Response to Anonymous Referee #1's comments:

General comments: The plateau pika (Ochotona curzoniae) is one of the main native soil faunas on the Qinghai-Tibet Plateau and plays a key role in the terrestrial ecosystem there. Previous studies have mainly focused on its active habits and the influence of population density on soil properties, plant communities, and so on. On contrast, the present study aims to study the effect of plateau pika disturbance and patchiness on ecosystem carbon emission at the plot scale (i.e. large bald patch, medium bald patch, small bald patch, intact grassland, above pika tunnel and pika pile). The results are critical for ecological restoration and environmental change on the Qinghai-Tibetan Plateau.

Our reply: We appreciate your positive comments. We have accepted all of the your suggestions and explained how we had revised the manuscript point by point.

Specific comments:

(1) Introduction: This section has not clarified clearly why we should study the effect of plateau pika disturbance and patchiness on ecosystem carbon emission at the plot scale, but not at other scales? What are the exact differences between this study and so many previous studies?

Our reply: Thank you for your careful review. We have revised the whole manuscript to eliminate the reader's confusion. In fact, we mainly focused on the effect of plateau pika disturbance and patchiness on ecosystem carbon emission of alpine meadow and we did not refer to any scales in this work. We have clarified clearly why we should study the effect of plateau pika disturbance and patchiness on alpine grassland at plot scale in our previous study (Yi et al., 2016).

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 homogeneous 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. We have revised this section as follow (Line 86-90).

"Previous studies have demonstrated that pikas disturbance and patchiness weaken the function of alpine meadow as a carbon sink (Liu et al., 13; Peng et al., 2015; Qin et al., 2018) and accelerated ecosystem carbon emission rate (Qin et al., 2015a). Nevertheless, most of these studies have mainly focused on ecosystem carbon emission rate under the homogeneous land surface rather than heterogeneous land surfaces."

Yi, S., Chen, J., Qin, Y., Xu, G.: The burying and grazing effects of plateau pika on alpine grassland are small: a pilot study in a semiarid basin on the Qinghai-Tibet Plateau, Biogeosciences, 13(22), 6273-6284, 2016.

(2) Materials and methods: Line 114-118: Is there any standard to distinguish the six representative underlying surfaces? Especially how to determine the threshold area for the division of large, medium and small bald patches (i.e. 9 m² and 1 m²)?

Our reply: Thank you for your careful review. Six representative underlying surfaces were selected according to the previous work in our study site (Yi et al., 2016; Qin et al., 2018). They were distinguished easily in aerial photographs. Large bald patches had less vegetation cover and the smallest side was larger than 3 m. Medium patches also covered by less vegetation cover and the larger side was in a range of 1 to 3 m and small bald patches were characteristic by less vegetation cover and the larger side was less than 1 m. Intact grassland was characteristic by high vegetation cover and no large and medium bare land was found. Pika tunnel and pika pile usually co-exited. Pika tunnel is approximately 6 cm in diameter and pika pile is in the front of pika tunnel, 60 cm in diameter and less vegetation cover.

We calculated the threshold area of large, medium and small patches by aerial photograph. Each aerial photograph has 12 million pixels. At a height of 20 m, the resolution of each pixel is ~1 cm and each photograph covers ~26 m × 35 m of ground. Pixels in each aerial image were first classified into two groups, i.e. vegetated or bare patches (Yi, 2016). Then patches with different sizes were created using OpenCv Library. And finally, fractions of vegetation and bare patches (large, medium and small patches) were calculated. We revised this part as follow (Line 114-118).

"At early June 2016, three 100 m \times 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)."

Yi, S.H., 2016. FragMAP: a tool for long-term and cooperative monitoring and analysis of small-scale habitat fragmentation using an unmanned aerial vehicle. Int. J. Remote Sens. 1-12. http://dx.doi.org/10.1080/01431161.2016.1253898.

Yi, S., Chen, J., Qin, Y., Xu, G.: The burying and grazing effects of plateau pika on alpine grassland are small: a pilot study in a semiarid basin on the Qinghai-Tibet Plateau, Biogeosciences, 13(22), 6273-6284, 2016.

Qin, Y., Yi, S., Ding, Y., Xu, G., Chen, J., Wang, Z.: Effects of small-scale patchiness of alpine grassland on ecosystem carbon and nitrogen accumulation and estimation in northeastern qinghai-tibetan plateau, Geoderma, 318, 52-63, 2018.

(3) Line 124-136: Were the soil temperature and moisture measured at all three 100 m \times 100 m plots or only one 100 m \times 100 m plots?

