

# Warming and grazing increase mineralization of organic P in an alpine meadow ecosystem of Qinghai-Tibet Plateau, China

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## Abstract

**Background and aims** Little is known about the soil phosphorus (P) biogeochemical cycling in response to combined warming and grazing, especially in the alpine meadow ecosystem of the Qinghai-Tibet Plateau. Here, we used a free-air temperature enhancement

system in a controlled warming-grazing experiment to test the hypothesis that combined warming and grazing would significantly accelerate mineralization of soil organic P.

**Methods** A two factorial design of warming (1.2–1.7°C temperature increase) and moderate grazing was utilized. A fractionation method was applied to investigate the sizes of different soil inorganic and organic P fractions.

**Results** Results showed that both warming and grazing significantly decreased the quantity of organic P extracted by first NaOH (N(I)Po), as well as the total extractable organic P (TPo) at the 0–10 cm depth. Warming also decreased the total P of soil at 0–10 cm. The combined warming and grazing treatment (WG) led to the reduction of major soil organic P fractions (N(I)Po, TPo) by 40–48% and 28–32%, respectively compared with other treatments at 0–10 cm. The activities of acid and alkaline phosphomonoesterase (AcPME and AlPME) were both enhanced by warming and grazing, and their interaction. Decreased concentrations of soil N(I)Po and TPo were accompanied by increased AcPME activity ( $P < 0.01$ ) and soil temperature ( $P < 0.05$ ), indicating the enhanced mineralization of organic P under rising temperature. Meanwhile, leaf biomass P of two major species (*Potentilla anserine* and *Gentiana straminea*) within these plots were significantly enhanced by either grazing or warming.

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**Conclusions** The microbial mineralization of soil organic P could be strongly increased under combined warming and grazing conditions as driven by increasing plant demand for P and enhanced microbial activities.

**Keywords** Warming · Grazing · Alpine meadow · Soil phosphorus cycling · Soil phosphatase activities

## Introduction

Phosphorus (P), one of the major soil nutrients in terrestrial ecosystems, is essential to plant growth. Under natural conditions, the loss of P can be replenished only by the release of P from primary minerals such as apatite, resulting in a gradual decrease of total P in the soil with time (Filippelli 2008). In many ecosystems P is in short supply and tends to limit the net primary production (NPP), nitrogen (N) fixation and carbon (C) storage in the long term (Vitousek and Howarth 1991; Tiessen 2008; Wang et al. 2010).

Global surface temperature is predicted to increase by 1.1 to 6.4°C by the end of this century (IPCC 2007). Soil P cycling is controlled mainly by geochemical and biological processes (Frossard et al. 1995), and can be vulnerable to global climate change and land-use management (Williams and Haynes 1990; Papanikolaou et al. 2010; Jouany et al. 2011). Microbial mineralization of organic P, producing available inorganic P for plants, is thought to be the key process of soil P cycling in unfertilized/natural ecosystems (Chen et al. 2004). Soil phosphatases, produced by both microbes and plants, are involved in the P mineralization. Increased temperature can stimulate soil phosphatase activity (Papanikolaou et al. 2010), and accelerate P cycling by enhancing plant growth which increases the demand for nutrients, quantity and quality of litter inputs, root turnover and exudation (Sardans et al. 2006), and by increasing microbial biomass and activity (Raghothama 1999). Meanwhile, the effects of warming can be controlled by soil water availability (Sardans et al. 2006). In grassland ecosystems, land-use management such as grazing can substantially affect P cycling through its direct control on plant and animal residues (Williams and Haynes 1990). Grazing also promotes the nutrient cycling rates, and increases soil nutrient heterogeneity through the uneven deposition of faeces and urine

(O'Connor 1981; Carline et al. 2005), which can modify the proportions of organic and inorganic soil P fractions (Galvao and Salcedo 2009; Hao et al. 2008). The combined direct (grass consumption) and indirect (recycling through urine and/or faeces deposition) effects of grazing have been studied extensively (O'Connor 1981; Williams and Haynes 1990; Carline et al. 2005). However, the net effect of grazing on P cycling and its consequence for primary productivity seem to vary with locations and to be dependent on other factors such as the climate (Jouany et al. 2011). Exploring the mechanisms driving P availability can provide insights into the coupling of C, water and nutrient cycles, and ultimately the responses of ecosystems to climate change (Vandecastel et al. 2009).

The Qinghai-Tibet alpine meadow has been reported to be an extremely sensitive region to global climate change (Thompson et al. 1993; Thompson et al. 2000; Wang and French 1994). Moreover, 40% greater than average increase in surface temperature (1.3 to 6.9°C) was predicted to occur in this region in the future (Giorgi et al. 2001). Concurrent with climate change, increased grazing pressure is also causing profound changes to pastoral land-use dynamics in this alpine meadow ecosystem (Zhou et al. 2005). Such changes, together with increased temperature, will strongly influence litter decomposition and nutrient mineralization, and all other important soil processes (Ineson et al. 1998a, b; Schmidt et al. 2004). Although climate change experiments in alpine ecosystems in the temperate zone are sparse (Luo et al. 2009), many studies from tundra ecosystems suggest that the altered nutrient cycling in alpine ecosystems may be a key response to climate and grazing perturbations (Jonasson et al. 1993; Shaver et al. 1998). There have been a number of studies on the responses of P cycling to warming or grazing, but in this region, the combined influence of warming and grazing on P cycling has never been investigated.

We experimentally manipulated temperature by actively warming plots using infrared heaters in a free-air temperature enhancement (FATE) system in an alpine meadow on the Qinghai-Tibet Plateau since May 2006, as described by Kimball et al. (2008), and applied a moderate grazing treatment to it. We hypothesized that warming and grazing would decrease the sizes of P pools by stimulating P cycling and increasing plant and microbial demands for P, and increase phosphatase activities; and grazing could

modify the response of P cycling to warming through decreased litter inputs and uneven faeces and urine deposition. The objectives of this study were to investigate through a controlled warming-grazing experiment: 1) the effects of warming and grazing on various inorganic and organic soil P fractions obtained through a sequential extraction scheme; 2) the effects of warming and grazing on the activities of soil acid and alkaline phosphatase; and 3) the relationships between soil P fractions, phosphatase activities and other environmental factors and soil biochemical properties.

