

# Effects of zokors (*Myospalax baileyi*) on plant, on abiotic and biotic soil characteristic of an alpine meadow



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## ABSTRACT

The population of plateau zokor (*Myospalax baileyi*) has markedly increased on the degraded alpine meadow of Qinghai-Tibetan plateau. Zokors build mounds that alter plant and soil nutrients as well as biotic communities. This study explored changes in biotic and abiotic features of the community on zokor mounds of different ages (one year, and five-six year, referred to as ZM-1, and ZM-5-6) and undisturbed alpine meadow (CM) in the eastern Qinghai-Tibetan Plateau. Significant difference of four plant functional groups on the CM, ZM-1 and ZM-5-6 indicated the grass and sedge plants have led the recovery, and the forb plants lag behind the legumes in the process of restoration in disturbed alpine meadow. Redundancy analysis (RDA) identified that variance in soil microbial communities are mainly explained by the interacting effects of soil physicochemical properties and plant community characteristics. The results also demonstrate that influence of zokors on alpine meadow restoration is complex. While it is clear that intermediate concentrations of zokors appear to enhance the recovery process, it is also clear that the positive feedback between degradation and zokor population dynamics can lead to zokor population levels that cause degradation and retard recovery. A management challenge then becomes how to maintain these intermediate zokor populations and thus maximize meadow recovery.

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## 1. Introduction

Over the past decades, on the Qinghai-Tibetan Plateau, anthropogenic perturbations and climate change have led to the degeneration of alpine meadows (Li et al., 2008; Li et al., 2009), and this degradation has allowed rodents to invade the alpine meadow. These rodent invasions lead to positive feedbacks with the meadow ecosystems; degraded meadows are vulnerable to invasion, and rodent invasion causes further degradation, increasing vulnerability (Zhou et al., 2010; Hu et al., 2015).

The most important of these rodent invaders is the plateau zokor (*Myospalax baileyi*), a highly specialized fossorial muroid in the family Spalacidae with adults that weigh in the range of 200–250 g. Despite their relatively small size, these subterranean rodents can influence soil quality as well as ecosystem structure and function. The plateau zokor (*Myospalax baileyi*) is highly adapted to its subterranean life and also the only subterranean rodent species on the

Qinghai-Tibetan Plateau (Wei et al., 2006a; Wang et al., 2008). They are non-social animals that are broadly distributed in farm, prairie, alpine prairie and meadow at an average density of 15 animals per hectare (Zhang and Liu, 2003). In a healthy ecosystem, plateau zokors mainly consume forbs, i.e. *Potentilla anserine*, *Saussurea likiangensis* and *Oxytropis coerulea*, but they compete with livestock for scarce *Graminae* and *Cyperaceae* plants in degraded grasslands (Wang et al., 2000). The most important ecosystem-scale characteristics of plateau zokors are their foraging and burrowing activities, which can disrupt alpine meadows and cause degeneration. Plateau zokors excavate tunnels from deep nests to subsurface in the course of foraging and then move this loosened soil to the surface (Canals et al., 2013; Li et al., 2009). Each zokor can move several thousand times its body weight of soil to the surface annually, causing major disruptions to the plant community. (Wang and Fan, 1987).

There is some evidence that plateau zokors' burrowing activities lead to grassland degradation (Zhang et al., 2014a,b) by decreasing plant species diversity (Zong et al., 2006) and biomass (Li et al., 2009). Zokor invaded sites also have lower soil water content, less soil organic matter (Wang et al., 2007), and higher gravel concentrations (Wei et al., 2006b). Their burrowing activities significantly reduce soil density, which provides improved conditions for further

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**Table 1**  
Geographical characteristics and dominant plants from the disturbed and control plots.

Zokor mounds	Geographic coordinate	Altitude (m)	Dominant species
ZM-1	32°49.802'N,102°35.276'E	3491	<i>Koeleria cristata</i> + <i>Elymus nutan</i> + <i>Poa pratensi.</i> + <i>Festuca rubra.</i>
ZM-5-6	32°49.825'N,102°35.270'E	3484	<i>Koeleria cristata</i> + <i>Elymus nutan</i> + <i>Poa pratensis.</i> + <i>Scripus distigmaticus</i>
CM	32°49.801'N,102°35.214'E	3489	<i>Saussurea nigrescens</i> + <i>Festuca rubra.</i> + <i>Artemisia annua</i> + <i>Avena fatua</i>

ZM-1: the plots with one-year zokor mounds, ZM-5-6: the plots with five-six-year zokor mounds, CM: the plots without zokor mounds. The same below.

zokors invasion (Chu et al., 2016). Zokors also shift the successional trajectory and disrupt the accumulation of higher biomass taxa (Liu et al., 2014). Furthermore, the increasing distribution area of zokor mounds stimulates the emission of nitrous oxide (N<sub>2</sub>O) and weakens the effect alpine meadow ecosystems as greenhouse sinks (Zhang et al., 2014a,b). Other researchers have argued, however, that intermediate zokor population densities promote ecosystem function (Schiffman, 1994; Huntly and Inouye, 1988). Low to medium densities of zokors are associated with higher water availability and increased nutrient levels (Li and Zhang, 2006; Karsten et al., 2007). These physiochemical changes can enhance seed germination and survival (Bao et al., 2016).

Soil microbes are very sensitive to the environment, and changes to these organisms result in rapid alterations in soil microbial species and functional diversity patterns (Wang et al., 2013). Researches have shown that the distribution of soil microbes is closely related to plant communities and that it directly controls ecosystem function (Cai et al., 2010). The effects of anthropogenic perturbations, such as land use (Drenovsky et al., 2010) and nutrient disturbance (Bell et al., 2013), and natural factors, such as wild-fire (Ferrenberg et al., 2013) on microbial community structure and function have been studied. However, the ways in which the microbial community changes in response to plateau zokor mounds resulting from the effects of both anthropogenic perturbations and natural factors in alpine meadow have not been adequately described.

Recently, plateau zokors have become a substantial pest species in a large area of the Qinghai-Tibetan plateau because their population densities have grown, often reaching to 40–42 individuals per ha found in the experimental plots, a density that leads to serious damage to the pastures (Yang et al., 2016). Despite all of the attention paid to the physiochemical effects of zokors, previous studies have focused less (or not at all) on the soil microbial community. This study assesses the impact of zokor mounds on plant communities, nutrients, and the soil microbial community by comparing disturbed and control meadows. We hypothesized that tunnel excavation and mound forming would change each of these three parameters, and the microbial effects would be particularly large because of the dynamic natures of these communities; and the intermediate zokor populations could enhance the degraded alpine meadow recovery.

## 2. Materials and methods

### 2.1. Experimental design

The study was conducted at Hongyuan county, located in the eastern edge of Qinghai-Tibetan Plateau (N: 32°50'–33°22', E: 102°01'–103°23'). The study site has an average altitude of 3500 m, with a mean annual temperature of 1.1 °C, mean annual precipitation of 650 mm–730 mm, with 80% falling during May to August, and the annual mean duration of snow cover is 76 days (Li et al., 2011).

