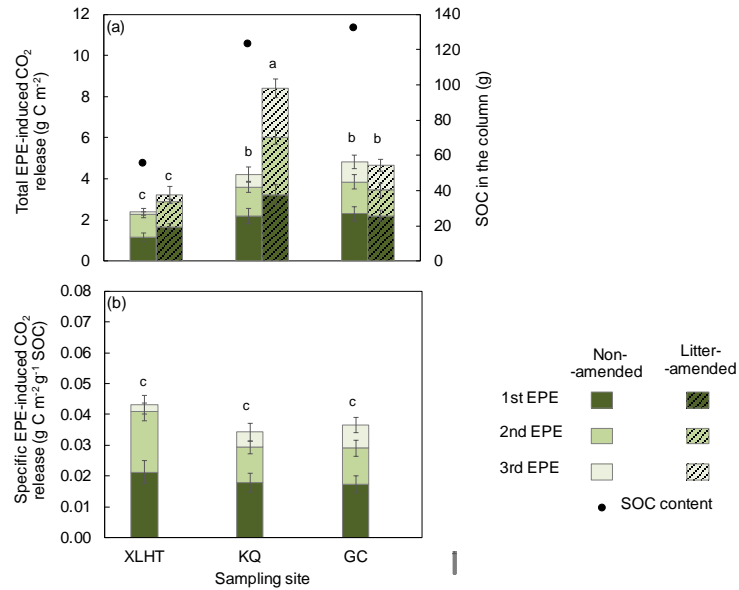


Figure 2: The $\delta^{13}\text{C}$ values of respired CO_2 in the litter-amended Gangcha (GC) soils after the first extreme precipitation event (EPE). Mean values are shown with standard error (n = 3).



5 **Figure 3: Total (a) and specific (b) extreme precipitation event (EPE)-induced CO₂ release in the litter-amended and non-amended grassland soils during three EPEs. Mean values are shown with standard deviation (n = 3). Lower-case letters (a, b, c) indicate significantly different levels among the litter-amended and non-amended soils determined by Duncan's multiple range test (one-way ANOVA, $p < 0.05$).**