## Materials and methods

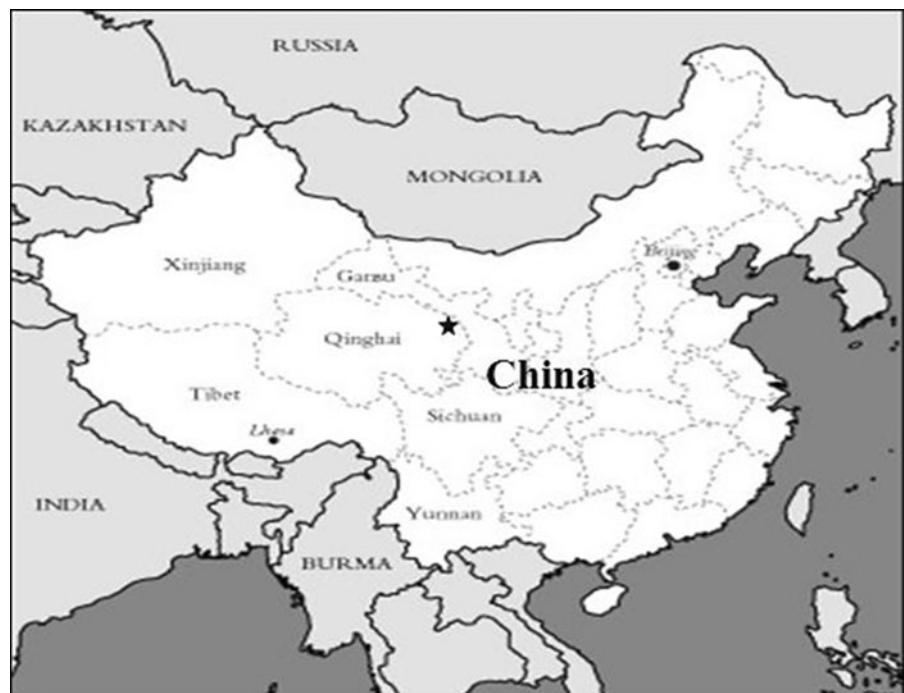
### Experimental site

The experimental site and design were described by Kimball et al. (2008) and Luo et al. (2009, 2010). It is located at the Haibei Alpine Meadow Ecosystem Research Station (HBAMERS) (Fig. 1), a facility run by the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. The station is situated at latitude 37° 37'N, longitude 101° 12'E, and the mean elevation of the valley bottom is 3,200 m. Lying in a

large valley surrounded by the Qilian Mountains on the northeast of the Qinghai-Tibetan Plateau, the station experiences a typical plateau continental climate which is dominated by the southeast monsoon in summer, from May to September and high pressure systems from Siberia in winter. Summers are short and cool, and winters are long and severely cold. Mean annual temperature is  $-2^{\circ}\text{C}$ , while mean annual precipitation is 500 mm, over 80% of which falls during the summer monsoon season. The soil is classified as *Mat-Crylic Cambisols*, as the mean organic carbon contents for the 0–8 and 8–32 cm soil depths are 7.27% and 3.32%, respectively (Wu et al. 2005).

The infrared heating system, herein called a free-air temperature enhancement (FATE), has been set up since May 2006. As the effects of global warming was predicted to be larger at night than during daytime, the setpoint differences between heated and corresponding reference plots were set as  $1.2^{\circ}\text{C}$  during daytime and  $1.7^{\circ}\text{C}$  at night in summer, which fell within limits of predicted temperature increases for this century ( $1.1\text{--}6.4^{\circ}\text{C}$ ) (IPCC 2007). The soil temperature at depths of 5, 10 and 20 cm were measured automatically using type-K thermocouples (Campbell Scientific, Logan, Utah, U.S.A), which were connected to a CR1000 datalogger. Meanwhile the soil

**Fig. 1** Location of the experimental site: Haibei Alpine Meadow Ecosystem Research Station (“★” in the map)



temperatures at 0 and 40 cm depth were manually monitored using mercury-in-glass thermometers. Warming significantly increased soil temperatures at the 0–40 cm soil depths in 2006, 2007 and 2008 (Hu et al. 2010).

A two factorial design (warming and grazing) was used with four replicates for each of four treatments, i.e. no warming with no grazing (NWNG); no warming with grazing (NWG); warming with no grazing (WNG); and warming with grazing (WG). In total, 16 circular plots of 3-m diameter were used in a randomized block design in the field. Initially, one adult Tibetan domestic sheep (*Ovis aries*) was fenced in the grazing plots on the morning of 15 August 2006 for approximately 2 h. The canopy height was 8–9 cm and 4–5 cm before and after grazing, respectively. Two sheep were fenced for approximately 1 h in the grazing plots on the mornings of 12 July, 3 August and 12 September 2007, and 8 July and 20 August in 2008. The canopy height of the vegetation was measured at 50 points within the plots before and after grazing, and the sheep were removed from the grazing plots when the canopy height was reduced to approximately half of the initial height, which generally corresponded to a moderate stocking rate in the region. All experimental sheep were fenced into 3 additional 5×5 m fenced plots for a day before commencement of the grazing experiment to help them adapt to small plots. Outside the fenced warming-grazing plots, there was an area under free grazing (FG) condition. This area was grazed by animals freely during the winter season which resulted in approximately complete removal of litter.

### Soil sampling

Soil samples from each plot were collected on 2 August 2009 using a 5 cm-diameter corer. Five soil cores were randomly collected within each plot and bulked as a composite sample. Soil samples from the soil depths of 0–10 and 10–20 cm were taken. In addition, soil samples were also taken from the free grazing area in order to study the effects of FG on soil P. All soil samples were sent to the laboratory and sieved through a 2 mm screen and stored in a refrigerator at 4°C prior to analyses of soil P fractions and enzymatic activities. Soil temperature, soil moisture,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , microbial biomass C and N (MBC and MBN), soluble organic C and N (SOC and SON), pH and total N of soil were analyzed and had already been reported by Rui et al. (2011).

### Soil P fractionation

A modified version of Hedley and Stewart (1982) fractionation scheme was used to sequentially extract various forms of inorganic (Pi) and organic (Po) soil P. In brief, 1.0 g of finely ground (<150  $\mu\text{m}$ ) air-dried soil was extracted with 30 ml of each extractant solution in an end-to-end shaker for 16 h. Tubes were then centrifuged at 10,000 RPM for 10 min, and the supernatant was decanted and saved for measurement of P.

The extractant solutions were 1 M ammonium chloride ( $\text{NH}_4\text{Cl}$ ) (APi), 0.5 M sodium bicarbonate ( $\text{NaHCO}_3$ ) (pH 8.5) (BPi, BPo), first 0.1 M sodium hydroxide (NaOH) (N(I)Pi, N(I)Po), 1 M hydrochloric acid (HCl) (HPi) and second 0.1 M sodium hydroxide (NaOH) (N(II)Pi, N(II)Po). The concentration of inorganic P in the extracts was determined after the precipitation of organic matter by acidification, and the concentration of total P in the extracts was determined after persulfate oxidation, while the concentration of organic P in the extracts was calculated as the difference between total P and inorganic P (Tiessen and Moir 1993). The P in the extracts was determined by the method of Murphy and Riley (1962). Soil total P (TP) was determined using nitric acid ( $\text{HNO}_3$ )—perchloric acid ( $\text{HClO}_4$ ) digestion (Olsen and Sommers 1982).

