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Soil Enzyme Activity Changes in Different-Aged Spruce Forests of the Eastern Qinghai-Tibetan Plateau*1

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ABSTRACT

Activities of selected soil enzymes (invertase, acid phosphatase, proteinase, catalase, peroxidase and polyphenoloxidase) were determined under different spruce forests with restoration histories of 5, 13, 18, 23, 27 years and an old growth forest over 400 years old in the eastern Qinghai-Tibetan Plateau, China, and their possible use as indicators of ecosystems health were analyzed. Plots 10 × 10 m with 4 replications were established to investigate three hypotheses: soil enzyme activities a) would increase with the restoration process; b) would be greater in surface soils than at lower depths; and c) would be correlated to selected physicochemical properties. Results showed that as the forests developed after restoration, invertase and peroxidase activities usually increased up to the 23 year point. Also soil enzyme activities were associated with surface soils and decreased with depths, suggesting that in earlier restoration stages surface addition of organic fertilizer to soils might be more effective than additions at depth. In the 0–20 cm soil, there were significant correlations ($P < 0.01$ or < 0.05) between some soil enzyme activities and some selected chemical properties. Therefore, temporal changes in enzyme activities should be included as an indicator when evaluating sustainable forest management practices.

Key Words: acid phosphatase, catalase, invertase, peroxidase, spruce forest (*Picea* spp.)

INTRODUCTION

Soil enzymes play an essential role in catalyzing reactions necessary for organic matter decomposition and nutrient cycling in ecosystems (Taylor *et al.*, 1989; Johansson *et al.*, 2000). Agricultural management practices (*e.g.* crop rotation, mulching, burning, tillage and application of fertilizers and pesticides) have diverse effects on the various soil enzyme activities (Bandick and Dick, 1999; Aon and Colaneri, 2001; Ajwa *et al.*, 1999; Xu *et al.*, 2002). So, enzymatic activities are candidate "sensors" of soil stress for management practices that may timely forewarn soil degradation (Bergstrom *et al.*, 1998; Margesin *et al.*, 2000).

The Qinghai-Tibetan Plateau is one of the special geographical territories in western China. The cryosphere of the plateau (*i.e.* glaciers, snow deposits and frozen ground) responds noticeably to climate change and is closely correlated with global environmental changes. However, in the last few decades overexploitation and irrational utilization of water, land and biological as well as mineral resources have led to quality decline in certain resources and deterioration of the environment. Degradation of grasslands, reduction of forest coverage, intensification of land desertification, as well as the obvious environmental pollution in some major urban and rural areas all attest to this. Since the beginning of the 1990s a great deal of special attention has been paid to ecological restoration in this area.

The subalpine coniferous forests in China are mostly concentrated in high altitude areas with a geographical range of N 25°–34° and E 95°–111° (Yang and Li, 1992). This forest type is one of the most important indigenous forests for timber products in China. It also plays a key role in the composition of the ecological barrier at the eastern fringe of the Qinghai-Tibetan Plateau and on the

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upper reaches of the Yangtze River. To date little is known about changes in soil enzyme activities on the Qinghai-Tibetan Plateau for spruce forests with different restoration ages. A better understanding of changes in soil enzyme activities in the restoration process and pristine ecosystem would allow greater understanding of ecosystem functions and the effects of disturbances on forest ecosystem. In addition, they could aid in developing sustainable forest management practices.

The objective of this study was to determine the changes in soil enzyme activities at different restoration stages of a spruce forest in the eastern Qinghai-Tibetan Plateau and their possible use as an indicator of ecosystems health. There were three hypotheses: soil enzyme activities a) would increase with the restoration process; b) would be greater in surface soils than at lower depths; and c) would be correlated to the various soil enzymes and selected physicochemical properties. In order to test these hypotheses, the soil chemical properties, activities of invertase, acid phosphatase, proteinase, catalase, peroxidase and polyphenoloxidase and the vertical distribution of soil enzymes of spruce forests with different restoration ages in the eastern Qinghai-Tibetan Plateau were determined.

MATERIAL AND METHODS

Site and treatments

The study area was located at Jinchuan County of Western Sichuan, China (N 32°19′–32°21′, E 100°42′–100°44′). The mean annual temperature was 8–13 °C and the mean annual precipitation was 750–889 mm with most of rainfall generally occurring from May to October. The mean annual evaporation was 1 200–1 544 mm, but the mean annual solar radiation was as high as 51.9×10^8 J m^{-2} (Bao *et al.*, 2002). Originally spruce forests (*Picea* spp.) dominated the study area, but in some locations they were destroyed for commercial purposes. Thus, local governments began reforestation with spruce seedlings in the 1970s.

This experiment was conducted on a brown subalpine coniferous soil. The 10 × 10 m experimental plots were located in spruce forests of different restoration ages and a natural, old-growth spruce forest. The experiment was designed with six treatments: a) 5-year-old, b) 13-year-old, c) 18-year-old, d) 23-year-old, e) 27-year-old, and f) an old-growth spruce forest that was more than 400 years in age (Table I), with four replications of each treatment. The canopy coverage for each of the different restoration spruce forests was 85%, and the tree density was 3 300–3 600 stems ha^{-1}.

