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Response of soil enzyme activity to long-term restoration of desertified land☆

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ABSTRACT

Low extracellular enzyme activity in desert soil can be recovered during the succession of re-vegetation, especially in soils forming under shrubs (microsite soil), which closely reflects desert restoration conditions. However, not much is known about the restoration of soil enzyme activity at these microsites. By using the space-for-time substitution method, soils on moving sand dunes that had been stabilized at different dates over a fifty year period at the southeastern fringe of the Tengger Desert were selected to investigate the enzyme activities in the surface soil crust and three other soil depths at microsites to demonstrate the evolution of enzymatic activity at different stages from bare soil to complex vegetation over a fifty year sequence. The results showed that organic C and total and available N, P, and enzyme activities (dehydrogenase, catalase, α- and β-glucosidase, protease, and phosphatase) were progressively enhanced in each microsite soil in the 50-year chronosequence and had effect down to 35 cm depth. Soil enzyme activities of the crust and the 0–5 cm soil layer were higher than in deeper soil layers. The observed increase over time of the values of the measured soil properties, such as organic C, total and available N, was much larger in the crust and the 0–5 cm soil layer in comparison to the deeper layers. The improvement of desert soil quality indicated that desertification can be mitigated to a certain extent under human controls.

1. Introduction

Intensive cultivation and overgrazing lead to the loss of vegetative cover, followed by the loss of most topsoil. Millions of hectares of cultivated land have been lost in this way (Wang et al., 2012). Re-vegetation has been reported as one effective method to control soil erosion and restore a healthy ecosystem in the desert region (Li et al., 2003). Soil nutrient levels increased during the restoration process and were used as an indication of successful restoration (Cao et al., 2008; Chen and Duan, 2009; Tongway et al., 2003). He et al. (2009) reported that the storage of C and N in bulk soil and soil particle-size fractions increased during a soil restoration time sequence measured at 3, 8, 20, 24, and 28 years from planting. However, soil microbial properties, such as soil enzyme activities, which are the driving force in nutrient cycling, are more sensitive than physical and chemical indicators (Tabatabai., 1994). Soil enzyme levels were closely related to crust development, nutrient transformation, soil formation on stabilized dunes, and soil fertility in rehabilitated sandy soils (Consuelo and Teodoro, 2002). Sampling of sites 5, 10, and 23 years after replanting showed a progression of increasing leaf deposition, net primary productivity and electrical conductivity, and consequently elevated nutrient content and microbial biomass (Cao et al., 2008). Over time, vegetation restoration improved activities of polyphenol oxidase, dehydrogenase urease, protease, and phosphomonoesterase (Cao et al., 2008; Consuelo and Teodoro, 2002; Dick et al., 1996; Nannipieri et al., 2002). Blank (2002) noted increased activities of N-mineralizing enzymes at inter-shrub areas (microsites) after restoration. Re-planting of sandy soils has resulted in the restoration of stable vegetation to combat desertification and has significantly improved physical, chemical, and biological soil properties (Bolling and Walker, 2000; Cao et al., 2008; Ffolliott et al., 1995; Zhang et al., 2008; Zuo et al., 2009).

The progressive response of soil enzymes after replanting can indicate the degree of success of desert restoration projects (Cao et al., 2008). Plant restoration enhanced physical, chemical and microbial properties from top-soil to deeper layers (Cao et al., 2008), meaning that sandy soil recovered biological activity from the surface downwards. Tongway et al. (2003) demonstrated that the enhancement trend was most apparent in the top 1 cm of soil rather than deeper layers. Usually, enzyme activities decrease with increasing soil depth

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(Blank, 2002). Crusts develop slowly from barren or disturbed soils and restoration of desertified sands is a long process, during which natural succession resulted in the disappearance of some replanted species at a later stage (Thompson et al., 2006). Soil nutrients and biological properties in soils beneath shrubs were higher than those between shrubs in dry soils (Jackson et al., 1988; Sarig and Steinberger, 1994; Schlesinger et al., 1996). Sarig et al. (1994) considered that nutrient heterogeneity was mainly controlled by the presence of plant cover as such, not species forming that cover. Thompson et al. (2006) documented that microsites can reflect degree of desert restoration, yet much remains unknown about microsite soil.

