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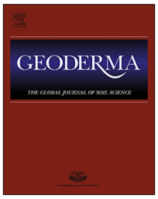

Effect of disturbance by plateau pika on soil nitrogen stocks in alpine meadows

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ABSTRACT

The presence of small burrowing herbivores often cause extensive disturbance to the grassland soil nutrient stocks. This study investigated the effect of small burrowing herbivore presence, the plateau pika (*Ochotona curzoniae*), and variation in disturbance intensity on soil nitrogen stocks at five alpine meadow sites. Disturbed plots occupied by plateau pikas consist of burrows and their associated bare soil patches in a matrix of continuous alpine meadow. The percentage of the bare soil area in a disturbed plot was used as a proxy for disturbance intensity of plateau pikas. This study showed that the soil total nitrogen stock was lower of bare soil than vegetated soil within disturbed plots, while ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), inorganic nitrogen and ammonium nitrogen/nitrate nitrogen ratio (ANR) were higher in bare soil than vegetated soil within the disturbed plots. This study further showed that the NH_4^+-N, inorganic nitrogen and ANR of disturbed plots were higher than those of undisturbed plots, whereas soil total nitrogen and the NO_3^--N stock was not different between disturbed plots and undisturbed plots. This study also showed that the soil total nitrogen, NH_4^+-N and inorganic nitrogen stocks of disturbed plots were higher at intermediate disturbance intensities of plateau pikas, whereas the NO_3^--N stock and ANR were not related to disturbance intensities. These results suggest that the NH_4^+-N and inorganic nitrogen stocks were higher in the presence of plateau pikas, and intermediate disturbance intensity of plateau pikas was beneficial for soil quality due to higher soil total nitrogen, NH_4^+-N and inorganic nitrogen stocks. The findings of this study present a possible approach for estimating how the presence of a small burrowing herbivore influences grassland soil nitrogen stock.

1. Introduction

Grassland covers approximately 40% of the terrestrial area on earth (Suttie et al., 2005), and its soil is a vital nitrogen pool in terrestrial ecosystems (Whitehead, 1995). Grassland soil nitrogen not only plays essential roles in improving soil quality, sustaining plant growth and biogeochemical cycling of elements (Galloway et al., 2004; Tian et al., 2006; Inselsbacher, 2014; Srivastava et al., 2016; Tian et al., 2019), but also relates to global climate change because it influences the carbon cycle of grassland ecosystems (Tian et al., 2014; Srivastava et al., 2016). However, grassland soil nitrogen has been demonstrated to be affected by many biotic factors, such as plant diversity (Palmborg et al., 2005; Yan et al., 2019; Wei et al., 2019), livestock grazing (Gao et al., 2009; Rui et al., 2011; Zhang et al., 2018), disturbance by small herbivores (Sherrod and Seastedt, 2001; Yurkewycz et al., 2014; Clark et al., 2016; Yu et al., 2017a,b), etc.

Small burrowing herbivores are underappreciated components of many grassland ecosystems (Davidson et al., 2012) and often cause extensive disturbance to grasslands (Desmet and Cowling, 1999; Canals et al., 2003; Yi et al., 2016; Yu et al., 2017a; Zhao et al., 2019). Disturbance by small burrowing herbivores often influences grassland soil nitrogen through bioturbation, movement of soil and litter, and burial and deposition of uneaten plants and waste material (Yurkewycz et al., 2014; Clark et al. 2016; Zhang et al. 2016; Lindtner et al., 2019). Previous studies have found that disturbance by small burrowing herbivores increases (Malizia et al., 2000; Hagenah and Bennett, 2013; Yurkewycz et al., 2014; Yu et al., 2017a), decreases (Sherrod and Seastedt, 2001), or has no impact on total soil nitrogen (Canals et al.,

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Table 1
Basic data at the five survey sites.

Location	MAT	MAP	Dominant species	Main associate species	Bare soil area (%)
Luqu (101.6°–103°E, 34°–34.8°N; 3550 m)	2.3 °C	600–800 mm	*Kobresia pygmaea*	*Elymus nutans* *Poa pratensis* *Anemone obtusiloba*	11.41 ± 3.90b
Maqu (100.8°–102.5°E, 33.1°–34.5°N; 3530 m)	1.2 °C	600–800 mm	*K. pygmaea*	*E. nutans* *P. pratensis* *A. obtusiloba*	11.39 ± 5.23b
Gonghe (99°–101.5°E, 35.5°–37.2°N; 3750 m)	4.1 °C	250–500 mm	*K. pygmaea*	*E. nutans* *P. pratensis* *A. obtusiloba*	9.78 ± 1.86b
Gangcha (99.3°–100.6°E, 36.9°–38°N; 3265 m)	−0.6 °C	370.5	*K. humilis*	*P. pratensis* *Leontopodium nanum* *Potentilla bifurca*	8.20 ± 2.85b
Zhiduo (33°–36.3°E, 89.4°–96.4°N; 4640 m)	−3.8 °C	290.9 mm	*Carex moorcroftii*	*K. pygmaea* *Poa poophagorum* *L. nanum*	16.41 ± 2.42 a

Note: MAT is mean annual temperature; MAP is mean annual precipitation; Percentage of the total plot area (35 m by 35 m) in the disturbed plot is presented as the mean ± SD for the five sites; Different letters denote significant differences at $P < 0.05$.

