

Response to Anonymous Referee #2's comments:

General comments: This study aimed to address the impact of patchiness and pika disturbance on ecosystem respiration at an alpine meadow grassland. The topic is interesting and meaningful and they have presented a good dataset that is sufficient to address the questions they brought up. However, I think the storyline can be better organized and many technical details still need to be added. General comments: 1. According to the title of the article, the whole story should be centered on the ecosystem respiration. Therefore, I suggest the authors to re-organize the storyline by: (1) using the “intact grassland” type as a reference, which is the natural status of the site, and compare other types to IG to indicate the effects of patchiness or pika disturbance. (2) presenting the CO₂ flux first, then environmental conditions and use the differences in soil conditions to explain the flux differences. This applies to abstract, result, order of the figures and discussions. Particularly for discussion, consider separating the sections based on different effects (patchiness and pika disturbance) and explain what factors caused the difference in fluxes compared to the reference type (IG). 2. Method section needs to be expanded with more information on the details. See my comments on each specific line.

Our reply: Thank you for your suggestion. The storyline were re-organized and the whole manuscript has been revised according to your suggestion in the section of abstract, result, order of the figures and discussions.

Abstract (Line 21-41)

“Plateau pikas (*Ochotona curzoniae*) disturbance and patchiness intensify the spatial heterogeneous distribution of vegetation productivity and soil physicochemical properties, which may alter ecosystem carbon emission process. Nevertheless, previous researches have mostly focused on the homogeneous vegetation patches rather than heterogeneous land surface. Thus, this study aims to improve our understanding of the difference in ecosystem respiration (Re) over heterogeneous land surface in an alpine meadow grassland. Six different land surface: large bald patch, medium bald patch, small bald patch, intact grassland, above pika tunnel and pika pile were selected to analyze the response of Re to pikas disturbance and patchiness, and

the key controlling factors. The results showed that (1) Re under intact grassland were 0.22-1.07 times higher than pika pile and bald patches; (2) soil moisture (SM) of intact grassland was 2-11% higher than those of pika pile and bald patches despite pikas disturbance increased water infiltration rate, while soil temperature (ST) under intact grassland was 1-3°C less than pika pile and bald patches; (3) Soil organic carbon (SOC) and total nitrogen (TN) under intact grassland were approximate 50 % and 60 % less than above pika tunnel, whereas 10-30 % and 22-110 % higher than pika pile and bald patches; and (4) Re was significantly correlated with SM, TN and vegetation biomass ($P < 0.05$). Our results suggested that pikas disturbance and patchiness altered ecosystem carbon emission pattern, which was mainly attributed to the reduction of soil water and supply of substrates. Given that the wide distribution of pikas and large area of bald patches, the varied Re under heterogeneous land surfaces should not be neglected for estimation of ecosystem carbon emission at plot or region scale.”

Results (Line 194-233)

“Ecosystem respiration

Pikas disturbance and patchiness had significant effect on ecosystem respiration (Table 1, $P < 0.001$). During the growing season, ecosystem respiration has a maximum value in August and minimum value in June (Figure 2). In June, ecosystem respiration under intact grassland, above pika tunnel, small patch and pika pile had no significant difference and the lowest ecosystem respiration were found under large and medium patch (Figure 2). Average ecosystem respiration under intact grassland was $4.03 \mu\text{mol m}^{-2} \text{s}^{-1}$, which were 6.90 % to 102.50 % higher than other surface types both in July and August (Figure 2).

Microclimate and soil hydrothermal characteristics

Mean temperature and total rainfall during the growing seasons from 1 May to 30 September in 2016 were $6.18 \text{ }^\circ\text{C}$ and 343.4 mm, respectively (Figure 3). Soil temperature and moisture were significantly different ($P < 0.001$) among various surface types (Table 1). The monthly average soil temperature was in a range of $8.20\text{-}13.72 \text{ }^\circ\text{C}$ during June to August, which was approximate $1\text{-}3 \text{ }^\circ\text{C}$ higher under pika pile and bald

patches than the intact grassland (Figure 4a, $P < 0.05$). The monthly mean soil moisture from June to August was approximate 30 % for intact grassland and above pika tunnel, 25 % for small patch and pika pile, and 20 % for larger and medium patch (Figure 4b). Soil saturated hydraulic conductivity also showed significant variation under different land surface types ($P = 0.027$, Table 2). Soil saturated hydraulic conductivity of intact grassland had no significant difference with small patch and above pika tunnel ($P > 0.05$), while it was approximate 40 % higher than medium and large patches and 17 % lower than pika pile (Figure 5).

Soil and vegetation properties

Both pikas disturbance and patchiness significantly affected soil compactness, SOC density, TN density and vegetation biomass (Table 2) ($P < 0.001$). Soil compactness was over 0.30 Pa in intact grassland and above pika tunnel, approximate 0.20 Pa for bald patches and less than 0.10 Pa for pika pile (Figure 6), respectively. Mean SOC and TN density under intact grassland were 52.45 % and 59.14 % less than above pika tunnel, whereas they were 9.69-30.12 % and 22.47-109.62 % higher than pika pile and bald patches (Figure 7). Aboveground and belowground biomass under intact grassland were approximate 30 % higher than above pika tunnel, 90 % higher than pika pile, 123-252 % and 134-289 % higher than bald patches (Figure 8a, b).

Factors regulate ecosystem respiration

We analyzed the relationships of ecosystem respiration with biotic and abiotic factors for six land surface types (Figure 9). Correlation analysis showed that ecosystem respiration had no significant correlation with soil temperature ($P > 0.05$, Figure 9). However, ecosystem respiration was significantly and positively related to soil moisture ($P < 0.01$), soil total nitrogen ($P < 0.05$), aboveground ($P < 0.05$) and belowground biomass ($P < 0.05$) (Figure 9). ”

Discussion (Line 236-326)

“Effect of pikas disturbance on ecosystem respiration

Pikas burrowing activities increased oxygen content in deep soil, which contributed to the decomposition of soil organic matter (Martin, 2003). The deposition of urine and feces by small herbivorous mammals could also promote ecosystem nutrition

circulation (Clark et al., 2005). It was suggested that excreta deposited by pikas and frequently haunted in or near their burrows supplied organic C available to microbial decomposition with an increase in ecosystem CO₂ emission (Cao et al., 2004). Indeed, SOC and TN densities reached up to 14.54 and 0.98 kg m⁻² in above pika tunnel, which was 2.45 and 2.10 times higher than that of intact grassland (Figure 7), respectively. The consistent results reported that the contents of available soil nutrients around the pikas burrow were higher than those in control sites on an alpine meadow (Zhang et al., 2016). We also found that SOC and TN densities under pika pile decreased 13.35 % and 42.93 % than intact grassland. However, no significant difference of Re was found between intact grassland and above pika tunnel, while Re under pika pile were 42.08 % less than intact grassland (Figure 2). The similar result was also found in an alpine meadow on the QTP (Peng et al., 2015), which indicated that ecosystem respiration decreased with increasing of pika holes because of grassland biomass regulated soil C and N with increasing number of pika holes. These results confirmed that pikas disturbance did not increase ecosystem carbon emission directly, but facilitated CO₂ emission into the atmosphere through pika holes (Qin et al., 2015a). The difference of ecosystem respiration between intact grassland and pika piles was mainly related to changes in vegetation biomass and soil moisture. For example, both aboveground and belowground biomass decreased 244.62 % and 279.89 % under pika piles compared with the intact grassland (Figure 8). The reduction of vegetation biomass production decreased aboveground plant respiration and root respiration by decreasing carbon allocation (e.g., root exudates and litter, and available SOC) (Raich and Potter, 1995; Högberg et al., 2002; Yang et al., 2018). Consistent with previous studies which demonstrated that pikas burrowing activity increased water infiltration rate (Hogan, 2010; Wilson and Smith, 2015), our results also showed that soil saturated hydraulic conductivity in pika pile was significantly higher than bald and vegetation patches (Figure 5). Nevertheless, the increased water infiltration was unable to increase soil moisture under pika piles. For example, soil moisture under pika piles was approximate 5 % lower than intact grassland (Figure 4). Our result was discrepant with previous studies which reported old pika mound had

the highest soil moisture during the summer (Ma et al., 2018) and moderate pika burrowing activities increased surface soil moisture (Li and Zhang, 2006). This difference may be contributed to the high pika density in alpine meadow (Guo et al, 2017). Moreover, pika piles were loose (Figure 6) with less vegetation cover (Figure 8), which was not beneficial for soil moisture storage.

Effect of patchiness on ecosystem respiration

Our results clearly showed that patchiness resulted in significant reduction of ecosystem carbon emission. Compared with the intact grassland, ecosystem respiration decreased approximate 17-48 % for bald patches (Figure 2). Two possible mechanisms could account for the effects of patchiness on ecosystem respiration. On one hand, the reduction of SOC and TN decreased microbial respiration by decreasing substrate supply to microbes in the rhizosphere (Nobili et al., 2001; Scott-Denton et al., 2010). Our results indicated that patchiness caused evident loss of SOC and TN (Figure 7) due to reduction in C input from vegetation and increasing in C output from soil erosion (Qin et al., 2018). Previous study have shown that the spatial heterogeneity of soil respiration was attributed to uneven soil organic carbon and total nitrogen content (Xu and Qi, 2010). Soil organic carbon was considered as the basic substrate of CO₂ emission by microbial decomposition (Sikora and Mccoy, 1990) and soil total N enhanced ecosystem CO₂ emission by providing a source of protein for microbial growth (Tewary et al., 1982). On the other hand, low moisture availability would limit microbial respiration by restricting access to C substrates, reducing the diffusion of C substrates and extracellular enzymes, and limiting microbial mobility (Yuste et al., 2003; Wang et al., 2014). Our results showed that soil moisture under large and medium patches decreased 10 % than intact grassland (Figure 4). Previous studies had reported that the soil compaction of bald patches decreased the rate of water infiltration (Wuest et al., 2006; Wilson and Smith, 2015), which was similar with our results showed that bald patches had less saturated soil hydraulic conductivity (Figure 5). Low vegetation cover under bald patches was not beneficial for water retention and utilization, where most of soil water was mainly lost as a way of evaporation (Yi et al., 2014). We have measured evaporation of the intact grassland,

isolate grassland, large patches, medium patches and small patches since the early June 2016. Three years results indicated that evaporation under bald patches were higher than the intact grassland (data were not shown here).

