Plant Soil (2010) 327:403–411
DOI 10.1007/s11104-009-0065-3

REGULAR ARTICLE

Establishment of *Elymus natans* improves soil quality of a heavily degraded alpine meadow in Qinghai-Tibetan Plateau, China

Ruizhang Feng · Ruijun Long · Zhanhuan Shang · Yushou Ma · Shikui Dong · Yanlong Wang

Received: 7 February 2009 / Accepted: 4 June 2009 / Published online: 20 June 2009

Abstract *Elymus natans* is a dominant native species widely planted to restore the heavily degraded alpine meadows in Qinghai-Tibetan plateau. The objective of this study was to determine how *E. natans* establishment affected the quality and fertility of a heavily degraded soil. Soil samples (at depths of 0–10, 10–20 and 20–30 cm) were collected from the 3- and 7-year-old *E. natans* re-vegetated grasslands, and in the heavily degraded alpine meadow (control). The establishment of *E. natans* promoted plant cover and aboveground biomass. Compared to the non-reseeded meadow, the concentration of total organic C increased by 13% in the soil under 3-year-old reseeded *E. natans* grassland at 0–10 cm, and by 7–33% in the soil under 7-year-old reseeded *E. natans* grassland at 0–10, 10–20 and 20–30 cm depths. Rapid increases in total and available N were also observed in two *E. natans* re-vegetated grasslands, especially in the 0–10 cm soil layer. Across three sampling depths, total P concentration was increased by 17–35% and 18–54% in 3- and 7-year-old reseeded soil respectively, compared to the soil of control. After 3 years of *E. natans* growth, microbial biomass C increased by 13–58% at 0–10 and 10–20 cm layers; while it increased by 43–87% in 7-year-old reseeded treatment at 0–10, 10–20 and 20–30 cm depths relative to control. A similar increasing trend was observed for microbial biomass N and P generally. Significant increase in neutral phosphatase, urease, catalase and dehydrogenase was also found in 3- and 7-year-old re-vegetated grasslands compared with heavily degraded meadow. Our results suggest a significant positive impact of *E. natans* establishment on soil quality. Thus, *E. natans* establishment could be an effective and applicable measure in restoring heavily degraded alpine meadow in the region of Qinghai-Tibetan Plateau.

Responsible Editor: Tim Simon George.

R. Feng
Northwest Plateau Institute of Biology,
Chinese Academy of Sciences,
Xining 810008, China

R. Long (✉) · Z. Shang
International Centre for Tibetan Plateau Ecosystem
Management, Lanzhou University,
Lanzhou 730070, China
e-mail: longrj@lzu.edu.cn

Y. Ma · Y. Wang
Qinghai Academy of Animal and Veterinary Sciences,
Xining 810003, China

S. Dong
Environmental School, Beijing Normal University,
Beijing 100875, China

R. Feng
Graduate School of the Chinese Academy of Sciences,
Beijing 100039, China

Keywords Plantation age · Plant biomass · Soil organic carbon · Soil nutrients · Microbial biomass · Soil enzymes

Introduction

Qinghai-Tibetan Plateau, the highest and largest plateau on earth, has an average altitude of 4000 m a. s. l., is an important component of the global terrestrial ecosystem, and one of the main areas of animal husbandry in China. Alpine meadow and steppe covers about 1.5 million km^2, accounting for two thirds of the total plateau area (Sun and Zheng 1998). However, this important ecosystem has been severely degraded since the 1970s, primarily due to overgrazing (Li and Zhou 1998), increased rodent populations (Liu et al. 1999), cryoturbation and warming climate (Ma 1999). There are currently 425,100 km^2 of degraded and 70,319 km^2 of heavily degraded alpine meadow and steppe in the region, making up 33.0 and 16.5% of the total meadow and steppe area, respectively (Wang 1997; Ma 1999). Evidence indicates that lightly and moderately degraded meadows can revert to their initial state within 10–20 years, by reducing the grazing pressure and controlling the rodent damage (Bao and Gao 1994; Ma et al. 2008). However, for heavily degraded meadow with no remaining native vegetation, the only possible strategy for restoring is re-vegetation and reestablishment, followed by appropriate management (Ma et al. 2007; Wang et al. 2006).