2003). In addition to soil total nitrogen, soil inorganic nitrogen is a more accurate indicator of nitrogen availability for plants than soil total nitrogen because plants can absorb inorganic nitrogen directly through the rooting system (Nguyen et al., 2017; Li et al., 2019). Soil inorganic nitrogen mainly consists of soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) (Zhong et al., 2015). Soil NH_4^+-N and NO_3^--N are very sensitive to disturbance by small burrowing herbivores because they are significantly correlated with soil physicochemical and biological properties (Srivastava et al., 2016; Zhang et al., 2018). However, there is no consensus concerning the response of soil inorganic nitrogen (including NH_4^+-N and NO_3^--N) to disturbance by small burrowing herbivores (Clark et al. 2016; Yu et al., 2017a,b; Zhao et al., 2019). Recent studies propose that the NH_4^+-N/NO_3^--N ratio (ANR) is a better indicator of relative availability of soil inorganic nitrogen than either NH_4^+-N or NO_3^--N (Li et al., 2019). However, how disturbance by small burrowing herbivores influences ANR is not yet well documented. Therefore, more studies are needed to simultaneously investigate the effect of disturbance by small burrowing herbivores on the total nitrogen, inorganic nitrogen (including NH_4^+-N and NO_3^--N) stocks and ANR in grassland ecosystems.

The plateau pika (*Ochotona curzoniae*) is a common, social, semi-fossorial herbivore with averaging 150 g and native to grasslands in Asia (Dobson et al., 1998; Fan et al., 1999; Davidson et al., 2012), especially to alpine meadows in the Qinghai-Tibetan Plateau (Zhang et al., 2016; Yi et al., 2016; Yu et al., 2017a,b). This small burrowing herbivore prefers an open habitat and avoids thick vegetation (Fan et al., 1999). Once plateau pikas occupy some suitable alpine meadows, and their disturbance will further enable these alpine meadows to become more open and short-statured, leading alpine meadows to be discrete mosaics of vegetated soil area and bare soil patches over a range of spatial scales (Wilson and Smith, 2015; Yu et al. 2017b; Zhao et al., 2019). Many studies have focused on differences in soil nitrogen between vegetated soil and bare soil in the presence of plateau pikas (Zhang et al., 2016; Yi et al., 2016; Yu et al., 2017b; Zhao et al., 2019), neglecting to select areas lacking plateau pikas as reference; other studies have compared soil nitrogen in vegetated soil areas with and without plateau pikas (Sun et al., 2015; Pang and Guo, 2017; Yu et al., 2017a), ignoring the bare soil patches in the presence of plateau pikas. These approaches lead to uncertainty in estimating the effects of disturbance by plateau pikas on soil nitrogen stocks of alpine meadows. A plot-scale approach is a possible way to completely estimate the responses of total nitrogen, inorganic nitrogen, and ANR to disturbance by plateau pikas and variation in disturbance intensity, because it can simultaneously consider the heterogeneity of soil nitrogen stocks in the presence of plateau pikas and the difference in soil nitrogen between areas with and without plateau pikas.

Here, this study employs the plateau pika as an example study animal for investigating variation of soil nitrogen stock and ANR in relation to the presence of a small burrowing herbivore across five sites. This study hypothesizes that (1) the soil total nitrogen is higher in the presence of plateau pikas; (2) the inorganic nitrogen stocks (including NH_4^+-N and NO_3^--N) and ANR are lower in the presence of plateau pikas; and (3) soil total nitrogen and inorganic nitrogen stocks has different responses to the disturbance intensity of plateau pika. This represents a possible approach for estimating the influence of the presence of a small burrowing herbivore on soil nitrogen stocks and is also helpful for comprehensively elucidating the role of plateau pikas in alpine meadow ecosystems of the Qinghai-Tibetan Plateau.

2. Materials and methods

2.1. Study sites

Where plateau pikas live is mainly regulated by habitats conditions (Fan et al., 1999; Smith and Foggin 1999; Guo et al., 2012). Thus, plateau pikas can live in many soil types, topography and microclimates. To identify a general pattern how soil nitrogen stocks change in relation to the presence of plateau pikas of alpine meadows across different environments, we selected five study sites on the Qinghai-Tibetan Plateau, at Zhiduo, Gangcha, Luqu, Maqu and Gonghe Counties in a large scale (Appendix: Fig. S1). These study sites experience the same cold, humid continental plateau climate and include similar alpine meadows dominated by sedges (Appendix: Table S1). The five study sites range in elevation from 3000 m to 4650 m, with average annual precipitation varying from 290 mm to 800 mm (Table 1). According to the Chinese Soil Classification System (Gong, 2001), the soils at the five study sites are classified as alpine meadow soils (similar to Cambisols in the WRB soil classification system) (Appendix: Table S2) with root mats (consisting of compact plant roots) approximately 7–11 cm thick in the topsoil horizons, which impede water infiltration. Generally, the burrowing activities of plateau pika can break the root mats, resulting in an increase in water infiltration (Wilson and Smith, 2015).

Within each site, the plateau pika is only small burrowing herbivore in alpine meadows, and its population increases rapidly within a relatively short period of time because a female plateau pika can produce 3 to 5 litters within a three-week interval (Fan et al., 1999), and generally peaks in August. This implies that soil had better sample in August, which can truly reflect the effect of plateau pika presence on soil nitrogen stcoks.

2.2. Survey design

Plateau pikas are social animals and live in family groups that often contain approximately 2 to 5 adults per family (Qu et al., 2013), and young offspring do not disperse in their year of birth (Dobson et al., 1998). Thus, plateau pikas were distributed territorially and patchily on alpine meadows. The sites with absence of plateau pikas might be potential suitable habitats. Therefore, sites lacking plateau pikas were easily found because the diffusion of plateau pikas is a gradual process.

A random stratified and paired design was used to select the plots (Appendix: Fig. S1). At each of five sites, 10 disturbed plots with plateau pikas presence were selected (Appendix: Fig. S2), and these disturbed plots were 3 km to 5 km from each other. Next, a paired adjacent undisturbed plot lacking plateau pikas was selected for each disturbed plot (Appendix: Fig. S3). The size of each plot was 35 m × 35 m, which is approximately equivalent to the average area of plateau pika's home range with the average size of 1,262.5 m^2 (Fan et al., 1999). The distance between each disturbed plot and its paired undisturbed plot ranged from 500 m to 1000 m to ensure that the undisturbed plot was a true reference area. If the distance between each disturbed plot and its paired undisturbed plot was too close, there will be overlap between disturbed and undisturbed plots. In addition, each paired plots were ensured to share the same alpine meadow, with no obvious differences in soil type, topography, microclimate and vegetation composition (Appendix: Table S1), which can ensure that each disturbed plot has a paired undisturbed plot to the utmost extent. In total, we examined 10 pairs of plots at each site and thus 100 plots across five sites, consisting of 50 disturbed plots and 50 undisturbed plots. Each paired plots was used as a single management unit. All plots were fenced to exclude summer grazing by large herbivores. Gates were opened during the winter to allow livestock grazing.

