Response to Anonymous Referee #1's comments:

Most questions raised by previous reviews have been answered. However, there are still some corrections to be made (as follows), which should be revised before this paper could be accepted.

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.

Line 91-92: The land surface type “pika tunnel” was lost.

Our reply: Thanks for your careful review. We have added “pika tunnel” according to your suggestion (Line 91-95).

“(1) investigate the spatial heterogeneity of Re among different surface types (plateau pika pile, above pika tunnel, different sizes of bald patches and vegetation) of alpine grassland; (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).”

Line 159: What is the value and unit of R (ideal gas constant)?

Our reply: Thanks for your careful review. The unit of R (ideal gas constant) had been added according to your suggestion (Line 159-162).

“where Fc is the soil CO_2 efflux rate (μmol m^{-2} 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}), R is the ideal gas constant (J $K^{-1}mol^{-1}$), S is soil surface area (cm^2), T_0 is initial air temperature (°C), and $\partial C'/\partial t$ is the initial rate of change in water-corrected CO_2 mole fraction (μmol^{-1} s^{-1}). ”

Line 214-216: The description was inconsistent with Table 1.

Our reply: Thanks for your careful review. We have revised this part as follow (Line 215-217).

“Ecosystem respiration showed significant difference among varied land surface types during the growing season (Table 1, $P<0.001$). Except for the pika pile, ecosystem respiration maximized in August and minimized in June (Figure 2).”

Line 216-217: I don’t think the description is correct. For example, the Re of PP in June was higher than that in August.

Our reply: Thanks for your careful review. We completely agree with you that Re of PP in

June was higher than that in August. To eliminate the confusion, we have revised this part as follow (Line 215-217).

"Ecosystem respiration showed significant difference among varied land surface types during the growing season (Table 1, $P<0.001$). Except for the pika pile, ecosystem respiration maximized in August and minimized in June (Figure 2)."

Line 234-236: Please add the value. I think there may be some mistakes, because the description was inconsistent with Figure 5.

Our reply: Thanks for your careful review. We have added the value of soil saturated hydraulic conductivity under different surface types according to your suggestion (Line 234-241).

"Soil saturated hydraulic conductivity also showed significant variation under different land surface types ($P=0.027$, Table 2). For example, soil saturated hydraulic conductivity under large bald patch, medium bald patch, small bald patch, intact grassland patch, above pika tunnel and old pika pile were 1.54, 1.53, 2.14, 2.13, 2.12 and 2.58 cm h^{-1}, respectively (Figure 5). Soil saturated hydraulic conductivity under intact grassland patch was approximate 40 % higher than medium and large patches and 17 % lower than pika pile, while there was no significant difference among intact grassland patch, small patch and above pika tunnel ($P>0.05$)."

Line 256-378: This study was conducted only in growing season (June-August), however, some conclusions of cited references in the "Discussion" section were done all the year round. This may lead to lots of inconsistency, which should be clarified.

Our reply: Thanks for your careful review. We completely agree with you that some of conclusions of cited references in the "Discussion" section were done all the year round and we get them in growing season. Due to harsh environment on the QTP, it is difficult to conduct long-term field observation of ecosystem respiration all the year round. However, the main conclusions of cited references about the effect of patchiness and plateau pika on ecosystem respiration (Liu et al., 2013; Peng et al., 2015; Qin et al., 2015a), soil nutrition (Li and Zhang, 2006; Chen et al., 2017; Qin et al., 2018), soil temperature and soil moisture (Ma et al., 2018), vegetation biomass (Yi et al., 2016), soil saturated hydraulic conductivity (Wilson and Smith, 2015) at alpine grasslands in this manuscript were all conducted in the growing season. We therefore believe our conclusions are comparable with the previous studies.

Chen, J., Yi, S., Qin, Y.: The contribution of plateau pika disturbance and erosion on patchy alpine grassland soil on the Qinghai-Tibetan Plateau: Implications for grassland restoration, Geoderma, 297, 1-9, 2017.

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Liu, Y.S., Fan, J.W., Harris, W., Shao, Q.Q., Zhou, Y.C., Wang, N., Li, Y.Z.: Effects of plateau pika (Ochotona curzoniae) on net ecosystem carbon exchange of grass-land in the Three Rivers Headwaters region, Qinghai-Tibet, China, Plant. Soil., 366,491-504, 2013.

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.