Factors affected ecosystem respiration

Most previous studies showed that soil temperature explained most of the temporal variation of ecosystem respiration on the alpine grassland on the QTP (Lin et al, 2011; Qin et al., 2015c; Zhang et al., 2017). Our results indicated that soil temperature under pika piles and bald patches was approximate 1 to 3 °C higher than intact grassland (Figure 4), which mainly resulted from the heterogeneity of surface albedo, surface soil water retention, heat conduction properties and radiation (Beringer et al., 2005; Pielke, 2005; Yi et al., 2013; You et al., 2017). It was suggested that pikas disturbance create a better soil temperature buffer for them to avoid the extreme cold in winter (Ma et al., 2018), whereas high soil temperature under bald patch was a disadvantage for the recovery of vegetation because patch surface had the smallest soil moisture content (Figure 4) and the largest daily range of soil temperature (Ma et al., 2018). However, no an obvious relationship between R_e and soil temperature was found in the present study (Figure 9), which suggested that other factors involved in controlling R_e induced by pikas disturbance and patchiness. Our results showed that R_e were positively correlated with soil moisture, soil total nitrogen, aboveground and belowground biomass (Figure 9). Pikas disturbance and patchiness led to the drying and loosening of soil (Figure 4 and 6). It was considered that loose, dry surface sediments and strong winds were the primary factors responsible for soil erosion (Dong et al., 2010b) and wind erosion was especially common in arid and semi-arid regions (Zhang and Dong, 2014). This resulted in the reduction of soil organic carbon, total nitrogen and vegetation biomass (Figure 7 and 8). The alteration of these biotic and abiotic factors induced by pikas disturbance and patchiness led to the decline of ecosystem respiration. Nevertheless, the decline of ecosystem respiration did not completely offset the sequestration of C fixed by photosynthesis because of the lower vegetation cover under bald patches and pika piles. Given the large area covered by bald patches in alpine grasslands, patchiness was more susceptible to erosion and

exert greater influence on ecosystem respiration than pikas disturbance. Recent study has also reported that bald patches of various sizes on the grasslands played a much more important role than pikas direct disturbance in reducing vegetation cover, aboveground biomass, soil carbon and nitrogen (Yi et al., 2016). ”

We also added more information in the section of field observation and soil and vegetation sampling according to your suggestion.

Field observation

At early June 2016, three 100 m × 100 m plots were established as replicates. In each plot, six representative land surfaces were selected: (1) large bald patch with size larger than 9.0 m² (LP), (2) medium bald patch with size of 1.0-9.0 m² (MP), (3) small bald patch with size of less than 1.0 m² (SP), (4) intact grassland patch (IG), (5) above pika tunnel (PT), (6) old pika pile (PP) (Figure 1) (Yi et al., 2016; Qin et al., 2018). For each surface type, nine 1 m × 1 m quadrats were set up, of which three was used for soil temperature and soil moisture measurement, three for soil saturated hydraulic conductivity measurement and three for soil compactness measurement, soil and vegetation sampling. We also set up three 2 m × 2 m quadrats in each surface type in a 100 m × 100 m plot for measuring ecosystem respiration.

(Insert Figure 1 here)

Soil temperature and moisture at 10 cm were measured in a 100 m × 100 m plot where ecosystem respiration was measured by using an auto-measurement system (Decagon Inc., USA) from early June to the late August. The system consisted of an EM50 logger and five 5TM sensors. The data were logged automatically every 30 min. Soil saturated hydraulic conductivity and compactness were measured one time in each month from June to August. Soil saturated hydraulic conductivity was measured by Dual Head infiltrometer (Decagon Inc., USA). The measurement process included soak time 15 min, hold time 20 min at low pressure head (5 cm) and high pressure head (15 cm) with 2 cycles. Each measurement takes 95 min altogether. Soil compactness was measured with TJSD-750 (Hangzhou Top Instrument co., LTD, Hangzhou, China) from the soil surface to 10 cm depth. Ecosystem respiration rates were measured using the LICOR-8150 Automated Soil CO₂ Flux System, which was

an accessory for the LI-8100A could connect 16 individual chambers at one time and were sampled and controlled by the LI-8100A Analyzer Control Unit. The air temperature inside of the chamber was measured using the internal thermistor of the chamber. The ecosystem CO₂ fluxes were calculated by the equation as follow.

$$F_c = \frac{10VP_0 \left(1 - \frac{W_0}{1000}\right) \partial C'}{RS(T_0 + 273.15) \partial t}$$

where F_c is the soil CO₂ efflux rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), V is volume (cm^3), P_0 is the initial pressure (kPa), W_0 is the initial water vapor mole fraction (mmol mol^{-1}), S is soil surface area (cm^2), T_0 is initial air temperature ($^{\circ}\text{C}$), and $\partial C'/\partial t$ is the initial rate of change in water-corrected CO₂ mole fraction ($\mu\text{mol}^{-1} \text{mol s}^{-1}$).

Six LICOR-8100-104 long-term opaque chambers (20cm in diameter LICOR, Inc., Lincoln, NE, USA) were used to measure alternately between three replicates for six land surface types. Therefore, 3 days at least were required to complete one rotation measurements of ecosystem respiration. To measure ecosystem respiration, eighteen polyvinyl chloride collars with a 20 cm inner diameter and a 12 cm height were inserted into the soil with 3-4 cm exposed to the air (Qin et al., 2013). All of the collars were installed at least 24 h before the first measurement to reduce disturbance-induced ecosystem CO₂ effluxes. Ecosystem respiration rates were measured every 7-10 days from June 16 to August 20 in 2016 depending on weather conditions. A round-the-clock measurement protocol was carried out and ecosystem respiration rates were measured every 30 minutes. Each measurement takes 1 minute and 45 seconds, including pre-purge 10 seconds, dead band 15 seconds, observation length 1 minute and post-purge 20 seconds.

Soil and vegetation sampling

Soil samples were collected during the periods of late July to early August 2016. In each surface type of each plot, five soil cores were collected using a stainless-steel auger (5 cm in diameter) at depths of 0-10, 10-20, 20-30 and 30-40 cm, and bulked as one composite sample for each depth in each quadrat. Another five soil cores were sampled by cylindrical cutting ring (7 cm in diameter and 5.2 cm in depth) to

determine soil bulk density from each land surface type. Pika tunnel was approximate 6 cm in diameter and 40 cm in depth. Therefore, soil samples were available to collect at depth of 40cm. Totally, 512 soil samples were collected. Soil samples were firstly air-dried, then removed gravel and stone with manual sieving and finally weighed. The remaining soil samples with diameter less than 2 mm were ground to pass through a 0.25 mm sieve for analysis of soil organic carbon (SOC) and soil total nitrogen (TN) concentration. SOC was measured by dichromate oxidation using Walkley-Black acid digestion (Nelson and Sommers, 1982). TN was determined by digestion and then tested using a flow injection analysis system (FIAstar 5000, Foss Inc., Sweden). Aboveground and belowground biomasses were determined within a 1 m × 1 m quadrat on 4 August 2016 during peak biomass and species diversity. There were a total of 108 aboveground and belowground vegetation samples (3 plots × 6 land surface types × 3 replicates) from the study area. Aboveground biomass was determined by clipping all above-ground living plants at ground level, drying (oven-dried at 65°C for 48 h) and weighing. Belowground biomass was sampled by collecting five soil columns, and each soil column was 5 cm in diameter and 40 cm in depth. Soil cores were washed with a gentle spray of water over a fine mesh screen until soil separated from the roots, and then drying (oven-dried at 65°C for 48 h) and weighing.

Specific comments:

(1) L52, other substrates? Such as?

Our reply: Thank you for your question. The substrates affected ecosystem respiration included carbohydrate fixed by leaves, vegetation litter and soil organic matter. We have revised the manuscript as follow (Line 49-53).

“Dependent on autotrophic (plant) and heterotrophic (microbe) activity, ecosystem respiration is mainly controlled by abiotic factors (primarily temperature and water availability) (Chimner and Welker, 2005; Flanagan and Johnson, 2005; Nakano et al., 2008; Buttler et al., 2018), and supply of carbohydrate fixed by leaves, vegetation litter and soil organic matter (Janssens et al., 2001; Reichstein et al., 2002).”

(2) L57, ecological system? Ecosystem!

Our reply: Thank you for your suggestion. We have changed ecological system to ecosystem according to your suggestion (Line 57).

(3) L68, this definition of patchiness need to be referred to earlier in the paragraph.

Our reply: Thank you for your suggestion. The definition of patchiness has been moved to earlier in the paragraph according to your suggestion. We revised this part as follow (Line 56-77).

“One of the basic function of terrestrial ecosystem is to regulate carbon balance between the atmosphere and ecosystem (Canadell et al., 2007; Le Quéré et al., 2014; Ahlström et al., 2015). However, this balance would be broken by widespread land degradation (Post and Kwon, 2000; Dregne, 2002), which accompanied with the reduction of photosynthetic fixed carbon dioxide from atmosphere and carbon sequestration by soils (Defries et al., 1999; Upadhyay et al., 2005). It was estimated that land degradation had resulted in 19-29 Pg C loss worldwide (Lal, 2001). Over the past decades, grasslands have experienced patchiness throughout the world and this process is still ongoing (Baldi et al., 2006; Wang et al., 2009; Roch and Jaeger, 2014). Patchiness generally refers to a landscape that consists of remnant areas of native vegetation surrounded by a more heterogeneous and patchy situation (Kouki and Löfman, 1998). Other than climate change (Yi et al., 2014), vegetation self-organization (Rietkerk et al., 2004; Venegas et al., 2005; McKey et al., 2010) or anthropogenic disturbances (Kouki and Löfman, 1998; Yi et al., 2016), rodents burrowing activities were also considered as the origin of the patchiness (Wei et al., 2007; Davidson and Lightfoot, 2008). This patchiness intensified spatial heterogeneity of land surface and led to the changing of the structure and function of the original ecosystem (Herkert et al., 2003; Bestelmeyer et al., 2006; Lindenmayer and Fischer, 2013). For instance, there is abundant evidence that patchiness not only intensified the spatial heterogeneous distribution of ecosystem organic carbon (C) and vegetation productivity (Yan et al., 2016; Qin et al., 2018) but also altered the pattern of coupled water and heat cycling between the land surface and the atmosphere (Saunders et al., 1991; You et al., 2017; Ma et al., 2018). Consequently, this may alter ecosystem carbon emission process (Juszczak et al., 2013).”