Although there were many kinds of bare soil patches in alpine meadow, the bare soil patches caused by plateau pika were easily visible and differed from the signs of disturbance caused by factors (Yu et al., 2017b). Previous studies have found that soil nitrogen concentration is different between bare soil and vegetated soil in the presence of plateau pika (Yu et al., 2017b; Zhao et al., 2019). To accurately estimate the soil nitrogen stock of disturbed plot, each disturbed plot was further divided into vegetated soil area and bare soil area (Appendix: Fig. S2). In this study, the bare soil patches were constricted to those produced by plateau pika. Thus, the bare soil area in undisturbed plots was zero and the vegetated area in undisturbed plot was 100% because this study just focused on bare soil patches induced by plateau pika. Bare soil area consisted of all bare soil patches in a disturbed plot, and vegetated soil areas were covered with vegetation. The bare soil area of each disturbed plot was estimated by summing the areas of all bare soil patches.

The volume of loose soil generated by wild boar has been used to estimate the disturbance intensity of this ungulate species (Bueno et al., 2013), and the area of bare soil induced by plateau pikas is positively related to the population density of the lagomorph species (Yu et al., 2017b). Therefore, this study used the percentage of bare soil area in each disturbed plot as a proxy for intensity of disturbance by plateau pika.

2.3. Sampling and analysis

Field surveys were conducted in early August 2017 because plateau pikas disturb alpine meadows mostly during this time period (Qu et al., 2013).

In each disturbed plot, five vegetated soil subplots (1 m × 1 m) were placed on the vegetated soil area approximately 8 m apart along a W pattern and were moved slightly to avoid bare soil patches if needed. Additionally, five bare soil patches, as bare soil subplots, were determined by the corresponding positions of the vegetated soil subplots to ensure that distances between vegetated and bare soil subplots were as short as possible, and this distance was < approximately 1 m. Within each undisturbed plot, five subplots were also placed approximately 8 m apart along a W pattern. Therefore, we collected samples from three soil contexts: undisturbed soil without plateau pikas, bare soil and vegetated soil with plateau pikas. Although soil nitrogen in bare soil patches was dependent on the "age" of the exposed soil, this study selected 250 bare soil patches across all disturbed plots consisted of old and new bare soil patches, which can include the effect of age of bare patches on soil nitrogen in a large scale and facilitate the discovery of a general pattern about the effects of disturbance by plateau pikas on soil nitrogen.

Before collecting the soil samples, plant and litter were cleared from the soil surface. Previous studies have shown that most burrows dug by plateau pikas generally do not extend below 20 cm (Yu et al., 2017b), although sometimes a few burrows can reach depths of 60 cm (Fan et al., 1999). The majority of the plant roots in these alpine meadows are in the top 20 cm of the soil. Therefore, in this study soil samples were collected at a depth of 20 cm with a 5-cm diameter soil corer to measure soil nitrogen concentrations. Meanwhile, soil profiles of 20 cm depth were dug using a stainless-steel cutting ring (the volume is 100 cm^3) to collect soil cores to determine soil bulk density. All soil samples were transported and stored at 4 °C prior to analysis. Soil bulk density was calculated by dividing the weight of the dry soil by the volume of each core occupied by soil.

Soil nitrate nitrogen ($NH_4{}^+$-N) and ammonium nitrogen ($NO_3{}^-$-N) were extracted with potassium chloride (KCl, 2 mol L^{-1}), and the concentrations were measured by the flow injection method (FIA star 5000 Analyzer, FOSS, DK). Inorganic nitrogen concentration was calculated as the sum of $NH_4{}^+$-N and $NO_3{}^-$-N concentrations. Total nitrogen concentration was measured via the Kjeldahl procedure (Foss Kjeltec 8400, FOSS, DK). To determine soil nitrogen stocks for each plot, we averaged the data from the 5 subplots.

2.4. Soil nitrogen stock calculations

Firstly, this study examined the difference in soil nitrogen stock (including total nitrogen, inorganic nitrogen, $NH_4{}^+$-N and $NO_3{}^-$-N) between bare soil and vegetated soil within disturbed plot. Soil nitrogen stocks at soil depths of 20 cm of bare soil and vegetated soil within disturbed plots were calculated as follows:

$$N_{stock-VS/BS} = N_{VS/BS} \times BD_{VS/BS} \times T \times (1 - \delta_{VS/BS}) \times 0.01 \tag{1}$$

where $N_{stock\text{-}VS/BS}$ is each soil nitrogen stock (including total nitrogen, inorganic nitrogen, $NH_4{}^+$-N and $NO_3{}^-$-N) of vegetated soil or bare soil within disturbed plots (kg m^{-2}); $N_{VS/BS}$, $BD_{VS/BS}$ and $\delta_{VS/BS}$ are soil nitrogen concentration (g kg^{-1}), soil bulk density (g cm^{-3}), soil fraction of gravel larger than 2 mm of vegetated soil or bare soil within disturbed plots. T is the soil thickness (20 cm).