Peng, F., Quangang, Y., Xue, X., 111, J., Wang, T.: Effects of rodent-induced land degradation on ecosystem carbon fluxes in alpine meadow in the Qinghai-Tibet Plateau, China, Solid. Earth., 6, 303-310, 2015.

Qin, Y., Chen, J.J., Yi, S.H.: Plateau pikas burrowing activity accelerates ecosystem carbon emission from alpine grassland on the Qinghai-Tibetan Plateau, Ecol. Eng., 84, 287-291, 2015a.

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.

Wilson, M.C. and Smith, A.T.: The pika and the watershed: The impact of small mammal poisoning on the ecohydrology of the Qinghai-Tibetan Plateau, Ambio, 44(1), 16-22, 2015.

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.

Line 434: Change “qinghai-tibet plateau” to “Qinghai-Tibet Plateau”.

Our reply: Thanks for your careful review. We have changed "qinghai-tibet plateau" to "Qinghai-Tibet Plateau" according to your suggestion (Line 437-438).

Cheng, G., Wu, T.: Responses of permafrost to climate change and their environmental significance, Qinghai-tibet plateau, J. Geophys. Res., 112(F2), 1-10, 2007.

Line 486: Add all author name instead of "et al".

Our reply: Thanks for your careful review. We have added all author names in this reference (Line 490-497).

"Janssens, I. A., Lankreijer, H., Matteucci, G., Kowalski, A. S., Buchmann, N., Epron, D., Pilegaard, K., Kutsch, W., Longdoz, B., Grünwald, T., Montagnani, L., Dore, S., Rebmann, C., Moors, E.J., Grelle, A., Rannik, Morgenstern, K., Oltchev, S., Clement, R., Gudmundsson, J., Minerbi, S., Berbigier, P., Ibrom, A., Moncrieff, J., Aubinet, M., Bernhofer, C., Jensen, N.O., Vesala, T., Granier, A., Schulze, E.D., Lindroth, A., Dolman, A.J., Jarvis, P.G., Ceulemans, R., Valentini, R.: Productivity overshadows temperature in determining soil and ecosystem respiration across european forests, Global. Change. Biol., 7(3), 269-278, 2001."

Line 677: Add the depth of soil temperature and soil moisture.

Our reply: Thanks for your careful review. We have added the depth of soil temperature and soil moisture in Figure 4 (Line 685-687).

"Figure 4. Monthly average soil temperature and soil moisture at 10 cm depth 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)."

Response to Anonymous Referee #2's comments:

The authors addressed most of my concerns.

Our reply: We appreciate your positive comments.

Response to Anonymous Referee #3's comments:

The quality of manuscript has been improved greatly in the revision. However, in the response, I find that you mentioned that the specific aims of this study were to (1) investigate the spatial heterogeneity of Re among different surface types (plateau pika pile, different sizes of bald patches and vegetation) of alpine grassland; ……this is ok, you say that plateau pika pile is just one of the surface types, so your question is one factor: surface types. But in data analysis you consider plateau pika disturbance as an independent factor to do TWO-WAY ANOVA. Consequently, the statistics is wrong.

Our reply: We appreciate your constructive comments. We completely agree with you that we have made wrong statistics by using TWO-WAY ANOVA. Actually, we used One-way analysis of variance (ANOVA) to determine the differences of variables among six surface types in the first revision. However, we misunderstood your comments in second revision and improperly used TWO-WAY ANOVA. Therefore, we reanalyzed the data by using ANOVA and revised the related section of "Statistical analysis", "Results" and "Table list".

Statistical analysis (Line 208-210)

"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."

Results

Ecosystem respiration (Line 215-217)

"Ecosystem respiration showed significant difference among varied land surface types during the growing season (Table 1, $P<0.001$). Except for the pika pile, ecosystem respiration maximized in August and minimized in June (Figure 2). "

Microclimate and soil hydrothermal characteristics (Line 234-241)

"Soil saturated hydraulic conductivity also showed significant variation under different land surface types ($P=0.027$, Table 2). For example, soil saturated hydraulic conductivity under large bald patch, medium bald patch, small bald patch, intact grassland patch, above pika tunnel and old pika pile were 1.54, 1.53, 2.14, 2.13, 2.12 and 2.58 cm h^{-1}, respectively (Figure 5). Soil saturated hydraulic conductivity under intact grassland patch was approximate 40 % higher than medium and large patches and 17 % lower than pika pile, while it was no significant difference among intact grassland patch, small patch and above pika tunnel ($P>0.05$)."

