Ecological Engineering 94 (2016) 88–94

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Influence of ecological restoration on vegetation and soil microbiological properties in Alpine-cold semi-humid desertified land

Yu-Fu Hu*,[1], Jia-Jia Peng[1], Shu Yuan[1], Xiang-Yang Shu, Shuang-Long Jiang, Qin Pu, Ke-Ya Ma, Cheng-Ming Yuan, Guang-Deng Chen, Hai-Hua Xiao

College of Resources Science and Technology, Sichuan Agricultural University, Chengdu 611130, China

A R T I C L E I N F O

Article history:
Received 6 January 2016
Received in revised form 28 March 2016
Accepted 22 May 2016

Keywords:
Desertification
Ecological restoration
Vegetation
Soil microbial
Northwest Sichuan

A B S T R A C T

Recently desertification of Alpine-cold semi-humid grassland has become increasingly serious on the eastern edge of the Qinghai-Tibet plateau. However, the restoration and control of desertified land in these areas have not received enough attention as in arid and semi-arid areas, and little is known about the vegetation community and soil microbiological properties during the ecological restoration in Alpine-cold semi-humid desertified areas. In this paper, the method of fencing, removing grazing and planting *Tamarix ramosissima* was taken as the measure for ecological restoration of Alpine-cold desertified land in Northwest Sichuan. The results showed ecological restoration resulted in significant improvement in the height, coverage, density, biomass, and diversity of vegetation communities, numbers of soil microorganisms (including bacteria, actinomycetes and fungi). Microbial biomass carbon and nitrogen, and urease, invertase and protease activities increased after the restoration, especially in the 0–20 cm layer. These trends increased with increasing restoration age but decreased with increasing soil depth. Ecological restoration by fencing, non-grazing and planting *T. ramosissima* is therefore considered an effective and applicable measure to restore vegetation and soil microbiological properties and control desertification in the Northwest Sichuan, and is recommended for adoption in Alpine-cold semi-humid sandy areas on a large scale.

1. Introduction

Plants and soil microorganisms are an important component of soil ecosystems, and play a critical role in soil nutrient cycling and transformation and soil fertility succession (Duan et al., 2015; Zhang et al., 2015). Investigations into the surface vegetation community and soil microbiological properties during the ecological restoration of desertified lands are necessary for in-depth understanding of restoration mechanisms and the interactions between soil and plant communities. Many intensive studies concerning the effects of desertification control and rehabilitation measures on protection benefits have been carried out in desertified lands (Zhang et al., 2004; Li et al., 2009; Slimani et al., 2010; Chang et al., 2015), but very few have focused on soil biochemical and ecological properties during the ecological restoration.

The Northwest Sichuan Alpine-cold semi-humid grassland locates on the eastern edge of the Tibetan Plateau (31°51′–33°19′ N, 101°51′–103°23′ E). It is the world's largest highland peat swamp wetland grassland, and one of the China's five major pastoral areas. Since the 1970s, the grassland ecosystem and local environment have degraded, and the desertified land area has enlarged gradually because of overgrazing and intensive farming. Studies reported that the desertified land area increased by 28.1% in 1994–2009, and reached 0.8219 million ha up to year 2009 (Yong et al., 2003; Liao et al., 2011). The restoration and control of desertified land is an urgent problem that needs to be solved in these regions; however, the grassland desertification in Alpine-cold semi-humid areas is not as well studied as the arid and semi-arid areas. There has been considerable researches about ecological restoration and control for the desertified land in arid and semi-arid areas (Guo et al., 2003; Verdoodt et al., 2010; Park et al., 2013; Liu et al., 2014a,b), but little research has been done in Northwest Sichuan Alpine-cold semi-humid desertified land. In addition, no report is available for the ecological properties during the ecological restoration of Alpine-cold semi-humid desertified areas.

Tamarix ramosissima, a *Tamaricaceae* shrub, is widely distributed in Northwest Sichuan Alpine-cold semi-humid grassland. It shows a relatively strong ability to tolerate drought, cold, and low-nutrient

* Corresponding author.
E-mail address: huyufu@sicau.edu.cn (Y.-F. Hu).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.ecoleng.2016.05.061
0925-8574/

conditions. It also serves as a restoration plant for desertified lands. In this paper, fencing, non-grazing and planting *T. ramosissima* have been adopted as the methods for the ecological restoration of desertified lands in Northwest Sichuan. The objective of this study is to evaluate the changes of surface vegetation community and soil microbiological properties due to the ecological restoration of Alpine-cold semi-humid desertified land in Northwest Sichuan. Therefore, we examined the desertified land with ecological restoration by fencing, non-grazing and *T. ramosissima* planting methods for 0, 4, 7, 15, and 21 years. The coverage, biomass, and diversity of surface vegetation, soil microbial quantity, microbial biomass carbon, and nitrogen content and enzyme activities of urease, invertase, and protease were surveyed.

2. Study area and research methodology

2.1. Outline of study area

This study was conducted at the Restoration Demonstration Area of Desertification Land in Sichuan Province (33°1′ N and 102°37′ E), east edge of Qinghai Tibet Plateau, northwest of Sichuan Province, China. This average elevation of the area is over 3600 m and it belongs to the continental plateau cold semi-humid monsoon climate, characterized by short spring and long winter. The average annual precipitation is 791.95 mm and the precipitation is mainly concentrated in May–October. The annual land evaporation is about 684.2 mm. The average annual windspeed is 1.6–2.4 m s^{-1}. The annual average temperature is 0.9 °C, the average temperature is −10.3 °C in the coldest month, and 10.9 °C in the hottest month. With longer sunshine hours and strong solar radiation, the annual average sunshine time is 2158.7 h and the total annual solar radiation is 6194 MJ m^{-2}. The landscape is characterized by gently undulating moving and semi-moving sand dunes with inter-dune bottomlands. The soils were classified as Cambic Arenosols (FAO, 2006); sandy in texture, light yellow in color, loose in consistency, and low in organic matter content. Because of these characteristics, the soil is particularly susceptible to wind erosion. The vegetation on desertified grassland is dominated by *Leymus secalinus*, *Elymus nutans* and *Festuca ovina*, *Kobresia pygmaea* and *Tamarix ramosissima*. The main livestock present in the area are yak and Tibetan sheep. More than 80% of the local people are Tibetans whose livelihoods depend mainly on livestock production.