Second, if there was no difference in soil nitrogen stocks between bare soil and vegetated soil within disturbed plot, we can use the mean of soil nitrogen stock of vegetated soil and bare soil to calculate the soil nitrogen stocks of disturbed plot. If there was difference in soil nitrogen stocks between bare soil and vegetated soil within disturbed plot, the soil nitrogen stock of bare soil or vegetated soil could not represent the soil nitrogen of disturbed plot. Thus, the soil nitrogen stock of disturbed plot should be calculated on the basis of soil nitrogen stock of bare soil and vegetated soil and their percentage within disturbed plot. Soil nitrogen stocks of disturbed and undisturbed plots were calculated as follows:

$$N_{stock-dist} = N_{stock-BS} \times BA + N_{stock-VS} \times VA \tag{2}$$

where, $N_{stock\text{-}dist}$ is each soil nitrogen stock of disturbed plots (kg m^{-2}); $N_{stock\text{-}BS}$ is each soil nitrogen stock of bare soil within disturbed plots (kg m^{-2}); $N_{stock\text{-}VS}$ is each soil nitrogen stock of vegetated soil within disturbed plots (kg m^{-2}); BA and VA are area (%) of vegetated soil and bare soil within the disturbed plots.

$$N_{stock-undist} = [N_{US} \times BD_{US} \times T \times (1 - \delta_{US}) \times 0.01] \times 100\% \quad (3)$$

where, $N_{stock\text{-}undist}$ is each soil nitrogen stock of undisturbed plots (undisturbed soil) ($kg\ m^{-2}$), N_{US}, BD_{US}, δ_{US} are soil nitrogen concentration ($g\ kg^{-1}$), soil bulk density ($g\ cm^{-3}$), and soil fraction of gravel larger than 2 mm of undisturbed soil. The bare soil area in undisturbed plots without plateau pikas was 0 and the undisturbed area was 100% of the total area because only bare soil areas resulting from plateau pika activity were considered in this study.

$$ANR = NH_4^+ \text{ - N stock}/NO_3^- \text{ - N stock}$$

2.5. Data analysis

All statistical analyses were performed with R 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria). A Generalized Linear Mixed Model (GLMM) with the function “lmer” from the lme4 package was used to examine differences in soil nitrogen stocks (including total nitrogen, inorganic nitrogen, NH_4^+-N and NO_3^--N) and ANR between vegetated soil and bare soil within disturbed plots across five sites, where the paired sampling points nested within each site constituted a random factor included in the analysis. In addition, a GLMM with the paired sampling points as random factor was used to evaluate differences in soil nitrogen stocks and ANR between vegetated soil and bare soil within disturbed plots at each site because there were 10 paired plots at each site.

A GLMM was also applied to examine the effects of disturbance by plateau pikas on soil nitrogen stocks and ANR across five sites for presenting a general pattern of soil nitrogen stocks in relation to the plateau pika presence, where the paired plots nested within each site constituted a random factor. Additionally, a GLMM with the paired plots as random factor was used to evaluate the effects of the disturbances by plateau pikas on soil nitrogen stocks and ANR at each of five sites, which can support the general pattern.

To clarify the responses of soil nitrogen stocks and ANR to the disturbance intensity of plateau pikas, linear model (LM) was used to examine the relationships of soil nitrogen stocks and ANR with percentage of bare soil area in the disturbed plot. The percentage of bare soil area was considered to be the fixed factor, and it was used to construct a regression analysis. Regression curves of soil nitrogen stocks and ANR on percent of bare soil area were obtained.

3. Results

3.1. Soil bulk density in undisturbed soil, vegetated soil and bare soil

When the data from the five sites was analyzed together or the data from each site was analyzed separately, soil bulk density was not significantly different among the three surface types and averaged $0.95 \pm 0.20\ g\ cm^{-3}$, $0.94 \pm 0.16\ g\ cm^{-3}$, $0.95 \pm 0.19\ g\ cm^{-3}$ for undisturbed soil, vegetated soil and bare soil (Appendix: Table S3).

3.2. Soil nitrogen stocks of undisturbed and disturbed plots

When data from the five sites were analyzed together, there were no differences in total nitrogen or NO_3^--N stocks between disturbed and undisturbed plots (total nitrogen: $F = 3.08$, $P = 0.086$; NO_3^--N: $F = 0.10$, $P = 0.755$) (Fig. 1), whereas the NH_4^+-N, inorganic nitrogen stocks and ANR were all higher in disturbed plots than in undisturbed plots (NH_4^+-N: $F = 83.81$, $P < 0.001$; inorganic nitrogen: $F = 38.63$, $P < 0.001$ and ANR: $F = 45.55$, $P < 0.001$).

When data from each site were analyzed separately, the responses of NH_4^+-N, NO_3^--N, inorganic nitrogen stocks and ANR to disturbance by plateau pikas were consistent among five sites (Appendix: Table S4). However, the effect of disturbance by plateau pikas on total nitrogen stock was site-dependent. Disturbance by plateau pikas had no impact on total nitrogen stock at Gangcha, while it decreased total nitrogen stock at Zhiduo, and increased total nitrogen stocks at Luqu, Maqu and Gonghe.

3.3. Soil nitrogen stocks and ANR of vegetated soil and bare soil within disturbed plots

Accounting for an overall scale effect, total nitrogen stock in bare soil was lower than that in vegetated soil ($F = 63.08$, $P < 0.001$) (Fig. 2), whereas NH_4^+-N, NO_3^--N, inorganic nitrogen stocks and ANR were all higher in bare soil than in vegetated soil (NH_4^+-N: $F = 66.84$, $P < 0.001$; NO_3^--N: $F = 23.03$, $P < 0.001$; inorganic nitrogen: $F = 59.79$, $P < 0.001$ and ANR: $F = 12.99$, $P = 0.001$).

Considering site-scale effects, total nitrogen, NH_4^+-N and inorganic nitrogen stocks of bare soil and vegetated soil were similar at each site, whereas the NO_3^--N stock and ANR differed between bare soil and vegetated soil at each of five sites. The bare soil had lower total nitrogen stocks and higher NH_4^+-N and inorganic nitrogen stocks than vegetated soil at each site (Appendix: Table S5), similar to results from study-wide comparisons. The NO_3^--N stock was not different between vegetated and bare soils at Gangcha, while NO_3^--N stocks were lower in vegetated soil than in bare soil at Zhiduo, Luqu, Maqu and Gonghe. The ANR was higher in vegetated soil than in bare soil at Gangcha and Luqu, whereas it was not different between vegetated soil and bare soil at Gonghe, Zhiduo or Maqu.