Soil and vegetation properties (Line 244-245)

"Soil and vegetation properties showed significant variation under different land surface types (Table 2) ($P<0.001$)."

Table list (Line 669-672)

Table 1. ANOVA results of soil temperature, soil moisture and ecosystem respiration under

different land surface types.

	Soil temperature			Soil moisture			Ecosystem respiration		
	June	July	August	June	July	August	June	July	August
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 soil compactness, aboveground biomass, belowground biomass, soil hydraulic conductivity, SOC and TN density under different land surface types.

	Soil compactness	Aboveground biomass	Belowground biomass	Saturated hydraulic conductivity	SOC density	TN density
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

By the way, if you consider patchiness and pika disturbance as two different independent factors, your table 1 and table 2 were incomplete, how about interaction of two factors.

Our reply: We appreciate your constructive comments. The treatments in this study do not meet the criteria for analyzing the interaction of patchiness and pika disturbance using TWO-WAY ANOVA. To eliminate the confusion, we have reanalyzed the data by ANOVA and revised the related part in the manuscript.

In conclusion, experimental design and data statistics at all are mismatching.

Our reply: We appreciate your constructive comments. The data were reanalyzed by ANOVA and the related sections were also revised. We believe experimental design and data statistics are consistent now.

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

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Abstract

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.

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

Introduction

Ecosystem respiration (Re) is the key process to determine the carbon budget in the terrestrial ecosystem. Thus, even a small imbalances between CO_2 uptake via photosynthesis and CO_2 release by ecosystem respiration can lead to significant interannual variation in atmospheric CO_2 (Schimel et al., 2001; Cox et al., 2000; Grogan and Jonasson, 2005; Oberbauer et al., 2007; Warren and Taranto, 2011). 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; Buttlar et al., 2018), and supply of carbohydrate fixed by leaves, vegetation litter and soil organic matter (Janssens et al., 2001; Reichstein et al., 2002). Therefore, any external disturbance altering environmental conditions and affecting vegetation growth would exert profound influence on ecosystem carbon emission.

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., 2006; 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).

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. Thus, the specific aims of this study were to (1) investigate the spatial heterogeneity of Re among different surface types (plateau pika pile, above pika tunnel, different sizes of bald patches and vegetation) of alpine grassland; (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).

Materials and methods

Site description

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 × 100 m plots were established as replicates. Each 100 × 100 m plot was in a distance of less than 50 m, which has the similar plant and terrain. In each plot, six representative land surfaces were selected: (1) large bald patch with size larger than 9.0 m^2 (LP), (2) medium bald patch with size of 1.0-9.0 m^2 (MP), (3) small bald patch with size of less than 1.0 m^2 (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). There were no other mammals, e.g. marmot and zokor in our study plots. All of the piles in each plot were created by plateau pikas. 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 largest side was in a range of 1 to 3 m and small bald patches were characterized by less vegetation cover and the largest side was less than 1 m. Intact grassland was characterized by high vegetation cover and no large and medium bare land was found. Pika tunnel and pika pile usually co-existed. 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, 2017). 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. For each surface type in each plot, six 1 m × 1 m quadrats were set up, of which three was used for soil saturated hydraulic conductivity measurement and three for soil compactness measurement, soil and vegetation sampling. We also set up another three 1 m × 1 m quadrats and three 2 m × 2 m quadrats in each surface type in a 100 m × 100 m plot for measuring soil temperature, soil moisture and ecosystem respiration.

(Insert Figure 1 here)

A meteorological tower was established in our observation station since 2008. Air temperature (°C) at 2.0m was measured by HMP45C (Vaisala, Helsinki, Finland), and precipitation was measured using an all-weather precipitation gauge (Geonor T-200B, Norway) (Wu et al., 2015). Soil temperature and moisture at 10 cm were 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 CO_2 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 CO_2 fluxes were calculated by the equation as follow.

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

where Fc is the soil CO_2 efflux rate (μmol m^{-2} 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}), R is the ideal gas constant (J $K^{-1}mol^{-1}$), S is soil surface area (cm^2), T_0 is initial air temperature (°C), and $\partial C'/\partial t$ is the initial rate of change in water-corrected CO_2 mole fraction (μmol^{-1} 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 CO_2 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

The soil organic C and total N densities in different land surface were calculated using the equation (2) and (3):

$$SOC = \sum_{i=1}^{n} \rho * (1 - \sigma_{gravel}) * C_{SOC} * D_i \tag{2}$$

$$TN = \sum_{i=1}^{n} \rho * (1 - \sigma_{gravel}) * C_{TN} * D_i \tag{3}$$

where SOC is soil organic C density (kg m^{-2}), TN is soil total N density (kg m^{-2}), 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, 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 Pearson correlation analysis using R.