The meadow is of the continental cold type characterized by a short spring and autumn, a long winter, and a mild-to-cool summer, and has a growing season of 120–140 days from early May to late September (Wu et al., 2011). The plant coverage was higher than

80% and plant height was about 45–60 cm for the tallest grasses. The dominant species at the study site included sedges such as *Kobresia setchwanensis*, *K. pygmaea*; and the grasses *Agrostis clavata*, and *Elymus nutans*. Some forb species, including *Anemone trullifolia*, *Potentilla anserine*, *Saussurea nigrescens*, *Anemone trullifolia* are also abundant in the alpine meadow (Li et al., 2011). The soils at this site are Cambisols, with low pH (4.6–6.0), total nitrogen (4.78 g kg<sup>-1</sup>) and phosphorus (1.02 g kg<sup>-1</sup>).

Located in the grasslands of the research station of the Southwest University for Nationalities in Hongyuan County, the site has been used as a winter pasture for decades. Yaks (*Bos grunniens*) are only allowed in the period from October to April. The estimated population density is 10 yaks/ha (Wu et al., 2011). The population density of plateau zokors is about 39.7–42.1 individuals ha<sup>-1</sup> (Yang et al., 2016). The zokor mounds were categorized into two types depending on the year of excavation. The one-year zokor mounds were excavated in September 2010, and the five-six-year zokor mounds had been built since 2007.

Three plots of 50m × 50 m were established in early September 2011, containing grassland with one-year mounds, five-six-year mounds and undisturbed alpine meadow (hereafter referred to as ZM-1, ZM-5-6, and CM), respectively (Table 1). Ten replicates of each mound type were randomly selected, and each was approximately 1 m<sup>2</sup>.

### 2.2. Plant sampling

On five randomly selected zokor mounds from the ten ones, the aboveground plant biomass was harvested and separated into four functional groups (grasses, sedges, legumes and forbs). For each subplot, the height, frequency and coverage of each species were recorded (Wang et al., 2010).

The plant coverage, species richness and biomass were obtained to characterize the aboveground plant community. The importance value (IV) of individual plant species was calculated by the following equation to value the dominance of one species in the community:

$$IV = (Cr + Hr + Fr) / 3$$

Where *Cr*, *Hr* and *Fr* are the relative coverage, relative height and relative frequency, respectively (Tan et al., 2008).

The importance value was used to calculate the Shannon-Wiener index (*H*), the Pielou *J* index (*E*) and Simpson index (*P*) using the following equations:

$$H = - \sum_{i=1}^S P_i \ln P_i$$

$$E = H / \ln S$$

$$P = 1 - \sum_{i=1}^S P_i^2$$

where *P<sub>i</sub>* is the important value. *S* is the plant species richness (Fang et al., 2009).

**Table 2**  
Available PLFAs of topsoil (0–10 cm) from the disturbed and control plots.

Fatty acid	Source	Fatty acid	Source
Normal saturated		Terminally branched saturated	
14:0	Bacteria <sup>abc</sup>	a15:0	Bacteria, Gram (+) <sup>cdei</sup>
15:0	Bacteria <sup>abc</sup>	i15:0	Bacteria, Gram (+) <sup>defi</sup>
16:0	Bacteria <sup>abc</sup>	a17:0	Bacteria, Gram (+) <sup>cdefi</sup>
18:0	Bacteria <sup>abc</sup>	i16:0	Bacteria, Gram (+) <sup>cdefi</sup>
20:0	Nematodes <sup>e</sup>	i17:0	Bacteria, Gram (+) <sup>cdefi</sup>
Monounsaturated		Mid-chain branched saturated	
cy17:0	Bacteria, Gram (–) <sup>cdei</sup>	2Me18:0	Prokaryotic <sup>e</sup>
cy19:0	Bacteria, Gram (–) <sup>dfi</sup>	10Me17:0	Actinomycetes <sup>efh</sup>
16:1 $\omega$ 9c	Bacteria, Gram (–) <sup>cdf</sup>	10Me18:0	Actinomycetes <sup>defh</sup>
18:1 $\omega$ 7c	Bacteria, Gram (–) <sup>defi</sup>	Polyunsaturated	
18:1 $\omega$ 8t	Bacteria, Gram (–) <sup>e, f</sup>	18:2 $\omega$ 9,12	Fungi <sup>cdfi</sup>
18:1 $\omega$ 9c	Fungi <sup>cdg</sup>		

Gram(+) and Gram(–) for gram-positive and gram-negative bacteria, respectively. Only dominant PLFAs were shown. The same below.

- a) Phillips et al. (2002).  
 b) Chinalia and Killham (2006).  
 c) Hu et al. (2014).  
 d) McKinley et al. (2005).  
 e) Zelles (1999).  
 f) Frostegård and Bååth (1996).  
 g) Madan et al. (2002).  
 h) Fierer et al. (2003).  
 i) Margesin et al. (2009).

### 2.3. Soil parameters analysis

After removal of aboveground plants, 8 soil samples were randomly collected from the 10 replicates and divided into 0–10 and 10–20 cm sections. These were transported in cooled boxes to the laboratory, sieved (<0.25 mm) and stored at 4 °C. Five of the replicates (0–10 and 10–20 cm) were used to measure physicochemical properties, and three replicates of the 0–10 cm soil samples were for used to estimate soil microbial structure diversity.

Soil bulk density was determined for each selected zokor mounds and CM using a cutting ring (inner diameter 5.03 cm, volume 100 cm<sup>3</sup>). And a soil column (200 × 200 mm at depth of 10 cm) was excavated on each selected zokor mounds and CM (Li et al., 2009). Fine roots were isolated by sieving 1 mm mesh. The total roots and soils in 0–10 and 10–20 cm were dried at 60 °C for 48 h and then weighed. Roots/soil contents (g/g) is the ratio of roots to soils. Five-gram soil samples were weighed, dried at 105 °C and re-weighed to obtain gravimetric soil water content.

Soil pH was determined with a glass electrode using a soil to water ratio of 1:1. The soil organic matter (SOM) was determined by a modification of the chromic acid titration method-Degtjareff method (Walkley and Black 1934). The total nitrogen (TN) was determined by dichromate oxidization. The total phosphorus (TP) and total potassium (TK) were digested by HF-HClO<sub>4</sub> (Jackson and Barak 2005), and determined by molybdenum-blue colorimetry and flame photometry, respectively. Available nitrogen (AN) in soil was extracted with KCl and quantified using an automated procedure (Skalar SANplus Segmented Flow Analyzer). The contents of available phosphorus (AP) and available potassium (AK) were extracted by sodium bicarbonate (Olsen et al., 1954) and ammonium acetate (Carson, 1980), respectively. The TP, AP and TK, AK concentrations were assayed by molybdenum blue colourimetry and flame photometry, and measured with a spectrophotometer (Hach DR2700, Hach Company, USA).