There have been many studies about specific areas under the canopy of specific species, and most studies have concentrated on the effects of the shrub on the chemical and physical properties of the soil as well as some enzyme activities (Cao et al., 2008; Zuo et al., 2009). Such studies cannot accurately reflect the situation over a large region. Little information has been reported about the progressive changes in soil rehabilitation projects during periods as long as 50 years. The objectives of the present study were 1) to determine the rate of change in various chemical and biochemical soil properties during sand land rehabilitation, including nutrient contents and activities of dehydrogenase, catalase, α- and β-glucosidase, protease, and phosphatase, and 2) to identify the rates of change of soil properties in inter-shrub areas over a span of 50 years, when sand-fixing was first used on desertified land.

2. Materials and methods

2.1. Site description

Field investigation and sample collection were conducted at the Shapotou Desert Research and Experiment Station (SDRES) of the Chinese Academy of Sciences (CAS) (37°32′N, 105°02′E, 1300 masl). The station is located in the Shapotou–Hongwei area at the southeastern edge of the fourth largest desert in China (Tengger Desert with 36,000 km^2 area), near the semi-arid agro-pastoral transitional zone of Northwest China. The Shapotou region has evolved complex, stable, productive, rain-fed, artificial, and natural ecosystems capable of reversing desertification from the simple, artificial vegetation system in the arid desert region (Chen and Duan, 2009; Duan et al., 2004; Li et al., 2004; SDRES, CAS, 1991; Zhang et al., 2004). Shapotou is referred to as one of the most successful models for desert control and ecological restoration in the arid desert region of China and probably of the world (Li et al., 2010).

The SDRES area (37°27′N, 104°57′E) is at an elevation of 1339 m a.m.s.l., in the steppe desert zone and can also be described as an ecotone between desert and oasis (Li et al., 2010). The annual mean temperature is 10.0 °C, the mean low temperature is −6.9 °C in January, and the high is 24.3 °C in July. The mean annual precipitation was 186 mm, and approximately 80% of this occurs between May and September. The annual potential evaporation is approximately 2900 mm. The mean annual wind velocity is 2.9 m/s. The growing season ranges from 150 to 180 days between the last frost in mid-April and the first frost in late September. The primary soils in the SDRES area are Orthic Sierozem and Typic Psammaquent (Chen et al., 1998; FAO/UNESCO, 1974). The dominant vegetation species are annual plants (*Agriophyllum squarrosum* Moq. and *Hedysarum scoparium* Fisch) and shallow-rooting shrubs (*Artemisia ordosica*), and natural vegetation has approximately 1% surface coverage (Li et al., 2004).

The fields investigated in this study lie in the artificially re-vegetated desert area (Li et al., 2002). The non-irrigated vegetation protective system was established in the 1950s. Initially, barriers to sand movement were established using a matrix of straw ropes in a checkerboard pattern. The straw checkerboards held in place almost 99% of the quantity of sand previously transported over the mobile dunes. Shrubs were planted in the checkerboards in different years (1956, 1964, 1975, and 1987) and the successful revegetation over this time period has attracted the attention of many scientists who now seek to study the ecological patterns and processes. The stabilized sand surface provided better conditions for all plants and promoted airborne dust deposition onto the surface, which led to the formation of soil bio-crust (Li et al., 2002). The mobile dune-dominated landscape has been turned into a stable, complex desert ecosystem and the evolution of micro-habitats has been promoted (Wang et al., 2006).