Results

Ecosystem respiration

Ecosystem respiration showed significant difference among varied land surface types during the growing season (Table 1, $P<0.001$). Except for the pika pile, ecosystem respiration maximized in August and minimized 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 was found under large and medium patches (Figure 2).

Average ecosystem respiration under intact grassland was 4.01 μmol m^{-2} s^{-1} in July, which was 24.35 % to 137.39 % higher than other surface types (Figure 2). In August, average ecosystem respiration were 4.07 μmol m^{-2} s^{-1} and 4.85 μmol m^{-2} s^{-1} for intact grassland and above pika tunnel, 2.59-3.81 μmol m^{-2} s^{-1} for bald patches and 1.18 μmol m^{-2} s^{-1} for pika pile (Figure 2).

(Insert Table 1, 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 among various land surface types (Table 1, $P<0.05$). 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). For example, soil saturated hydraulic conductivity under large bald patch, medium bald patch, small bald patch, intact grassland patch, above pika tunnel and old pika pile were 1.54, 1.53, 2.14, 2.13, 2.12 and 2.58 cm h^{-1}, respectively (Figure 5). Soil saturated hydraulic conductivity under intact grassland patch was approximate 40 % higher than medium and large patches and 17 % lower than pika pile, while it was no significant difference among intact grassland patch, small patch and above pika tunnel ($P>0.05$).

(Insert Table 2, Figure 3 to 5 here)

Soil and vegetation properties

Soil and vegetation properties showed significant variation under different land surface types (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).

(Insert 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).

(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 CO_2 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. This was because pika burrowing activity transferred of deeper, nutrient-poor soil to the soil surface, improved soil aeration increased rate of organic carbon mineralization and soil erosion took away soil nutrition (Wei et al., 2006; Qin et al., 2015a; Chen et al., 2017). However, except July, no significant difference of Re was found between intact grassland and above pika tunnel, while Re under pika pile was 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 CO_2 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 CO_2 emission by microbial decomposition (Sikora and Mccoy, 1990) and soil total N enhanced ecosystem CO_2 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). It was well known that rising of soil temperature under natural condition enhanced ecosystem respiration by stimulating decomposition of soil organic matter (Conant et al., 2008), increasing plant biomass (Yi et al., 2014) and activity of microbial enzymes

(Bond-Lamberty and Thomson, 2010). However, obvious relationship between Re and soil temperature was not 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).

Effect of pikas disturbance on patchiness

Natural vegetation patches, bald patches with different sizes and pikas piles coexisted on the alpine meadow (Figure 1), which supported that alpine grassland had also experienced fragmentation (Qin et al., 2018). Several proposed mechanisms may be accounted for the formation and development of patchiness in alpine grassland. As one of dominant form of land utilization, alpine grasslands are widely used for grazing. Previous studies suggested that overgrazing destroyed the original vegetation and led to decrease in the coverage and looseness of soil (Dong et al., 2013), which was prone to form bald patch due to soil erosion (Fécan et al., 1998; Zhang and Dong, 2014). Other than livestock, alpine grassland is also habitats for many small mammals such as plateau pika, zokor (*Eospalax fontanierii*), marmot (*Marmota himalayana*) and fox (*Vulpes ferrilata*). Pikas were considered to create a patchy matrix by changing soil properties (Chen et al., 2017), digging tunnels and burying activities

(Dong et al., 2013). On one hand, pikas bury vegetation by fresh excavated soil, then small bare soil patches are formed and further large soil patches are then formed by linking small bare soil patches by wind and/or water (Wei et al., 2007; Ma et al., 2018). On the other hand, pikas dig tunnel underground. Although pikas make burrows are the primary homes to a wide variety of small birds and lizards (Smith and Foggin, 1999), the collapse of pika tunnels results in the emergence of bald soil patches (Zhou et al., 2003; Cao et al., 2010). Moreover, alpine grassland is underlain by extensive permafrost (Chen and Wu, 2007). The repeated freeze and thaw cause the crack of the sod around the barren area (Yang et al. 2003) and create precondition for forming bald patch. However, to date, there are no direct evidences to demonstrate the potential mechanism for forming and developing of patchiness for alpine grassland on the QTP. It is, therefore, critical to perform long-term repeated monitoring studies to determine whether bald patches are developed from pika piles or burrow tunnels and what the major factors affecting bald patch expansion are (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 soil temperature, soil moisture and ecosystem respiration under different land surface types.