### 2.4. Soil microbial community structure

Soil microbial community compositions for each soil sample were assessed by measuring the composition of microbial phospholipids acids using procedures described by Frostegård et al. (1993) and Bardgett et al. (1996). Based on the results of exist-

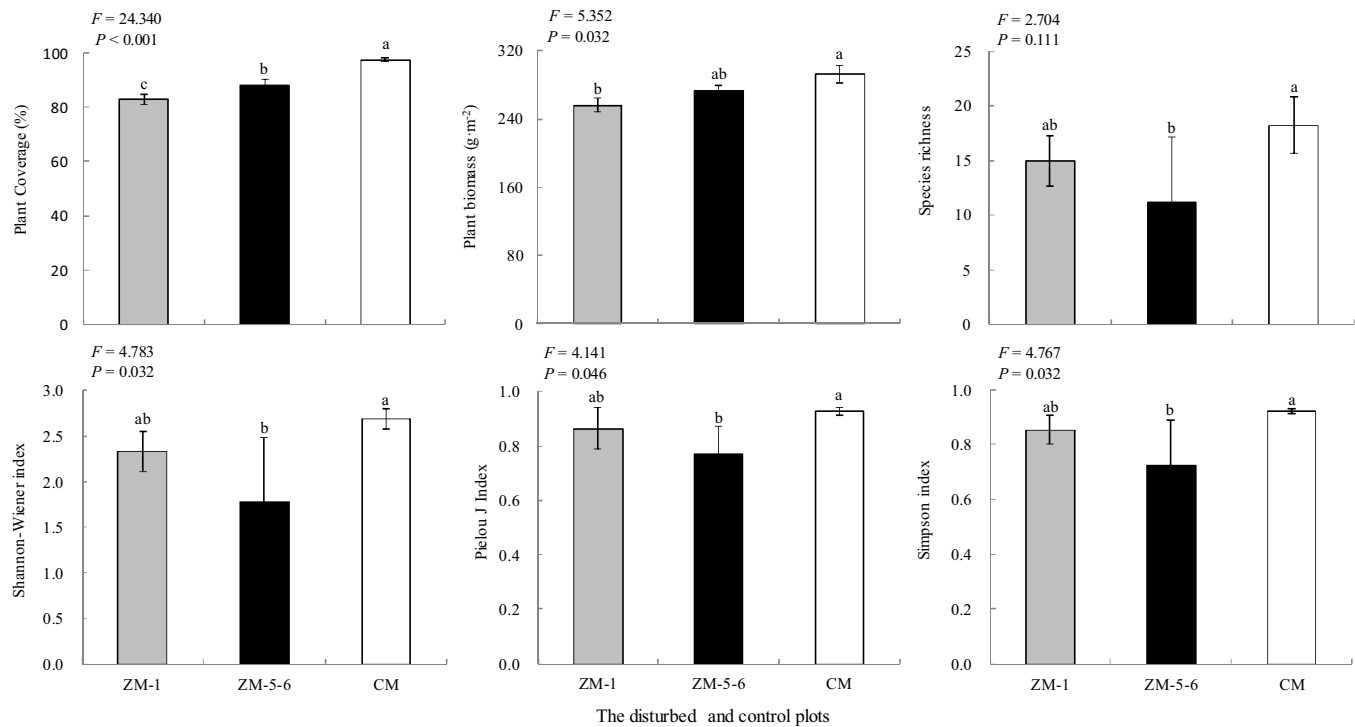
ing research and using the method of PLFA biomarkers, the present study showed that the appraised carbon chain length of PLFAs was C11 to C21 for rich species, including several kinds of saturated, unsaturated and cyclic PLFAs, of which there were 27 types in total (Table 2).

Nowadays, the PLFA method for assaying the composition of microbial communities has to a large extent been replaced by techniques based on nucleic acid extraction and analysis, particularly genes coding for ribosomal RNA (rRNA). And there are also some limitations to PLFA-based methods according to Frostegård et al. (2011). Such as the indicator of PLFAs, the rate of turn-over of PLFAs, and the diversity indices. However, PLFA quantification is probably suitable for assaying the biomasses and composition of microbial communities in soils, and may even be more sensitive in detecting shifts in microbial community composition when compared to nucleic acid based methods (Ramsey et al., 2006). Ramsey et al. (2006) also found that PLFA maximizes power compared to CLPP and PCR-based approaches. In 44% of studies, PLFA differentiated treatments that were not resolved by CLPP analysis; in 20% of studies, PLFA differentiated treatments that were not resolved by PCR-based methods.

### 2.5. Data analysis

The data are analysed with multivariate analysis of variance (ANOVA) with LSD tests at the significance levels of  $P=0.05$  and 0.01. In addition, both the importance value of plant species and soil microbial PLFA are analysed using principal component analysis (PCA) conducted by CANOCO 4.5 software (Micro-computer Power, Ithaca, NY) (Braak and Šmilauer, 2002) to identify changes in plants and soil microbial community found on zokor mounds and CM.

The correlation analyses for soil physicochemical properties, plant community characteristics, restoration years and soil microbial community are performed with the 'Vegan' package (Dixon 2003) of the R-language environment (version 3.3, R Foundation for Statistical Computing, Vienna, Austria). To identify the individual influences of soil physicochemical properties, plant community characteristics, and restoration year, the unique and shared influences of the variables are disentangled using variation partitioning (Borcard et al., 1992; Okland 2003).



**Fig. 1.** Plant community characteristics and plant community diversity from disturbed and control plots. Different lowercase letters indicate significant difference in the same plant functional group. The same below.

### 3. Results

#### 3.1. Plant community quantitative characteristics

Plateau zokor disturbance significantly reduced the plant coverage ( $F = 24.340$ ,  $P < 0.001$ ) and biomass ( $F = 5.352$ ,  $P < 0.024$ ). The plant coverage and biomass in CM were 97.50% and  $292.35 \text{ g m}^{-2}$ , significantly higher than 82.80% and  $255.78 \text{ g m}^{-2}$  in ZM-1, and 88.00% and  $273.42 \text{ g m}^{-2}$  in ZM-5-6. The same pattern occurred in the plant richness, although this effect was not statistically significant (Fig. 1). There were also significantly negative effects of zokor mounds on the plant community diversity. The Shannon-Wiener index, Pielou J index, and Simpson index declined in ZM-1, and decreased significantly in ZM-5-6 compared with CM (Fig. 1).

In comparison with that in ZM-1, plant functional groups characteristics, especially the coverage and biomass of grass and sedge plants, increase 5–6 years later, while the coverage and biomass of forb plants decline (Fig. 2). The legume community on zokor mounds show significantly lower richness, coverage and biomass, as compared to CM (Table 3, Fig. 2).