Our reply: Thank you for your question. Soil temperature and moisture were measured in one 100 m × 100 m plot where ecosystem respiration was measured. Both soil temperature and moisture were measured with three replicates under each underlying surface type. We revised this part to eliminate the confusion (Line 124-127).

"Soil temperature and moisture at 10 cm were measured in a $100 \text{ m} \times 100 \text{ m}$ 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"

(4) Were the soil saturated hydraulic conductivity, soil hardness and ecosystem respiration rates measured for only one time or many times during the study periods? These key questions should be clarified.

Our reply: Thanks for your suggestion. Soil saturated hydraulic conductivity and soil hardness under each surface type were measured one time every month from June to August. Ecosystem respiration was measured every 7-10 days from June 16 to August 20 depending on weather conditions. We therefore revised this part as follow (Line 124-155).

"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 once each month from June to August. Soil saturated hydraulic conductivity was measured by Dual Head infiltrometer (Decagon Inc., USA). The measurement process included 15 min soak time, 20 min hold time 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 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.

$$Fc = \frac{10VP_{\theta} \left(1 - \frac{W_{\theta}}{1000} \right)}{RS(T_{\theta} + 273.15)} \frac{\partial C'}{\partial t}$$

where Fc is the soil CO₂ efflux rate (µmol m⁻² s⁻¹), V is volume (cm³), P_{θ} is the initial pressure (kPa), W_{θ} is the initial water vapor mole fraction (mmol mol⁻¹), S is soil surface area (cm²), T_{θ} is initial air temperature (°C), and $\partial C'/\partial t$ is the initial rate of change in water-corrected CO₂ mole fraction (µmol⁻¹ 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."

(5) Line 138-141: How depth was the pika tunnel? Did this depth limit the collection of soil core to 40 cm?

Our reply: Thanks for your question. We investigated pika tunnel by digging soil pole and the depth of pika tunnel was about 40cm. Therefore, it wasn't difficulty to collect soil core at depth of 40cm. We have revised this part as follow (Line 157-164). "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."

(6) Discussion: Line 216-217: "Nevertheless, the increased water infiltration was unable to increase soil moisture under pika pile." Why? The potential reasons should be discussed.

Our reply: Thanks for your suggestion. We discussed the reason why the increased water infiltration was unable to increase soil moisture under pika pile as follow (Line 263-271).

"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."

(7) Line 227-229: The explanation for the low soil moisture under bald patches was not convincing, because the vegetation transpiration at intact grassland may be higher than the corresponding soil evaporation under bald patches at the same periods.

Our reply: Thanks for your comment. In fact, we have measured evaporation under different surfaces of the intact grassland, isolate grassland, large patches, medium patches and small patches since the early June 2016. It is difficult to measure evaporation from pika tunnle and pika pile due to their small sizes. Therefore, these data were not presented in this manuscript. We found that the evaporation under bald patches were higher than the intact grassland in our study sites through three years observation. We have revised this part as follow (Line 288-297).

"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)."

(8) Line 230-233: More details about the reason for the different soil temperature patterns should be added.

Our reply: Thank you for your suggestion. We have added more detailed information about the difference of soil temperature between intact grassland and pika pile and bald patches. This part has been revised as follow (Line 301-309).

"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)."

(9) Line 234-235: What is the reason for the description of "high soil temperature under bald patch was a disadvantage for the recovery of vegetation"?

Our reply: Thank you for your question. Our study site belongs to semi-arid region, where water was one of dominant limit factors for vegetation growth. Patch surface had the smallest soil moisture content and the largest daily range of soil temperature, which was not beneficial for soil water retention. We have changed this part as follow (Line 305-309).

"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)."

Ma, Y.J., Wu, Y.N., Liu, W.L., Li, X.Y., Lin, H.S.: Microclimate response of soil to plateau pika's disturbance in the northeast qinghai-tibet plateau, European Journal of Soil Science, 69(2), 232-244, 2018.

Technical corrections:

(1) Line 33: Delete "under".

Our reply: Thank you for your suggestion. We have deleted "under" according to your suggestion.

(2) Line 88-90: This sentence is not exact, because lots of previous researches have studied the heterogeneous underground vegetation and belowground soil properties.

Our reply: Thank you for your suggestion. 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. 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." (Line 88-90)

(3) Line 188-189: This sentence has the same mean with the sentence in line 185-186.

Our reply: Thank you for your suggestion. We have deleted this sentence according to your suggestion.

(4) Line 197-198: According to the description in line 172, the growing season in the study is from May to September. Please add the data about ecosystem respiration in May and September.