### Determination of soil acid and alkaline phosphatase activities

Acid phosphomonoesterase (AcPME) (EC 3.1.3.2) and alkaline phosphomonoesterase (AIPME) (EC 3.1.3.1) activities were determined by the methods described by Tabatabai and Bremner (1969) using para-nitrophenyl phosphate (*p*NPP) as an orthophosphate monoester substrate. In brief, soil sample was incubated at 37°C for 1 h (AcPME-pH 6.5, AIPME-pH 11). The enzymatic reaction was stopped by addition of NaOH and  $\text{CaCl}_2$ , and the absorbance of the *p*NPP produced was measured spectrophotometrically at a wavelength of 400 nm.

### Plant leaf biomass P measurement

Two major species within the plots, *Potentilla anserina* and *Gentiana straminea*, were selected for plant leaf biomass P measurements. The aboveground biomass was estimated by a non-destructive sampling

method described by Klein et al. (2007) within  $1 \times 1$  m quadrats of these plots. Leaf biomass of each species was determined as total aboveground biomass multiplied by proportion of this species within all species and the proportion of leaf biomass in total plant biomass of individual species. The leaf P concentration was determined after the  $\text{HNO}_3 - \text{HClO}_4$  digestion of 1.0 g dry leaf (Olsen and Sommers 1982). The total leaf biomass P was calculated as leaf biomass multiplied by leaf P concentration.

### Statistical analyses

Statistical significances of the effects of warming and grazing on soil P fractions and phosphatase activities at different soil depths and leaf biomass P were determined separately by two-way analysis of variance (ANOVA) using Statistix for Windows version 8.0 (Analytical Software, Tallahassee, FL), with warming and grazing as the main factors. Multi-comparison of each treatment was conducted using one-way ANOVA. Least Significant Difference (LSD) was used to separate the means when differences were significant at the  $P < 0.05$  level. Linear regression and redundancy analysis (RDA) was used to examine the relationship between environmental and biochemical parameters (soil moisture, pH, total N, C/N ratio,  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N, MBC, MBN, SOC and SON), soil P fractions and phosphatase activities, using Canoco Software 4.5 (Microcomputer Power, USA). Principal component analysis (PCA) was applied with a correlation similarity matrix to determine the major factors influencing all these P-associated parameters, using IBM SPSS Statistics 19 (SPSS Inc., USA).

## Results

### Soil P fractions

The sizes of soil P fractions at the 0–10 cm depth were generally larger than at 10–20 cm (Fig. 2). The soil TP ranged from 786 to 833  $\mu\text{g g}^{-1}$  and 721 to 746  $\mu\text{g g}^{-1}$  for the 0–10 and 10–20 cm depths, respectively (Fig. 3). N(I)Po, ranging from 101 to 260  $\mu\text{g g}^{-1}$ , comprised the largest proportion (12.6%–32.7% of TP) among all extracted fractions for both soil depths, followed by the N(II)Po (77 to 161  $\mu\text{g g}^{-1}$ ) and HPi (65 to 184  $\mu\text{g g}^{-1}$ ) while the APi (1.6 to 8.5  $\mu\text{g g}^{-1}$ )

constituted the smallest portion (0.22%–1.03%) of TP (Fig. 2).

Warming and grazing affected the soil P fractions mainly at 0–10 cm depth, while at 10–20 cm these fractions were less affected (Table 1 and Figs. 2 and 3). Warming and grazing significantly affected soil organic P fractions. The ANOVA results have shown that both warming and grazing reduced concentrations of N(I)Po ( $P=0.01$ , Table 1). There was no significant interaction between warming and grazing on N(I)Po ( $P=0.16$ ), but the WG treatment caused the largest decrease of it, by 48.3% compared with NWNG (Fig. 2), suggesting that the effects of warming and grazing on N(I)Po were additive. WG also significantly decreased N(II)Po compared with WNG at 0–10 cm (Fig. 2a). In addition, total extractable Po (TPo) was significantly reduced by both warming and grazing treatments at 0–10 cm ( $P=0.01$  and  $P<0.01$ , respectively) (Table 1 and Fig. 3a). Significant interaction between warming and grazing was found on TPo ( $P=0.01$ ) at 0–10 cm, and WG decreased it by 31.6% compared with NWNG. Among all treatments, WG led to the reduction of major soil organic P (N(I)Po, TPo) fractions by 40–48% and 28–32%, respectively. Moreover, soil TP was significantly reduced by warming at 0–10 cm ( $P=0.045$ ) (Table 1), and TP under WNG was significantly lower than NWNG and FG (Fig. 3a).

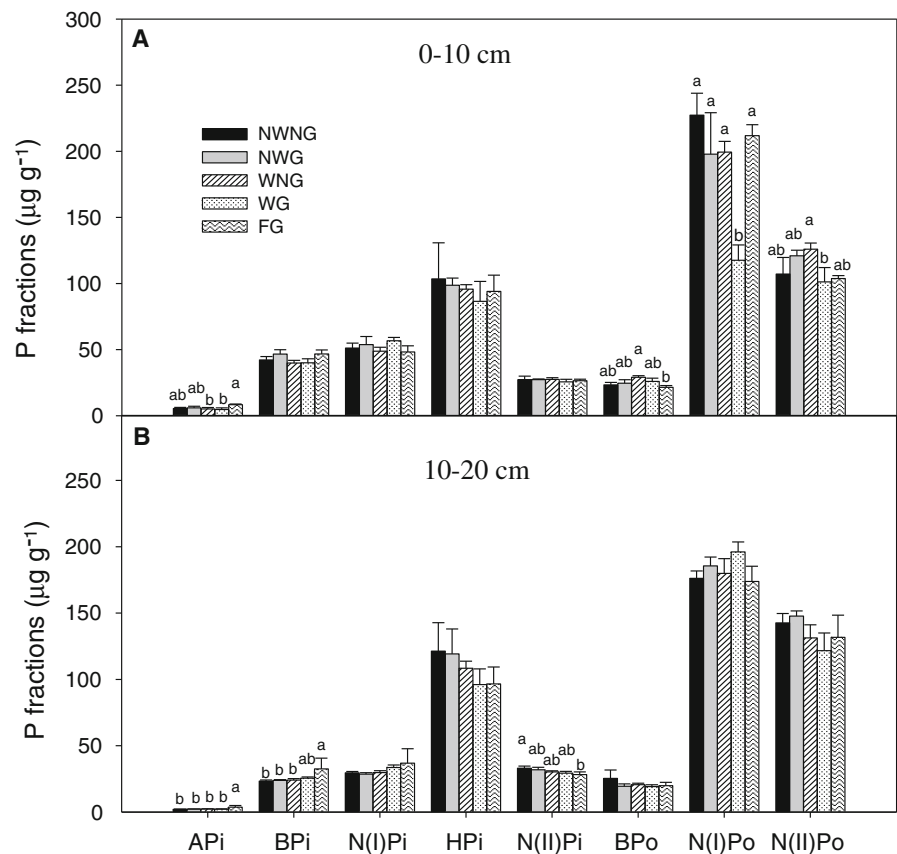
Inorganic P fractions and total extractable Pi (TPi) were generally less affected by warming and grazing treatments (Table 1 and Figs. 2 and 3) compared with the organic P fractions. APi, the solution of Pi extracted by  $\text{NH}_4\text{Cl}$ , had the minimum P concentration, ranging from 1.56 to 8.48  $\mu\text{g g}^{-1}$ . BPi, N(IPi), HPi and N(II)Pi were larger fractions compared with APi, but were also little affected by warming and grazing (Fig. 2). However, FG had significant influence on these labile P fractions, as FG significantly increased APi at 0–10 cm compared with WNG and WG, and increased APi and BPi at 10–20 cm compared with NWNG, NWG and WNG (Fig. 2). FG also decreased BPo at 0–10 cm compared with WNG and N(II)Pi at 10–20 cm compared with NWNG (Fig. 2).