Cultivars of native species such as *Elymus natans, Poa crymophila* and *Festuca sinensis* have been used widely in degraded meadow rehabilitation in Qinghai-Tibetan Plateau region because of their good adaptation, forage quality and high availability (Wang et al. 2006). With financial support from central and provincial governments, approximately 2,000 km^2 of degraded alpine meadow was artificially established in Guoluo Prefecture of Qinghai Province in 1998–2002 (Wang et al. 2006). Furthermore, intensive studies concerning the effects of artificial restoring measures on vegetation, the ecological and economic benefits have been carried out in the alpine region (Zhang and Zhao 2002; Zhou et al. 2007; Wang et al. 2007), but very few have focused on soil quality. The objective of this study was to investigate the changes of soil physical, chemical and microbiological properties due to the establishment *Elymus natans* on severely degraded alpine meadow. Information on the changes of these properties is very important to better understand phytoremediation mechanisms and interactions between soil and plant communities, and for appropriate management and protection of the eco-environment.

Materials and methods

Study sites

This study was conducted at Maqeen Zang Autonomous County of Qinghai Province. The study site (34°28′N, 100°09′E) has an altitude of 4100 m, with annual average air temperature minus 4°C, and no frost-free season. The mean annual precipitation range is 513–560 mm and the annual mean pan-evaporation is approximately 3000 mm. Based on data from China's second national soil survey, the soil is classified as Alpine Meadow Soil (National Soil Survey Office 1998), similar to a Inceptisols soil according to USDA classification. The natural vegetation in this region was mainly alpine Kobresia meadow, which was dominated by *Kobresia humilis*, *K. capillifolia*, *K. pygmaea*, *K. tibetica*, *Poa crymophila* and *Festuca rubra*. However, the vegetation of heavily degraded meadow was dominated by *Artemisia nanschanica* and *Aster flaccidus*, being characterized by low vegetation cover and low edible forage production (Ma 1999).

Elymus natans was gradually planted on heavily degraded meadow with a series of agronomic measures, beginning in the spring of 1998. Botulinum-C was applied to kill wild rodents (especially *Ochotona curzoniae*) in the winter of 1997. In re-vegetation of heavily degraded areas by *E. natans*, the soil was ploughed and harrowed; diammonium phosphate (45 kg ha^{-1}) was applied as basal fertilizer. The seeding rate was 43–45 kg ha^{-1}. The seeded grasslands were fertilized with urea (75 kg ha^{-1}) at the jointing stage from the second year. Metsulfuron-methyl and 2, 4-D-butyl were used as herbicides to control weeds in the third, fourth and sixth year after re-vegetation. In addition, seeded grasslands were enclosed to prevent grazing in the first year of rehabilitation and light rotational grazing was permitted in winter from the second year. To date, there are 3- and 7-year-old grasslands of *E. natans* re-vegetated on the heavily

degraded alpine in the study site, with the areas of each grassland >10 ha.

Experimental design

In August 2006, we selected an area (about 1000× 1000 m) that included three treatments: 3-year-old grassland of *E. natans* re-vegetated; 7-year-old grassland of *E. natans* re-vegetated, and heavily degraded meadow (control). The three treatments adjoined each other and have gently rolling topography. Three plots (50×50 m) were randomly enclosed in each of the three treatments for vegetation and soil sampling.

Vegetation survey

Plant community characteristics were investigated using 10 quadrats of 0.25 m^2 (50×50 cm) in each plot. The total ground coverage was estimated from these quadrats. Plant species were identified and recorded, then split into three groups (grass, sedges and forbs). In each plot, vegetation was clipped off flush with the ground from five randomly selected quadrats of area 0.25 m^2 for determination of above-ground biomass. Below-ground biomass was sampled with ten soil cores (diameter 5 cm) per plot at 0–30 cm depths, and collected by washing off soil in a mesh bag (mesh size of 0.5 mm). The above- and below-ground plant biomass was dried at 70°C for 48 h and weighed (Wang et al. 2006).

Soil sampling

In each plot, five soil columns were randomly excavated to 30 cm depth and cut into three segments to represent three soil depths of 0–10, 10–20 and 20–30 cm. The subsamples at the same depth from different columns in each plot were mixed to provide a composite sample. Overall, 27 composite soil samples were collected comprising three grasslands, three field replicate plots and three depths. After visible roots and other debris were picked out, each composite soil sample was sieved to pass a 2-mm screen, and then split into two sub-samples. One sub-sample was kept at field-moisture in a cooler at 4°C for a week before soil microbial biomass C, N, and P analysis. The other sub-sample was air-dried and stored for 2 months at room temperature for soil pH, organic C, nutrients, and soil enzymes analysis.

Analytical methods

Soil bulk density and maximum water-holding capacity were determined using a cutting ring (volume of 100 cm^3; inner diameter of 5 cm) (Institute of Soil Science, Chinese Academy of Sciences (ISSCAS) 1978). For determining the maximum water-holding capacity of soil, the cutting rings were closed at one end with a fine mesh and open at the other end, and saturated with water for 3 h. The water surplus was drawn off using a sand bed greater than 20 cm deep and consisted of sand with 0.1–2 mm particle size, the remaining water in the soil represented the maximum water-holding capacity (ISSCAS 1978).