3.4. Relationships between soil nitrogen stocks, ANR with intensity of disturbance by plateau pikas

The bare soil area percentages within disturbed plots ranged from 3% to 21% across the five sites (Table 1). The bare soil area was significantly higher in Zhiduo than in the other four sites ($F = 7.90$, $P < 0.001$). Total nitrogen, inorganic nitrogen and NH_4^+-N stocks exhibited hump-shaped trends with increasing disturbance intensity (Fig. 3), while NO_3^--N stocks and ANR showed no obvious relationships with disturbance intensity. These results present a general pattern concerning the effect of plateau pika disturbance on total nitrogen, inorganic nitrogen and NH_4^+-N stocks.

4. Discussion

Although the disturbance by small burrowing herbivores often modifies soil nitrogen and its processes (Canals et al., 2003; Clark et al., 2016; Chen et al., 2017; Yu et al., 2017a,b), this study finds that there are no differences in soil bulk density among undisturbed soil, vegetated soil and bare soil. The slight body weight (approximate 150 g) of plateau pikas cannot compact topsoil layer, which is different from large herbivores (Stavi et al., 2008). As a semi-fossorial herbivore, plateau pika cannot construct the foraging tunnels, and further cannot loosen the topsoil layer. Plateau pikas can transfer an amount of deeper soil to the soil surface, which may form a surface of loosen soil layer, however, the raindrop impacts enable the surface of bare soil to gradually become compact (Guo et al., 2012).

This study uses plot-scale data to address the variation of soil nitrogen stocks between disturbed plots and undisturbed plots, finding that soil total nitrogen in disturbed plots is not significantly different from that in undisturbed plots when data from five sites are analyzed together, contrary to the first hypothesis and the result of soil organic carbon (Appendix: Table S6; Fig.S4). It seems that the presence of plateau pikas induces difference in soil total nitrogen within disturbed plots, rather than actually alters total soil nitrogen stock when compared to undisturbed plots. However, the response of soil total nitrogen stock to disturbance by plateau pikas is site-dependent. Higher soil total nitrogen stock in disturbed plots at Luqu, Maqu and Gonghe can be explained by three mechanisms: first, disturbance by plateau pikas can increase deposition rate of uneaten food and tall plant clippings,

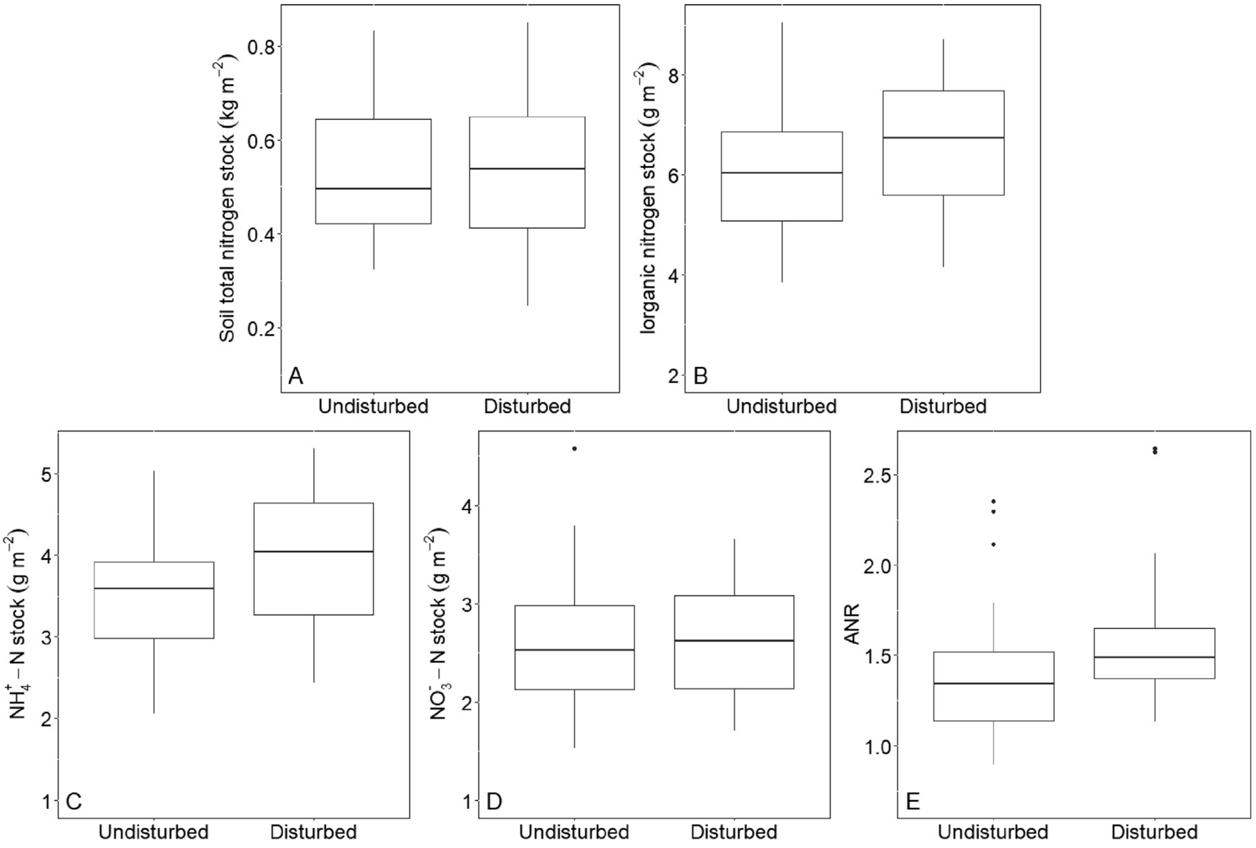

Fig. 1. Total nitrogen (A, $F = 3.08$, $P = 0.086$), inorganic nitrogen (B, $F = 38.63$, $P < 0.001$), NH_4^+-N (C, $F = 83.81$, $P < 0.001$) and NO_3^--N (D, $F = 0.10$, $P = 0.755$) stocks and ANR (E, $F = 45.55$, $P < 0.001$) of undisturbed and disturbed plots across five sites. From top to bottom of each standard boxplot are the maximum, the third quartile, median, first quartile, and minimum. The points outside the standard boxplots are outliers.