#### 3.2. Importance values of individual plant species

Zokor mounds significantly affected the plant community composition (Table 4). The dominant plant species were fell into grass and sedge families among different plots, with no annual species

being found in CM, and the dominant perennial species were evenly distributed because of their *IV* close to each other. It is noteworthy that some plants species did not occur at all on zokor mounds.

The ordination analysis showed that some plant species were particularly important in separating the disturbed and CM plots (Fig. 3). With formation time of zokor mounds, the disturbed plots tended to be close to CM plots. The annual species *Plantago depressa* willd, *Artemisia annua* and *Gentianopsis paludosa* had increasing *IV* in ZM-1. The perennial species *Leontopodium nanum*, *Carex tristachya*, and *Scirpus distigmaticus* were most closely associated in ZM-5-6, while nine perennial species in the *Gramineae*, *Leguminosae*, *Compositae*, *Gentianaceae* and *Ranunculaceae* families, were associated with CM (Fig. 3).

#### 3.3. Soil physicochemical properties

Plateau zokor mounds, soil depth and their interaction had significant effects on soil physical properties, but there was no effect of zokor mounds on soil water content (Table 5). Zokor mounds significantly decreased the ratio of roots to soils (R/S), while the soil bulk density showed a significantly opposite trend ( $P < 0.001$ ). The R/S and soil water content significantly decreased with soil depth ( $P < 0.05$ ) (Table 5, Fig. 4).

Except for the TP and TK contents, the MANOVA performed over the data set of soil chemical variables all exhibited a highly significant overall interaction between zokor mounds and soil depth.

**Table 3**  
ANOVA for plant functional groups quantitative characteristics from the disturbed and control plots.

Response Variables	Richness		Coverage (%)		Biomass( $\text{g m}^{-2}$ )	
	F	P	F	P	F	P
Legume	5.807	0.019	7.235	0.010	7.523	0.009
Grass	0.678	0.528	7.177	0.010	4.268	0.042
Sedge	2.946	0.094	1.342	0.301	2.403	0.136
Forb	1.741	0.220	11.598	0.002	15.418	0.001

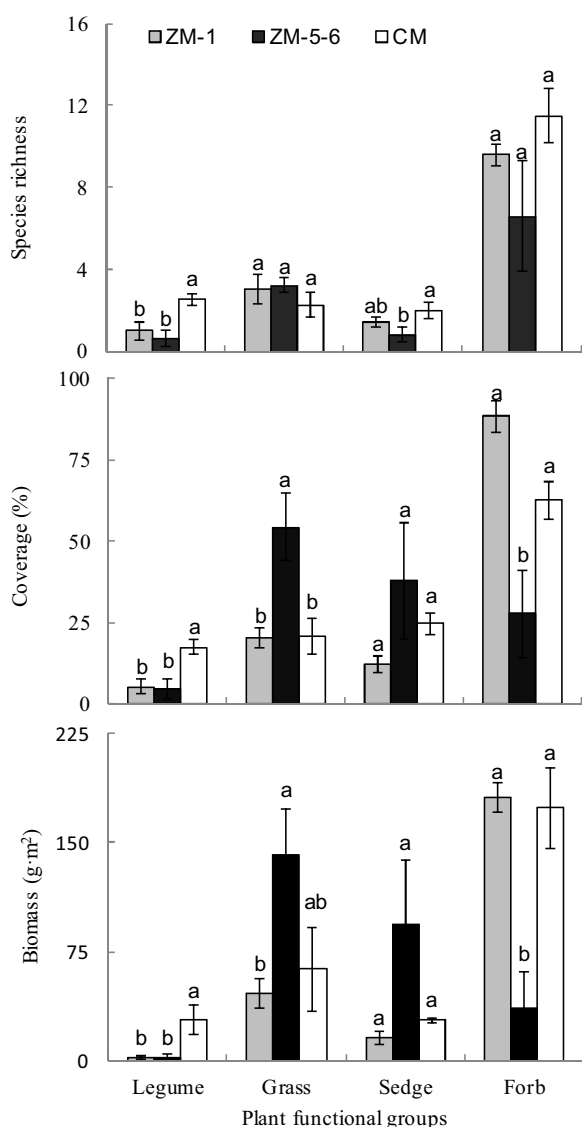


Fig. 2. Plant functional groups characteristics from the disturbed and control plots.

These chemical properties were also correlated with both zokor mounds and soil depth, while soil pH was not affected by zokor mounds (Table 5). Soil pH, SOM and contents of available soil nutrients decreased with soil depth, while the TN increased. Contents of AP, AN, TN and SOM at topsoil (0–10 cm) all significantly decreased

on zokor mounds compared to CM. In contrast, the soil pH markedly increased.

With disturbance time, the R/S, soil water content, and the contents of AP and AK in topsoil increase; the soil pH, the contents of AN, TN and SOM, however, decline (Figs. 3 & 4). Plant community composition in the ordination analysis was related to spatial variability, particularly the R/S, SOM and the contents of AN and TN (Fig. 3).

### 3.4. Soil microbial biomass and characteristics

Plateau zokor mounds significantly reduced the total PLFAs ( $F=11.732$ ,  $P=0.008$ ), bacterial PLFAs ( $F=17.957$ ,  $P=0.003$ ) and Gram-positive (Gram (+)) PLFAs ( $F=53.372$ ,  $P<0.001$ ) indicating soil microbial biomass, bacterial biomass, and Gram (+) biomass, respectively, while significantly increasing the soil microbial richness ( $F=29.400$ ,  $P=0.001$ ), bacterial richness ( $F=31.200$ ,  $P=0.001$ ). There were only two kinds of fungal and actinomycetes PLFAs found in the disturbed and CM plots (data not shown), with no significant difference of actinomycetes biomass, but the fungal biomass in ZM-5-6 was significantly higher than that in the ZM-1 and CM ( $F=15.368$ ,  $P=0.004$ ), which were related to the ratio of bacterial PLFAs to fungal PLFAs (B/F). Changes in Gram (+) and Gram (-) biomass also resulted in a significant decline of the ratio of Gram (+) to Gram (-) signature PLFAs ratio value. The iso to anteiso PLFA ratio values, indicative of nutrient stress, showed a similar pattern, with a decrease on zokor mounds compared to CM (Table 6, Fig. 5).

The soil microbial structure determined through PCA of the PLFA data showed significant overall responses to zokor mounds (Fig. 6). Some PLFA species were crucial in differentiating ZM-1, ZM-5-6 and CM. The ZM-1 and ZM-5-6 were negative with the PC1 axis, while the CM positive with it. Both the bacterial PLFAs (e.g. 18:1 $\omega$ 7c, 15:0, 16:1 $\omega$ 9c) and the dominant fungal PLFA 18:1 $\omega$ 9c, as well as the actinomycetes 10Me17:0 demonstrated as important discriminators among ZM-1, ZM-5-6 and CM (Fig. 6). Some soil abiotic variables, such as soil pH, bulk density, available nutrients, and R/S, had effects on the soil microbial composition (Fig. 6).