Soil pH was determined using 1:2.5 soil: water ratio (ISSCAS 1978). Total organic carbon (C) was determined by the dichromate oxidation method (ISSCAS 1978), and total nitrogen (N) was measured by a semi-micro Kjeldahl digestion procedure (ISSCAS 1978). Total phosphorus (P) content was determined by colorimetry after digesting soil with perchloric acid (ISSCAS 1978), and total potassium (K) was fused by NaOH, then measured by flame photometry (ISSCAS 1978). Available N was hydrolyzed by 1.0 M NaOH, absorbed by H_3BO_3, then titrated with 0.01 M HCl (ISSCAS 1978).

Soil microbial biomass C (MBC), N (MBN) and P (MBP) were determined by $CHCl_3$ fumigation extraction (Wu et al. 1990; Brookes et al. 1982, 1985). Three portions (30 g each) of 2 mm sieved fresh soil were exposed to alcohol-free $CHCl_3$ vapor in a vacuum desiccator for 24 h at 25°C; then placed them in a clean empty desiccator, and the residual $CHCl_3$ was removed from the fumigated soil by repeated evacuation. The fumigated samples were shaken for 30 min in 60 ml 0.5 M K_2SO_4 and extracted. Afterward, the solution was filtered through Whatman No. 42 filter paper. The other three portions of fresh soil were not fumigated, but were extracted the same way as above. The filtrates from fumigated and non-fumigated soil samples were used for analyzing extracted organic C and extractable N by using dichromate oxidation and micro-Kjeldahl digestion, respectively. Microbial biomass C and N was calculated as the difference of total extractable C (or N) between fumigated and non-fumigated samples by the conversion factor of 0.45 (Wu et al. 1990, Jenkinson 1988).

For microbial biomass P measurement, 10 g fresh moist soil was fumigated with $CHCl_3$ for 24 h at 25°C

in the dark. The fumigated soil and non-fumigated soil samples were extracted with 0.5 M $NaHCO_3$ for 30 min (Brookes et al. 1982). Phosphorus concentration in the filtrate was colorimetrically determined. MBP was then calculated as the increase of extractable P in the fumigated and non-fumigated soil using a conversion factor of 0.4 (Brookes et al. 1982, He et al. 1997).

Soil urease activity was measured by using urea as the substrate, and the released ammonium was assayed colorimetically at 578 nm (Guan 1986). The enzyme activity is expressed as *n* kat g^{-1} dry soil. One katal is defined as 1 mol of NH_3-N formed per second (Shi et al. 2006). For measuring catalase activity, 2 g of air-dried soil with 40 mL distilled water and 5 mL 0.3% H_2O_2 was shaken for 20 min at 150 rpm, the surplus H_2O_2 in filtrate was titrated with 0.02 mol L^{-1} $KMnO_4$ (Guan 1986). The catalase activity is expressed as *n* kat g^{-1} dry soil. One katal is defined as 1 mol of $KMnO_4$ was deoxidated per second (Shi et al. 2006). Soil dehydrogenase was measured as described by Pepper et al. (1995); 5 g of air-dried soil was incubated with 2,3,5- triphenyltetrazolium chloride substrate at 37°C for 24 h and the concentration of the triphenyl formazan (TPF) product was colorimetrically (485 nm, UV330, Unicam UV-vis) measured. The dehydrogenase enzymatic activity is expressed as *p* kat g^{-1} dry soil. One katal is defined as 1 mol of TPF formed per second (Shi et al. 2006). Neutral phosphatase was assayed using a modified disodium phenylphosphate method; 3 g of air-dried soil was incubated in 5 mL disodium phenyl phosphate, 5 mL citrate buffer at pH 7 and 0.5 mL toluene at 37°C for 12 h and the concentration of phenol released by phosphatase was colorimetrically (570 nm, UV330, Unicam UV-vis) measured (Zhao and Jiang 1986). The enzyme activity is expressed as *n* kat g^{-1} dry soil. One katal is defined as 1 mol of phenol formed per second (Shi et al. 2006).

Statistical analysis

All results are reported as means ± standard deviations. Differences in vegetation data between treatments were assessed by one-way analysis of variance (ANOVA). The least significant difference (LSD) test was used to compare the means at $P \leq 0.05$. All the data for soil attributes were analyzed by two-way ANOVA with plantation age and soil depth as fixed factors. If significant F-tests were obtained for the main (grassland and depth) or interaction effects, a LSD test was used to compare means at $P \leq 0.05$ (Li et al. 2007). The significance of a linear relationship between various parameters was expressed by the Pearson's product moment correlation coefficient (Li et al. 2007). All statistical analyses were conducted by using SPSS 13.0.

