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#### **Original Research Paper**

# Response of Soil Chemical and Microbial Properties to Vegetation Restoration on the Loess Plateau, China

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# ABSTRACT

Revegetation has been reported as one of the most effective measures for reducing soil erosion on the Loess Plateau in China. We evaluated the effect of natural revegetation restoration on soil chemical nutrient, microbial biomass and enzyme activities on the Loess Plateau of China. The vegetation types studied, in order from the shortest to the longest enclosure duration, were abandoned grazed grassland (Ab.G3, 3 years), *Hierochloe odorata* Beauv. (Hi.O7, 7 years) communities, *Thymus mongolicus* Ronnm (Th.M15, 15 years), *Artemisia sacrorum* Ledeb (At.S25, 25 years), *Stipa bungeana* Trin Ledeb (St.B36, 36 years), and *Stipa grandis* P. Smirn (St.G56, 56 years). The results showed that soil organic carbon (SOC) and total nitrogen (N) was 9.2-32.2 and 1.2-3.5 g/kg during vegetation restoration, respectively. Except from Th.M15, SOC and total N increased with restoration time. Also, soil microbial biomass carbon (Cmic), phosphorus (Pmic), invertase activities, and alkaline phosphatase activities increased with restoration time. Whereas, soil microbial biomass nitrogen (Nmic) and urease activity was the highest in the early succession period and lowest in the mid succession period (under Th.M15 and At.S25). Revegetation resulted in more abundant and stable soil ecological environment by enhancing soil nutrients and biological properties. Thus, this is a beneficial measure for the recovery of degraded soils on the Loess Plateau of China.

# INTRODUCTION

Revegetation has been reported as the most effective and useful way to abate soil erosion and soil degradation (Hou et al. 2002) and to restore the ecological integrity of disturbed ecosystems (Montalvo et al. 1997). However, in many ecosystems, vegetation productivity may be limited by nutrient availability (Aaron et al. 2001, Aerts & Chapin 2000). Microorganisms play an important role in organic matter decomposition and nutrient cycling. In both, natural and agro-ecosystems, microbial biomass and enzymatic activities have often been proposed as the early and sensitive indicators of soil ecological stress or indicators of restoration processes (Doran 1980, Dick & Tabatabai 1993). Soil microbial biomass is an important source of soil organic carbon (SOC) and plays a fundamental role in carbon and nitrogen cycling (Spence et al. 2011). Although the effect of vegetation restoration on soil biochemical properties have been well studied (An et al. 2009, Huang et al. 2007, 2009, Wang et al. 2011, Jia et al. 2012), soil microbial properties in relation to vegetation restoration is still not well documented. However, it is essential for understanding the effect of vegetation restoration on soil ecological environment.

The objective of this study was to assess the potential impact of natural vegetation restoration on soil nutrient cycling and biochemical properties on the Loess Plateau. We investigated the concentration of soil nutrients, microbial biomass and enzyme activity in soils at depths of 0-20 cm and 20-40 cm from restored grasslands of different vegetation types on the Loess Plateau of China. The following hypotheses were tested in this study: (1) revegetation would result in increase in soil nutrients because of the additional input of plant residue; (2) vegetation restoration would improve soil microbial activities and enhance more microbial biomass.

# MATERIALS AND METHODS

The study sites and study approach: The method of spacefor-time substitution, as an effective way of study changing over time (Sparling et al. 2003), was used to monitor plant and soil changes occurring along a vegetative chronosequence developed on similar soils under similar climatic conditions (Bhojvaid & Timmer 1998). Sites that have been stabilized by revegetation at different times offer an ideal opportunity to understand vegetation succession process, as soil conditions before revegetation are largely driven by geomorphological processes, and vegetation succession commences after the establishment of sand-binding vegetation.