Our reply: Thank you for your suggestion. Actually, our field observation started at the early June and finished at the late August in 2016. It's pity we can't add the data of ecosystem respiration in May and September.

(5) Line 214: Change "Figure 3" to "Figure 4".

Our reply: Thank you for your suggestion. We have changed "Figure 3" to "Figure 4" according to your suggestion.

(6) Line 311: Some references cited in the text were not listed in the "Reference" section.

Our reply: Thank you for your suggestion. The references have been checked carefully through manuscript according to your suggestion. And now all the references cited in the manuscript are also included in the "Reference" section.

(7) Line 518-520: Six small photos below the aerial photo are not clear. Moreover, add "MP" after "2".

Our reply: Thank you for your suggestion. We have redrawn Figure 1 according to your suggestion. We also add "MP" after "2". We believe that the photos are clear now.

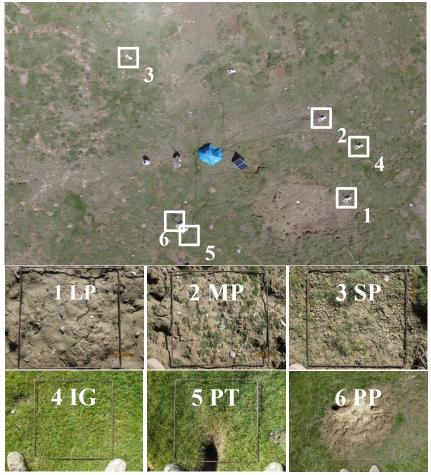


Figure 1. An aerial photo of field observation of ecosystem respiration at six surface types: (1) Large bald patch (LP), (2) Medium bald patch (MP), (3) Small bald patch (SP), (4) Intact grassland patch (IG), (5) above pika tunnel (PT) and (6) old Pika pile (PP).

(8) Line 539: The regression analysis was used to analyze the relationships of ecosystem respiration with biotic and abiotic factors (line 168-169). However, the result in figure 9 was only the correlation coefficient between them.

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 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 < 0.001, indicates significant differences at P < 0.05.). The bold bigger numbers mean the higher correlation."

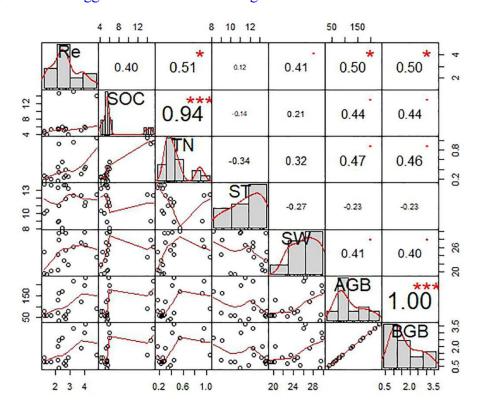


Figure 9. The correlation coefficient charts between ecosystem respiration (Re) and biotic and abiotic factors for all six land surfaces. The diagonal line in the figure shows the distributions of the variables themselves. The lower triangle (the left bottom of the diagonal) in the figure shows scatter 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 < 0.05.). 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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Abstract

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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 patched; 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.

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

Introduction

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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 CO₂ (Schimel et al., 2001; Cox et al., 2000; Grogan and Jonasson, 2005; Oberbauer et al., 48 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; Nakano et al., 2008; Buttlar et al., 2018), and supply of carbohydrate fixed by leaves, 52 vegetation litter and soil organic matter (Janssens et al., 2001; Reichstein et al., 2002). 53 54 Therefore, any external disturbance altering environmental conditions and affecting vegetation growth would exert profound influence on ecosystem carbon emission. 55 One of the basic function of terrestrial ecosystem is to regulate carbon balance between 56 the atmosphere and ecosystem (Canadell et al., 2007; Le Quéré et al., 2014; Ahlström et al., 57 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; Upadhyay et al., 2005). It was estimated that land degradation had resulted in 19-29 Pg C loss 61 worldwide (Lal, 2001). Over the past decades, grasslands have experienced patchiness 62 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 and Löfman, 1998). Other than climate change (Yi et al., 2014), vegetation self-organization 66 (Rietkerk et al., 2004; Venegas et al., 2005; McKey et al., 2010) or anthropogenic 67 disturbances (Kouki and Löfman, 1998; Yi et al., 2016), rodents burrowing activities were 68 also considered as the origin of the patchiness (Wei et al., 2006; Davidson and Lightfoot, 69 70 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; 71 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 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).