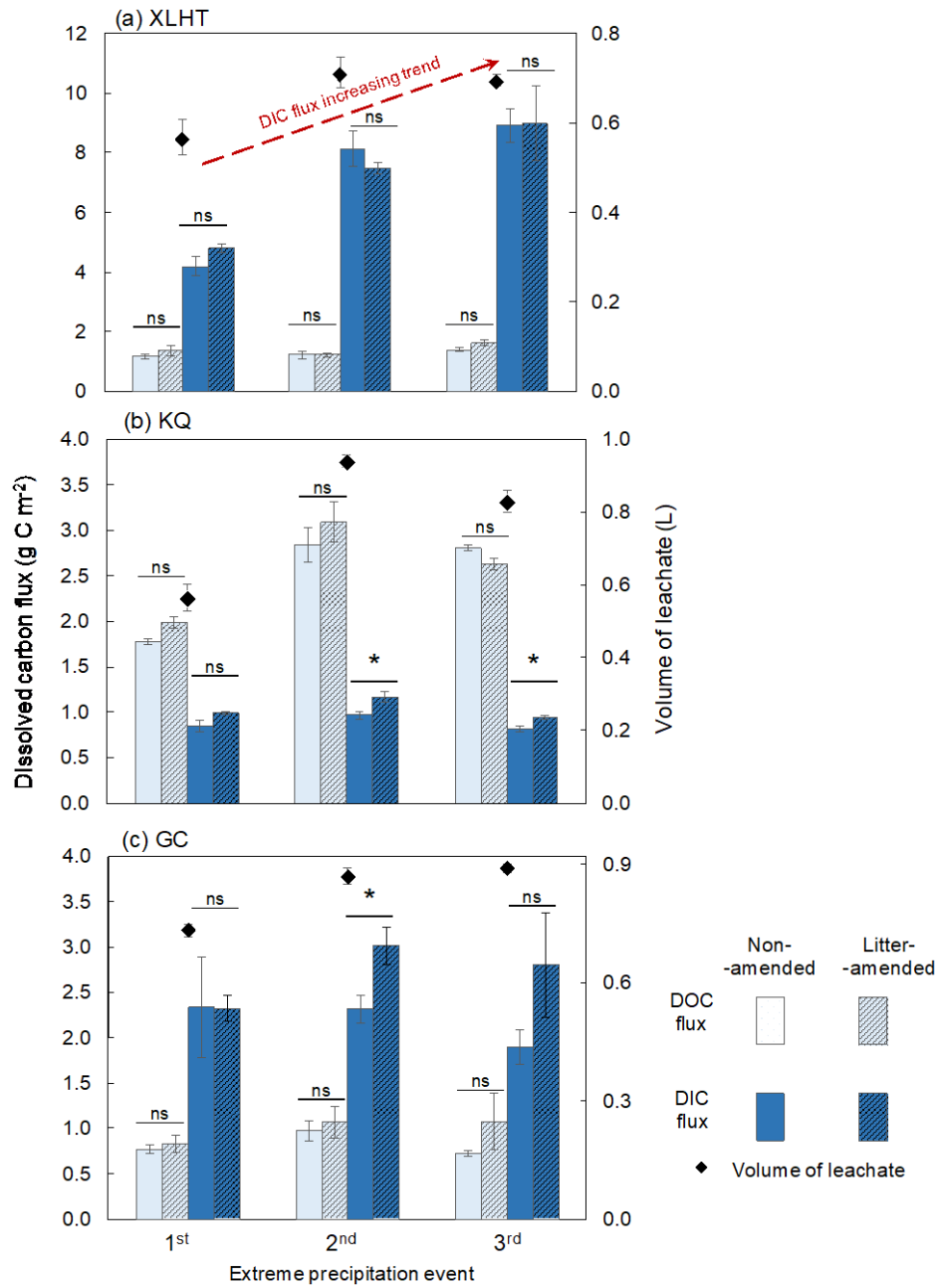


Figure 4: Fluxes of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) and volume of leachates from soil columns after extreme precipitation events (EPEs). Mean values are shown with standard error (n = 3). * and ns denote significant and no difference between the litter-amended and non-amended soils determined by independent samples T test, respectively ($p < 0.05$).

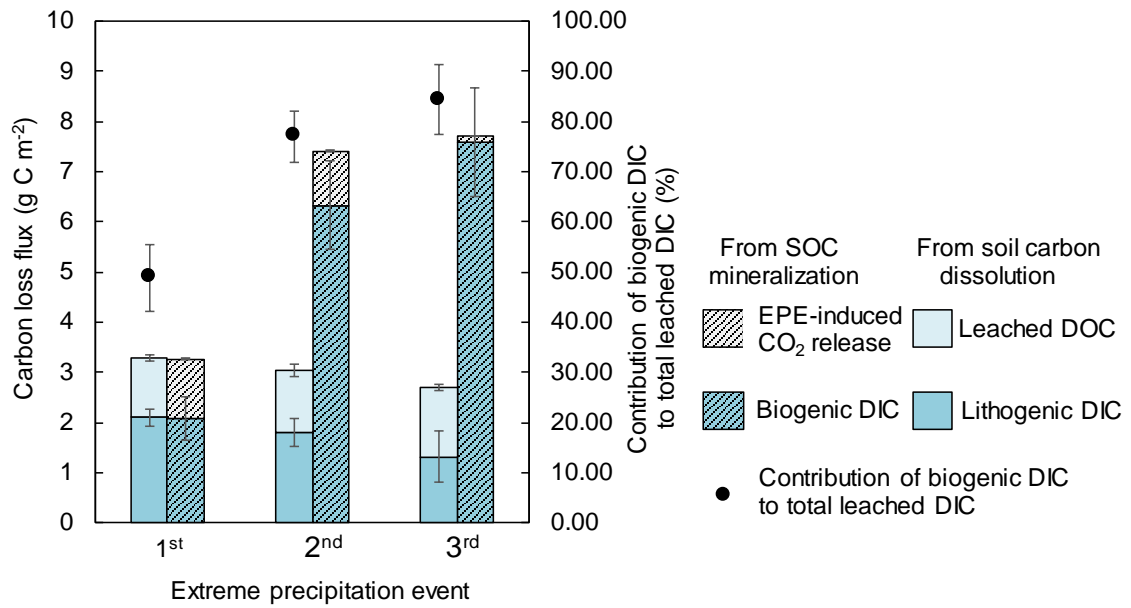


Figure 5: Carbon loss fluxes from soil organic carbon (SOC) mineralization in the non-amended XLHT soils. Fluxes include extreme precipitation event (EPE)-induced CO₂ release and leaching of biogenic dissolved inorganic carbon (DIC), dissolved organic carbon (DOC) and lithogenic DIC. Mean values are shown with standard error (n = 3).