Results

Vegetation characteristics

E. natans establishment practices including ploughing up the soils and application of herbicides killed species (mainly forbs) that previously inhabited in the degraded meadows (control), such that *E. natans* dominated over re-established grasslands (Table 1). *E. natans* establishment on the degraded vegetation resulted in significant increases in plant cover by 62–66% and total aboveground biomass by 48–51% (Tables 1 and 2). Consistent with the change in species, a great change in aboveground biomass composition was observed, that is, aboveground biomass was mainly contributed

Table 1 Vegetation compositions under 3- and 7-year-old re-vegetated grasslands and heavily degraded meadow (Control) (means ± SD)

Plantation age	Species number	Dominant species	Dominance (%)	Sub-dominan species	Sub-dominance (%)	Plant cover (%)
Control	27±3 a	*Artemisia nanschanica*, *Aster flaccidus*	23.6±1.7 c	*Ajania tenuifolia*, *Morina chinensis*	16.9±0.8 b	58±4 b
3-year	7±1 c	*Elymus natans*	62.2±3.6 a	*Poa crymophilia*	10.7±1.3 c	96±3 a
7-year	11±2 b	*Elymus natans*	50.2±2.9 b	*Poa crymophilia*, *Festuca sinensis*, *Koeleria cristata*	30.1±1.7 a	94±4 a

Values in the same column sharing the same letters are not significantly different from each other ($P \leq 0.05$)

Table 2 Above- and below-ground biomass (dry matter based) under 3- and 7-year-old re-vegetated grasslands and heavily degraded meadow (Control) (means ± SD)

Plantation age	Above-ground biomass (g m^{-2})				Below-ground biomass in the 30-cm soil layer (g m^{-2})
	Total	Grass	Sedge	Forbs	
Control	250±31 b	4±2 b	1±1	245±34 a	1360±237 a
3-year	377±49 a	374±48 a	–	3±2 b	1165±279 b
7-year	371±45 a	370±45 a	–	1±1 b	1313±121 a

Difference in sedge biomass was not assessed

Values in the same column sharing the same letters are not significantly different from each other ($P \leq 0.05$)

by grass (*E. natans*) on reestablished grasslands whereas mainly by forbs on non-reseeded land (Table 2). It would take seven years for sowed *E. natans* to catch up with the degraded vegetation in belowground biomass (Table 2).

Soil bulk density, water-holding capacity and pH

Sowing *E. natans* on degraded meadows decreased soil bulk density by 13 and 10% and increased soil water-holding capacity by 9 and 4% at depth 0–30 cm in 3- and 7-year old reestablished vegetation compared to non-reseeded vegetation, respectively (Table 3). In addition, soil pH was significantly lowered by *E. natans* establishment at the depths of 0–10 and 10–20 cm but not at the depth of 20–30 cm compared to non-reseeded vegetation (Table 3, significant interaction of vegetation by depth).

Soil total organic C and nutrients

Total soil organic C (TOC) concentrations were greater by 7–33% in 7-year old reseeded soil at three sampling depths of 0–10, 10–20 and 20–30 cm and by 13% in 3-year old seeded soil only at depth 0–10 cm, than those in the soil of control (Table 4, significant interaction of vegetation by depth). Total N concentration changed in a similar pattern as TOC under different treatments (Table 4). Available N showed a consistent increase at depth 0–10 cm increasing with year after reestablishment of vegetation (Table 4). Total soil P concentration was increased by 17–35% in 3-year old reseeded soil and increased by 18–54% in 7-year old reseeded soil compared with the soil of control across three sampling depths, respectively. However total soil K concentration was not affected by *E. natans* reestablishment (Table 4).

Table 3 Soil bulk density, water-holding capacity and pH at three soil depths under 3- and 7-year-old re-vegetated grasslands and heavily degraded meadow (Control) (means±SD)