Beginning in 1994, *T. ramosissima* was gradually planted on desertified land in Restoration Demonstration Area of Desertification Land in Sichuan Province with the help of grazing blocked by fencing and grids as sand binders. The grid was composed of 2 × 2 m squares made of *T. ramosissima* branch. The *T. ramosissima* seedings were planted with about 2 m spacing between plants. No fertilization or irrigation was applied during the growth of *T. ramosissima*. Before planting, the vegetative cover was generally less than 5%, and wind erosion often occurred during the dry winter and spring seasons. *T. ramosissima* grew to form 1 m high shrubby belts 2–4 years after planting. With gradual stabilization of the sandy land, some short grasses, legumes, and forbs colonized, and a stabilized shrubby-grass vegetation system was gradually established. To date, an age sequence of 4, 7, 15, and 21 year-old *T. ramosissima* plantations is distributed on the sandy land. The *T. ramosissima* plantations were enclosed with wire fence to exclude grazing animals.

2.2. Experimental design and soil sampling

Four types of desertified land with ecological restoration by fencing, non-grazing and *T. ramosissima* planting for 4, 7, 15, and 21 years were selected from Restoration Demonstration Area of Desertification Land in Sichuan Province. We selected randomly five 50 × 50 m sites from each restoration-year for sampling. Also five 50 × 50 m non-restored areas of desertified land were chosen as control (0 year) sites (CTRL). For each restoration-year, the site conditions and soil characteristics were relatively consistent prior to ecological restoration.

Vegetation investigation and soil sampling were completed in June 2014. For each restoration-year, three 10 × 10 m plots were selected randomly for investigating shrubby vegetation features. Five *T. ramosissima* shrubs were selected at the same growth status from each plot, the plant height and coverage were investigated and recorded, and the aboveground parts were cut, and packed in bags for measuring aboveground biomass. Plots (1 × 1 m) were selected randomly for surveying species, numbers of individuals of herbaceous plant from each 10 × 10 m plot. Typical quadrats (0.5 × 0.5 m) were used for herbaceous plant biomass determination from each 1 × 1 m plot. The aboveground portions of herbaceous plants were cut flush with the ground. Samples were rinsed with water, and dried in a constant-temperature oven at 85 °C until constant weight was attained. The biomass data was subsequently recorded. Soil sampling spots were placed in each 1 × 1 m plot, and the soil samples in 0–20, 20–40, and 40–60 cm soil depth were collected with a shovel. All samples were sieved through a 2 mm screen, and roots and other debris were removed and discarded. Half of each sample was kept field-moist in a cooler at 4 °C, and the other half was air-dried and stored at room temperature. Field-moist samples were analyzed within 2 weeks of sampling.

2.3. Laboratory analysis

Soil microbial biomass carbon and nitrogen were estimated using the chloroform fumigation–incubation method (Jenkinson and Powlson, 1976; Setia et al., 2012). The microbial quantity in soils was determined using the dilution plate count technique. For bacteria, fungi and actinomycetes, the following media were used: beef extract peptone medium, Martin's medium, and Gause's I medium, respectively (Hou et al., 2014). Soil enzymes were determined. The urease activity, 0.3–0.4 g of the moist soils was incubated with 1.5 ml of a 79.9 mM urea solution for 2 h at 37 °C. Released ammonium was extracted with 13.5 ml of 2 M KCl solution and determined colorimetrically using a modified Berthelot reaction (Kandeler et al., 2006). The invertase activity was determined using sucrose as substrate. Mixtures of 5 g of air-dried soil, 15 ml of 8% sucrose solution, 5 ml of phosphoric acid buffer (pH 5.5) and 5 drops of methylbenzene were incubated at 37 °C for 24 h and then filtered rapidly. Next, 1 ml of filtrate was mixed with 3 ml of 3, 5-dinitrosalicylic acid and heated in boiling water for 5 min. The mixture was diluted to 50 ml with distilled water and measured spectrophotometrically at 508 nm (Ye et al., 2015). For protease one gram of fresh soil was mixed with 2.5 ml of sodium caseinate (10 g ml^{-1}) in 0.1 mol l^{-1} of tri-sodium borate buffer at pH 8.1. The mixture was incubated at 37 °C for 1 h. The reaction was stopped with 2 ml of 17.5% tricloracetic acid and centrifuged. After centrifugation, 2 ml of the supernatant was mixed with 3 ml of 1.4 mol l^{-1} Na_2CO_3 and 1 ml of Folin–Ciocalteu reagent. Absorbance was recorded at 700 nm using a UV-spectrophotometer (Jamro et al., 2014).