(4) L89, not clear, others also studied the effect of pika disturbance and patchiness, which are what you meant as “heterogeneity” to my understanding. What makes your study different from theirs?

Our reply: Thank you for your question. We totally agree with your comment that lots of previous researches have studied the heterogeneous underground vegetation and belowground soil properties. However, few studies have investigated the difference of ecosystem respiration under the heterogeneous underlying surface. Here we mainly meant the heterogeneity of ecosystem respiration. Therefore, we have changed this sentence to “Nevertheless, most of these studies have mainly focused on ecosystem carbon emission rate under the homogeneous land surface rather than heterogeneous land surfaces.”

Typically, most of the previous studies compared carbon fluxes under intact vegetation at plots with different number of pika burrows. However, ecosystem carbon emissions from the heterogeneous land surface induced by pika piles and patchiness have yet to be quantified. These are the exact differences between this study and so many previous studies.

(5) L93, “underlying surface” sounds a little awkward. Change it to land surface or soil surface. Check this expression throughout the manuscript.

Our reply: Thank you for your suggestion. We have changed “underlying surface” to “land surface” in the whole manuscript according to your suggestion.

(6) L94, I think what you meant was “the spatial heterogeneity of Re” in aim.

Our reply: Thank you for your suggestion. We have revised the third aim according to your suggestion. We have revised the manuscript as follow (Line 92-95).

“Thus, the specific aims of this study were to (1) investigate the spatial heterogeneity of Re under the effect of pikas and patchiness; (2) illuminate the potential regulating mechanism of pikas disturbance and patchiness to ecosystem respiration (Re) in an alpine meadow grassland in the northeastern part of Qinghai-Tibetan Plateau (QTP).”

(7) L105 “plant” species

Our reply: Thank you for your suggestion. We have changed “species” to “plant species” according to your suggestion.

(8) L121, according to your description, seems the fluxes were measured in different plots from ones that measured environmental conditions, right? If yes, how far away are they? Are they comparable?

Our reply: Thank you for your question. Ecosystem respiration, soil temperature and moisture were measured in one 100 × 100 m plot and with three replicates under each land surface. Soil and vegetation were measured in all three 100 × 100 m plots. Each 100 × 100 m plot was in a distance of less than 50 m, which has the similar plant and terrain. We therefore believed they were comparable.

(9) L126, “were” logged . . .

Our reply: Thank you for your suggestion. We have changed “The Data logged automatically every 30 min” to “The data were logged automatically every 30 min” according to your suggestion.

(10) L129, soil hardness is not a very familiar concept. Explain it and what unit is used?

Our reply: Thank you for your suggestion. We have changed “soil hardness” to “soil compactness” according to your suggestion. We also added its unit both in result and Figure 5.

“Soil compactness was over 0.30 Pa in intact grassland patch and above pika tunnel, approximate 0.20 Pa for bald patches and less than 0.10 Pa for pika pile (Figure 5), respectively.”

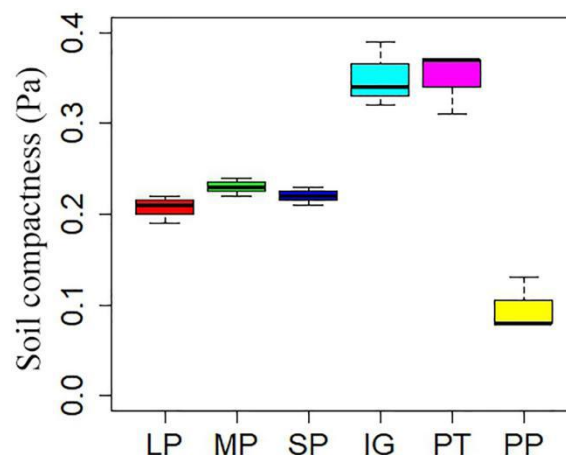


Figure 5. Soil compactness under different surface types: (1) large bald patch (LP), (2) medium bald patch (MP), (3) small bald patch (SP), (4) intact grassland patch (IG), (5) above pika tunnel (PT) and (6) old pika pile (PP).

(11) L131, since the respiration measurement is the key of this study, more details are needed. How big is the chamber? Transparent or opaque? How many replicates? Only one gas analyzer was used? How many minutes did one measurement take? What is the frequency of the data? During which period (specific dates) were the measurements taken? Also, how the fluxes were calculated? How the air temperature inside of the chamber was measured?

Our reply: Thank you for your suggestion. We have added more information regarding ecosystem respiration measurement according to your suggestion (Line 133-155).

“Ecosystem respiration rates were measured using the LICOR-8150 Automated Soil CO₂ Flux System, which was an accessory for the LI-8100A with at most 8 individual chambers at one time. Ecosystem CO₂ emission was sampled and controlled by the LI-8100A Analyzer Control Unit. The air temperature inside of the chamber was measured using the internal thermistor of the chamber. The ecosystem CO₂ fluxes were calculated by the equation as follow.

$$F_c = \frac{10VP_0 \left(1 - \frac{W_0}{1000}\right)}{RS(T_0 + 273.15)} \frac{\partial C'}{\partial t}$$

where F_c is the soil CO₂ efflux rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), V is volume (cm^3), P_0 is the initial pressure (kPa), W_0 is the initial water vapor mole fraction (mmol mol^{-1}), S is soil surface area (cm^2), T_0 is initial air temperature ($^{\circ}\text{C}$), and $\partial C'/\partial t$ is the initial rate of change in water-corrected CO₂ mole fraction ($\mu\text{mol}^{-1} \text{mol s}^{-1}$).

Six LICOR-8100-104 long-term opaque chambers (20cm in diameter LICOR, Inc., Lincoln, NE, USA) were used to measure alternately between three replicates for six land surface types. Therefore, 3 days at least were required to complete one rotation measurements of ecosystem respiration. To measure ecosystem respiration, eighteen polyvinyl chloride collars with a 20 cm inner diameter and a 12 cm height

were inserted into the soil with 3-4 cm exposed to the air (Qin et al., 2013). All of the collars were installed at least 24 h before the first measurement to reduce disturbance-induced ecosystem CO₂ effluxes. Ecosystem respiration rates were measured every 7-10 days from June 16 to August 20 in 2016 depending on weather conditions. A round-the-clock measurement protocol was carried out and ecosystem respiration rates were measured every 30 minutes. Each measurement takes 1 minute and 45 seconds, including pre-purge 10 seconds, dead band 15 seconds, observation length 1 minute and post-purge 20 seconds.”

(12) L138 change “determined” to “collected”.

Our reply: Thank you for your suggestion. We have changed “determined” to “collected” according to your suggestion.

(13) L142 from each surface type?

Our reply: Thank you for your careful review. The sentence has changed to “Another five soil cores were sampled by cylindrical cutting ring (7 cm in diameter and 5.2 cm in depth) to determine soil bulk density from each land surface type.” according to your suggestion.

(14) L149 how many replicates?

Our reply: Thank you for your careful review. Soil and vegetation samples were collected under six land surface types with three replicates in three 100 × 100 m plots. To eliminate the confusion, we have revised this part as follow (Line 171-176).

“There were a total of 108 aboveground and belowground vegetation samples (3 plots × 6 land surface types × 3 replicates) from the study area. Aboveground biomass was determined by clipping all above-ground living plants at ground level, drying (oven-dried at 65 ° C for 48 h) and weighing. Belowground biomass was sampled by collecting five soil columns, and each soil column was 5 cm in diameter and 40 cm in depth.”

(15) L150 change “sampled” to “determined”

Our reply: Thank you for your careful review. We have changed “sampled” to “determined” according to your suggestion (Line 173).

(16) L152 each type?

Our reply: Thank you for your careful review. It means each soil columns. To eliminate the confusion, this sentence was changed to “ There were a total of 108 aboveground and belowground vegetation samples (3 plots × 6 land surface types × 3 replicates) from the study area. Aboveground biomass was determined by clipping all above-ground living plants at ground level, drying (oven-dried at 65 ° C for 48 h) and weighing. Belowground biomass was sampled by collecting five soil columns, and each soil column was 5 cm in diameter and 40 cm in depth. ” (Line 171-176)

(17) L169, according to your figure, this seems like correlation analysis instead of regression.

Our reply: Thank you for your careful review. We have changed “regression analysis” to “correlation analysis” according to your suggestion.

(18) Figure 2, which year? Average Ta?

Our reply: Thank you for your careful review. All data in this manuscript were collected in 2016. Ta was daily average air temperature. To eliminate confusion, the title of Figure 2 has been changed to “Figure 2. Daily average air temperature and precipitation of the study site in 2016.”

(19) Figure 3, monthly average?

Our reply: Thank you for your question. Both soil temperature and soil moisture were monthly average. To eliminate confusion, the title of Figure 3 has been changed to “Figure 3. Monthly average soil temperature and soil moisture under different surface types: (1) large bald patch (LP), (2) medium bald patch (MP), (3) small bald patch (SP), (4) intact grassland patch (IG), (5) above pika tunnel (PT) and (6) old pika pile (PP).”

(20) Figure 8, μmol instead of umol

Our reply: Thank you for your suggestion. We have replaced “ umol ” by “ μmol ” according to your suggestion.