2.2. Experimental design and soil sampling

Soil samples were collected from different sites representing different time sequences from 20 m × 20 m plots in August of 2007. Soil samples (superficial crust plus three subsurface layers at depths of 0–5 cm, 5–15 cm, and 15–35 cm) were collected with four replicates from five sites including one shifting sand site and the dunes restored in 1956, 1964, 1975, and 1987.(i.e., dunes stabilized and replanted for 50, 43, 32, and 20 years). A shifting sand site was chosen outside the stabilized area, where the main landscape type was high and dense reticulate chains of barchan dunes. There were no soil crusts and plants in mobile dunes (Li et al., 2014). The replanting dates were chosen to examine the effect of time on sand soil development. These sites were restored with very similar treatments by planting seedlings of the same shrub species with the same density in similar straw checkerboards. The selected sequence represents the different stages of transformation from the relatively simple primary sand-binding vegetation into a functional ecosystem with a complex structure and composition (Li et al., 2002). Previous research papers have noted that in addition to the surface crust, there have also been differentiations of the subsurface sands into separate horizons (Duan et al., 2004). As a result, we set three depths down to 35 cm.

All samples were gently mixed, sieved through a 2 mm screen to remove the root material and other debris, and stored in polyethylene bags. Half of each sample was air-dried and stored at room temperature for analysis of chemical and physical soil properties. The other half of each sample was kept field-moist in a cooler at 4 °C for analysis within two weeks to allow determination of soil biological properties.

2.3. Measurements

2.3.1. Soil chemical properties

All methods for pH, total C, N, and P and available N, P, and soil moisture were described in detail in Zhang et al. (2010). Briefly, the pH of soil samples was measured with a suspension of soil in distilled water (1:2.5) by a glass electrode. Total C and N were determined using an Elementar Vario EL analyzer (Matejovic, 1995), and total P was determined by the H_2SO_4–$HClO_4$ digestion method (Kuo, 1996). Available P was determined using the Olsen method with $NaHCO_3$ as an extractant (Kuo, 1996), and available N was extracted with KCl (2 $mol \cdot L^{-1}$) and analyzed by one Continued Flow Analysis (CFA) (Miller and Keeney, 1982).

2.3.2. Soil enzyme activities

Soil enzyme activities were measured with colorimetric determination methods described in Zhang et al. (2010). Briefly, the soil dehydrogenase activity was measured by using triphenyltetrazolium chloride (TTC) (Sigma-Aldrich Inc., US) as the substrate (3:100 substrate:water, w/v) and expressed as μg TPF g^{-1} soil 24 h (Tabatabai, 1994). The catalase activity was determined by spectrophotometry via the measurement of hydrogen peroxide breakdown (Trasar-Cepeda, 1999). The protease activity was determined using casein (Sigma-Aldrich Inc., US) as the substrate (Ladd and Butler, 1972). The phosphatase, α-D-glucosidase, and β-D-glucosidase activities were measured by colorimetric determination of the released *p*-nitrophenol (Tabatabai, 1994), with sodium *p*-nitrophenyl phosphate (Seebio Biotech Inc., China), *p*-nitrophenyl α-D-glucoside (J&K China Chemical Ltd.,) and *p*-nitrophenyl β-D-galactoside (Sigma-Aldrich Inc.) as substrates. For phosphatase, α-D-glucosidase, and β-D-glucosidase activity measurements, controls were also included, in which substrates were added after the soil sample incubation and prior to analysis.

2.4. Statistical analysis

All evaluations were conducted in triplicate and all data were expressed per gram of oven-dried (105 °C) soil. 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). *P* and *F* values were shown from ANOVA. The Pearson correlation analysis was carried to study the relationship between enzymatic and chemical data of soils, which were analyzed with principal component analysis (PCA). Relationships among soil samples were evaluated on the two most significant principal component dimensions by calculated scores. Treatment and statistical analysis of data were conducted using the SPSS 19.0 software.

3. Results

3.1. Soil chemical properties

Significant differences were found for soil organic C and total N between plots stabilized and replanted at different years (restoration period) and at different soil depths (Fig. 1). Soil organic C, total N, and total P increased after restoration and had notable increases after 30 years. They were also higher in the crust and 0–5 cm soil layer than in deeper soil layers (Fig. 1). Similarly, available N and P differed significantly between restoration periods and soil depths (Fig. 1). The soil pH value increased slightly at first and then decreased slightly, while pH increased with soil depth.