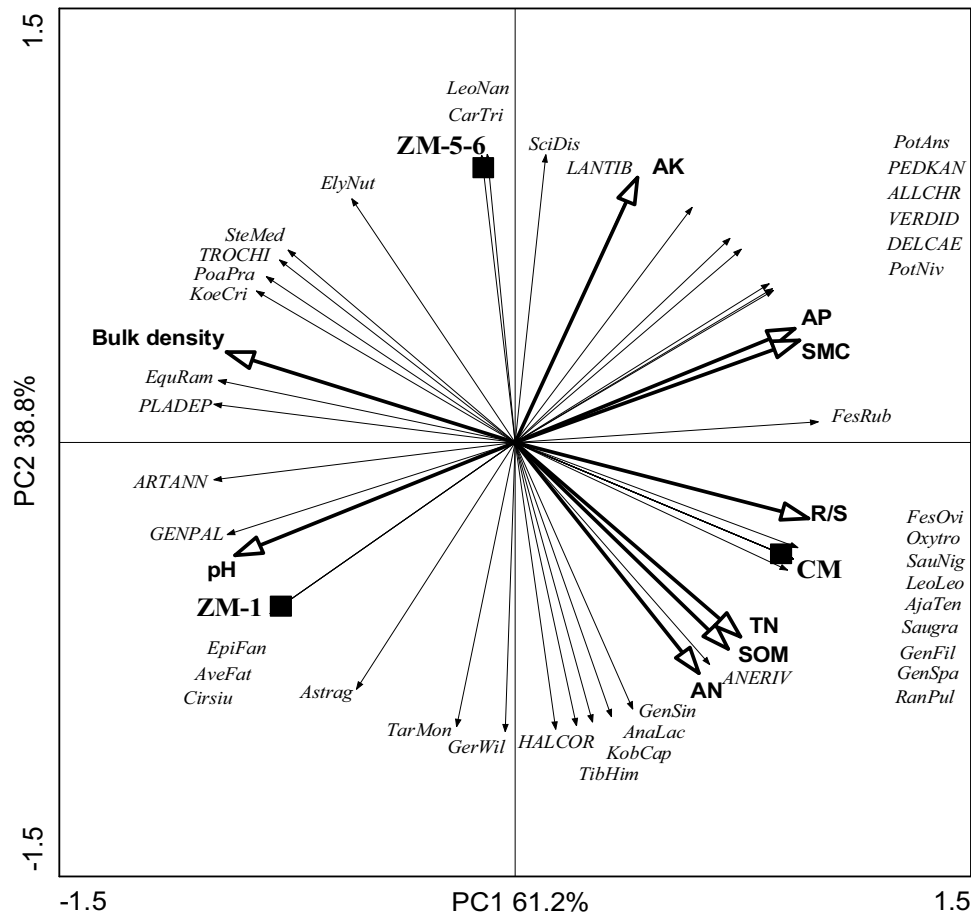
### 3.5. The relationships among soil microbial community and environmental factors

The soil microbial communities in this study were influenced by restoration year for changes in plant community characteristics and soil physicochemical properties. PCA analyses showed that soil physicochemical properties have effects on the soil microbial composition (Fig. 6). The restoration year has a very weak effect on soil microbial communities (Fig. 7). Results show that soil physicochemical properties explain 95.93% of microbial variance, while restoration year explains 53.35%, and plant community character-

Table 4  
Important value of annual (A) and perennial (P) species from the disturbed and control plots.

Species	Biotypes present	Important value (IV)		
		ZM-1	ZM-5-6	CM
<i>Elymus nutan</i>	P, Gramineae	5.26	7.06	4.12
<i>Poa pratensis</i> L.	P, Gramineae	5.14	5.59	3.68
<i>Koeleria cristata</i>	P, Gramineae	5.93	6.38	3.83
<i>Festuca rubra</i> L.	P, Gramineae	3.77	4.33	5.05
<i>Festuca ovina</i>	P, Gramineae	absent	absent	3.61
<i>Avena fatua</i>	P, Gramineae	5.06	absent	absent
<i>Scripus distigm aticus</i>	P, Cyperaceae	3.66	9.93	4.62
<i>Carex tristachya</i>	P, Cyperaceae	3.64	4.13	3.65
<i>Kobresia capillifolia</i>	P, Cyperaceae	absent	absent	4.91
<i>Anaphalis lactea</i>	P, Compositae	absent	absent	3.50
<i>Saussurea nigrescens</i>	P, Compositae	absent	absent	5.16
<i>Allium chrysanthum</i>	A, Liliaceae	absent	3.69	absent
<i>Artemisia annua</i>	A, Compositae	9.08	absent	absent





**Fig. 3.** The important value of all the plant species and soil characteristics biplot diagram from the PCA.

R/S and SMC are ratio of roots to soils and soil water content. Species codes are as follows: perennial species, *Elymus nutan* = ElyNut, *Poa pratensis* L. = PoaPra, *Koeleria cristata* = KoeCri, *Festuca rubra* L. = FesRub, *Festuca ovina* = FesOvi, *Avena fatua* = AveFat, *Scirpus distigmaticus* = SciDis, *Carex tristachya* = CarTri, *Kobresia capillifolia* = KorCap, *Tibetia himalaica* = TibHim, *Astragalus* sp. = Astrag, *Oxytropis* = Oxytro, *Anaphalis lacteal* = AnaLac, *Saussurea nigrescens* = SauNig, *Taraxacum mongolicum* = TarMon, *Cirsium* = Cirsiu, *Leontopodium leontopodioides* = LeoLeo, *Leontopodium nanum* = LeoNan, *Ajania tenuifolia* = AjaTen, *Saussurea graminea* = SauGra, *Epilobium fangii* = EpiFan, *Gentiana sino-ornata* = GenSin, *Gentiana filistyla* = GenFil, *Gentiana spathulifolia* var. *ciliate* = GenSpa, *Ranunculus pulchellus* = RanPul, *Geranium wilfordii* = GerWil, *Potentilla anserine* = PotAns, *Potentilla nivea* = PotNiv, *Equisetum ramosissimum* = EquRam, *Stellaria media* = SteMed; Annual species, *Plantago depressa* Willd = PLADEP, *Allium chrysanthum* = ALLCHR, *Artemisia annua* = ARTANN, *Halenia corniculata* = HALCOR, *Gentianopsis paludosa* = GENPAL, *Delphinium caeruleum* = DELCAE, *Anemone rivularis* = ANERIV, *Trollius chinensis* = TROCHI, *Veronica didyma* = VERDID, *Lancea tibetica* = LANTIB, *Pedicularis kansuensis* = PEDKAN.