### Soil phosphatase activities

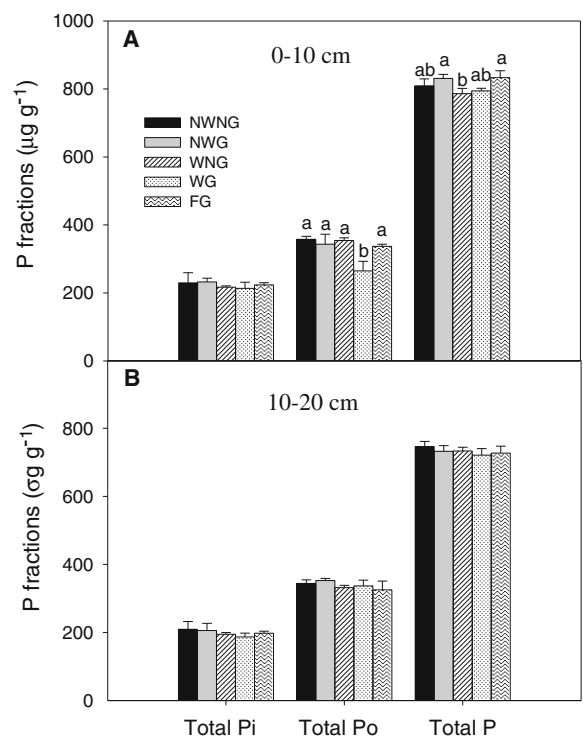
The activities of soil alkaline phosphatase were ranging from 8.7 to 26.9  $\mu\text{g p-NP g}^{-1} \text{ h}^{-1}$  for the 0–10 and 10–20 cm depths, generally higher than soil acid



**Fig. 2** Inorganic and organic phosphorus (P) fractions ( $\mu\text{g g}^{-1}$ ) of the 0–10 cm (a) and 10–20 cm (b) soil depths extracted using a P fractionation scheme. NWNG: no warming with no grazing; NWG: no warming with grazing; WNG: warming with no grazing; WG: warming with grazing; and FG: free grazing. Different letters indicate significant differences between treatments at  $P < 0.05$  for each soil depth. Mean  $\pm$  SE values are shown



**Fig. 3** Total inorganic P and total organic P ( $\mu\text{g g}^{-1}$ ) extracted using a P fractionation scheme, and total P ( $\mu\text{g g}^{-1}$ ) measured with a digestion method for the 0–10 cm (a) and 10–20 cm (b) soil depths. Different letters indicate significant differences between treatments at  $P < 0.05$  for each soil depth. Mean  $\pm$  SE are shown. See Fig. 2 for explanations of the abbreviations



**Table 1** P values for soil at two depths (0–10 cm and 10–20 cm) from two-way ANOVAs of soil phosphorus (P) fractions using warming (W) and grazing (G) as main factors. APi: inorganic P extracted by 1 M ammonia chloride (NH<sub>4</sub>Cl); BPi: inorganic P extracted by 0.5 M sodium bicarbonate (NaHCO<sub>3</sub>); N(I)Pi: inorganic P extracted first by 0.1 M sodium hydroxide (NaOH); HPi: inorganic P extracted by 1 M hydrochloric acid

(HCl); N(II)Pi: inorganic P extracted second by 0.1 M NaOH; BPo: organic P extracted by 0.5 M NaHCO<sub>3</sub>; N(I)Po: organic P extracted first by 0.1 M NaOH; N(II)Po: organic P extracted second by 0.1 M NaOH; Total Pi: total inorganic P; Total Po: total organic P; AcPME: acid phosphomonoesterase; and ALPME: alkaline phosphomonoesterase

	APi	BPi	N(I)Pi	HPi	N(II)Pi	BPo	N(I)Po	N(II)Po	Total Pi	Total Po	Total P	AcPME	ALPME
0–10 cm													
W	0.49	0.16	0.95	0.58	0.72	0.07	0.01*	0.96	0.43	0.01*	0.045*	<0.01*	0.02*
G	0.97	0.45	0.20	0.69	0.68	0.60	0.01*	0.59	0.99	<0.01*	0.28	<0.01*	0.06
W×G	0.67	0.49	0.50	0.90	0.66	0.25	0.16	0.08	0.88	0.01*	0.61	<0.01*	<0.01*
10–20 cm													
W	0.84	0.21	0.06	0.31	0.15	0.50	0.35	0.11	0.38	0.22	0.51	0.92	0.40
G	0.81	0.31	0.27	0.68	0.54	0.32	0.12	0.83	0.75	0.55	0.48	0.01*	0.09
W×G	0.67	0.64	0.07	0.77	0.94	0.54	0.66	0.50	0.91	0.87	0.97	0.07	0.60

“\*” indicates significance at  $P < 0.05$

phosphatase (5.5–14.5  $\mu\text{g p-NP g}^{-1} \text{h}^{-1}$ ). Both warming and grazing strongly affected the activities of soil acid and alkaline phosphatase. At the 0–10 cm soil depth, warming, grazing and their interaction increased AcPME activity significantly ( $P < 0.01$ ), while warming treatments and the interaction between warming and grazing also significantly increased ALPME activity ( $P = 0.02$  and  $P < 0.01$  respectively) (Table 1 and Fig. 4). WG caused the largest increases in both AcPME and ALPME activities at 0–10 cm, by 117.8 and 36.8% compared with the other treatments, respectively (Fig. 4). At 10–20 cm, grazing still had a strong influence on the activities of AcPME ( $P = 0.01$ ), while little effect of warming was found (Table 1). FG also increased the activity of AcPME compared with NWNG, NWG and WNG at 0–10 cm (Fig. 4).