3.2. Soil enzyme activities

The soil enzyme activities differed significantly among sand restoring sites (Fig. 2, Table 1). The activities of soil dehydrogenase, α-D-glucosidase, β-D-glucosidase, protease and phosphatase increased significantly as the period of sand land restoration increased, especially in the crust and 0–5 cm soil layer; however, the catalase activity increased only slightly. The activities of test enzymes other than catalase in sand-fixed sites were several times higher than those in the control site (mobile dune without restoring). Soil microbial enzyme activities changed with different stages of restoration and had positive relationships with soil nutrients (Table 2).

3.3. Principal component analysis among restoring ages, soil properties and soil enzyme activities

Principal component analysis (PCA) was performed on a correlation matrix of the data obtained of biological activities and soil chemical parameters, which ordinated the variation of the data on the two most significant components (factors 1 and 2) (Fig. 3). Two factors altogether

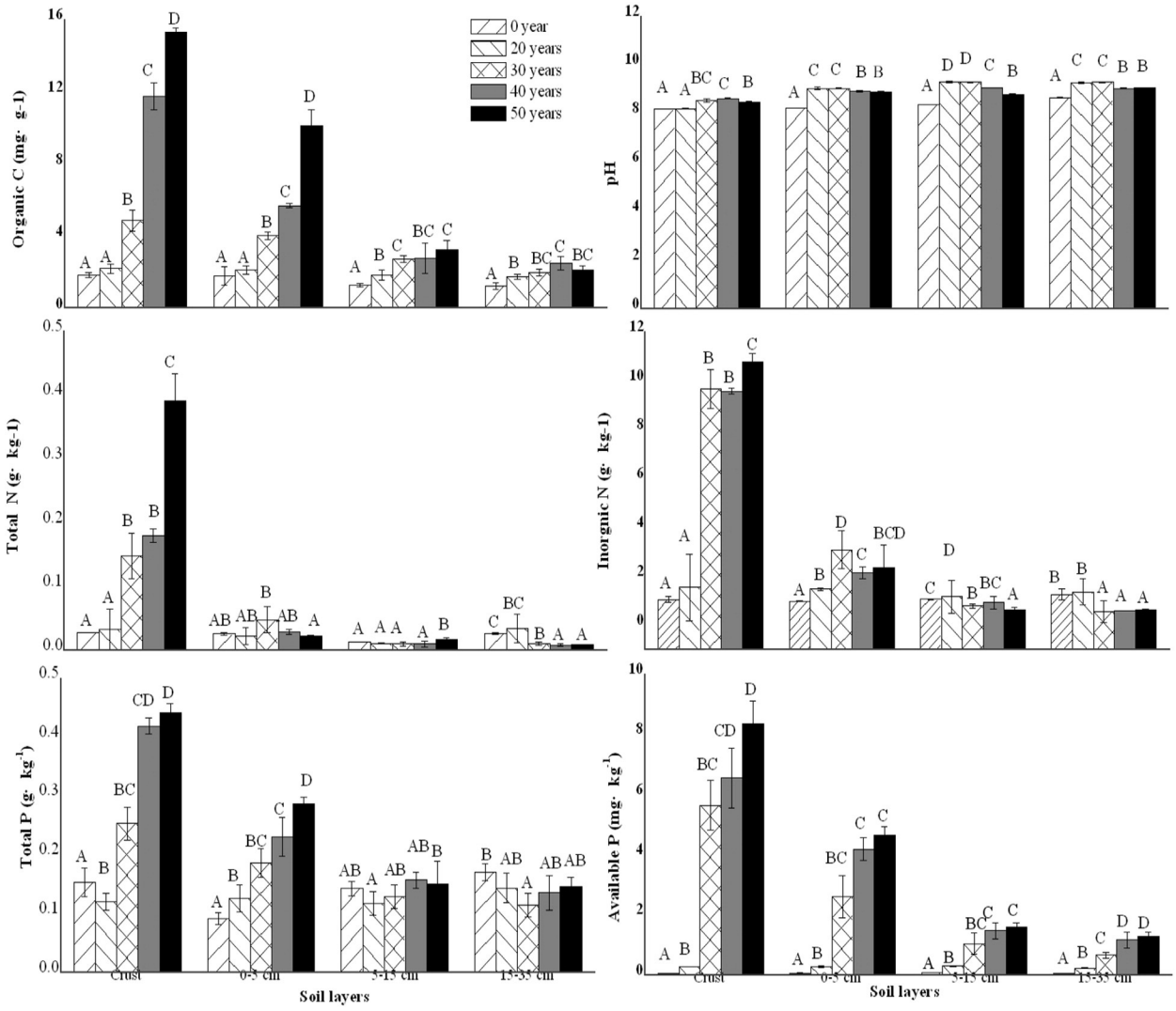

Fig. 1. Changes of organic C, pH, total and available N, P contents in soils collected from microsites with 20,30, 40 and 50 years of restoration and mobile dune (Values are the means ± S.D., Values having the different letters differ significantly ($p < 0.05$) as determined by the LSD test).

