

RESEARCH ARTICLE

# Impact of plateau pikas (*Ochotona curzoniae*) on soil properties and nitrous oxide fluxes on the Qinghai-Tibetan Plateau

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## Abstract

This paper demonstrates the impact of an endemic fossorial animal, plateau pika (*Ochotona curzoniae*), on soil properties and N<sub>2</sub>O flux at the Zoige Wetland. Pika burrow and control sites without disturbance by pika were selected to measure the soil water content, bulk density, soil organic matter (SOM), NH<sub>4</sub>-N content and NO<sub>3</sub>-N content in August 2012. N<sub>2</sub>O fluxes were measured with static opaque chambers at these sites in June and August 2012. Pika burrowing altered soil aeration by transferring deeper soil to the surface and by constructing underground burrows, which significantly increased bulk density, and reduced soil water content, SOM and NH<sub>4</sub>-N content at 0–10 cm and 10–20 cm soil depth. N<sub>2</sub>O flux had a significant correlation with bulk density, SOM and NH<sub>4</sub>-N content. Pika burrowing significantly influenced N<sub>2</sub>O flux by increasing N<sub>2</sub>O flux at the control site from near zero to 0.063±0.011 mg m<sup>-2</sup> h<sup>-1</sup>. Our findings described how pika burrowing influences the soil traits and significantly increases the principal greenhouse gas N<sub>2</sub>O emission. As plateau pika was commonly considered as a pest, our findings give a novel clue to effectively manage populations of plateau pika on the Qinghai-Tibet Plateau from the perspective of greenhouse gas emission.

## Introduction

Animal activity plays an important role in modulating biogeochemical cycles of water, carbon and nutrients, primarily through disturbance and altering soil properties [1,2]. In meadow ecosystems, burrowing activities of rodents, lagomorphs and other herbivorous mammals cut grass roots, dig and transfer soils from the subterranean to the surface, and excavate mounds [3,4]. These activities can profoundly change the structure and functional dynamics of meadow soils [5–7]. The altered soil properties and changed underground aerobic/anaerobic

environment are expected to influence carbon and nitrogen cycles and the ecosystem release of greenhouse gases to the atmosphere [8–10].

Nitrous oxide (N<sub>2</sub>O) is an important greenhouse gas whose warming potential is 298 times that of the same mass of carbon dioxide (CO<sub>2</sub>) [11]. The concentration of N<sub>2</sub>O in the atmosphere has increased from a preindustrial concentration of approximately 270±7 ppb in 1750 to 324.2 ppb in 2011 [12], and anthropogenic and natural soil disturbance has been an important sources for the increased atmospheric N<sub>2</sub>O [13,14]. N<sub>2</sub>O is produced in soils via the chemical processes of denitrification and nitrification, which are regulated by soil moisture, soil nitrogen (N) content, and temperature [15]. Burrowing activities can definitely influence N<sub>2</sub>O emissions through changing soil physical and chemical properties and modifying soil aeration conditions [16,17]. However, direct evidence on how and to what extent lagomorph burrowing activities impact soil properties and N<sub>2</sub>O emission of meadow ecosystems is still obscure [6]. Although the plateau pika is considered to be a harmful mammal for its burrowing and foraging, previous work has revealed that pikas are important for maintaining or restoring native plant communities by improving the soil quality [18]. Furthermore, due to the alteration of soil properties, monitoring the greenhouse gas emission in the increasing distribution area of pika burrow will improve understanding ecological role of pikas and then enhance the wildlife management in the Zoige Wetland.

In this study, we quantitatively investigate the impacts of the burrowing activities of plateau pika (*Ochotona curzoniae*) on soil properties and N<sub>2</sub>O emissions of an alpine meadow ecosystem in southeastern Qinghai-Tibetan Plateau. Plateau pika is an endemic species to the Qinghai-Tibetan Plateau widely distributed from the Himalayan to the Hengduan Mountains [19]. These animals live underground throughout their lifetime and are active burrowers, sometimes emerging for mating and food collection on the ground [20]. The burrowing reduces grass yield essential for local husbandry, e.g. yaks, goats etc., for which plateau pikas have been viewed as pests and are subject to pest controls [21]. However, others argue that plateau pikas are keystone species performing essential ecosystem roles, such as increasing plant diversity, in the alpine meadow ecosystem [22]. A better understanding of the role of plateau pikas' burrowing activity on soil properties and N<sub>2</sub>O, therefore, is important for assessing the ecosystem role of this endemic species.