	Soil temperature			Soil moisture			Ecosystem respiration		
	June	July	August	June	July	August	June	July	August
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 soil compactness, aboveground biomass, belowground biomass, soil hydraulic conductivity, SOC and TN density under different land surface types.

	Soil compactness	Aboveground biomass	Belowground biomass	Saturated hydraulic conductivity	SOC density	TN density
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

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). The upper solid lines, the bottom solid lines, the bold solid horizontal line and the empty dot mean the maximum value, minimum value, median and abnormal value. Letters on the error bars indicate significant differences among different surface types at $P < 0.05$.

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

Figure 4. Monthly average soil temperature and soil moisture at 10 cm depth 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).

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).

Figure 6. 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).

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 (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 red line means the frequency distribution of

variables. 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.

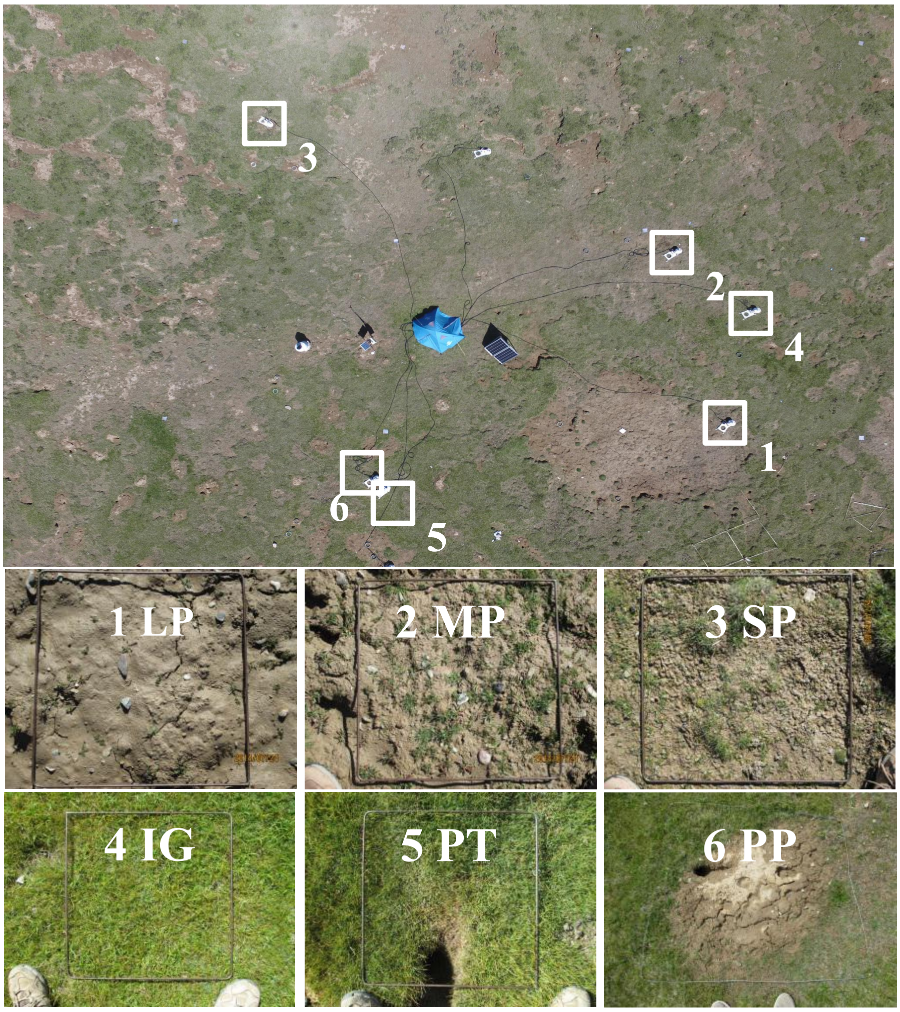
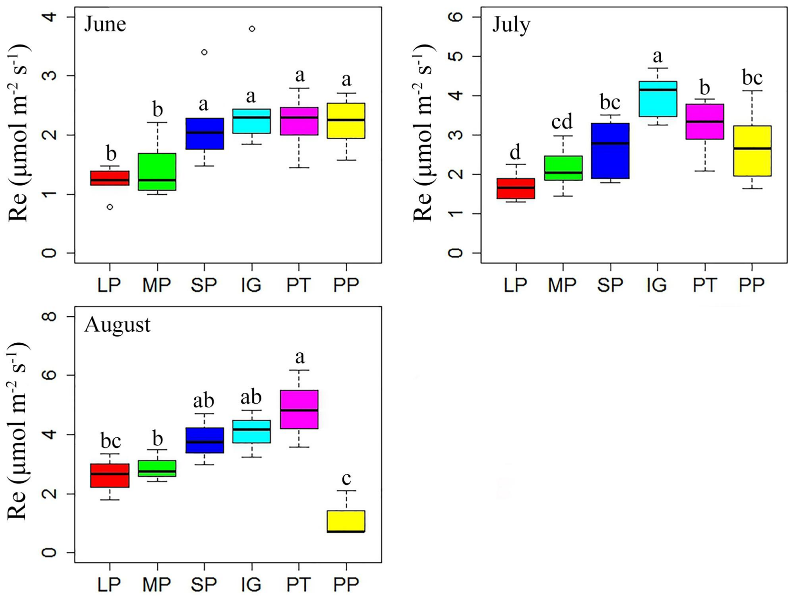
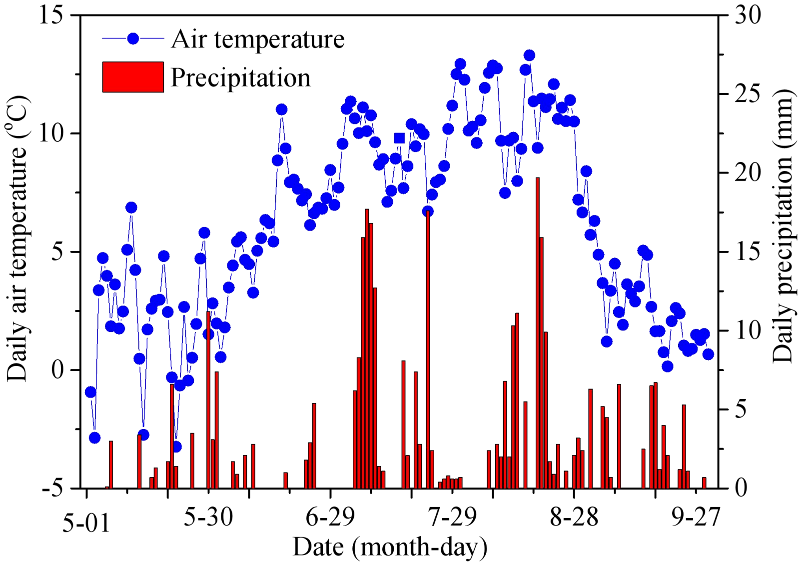