2.4. Statistical analysis

The Margalef species richness index (*D*), Shannon-Wiener diversity index (*H*) and Pielou evenness index (*E*) were used to calculate plant diversity. The following equations were used for these calculations (Ahmed et al., 2015): $D = (S-1)/LnN$; $H = -\sum P_i InP_i$; $P_i = N_i/N$; $E = H/InS$. Where *S* is the number of species observed in

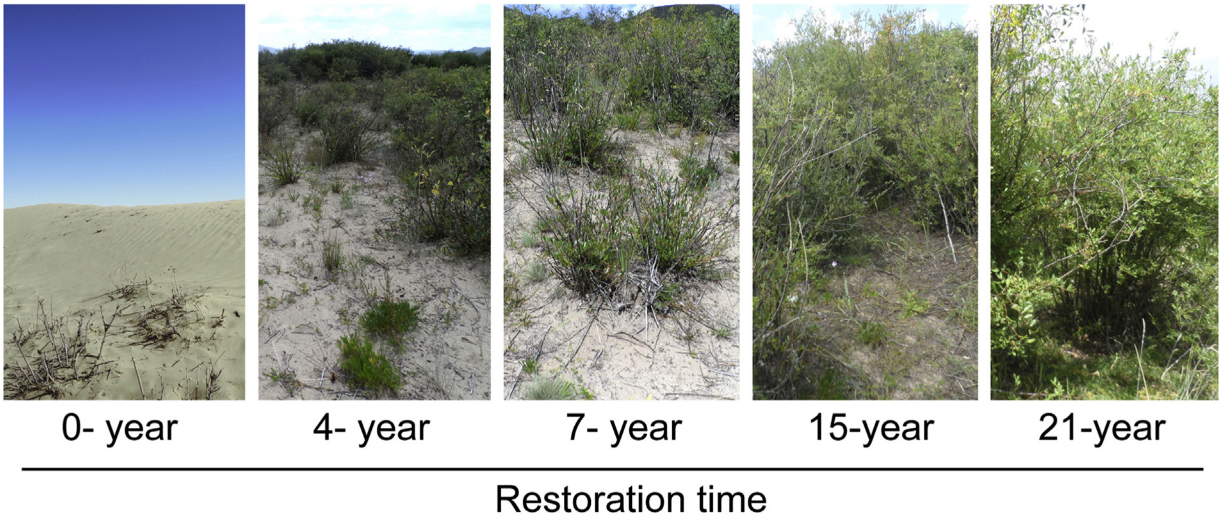

Fig. 1. Shrubs and herbs on desertified land of different restoration ages.

Table 1
Vegetation characteristics in different ecological restoration age of desertified land.

Vegetation	Index	0-year	4-year	7-year	15-year	21-year
Shrub	Height (m)	0	0.5 ± 0.1D	1.0 ± 0.2C	1.8 ± 0.3B	2.5 ± 0.6A
	Coverage (%)	0	31.2 ± 3.6C	35.6 ± 5.2C	52.2 ± 5.1B	85.3 ± 6.2A
Herb	Height (cm)	5.2 ± 0.8D	10.3 ± 2.3C	14.6 ± 3.1BC	17.8 ± 3.5B	23.9 ± 4.1A
	Coverage (%)	6.5 ± 1.6E	25.7 ± 3.2D	48.6 ± 4.5C	59.4 ± 5.2B	87.5 ± 7.3A
	Density (ind m^{-2})	24.4 ± 3.2D	192.3 ± 12.6C	420.6 ± 23.5 B	476.4 ± 27.8B	684.6 ± 34.4A

Multiple comparisons using the least significant difference (LSD) method, uppercase letters represent a significant level of 0.05. Values with the same letter are not significantly different between restoration ages.

the plot, N is the total number of individuals, and N_i is the number of individuals of species i.

All results are reported as means ± standard deviations. The data of each variable measured were analyzed by one-way ANOVA and the means were calculated using the least significant difference (LSD) method (at $p = 0.05$) (SPSS 19.0 software). The Pearson correlation analysis was done in order to study the relationship between vegetation characteristics and soil microbiological properties, Figures were drawn using Microsoft Excel software.

3. Results

3.1. Vegetation height, coverage, and density

There were few herbaceous plants and no shrubs in the areas of non-restored desertified land (CTRL). The average height, coverage and density of herbaceous plants were 5 cm, 6% and 24 and m^{-2}, respectively (Fig. 1 and Table 1). This result suggested that the ground vegetation was seriously damaged by overgrazing and wind erosion in the desertified land. With the increase of ecological restoration age, the height and coverage of vegetation increased significantly. After 21 years of ecological restoration, the height and coverage of shrub vegetation increased to 2.5 m and 85%, and the height, coverage, and density of herbaceous vegetation increased to 23 cm, 87% and 684 ind m^{-2}, respectively, significantly higher than CTRL ($p < 0.05$). This shows that fencing, non-grazing and *T. ramosissima* planting were effective in promoting vegetation restoration.

3.2. Vegetation species richness and diversity

The Margalef species richness index and Shannon–Wiener diversity index of surface vegetation were 0.417 and 0.656, respectively, in non-restored desertified land (Fig. 2). The surface vegetation community degraded seriously, the number of species reduced and the structure became simple. The richness index, Shannon–Wiener diversity index and Pielou's evenness index of the herbaceous vegetation increased significantly with increasing years under ecological restoration. After 21 years of ecological restoration, the richness index, Shannon–Wiener diversity index and Pielous evenness index increased to 2.642, 2.147, and 1.123, respectively, and were significantly higher than the CTRL ($p < 0.05$). This suggests that the fencing, non-grazing and *T. ramosissima* planting had a positive effect on the restoration and succession of the ground vegetation communities in the desertified land.