The studied area was the Yunwu Observatory for Vegetation Protection and Eco-environment within the Loess Plateau in China. The observatory is permanent grassland located in Guyuan in Ningxia province between 106°24' and 106°28' longitude and between 36°13' and 36°19' latitude. Soil type of the area is typic loessic orthic primosols, and soil texture is medium loam. It is a protected grassland area and is more than 100 years old. In this study, an existing successional sere, a series of stages of a particular plant succession was selected in a relatively homogeneous field. According to the process of plant succession in this area, we studied six successional stages (3, 7, 15, 25, 36 and 56 years) as given in Table 1. Depending on the time of natural succession, the plant communities were: (a) Ab.G3: 3year-old abandoned overgrazing grassland, (b) Hi.O7: Hierochloe odorata Beauv. (7 year), (c) Th.M15: Thymus mongolicus Ronnm (15 year), (d) At.S25: Artemisia sacrorum Ledeb (25 year), (e) St.B36: Stipa bungeana Trin Ledeb (36 year), and (f) St.G56: Stipa grandis P. Smirn (56 year).

The study area has a sub-arid climate that is characterized by heavy seasonal rainfall. The mean annual temperature is 7°C. Average annual rainfall is 400 mm (1941-2000, C.V. 18%) with distinct wet and dry seasons. The rainy season starts in July and ends in September. The rainfall in July accounts for 24% of the total annual rainfall.

**Soil sampling and processing:** Soil samples were collected in July 2012. For each vegetation type, contours at 3 elevations (an upper slope, a middle slope, and a lower slope) were drawn. At each contour, 3 areas of  $60 \text{ m} \times 60 \text{ m}$  were laid out. From each area, soil samples were obtained at depths of 0-20 cm and 20-40 cm. Five core samples were taken from each area and mixed to form a bulk sample of about 1 kg. A total of 108 soil samples were obtained for all vegetation types. Part of the fresh samples was sealed in plastic bags and saved in the refrigerator at -20°C for soil microbial biomass analyses, and the remaining samples were air dried at room temperature in the laboratory. Each sample was passed through a 2-mm sieve to remove large roots, stones, and macrofauna.

The analysis of soil microbial properties: Soil microbial biomass carbon (Cmic), nitrogen (Nmic), and phosphorus

Sample site name	Revegetation years (a)	Geographical coordinates $\psi(N), \lambda(E)$	Elevation (m)	Slope gradient (°)	Dominant species	Accompanying species	Total fresh mass (g)
Ab.G3	3	36°15.8072 106°23.2262	2078	5	<i>Leymus secalinus</i> (Georgi) Tzvel.	Artemisia scoparia Thymus mongolicus Potentilla bifurca	86.00
Hi.O7	9	36°15.8072 106°24.5922	2080	6	Hierochloe ordorata Leymus secalinus	Artemisia scoparia Thymus mongolicus	179.55
Th.M15	15	36°15.1012 106°23.4152	1903	10	Thymus mongolicus Romm	Stipa gradiss Artemisia sacrorum Potentilla bifurca Linn.	539.00
At.S25	25	36°15.7512 106°23.4152	2082	12	Artemisia sacrorum Ledeb.	Stipa bungana Heteropappus altaicus Thymus mongolicus	545.60
St.B36	36	36°15.1012 106°23.9352	2097	14	<i>Stipa bungean</i> Trin Ledeb	Artemisia sacrorum Thymus mongolicu Leymus secalinu Potentilla bifurca	618.40
St.G56	56	36°15.1432 106°23.2042	2058	10	<i>Stipa grandis</i> P. Smirn	Artemisia frigida Willd. Potentilla acaulis L. Medicago ruthenica Potentilla angustiloba	805.60

Table 1: Geographical and vegetation characteristics.

Note: Ab.G3: recently abandoned grazing on grassland (3 year); Hi.O7: *Hierochloe ordorata* Beauv. (7 year); Th.M15: *Thymus mongolicus* Ronnm. (15 year); At.S25: *Artemisia sacrorum* Ledeb. (25 year); St.B36: *Stipa bungeana* Trin Ledeb. (36 year); St.G56: *Stipa grandis* P. Smirn. (56 year)

(Pmic) were determined by the fumigation-extraction method using 15 g of oven-fried, field moist-equivalent soil sample (<2 mm) and 0.5 M K<sub>2</sub>SO<sub>4</sub> (Brookes et al. 1984, Vance et al. 1987, Zhou & Li 1998). Cmic was determined by a TOC analyser (Phoenix 8000, Tekmar Dohrman, Mason, OH, U.S.A.), and Nmic was determined colorimetrically with a spectrophotometer (Hitachi, Tokyo, Japan, UV2300) at 220 and 275 nm. The Cmic and Nmic were calculated using a k<sub>EC</sub> factor of 0.45 (Wu et al. 1990) and a k<sub>EN</sub> factor of 0.54 (Vance et al. 1987), respectively. Soil Pmic was determined colorimetrically with a spectrophotometer at 700 nm. The Pmic was calculated using the k<sub>EP</sub> factor of 0.40 (Hedley & Stewart 1982).