Figure 2.


June
July
August
Re (µmol m-2 s-1)
0
1
2
3
4
5
6
8
LP
MP
SP
IG
PT
PP
b
b
a
a
a
a
d
cd
bc
a
b
bc
bc
b
ab
ab
a
c

716 **Figure 3**.



717

718

Figure 4.

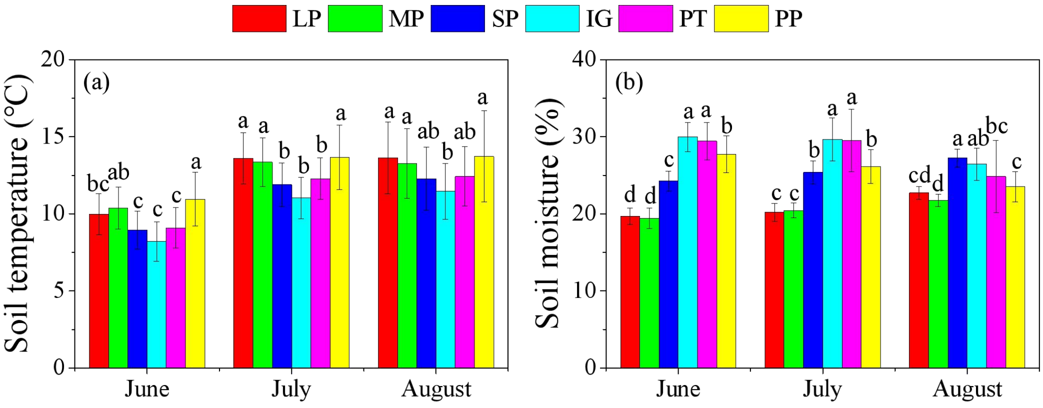

LP
MP
SP
IG
PT
PP
(a)
Soil temperature (°C)
0
5
10
15
20
bc ab c c c a
a a b b b a
a a ab b ab a
June
July
August
(b)
Soil moisture (%)
0
10
20
30
40
d d c a a b
c c b a a b
cd d a ab bc c
June
July
August

Figure 5.

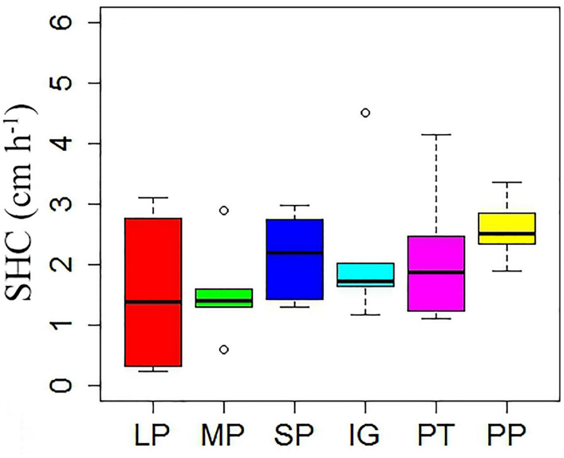


Figure 6.

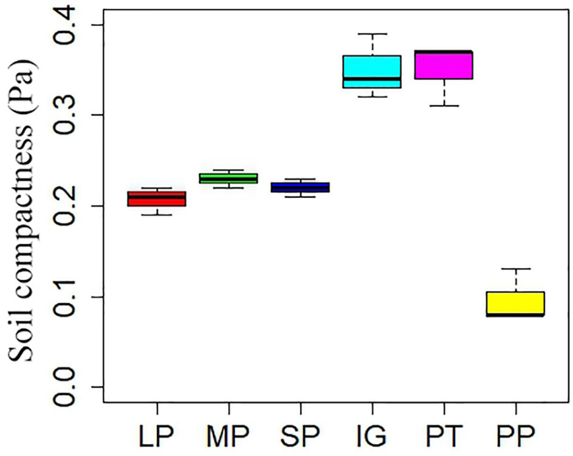


Figure 7.

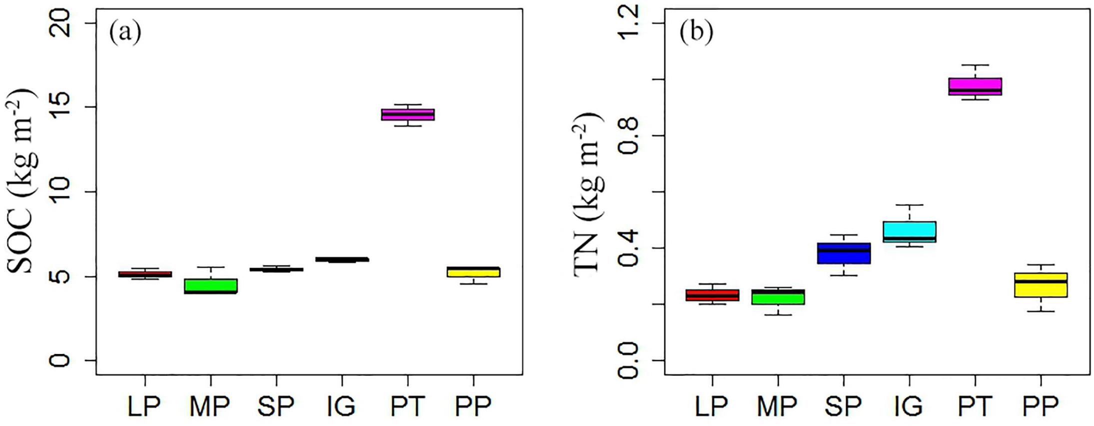

(a)
SOC (kg m^{-2})
0
5
10
15
20
LP
MP
SP
IG
PT
PP
(b)
TN (kg m^{-2})
0.0
0.4
0.8
1.2
LP
MP
SP
IG
PT
PP

Figure 8.