3.3. Vegetation biomass

The plant biomass was very low in non-restored desertified land (Fig. 3). With the increase of ecological restoration age, the shrub and herbaceous plant aboveground biomass all increased significantly. After 21 years of ecological restoration, the shrub aboveground biomass increased from 0 kg m^{-2} to 1514.8 kg m^{-2}, and the herbaceous plant aboveground biomass increased from 170.91 g m^{-2} to 1236.12 g m^{-2}. During the 21-year ecological restoration, the total aboveground vegetation biomass in desertified land increased by 17.27 times of CTRL.

3.4. Soil microbial quantity

The soil microbial quantity in the 0–60 cm layer increased with years under ecological restoration, and decreased with soil depth (Table 2). During the ecological restoration, the number of bacteria, actinomycetes and fungi in desertified land significantly increased. After 21 years of ecological restoration, the number of bacteria,

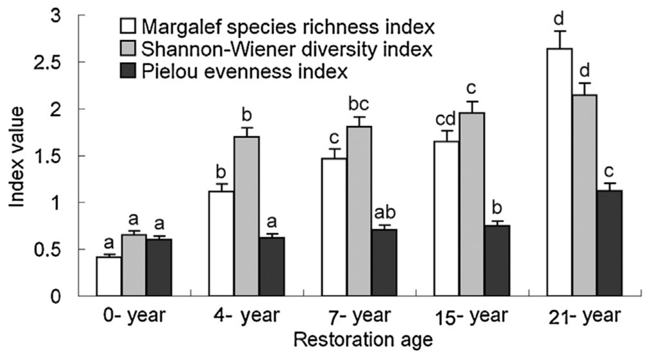

Fig. 2. Vegetation diversity of desertified land of different restoration ages. Error bars show standard deviations ($n = 5$). Multiple comparisons using the least significant difference (LSD) method, uppercase letters represent a significant level of 0.05. With the same letter are not significantly different between restoration ages.

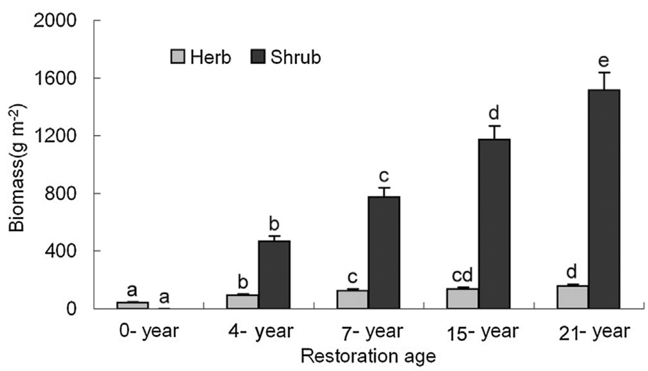

Fig. 3. Vegetation biomass of desertified land of different restoration ages. Error bars show standard deviations ($n = 5$). Multiple comparisons using the least significant difference (LSD) method, uppercase letters represent a significant level of 0.05. With the same letter are not significantly different between restoration ages.

actinomycetes and fungi increased by 9.71, 7.54, and 5.58 times at 0–20 cm, compared with CTRL, respectively. With increased soil depth, the number of bacteria, actinomycetes and fungi and their variation decreased. This results suggested that ecological restoration of desertified land resulted in a significant increase of soil microbial quantity, especially in the 0–20 cm layer, and the increase of bacteria was greatest, followed by the actinomycetes and fungi.

3.5. Soils microbial biomass carbon and nitrogen

During the ecological restoration process, the soil microbial biomass carbon and nitrogen contents increased significantly (Table 3). The increase of microbial biomass carbon and nitrogen contents was greatest in the 0–20 cm soil layer. With increased soil depth, soil microbial biomass carbon and nitrogen contents and their variation decreased. After 21 years of ecological restoration, soil microbial biomass carbon and nitrogen contents increased by 6.41 and 5.36 times at 0–20 cm, respectively, compared with CTRL ($p < 0.05$). These results showed that ecological restoration led to a significant increase of soil microbial biomass carbon and nitrogen. Basically, the increase of soil microbial biomass carbon was greater than that of microbial biomass nitrogen.

3.6. Soil enzyme activity

The activities of soil urease, invertase, and protease in desertified land increased gradually with years under ecological restoration (Table 4). After 21 years of ecological restoration, the activities of urease, invertase, and protease increased by 10.16, 27.03, and 3.3 times at 0–20 cm, respectively, compared with CTRL ($p < 0.05$). With increased soil depth, the activities of soil urease, invertase, and protease decreased. At 20–40 cm and 40–60 cm soil depth, the activities of urease, invertase and protease also increased significantly with increasing years under ecological restoration, but their increment was less than that at 0–20 cm. This result suggested that ecological restoration significantly promoted activities of soil urease, invertase, and protease in the desertified land.

3.7. Correlation between vegetation characteristics and soil microbiological properties

The height, coverage, biomass and Margalef species richness index of vegetation community were significantly positively correlated to soil microbial biomass carbon and nitrogen contents, activities of invertase and protease and quantities of bacteria and actinomycetes (Table 5). Microbial biomass carbon and nitrogen contents had significant positive correlation with quantities of bacterial, actinomycetes and fungi and activities of urease, invertase and protease. Bacterial, actinomycetes, and fungi quantities were positively correlated to activities of urease, invertase and protease. It indicated that there were closely correlation between the surface vegetation and the soil microbiological properties.

4. Discussion

4.1. The influence of ecological restoration on vegetation

Vegetation is a key index of ecosystem quality, and the ecosystem restoration is largely based on the vegetation restoration (Muys et al., 2003). The plant diversity improves the biodiversity of other species in the ecosystem (Zhang and Dong, 2010). The results of the current study showed that the ecological restoration by fencing, non-grazing and *T. ramosissima* planting is effective. The height, coverage, density, biomass, and diversity of surface vegetation significantly increased during the ecological restoration. Our results supported the conclusions of other studies conducted in the arid

Table 2
Soil microbial number in different ecological restoration age of desertified land.