**Table 5**  
ANOVA for soil physicochemical properties (0–10 and 10–20 cm) from the disturbed and control plots.

Items	Zokor mounds (Z)		Soil depth (D)		Z × D interaction	
	F	P	F	P	F	P
R/S	32.483	<0.001	55.34	<0.001	26.61	<0.001
SMC (g kg <sup>-1</sup> )	1.008	0.368	5.897	0.032	11.668	0.002
Bulk (g cm <sup>-3</sup> )	28.801	<0.001	29.564	<0.001	9.338	0.004
Soil pH	2.722	0.114	5.280	0.044	9.927	0.004
SOM (g kg <sup>-1</sup> )	34.961	<0.001	18.850	0.001	13.234	0.002
TN (g kg <sup>-1</sup> )	19.827	<0.001	2.352	0.156	7.784	0.009
TP (g kg <sup>-1</sup> )	0.894	0.439	0.135	0.721	0.087	0.917
TK (g kg <sup>-1</sup> )	2.545	0.128	3.174	0.105	0.106	0.900
AN (mg kg <sup>-1</sup> )	37.665	<0.001	6.469	0.029	7.774	0.009
AP (mg kg <sup>-1</sup> )	22.542	<0.001	5.056	0.048	1.665	0.238
AK (mg kg <sup>-1</sup> )	47.119	<0.001	84.988	<0.001	12.714	0.002

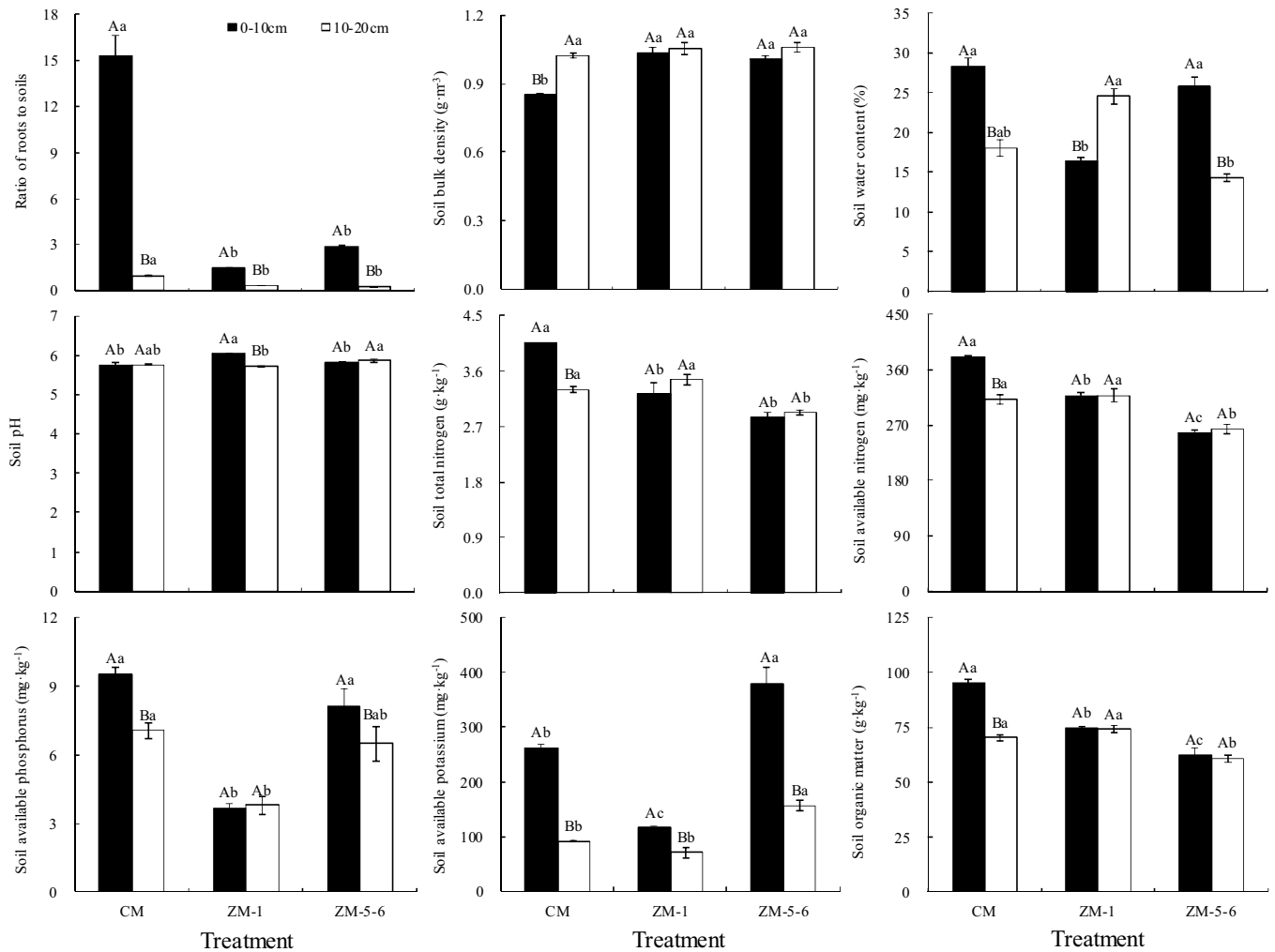
SOM: soil organic matter, TN: total nitrogen, TP: total phosphorus, TK: total potassium, AN: available nitrogen, AP: available phosphorus, AK: available potassium.

istics explains 32.54% (Fig. 7). The effects of soil physicochemical properties and restoration year explain as much as 51.83% of soil microbial variance, and the effects of soil physicochemical properties and plant community characteristics explain 31.05%. Thus soil physicochemical properties are the most influential on the soil microbial communities.

## 4. Discussion

### 4.1. Responses of the aboveground vegetation to zokor mounds

Plateau zokor mounds changed the plant species composition and significantly reduced the vegetation coverage and biomass. This conclusion is consistent with findings of Wang et al. (2008),



**Fig. 4.** Soil physicochemical properties from disturbed and control plots.

Different capital letters indicate significant difference between 0 and 10 and 10–20 cm in the same disturbed plots; Different lowercase letters indicate significant difference between disturbed and control plots at the same soil depth. Soil total phosphorus and soil total potassium had no significant difference between disturbed and control plots.

**Table 6**

ANOVA for soil microbial biomass and characteristics (0–10 cm) from disturbed and control plots.