	Depth (cm)	Grassland			ANOVA		
		Control	3-year	7-year	Source	*P*	$LSD_{0.05}$
Bulk density (g cm^{-3})	0–10	1.46±0.05	1.30±0.02	1.37±0.04	Plantation age (P)	**	0.02
	10–20	1.57±0.06	1.38±0.02	1.42±0.07	Depth (D)	**	0.02
	20–30	1.67±0.05	1.42±0.07	1.45±0.02	P × D	ns	
Water-holding capacity (%)	0–10	20.8±0.8	23.3±1.5	21.4±1.1	Plantation age (P)	*	0.62
	10–20	19.6±1.1	21.1±1.2	20.8±0.7	Depth (D)	**	0.62
	20–30	19.2±0.3	20.3±1.1	19.7±1.4	P × D	ns	
pH	0–10	7.7±0.1	7.2±0.0	7.0±0.0	Plantation age (P)	**	
	10–20	8.0±0.0	7.6±0.0	7.7±0.1	Depth (D)	**	
	20–30	8.0±0.0	8.0±0.1	8.0±0.0	P × D	*	0.1

ns, not significant, * $p \leq 0.05$, ** $p \leq 0.01$

Table 4 Concentrations of total soil organic C and nutrients at three depths under 3- and 7-year-old re-vegetated grasslands and heavily degraded meadow (Control) (means±SD)

	Depth (cm)	Grassland			ANOVA		
		Control	3-year	7-year	Source	*P*	LSD $_{0.05}$
Total organic C (g kg^{-1})	0–10	33.4±1.7	37.8±0.8	44.4±1.3	Plantation age (P)	**	
	10–20	33.5±2.4	27.2±1.2	37.1±0.5	Depth (D)	**	
	20–30	36.1±0.4	24.7±0.2	38.8±1.2	P × D	**	1.8
Total N (g kg^{-1})	0–10	3.41±0.02	4.52±0.02	4.50±0.09	Plantation age (P)	**	
	10–20	3.17±0.03	3.04±0.02	3.75±0.09	Depth (D)	**	
	20–30	3.11±0.09	2.98±0.08	3.46±0.03	P × D	**	0.07
Total P (g kg^{-1})	0–10	0.52±0.00	0.70±0.03	0.80±0.01	Plantation age (P)	**	
	10–20	0.58±0.01	0.62±0.02	0.75±0.01	Depth (D)	*	
	20–30	0.55±0.00	0.67±0.01	0.65±0.01	P × D	**	0.02
Total K (g kg^{-1})	0–10	18.9±0.3	18.9±0.3	18.7±0.5	Plantation age (P)	ns	
	10–20	18.7±0.4	18.8±0.2	18.9±0.9	Depth (D)	ns	
	20–30	18.6±0.3	18.8±0.3	18.6±0.7	P × D	ns	
Available N (mg kg^{-1})	0–10	209±3	247±1	271±3	Plantation age (P)	**	
	10–20	213±4	166±1	222±3	Depth (D)	**	
	20–30	217±4	147±4	160±3	P × D	**	9

ns, not significant, * p≤0.05, ** p≤0.01

Soil microbial biomass

Soil MBC concentration increased by 13–58% in 3-year old reseeded land at depths of 0–10 and 10–20 cm, and by 43–87% in 7-year old reseeded land at depths of 0–10, 10–20 and 20–30 cm compared with the non-reseeded meadow, respectively (Table 5). Soil MBN concentration showed a similar change pattern to MBC as affected by re-vegetation. MBP generally showed a consistent increase with plantation age at three sampling depths (Table 5). MBC, MBN and MBP were all much higher in the surface (0–10 cm) than in deeper soil (10–30 cm) (Table 5).

Table 5 Concentrations of soil microbial biomass at three depths under 3- and 7-year-old re-vegetated grasslands and heavily degraded meadow (Control) (means ± SD)

	Depth (cm)	Grassland			ANOVA		
		Control	3-year	7-year	Source	*P*	LSD $_{0.05}$
Microbial biomass C (mg kg^{-1})	0–10	556±38	878±70	807±38	Plantation age (P)	**	
	10–20	307±5	348±9	573±12	Depth (D)	**	
	20–30	254±15	247±12	364±17	P × D	**	36
Microbial biomass N (mg kg^{-1})	0–10	126±6	168±7	152±19	Plantation age (P)	*	
	10–20	69±2	81±8	116±7	Depth (D)	**	
	20–30	60±2	65±9	71±5	P × D	*	5
Microbial biomass P (mg kg^{-1})	0–10	21±2	23±2	31±1	Plantation age (P)	**	
	10–20	13±1	14±1	21±1	Depth (D)	**	
	20–30	11±1	15±1	13±1	P × D	*	2

ns, not significant, * p≤0.05, ** p≤0.01

Soil enzymes

Three or seven years after re-vegetation, activities of soil neutral phosphatase, catalase and urease were generally greater at three sampling depths of 0–10, 10–20 and 20–30 cm in re-vegetated soils than those in non-revegetated soil (Fig. 1). Seven years' growth of reseeded *E. natans* had soil dehydrogenase activity increased at three sampling depths, whereas 3 years' growth of reseeded *E. natans* only increased dehydrogenase activity at the depth of 0–10 cm, compared to non-reseeded vegetation.