Microbial species	Soil depth (cm)	0-year	4-year	7-year	15-year	21-year
Bacteria (10^6 cfu g^{-1})	0–20	0.35 ± 0.02 E	1.35 ± 0.11D	1.65 ± 0.12C	2.26 ± 0.18B	2.99 ± 0.25A
	20–40	0.48 ± 0.03D	0.94 ± 0.09C	1.29 ± 0.10C	1.64 ± 0.14AB	2.15 ± 0.19A
	40–60	0.44 ± 0.02C	0.78 ± 0.06BC	0.95 ± 0.08B	1.14 ± 0.09B	1.67 ± 0.15A
Actinomycetes (10^6 cfu g^{-1})	0–20	0.07 ± 0.01C	0.31 ± 0.02BC	0.41 ± 0.03B	0.57 ± 0.05B	0.75 ± 0.06A
	20–40	0.06 ± 0.01D	0.20 ± 0.01C	0.31 ± 0.02BC	0.35 ± 0.02B	0.54 ± 0.04A
	40–60	0.05 ± 0.01D	0.12 ± 0.01CD	0.20 ± 0.01BC	0.23 ± 0.01B	0.38 ± 0.03A
Fungi (10^6 cfu g^{-1})	0–20	0.36 ± 0.02D	1.11 ± 0.10C	1.65 ± 0.15B	2.01 ± 0.15AB	2.37 ± 0.20A
	20–40	0.45 ± 0.03D	0.69 ± 0.06CD	0.87 ± 0.08BC	1.05 ± 0.09AB	1.23 ± 0.11A
	40–60	0.30 ± 0.02C	0.46 ± 0.03BC	0.63 ± 0.05BC	0.84 ± 0.07AB	1.14 ± 0.10A

Multiple comparisons using the least significant difference (LSD) method, uppercase letters represent a significant level of 0.05. Values with the same letter are not significantly different between restoration ages within the same depth.

Table 3
Microbial biomass carbon and nitrogen in different ecological restoration age of desertified land.

Index	Soil depth (cm)	0-year	4-year	7-year	15-year	21-year
Microbial carbon	0–20	17.38 ± 1.64 E	51.08 ± 4.48 D	77.63 ± 6.52C	93.01 ± 7.71 B	128.94 ± 9.83 A
($mg\,kg^{-1}$)	20–40	19.03 ± 1.58 E	36.00 ± 3.20 D	51.62 ± 5.07C	73.25 ± 6.30 B	86.05 ± 6.09 A
	40–60	19.56 ± 1.67 D	25.87 ± 1.76 CD	32.52 ± 2.92 BCE	40.98 ± 3.64 B	51.28 ± 3.81 A
Microbial nitrogen	0–20	2.58 ± 0.23 D	5.65 ± 0.58C	7.43 ± 0.65C	12.93 ± 1.06 B	16.40 ± 1.29 A
($mg\,kg^{-1}$)	20–40	3.22 ± 0.24C	4.19 ± 0.31C	5.14 ± 0.52 BCE	6.84 ± 0.71 B	9.04 ± 0.58 A
	40–60	3.62 ± 0.27 B	4.09 ± 0.26 B	4.43 ± 0.37 AB	5.25 ± 0.48 AB	7.42 ± 0.63 A

Multiple comparisons using the least significant difference (LSD) method, uppercase letters represent a significant level of 0.05. Values with the same letter are not significantly different between restoration ages within the same depth.

Table 4
Soil enzyme activities in different ecological restoration age of desertified land.

Soil enzyme	Soil depth/cm	0-year	4-year	7-year	15-year	21-year
Urease activity	0–20	1.21 ± 0.11 D	4.19 ± 0.36C	5.82 ± 0.48C	11.32 ± 1.03 B	13.51 ± 1.06 A
($\mu g\,g^{-1}\,h^{-1}$)	20–40	1.08 ± 0.08 D	1.42 ± 0.11C	2.97 ± 0.22 BCE	3.89 ± 0.29 B	4.77 ± 0.38 A
	40–60	1.02 ± 0.06C	1.09 ± 0.09C	2.17 ± 0.15 B	3.62 ± 0.27 A	3.94 ± 0.31 A
Invertase activity	0–20	0.28 ± 0.02 E	2.30 ± 0.14 D	3.28 ± 0.28C	5.21 ± 0.45 B	7.85 ± 0.63 A
($\mu g\,g^{-1}\,h^{-1}$)	20–40	0.21 ± 0.02 E	1.21 ± 0.09 D	1.97 ± 0.11 CD	3.32 ± 0.24 B	4.70 ± 0.34 A
	40–60	0.28 ± 0.02 D	0.96 ± 0.08C	1.37 ± 0.10C	2.47 ± 0.19 AB	3.41 ± 0.26 A
Protease activity	0–20	0.20 ± 0.02 E	0.36 ± 0.03 D	0.50 ± 0.03C	0.75 ± 0.05 B	0.86 ± 0.07 A
($\mu g\,g^{-1}\,h^{-1}$)	20–40	0.19 ± 0.01 D	0.30 ± 0.02C	0.40 ± 0.03 CB	0.62 ± 0.04 A	0.66 ± 0.05 A
	40–60	0.12 ± 0.01 D	0.21 ± 0.02 DC	0.31 ± 0.03 CB	0.49 ± 0.04 A	0.55 ± 0.04 A

Multiple comparisons using the least significant difference (LSD) method, uppercase letters represent a significant level of 0.05. Values with the same letter are not significantly different between restoration ages within the same depth.