thereby increasing organic matter input (Liu et al., 2013; Zhang et al., 2016; Pang and Guo, 2017); second, plateau pikas excrete urine and feces, contributing to the input of soil organic matter (Yu et al., 2017a); third, the burial behavior of plateau pikas can protect soil organic matter pools, mainly consisting of litter and waste material, from decomposition and removal (Yurkewycz et al., 2014; Clark et al., 2016). However, soil total nitrogen stocks were lower in disturbed plots than in undisturbed plots at Zhiduo, and this is related to bare soil area. The bare soil area is greater at Zhiduo than at the other four sites and is over 13% (Table 1); higher percentage of bare soil area may cause low soil total nitrogen stock at Zhiduo. This study also found that the NO_3^--N stock did not differ between disturbed and undisturbed plots at both the individual site scale and the study-wide scale; however, the NH_4^+-N, inorganic nitrogen stocks and ANR were higher in disturbed than undisturbed plots at both scales, and these results do not support the second hypothesis. Higher NH_4^+-N stock in disturbed plots can be explained by soil nitrogen mineralization and utilization by plants. Decomposition and mineralization of soil organic matter is higher in disturbed plots (Clark et al., 2016; Zhao et al., 2019), resulting in an increase in NH_4^+-N stock, while bare soil areas in disturbed plots can decrease nitrogen absorption and utilization by plants (Canals et al., 2003; Clark et al., 2016), contributing to higher NH_4^+-N stock in soil. The ANR was greater than 1 at all five sites, indicating that NH_4^+-N is the predominant form of inorganic nitrogen in soils. Thus, the response of inorganic nitrogen to disturbance by plateau pikas is similar to that of NH_4^+-N stock. The higher ANR in disturbed plots may be explained in two ways. Low nitrification in grasslands (Yu et al., 2017a,b) encourages more inorganic nitrogen to be stored as NH_4^+-N stock in soil. In addition, higher water infiltration (Liu et al., 2013; Wilson and Smith, 2015) increases leaching of NO_3^--N from topsoil to deep soil in bare soil areas, contributing to higher ANR in disturbed plots. Therefore, disturbance by plateau pikas can improve soil nitrogen availability in alpine meadows of the Qinghai-Tibetan Plateau. These results indicate that disturbance by plateau pikas actually increases NH_4^+-N and inorganic nitrogen stocks, and redistributes soil total nitrogen and NO_3^--N stocks in alpine meadows. These findings present a possible approach for estimating how disturbance by plateau pikas influences soil nitrogen stocks.

This study also finds that soil nitrogen stocks and ANR are different between bare soil and vegetated soil within disturbed plots, similar to soil organic carbon stock within disturbed plot (Appendix: Table S7; Fig. S5). Compared to vegetated soil, the lower soil total nitrogen stock in bare soil may be caused by several mechanisms: first, the lack or minus of vegetation in bare soil area can reduce the input of organic matter (Yu et al., 2017a); second, the burrowing activities of plateau pikas move deeper soils with low organic matter content to overlay the original topsoil (Clark et al., 2016); third, bare soil encourages more dissolved nitrogen to leach from topsoil to deep soil layers than do vegetated soil because of the higher water infiltration in bare soil areas (Wilson and Smith, 2015; Chen et al., 2017); fourth, higher mineralization of soil organic matter in bare soil (Yu et al., 2017b) results in a reduction of soil total nitrogen. However, the NH_4^+-N, NO_3^--N and inorganic nitrogen stocks were higher in the bare soil than in the vegetated soil, in agreement with the disturbance by pocket gophers (Canals et al., 2003) and pack rats (Whitford and Steinberger, 2010). Relatively high temperature and oxygenation (lower soil hardness) in bare soil are favorable for soil microbial activity (Liu et al., 2013), which encourages organic matter, including buried litter and waste materials, to mineralize faster, contributing to increases in the NH_4^+-N, NO_3^--N and inorganic nitrogen stocks. Furthermore, paucity or lack of plants in bare soil areas reduces plant nitrogen uptake from soils (Canals et al., 2003; Clark et al., 2016; Yu et al., 2017b). These two