Discussion

The present study indicated that *E. natans* establishment on heavily degraded alpine meadow in the Tibetan Plateau has significantly improved soil quality and fertility, which was indicated by reduced soil bulk density, increased water-holding capacity, enrichments in total soil organic C, N, P and available N, and increased microbial mass and activity flourish after 3 to 7 growing seasons. Results clearly suggest that reseeding perennial species such as *E. natans* can be an effective strategy to rehabilitate seriously degraded meadows in the Tibetan Plateau.

Soil organic C content is a result of the equilibrium between organic matter input from plant biomass and loss during decomposition. In this study, the above-ground biomass in 3- and 7-year re-vegetated grasslands was 377 and 371 $g m^{-2}$ of dry matter (DM); in contrast, it was 250 $g m^{-2}$ of DM in control (Table 2). Therefore, the increase in soil organic C concentration in the two re-vegetated treatments relative to heavily degraded meadow are almost certainly a direct consequence of more plant litter inputs to soils. Additionally, although below-ground biomass in 3-year-old re-vegetated grassland was lower than that in 7-year grassland and degraded meadow, it was dominated by fibrous roots in two re-vegetated grasslands, whereas this was dominated by tap roots in control soil. It is well known that the fibrous roots with diameter≤1 mm provide more organic C because of their fast growth, decomposition rate and greater biological activities than tap roots in soil (Wu et al. 1997). Thus, the enrichment of soil organic C in two *E. natans* reseeded grasslands was probably also contributed to by the change in the morphology of root system. The increase in total N in re-vegetated grasslands could be attributed to the

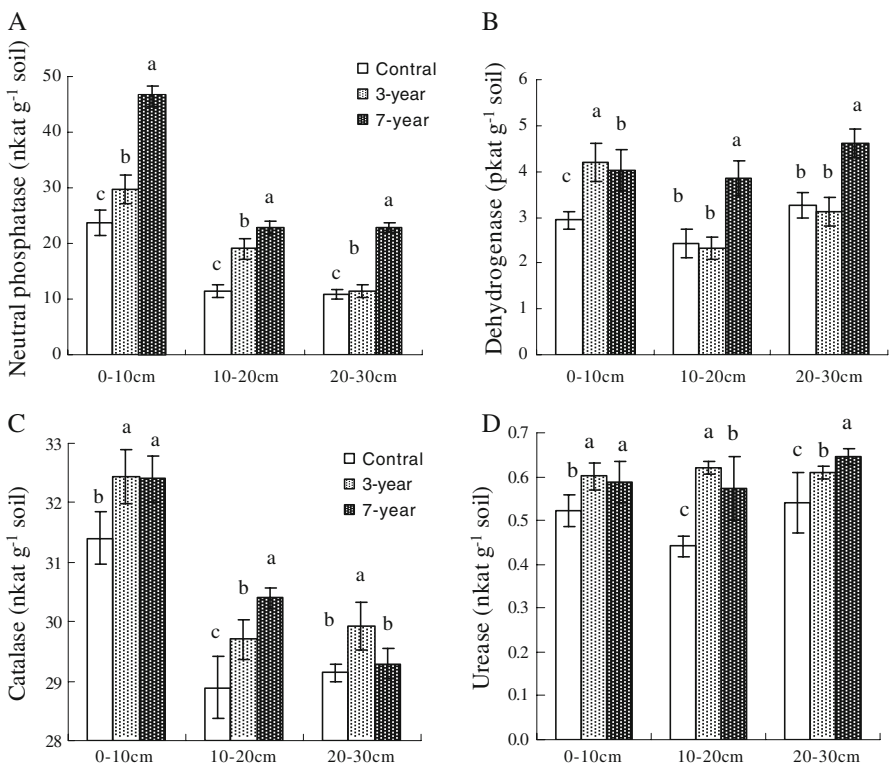

Fig. 1 The activities of neutral phosphatase **a**, dehydrogenase **b**, catalase **c**, and urease **d** at three soil depths under 3- and 7-year re-vegetated grasslands and heavily degraded alpine meadow (Control). Bars indicate ± SD; columns with different letters represent significant differences at $P \leq 0.05$

accumulation of organic matter, as evidenced by the significantly positive relations between total N and organic C (Total N=0.076 TOC + 0.90, n=27, r=0.77, p<0.01). In this study, a significant linear relationship between total P concentration and organic C (Total P= 0.0069 TOC + 0.41, n=27, r=0.45, p<0.05) indicates that the increase in total soil P was closely linked to the enrichment in soil organic matter. It is known that 20–76% of soil P is present as organic forms (Huang 2000). Furthermore, applications of diammonium phosphate before sowing and urea at jointing stage from the second year would also contribute to increases in total soil N and P.