(21) Figure 9, this is not a good way to present correlation results. First, specify what analysis in the caption. Second, the full correlation table looks redundant as it presents

two copies of each pair of variables. Also, correlation coefficients and P value need to be included. Was the correlation done across the different surface types?

Our reply: Thank you for your suggestion. We have redrawn Figure 9 according to your suggestion. And now it contained both the correlation coefficients and P value in one figure. The correlation of ecosystem respiration with biotic and abiotic factors were done across the different surface types. The title of Figure 9 was changed to “Figure 9. The correlation coefficient charts between ecosystem respiration (Re) and biotic and abiotic factors for all six land surfaces. The diagonal line in the figure shows the distributions of the variables themselves. The lower triangle (the left bottom of the diagonal) in the figure shows scatter plots of the two properties. The upper triangle (the upper right of the diagonal) in the figure indicates the correlation values of the two parameters; the asterisk indicates the degree of significance (***) indicates significant differences at $P < 0.001$, * indicates significant differences at $P < 0.01$, * indicates significant differences at $P < 0.05$.) The bold bigger numbers mean the higher correlation.”

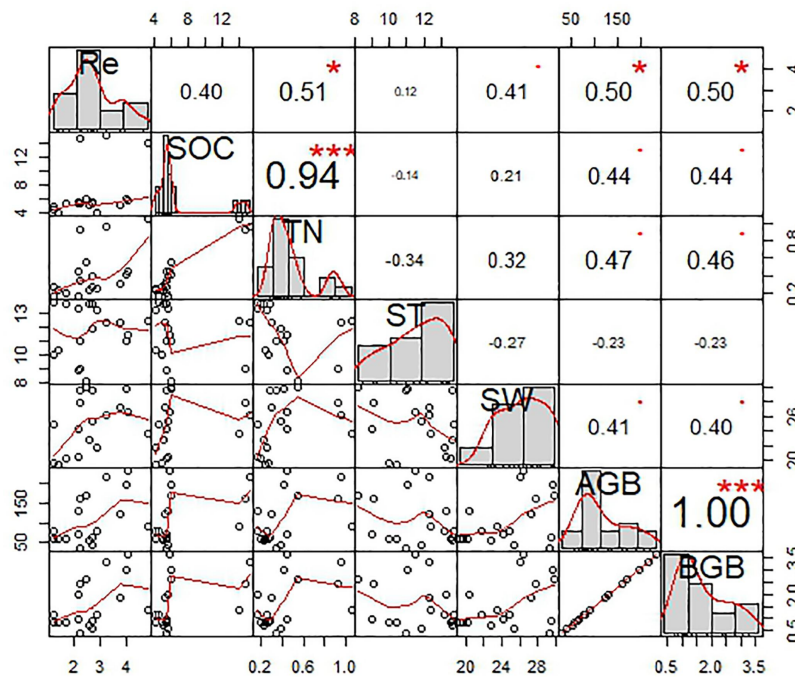


Figure 9. The correlation coefficient charts between ecosystem respiration (Re) and biotic and abiotic factors for all six land surfaces. The diagonal line in the figure shows the distributions of the variables themselves. The lower triangle (the left

bottom of the diagonal) in the figure shows scatter plots of the two properties. The upper triangle (the upper right of the diagonal) in the figure indicates the correlation values of the two parameters; the asterisk indicates the degree of significance (***) indicates significant differences at $P < 0.001$, * indicates significant differences at $P < 0.01$, * indicates significant differences at $P < 0.05$). The bold bigger numbers mean the higher correlation.

1 **Effect of plateau pikas disturbance and patchiness on ecosystem carbon emission of**
2 **alpine meadow on the northeastern part of Qinghai-Tibetan Plateau**

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20 **Abstract**

21 Plateau pikas (*Ochotona curzoniae*) disturbance and patchiness intensify the spatial
22 heterogeneous distribution of vegetation productivity and soil physicochemical properties,
23 which may alter ecosystem carbon emission process. Nevertheless, previous researches have
24 mostly focused on the homogeneous vegetation patches rather than heterogeneous land
25 surface. Thus, this study aims to improve our understanding of the difference in ecosystem
26 respiration (Re) over heterogeneous land surface in an alpine meadow grassland. Six different
27 land surface: large bald patch, medium bald patch, small bald patch, intact grassland, above
28 pika tunnel and pika pile were selected to analyze the response of Re to pikas disturbance and
29 patchiness, and the key controlling factors. The results showed that (1) Re under intact
30 grassland were 0.22-1.07 times higher than pika pile and bald patches; (2) soil moisture (SM)
31 of intact grassland was 2-11% higher than those of pika pile and bald patches despite pikas
32 disturbance increased water infiltration rate, while soil temperature (ST) under intact
33 grassland was 1-3°C less than pika pile and bald patches; (3) Soil organic carbon (SOC) and
34 total nitrogen (TN) under intact grassland were approximate 50 % and 60 % less than above
35 pika tunnel, whereas 10-30 % and 22-110 % higher than pika pile and bald patches; and (4)
36 Re was significantly correlated with SM, TN and vegetation biomass ($P < 0.05$). Our results
37 suggested that pikas disturbance and patchiness altered ecosystem carbon emission pattern,
38 which was mainly attributed to the reduction of soil water and supply of substrates. Given that
39 the wide distribution of pikas and large area of bald patches, the varied Re under
40 heterogeneous land surfaces should not be neglected for estimation of ecosystem carbon
41 emission at plot or region scale.

42 **Keywords:** pikas disturbance; patchiness; ecosystem respiration; alpine meadow; the
43 Qinghai-Tibetan Plateau

44 **Introduction**

45 Ecosystem respiration (Re) is the key process to determine the carbon budget in the terrestrial
46 ecosystem. Thus, even a small imbalances between CO₂ uptake via photosynthesis and CO₂
47 release by ecosystem respiration can lead to significant interannual variation in atmospheric
48 CO₂ (Schimel et al., 2001; Cox et al., 2000; Grogan and Jonasson, 2005; Oberbauer et al.,
49 2007; Warren and Taranto, 2011). **Dependent on autotrophic (plant) and heterotrophic**
50 **(microbe) activity, ecosystem respiration is mainly controlled by abiotic factors (primarily**
51 **temperature and water availability) (Chimner and Welker, 2005; Flanagan and Johnson, 2005;**
52 **Nakano et al., 2008; Buttlar et al., 2018), and supply of carbohydrate fixed by leaves,**
53 **vegetation litter and soil organic matter (Janssens et al., 2001; Reichstein et al., 2002).**
54 Therefore, any external disturbance altering environmental conditions and affecting
55 vegetation growth would exert profound influence on ecosystem carbon emission.

56 One of the basic function of terrestrial ecosystem is to regulate carbon balance between
57 the atmosphere and **ecosystem** (Canadell et al., 2007; Le Quéré et al., 2014; Ahlström et al.,
58 2015). However, this balance would be broken by widespread land degradation (Post and
59 Kwon, 2000; Dregne, 2002), which accompanied with the reduction of photosynthetic fixed
60 carbon dioxide from atmosphere and carbon sequestration by soils (Defries et al., 1999;
61 Upadhyay et al., 2005). It was estimated that land degradation had resulted in 19-29 Pg C loss
62 worldwide (Lal, 2001). **Over the past decades, grasslands have experienced patchiness**
63 **throughout the world and this process is still ongoing (Baldi et al., 2006; Wang et al., 2009;**
64 **Roch and Jaeger, 2014). Patchiness generally refers to a landscape that consists of remnant**
65 **areas of native vegetation surrounded by a more heterogeneous and patchy situation (Kouki**
66 **and Löfman, 1998). Other than climate change (Yi et al., 2014), vegetation self-organization**
67 **(Rietkerk et al., 2004; Venegas et al., 2005; McKey et al., 2010) or anthropogenic**
68 **disturbances (Kouki and Löfman, 1998; Yi et al., 2016), rodents burrowing activities were**
69 **also considered as the origin of the patchiness (Wei et al., 2006; Davidson and Lightfoot,**
70 **2008). This patchiness intensified spatial heterogeneity of land surface and led to the**
71 **changing of the structure and function of the original ecosystem (Herkert et al., 2003;**
72 **Bestelmeyer et al., 2006; Lindenmayer and Fischer, 2013). For instance, there is abundant**
73 **evidence that patchiness not only intensified the spatial heterogeneous distribution of**

74 ecosystem organic carbon (C) and vegetation productivity (Yan et al., 2016; Qin et al., 2018)
75 but also altered the pattern of coupled water and heat cycling between the land surface and the
76 atmosphere (Saunders et al., 1991; You et al., 2017; Ma et al., 2018). Consequently, this may
77 alter ecosystem carbon emission process (Juszczak et al., 2013).