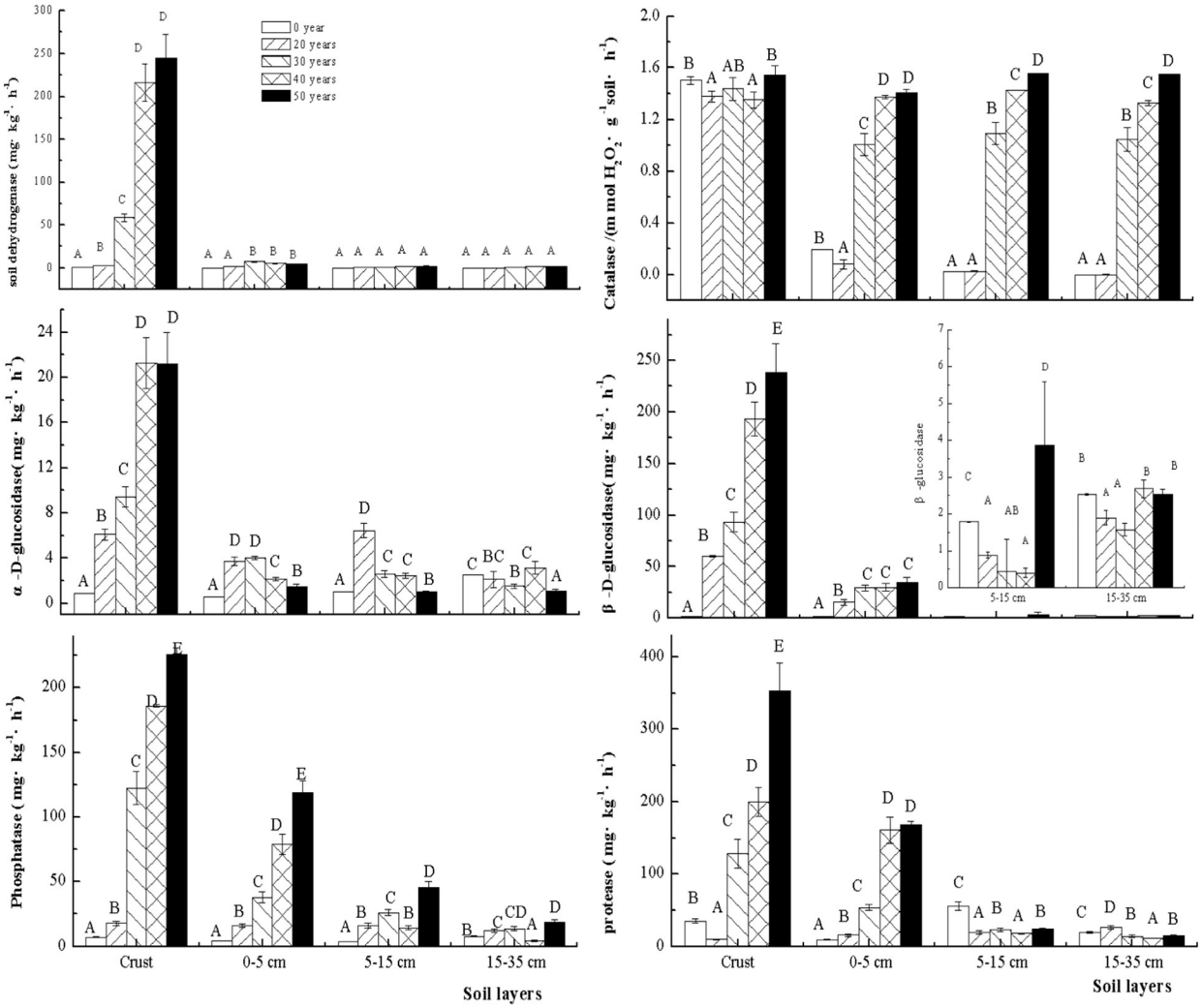

Fig. 2. Changes of soil enzyme activities with 20, 30, 40 and 50 years of restoration and mobile dune (Values are the means ± S.D., values having the different letters differ significantly ($p < 0.05$) as determined by the LSD test).

accounted for 95.4% of the total variance. Factor 1 explained 91.7% of the total variability and was related to available P, soil phosphomonoesterase, total carbon, total phosphorus, total nitrogen, inorganic nitrogen, soil protease, soil β-glucosidase, soil α-glucosidase and dehydrogenase activity. This factor discriminated among soils of different restoring age areas showing that these parameters have been seriously affected by restoration.

Table 1
Analyses of variance for test parameters.

Index	Restoring age		Soil depth		Restoring age × soil depth	
	F	*P*	*F*	*P*	*F*	*P*
Organic C	81.72	<0.001	28.10	<0.001	5.55	<0.001
Total N	11.99	<0.001	53.96	<0.001	16.52	<0.001
Total P	6.84	<0.001	5.23	0.002	1.88	0.043
Available N	4.36	0.003	21.15	<0.001	4.58	<0.001
Available P	38.67	<0.001	42.72	<0.001	8.65	<0.001
pH	59.24	<0.001	35.37	<0.001	8.85	<0.001
Catalase	67.68	<0.001	5.63	0.001	1.31	0.218
Dehydrogenase	73.39	<0.001	225.92	<0.001	74.06	<0.001
β-Glucosidase	27.09	<0.001	75.94	<0.001	17.47	<0.001
α-Glucosidase	5.85	<0.001	29.74	<0.001	7.14	<0.001
Protease	8.10	<0.001	8.69	<0.001	5.24	<0.001
Phosphatase	24.30	<0.001	119.62	<0.001	20.83	<0.001

