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

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ABSTRACT

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

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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