## Material and methods

This study was authorized by the Management Bureau of Zoige National Nature Reserve. The field study did not involve endangered or protected species, and no specific permissions were required for the study.

## Site description and site selection

This study was conducted at Lake Huahu (33.5°N, 102.5°E, 3430 m a.s.l.), the Sichuan Zoige wetland National Nature Reserve, approximately 40 km north of Ruergai County town, Sichuan province, China. It has a typical Tibetan plateau cold climate. The annual mean precipitation is 650 mm and the mean temperature is 1.7 °C with a three-month growing season from June to August where most of the precipitation occurs [23].

As the groundwater table descends with the increase of the distance to the Huahu Lake water surface, plateau pikas got more possibility to inhabit in the area with low groundwater table to reduce the risks of being inundated. According to the groundwater table and distribution of pika burrow, two plots were selected for N<sub>2</sub>O emission studies. The distance from plot PIK (33.929°N, 102.820°E; 3439m a.s.l.) to plot LIT (33.918°N, 102.818°E; 3435m a.s.l.) is approximately 1200m. The plot LIT is very close to the Huahu Lake with a higher groundwater

table than that at the plot PIK. Three sets of samples were taken the BUR set at the plot PIK was placed on pika burrow with the whole burrow involving in the chamber base, to measure N<sub>2</sub>O emission from pika burrow., whilst the CON set at the plot PIK and the LIT set at the plot LIT representing the control samples for BUR measure N<sub>2</sub>O emission from meadow without burrow on the surface. When measuring, samples of BUR, CON and LIT were selected along sampling lines which are determined by random start points and random directions. Each sample located from others at least 5 m. *Carex muliensis* and *Potentilla anserina* are the predominant plant species covering about 50%-90% of the area at the sites LIT, CON and BUR. Aboveground vegetation was removed by cutting down along the ground surface to measure N<sub>2</sub>O flux without the impact of aboveground vegetation.

### N<sub>2</sub>O flux measurement

Due to the much lower ambient temperature, Greenhouse gas fluxes emitted during the non-growing season stay at low magnitude on the Qinghai-Tibet Plateau [9], while pika burrowing is more active in growing season. Consequently, the impact of pika burrowing on N<sub>2</sub>O flux mainly occurs in the short growing season. To precisely evaluate impact of pika burrowing, N<sub>2</sub>O flux was measured in June and August 2012. The static opaque chamber technique was used to determine the N<sub>2</sub>O flux [24]. When measuring N<sub>2</sub>O flux, six chambers (volume: 125 L, surface area: 0.25 m<sup>2</sup>) were placed randomly at the site LIT and CON. When measuring N<sub>2</sub>O flux at the site BUR, five chambers were placed on pika burrows that cover about 30–50% surface area of each chamber.

The stainless steel-chambers were surrounded by polyethylene foam to avoid any temperature fluctuations inside. Two fans were installed in the chamber to mix the air. Each chamber has a 20 cm length base which was installed 15 cm into the ground to mediate pressure pumping in chamber during wind gusts. Furthermore, each chamber was installed at least 2 days before gas samples collecting each time to avoid disturbing to greenhouse gas production in soil. Using 100 ml polypropylene syringes, four gas samples (200ml of each) from each chamber were taken at 10 minutes intervals over a 30 min period and then stored in 500 ml plastic and aluminum membrane gas sampling bags. All of the chambers started gas sample collecting at 09:00 (GMT+8), then ended at 09:30 (GMT+8), to avoid the diurnal fluctuation of N<sub>2</sub>O flux. In order to clarify N<sub>2</sub>O flux without impact of aboveground vegetation at the three sites, N<sub>2</sub>O flux was measured again after aboveground vegetation removed. The N<sub>2</sub>O concentration was then analyzed within one week by gas chromatography (7890A, Agilent, California, USA), equipped with an electron capture detector (ECD) for N<sub>2</sub>O.

### Soil properties

Soil samples were collected in August, 2012. Close to each chamber at the site LIT and CON, soil samples at 0–10, 10–20, 20–30 cm depth were collected. At the site BUR, the chambers were removed after gas sampling. Soil samples from three layers were collected at the place where the chambers were installed to ensure the soil samples reflect the condition of the pika burrows. Because of the burrows were at 10–20 cm soil depth, soil samples from the entrance or near it were collected, representing soil at the 10–20 cm depth at the site BUR.