#### Plant leaf P concentration and leaf biomass P

Both *Potentilla anserine* and *Gentiana straminea* were important species within these plots. However, grazing favored the abundance of *Potentilla anserine*, while warming tended to favor *Gentiana straminea*. The leaf P concentration of *Potentilla anserine* was generally higher than *Gentiana straminea* (Fig. 5a). WNG led to the lowest leaf P concentration of *Potentilla anserine* (1.08  $\text{mg g}^{-1}$ ), which was significantly lower compared with NWNG (1.33  $\text{mg g}^{-1}$ ) (Fig. 5a). Grazing (NWG and WG) significantly increased leaf biomass P of

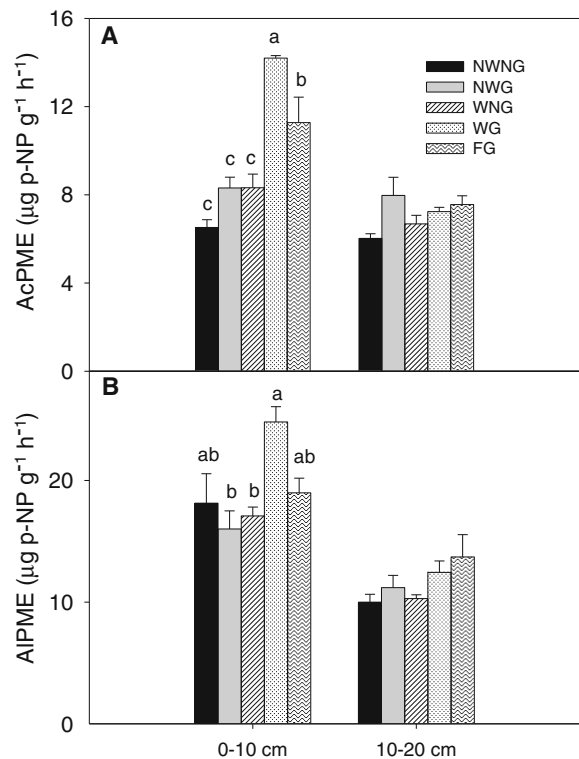
*Potentilla anserine*, which rose from 13.4 to 15.2  $\text{mg m}^{-2}$  under NWNG and WNG treatments to 40.3 and 36.9  $\text{mg m}^{-2}$  under NWG and WG treatments (Fig. 5b). WNG significantly affected leaf biomass P of *Gentiana straminea* compared with NWG, by increasing it from 12.6 to 24.0  $\text{mg m}^{-2}$  (Fig. 5b).

#### Relationships between environmental factors and soil P fractions and phosphatase

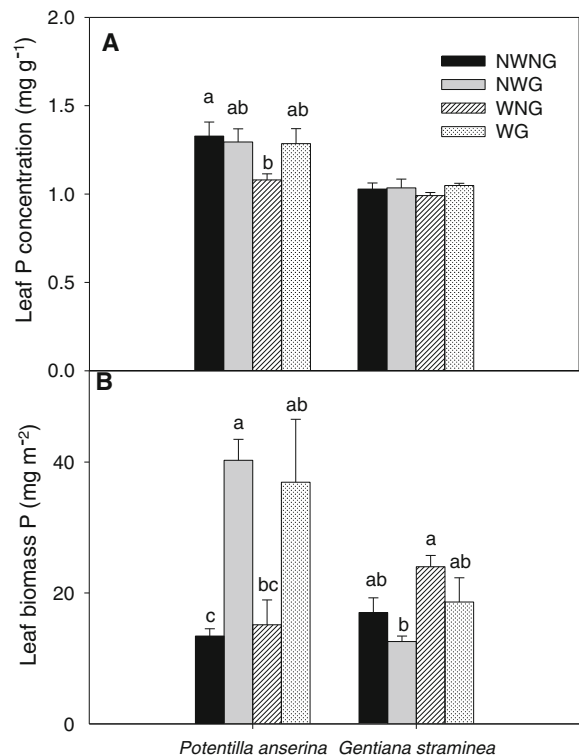
N(I)Po and TPo were negatively correlated with the AcPME activity and soil temperature at 0–10 cm. The activity of AcPME and soil temperature explained 44% and 38% of the variation of N(I)Po (Fig. 6a and b), and 54% and 29% of the variation of TPo (Fig. 6c and d), respectively.

Figure 7 demonstrates the relationships between environmental and soil biochemical factors, and soil P fractions and phosphatase activities. The approximately same direction between the arrows that soil P parameters are pointing and that of environmental and soil biochemical parameters indicates a high positive correlation (the longer the arrow, the stronger the relationship; Kennedy et al. 2004). TP was highly and positively correlated with soil moisture, as their arrows have approximately same direction and length. ALPME was highly and positively correlated with TN, NH<sub>4</sub><sup>+</sup>-N and MBN, while AcPME was highly and positively correlated with NO<sub>3</sub><sup>-</sup>-N and SOC. The N

**Fig. 4** Acid phosphomonoesterase (AcPME) (**a**) and alkaline phosphomonoesterase (AIPME) (**b**) activities in soil from 0–10 to 10–20 cm depths. Different letters indicate significant differences between treatments at  $P < 0.05$  for each soil depth. Mean  $\pm$  SE values are shown. See Fig. 2 for explanations of the abbreviations