Soil microorganisms play a crucial role in increasing the level of soil fertility and accelerating re-vegetation process through their activities in soils (Visser et al. 1983). In this study, MBC, MBN and MBP at the three sampling depths (except for MBC at the 3-year soil at 20–30 cm) increased after 3 to 7 year of *E. natans* reestablishment on heavily degraded meadows. Soil microbial biomass closely correlates with changes in vegetation cover (Baldrian et al. 2008). Such cover will increase the input litters and root exudates, secretions, sloughed cells and senescent tissues into soil (Kuzyakov and Domanski 2000), which provide carbon and energy sources for the growth of microorganisms (Li et al. 2006). Therefore, increased microbial C, N, and P could be caused by the enrichment of soil organic C related to the improved vegetation in re-vegetated treatments. This was supported by the close correlation between the concentrations of organic C to MBC, MBN and MBP (Table 6). The increase in microbial biomass suggested that soils under two rehabilitated grasslands can supply more readily decomposable organic matter and available N for vegetation, indicated by an enrichment of available N after *E. natans* establishment.

Soil enzymes are known to correlate with the response of microbial biomass to change in vegetation (Acosta-Martinez et al. 2008). The establishment of *E. natans* on heavily degraded meadow increased total organic C and soil microbial biomass, which in turn, increased the activities of soil neutral phosphatase, urease, catalase and dehydrogenase. This was consistent with the observed correlations of neutral phosphatase, catalase and dehydrogenase activities with MBC, MBN and MBP (Table 6).

Li et al. (2007) found that soil bulk density was negatively correlated to changes in soil organic matter; therefore, the enrichment in organic C content in two re-vegetated grasslands decreased soil bulk density in present study. Water in soil is retained in pore spaces and adsorbed onto the surface of mineral and organic matter particles, and the increase in organic matter content increased soil porosity (Li et al. 2007); as a result, 3- and 7-year-old re-vegetated grasslands increased soil water-holding capacity due to the enrichment of organic C. Decreased soil bulk density can improve plant root growth and development, which accordingly permits increased root penetration and exploration of a great volume of soil (Izquierdo et al. 2003). The increase in soil water-holding capacity suggests that re-vegetated soils store more water than the control soil, which will be favorable for palatable herbages (*E. natans*, *P. crymophila, K. cristata* and *F. sinensis*) to develop after *E. natans* rehabilitation (Wang et al. 2007), and may affect soil biochemical and microbiological activities (Tejada et al. 2006).

Conclusion

The present study demonstrates that establishment of *E. natans* on heavily degraded alpine meadow significantly improved soil quality. Thus, establishing suitable vegetation on severely degraded alpine meadow could be one effective measure to rehabilitate degraded soils in Qing-hai Tibetan plateau.

Table 6 Correlations between microbial biomass C (MBC), N (MBN), P (MBP) and total soil organic C and enzyme activities

	Total organic C	Neutral phosphatase	Urease activity	Catalase activity	Dehydrogenase activity
MBC	0.69**	0.86**	−0.05	0.89**	0.51**
MBN	0.45**	0.69**	0.06	0.81**	0.33*
MBP	0.55**	0.83**	0.19	0.89**	0.43*

**, Correlation is significant at P≤0.01, *, significant at P≤0.05

Acknowledgements This work was supported by the Key Project of the National Natural Science Foundation of China (No. 30730069) and National Natural Science Foundation of China (No. 30600083). We are grateful to Prof. Xiaogang Li from Lanzhou University, China, and Prof. Guijun Yan from the University of Western Australia for their valuable comments and suggestions on the manuscript.

References

Acosta-Martinez V, Acosta-Mercado D, Sotomayor-Ramrez D, Cruz-Rodrguez L (2008) Microbial communities and activities under different management in semiarid soils. Soil Biol Biochem 38:249–260

Baldrian P, Trögl J, Šnajdr J, Valášková V, Merhautvá V, Cajthaml T, Herinková J (2008) Enzyme activities and microbial biomass in topsoil layer during spontaneous succession in spoil heaps after brown coal mining. Soil Biol Biochem 40:2107–2115

Bao XK, Gao YX (1994) Character and distinguish standard of mattic epipedon. In: New theory of Chinese Soil System Classification. Science Press, Beijing

Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. Soil Biol Biochem 14:319–329

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and release of soil nitrogen: a rapid direct extraction method to measure microbial biomass N in soil. Soil Biol Biochem 17:837–842