78 Plateau pikas (*Ochotona curzoniae*, hereafter pikas) are small mammals endemic to the
79 alpine grasslands on the Qinghai-Tibetan Plateau (QTP) (Smith and Foggin, 1999; Lai and
80 Smith, 2003). Living in underground, they excavated deep layer soil to surface through
81 foraging and digging activities (Lai and Smith, 2003) and led to substantial bald piles on the
82 ground. The bald pile was considered to gradually become bald patches under soil erosion,
83 gravity, freeze-thaw and other factors (Chen et al., 2017; Ma et al., 2018). As a consequence,
84 natural vegetation patches and adjacent bald patches with different sizes, and pikas piles
85 represent the most common landscape pattern in the alpine meadow grassland on the QTP.
86 Previous studies have demonstrated that pikas disturbance and patchiness weaken the function
87 of alpine meadow as a carbon sink (Liu et al., 13; Peng et al., 2015; Qin et al., 2018) and
88 accelerated ecosystem carbon emission rate (Qin et al., 2015a). **Nevertheless, most of these**
89 **studies have mainly focused on ecosystem carbon emission rate under the homogeneous land**
90 **surface rather than heterogeneous land surfaces.** It remains unclear what the differences of R_e
91 are among heterogeneous land surfaces, especially under the disturbance of pikas and
92 patchiness. **Thus, the specific aims of this study were to (1) investigate the spatial**
93 **heterogeneity of R_e under the effect of pikas and patchiness; (2) illuminate the potential**
94 **regulating mechanism of pikas disturbance and patchiness to ecosystem respiration (R_e) in an**
95 **alpine meadow grassland in the northeastern part of Qinghai-Tibetan Plateau (QTP).**

96 **Materials and methods**

97 **Site description**

98 This study was conducted at the permanent plots at Suli Alpine Meadow Ecosystem
99 Observation and Experiment Station (98°18'33.2", 38°25'13.5", 3887 m a.s.l.), Northwest
100 Institute of Eco-Environment and Resources, Chinese Academy of Science. The study area is
101 characterized by a continental arid desert climate, with low mean annual air temperature, little
102 rainfall, and high evaporation (Wu et al., 2015). The mean annual air temperature was
103 approximately -4°C and the annual precipitation ranged from 200 to 400mm, respectively

104 (Chang et al., 2016). The permafrost type at our site is transition and the active layer depth is
105 2.78 ± 1.03 m (Chen et al., 2012). The dominant plant species in the study area were *Kobresia*
106 *capillifolia*, *Carex moorcroftii* (Qin et al., 2014). Soils was classified as “felty” with a pH of
107 8.56, 30.96 % silt and fine, 57.52 % fine sand and 10.68 % coarse sand, and soil bulk density
108 is 1.41 g cm^{-3} within a 0-40 cm depth of the soil layer (Qin et al., 2015b). The grassland in
109 this area suffered from degradation due to permafrost degradation and external disturbance
110 from grazing livestock and small mammals, i.e. plateau pikas (Yi et al., 2011, Qin et al.,
111 2015a). As a result, a mosaic pattern of vegetation patches, bald patches with different sizes
112 and pika piles was common.

113 **Field observation**

114 At early June 2016, three $100 \text{ m} \times 100 \text{ m}$ plots were established as replicates. In each plot, six
115 representative land surfaces were selected: (1) large bald patch with size larger than 9.0 m^2
116 (LP), (2) medium bald patch with size of $1.0\text{-}9.0 \text{ m}^2$ (MP), (3) small bald patch with size of
117 less than 1.0 m^2 (SP), (4) intact grassland patch (IG), (5) above pika tunnel (PT), (6) old pika
118 pile (PP) (Figure 1) (Yi et al., 2016; Qin et al., 2018). For each surface type, nine $1 \text{ m} \times 1 \text{ m}$
119 quadrats were set up, of which three was used for soil temperature and soil moisture
120 measurement, three for soil saturated hydraulic conductivity measurement and three for soil
121 compactness measurement, soil and vegetation sampling. We also set up three $2 \text{ m} \times 2 \text{ m}$
122 quadrats in each surface type in a $100 \text{ m} \times 100 \text{ m}$ plot for measuring ecosystem respiration.

123 (Insert Figure 1 here)

124 Soil temperature and moisture at 10 cm were measured in a $100 \text{ m} \times 100 \text{ m}$ plot where
125 ecosystem respiration was measured by using an auto-measurement system (Decagon Inc.,
126 USA) from early June to the late August. The system consisted of an EM50 logger and five
127 5TM sensors. The data were logged automatically every 30 minutes. Soil saturated hydraulic
128 conductivity and compactness were measured one time in each month from June to August.
129 Soil saturated hydraulic conductivity was measured by Dual Head infiltrometer (Decagon Inc.,
130 USA). The measurement process included soak time 15 minutes, hold time 20 minutes at low
131 pressure head (5 cm) and high pressure head (15 cm) with 2 cycles. Each measurement takes
132 95 minutes altogether. Soil compactness was measured with TJS-750 (Hangzhou Top
133 Instrument co., LTD, Hangzhou, China) from the soil surface to 10 cm depth. Ecosystem

134 respiration rates were measured using the LICOR-8150 Automated Soil CO₂ Flux System,
135 which was an accessory for the LI-8100A could connect 16 individual chambers at one time
136 and were sampled and controlled by the LI-8100A Analyzer Control Unit. The air
137 temperature inside of the chamber was measured using the internal thermistor of the chamber.
138 The ecosystem CO₂ fluxes were calculated by the equation as follow.

$$139 \quad F_c = \frac{10VP_0 \left(1 - \frac{W_0}{1000}\right) \frac{\partial C'}{\partial t}}{RS(T_0 + 273.15)}$$

140 where F_c is the soil CO₂ efflux rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), V is volume (cm^3), P_0 is the initial pressure
141 (kPa), W_0 is the initial water vapor mole fraction (mmol mol^{-1}), S is soil surface area (cm^2), T_0
142 is initial air temperature ($^{\circ}\text{C}$), and $\partial C'/\partial t$ is the initial rate of change in water-corrected CO₂
143 mole fraction ($\mu\text{mol}^{-1} \text{mol s}^{-1}$).

144 Six LICOR-8100-104 long-term opaque chambers (20cm in diameter LICOR, Inc.,
145 Lincoln, NE, USA) were used to measure alternately between three replicates for six land
146 surface types. Therefore, 3 days at least were required to complete one rotation measurements
147 of ecosystem respiration. To measure ecosystem respiration, eighteen polyvinyl chloride
148 collars with a 20 cm inner diameter and a 12 cm height were inserted into the soil with 3-4 cm
149 exposed to the air (Qin et al., 2013). All of the collars were installed at least 24 h before the
150 first measurement to reduce disturbance-induced ecosystem CO₂ effluxes. Ecosystem
151 respiration rates were measured every 7-10 days from June 16 to August 20 in 2016
152 depending on weather conditions. A round-the-clock measurement protocol was carried out
153 and ecosystem respiration rates were measured every 30 minutes. Each measurement takes 1
154 minute and 45 seconds, including pre-purge 10 seconds, dead band 15 seconds, observation
155 length 1 minute and post-purge 20 seconds.

156 **Soil and vegetation sampling**

157 Soil samples were collected during the periods of late July to early August 2016. In each
158 surface type of each plot, five soil cores were collected using a stainless-steel auger (5 cm in
159 diameter) at depths of 0-10, 10-20, 20-30 and 30-40 cm, and bulked as one composite sample
160 for each depth in each quadrat. Another five soil cores were sampled by cylindrical cutting
161 ring (7 cm in diameter and 5.2 cm in depth) to determine soil bulk density from each land

162 surface type. Pika tunnel was approximate 6 cm in diameter and 40 cm in depth. Therefore,
 163 soil samples were available to collect at depth of 40cm. Totally, 512 soil samples were
 164 collected. Soil samples were firstly air-dried, then removed gravel and stone with manual
 165 sieving and finally weighed. The remaining soil samples with diameter less than 2 mm were
 166 ground to pass through a 0.25 mm sieve for analysis of soil organic carbon (SOC) and soil
 167 total nitrogen (TN) concentration. SOC was measured by dichromate oxidation using
 168 Walkley-Black acid digestion (Nelson and Sommers, 1982). TN was determined by digestion
 169 and then tested using a flow injection analysis system (FIAstar 5000, Foss Inc., Sweden).
 170 Aboveground and belowground biomasses were determined within a 1 m × 1 m quadrat on 4
 171 August 2016 during peak biomass and species diversity. There were a total of 108
 172 aboveground and belowground vegetation samples (3 plots × 6 land surface types × 3
 173 replicates) from the study area. Aboveground biomass was determined by clipping all
 174 above-ground living plants at ground level, drying (oven-dried at 65°C for 48 h) and weighing.
 175 Belowground biomass was sampled by collecting five soil columns, and each soil column was
 176 5 cm in diameter and 40 cm in depth. Soil cores were washed with a gentle spray of water
 177 over a fine mesh screen until soil separated from the roots, and then drying (oven-dried at
 178 65°C for 48 h) and weighing.

179 **Statistical analysis**

180 The soil organic C (kg m⁻²) and total N (kg m⁻²) densities in different land surface were
 181 calculated using the equation (1) and (2):

$$182 \quad \text{SOC} = \sum_{i=1}^n \rho * (1 - \sigma_{\text{gravel}}) * C_{\text{SOC}} * D_i \quad (1)$$

$$183 \quad \text{TN} = \sum_{i=1}^n \rho * (1 - \sigma_{\text{gravel}}) * C_{\text{TN}} * D_i \quad (2)$$

184 where SOC is soil organic C density, TN is soil total N density, ρ is the soil bulk density (g
 185 cm⁻³), σ_{gravel} is the relative volume of gravel (% w/w), C_{SOC} is soil organic C content (g kg⁻¹),
 186 C_{TN} is soil total N content (g kg⁻¹) and D_i is soil thickness (cm) at layer i , respectively; $i=1, 2,$
 187 3 and 4.

188 The data were presented as mean ± standard deviation. Statistical analyses were performed
 189 using the SPSS 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). One-way

190 analysis of variance (ANOVA) and a multi-comparison of a least significant difference (LSD)
191 test were used to determine differences at the $p=0.05$ level. The relationships of ecosystem
192 respiration with biotic and abiotic factors were analyzed by correlation analysis using R.

193 **Results**

194 **Ecosystem respiration**

195 Pika disturbance and patchiness had significant effect on ecosystem respiration (Table 1,
196 $P<0.001$). During the growing season, ecosystem respiration has a maximum value in August
197 and minimum value in June (Figure 2). In June, ecosystem respiration under intact grassland,
198 above pika tunnel, small patch and pika pile had no significant difference and the lowest
199 ecosystem respiration were found under large and medium patches (Figure 2). Average
200 ecosystem respiration under intact grassland was $4.03 \mu\text{mol m}^{-2} \text{s}^{-1}$, which were 6.90 % to
201 102.50 % higher than other surface types both in July and August (Figure 2).