Readings from the surface of unstabilized dunes and all sample layers below it were similar to the 0–5 cm layer of soils stabilized for 20 years, the 5–15 cm and 15–30 cm layers of soils stabilized for 30 years, and the 15–30 cm layer of soils stabilized for 40 years. The PCA evidenced that 0-year-crust, 0 year-0–5 cm. 20 years-0–5 cm, 0 years-5–15 cm, 30 years-5–15 cm, 0 year-15–30 cm, 30 years-15–30 cm and 40 years-15–30 cm samples had higher similarity, and 30 years-0–5 cm, 20 years-5–15 cm, 20 years-15–30 cm, and 50 years-15–30 cm treatments had higher similarity.

4. Discussion

The pH of soil crusts after 20–30 years of rehabilitation was higher than that of the control most likely because of the salinity added by plant branches and leaves (Wang et al., 2006). Soil pH can be affected by biotic crust and vascular plant species (Thompson et al., 2005). Decrease of crust pH after 40 years may be due to the effects of branches and roots, whereas increased soil pH with depth may be because of less organic matter inputs to these soils.

Total C concentrations in the soil surface layer were higher than deeper layers, which was probably due to several factors. These factors include higher litter inputs and reduced soil erosion rate of the soil surface layer, low incorporation rate of surface litter into the soil by soil fauna, and low mineralization rate of C due to the high phenol and lignin contents in litter (Cao et al., 2008). The C input from vegetation to the

Table 2
Correlations between the soil chemical properties and soil hydrolase activities.