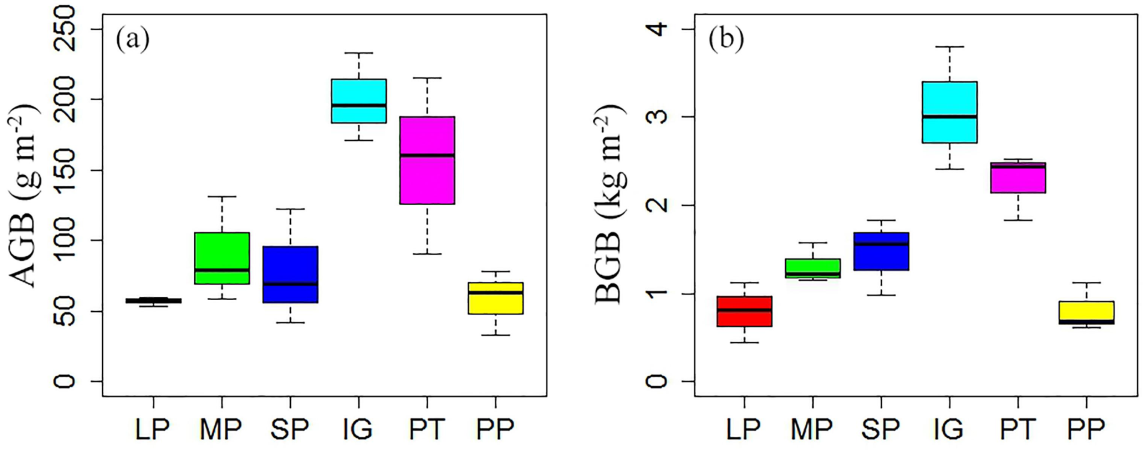
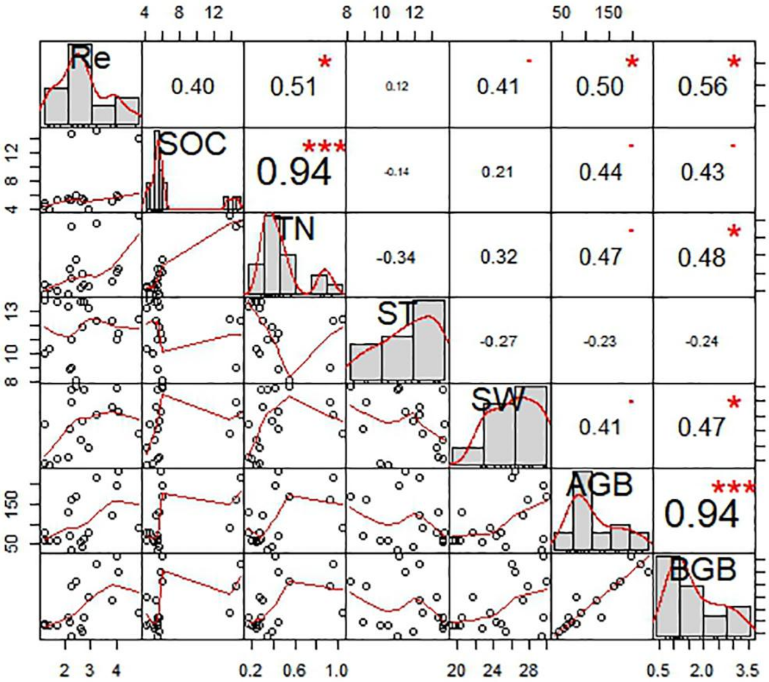

(a)
AGB (g m-2)
0
50
100
150
200
250
LP
MP
SP
IG
PT
PP
(b)
BGB (kg m-2)
0
1
2
3
4
LP
MP
SP
IG
PT
PP

Figure 9.


Re
SOC
TN
ST
SW
AGB
BGB
0.40
0.51 *
0.12
0.41 -
0.50 *
0.56 *
0.94 ***
-0.14
0.21
0.44 -
0.43 -
-0.34
0.32
0.47 -
0.48 *
-0.27
-0.23
-0.24
0.41 -
0.47 *
0.94 ***
4
8
12
8
10
12
50
150
2
3
4
0.2
0.6
1.0
20
24
28
0.5
2.0
3.5
13
10
8
26
20
0.8
0.2