In this study, invertase and urease activities were selected to indicate soil C and N turnover, and alkaline phosphatase activity was selected to indicate soil phosphorus (P) turnover. The method of 3, 5-dinitrosalicylic acid colorimetry was used for the determination of invertase activity (Yao & Huang 2006). A 5g sample of air-dried soil (<1 mm) was incubated in 5 mL of citrate solution (pH 6.7) and 5 mL of 10 % urea solution at 37°C for 3 hours. The soil solution was diluted to 50 mL with distilled water, and the suspension was filtered with 1 mL aliquot treated with 4 mL of sodium phenol solution (mixture of 100 mL 6.6 M phenol solution and 100 mL 6.8 M NaOH) and 3 mL of 0.9 % sodium hypochlorite. The released ammonium was quantified colorimetrically with a spectrophotometer (Hitachi UV2300) at 578 nm.

For determination of alkaline phosphatase activity, 10 g of air-dried soil (<1 mm) and 2 mL of toluene were incubated in 10 mL of disodium phenyl phosphate and 10 mL of

0.05 M borate buffer (pH 9.6) at 37°C for 3 hours. The samples were filtered, and the filtrate was coloured with 0.5 mL of 2 % 4-aminoantipyrine and 8 % potassium ferrocyanide, and the released phenol was determined colorimetrically with a spectrophotometer (Hitachi, UV2300) at 510 nm (Guan et al. 1991).

The analysis of soil chemical properties: Soil samples were grinded to pass through a 0.15 mm sieve to measure the concentration of soil organic carbon (SOC) and total nitrogen. The SOC concentration (g/kg) of soil samples was analysed by potassium dichromate volumetry, and total N was measured by the semi-micro Kjeldahl method.  $NH_4$ -N and  $NO_3$ -N were extracted from moist samples (5g oven-dry equivalent) by shaking with 1M KCl at a soil solution ratio of 1:10, followed by centrifuging at 2000 rpm for 30 min. The supernatant for  $NH_4$ -N and  $NO_3$ -N was determined using a flow autoanalyzer. Available phosphorus (Available-P) was extracted and measured in a buffered alkaline solution with 0.5 M sodium bicarbonate. The extracts were quantified colorimetrically with a spectrophotometer at 660 nm.

**Statistical analysis:** Data were analysed to provide mean and standard deviation for each variable measured at every depth for sites representing the different stages of revegetation. SPSS 11.0 was used for statistical analysis. Analyses of variance were performed using the ANOVA procedure in the SPSS statistical software. The Student-Newman-Keuls method (P<0.05) was used to assess the differences among revegetation types and soil depths.

# RESULTS

Soil chemical properties: There were substantial differences

Vegetation SOC Total N  $NH_4-N$ NO<sub>2</sub>-N Available-P Soil Depth (cm) (mg/kg) (g/kg) (g/kg) (mg/kg) (mg/kg) types AbG3 0-20 9.62±0.83Da 1.22±0.07Ea 4.12±0.22Ba 14.47±0.04Ba 2.28±0.16Da 2.35±0.29Ca 20 - 409.23±0.87Ea 1.25±0.14Ea 3.67±0.02Ba 11.98±0.39DEa HiO7 2.20±0.04Da 3.39±0.25Ca 0-20 20.41±1.80Ca 5.03±0.084Ba 10.29±0.41Ca 20 - 4017.49±0.34Da 2.03±0.03Db 4.26±0.02Bb 8.88±0.24Eb 2.53±0.37Cb ThM15 29.82±0.09ABa 0 - 203.19±0.04Ba 6.94±0.49Aa 14.37±0.13Ba 4.39±0.26Aa 24.39±1.49Bb 5.05±0.22ABa 20 - 402.74±0.11ABb 19.88±0.84Aa 3.26±0.08Ab AtS25 23.57±0.95Ca 0-202.61±0.03Ca 6.78±0.51Aa 14.13±0.45Ba 3.65±0.10BCa 20-40 21.30±0.09Cb 2.27±0.12Cb 6.08±0.02Aa 17.19±0.17ABa 3.02±0.07ABb StB36 0-20 28.00±0.09Ba 3.15±0.04Ba 3.97±0.07Ba 16.88±0.52Aa 3.55±0.06Ca 20 - 40 $21.29 \pm 0.14$ Cb 2.66±0.04Bb 4.82±0.81ABa 13.76±1.79CDa 2.74±0.13BCb StG56 0-20 32.25±0.18Aa 3.45±0.02Aa 4.90±0.47Ba 18.18±0.22Aa 4.06±0.30ABa 20-40 25.58±0.19Ab 2.90±0.05Ab 4.42±0.32Ba 15.69±0.09BCa 3.21±0.17ABb