Table 5
Correlation between vegetation community characteristics and microbiological properties.

	Height	Coverage	Biomass	*D*	*H*	*E*	MBC	MBN	Bacterial	Actinomycetes	Fungi	Urease	Invertase	Protease
Height	1													
Coverage	0.998**	1												
Biomass	0.945*	0.935*	1											
D	0.989**	0.985**	0.941*	1										
H	0.889*	00.87	0.988**	0.882*	1									
E	0.902*	0.905*	00.753	0.931*	00.651	1								
MC	0.999**	0.998**	0.956*	0.988**	0.905*	0.888*	1							
MN	0.981**	0.975**	0.898*	0.956*	0.847	0.885*	0.974**	1						
Bacterial	0.992**	0.984**	0.962**	0.982**	0.924*	0.87	0.992**	0.982**	1					
Actinomycetes	0.995**	0.988**	0.959*	0.980**	0.918*	0.87	0.994**	0.985**	0.999**	1				
Fungi	0.982**	0.979**	0.972**	0.955*	0.940*	0.81	0.987**	0.959**	0.984**	0.988**	1			
Urease	0.971**	0.964**	0.889*	0.937*	0.844	0.86	0.964**	0.998**	0.975**	0.979**	0.958*	1		
Invertase	0.993**	0.988**	0.919*	0.985**	0.862	0.921*	0.988**	0.992**	0.990**	0.991**	0.962**	0.982**	1	
Protease	0.980**	0.976**	0.909*	0.943*	0.863	0.85	0.976**	0.994**	0.979**	0.985**	0.975**	0.996**	0.981**	1

D, Margalef species richness index; H, Shannon-Wiener diversity index; E, Pielou evenness index; MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen. A single asterisk (*) indicates $p \leq 0.05$. The double asterisk (**) indicates $p \leq 0.01$.

and semi-arid desertified land (Verdoodt et al., 2010; Yi et al., 2012; Park et al., 2013). Moreover, in our study, we found that the increases of coverage, biomass and diversity of vegetation in the Alpine-cold semi-humid desertified area are greater than those in the arid and semi-arid areas (Verdoodt et al., 2010; Park et al., 2013). This might be because the study area was located in semi-humid area with more favorable water conditions than the arid and semi-arid areas and thus is more favorable for the plant growth. In addition, the ecological restoration measure for desertified land in the present study is different from previous studies in which the fencing and non-grazing was used mainly for natural restoration of desertified land ecosystems (Verdoodt et al., 2010; Park et al., 2013). In this study, we suggest that the ecological restoration method of fencing and *T. ramosissima* planting may be more effective than the fencing and non-grazing method. The *T. ramosissima* functions as a pioneer plant to fix moving sands. In addition, it improves the surface environment and enhances soil physico-chemical and microbial properties, thus providing a favorable condition for the growth of other herbaceous plants.

4.2. The influence of ecological restoration on soil microbiological properties

Soil microorganisms are the most active component of the soil ecosystem and are directly involved in many ecological processes, such as nutrient cycling and organic matter decomposition. Soil microbial diversity is a key biological indicator that reflects the soil ecosystem health and soil quality and has been widely used to evaluate the relationship between biological community and restoration function in degraded ecosystems (Smith and Paul, 1990; Harris, 2003; Fitter et al., 2005; Gil-Sotres et al., 2005; Zhang et al., 2013).

The development of annual and perennial herbs forms an important component of net primary productivity, and the plant residues provide an important input of soil organic matter, carbon, nitrogen, and other nutrients for microbial utilization (Kaur et al., 2002; Cao et al., 2008, 2011; Li et al., 2013; Miao et al., 2015). In addition, we noted that in the same ecological restoration year, the increase of soil microbial biomass carbon and nitrogen in the present study was greater than that in semi-arid desertified land in Northeast China (Cao et al., 2008), which may be due to the water conditions as mentioned above.

Soil enzyme activity can be used as an indicator of soil fertility and microbial activity (Badiane et al., 2001), and there is considerable evidence that enzyme activity can be used to evaluate the influence of land-use on soil properties (Saggar et al., 1999; Zhang et al., 2015). Soil enzyme levels are closely related to nutrient transformations and soil fertility in rehabilitated sandy soils (Consuelo and Teodoro, 2002). Su et al. (2005) reported that the enzyme activities of urease, invertase, phosphatase and catalase in soils increased with years under vegetation restoration in abandoned land in Loess hilly areas, which are consistent with our current studies. Increased soil enzyme activities might be due to increased organic matter input and C and N immobilization during the process of organic matter decomposition (Kushwaha et al., 2000; Plaza et al., 2004; Cao et al., 2008; Zhang et al., 2015). These changes may also be attributed to improvement of the soil environment for soil microorganism growth (Aon et al., 2001; Cao et al., 2008; Liu et al., 2014a,b).

The soil depth is one of the factors that influence the microorganisms in soil. Studies have shown that the microorganism number varies with soil layers even in the same vegetation condition (Tanon et al., 2005; Cao et al., 2008, 2011). The results of our study show that the number of microorganisms, microbial carbon and nitrogen content and enzyme activity in soil declined with depth in the soil profile. The asymmetrical inputs of litter throughout the soil profile due to the aboveground litter incorporation on the soil surface, together with better soil aeration, account for the higher soil enzyme activities in the upper 0–20 cm layer than in the lower 20–60 cm layer (Belay-Tedla et al., 2009; Laik et al., 2009; Zhang et al., 2015).