Plateau pikas (Ochotona curzoniae, hereafter pikas) are small mammals endemic to the alpine grasslands on the Qinghai-Tibetan Plateau (QTP) (Smith and Foggin, 1999; Lai and Smith, 2003). Living in underground, they excavated deep layer soil to surface through foraging and digging activities (Lai and Smith, 2003) and led to substantial bald piles on the ground. The bald pile was considered to gradually become bald patches under soil erosion, gravity, freeze-thaw and other factors (Chen et al., 2017; Ma et al., 2018). As a consequence, natural vegetation patches and adjacent bald patches with different sizes, and pikas piles represent the most common landscape pattern in the alpine meadow grassland on the QTP. Previous studies have demonstrated that pikas disturbance and patchiness weaken the function of alpine meadow as a carbon sink (Liu et al., 13; Peng et al., 2015; Qin et al., 2018) and accelerated ecosystem carbon emission rate (Qin et al., 2015a). Nevertheless, most of these studies have mainly focused on ecosystem carbon emission rate under the homogeneous land surface rather than heterogeneous land surfaces. It remains unclear what the differences of Re are among heterogeneous land surfaces, especially under the disturbance of pikas and patchiness. 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 Oinghai-Tibetan Plateau (OTP).

Materials and methods

Site description

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

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

Field observation

At early June 2016, three 100 m \times 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 \times 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 \times 2 m quadrats in each surface type in a 100 m \times 100 m plot for measuring ecosystem respiration.

123 (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 minutes. 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 minutes, hold time 20 minutes at low pressure head (5 cm) and high pressure head (15 cm) with 2 cycles. Each measurement takes 95 minutes 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.

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$$Fc = \frac{10VP_{\theta}\left(1 - \frac{W_{\theta}}{1000}\right)}{RS(T_{\theta} + 273.15)} \frac{\partial C'}{\partial t}$$

where Fc is the soil CO₂ efflux rate (µmol m⁻² s⁻¹), V is volume (cm³), P_{θ} is the initial pressure (kPa), W_{θ} is the initial water vapor mole fraction (mmol mol⁻¹), S is soil surface area (cm²), T_{θ} is initial air temperature (°C), and $\partial C'/\partial t$ is the initial rate of change in water-corrected CO₂ mole fraction (µmol⁻¹ 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.

Statistical analysis

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

$$SOC = \sum_{i=1}^{n} \rho * (1 - \sigma_{gravel}) * C_{SOC} * D_{i}$$
 (1)

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

where SOC is soil organic C density, TN is soil total N density, ρ is the soil bulk density (g

cm⁻³), σ_{garvel} is the relative volume of gravel (% w/w), C_{SOC} is soil organic C content (g kg⁻¹),

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.

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

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

Results

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 patches (Figure 2). Average ecosystem respiration under intact grassland was 4.03 μmol m⁻² s⁻¹, which were 6.90 % to 102.50 % higher than other surface types both in July and August (Figure 2).

202 (Insert Figure 2 here)

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 °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 8.20-13.72 °C during June to August, which was approximate 1-3 °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).

(Insert Table 1, Figure 3 to 5 here)

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).

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

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).

234 (Insert Figure 9 here)

Discussion

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

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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

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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 Re and soil temperature was

found in the present study (Figure 9), which suggested that other factors involved in controlling Re induced by pikas disturbance and patchiness. Our results showed that Re 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).

Conclusions

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

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

	Soil temperature			Soil moisture			Ecosystem respiration		
	June	July	August	June	July	August	June	July	August
\overline{F}	8.614	10.955	1.806	387.472	210.878	97.060	5.270	10.447	8.855
P	< 0.001	< 0.001	0.106	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001

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

	Soil	Soil Aboveground		Saturated hydraulic	SOC	TN
	compactness	biomass	biomass	conductivity	density	density
\overline{F}	81.506	6.193	12.925	2.752	145.942	50.567
P	< 0.001	0.002	< 0.001	0.027	< 0.001	< 0.001

577

- 583 Figure legends
- Figure 1. An aerial photo of field observation of ecosystem respiration at six 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).
- Figure 2. Ecosystem respiration of 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).
- Figure 3. Daily average air temperature and precipitation of the study site in 2016.
- Figure 4. 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
- 593 grassland patch (IG), (5) above pika tunnel (PT) and (6) old pika pile (PP).
- Figure 5.Soil saturated hydraulic conductivity (SHC) 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).
- 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
- pika tunnel (PT) and (6) old pika pile (PP).
- Figure 7. Soil organic carbon (SOC) (a) and total nitrogen (TN) (b) density of 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).
- Figure 8. Aboveground biomass (AGB) (a) and belowground biomass (BGB) (b) 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
- 606 (PP).
- 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 1.