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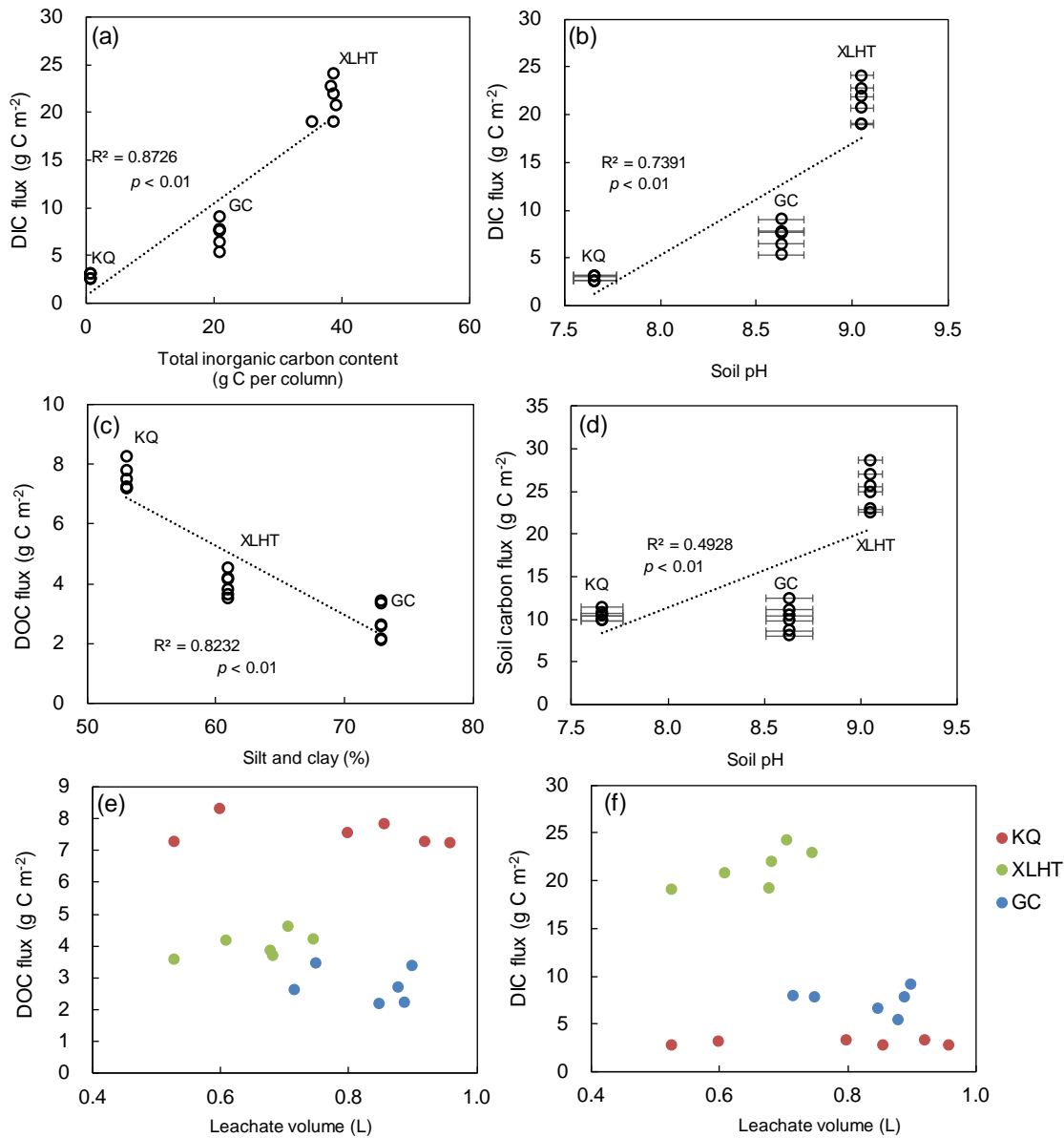


Figure 6: Relationship of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) fluxes with soil properties: (a) DIC flux with total inorganic carbon in the soil columns; (b) DIC flux with soil pH; (c) DOC flux with silt and clay content of soils; (d) total soil carbon flux with soil pH; (e) DOC flux with leachate volume; (f) DIC flux with leachate volume. Mean pH values are shown with standard error (n = 3).