Soil samples were separated into three sets. The first set of soil was air dried, passed through a 0.18 mm sieve first and then analyzed for soil organic matter (SOM), total N, and total C. The second set of soil was frozen immediately after collecting and reserved for NO<sub>3</sub>-N and NH<sub>4</sub>-N analysis, using a Discrete Auto Analyzer (Smartchem 300, AMS, Italy). The third set was collected by carefully cutting the soil at the edge of the sampling ring, made of stainless steel (5 cm diameter, 5 cm height) for measurement of the bulk density and water content

[25]. The SOM of dried samples was measured by the potassium-dichromate oxidation procedure after H<sub>2</sub>SO<sub>4</sub>-HClO<sub>4</sub> digestion [26]. Total N and total C of dried samples were analyzed using an elemental analyzer (vario MACRO cube, Elementar, Germany).

Since the whole soil samples are collected at very close sites, it is assumed that the densities of soil samples at the same depth are probably consistent. Consequently, soil bulk density and soil water content were taken as proxies to reflect soil aerobic condition.

## Statistical analysis

Nonparametric two-independent-samples test (Mann-Whitney Test) was used to analyze differences of soil properties between the sites at different soil depth. N<sub>2</sub>O flux differences between the sites were also tested this way. The impacts of soil depth and sampling location on soil properties were analyzed by the Repeated-measure ANOVA. The influence of water content on bulk density, SOM, total N, total C, NO<sub>3</sub>-N and NH<sub>4</sub>-N content were analyzed by the Spearman Correlation Analysis. The Spearman Correlation Analysis was also used to analyze the correlation between N<sub>2</sub>O flux and soil traits. All statistical analysis was done with software SPSS Statistics (19.0, IBM, USA).

## Results

### Soil properties

At a soil depth of 0–10 cm and 10–20 cm, soil water content at the site BUR was significantly lower than that at the site CON, whilst no significant difference was checked amongst soil water content at soil depth of 20–30cm which is located below the cavities of pikas (Fig 1).

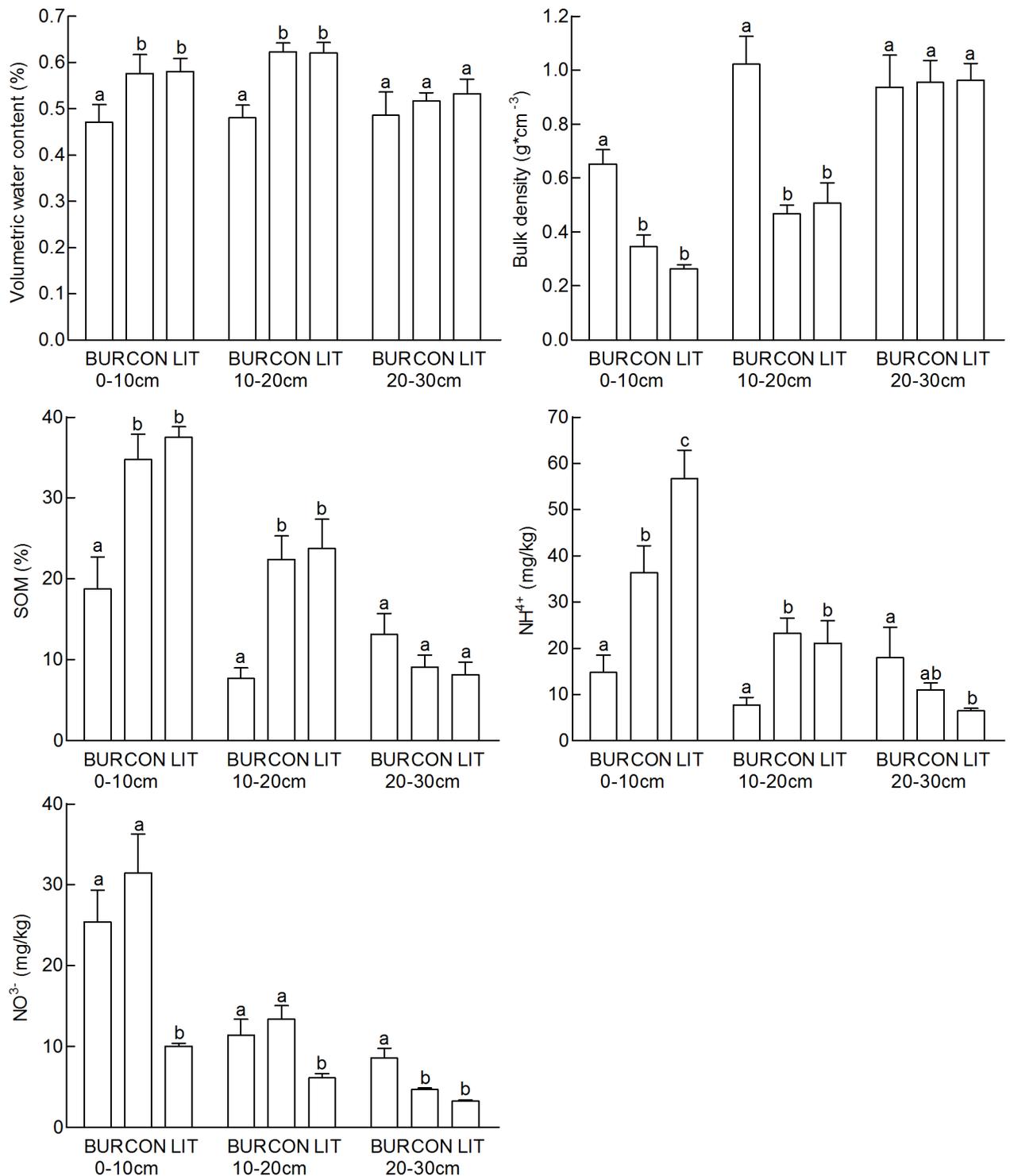
Besides the impact of soil depth on soil NH<sub>4</sub>-N, soil depth, sampling location and the interaction between soil depth and sampling location significantly influence the soil properties measured (Table 1).