Table 2: Effect of vegetation type on soil nutrient content (mean values with standard errors).

Note: (1) Ab.G3: recently abandoned grazing on grassland (3 year); Hi.O7: *Hierochloe ordorata* (7 year); Th.M15: *Thymus mongolicus* (15 year); At.S25: *Artemisia sacrorum* (25 year); St.B36: *Stipa bungeana* (36 year); St.G56: *Stipa grandis* (56 year). (2) Different capital letters indicate significant differences at P<0.05 among different vegetation types. Different lowercase letters indicate significant differences at P<0.05 between 0-20 cm depth and 20-40 cm depth. (3) SOC = soil organic carbon; N = nitrogen; P = phosphorus



Note: (1) Ab.G3: recently abandoned grazing on grassland (3 year); Hi.O7: *Hierochloe ordorata* (7 year); Th.M15: *Thymus mongolicus* (15 year); At.S25: *Artemisia sacrorum* (25 year); St.B36: *Stipa bungeana* (36 year); St.G56: *Stipa grandis* (56 year). (2) Different capital letters indicate significant differences at P<0.05 among different vegetation types. Different lowercase letters indicate significant differences at P<0.05 between 0-20 cm depth and 20-40 cm depth. (3) Cmic = soil microbial biomass carbon; Nmic = soil microbial biomass nitrogen; Pmic = soil microbial biomass phosphorus.



Fig. 2: Soil enzymes activities.

Note: (1) Ab.G3: recently abandoned grazing on grassland (3 year); Hi.O7: *Hierochloe ordorata* (7 year); Th.M15: *Thymus mongolicus* (15 year); At.S25: *Artemisia sacrorum* (25 year); St.B36: *Stipa bungeana* (36 year); St.G56: *Stipa grandis* (56 year). (2) Different capital letters indicate significant differences at P<0.05 among different vegetation types. Different lowercase letters indicate significant differences at P<0.05 between 0-20 cm depth and 20-40 cm depth.

Parameters	Cmic	Nmic	Pmic	Invertase	Urease	Alkaline phosphatas	SOC se	TN	NO <sub>3</sub> -N	NH <sub>4</sub> -N	Available-P
Cmic Nmic Pmic Invertase Urease Alkaline phospha SOC NO <sub>3</sub> -N	1.00 tase	-0.14 1.00	0.87** -0.35 1.00	0.93** -0.20 0.84** 1.00	-0.41 0.47 -0.50 -0.20 1.00	0.87** -0.22 0.84** 0.94** -0.15 1.00	0.92** -0.15 0.93** 0.89** -0.42 0.82** 1.00	0.90** -0.15 0.94** 0.85** -0.43 0.81** 0.99**	$\begin{array}{c} 0.48 \\ -0.59^* \\ 0.67^* \\ 0.47 \\ 0.53 \\ 0.56 \\ 0.52 \\ 1.00 \end{array}$	0.56 -0.41 0.54 0.50 -0.44 0.34 0.47 0.17	0.96** -0.21 0.83** 0.94** -0.36 0.81** 0.90** 0.39 0.64*
Available-P										1.00	1.00

Table 3: Correlation matrix for soil nutrient levels and soil microbial parameters.