202 (Insert Figure 2 here)

203 **Microclimate and soil hydrothermal characteristics**

204 Mean temperature and total rainfall during the growing seasons from 1 May to 30 September
205 in 2016 were $6.18 \text{ }^{\circ}\text{C}$ and 343.4 mm, respectively (Figure 3). Soil temperature and moisture
206 were significantly different ($P<0.001$) among various surface types (Table 1). The monthly
207 average soil temperature was in a range of $8.20\text{-}13.72 \text{ }^{\circ}\text{C}$ during June to August, which was
208 approximate $1\text{-}3 \text{ }^{\circ}\text{C}$ higher under pika pile and bald patches than the intact grassland (Figure
209 4a, $P<0.05$). The monthly mean soil moisture from June to August was approximate 30 % for
210 intact grassland and above pika tunnel, 25 % for small patch and pika pile, and 20 % for
211 larger and medium patch (Figure 4b). Soil saturated hydraulic conductivity also showed
212 significant variation under different land surface types ($P=0.027$, Table 2). Soil saturated
213 hydraulic conductivity of intact grassland had no significant difference with small patch and
214 above pika tunnel ($P>0.05$), while it was approximate 40 % higher than medium and large
215 patches and 17 % lower than pika pile (Figure 5).

216 (Insert Table 1, Figure 3 to 5 here)

217 **Soil and vegetation properties**

218 Both pika disturbance and patchiness significantly affected soil compactness, SOC density,
219 TN density and vegetation biomass (Table 2) ($P<0.001$). Soil compactness was over 0.30 Pa

220 in intact grassland and above pika tunnel, approximate 0.20 Pa for bald patches and less than
221 0.10 Pa for pika pile (Figure 6), respectively. Mean SOC and TN density under intact
222 grassland were 52.45 % and 59.14 % less than above pika tunnel, whereas they were
223 9.69-30.12 % and 22.47-109.62 % higher than pika pile and bald patches (Figure 7).
224 Aboveground and belowground biomass under intact grassland were approximate 30 %
225 higher than above pika tunnel, 90 % higher than pika pile, 123-252 % and 134-289 % higher
226 than bald patches (Figure 8a, b).

227 (Insert Table 2, Figure 6 to 8 here)

228 **Factors regulate ecosystem respiration**

229 We analyzed the relationships of ecosystem respiration with biotic and abiotic factors for six
230 land surface types (Figure 9). Correlation analysis showed that ecosystem respiration had no
231 significant correlation with soil temperature ($P>0.05$, Figure 9). However, ecosystem
232 respiration was significantly and positively related to soil moisture ($P<0.01$), soil total
233 nitrogen ($P<0.05$), aboveground ($P<0.05$) and belowground biomass ($P<0.05$) (Figure 9).

234 (Insert Figure 9 here)

235 **Discussion**

236 **Effect of pikas disturbance on ecosystem respiration**

237 Pikas burrowing activities increased oxygen content in deep soil, which contributed to the
238 decomposition of soil organic matter (Martin, 2003). The deposition of urine and feces by
239 small herbivorous mammals could also promote ecosystem nutrition circulation (Clark et al.,
240 2005). It was suggested that excreta deposited by pikas and frequently haunted in or near their
241 burrows supplied organic C available to microbial decomposition with an increase in
242 ecosystem CO₂ emission (Cao et al., 2004). Indeed, SOC and TN densities reached up to
243 14.54 and 0.98 kg m⁻² in above pika tunnel, which was 2.45 and 2.10 times higher than that of
244 intact grassland (Figure 7), respectively. The consistent results reported that the contents of
245 available soil nutrients around the pikas burrow were higher than those in control sites on an
246 alpine meadow (Zhang et al., 2016). We also found that SOC and TN densities under pika pile
247 decreased 13.35 % and 42.93 % than intact grassland. However, no significant difference of
248 Re was found between intact grassland and above pika tunnel, while Re under pika pile were
249 42.08 % less than intact grassland (Figure 2). The similar result was also found in an alpine

250 meadow on the QTP (Peng et al., 2015), which indicated that ecosystem respiration decreased
251 with increasing of pika holes because of grassland biomass regulated soil C and N with
252 increasing number of pika holes. These results confirmed that pikas disturbance did not
253 increase ecosystem carbon emission directly, but facilitated CO₂ emission into the atmosphere
254 through pika holes (Qin et al., 2015a). The difference of ecosystem respiration between intact
255 grassland and pika piles was mainly related to changes in vegetation biomass and soil
256 moisture. For example, both aboveground and belowground biomass decreased 244.62 % and
257 279.89 % under pika piles compared with the intact grassland (Figure 8). The reduction of
258 vegetation biomass production decreased aboveground plant respiration and root respiration
259 by decreasing carbon allocation (e.g., root exudates and litter, and available SOC) (Raich and
260 Potter, 1995; Högberg et al., 2002; Yang et al., 2018). Consistent with previous studies which
261 demonstrated that pikas burrowing activity increased water infiltration rate (Hogan, 2010;
262 Wilson and Smith, 2015), our results also showed that soil saturated hydraulic conductivity in
263 pika pile was significantly higher than bald and vegetation patches (Figure 5). Nevertheless,
264 the increased water infiltration was unable to increase soil moisture under pika piles. For
265 example, soil moisture under pika piles was approximate 5 % lower than intact grassland
266 (Figure 4). Our result was discrepant with previous studies which reported old pika mound
267 had the highest soil moisture during the summer (Ma et al., 2018) and moderate pika
268 burrowing activities increased surface soil moisture (Li and Zhang, 2006). This difference
269 may be contributed to the high pika density in alpine meadow (Guo et al, 2017). Moreover,
270 pika piles were loose (Figure 6) with less vegetation cover (Figure 8), which was not
271 beneficial for soil moisture storage.

272 **Effect of patchiness on ecosystem respiration**

273 Our results clearly showed that patchiness resulted in significant reduction of ecosystem
274 carbon emission. Compared with the intact grassland, ecosystem respiration decreased
275 approximate 17-48 % for bald patches (Figure 2). Two possible mechanisms could account
276 for the effects of patchiness on ecosystem respiration. On one hand, the reduction of SOC and
277 TN decreased microbial respiration by decreasing substrate supply to microbes in the
278 rhizosphere (Nobili et al., 2001; Scott-Denton et al., 2010). Our results indicated that
279 patchiness caused evident loss of SOC and TN (Figure 7) due to reduction in C input from

280 vegetation and increasing in C output from soil erosion (Qin et al., 2018). Previous study have
281 shown that the spatial heterogeneity of soil respiration was attributed to uneven soil organic
282 carbon and total nitrogen content (Xu and Qi, 2010). Soil organic carbon was considered as
283 the basic substrate of CO₂ emission by microbial decomposition (Sikora and Mccoy, 1990)
284 and soil total N enhanced ecosystem CO₂ emission by providing a source of protein for
285 microbial growth (Tewary et al., 1982). On the other hand, low moisture availability would
286 limit microbial respiration by restricting access to C substrates, reducing the diffusion of C
287 substrates and extracellular enzymes, and limiting microbial mobility (Yuste et al., 2003;
288 Wang et al., 2014). Our results showed that soil moisture under large and medium patches
289 decreased 10 % than intact grassland (Figure 4). Previous studies had reported that the soil
290 compaction of bald patches decreased the rate of water infiltration (Wuest et al., 2006; Wilson
291 and Smith, 2015), which was similar with our results showed that bald patches had less
292 saturated soil hydraulic conductivity (Figure 5). Low vegetation cover under bald patches was
293 not beneficial for water retention and utilization, where most of soil water was mainly lost as
294 a way of evaporation (Yi et al., 2014). We have measured evaporation of the intact grassland,
295 isolate grassland, large patches, medium patches and small patches since the early June 2016.
296 Three years results indicated that evaporation under bald patches were higher than the intact
297 grassland (data were not shown here).

298 **Factors affected ecosystem respiration**

299 Most previous studies showed that soil temperature explained most of the temporal variation
300 of ecosystem respiration on the alpine grassland on the QTP (Lin et al, 2011; Qin et al., 2015c;
301 Zhang et al., 2017). Our results indicated that soil temperature under pika piles and bald
302 patches was approximate 1 to 3 °C higher than intact grassland (Figure 4), which mainly
303 resulted from the heterogeneity of surface albedo, surface soil water retention, heat
304 conduction properties and radiation (Beringer et al., 2005; Pielke, 2005; Yi et al., 2013; You et
305 al., 2017). It was suggested that pikas disturbance create a better soil temperature buffer for
306 them to avoid the extreme cold in winter (Ma et al., 2018), whereas high soil temperature
307 under bald patch was a disadvantage for the recovery of vegetation because patch surface had
308 the smallest soil moisture content (Figure 4) and the largest daily range of soil temperature
309 (Ma et al., 2018). However, no an obvious relationship between Re and soil temperature was

310 found in the present study (Figure 9), which suggested that other factors involved in
311 controlling Re induced by pikas disturbance and patchiness. Our results showed that Re were
312 positively correlated with soil moisture, soil total nitrogen, aboveground and belowground
313 biomass (Figure 9). Pikas disturbance and patchiness led to the drying and loosening of soil
314 (Figure 4 and 6). It was considered that loose, dry surface sediments and strong winds were
315 the primary factors responsible for soil erosion (Dong et al., 2010b) and wind erosion was
316 especially common in arid and semi-arid regions (Zhang and Dong, 2014). This resulted in
317 the reduction of soil organic carbon, total nitrogen and vegetation biomass (Figure 7 and 8).
318 The alteration of these biotic and abiotic factors induced by pikas disturbance and patchiness
319 led to the decline of ecosystem respiration. Nevertheless, the decline of ecosystem respiration
320 did not completely offset the sequestration of C fixed by photosynthesis because of the lower
321 vegetation cover under bald patches and pika piles. Given the large area covered by bald
322 patches in alpine grasslands, patchiness was more susceptible to erosion and exert greater
323 influence on ecosystem respiration than pikas disturbance. Recent study has also reported that
324 bald patches of various sizes on the grasslands played a much more important role than pikas
325 direct disturbance in reducing vegetation cover, aboveground biomass, soil carbon and
326 nitrogen (Yi et al., 2016).