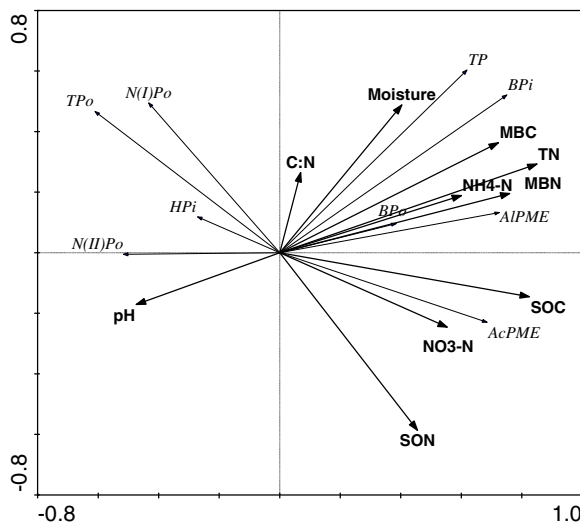
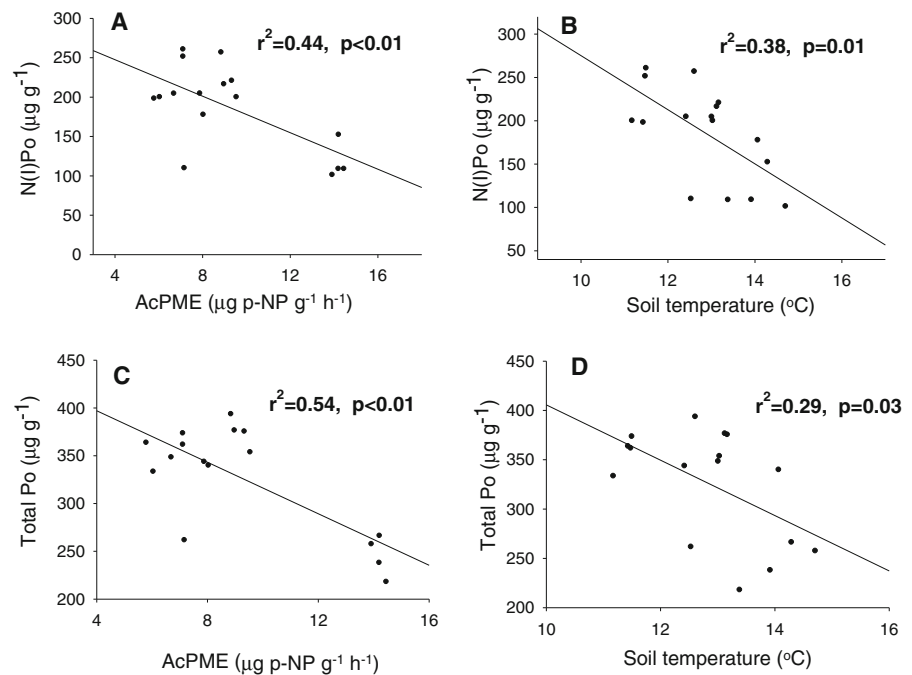


**Fig. 5** Plant leaf P concentration ( $\text{mg g}^{-1}$ ) (**a**) and leaf biomass P ( $\text{mg m}^{-2}$ ) (**b**) of two major species, *Potentilla anserina* and *Gentiana straminea* for different treatments. Different letters indicate significant differences between treatments at  $P < 0.05$ . Mean  $\pm$  SE are shown. See Fig. 2 for explanations of the abbreviations





**Fig. 6** Relationships between AcPME activity and N(I)Po (a), soil temperature and N(I)Po (b), AcPME activity and TPo (c), and soil temperature and TPo (d) at 0–10 cm soil depth



**Fig. 7** Biplot of redundancy analysis (RDA) of the relationships between environmental factors and soil P fractions and phosphatase activities. TN: soil total N; MBC: microbial biomass C; MBN: microbial biomass N; SOC: soluble organic C; SON: soluble organic N; TP: total P; TPo: total organic P; BPi: inorganic P extracted by 0.5 M sodium bicarbonate ( $\text{NaHCO}_3$ ); BPO: organic P extracted by 0.5 M  $\text{NaHCO}_3$ ; HPi: inorganic P extracted by 1 M hydrochloric acid (HCl); N(I)Po: organic P extracted first by 0.1 M sodium hydroxide (NaOH); N(II)Po: organic P extracted second by 0.1 M NaOH; AcPME: Acid phosphomonoesterase; and AIPME: alkaline phosphomonoesterase

(I)Po was negatively correlated with  $\text{NO}_3^-$ -N and SON. The PCA has showed that the clear separation of WG samples from other treatments along the PC2 axis. The PC1 and PC2 axes explained 37.6% and 21.8% of the variance of these parameters, respectively (Fig. 8).

## Discussion

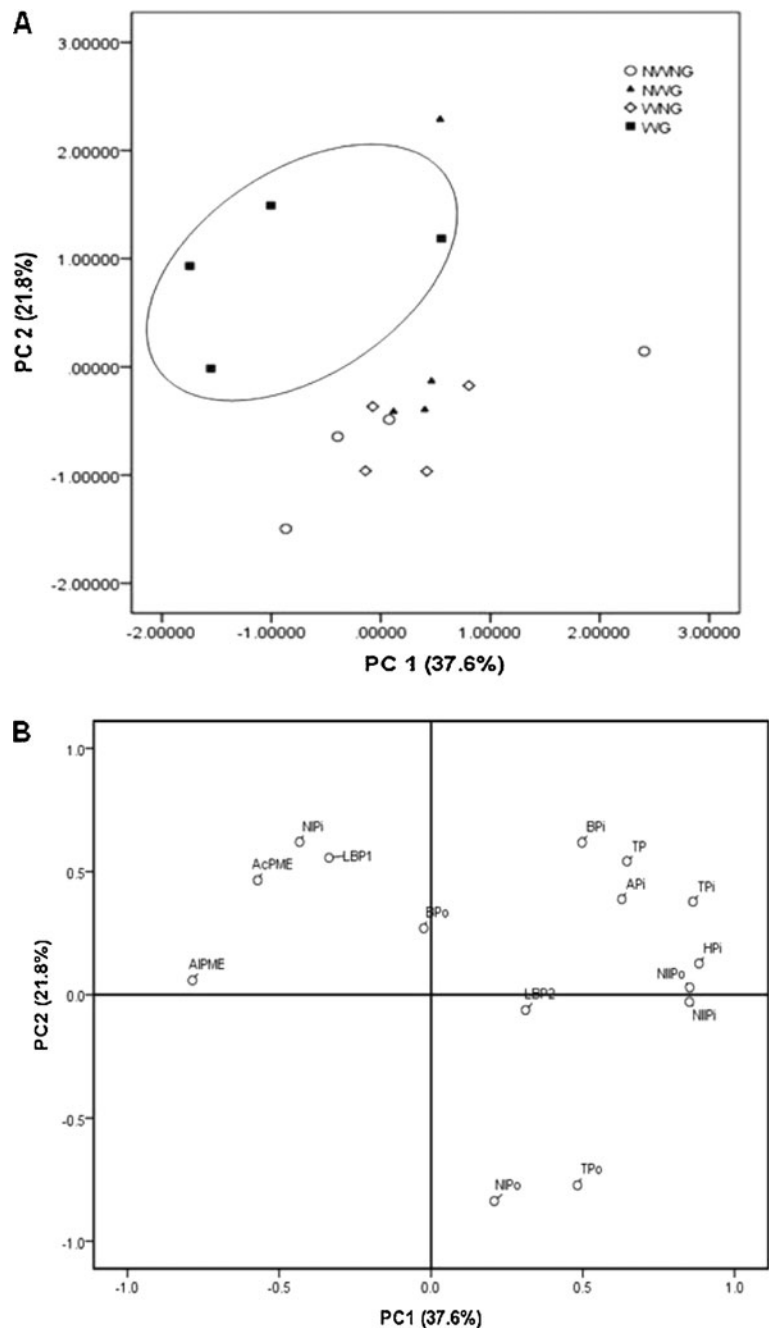
We have reported previously how warming and grazing affected soil temperature, moisture and C and N pools in 2009 (Rui et al. 2011). Basically, both WNG and NWG significantly increased average soil temperature of the growing season, while WG caused the largest increase compared with NWNG. Both WNG and WG decreased soil moisture significantly at 0–10 and 10–20 cm. Neither warming nor grazing significantly affected soil pH (7.02–7.85). Grazing significantly increased inorganic N concentration, while WG significantly increased total N and MBC at 10–20 cm and SOC at 0–10 cm (Rui et al. 2011).