Bulk density and SOM showed no significant difference at 20–30cm soil depth in the three sites (Fig 1). At 0–10cm and 10–20cm soil depth, SOM at the site BUR was significantly lower than that at the site CON, and bulk density at the site BUR was significantly higher than that at the site CON. The NH<sub>4</sub>-N content of the soil at the site BUR was significantly lower than that at the site CON at 0–10cm and 10–20cm soil depth, while the soil in the site LIT contained significantly more NH<sub>4</sub>-N than that at the site CON at 0–10cm soil depth. The NO<sub>3</sub>-N content of the soil at the site BUR was significantly higher than that at the site CON at 20–30cm soil depth, while the soil at the site LIT contained significantly less NO<sub>3</sub>-N than that at the site CON at 0–10cm and 10–20cm soil depth.

### N<sub>2</sub>O fluxes

N<sub>2</sub>O flux from the site with burrows (BUR) was significantly higher than that from the control site CON (Fig 2), irrespective of whether the aboveground vegetation was removed or not (Mann-Whitney test,  $P < 0.001$  with vegetation,  $P < 0.001$  with vegetation removed). The effect of removing vegetation was small compared to the effect of burrows being present in the soil. N<sub>2</sub>O flux from the BUR site increased from  $0.063 \pm 0.011 \text{ mg m}^{-2} \text{ h}^{-1}$  (mean  $\pm$  SE) to  $0.077 \pm 0.011 \text{ mg m}^{-2} \text{ h}^{-1}$  after removing the aboveground vegetation. N<sub>2</sub>O flux from the CON and LIT site was  $-0.009 \pm 0.011 \text{ mg m}^{-2} \text{ h}^{-1}$ ,  $-0.009 \pm 0.010 \text{ mg m}^{-2} \text{ h}^{-1}$ , and then increased to  $0.012 \pm 0.003 \text{ mg m}^{-2} \text{ h}^{-1}$ ,  $-0.002 \pm 0.010 \text{ mg m}^{-2} \text{ h}^{-1}$  with aboveground vegetation removed, respectively.

N<sub>2</sub>O fluxes were measured at the sites BUR, CON and LIT with aboveground vegetation and aboveground vegetation removed.