Response Variable	Soil microbial biomass		Response Variable	Soil microbial characteristics	
	F	P		F	P
Total PLFAs	11.732	0.008	Total PLFA richness	29.400	0.001
Bacteria	17.957	0.003	Bacteria richness	31.200	0.001
G <sup>+</sup> bacteria	52.372	<0.001	G <sup>-</sup> richness	7.000	0.027
G <sup>-</sup> bacteria	3.216	0.112	B/F	43.731	<0.001
Fungi	15.368	0.004	G <sup>+</sup> /G <sup>-</sup>	51.333	<0.001
Actinomyces	1.749	0.252	iso/anteiso	29.584	0.001

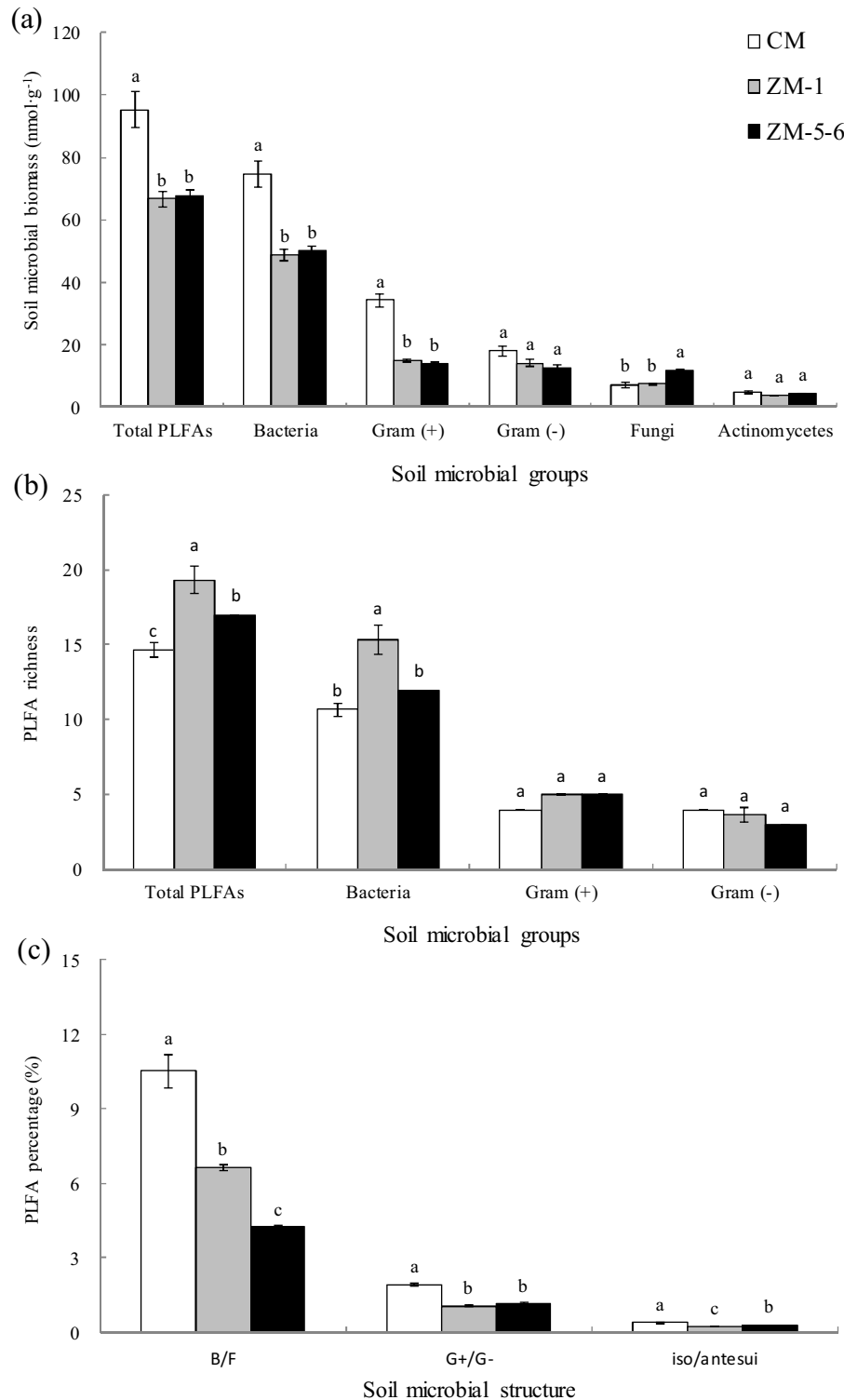
B/F: the ratio of bacterial PLFA to fungal PLFA, bacteria were estimated as the sum of G<sup>+</sup>, G<sup>-</sup>, 14:0, 15:0, 16:0 and 18:0; G<sup>+</sup>/G<sup>-</sup>: the ratio of gram-positive bacteria PLFA to gram-negative bacteria PLFA; iso/anteiso, the ratio of iso to anteiso branched PLFA, which were indicated using (i15:0+i17:0)/(a15:0+a17:0) [42].

Li et al. (2009) and Zhang et al. (2014a,b). After the original plants had been destroyed by plateau zokors, a high percentage of annual species and some new annual species gradually appeared in ZM-1. The perennial grasses increased, finally dominating plant community in alpine meadow (Xin et al., 2011). In conclusion, 5–6 years later, the grasslands degraded by plateau zokors had started to recover. The plant community composition and the dominant plant species on zokor mounds were changing towards to CM. The grasses and sedges, have led the recovery, and the forbs maybe lag behind the legumes in the process of long-time restoration. It seems to

need a longer time than 5 years to get the degraded alpine meadow to be completely recovered, although the plant biomass in ZM-5-6 was 94% of that for CM; the legumes, forbs characteristics and plant community coverage were significantly lower compared to CM.

#### 4.2. Responses of the soil properties to zokor mounds

The results demonstrate that plateau zokors with medium population densities could increase the contents of SOM and soil water content in topsoil (0–10 cm), changing the soil physicochemical



**Fig. 5.** Soil microbial biomass, richness and structure from disturbed and control plots. There were no significant difference between the disturbed and control plots for only two kinds of fungal PLFAs and actinomycetes PLFAs detected.

properties and promoting the material cycle in alpine meadow (Li and Zhang, 2006). However, when the population densities kept rising, the increasing numbers of zokor mounds resulted in serious erosion and desertification of soils (Zhou et al., 2010). Therefore plateau zokors had greatly direct impact on the soil physiochemical properties through their foraging and burrowing activities.