327 **Conclusions**

328 In this study, we investigated soil physicochemical properties, vegetation biomass and
329 ecosystem respiration (Re) under six land surfaces originating from pikas disturbance and
330 patchiness. We also analyzed the dominant factors regulated the Re. Our results showed that
331 pikas disturbance and patchiness decreased soil moisture but increased soil temperature,
332 which may be conducive to pikas survive in cold season but disadvantage for vegetation
333 growth. Patchiness caused evident decreasing in SOC and TN density, while both SOC and
334 TN density showed different response under pika piles and burrows. Both pikas disturbance
335 and patchiness decreased ecosystem carbon emission, and ecosystem respiration sharply
336 correlated with soil moisture, TN and vegetation biomass. Our results indicated that pikas
337 disturbance and patchiness led to the changing of ecosystem respiration process owing to the
338 drying of soil and the reduction of substrate supply. However, the decline of ecosystem
339 respiration may not able to offset the sequestration of C fixed by photosynthesis.

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577 **Table 1.** ANOVA results of the effect of patches fragmentation and small mammal
 578 activities on soil temperature, soil moisture and ecosystem respiration.

	Soil temperature			Soil moisture			Ecosystem respiration		
	June	July	August	June	July	August	June	July	August
<i>F</i>	8.614	10.955	1.806	387.472	210.878	97.060	5.270	10.447	8.855
<i>P</i>	<0.001	<0.001	0.106	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

579 **Table 2.** ANOVA results of the effect of patches fragmentation and small mammal activities
 580 on **soil compactness**, aboveground biomass, belowground biomass, soil hydraulic
 581 conductivity, SOC and TN density.

	Soil compactness	Aboveground biomass	Belowground biomass	Saturated hydraulic conductivity	SOC density	TN density
<i>F</i>	81.506	6.193	12.925	2.752	145.942	50.567
<i>P</i>	<0.001	0.002	<0.001	0.027	<0.001	<0.001

582

583 **Figure legends**

584 **Figure 1.** An aerial photo of field observation of ecosystem respiration at six surface types: (1)
585 Large bald patch (LP), (2) Medium bald patch (MP), (3) Small bald patch (SP), (4) Intact
586 grassland patch (IG), (5) above pika tunnel (PT) and (6) old Pika pile (PP).

587 **Figure 2.** Ecosystem respiration of different surface types: (1) large bald patch (LP), (2)
588 medium bald patch (MP), (3) small bald patch (SP), (4) intact grassland patch (IG), (5) above
589 pika tunnel (PT) and (6) old pika pile (PP).

590 **Figure 3.** Daily average air temperature and precipitation of the study site in 2016.

591 **Figure 4.** Monthly average soil temperature and soil moisture under different surface types:
592 (1) large bald patch (LP), (2) medium bald patch (MP), (3) small bald patch (SP), (4) intact
593 grassland patch (IG), (5) above pika tunnel (PT) and (6) old pika pile (PP).

594 **Figure 5.** Soil saturated hydraulic conductivity (SHC) under different surface types: (1) large
595 bald patch (LP), (2) medium bald patch (MP), (3) small bald patch (SP), (4) intact grassland
596 patch (IG), (5) above pika tunnel (PT) and (6) old pika pile (PP).

597 **Figure 6.** Soil compactness under different surface types: (1) large bald patch (LP), (2)
598 medium bald patch (MP), (3) small bald patch (SP), (4) intact grassland patch (IG), (5) above
599 pika tunnel (PT) and (6) old pika pile (PP).

600 **Figure 7.** Soil organic carbon (SOC) (a) and total nitrogen (TN) (b) density of different
601 surface types: (1) large bald patch (LP), (2) medium bald patch (MP), (3) small bald patch
602 (SP), (4) intact grassland patch (IG), (5) above pika tunnel (PT) and (6) old pika pile (PP).

603 **Figure 8.** Aboveground biomass (AGB) (a) and belowground biomass (BGB) (b) under
604 different surface types: (1) large bald patch (LP), (2) medium bald patch (MP), (3) small bald
605 patch (SP), (4) intact grassland patch (IG), (5) above pika tunnel (PT) and (6) old pika pile
606 (PP).

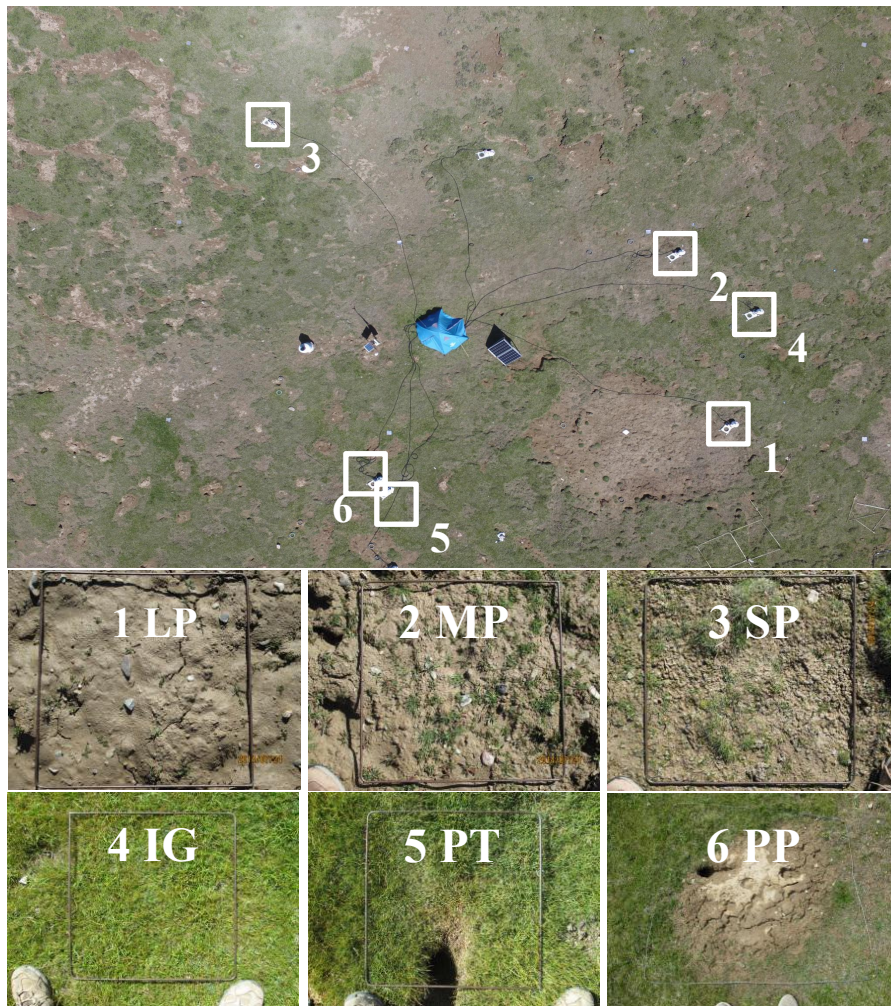
607 **Figure 9.** The correlation coefficient charts between ecosystem respiration (Re) and biotic
608 and abiotic factors for all six land surfaces. The diagonal line in the figure shows the
609 distributions of the variables themselves. The lower triangle (the left bottom of the diagonal)
610 in the figure shows scatter plots of the two properties. The upper triangle (the upper right of
611 the diagonal) in the figure indicates the correlation values of the two parameters; the asterisk
612 indicates the degree of significance (***) indicates significant differences at $P < 0.001$, *

- 613 indicates significant differences at $P < 0.01$, * indicates significant differences at $P < 0.05$).
- 614 The bold bigger numbers mean the higher correlation.