**Fig 1. Soil properties at different depth at the sites BUR, CON and LIT.** Water content, bulk density, SOM, NH<sub>4</sub>-N content and NO<sub>3</sub>-N content at soil depths of 0–10, 10–20 and 20–30cm in the BUR, CON and LIT sites. With the same lowercase letter did not differ significantly (P>0.05) based on the Mann-Whitney Test.

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**Table 1. Impacts of soil depth and sample location on soil water content, bulk density, SOM, NH<sub>4</sub>-N content and NO<sub>3</sub>-N content.**

Factors	Water Content (%)			Bulk Density (g*cm-3)			SOM (%)		
	df	F	P	df	F	P	df	F	P
Location	2	27.759	<0.001*	2	17.583	<0.001*	2	12.663	0.001*
Depth	2	6.711	0.004*	2	6.038	0.007*	2	15.407	<0.001*
Depth× Location	4	6.688	0.001*	4	5.058	0.003*	4	7.406	<0.001*
Factors	NH <sub>4</sub> -N(mg/kg)			NO <sub>3</sub> -N(mg/kg)					
	df	F	P	df	F	P			
Location	2	7.242	0.007*	2	13.801	<0.001*			
Depth	2	2.749	0.081	2	15.428	<0.001*			
Depth× Location	4	10.702	<0.001*	4	5.745	0.004*			

F and P values based on repeated-measure ANOVA are given.

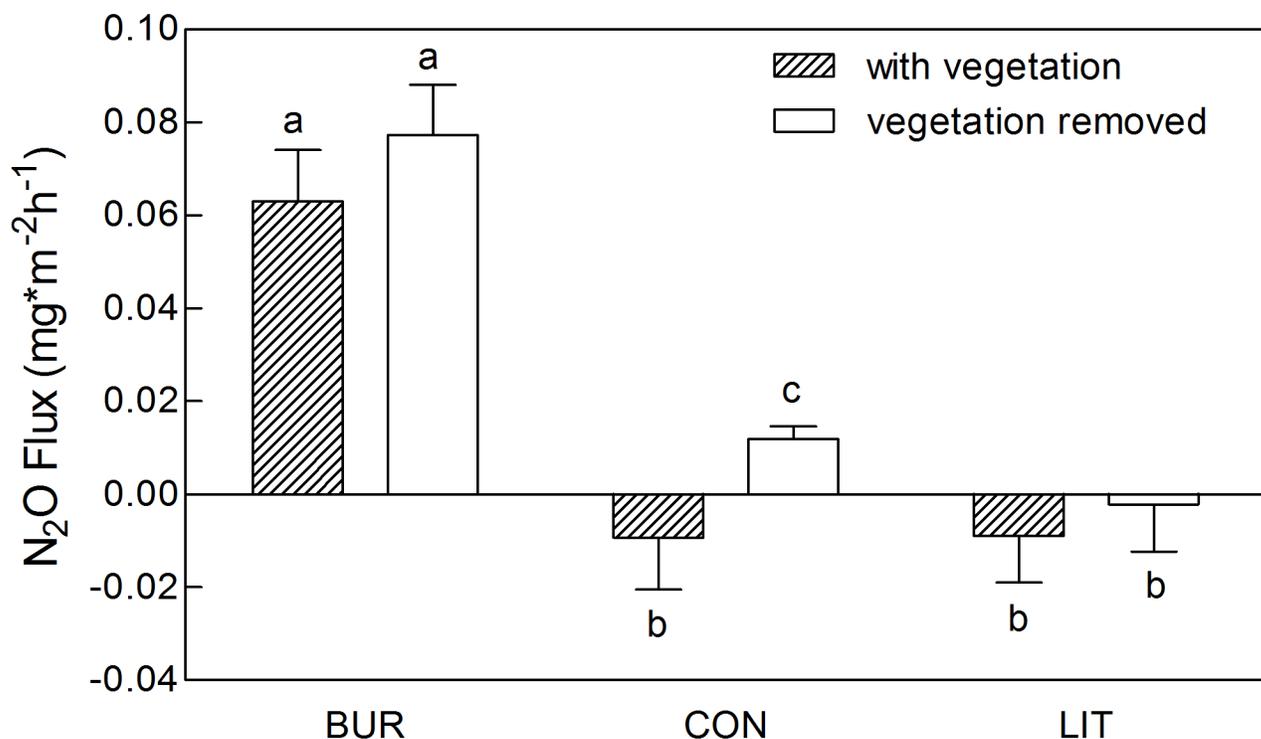
The asterisk (\*) means significant difference at P < 0.05

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The removal of aboveground vegetation at the sampling sites increased N<sub>2</sub>O flux, but only significantly increased the N<sub>2</sub>O flux at the site CON. With aboveground vegetation removed, the site CON changed from being a small N<sub>2</sub>O sink to being a source (Mann-Whitney test, P = 0.005).