Effects of warming on P cycling in the alpine meadow ecosystem on the Qinghai-Tibet Plateau

The effects of warming on terrestrial P cycling have been widely studied, and results from a range of sites

**Fig. 8** Ordination plot of principal component analysis (PCA) of all P-associated parameters under different warming and grazing regimes (a) and loading values of the individual parameter for PC1 and PC2 (b).

Numbers in parentheses are percentage variance for each principal component. LBP1: Leaf biomass P of *Potentilla anserina*; LBP2: Leaf biomass P of *Gentiana straminea*; APi: inorganic P extracted by 1 M ammonia chloride ( $\text{NH}_4\text{Cl}$ ); BPi: inorganic P extracted by 0.5 M sodium bicarbonate ( $\text{NaHCO}_3$ ); N(I)Pi: inorganic P extracted first by 0.1 M sodium hydroxide ( $\text{NaOH}$ ); HPi: inorganic P extracted by 1 M hydrochloric acid ( $\text{HCl}$ ); N(II)Pi: inorganic P extracted second by 0.1 M  $\text{NaOH}$ ; BPo: organic P extracted by 0.5 M  $\text{NaHCO}_3$ ; N(I)Po: organic P extracted first by 0.1 M  $\text{NaOH}$ ; N(II)Po: organic P extracted second by 0.1 M  $\text{NaOH}$ ; Total Pi: total inorganic P; Total Po: total organic P; AcPME: acid phosphomonoesterase; and ALPME: alkaline phosphomonoesterase. See Table 1 for explanations of other abbreviations



have reported that increased temperature could stimulate microbial and soil phosphatase activity, enhance microbial mineralization and plant uptake of P, hence result in an accelerated P cycling (Sardans et al. 2006; van Meeteren et al. 2007; Bell et al. 2010). Here we also found that warming had significant influences on soil phosphatase activities and soil organic P fractions, suggesting that on the Qinghai-Tibet Plateau, increased

temperature could lead to fundamental changes in soil P cycling.

Soil P cycling and availability are controlled by a combination of biological processes (mineralization—immobilization) and chemical processes (adsorption—desorption and dissolution—precipitation) (Chen et al. 2004). Soil pH is one of the main parameters determining the adsorption/desorption equilibrium of phosphate

in soil (Hinsinger 2001). However, as pH was little affected by warming and grazing in our study, due to the low rates of weathering of P-containing primary minerals in this region, the availability of P was controlled mainly by mineralization of mineralizable organic P (Schlesinger 1991). WG decreased N(I)Po, N(II)Po and TPo, and increased the activities of AcPME and AlPME at the 0–10 cm depth (Figs. 2a, 3a and 4). The N (I)Po, often associated with Fe and Al hydrous oxides, was considered to be moderately mineralizable. The enhanced mineralization of soil organic P in the warming treatments, as indicated by a significant decrease in N(I)Po, can be attributed to increased plant and microbial demands under the warming conditions. Several studies have reported that the mineralization of organic P is controlled mainly by demand for P (McGill and Cole 1981; Chen et al. 2004). It was well documented that warming could enhance plant and microbial growth, leading to an increased excretion of phosphatase to catalyze the mineralization of organic P and to meet their demands for available P (Sardans et al. 2006). In our study, soil temperature explained 38% and 29% of the variation of N(I)Po and TPo, respectively. WNG significantly increased leaf biomass P compared with NWG in *Gentiana straminea* (Fig. 5b), while NWG and WG significantly increased leaf biomass P compared with NWNG in *Potentilla anserina*. As the leaf P concentration of the two species was not affected or reduced by WNG (*Potentilla anserina*), the increases in leaf biomass P were mainly due to the increase in the leaf biomass under warming, considering that warming tended to favor the *Gentiana straminea* population while *Potentilla anserina* became dominant in grazing plots. However, warming also significantly increased total P content in plant litter in 2009 (Data not published), directly revealing the increased plant demand for P under the warming conditions. Microbial activity was undoubtedly a key regulator of P availability and both microbial mineralization and immobilization occurred rapidly, and their net effect on P availability varied with soil moisture, temperature, and soil organic matter quantity and quality (Vandekar et al. 2009). Microbes were thought to outcompete plants for available P over short timescales due to their high surface area to volume ratio, rapid growth, and high turnover rates (Cole et al. 1977; Schimel et al. 1989). Olander and Vitousek (2004) reported that microbial demand determined the partitioning of newly added P into biological vs geochemical sinks. Microbial biomass could act as a

source (mineralization) or a sink (immobilization) for plant-available nutrients. In another study (Rui et al. 2011) we have reported that WG significantly increased MBC at 10–20 cm and SOC at 0–10 cm, suggesting a stronger demand for P by the microbes under the WG treatment. Nevertheless, the effects of warming in increasing P mineralization and plant and microbial uptake of P could be controlled simultaneously by the decreased soil moisture under the warming conditions. Lower water supply could limit P diffusion at the root interface and uptake by plants, and also hinder microbial activity (Bradford and Hsiao 1982). However, although warming significantly decreased soil moisture in our study, the lowest value of soil moisture of the two depths was still approximately 30%, indicating water was not a limiting factor in this region. Sardans et al. (2007) suggested that the greatest values of phosphatase activity were observed when intermediate values of soil water content coincided with intermediate values of temperatures in soil and air, which were the most suitable for active plant metabolism and soil microbial activity. However, the decreased soil moisture might restrict the solubility of P, and limit its availability for plants.