	pH	TC	IN	TN	AP	TP	Deh	Cata	β-Glu	α-Glu	PR	AP
pH	1.000											
TC	−0.188	1.000										
IN	−0.262	0.925**	1.000									
TN	−0.230	0.821**	0.817**	1.000								
AP	−0.246	0.905**	0.898**	0.983**	1.000							
TP	−0.287	0.968**	0.861**	0.882**	0.934**	1.000						
DEH	−0.194	0.989**	0.875**	0.824**	0.898**	0.976**	1.000					
CATA	−0.405	−0.312	−0.128	−0.274	−0.255	−0.311	−0.371	1.000				
β-GLU	−0.019	0.971**	0.905**	0.845**	0.914**	0.932**	0.962**	−0.34	1.000			
α-GLU	−0.012	0.951**	0.837**	0.840**	0.897**	0.936**	0.960**	−0.354	0.979**	1.000		
PR	−0.301	0.963**	0.969**	0.855**	0.927**	0.933**	0.936**	−0.267	0.923**	0.867**	1.000	
AP	−0.206	0.959**	0.910**	0.948**	0.986**	0.972**	0.954**	−0.297	0.959**	0.946**	0.950**	1.000

SOC, soil organic C; TN, total N; TP, total P; AN, available N; AP, available P; DEH, dehydrogenase; CATA, catalase; β-GLU, β-glucosidase; α-GLU, α-glucosidase; PR, protease; AP, phosphomonoesterase.

soil increased with time after restoration, along with enhancement of N and P uptake by plants, which led to an increase in soil C, N and P and soil biological activity (Kaur et al., 2002). Biological soil crusts are one of the major components of desert ecosystems. Housman et al. (2006) reported that crusts have a greater ability to increase C and N inputs to desert ecosystems in the later successional stages and provided a well-developed habitat and more stable food-web than those in the early-successional stages. Similar patterns were observed in many studies conducted to evaluate the restoration effects of plantations on degraded sub-humid sites (Bhojvaid and Timmer, 1998; Wezel et al., 2000).

Increased soil enzyme activities might be due to increased organic matter input and C and N immobilization during the process of organic matter decomposition, as several studies have shown that soil enzyme activities can be affected by organic matter (Cao et al., 2008; Kushwaha et al., 2000; Plaza et al., 2004; Smith and Paul, 1990). These changes were also attributed to improvement of the soil environment (Cao et al., 2008; Aon et al., 2001; Chen, 2003). Availability of organic inputs increased because of revegetation, and as soil microorganisms increased, more enzymes were synthesized (An et al., 2009; Cao et al., 2008). The amount and catalytic ability of soil enzymes increased. Soil dehydrogenase, one intracellular enzyme, was tightly linked with microbial oxidation–reduction processes and the increased activity level indicated enhancements of soil microbial biomass. Enzyme activities could be used as an index of soil productivity and microbial activity. Changes of enzymes related to nutrient transformations indicated the potential to increase soil C, N and P storage after recovery. Soil pH has direct biochemical effects on extracellular enzyme activities immobilized in the soil matrix,