### Interactions between soil properties and N<sub>2</sub>O fluxes

Significant correlations were found between N<sub>2</sub>O flux and bulk density, SOM and NH<sub>4</sub>-N content; while N<sub>2</sub>O flux show no significant correlation with volume water content and NO<sub>3</sub>-N



**Fig 2. N<sub>2</sub>O fluxes from the sampling sites.**

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**Table 2. Correlation between N<sub>2</sub>O flux and soil properties.** The asterisks indicate significant correlation at  $p < 0.05$  based on the Spearman's Correlation Analysis,  $r$ .

	Water content	Bulk density	SOM	NH <sub>4</sub> -N	NO <sub>3</sub> -N
<i>r</i>	-0.38	0.63	-0.57	-0.79	0.23
Sig.	0.12	0.005*	0.014*	<0.001*	0.36
N	18	18	18	18	17

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(Table 2). N<sub>2</sub>O flux was positively correlated with bulk density, while negatively correlated with SOM and NH<sub>4</sub>-N content.

## Discussion

The Pika's burrowing transfers the deeper soil to the ground surface during the construction of a network of subterranean burrows. The burrows created by each individual may range from 8 m to 13 m with each branch of the burrow extending 1 m to 5 m [27]. Burrowing activity mixes soil from different soil depth and bring more air into soil, which makes more soil water releasing to the air, then decreases soil water content, especially in the upper layer of soil. The alteration of soil aeration may have also led to changes in soil properties such as the mineralization rate of soil organic matter [28]. In this study, we found that pika's burrowing significantly reduced soil water content at 0–10 cm and 10–20 cm soil depth (Fig 1). The alteration of soil water content could be also explained by the alteration of other soil properties including bulk density and SOM which shows significant correlations with soil water content (Table 3).

Pika activities including daily clearing of the entrance hole and seasonal maintenance of underground structures, such as burrow excavation and wall repair may increase bulk density by squeezing the soil [3], and then causing soil water content altering.

SOM is strongly related to soil water content [29]. Decreasing soil water content can accelerate SOM decomposition. It was reported that decomposition of SOM is greatly reduced in anaerobic environments such as marsh and swampland [30]. In this study, the water content at the site CON and LIT was significantly higher than that of BUR at 0–20 cm soil depth (Fig 1), which presumably made SOM at the site BUR easy to decompose. Pika's burrowing can also directly promote SOM decomposing by breaking organic matter into smaller particles and making soil fully mixed [31,32].

Nitrification generally occurs in aerobic environments while denitrification occurs in anaerobic environment. As Fig 1 shows, the different soil water content at the sites determined the NH<sub>4</sub>-N content and NO<sub>3</sub>-N content. At the site LIT, higher soil water content resulted in higher NH<sub>4</sub>-N content and lower NO<sub>3</sub>-N content, which is vice versa at the site BUR. NH<sub>3</sub> volatilization is enhanced in alkaline soil, which accelerate NH<sub>4</sub>-N loss at the site BUR.

The average N<sub>2</sub>O fluxes at the site CON and LIT were  $-0.009 \pm 0.011 \text{ mg m}^{-2} \text{ h}^{-1}$ ,  $-0.009 \pm 0.010 \text{ mg m}^{-2} \text{ h}^{-1}$ , representing weak N<sub>2</sub>O sinks. In previous studies, the average N<sub>2</sub>O fluxes from meadow soil were around  $0.03\text{--}0.04 \text{ mg m}^{-2} \text{ h}^{-1}$  in Haibei County [33],  $0.07 \pm 0.11 \text{ mg m}^{-2} \text{ h}^{-1}$  in the Zoige Wetland [34], representing weak N<sub>2</sub>O sources. The differences in N<sub>2</sub>O fluxes

**Table 3. Relationship between water content and bulk density, SOM, NH<sub>4</sub>-N, NO<sub>3</sub>-N content.** The asterisks indicate significant correlation at  $p < 0.01$  (Spearman's Correlation Analysis  $r$ ).