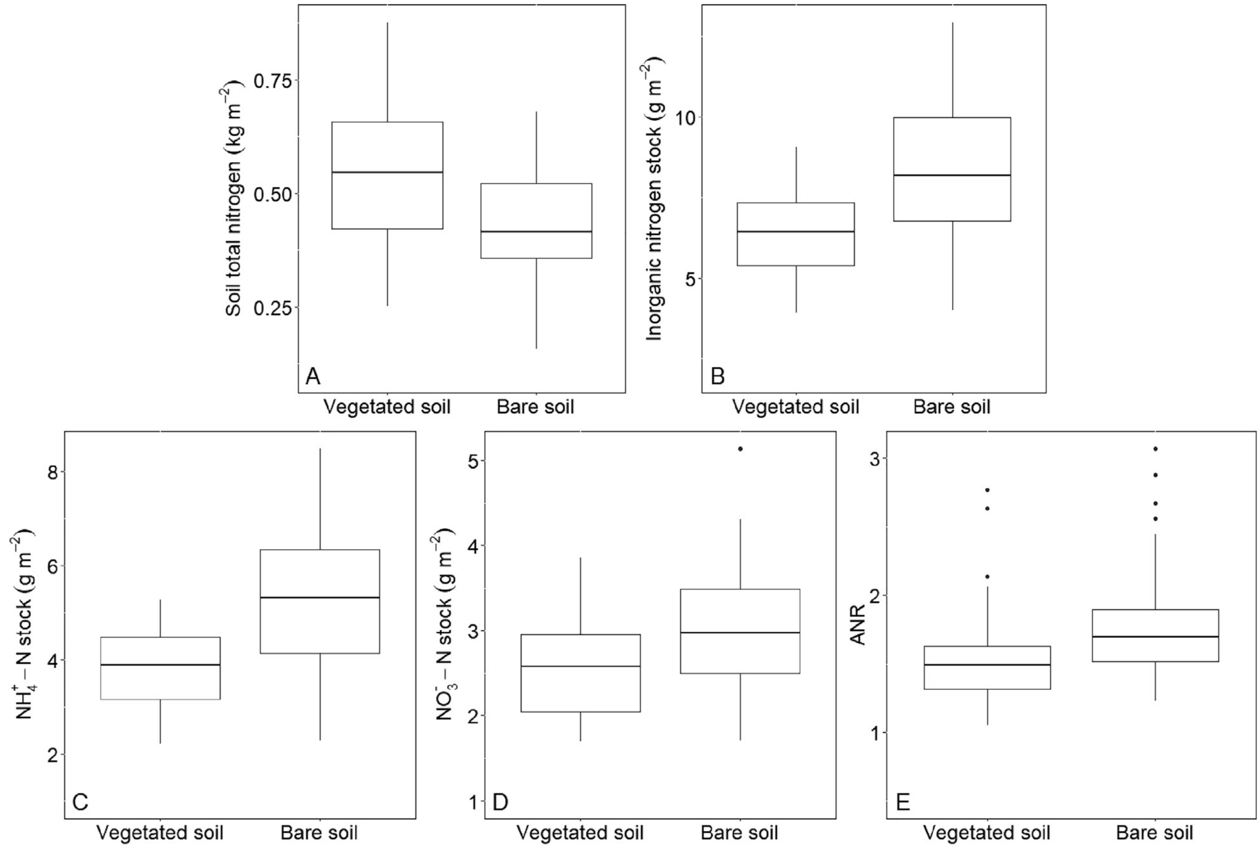

Fig. 2. Total nitrogen (A, $F = 63.08$, $P < 0.001$), inorganic nitrogen (B, $F = 66.84$, $P < 0.001$), NH_4^+-N (C, $F = 23.03$, $P < 0.001$) and NO_3^--N (D, $F = 59.79$, $P < 0.001$) stocks and ANR (E, $F = 12.99$, $P = 0.001$) of vegetated and bare soils within disturbed plots across five sites. From top to bottom of each standard boxplot are the maximum, the third quartile, median, first quartile, and minimum. The points outside the standard boxplots are outliers.

processes, while potentially reducing soil total nitrogen stock in the soil, can explain the slightly elevated rates of soil organic matter mineralization in bare soil (Canals et al., 2003), resulting in higher NH_4^+-N, NO_3^--N and inorganic nitrogen stocks. In addition to NH_4^+-N and NO_3^--N stocks, this study also shows that ANR is higher in bare soil than in vegetated soil, suggesting that bare soil may become productive because of higher available nitrogen (Li et al., 2019), which can provide a good habitat and “safe sites” for some plants (Hagenah and Bennett, 2013), especially opportunistic or pioneer species, to reoccupy the bare soil area. This may be the mechanism by which alpine meadow disturbed by small burrowing herbivores can naturally recover. Furthermore, the difference in NO_3^--N stock and ANR between vegetated soil and bare soil is site-dependent. The NO_3^--N stock at Gangcha did not differ between bare soil and vegetated soil, which is related to lower disturbance intensity than at the other four sites (Table 1). ANR is dependent on NH_4^+-N and NO_3^--N stocks simultaneously. ANR was not different between bare soil and vegetated soil at Zhiduo, Gonghe or Maqu, and this pattern is ascribed to the simultaneous elevation of both NH_4^+-N and NO_3^--N stocks in bare soil relative to vegetated soil, resulting in higher ANR at Gangcha. At Luqu, the relatively high temperature and precipitation may enhance soil organic matter mineralization when compared to other sites, which might increase NH_4^+-N stock (51%) more than NO_3^--N (27%), resulting an increase in ANR in bare soil. These results confirm that there are differences in soil nitrogen stock between bare soil and vegetated soil within disturbed plot; therefore, both of them cannot quantify the difference in soil nitrogen stocks between disturbed and undisturbed plots.

Many previous studies have verified that different levels of intensity of disturbance by plateau pikas have different impacts on soil organic carbon, soil total nitrogen concentrations, and plant species richness (Pang and Guo, 2017; Yu et al., 2017a,b). This study further finds that variation in disturbance intensity of plateau pikas also has different impacts on soil total nitrogen, inorganic nitrogen, NH_4^+-N, NO_3^--N and ANR, and this result partially supports the third hypothesis. This study suggest that there is a threshold of disturbance intensity of plateau pika for maximizing soil total nitrogen, NH_4^+-N, and inorganic nitrogen stocks. When disturbance intensity is below the threshold, the burial behavior by plateau pikas encourages greater deposition of litter, waste material and excrement into soil, which increases the input of organic matter (Liu et al. 2013; Zhang et al. 2016). When disturbance intensity exceeds the threshold, plateau pika activity decreases plant productivity (Liu et al., 2013) and increases soil nitrogen mineralization (Chen et al., 2017; Yu et al., 2017a,b), which reduces the soil total nitrogen stock. As far as NH_4^+-N and inorganic nitrogen stocks are concerned, they increased and then decreased with increasing disturbance intensity. Compared to lower disturbance intensity, intermediate disturbance intensity can improve soil water and temperature in the microenvironment for soil microbial activity (Liu et al., 2013), which may increase the mineralization rate of soil organic matter (Clark et al., 2016), resulting in elevated NH_4^+-N and inorganic nitrogen stocks. Compared to higher disturbance intensity, intermediate disturbance intensity increases the input of organic matter (Pang and Guo, 2017; Yu et al., 2017a), which enhances the source of NH_4^+-N and inorganic nitrogen stocks. The findings of this study clearly show that intermediate intensity of disturbance by plateau pikas benefits alpine meadows because this level of disturbance intensity relates to higher soil nitrogen stocks and improves nitrogen availability.