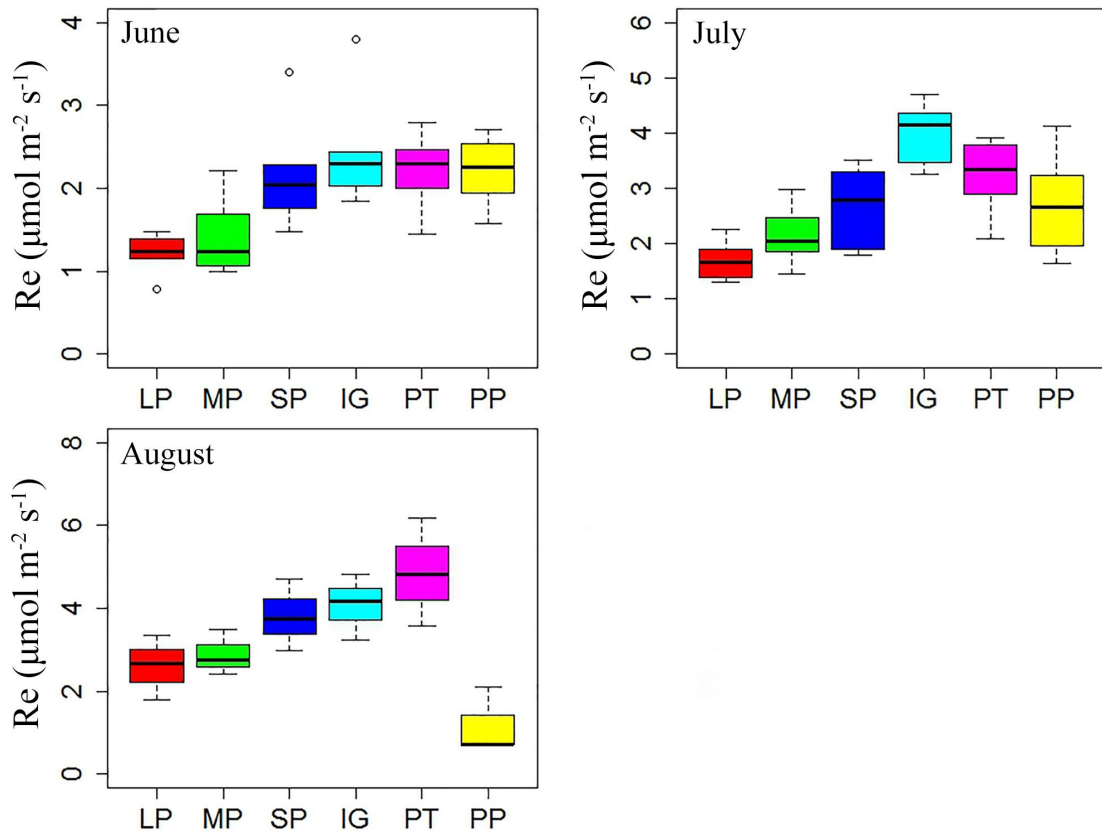
615 **Figure 1.**

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618 **Figure 2.**

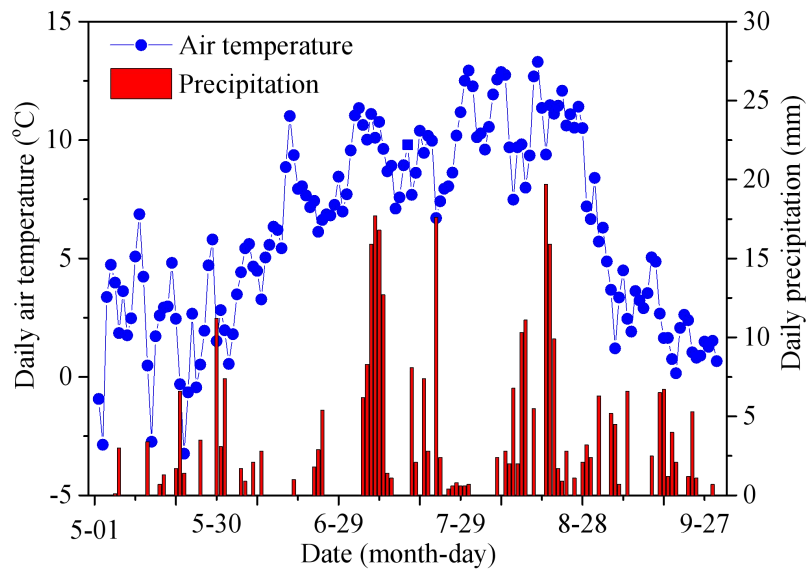


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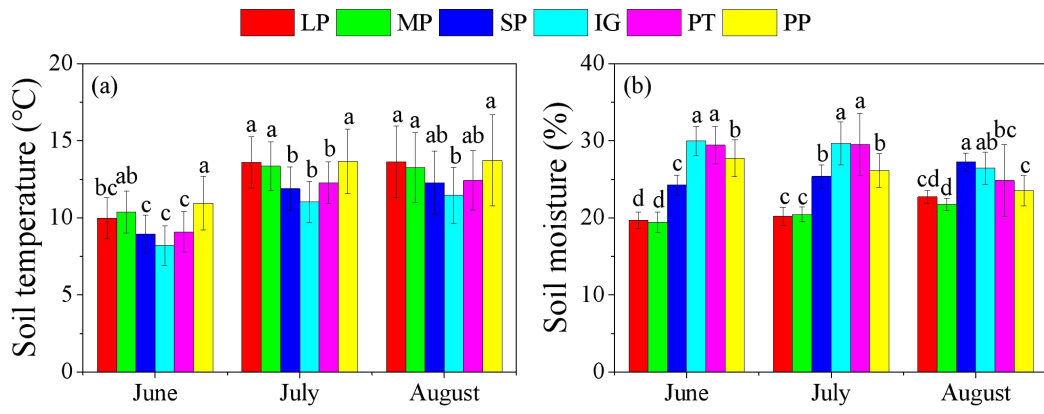
622 **Figure 3.**



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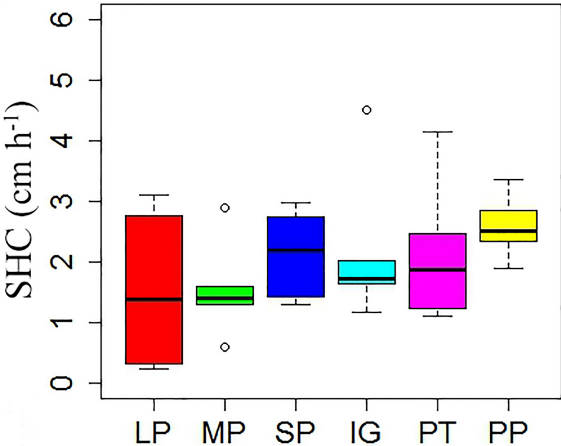
625 **Figure 4.**



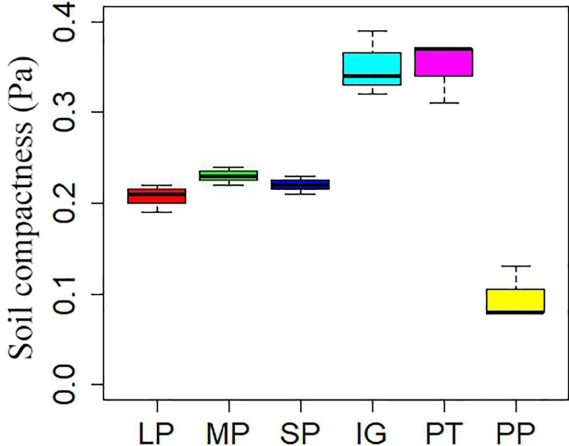
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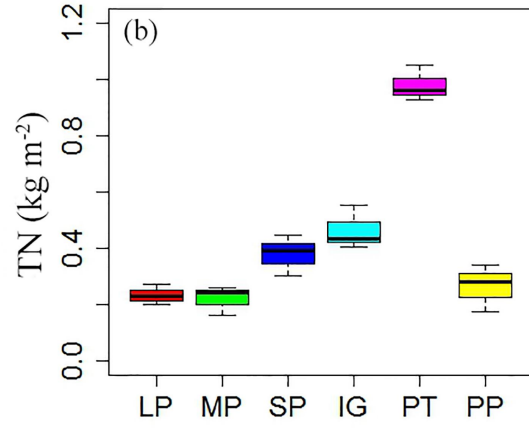
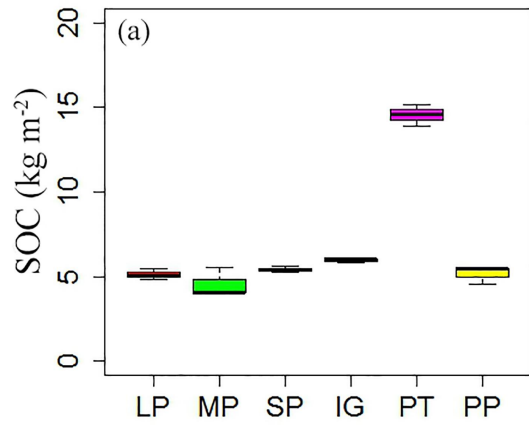


631 **Figure 6.**

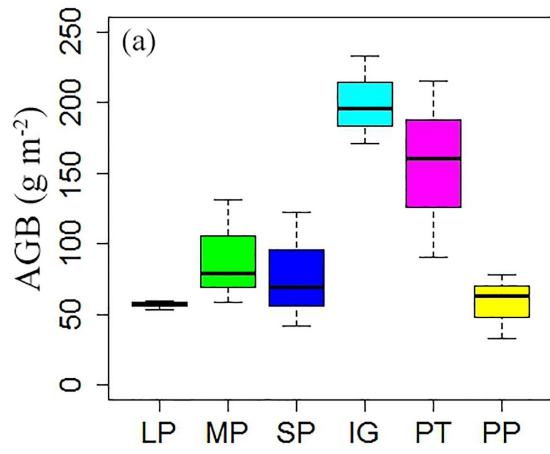


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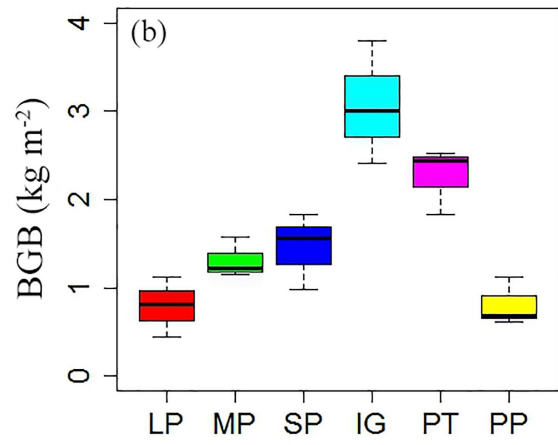
633 **Figure 7.**



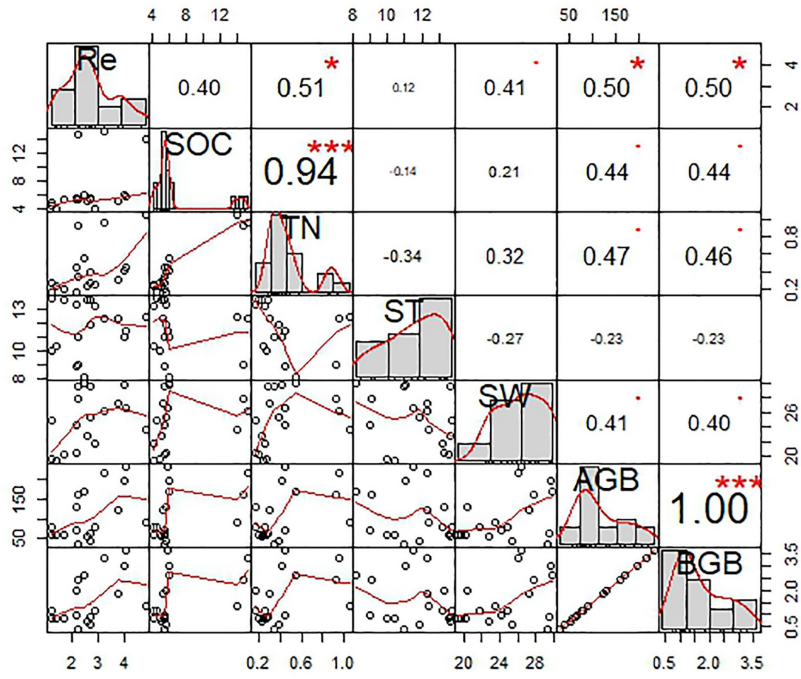
635 **Figure 8.**



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637 **Figure 9.**



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