Our study provides evidence that, compared to CM soils, the soils on zokor mounds are drier, more compact, and have lower R/S

(Fig. 4). The soil compactness was due to the trampling of grazing animals and water movement (Li et al., 2009). Plateau zokors excavated burrow systems, transferred the whole roots, and deposited soil on the ground surface, which increased the soil macroporosity, and quickly brought more water and soil nutrients to deeper soil layer (Wang et al., 2008). Therefore, the soil water content and R/S were lower compared to CM soils, and we could also deduce that the soil nutrients, including AP, AK, AN, TN and SOM, were lower on



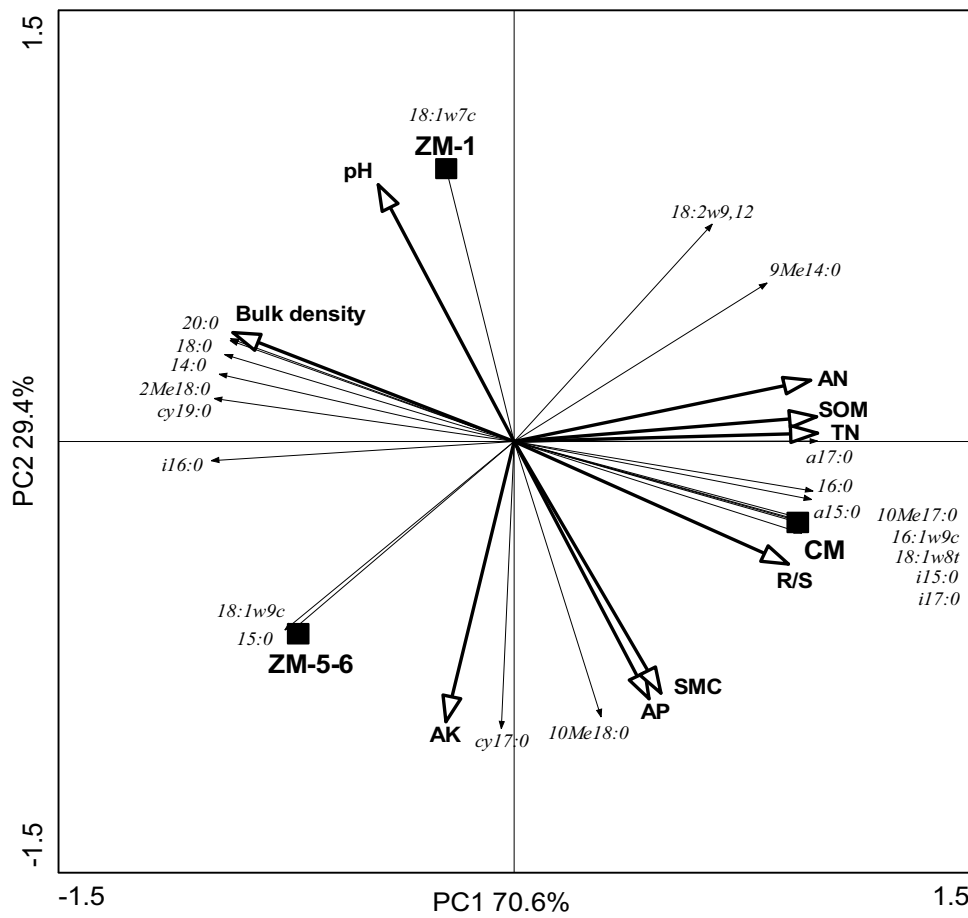


Fig. 6. Soil microbial structure and soil characteristics biplot diagram from the PCA.

Abbreviations: see Fig. 3.

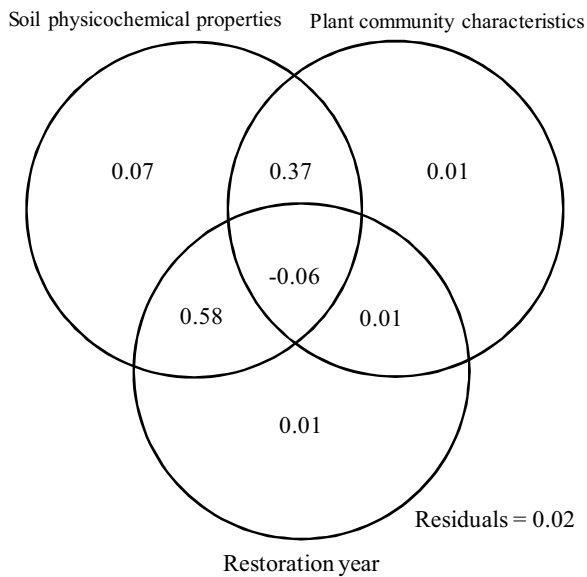


Fig. 7. Variation partitioning of RDA analyses for soil physicochemical properties, plant community characteristics and restoration year.

zokor mounds. A decrease in soil pH may result from an accumulation of SOM, and thus the production of organic acids (Cammeraat and Risch 2008). Therefore, there was the lower SOM, indirectly leading to a higher soil pH on zokor mounds (Fig. 4).

Previous studies have indicated that plateau zokors are important to changes in soil physicochemical properties by altering the plant community structure (Hogan 2010). In ZM-5-6, the soil nutrients were coordinated variation of changes in plant communities. There were the highest contents of AK and the lowest AN, TN and SOM in ZM-5-6 (Fig. 4), in which also has higher IV of forbs and sedges. Changes in plant species could alter the production and the composition of organic compounds and litter, and thus affect the quality and quantity of SOM and other nutrients (Hobbie et al., 2007; Aneja et al., 2006).

#### 4.3. Responses of soil microbial structure to zokor mounds

The soil microbial structure also significantly differed between the disturbed and CM plots, as measured by PLFA. First, soil microbial richness in disturbed plots was significantly higher than in CM, which implied that zokor disturbance may change the soil microbial composition. Second, zokor mounds changed soil microbial structure, deteriorating the microenvironment and stressing heavily soil nutrients.

Finally, there were also important effects of recovery time on microbial properties as revealed by the PCA analyses. The increasing dominant fungal PLFA 18:1w9c was located in ZM-5-6 (Fig. 6), which were also indicated higher fungal biomass and lower value of B/F than in ZM-5-6 (Fig. 5). This result showed that soil microbial composition has been changing and fungi biomass increased more than bacterial biomass in the progress of restoration.

#### 4.4. The relationships of restoration year, soil physicochemical properties, and plant community characteristics with soil microbial communities

The soil microbial communities in this study are influenced by soil properties, plant, and restoration year, and the most influential factors are soil physicochemical properties explaining 95.93% of soil microbial variance (Fig. 7), especially soil pH, SOM, AN and AP, contributed significantly to variations in soil microbial structure with restoration year (Fig. 6). The direct contribution of soil physicochemical properties, restoration years, and plant community characteristics to soil microbial community is 7.21%, 0.74% and 0.71%, respectively, which indicates that the soil and plant are the most important factors affecting soil microbial community with restoration years. Plant functional group richness are closely linked to soil microbial community structure and functions (Zhang et al., 2011). Meanwhile changes in soil physical properties provide the microenvironments for soil microbes, for instance an increase in soil N altering soil microbial biomass and function (Song et al., 2017). Therefore the combined effects of plant and soil could explain the most soil microbial variance, but the direct contribution of plant and soil is low.

In summary, the influence of zokors on alpine meadow restoration is complex. While it is clear from our data that intermediate concentrations of zokors appear to enhance the recovery process, it is also clear that the positive feedback between degradation and zokor population dynamics can lead to zokor population levels that cause degradation and retard recovery. A management challenge then becomes how to maintain these intermediate zokor populations and thus maximize meadow recovery.

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