Note that the mineral soil nitrogen changes very rapidly within a short time period. However, mineral soil nitrogen of each paired plots increases or decreases at the same time because each paired plots shared the same climatic conditions. In addition, soil nitrogen changes slowly because of low temperature and short growth season in the

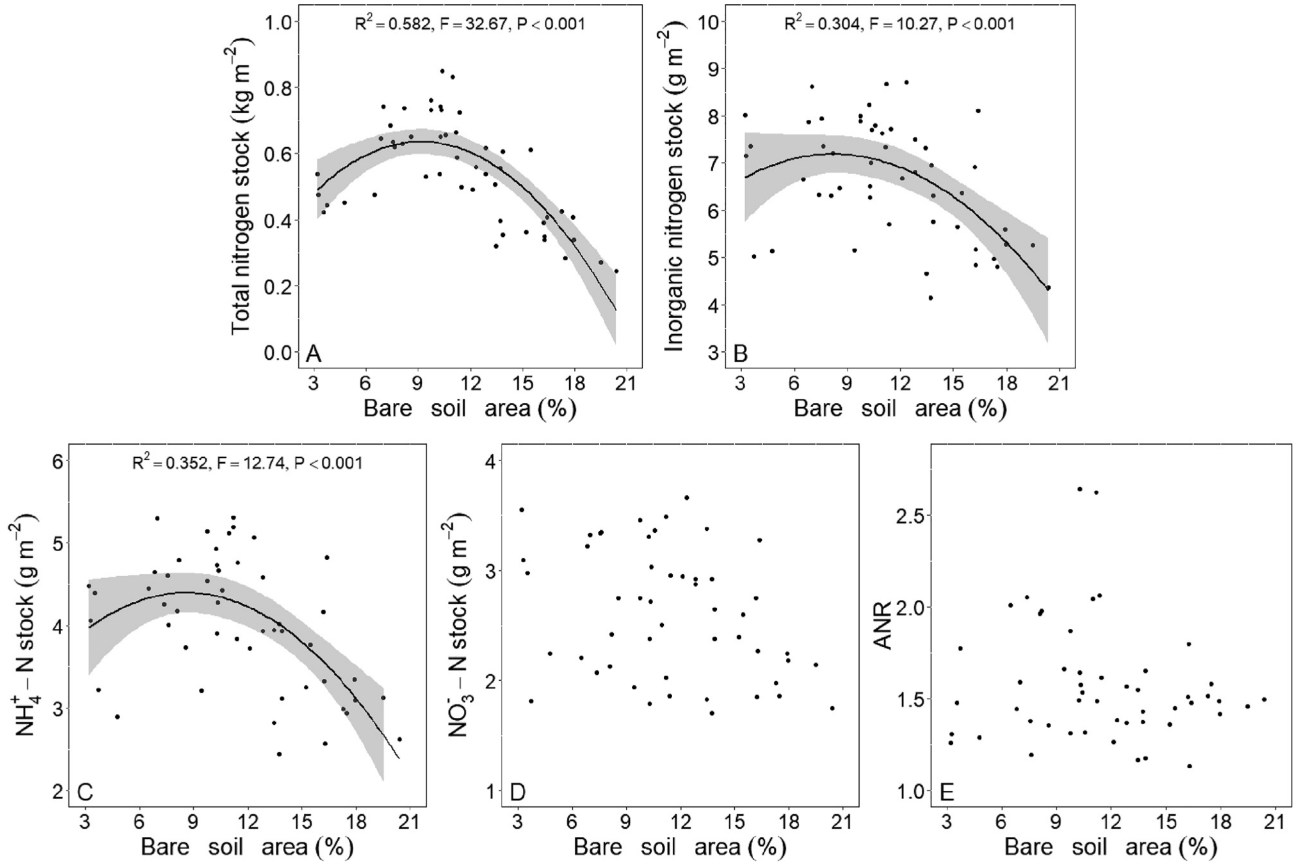

Fig. 3. Relationship between soil nitrogen stocks and ANR with the percent of bare soil area of disturbed plots across five sites based on linear models (LMs). For a detailed visualization of the relationship between the disturbance intensity and each soil nitrogen stocks, an adjusted local smoothed regression line (black) with its 95% confident interval (gray) was used.

Qinghai-Tibetan Plateau (Gong, 2001). Therefore, the findings of this study are credible to quantify the soil nitrogen stocks in relation to the presence of plateau pika and variation in disturbance intensity, even for that the mineral soil nitrogen changes very rapidly in a short time.

5. Conclusions

This study employs the plateau pika as an example animal for investigating the responses of soil nitrogen stocks to disturbance by a small burrowing herbivore and to variation in disturbance intensity. This study shows that the presence of plateau pikas relates to higher $NH_4{}^+$-N and inorganic nitrogen stocks, while has no correlation with the soil total nitrogen and $NO_3{}^-$-N stocks in alpine meadows of the Qinghai-Tibetan plateau. In addition, disturbance by plateau pikas can improve soil nitrogen availability due to higher ANR in disturbed plots. Furthermore, intermediate disturbance intensity of plateau pikas benefits alpine meadows because this level of disturbance intensity is relation to higher soil total nitrogen, $NH_4{}^+$-N and inorganic nitrogen stocks. These results describe a mechanism by which a small burrowing herbivore can influence soil nitrogen stocks.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114392.

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