	Bulk density	SOM	NH <sub>4</sub> -N	NO <sub>3</sub> -N
<i>r</i>	-0.573	0.531	0.398	0.107
Sig.	<0.001*	<0.001*	0.003*	0.454
N	54	54	54	51

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between these sites may be induced by the different soil water content. Due to most of precipitation occurring during the growing season under typical Tibetan plateau cold climate, the annual precipitation was taken as proxy to interpret how water content impact N<sub>2</sub>O flux. In 2012, the annual precipitation was 744mm in Zoige, compared with 526mm and 610mm in 2006 and 2007 in Zoige, and 450mm in 2005 in Haibei.

Soil animals are considered as soil ecosystem engineers, as they modify soil structure and interact with microbes through their burrowing activities [35]. N<sub>2</sub>O emissions from soil burrowed by earthworms can be up to three times greater than from control soil [36]. It is concluded that soil disturbance by animals usually alters the structure of the upper soil, and changes the oxidation-reduction condition of the soil. In this study, pika's burrowing brought deeper soil to the ground surface and exposed soil at surface of burrow to the atmosphere. The soil was thus vulnerable to soil denitrification and carbon oxidation, which was reflected by the decreased SOM and NO<sub>3</sub>-N from the upper soil at the site BUR. N<sub>2</sub>O flux at the site BUR significantly exceeded that at the site CON, suggesting that burrowing significantly increases the N<sub>2</sub>O emission in the Zoige Wetland. Spatial variation of N<sub>2</sub>O fluxes can be explained by the water content and soil nitrate content [37–39]. Due to the lower NO<sub>3</sub>-N content at upper layer of soil, N<sub>2</sub>O flux at the site LIT was much lower than that at the site CON with above-ground vegetation removed from both sites. On the other hand, N<sub>2</sub>O flux from the site BUR was much higher than that from the site CON under same magnitude of soil NO<sub>3</sub>-N content, mainly because of the lower water content at upper layer of soil (Fig 1) resulting more N<sub>2</sub>O produced from nitrification process. However, no significant correlation was found between N<sub>2</sub>O flux and soil water content, NO<sub>3</sub>-N content (Table 2), partly because soil water content and NO<sub>3</sub>-N content together determine N<sub>2</sub>O flux, causing each parameter could not be checked significant correlation with N<sub>2</sub>O flux.

It is also reported that CO<sub>2</sub> dissipates into pika tunnels could be easily emitted into the atmosphere through pika holes and then significantly increases the CO<sub>2</sub> flux from the area of pika burrows [40]. However, when measuring N<sub>2</sub>O flux from pika burrow in this study, it is difficult to distinguish the N<sub>2</sub>O released by underground burrows from the N<sub>2</sub>O flux released by the ground surface, which may amplify the N<sub>2</sub>O flux at the site BUR. The existing data cannot clearly clarify the N<sub>2</sub>O flux caused by pika burrowing, but the magnitude of N<sub>2</sub>O flux from pika burrow can be estimated by taking the density of pika burrow into account. The density of pika burrows around Huahu Lake was about 1100 per hectare (unpublished data). In other region of Qinghai-Tibet Plateau, the burrow density may achieve 1360 per hectare [41]. Consequently, N<sub>2</sub>O flux raised by pika burrowing could not be neglected because of the large magnitude.

The impact of fossorial animals on ecosystem function is beginning to attract more attention [4] and the debate on the role played by fossorial animals in ecosystem continues. To precisely clarify the function of plateau pika on the Qinghai-Tibet Plateau, the process and mechanism of how pika burrowing influences soil properties and greenhouse gas emission should be further investigated.

## Conclusions

In Zoige wetland, the burrowing activity of plateau pika altered the soil structure, resulting in decreasing soil water content, SOM and NH<sub>4</sub>-N content at the upper layer of soil around the burrow. With alteration of soil properties, especially decreasing of soil water content, pika burrow also caused significantly higher N<sub>2</sub>O flux, inducing the meadow from N<sub>2</sub>O sink to N<sub>2</sub>O source. In order to precisely evaluate the impact of pika burrowing on soil traits and N<sub>2</sub>O flux,

further work is required to investigate N<sub>2</sub>O flux released by subterranean burrows, mechanism of soil alteration.

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