5. Conclusion

The ecological restoration by fencing, non-grazing and *T. ramosissima* planting on the Alpine-cold semi-humid desertified land in Northwest Sichuan, China, resulted in significant improvement in surface vegetation and microbiological properties. This improvement was manifested in increased height, coverage, density, biomass, and diversity of the vegetation community, and remarkable enhancements to numbers of soil microorganisms, microbial biomass carbon and nitrogen, and soil enzyme activities. Our results indicate that ecological restoration by combining fencing, non-grazing and planting *T. ramosissima* is an effective and suitable measure to restore surface vegetation and soil microbiological properties and limit desertification in this area.

Acknowledgements

This research was supported by the National Key Technology Research Program of the Ministry of Science and Technology of China (2015BAC05B01, 2015BAC05B02), the national Natural Science Foundation of China (31372502) and the Science and Technology Plan Projects in Sichuan Province (2011SZZ023, 2013SZ0110, 2014SZ0057, 2014SZ0159). We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript and Dr. Shawn Gray (Northwestern University, USA) for critical reading of the manuscript and the language editing.

References

Ahmed, D.A., Fawzy, M., Saeed, N.M., Awad, M.A., 2015. Effect of the recent land use on the plant diversity and community structure of omayed biosphere reserve, Egypt. Global Ecol. Conserv. 38, 26–37.
Aon, M.A., Cabello, M.N., Sarena, D.E., Colaneri, A.C., Franco, M.G., Burgos, J.L., Cortassa, S., 2001. Spatio-temporal patterns of soil microbial and enzymatic activities in an agricultural soil. Appl. Soil Ecol. 18, 239–254.
Badiane, N.N.Y., Chotte, J.L., Pate, E., Masse, D., Rouland, C., 2001. Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions. Appl. Soil Ecol. 18, 229–238.
Belay-Tedla, A., Zhou, X., Su, B., Wan, S., Luo, Y., 2009. Labile recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the us great plains subjected to experimental warming and clipping. Soil Biol. Biochem. 41, 110–116.
Cao, C., Jiang, D., Teng, X., Jiang, Y., Liang, W., Cui, Z., 2008. Soil chemical and microbiological properties along a chronosequence of *Caragana microphylla* Lam. plantations in the Horqin sandy land of Northeast China. Appl. Soil Ecol. 40, 78–85.
Cao, C., Jiang, S., Ying, Z., Zhang, F., Han, X., 2011. Spatial variability of soil nutrients and microbiological properties after the establishment of leguminous shrub *Caragana microphylla* Lam. plantation on sand dune in the Horqin Sandy Land of Northeast China. Ecol. Eng. 37, 1467–1475.
Chang, I., Prasidhi, A.K., Im, J., Shin, H.D., Cho, G.C., 2015. Soil treatment using microbial biopolymers for anti-desertification purposes. Geoderma 253–254, 39–47.
Consuelo, Q., Teodoro, M., 2002. Dehydrogenase activity in Mediterranean forest soils. Biol. Fertil. Soil 35, 102–107.
Duan, B., Zhang, Y., Xu, G., Chen, J., Paquette, A., Peng, S., 2015. Long-term responses of plant growth: soil microbial communities and soil enzyme activities to elevated CO_2 and neighbouring plants. Agric. For. Meteorol. 213, 91–101.
Food and Agriculture Organization of the United Nations (FAO), 2006. FAO/IUSS working group WRB, world reference base for soil resources 2006. In: World Soil Resources Reports 103. FAO, Rome, Italy.
Fitter, A.H., Gilligan, C.A., Hollingworth, K., Kleczkowski, A., Twyman, R.M., Pitchford, J.W., 2005. Biodiversity and ecosystem function in soil. Funct. Ecol. 19, 369–377.
Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M.C., Seoane, S., 2005. Different approaches to evaluating soil quality using biochemical properties. Soil Biol. Biochem. 37, 877–887.
Guo, Z., Liang, T., Zhang, Z., 2003. Classification management for grassland in Gansu Province, China. New Zeal. J. Agric. Res. 46, 123–131.
Harris, J.A., 2003. Measurements of the soil microbial community for estimating the success of restoration. Eur. J. Soil Sci. 54, 801–808.
Hou, S., Xin, M., Wang, L., Jiang, H., Li, N., Wang, Z., 2014. The effects of erosion on the microbial populations and enzyme activity in black soil of northeastern china. Acta Ecol. Sin. 34, 295–301.
Jamro, G.M., Chang, S.X., Naeth, M.A., 2014. Organic capping type affected nitrogen availability and associated enzyme activities in reconstructed oil sands soils in Alberta, Canada. Ecol. Eng. 73, 92–101.
Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil V. A method for measuring soil biomass. Soil Biol. Biochem. 8, 209–213.
Kandeler, E., Mosier, A.R., Morgan, J.A., Milchunas, D.G., King, J.Y., Rudolph, S., Tscherkoa, D., 2006. Response of soil microbial biomass and enzyme activities to the transient elevation of carbon dioxide in a semi-arid grassland. Soil Biol. Biochem. 38, 2448–2460.
Kaur, B., Gupt, S.R., Singh, G., 2002. Carbon storage and nitrogen cycling in silvopastoral systems on a sodic soil in northwestern India. Agrofor. Syst. 54, 21–29.
Kushwaha, C.P., Tripathi, S.K., Singh, K.P., 2000. Variations in soilmicrobial biomass and N availability due to residue and tillage management in a dryland rice agroecosystem. Soil Till. Res. 56, 153–166.
Laik, R., Kumar, K., Das, D.K., Chaturvedi, O.P., 2009. Labile soil organic matter pools in a Calciorthent after 18 years of afforestation by different plantations. Appl. Soil Ecol. 42, 71–78.
Li, Y., Cui, J., Zhang, T., Okuro, T., Drake, S., 2009. Effectiveness of sand-fixing measures on desert land restoration in Kerqin Sandy Land northern China. Ecol. Eng. 35, 118–127.
Li, Y., Brandle, J., Awada, T., Chen, Y., Han, J., Zhang, F., Luo, Y., 2013. Accumulation of carbon and nitrogen in the plant–soil system after afforestation of active sand dunes in China's Horqin Sandy Land. Agric. Ecosyst. Environ. 177, 75–84.
Liao, Y.P., Wang, J.H., Fu, R., 2011. An analysis on dynamic changes and driving factors of the sandy land of Aba district in northwest Sichuan Province. Res. Soil Water Conserv. 18, 51–54.
Liu, R., Zhu, F., An, H., Steinberger, Y., 2014a. Effect of naturally vs manually managed restoration on ground-dwelling arthropod communities in a desertified region. Ecol. Eng. 73, 545–552.
Liu, Y., Yang, H., Li, X., Xing, Z., 2014b. Effects of biological soil crusts on soil enzyme activities in revegetated areas of the Tengger Desert, China. Appl. Soil Ecol. 80, 6–14.
Miao, R., Jiang, D., Musa, A., Zhou, Q., Guo, M., Wang, Y., 2015. Effectiveness of shrub planting and grazing exclusion on degraded sandy grassland restoration in Horqin sandy land in Inner Mongolia. Ecol. Eng. 74, 164–173.
Muys, B., Beckers, G., Nachtergale, L., Lust, N., Merckx, R., Granval, P., 2003. Medium-term evaluation of a forest soil restoration trial combining tree species change, fertilisation and earthworm introduction. Pedobiologia 47, 772–783.
Park, K.H., Qu, Z.Q., Wan, Q.Q., Ding, G.D., Wu, B., 2013. Effects of enclosures on vegetation recovery and succession in Hulunbeier steppe, China. For. Sci. Technol. 9, 25–32.
Plaza, C., Hernández, D., García-Gil, J.C., Polo, A., 2004. Microbial activity in pig slurry-amended soils under semiarid conditions. Soil Biol. Biochem 36, 1577–1585.