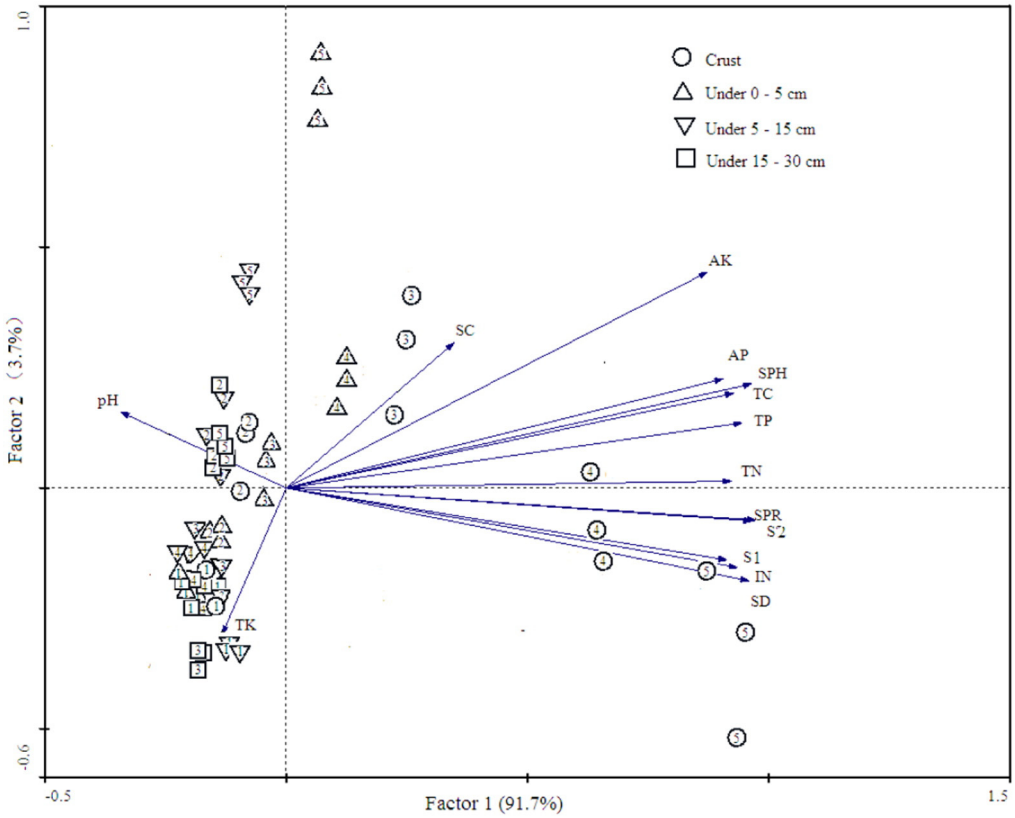

Fig. 3. Principal components analysis (PCA) of soil basic chemical characters and enzyme activities with 20, 30, 40 and 50 years of restoration and mobile dune (Values are the means ± S.D.). ○ crust, △ under 0–5 cm, ▽ under 5–15 cm. □ under 15–35 cm; 1. 50 year's restoration, 2. 40 year's restoration, 3. 30 year's restoration; 4. 20 year's restoration; 5. mobile dune without restoration.

while the present study did not show significant effects on soil enzymes from changes in proton concentration.

Nutrient contents and microbial activity showed similar trends for soil layers under the crust, which demonstrated that the restoration had an influence on the crust layer and the layers under the crust. The values of the soil properties decreased with increasing soil depth and Chen (2003) and Cao et al. (2008) observed similar results. The higher organic matter inputs (litter, dust, etc.) on the inter-shrub surface, together with better soil aeration, resulted in higher soil microbial activity in the crust and the 0–5 cm layers and heterogeneous distribution throughout the soil profile. Fig. 3 of the PCA showed that almost all chemical and biological parameters were affected by restoration, except total soil K.

It is realized that topsoil has been altered or lost and biodiversity was reduced or lost in many desert regions because of grazing, agriculture, mining or other means (Allen, 1993). The remaining topsoil had low nutrients, organic matter, and biological activity. Low soil enzyme activities were also reported in other sandy soils under rehabilitation (Cao et al., 2008; Li et al., 2007; Su and Zhao, 2003; Su et al., 2004). Restoration of soil nutrient levels and enzyme activity causes restoration of subsurface biodiversity as well.

The fact that chemical and biochemical soil properties in the study area improved with time in open areas undergoing restoration proved the viability of the straw rope checkerboard restoration process. The results suggested that revegetation can improve soil quality and the restoration of soil biological activity in these degraded ecosystems. However this restoration is a long-term process.

5. Conclusions

In the barren space between plants of the treated desert area, the nearby revegetation led to higher soil pH and nutrient contents, especially in the crust and the 0–5 cm under the crust compared with samples from under mobile sand dunes. Most test soil enzymes also increased significantly during the progression through successive stages of restoration, especially in the crust and the 0–5 cm soil layer. Soil microbial enzyme activities had positive relationships with available soil nutrients. Revegetation enhanced soil enzyme activity in quantity and catalytic properties. The improvement of soil chemical properties and soil enzyme activity occurred remarkably, and microsite soils were improved to 35 cm depth under crust, which indicated that desertification can be mitigated to a certain extent if human controls pressure.

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