Note: (1) Cmic = soil microbial biomass carbon; Nmic = soil microbial biomass nitrogen; Pmic = soil microbial biomass phosphorus; SOC = soil organic carbon; TN = total nitrogen; P = phosphorus. (2) \*means significant difference (P<0.05); \*\*means extremely significant difference (P<0.01).

in soil chemical properties among the six vegetation types (Table 2). Both, at the 0-20 cm depth and at 20-40 cm, apart from Th.M15, the concentration of SOC and total N increased with restoration time. The concentration of  $NH_4$ -N under At.S25 and Th.M15 was the highest, followed by soil under St.G56, St.B36, and Hi.O7, and was the lowest under Ab.G3-dominanted vegetation type. The concentration of  $NO_3$ -N was higher under lowest Hi.O7. Both, at the 0-20 cm depth and the 20-40 cm depth, the concentration of available P was 2.35-4.39 mg/kg, and decreased following this order: Th.M15 > St.G56 > At.S25 > St.B36 > Hi.O7 > Ab.G3.

Distribution of soil microbial biomass C, N, P: Soil microbial biomass C (Cmic), N (Nmic) and P (Pmic) varied with restoration time (Fig. 1). Cmic varied between 294 and 1228 mg/kg during vegetation restoration. Apart from Th.M15, Cmic increased with restoration time. The results showed that soil microbial biomass N (Nmic) was 13-46 mg/kg under different vegetation types. Nmic under Hi.O7dominated vegetation type was obviously higher than under other vegetation types, while under Th.M15-dominated vegetation type, which was the lowest, showed significant differences compared with other vegetation types (P < 0.05, n=9). Pmic showed a similar trend at the 0-20 cm depth and at the 20-40 cm depth. Under Th.M15-dominated vegetation type, a significant increases in Pmic occurred (P<0.05, n=3) and was >15 mg/kg in the following succession. Pmic under Hi.O7 was 2.5 times that Ab.G3 (calculated from data in Fig. 1).

Variations in soil enzyme activities: The activities of soil invertase, urease and alkaline phosphatase were significantly higher at the 0-20 cm depth than at the 20-40 cm depth (Fig. 2). With the exception of Th.M15, soil invertase activity showed an increasing trend with restoration time. Soil under Ab.G3 had the lowest invertase activity. There were significant differences for soil invertase activity under Ab.G3

and other vegetation types on the 0-20 cm depth. Soil urease activity under the different vegetation types was between 1.0 and 3.4 NH<sub>4</sub>-N mg/g. During the initial stage of natural recovery, soil urease activity was high under Ab.G3 and Hi.O7 (the average value was 3.3 and 3.6 NH<sub>4</sub>-N mg/g at the 0-20 cm depth and the 20-40 cm depth, respectively). Soil under Th.M15 had the lowest urease activity, and soil under St.G56, St.B36, and At.S25 had a level of urease activity at 2.45 NH<sub>4</sub>-N mg/g. At the 0-20 cm and 20-40 cm depth, soil alkaline phosphatase activity varied from 4.7 to 12.2 phenol mg/100g during revegetation succession. Soil under St.G56, St.B36, At.S25 and Th.M15 (restoration time > 15 yr) had significantly higher alkaline phosphatase activities than under Hi.O7 and Ab.G3.

**Relationships between soil microbial and biochemical parameters**: The correlation matrix for soil nutrient levels and soil microbial parameters is given in Table 3. During natural vegetation, SOC and TN were positively correlated with microbial properties related to C and P (Cmic, Pmic, invertase activity and alkaline phosphatase activity). There was no significant correlation between urease activity and other parameters. A significant correlation clearly existed between Nmic and NO<sub>3</sub>-N, whereas no significant correlation was found between NH<sub>4</sub>-N and other parameters.

### DISCUSSION

The effect of vegetation types on soil chemical properties: The accumulation of nutrients and organic matter in soils results from complex interactions between biotic processes moderated by plants and soil biota and abiotic processes driven by environmental processes (Hooper et al. 2000). The 56 years of revegetation not only concentrated more C in the soil but also had a positive influence on the accumulation of N. Apart from Th.M15, soil carbon concentration increased with vegetation recovery time. This finding is exactly consistent with Jia et al. (2012) who studied the dynamics of soil carbon in terrestrial systems and reported that soil carbon increased with vegetational restoration age. Carbon fixation via photosynthesis and the subsequent transfer of C to the soil via leaf litter and root turnover contribute to soil C accumulation (Leifeld & Kögel-Knabner 2005). We consider this to be a consequence of the increasing above ground biomass with revegetation years.