Saggar, S., Mcintosh, P.D., Hedley, C.B., Knicker, H., 1999. Changes in soil microbial biomass metabolic quotient, and organic matter turnover under *Hieracium* (*H. pilosella* L.). Biol. Fertil. Soil 30, 232–238.
Setia, R., Verma, S.L., Marschner, P., 2012. Measuring microbial biomass carbon by direct extraction – comparison with chloroform fumigation-extraction. Eur. J. Soil Biol. 53, 103–106.
Slimani, H., Aidoud, A., Rozé, F., 2010. 30 years of protection and monitoring of a steppic rangeland undergoing desertification. J. Arid Environ. 74, 685–691.
Smith, J.L., Paul, E.A., 1990. The significance of soil microbial biomass estimates in soil. Soil Biochem. 6, 357–396.
Su, Y.Z., Li, Y.L., Cui, J.Y., Zhao, W.Z., 2005. Influences of continuous grazing and livestock exclusion on soil properties in a degraded sandy grassland, Inner Mongolia, Northern China. Catena 59, 267–278.
Tanon, G., Boldreghini, P., Gioacchini, P., 2005. Seasonal changes in microbial nitrogen in an old broadleaf forest and in a neighboring young plantation. Biol. Fertil. Soil 41, 101–108.
Verdoodt, A., Mureithi, S.M., Ranst, E.V., 2010. Impacts of management and enclosure age on recovery of the herbaceous rangeland vegetation in semi-arid kenya. J. Arid Environ. 74, 1066–1073.
Ye, S., Yang, Y., Xin, G., Wang, Y., Ruan, L., Ye, G., 2015. Studies of the Italian ryegrass–rice rotation system in southern China: arbuscular mycorrhizal symbiosis affects soil microorganisms and enzyme activities in the *Lolium mutiflorum* L. rhizosphere. Appl. Soil Ecol. 90, 26–34.
Yi, X.S., Li, G.S., Yin, Y.Y., 2012. The impacts of grassland vegetation degradation on soil hydrological and ecological effects in the source region of the yellow river – a case study in Junmuchang region of Maqin country. Proc. Environ. Sci. 13, 967–981.
Yong, G.W., Shi, C.C., Qiu, P.T., 2003. Monitoring on desertification trends of the grassland and shrinking of the wetland in Ruoergai Plateau in north-west Siehuan by means of remote-sensing. J. Mt. Sci. 21, 758–762.
Zhang, J.T., Dong, Y., 2010. Factors affecting species diversity of plant communities and the restoration process in the loess area of China. Ecol. Eng. 36, 345–350.
Zhang, T.H., Zhao, H.L., Li, S.G., Li, F.R., Shirato, Y., Ohkuro, T., Taniyama, I., 2004. A comparison of different measures for stabilizing moving sand dunes in the Horqin Sandy Land of Inner Mongolia. China. Arid Environ. 58, 202–213.
Zhang, H., Li, G., Song, X., Yang, D., Li, Y., Qiao, J., Zhang, J., Zhao, S., 2013. Changes in soil microbial functional diversity under different vegetation restoration patterns for Hulunbeier Sandy Land. Acta Ecol. Sin. 33, 38–44.
Zhang, Y.L., Chen, L.J., Chen, X.H., Tan, M.L., Duan, Z.H., Wu, Z.J., Li, X.J., Fan, X.H., 2015. Response of soil enzyme activity to long-term restoration of desertified land. Catena 133, 64–70.