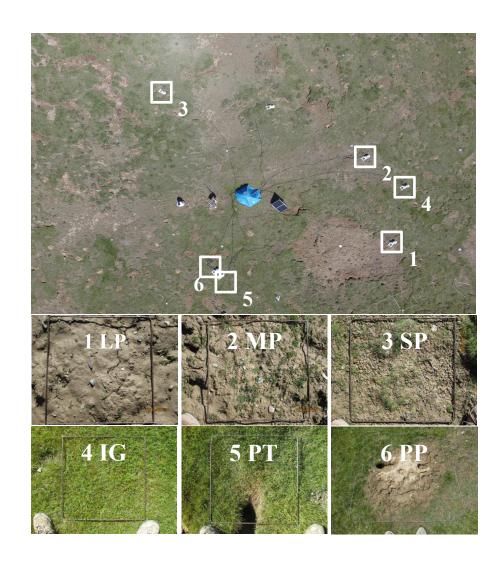


Figure 2.

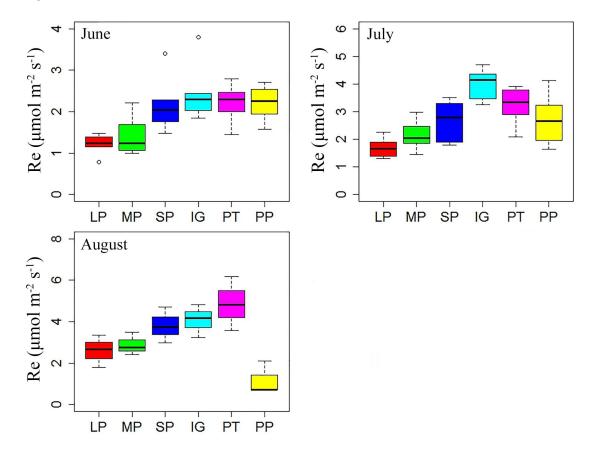


Figure 3.

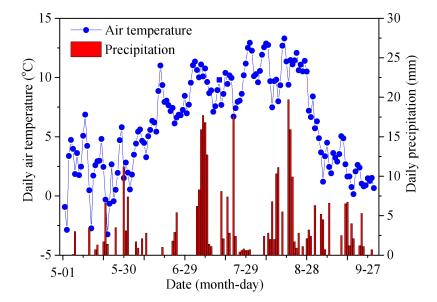


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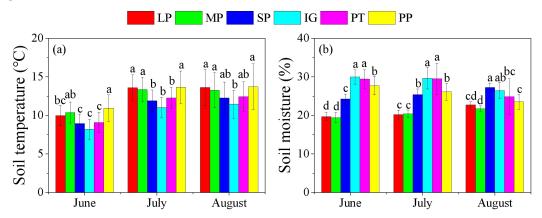


Figure 5.

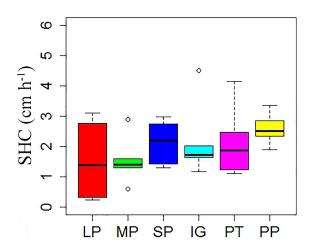


Figure 6.

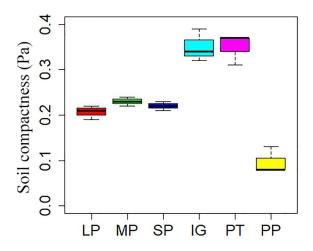


Figure 7.

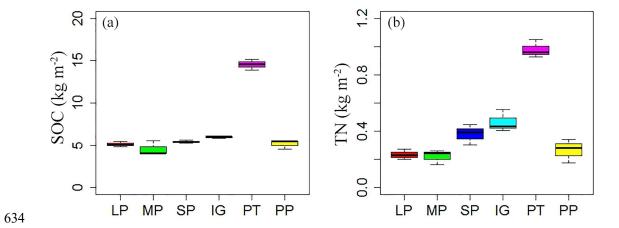


Figure 8.

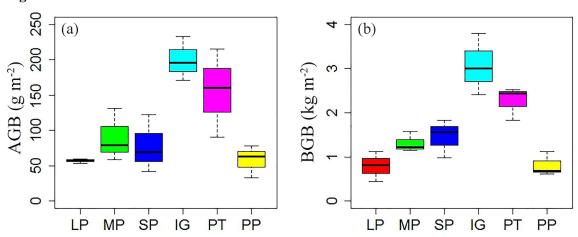


Figure 9.