Guan SY (1986) Soil enzymes and its methodology. Agricultural Press, Beijing

He ZL, Wu J, O'Donnell AG, Syers JK (1997) Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture. Biol Fertil Soils 24:421–428

Huang CY (2000) Soil science. Agricultural Press, Beijing

Institute of Soil Science, Chinese Academy of Sciences (ISSCAS) (1978) Physical and chemical analytical methods of soil. Shanghai Science Technology Press, Shanghai

Izquierdo I, Caravaca F, Alguacil MM, Roldán A (2003) Changes in physical and biological soil quality indicators in a tropical system (Havana, Cuba) in response to different agroecological management practices. Environ Manage 5:639–645

Jenkinson DS (1988) Determination of microbial biomass carbon and nitrogen in soil. In: Wilson JR (ed) Advances in nitrogen cycling in agricultural ecosystems. CAB International, Willingford, UK, pp 368–386

Kuzyakov Y, Domanski G (2000) Carbon input by plants into the soil: review. J Plant Nutr Soil Sci 163:421–431

Li WH, Zhou XM (1998) Ecosystems of Tibetan Plateau and approach for their sustainable management. Guang dong Science and Technology Press, Guangdong

Li YS, Wu LH, Lu XH, Zhao LM, Fan QL, Zhang FS (2006) Soil microbial biomass as affected by non-flooded plastic mulching cultivation in rice. Biol Fertil Soils 1:107–111

Li XG, Li FM, Rengel Z, Zhan ZY, Singh B (2007) Soil physical properties and their relations to organic carbon pools as affected by land use in an alpine pastureland. Geoderma 139:98–105

Liu W, Wang QJ, Zhou L (1999) Ecological process of forming "Black-Soil-type" degraded grassland. Acta Agrestia Sinica 4:300–307

Ma YS (1999) Review and prospect of the study on "Black Soil Type" degraded grassland. Pratacultural Science 2:5–8

Ma YS, Zhang ZH, Dong QM (2007) Application of restoration ecology in "black soil type" degraded grassland reconstruction. J Gansu Agr Aniv 2:91–97

Ma YS, Dong QM, Shi JJ, SunXD WYL, Sheng L, Yang SH (2008) Classification and control measure of "Black-soil-beach" degraded grassland in three river headwater region. Chin Qinghai J Ani Vet Sci 3:1–3

National Soil Survey Office (1998) Soil of China. China Agriculture Press, Beijing

Pepper IL, Gerba CP, Brendecke JW (1995) Dehydrogenase activity of soils. In: Environmental microbiology: a laboratory manual. Academic Press, NewYork

Shi W, Dell E, Bowman D, Iyyemperumal K (2006) Soil enzyme activities and organic matter composition in a turfgrass chronosequence. Plant Soil 288:285–296

Sun HL, Zheng D (1998) Formation, evolution and development of Qinghai-XiZang (Tibetan) Plateau. Guangdong Science and Technology Press, Guangdong

Tejada M, Garcia C, Gonzalez JL, Hernandez MT (2006) Use of organic amendment as a strategy for saline soil remediation: influence on the physical, chemical and biological properties of soil. Soil Biol Biochem 38:1413–1421

Visser S, Griffiths CL, Parkinson D (1983) Effects of surface mining on the microbiology of a prairie site in Alberta, Canada. Can J Soil Sci 63:177–189

Wang QJ (1997) The study of grassland resource, ecological environment and sustainable development on Qinghai-Tibet Plateau. Qinghai Prataculture 3:1–11

Wang WY, Wang QJ, Wang HC (2006) The effect of land management on plant community composition, species diversity, and productivity of Alpine Kobersia steppe meadow. Ecol Res 21:181–187

Wang CT, Cao GM, Wang QL (2007) Characteristics of artificial grassland plant communities with different establishment duration and their relationships with soil properties in the source region of Three Rivers in China. Chin J Appl Ecol 11:2426–2431

Wu J, Joergensen RG, Pommerning B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass by fumigation extraction: an automated procedure. Soil Biol Biochem 22:1167–1169

Wu Y, Liu SQ, Wang JX (1997) Effect of plant root system on soil anti-erosion. Chin J Appl Environ Biol 2:119–124

Zhang YS, Zhao XQ (2002) Quantitative characteristics of degenerative succession in Festuca sinensis sowing grassland in the alpine pastoral area. Chin J Appl Ecol 3:285–289

Zhao LP, Jiang Y (1986) An improved method to measure phosphatease activity in soils. Chin J Soil Sci 3:138–141

Zhou HK, Zhao XQ, Zhao L (2007) The community characteristics and stability of the Elymus nutans artificial grassland in alpine meadow. Chin J Grassland 2:13–25