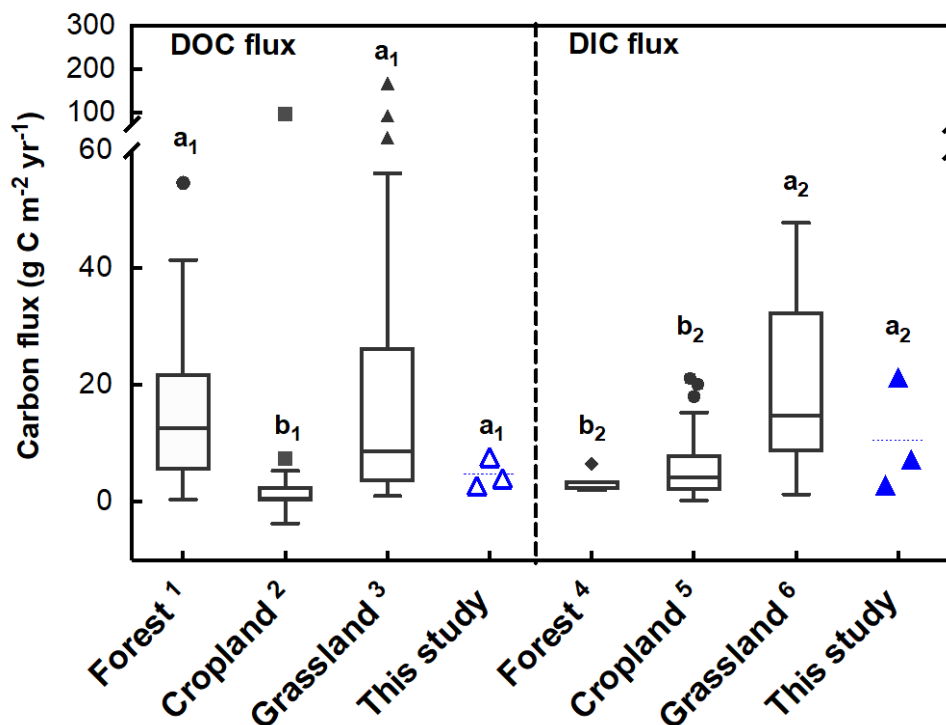


Figure 7: Leaching fluxes of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) in this study compared with that reported in the literature. ¹n = 110, data from Brooks et al., 1999; Froberg et al. 2005, 2006, 2011; Gielen et al., 2011; Kindler et al., 2011; Lu et al., 2013; Michalzik et al., 2000; Sanderman et al., 2008; ²n = 33, data from Brye et al., 2001; Kindler et al., 2011; Siemens et al., 2012; Walmsley et al., 2011; Wang and Alva, 1999; Gerke et al., 2016; Herbrich et al., 2017; Rieckh et al., 2014; Lenz, 2014; ³n = 46, data from Brooks et al., 1999; Brye et al., 2001; Ghani et al., 2010; Kindler et al., 2011; Mctiernan et al., 2001; Parfitt et al., 2009; Sanderman et al., 2008; Tipping et al., 1999; ⁴n = 8, data from Kindler et al., 2011; ⁵n = 32, data from Kindler et al., 2011; Siemens et al., 2012; Walmsley et al., 2011; Wang and Alva, 1999; Gerke et al., 2016; Herbrich et al., 2017; Rieckh et al., 2014; Lenz, 2014; ⁶n = 9, data from Brye et al., 2001; Kindler et al., 2011. Lower-case letters (a₁, b₁) and (a₂, b₂) represent significant different levels of DOC and DIC fluxes in different ecosystems determined by Duncan's multiple range test, respectively, (one-way ANOVA, *p* < 0.05). Dash lines represent mean values for the investigated soils.