Warming significantly decreased TP ( $P=0.045$ ) at the 0–10 cm soil depth. Studies of Mediterranean shrubland (Sardans et al. 2006) and subarctic heath ecosystems (Rinnan et al. 2008) also suggested that warming could decrease soil total P, mainly through the increased accumulation of P in plant biomass. As we have observed a higher P content in the litter under warming, presumably the P is getting returned in litter in the long term. However, the various inorganic P fractions, including A<sub>Pi</sub> (solution Pi), B<sub>Pi</sub> (labile Pi, which adsorbed on the surface of crystalline P compounds, sesquioxides or carbonate), N(I)<sub>Pi</sub> (moderately labile Pi, which was associated with amorphous and some crystalline Al, Fe hydrous oxides), H<sub>Pi</sub> (less labile Pi, which was associated with primary calcium minerals) and N(II)<sub>Pi</sub> (stable Pi, which was adsorbed into the mineral structure of soil components, or occluded by Fe and Al coatings), remained unchanged under the warming conditions. This implied that these inorganic P fractions were in transient turnover, and might not be controlled by only a single factor but by multiple factors, while the depletion of available inorganic P with relatively smaller size, could be quickly replenished by mineralization of organic P under the warming conditions.

Soil enzymes integrated information on soil microbial status and soil physical-chemical conditions and thus were a useful sensor to study the effects of environmental changes (Wick et al. 1998; Chen et al. 2003). It had been suggested that warming could increase soil phosphatase activities, not only through its direct influence in stimulating microbial activity, but also through its impacts on plant root exudation and mycorrhizal fungi. The activity of AcPME explained 44% and 54% of the variation of N(I)Po and TPo, respectively. Acid phosphatase was produced by bacteria, mycorrhizal fungi, yeasts, protozoa and plant roots. The symbiotic mycorrhizal fungi could coat plant rootlets, excreting phosphatase and organic acids to release P and provide an active uptake site of for rapid diffusion of P from soil pore spaces to the root surface (Filippelli 2008). Alkaline phosphatase was produced by bacteria, fungi and earthworms. Temperature increases by 5°C had been found to double the colonization of roots by mycorrhiza (Gavito et al. 2003). According to unpublished results of us, warming also significantly increased the concentration of total extractable glomalin (TEG) and easily extractable glomalin (EEG), glycoproteins produced by arbuscular mycorrhizal fungi which were important in enhancing soil aggregation, soil aeration, drainage and microbial activity (Jastrow and Miller 1997; Wright et al. 1999; Rillig et al. 2001), suggesting that the activities of soil phosphatase could be favored due to both direct and indirect effects of warming, therefore result in an accelerated P cycling.

#### Effects of grazing on P cycling in the alpine meadow ecosystem on the Qinghai-Tibet Plateau

The modern terrestrial P cycle is dominated by agriculture and human activity (Filippelli 2008). Grazing, the major land use mode of this meadow, can accelerate P cycling by promoting plant growth which will increase nutrients uptake and enhancing soil phosphatase activities (Williams and Haynes 1990; Hobbs 1996). Although NWG, the simulated moderate grazing treatment, did not affect various P fractions, WG decreased sizes of organic P fractions (N(I)Po, N(II)Po and TPo) significantly and increased the activities of soil acid and alkaline phosphatase, indicating that the combined warming and grazing treatment would augment the effects of warming or grazing alone. NWG also increases soil temperature due to enhanced solar radiation, but WG caused the largest temperature increase.

These results have profound implications on the alpine meadow in future warming conditions with continuous grazing, that P cycling may not be affected by warming or grazing alone, but can be significantly accelerated by combined effects of warming and grazing. Different from NWG, FG had significant influence on labile P fractions, as FG significantly increased APi and BPi at 0–10 and 10–20 cm, and decreased BPo at 0–10 cm and N(II)Pi at 10–20 cm, indicating that FG might be able to affect labile P fractions as a greater grazing intensity compared with NWG. These results in total suggested that the pattern or intensity of grazing was an important factor when evaluating its effect on P cycling.

In natural ecosystems, often both N and/or P availabilities are near limiting levels, but the dependence of biological N fixation on adequate P supply makes P the principal limiting element (Tiessen 2008). When P is limiting, nodule growth and N-fixation activity are limited (Haynes and Ludecke 1981), the diversity and productivity of plant community may be reduced (Falkengren-Grerup 1998; Wrage et al. 2010). Large amounts of N could stimulate phosphatase exudation and therefore plant uptake of P (Wrage et al. 2010). The activity of AcPME in our study was positively correlated with  $\text{NO}_3^-$ -N (Fig. 7), which was significantly stimulated by grazing as reported previously at this experimental site (Rui et al. 2011) and some other studies (e.g. Olofsson et al. 2001), suggesting that the grazing induced increase in  $\text{NO}_3^-$ -N could have a positive effect on phosphatase activity, consequently the P mineralization. Spiers and McGill (1979) also reported that acid phosphatase activity increased 6-fold in soils supplemented with glucose and  $\text{NH}_4\text{NO}_3$ . Turner et al. (2003) found that soil with a long history of N deposition in northern England had low P concentrations and most P was in the form of relatively stable organic P. However, P fertilization will favor the development of legumes on grazed grasslands and rangelands (Aydin and Uzun 2005; Martiniello and Berardo 2007). In another experiment within this region, we found that aboveground net primary production (ANPP) did not respond to the addition of N fertilizer but increased significantly in response to P fertilizer, implying that on this alpine meadow P rather than N could be a principal limiting factor (Data not published). The increased availability and total storage of N under warming and grazing conditions, and the possible depletion of organic P, could bring about

fundamental changes to the nutrients cycling and plant diversity in the long term on the plateau.

Unlike warming, grazing did not affect soil TP, which could be attributed to the quick return of P through deposition of animal faeces and urine, which contained both inorganic and organic P. The release of N and P was much faster via the animal decomposition pathway (Floate 1981), so that the accelerated P cycling might result in a transient turnover of all labile forms of N and P. However, as the effects of warming and grazing on soil P fractions and phosphatase were additive, the combined effects might lead to a gradual decrease of mineralizable and total P in the soil. As the availability and total content of N have been increased under the warming and grazing conditions, whether the depletion of organic P will result in a P deficiency and act as a limiting factor to the C and N cycling requires further investigation in this ecosystem in the long term.

## Conclusions

Our study demonstrated that in the alpine meadow ecosystem of the Qinghai-Tibet Plateau, the combined effects of warming and grazing could modify the P cycling by significantly increasing the mineralization of organic P and phosphatase activities. The effects of warming and grazing on soil P cycling were additive, and there could be a gradual decrease in soil-available and total P in the long term under future warming and grazing conditions. These results, together with the previously reported profound effects of warming and grazing on C and N cycling, would have significant implications for the long-term productivity and sustainability of this ecosystem.

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