In addition, subshrub which had a more extensive root system, occurred in the mid and later periods. More aboveground biomass, more litter, more belowground biomass, more root exudates from the 11th year to the 15th year (under Th.M15), the vegetation had entered a period of normal special succession. In this period, faster natural regeneration speed of grassland communities resulted in the thickest litter and most accumulation of litter (Cheng et al. 2006). Moreover, litter from softer stem and leaf of Th.M15 was more easily decomposed, which could supply more source of soil organic carbon, and promoted carbon cycle. For the above reasons, soil C concentration under Th.M15 was relatively higher during vegetation succession.

The effect of vegetation types on soil biochemical properties: A number of studies have focused on the potential of microbial biomass and enzymatic activity to serve as indices of soil productivity or as indicators of microbial activity in different systems (Dick et al. 1996, Acosta-Martínez et al. 2007, Bastida et al. 2008). In this study, we used Cmic, Nmic and Pmic and selected enzyme activities as indicators of soil biochemical properties. The current study showed that, except from Th.M15, Cmic and Pmic increased with time during vegetation succession. This changing trend suggested that revegetation would result in more microbial biomass and a higher utilization of C and P substrate resources by the dominant species. These results are in agreement with Huang et al. (2009) who reported that microbial biomass increased significantly as the vegetation rehabilitation time increased. This would be related to a higher belowground biomass. As vegetation succession progressed, more species emerged in the ecosystem in the mid to late stages, and more root exudates from the plant itself and from other surrounding plants were released, which may have enhanced the microbial biomass (Zhang et al. 2012). The increased nutrition resulted in more soil microbes; consequently, the growth of soil microbes could fix a considerable amount of C and P in microbial organisms. As the succession progressed, more SOC and available phosphorus showed more C accumulation and P availability. Soil enzymes participated in the material cycle of the soil biochemical process (Lü et al. 2005), and enzyme activity reflected the cyclic strength of the soil elements. Higher invertase and alkaline phosphatase activity indicated



Fig. 3: The ratio of Cmic to Nmic from different vegetation types.

more active C and P cycling in the later stage of vegetation succession.

Nitrogen (N) is an important factor limiting the growth of trees in many forest ecosystems (Vitousek 2004). The results showed that the range increase in total N, NO<sub>3</sub>-N and NH<sub>4</sub>-N was 260%, 120% and 130%, respectively after 56 years' natural succession. After revegetation, as Th.M15 was the dominant species, Nmic decreased. It is worth noting that although Nmic and urease activity was not significantly relevant, the two parameters appeared to have a similar change trend. This is contrary to what was expected as many studies have suggested that revegetation resulted in more microbial biomass, N and higher urease activity (An et al. 2005, Huang et al. 2007, 2009).

It was interesting to find that the soil under Th.M15 was unusual. When Th.M15 was the dominant species, a maximum was observed in the concentration of Cmic, and less Nmic was found. Moreover, a sharp increase in Pmic was found, and high concentration of Pmic was retained during later vegetation succession. This is probably related to the change in soil microbial community composition. The ratio of Cmic to Nmic could reflect the soil microbial community composition. The ratio of Cmic to Nmic during natural succession is shown in Fig. 3. Cmic:Nmic was <20 under Ab.G3 and Hi.O7, and the highest under Th.M15. The increasing of Cmic/Nmic showed that fungal growth was increasing and that bacterial growth was restrained (Li 2011). The increasing fungal abundance reflected more stable soil ecological environment (De Vries et al. 2006). However, the microbial processes involved are rather complicated, especially in the dynamic succession, and the simple explanation mentioned above can only provide clues. Additional effort should be made to identify the composition of the microbial community structures during vegetation succession.

# CONCLUSIONS

Our research demonstrated that vegetation recovery could improve soil chemical and microbial properties on the Loess Plateau of China. During vegetation succession, except from the Th.M15 community, the soil organic carbon, phosphorus and biochemical properties related to carbon and phosphorus increased with restoration time. These trends were likely due to the greater plant residues and root accumulation. Nmic and urease activity was the highest in the early succession period and lowest in the mid succession period (under Th.M15 and At.S25). Our study also indirectly showed the variation of soil microbial communities during vegetation restoration. In conclusion, vegetation restoration, which resulted in more abundant and stable soil ecological environment, is a beneficial measure for the recovery of degraded soils on the Loess plateau of China.

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