Soil

Soil samples were collected in September of 2002 from depths of 0–20 and 20–40 cm at 10 locations in each plot (10 × 10 m). One set of samples were sieved at field-moist conditions to pass a 4-mm screen and mixed to determine oven-dry weight. Samples were then air-dried for 10 d at room temperature, sieved to pass a 2-mm screen, mixed, and sub-sampled for a soil enzyme assay of available N, available P and available K. The other set of sub-samples were ground to pass through a 0.25-mm sieve to determine organic matter content, total N, and total P. The potassium dichromate heating method (SAS, 1988a), the semi-micro Kjeldahl method (SAS, 1988b), the hydrofluoric acid-perchloric acid colorimetry method (Liu, 1996), the diffusion absorption method (ISSCAS, 1978), the classical Olsen method (ISSCAS, 1978), and the ammonium acetate flame photometry method (SAS, 1988a) were utilized to determine organic matter, total N, total P, available N, available P, and available K, respectively.

Enzyme assays

Soil enzyme activities were assayed as described by Guan (1986). All enzyme activities were determined from air-dried samples in triplicate, and moisture content was measured after drying at 105 °C for 48 h. The invertase activity was expressed as mg glucose released g^{-1} h^{-1}; the phosphatase activity as mg P_2O_5 g^{-1} h^{-1}; the proteinase activity as mg NH_2-N g^{-1} h^{-1}; the catalase activity as mL 0.025 mol

TABLE I

Experimental plots in a spruce forest of Jinchuan County, Sichuan, China

Plot No.	Elevation m	Aspect	Slope position	Slope angle °	Soil type	Cutting time	Cultivation time	Restoration age Years
J1	3760	NW25°	Middle	24	Brown soil	1997	1998	5
J2	3750	NW25°	Middle	24	Brown soil	1997	1998	5
J3	3760	NW25°	Middle	24	Brown soil	1997	1998	5
J4	3751	NW25°	Middle	24	Brown soil	1997	1998	5
J5	3562	NW20°	Middle	22	Brown soil	1988	1990	13
J6	3550	NW20°	Middle	22	Brown soil	1988	1990	13
J7	3560	NW20°	Middle	22	Brown soil	1988	1990	13
J8	3551	NW20°	Middle	22	Brown soil	1988	1990	13
J9	3600	NW34°	Middle-lower	27	Brown soil	1982	1985	18
J10	3580	NW34°	Middle-lower	27	Brown soil	1982	1985	18
J11	3600	NW34°	Middle-lower	27	Brown soil	1982	1985	18
J12	3590	NW34°	Middle-lower	27	Brown soil	1982	1985	18
J13	3500	NW20°	Lower	18	Brown soil	Unsure	1980	23
J14	3490	NW20°	Lower	18	Brown soil	Unsure	1980	23
J15	3500	NW20°	Lower	18	Brown soil	Unsure	1980	23
J16	3491	NW20°	Lower	18	Brown soil	Unsure	1980	23
J17	3605	NW	Lower	8	Brown soil	Unsure	1976	27
J18	3595	NW	Lower	8	Brown soil	Unsure	1976	27
J19	3605	NW	Lower	8	Brown soil	Unsure	1976	27
J20	3585	NW	Lower	8	Brown soil	Unsure	1976	27
J21	3880	NW	Middle	17	Brown soil		Old-growth	> 400
J22	3870	NW	Middle	17	Brown soil		Old-growth	> 400
J23	3880	NW	Middle	17	Brown soil		Old-growth	> 400
J24	3865	NW	Middle	17	Brown soil		Old-growth	> 400

L^{-1} $KMnO_4$ g^{-1} h^{-1}; and the polyphenoloxidase and peroxidase activities as mL 0.005 mol L^{-1} I_2 g^{-1} h^{-1}.

Statistical analysis

One-way ANOVA tests were performed using SPSS computer language program to access the changes in soil enzyme activities of the spruce forests with different restoration ages. Bivariate correlations (Pearson, two-tailed) were used to analyze correlation among soil enzyme activities and soil chemical properties.

RESULTS

Soil enzyme activities and depth comparisons

Changes in enzyme activities differed greatly among the treatments (Fig. 1). For invertase activities in the 0–20 cm soil significant decreases ($P < 0.01$, $n = 4$) were found in soils of 5-, 13-, and 27-year-oldspruce forests, with 79%, 41% and 56% reductions, respectively, compared to the old growth spruce forest. However, invertase activity significantly increased ($P < 0.01$, $n = 4$) by 51% in the soil of 23-year-old spruce forest.

The lowest acid phosphatase activity in the 0–20 cm soil was also found in the 5-year-old restored spruce forests ($P \leq 0.05$, $n = 4$), while the greatest appeared in the 13-year-old spruce forests ($P < 0.01$, $n = 4$). The acid phosphatase activities in 0–20 cm soils of 5-, 18-, 23- and 27-year-old spruce forests decreased by 37% ($P < 0.01$, $n = 4$), 14%, 18% ($P < 0.05$, $n = 4$) and 21% ($P < 0.05$, $n = 4$), respectively, whereas the 13-year-old spruce forest increased by 28% ($P < 0.01$, $n = 4$).

Unlike other soil enzymes activities, however, the highest value of polyphenoloxidase activity was

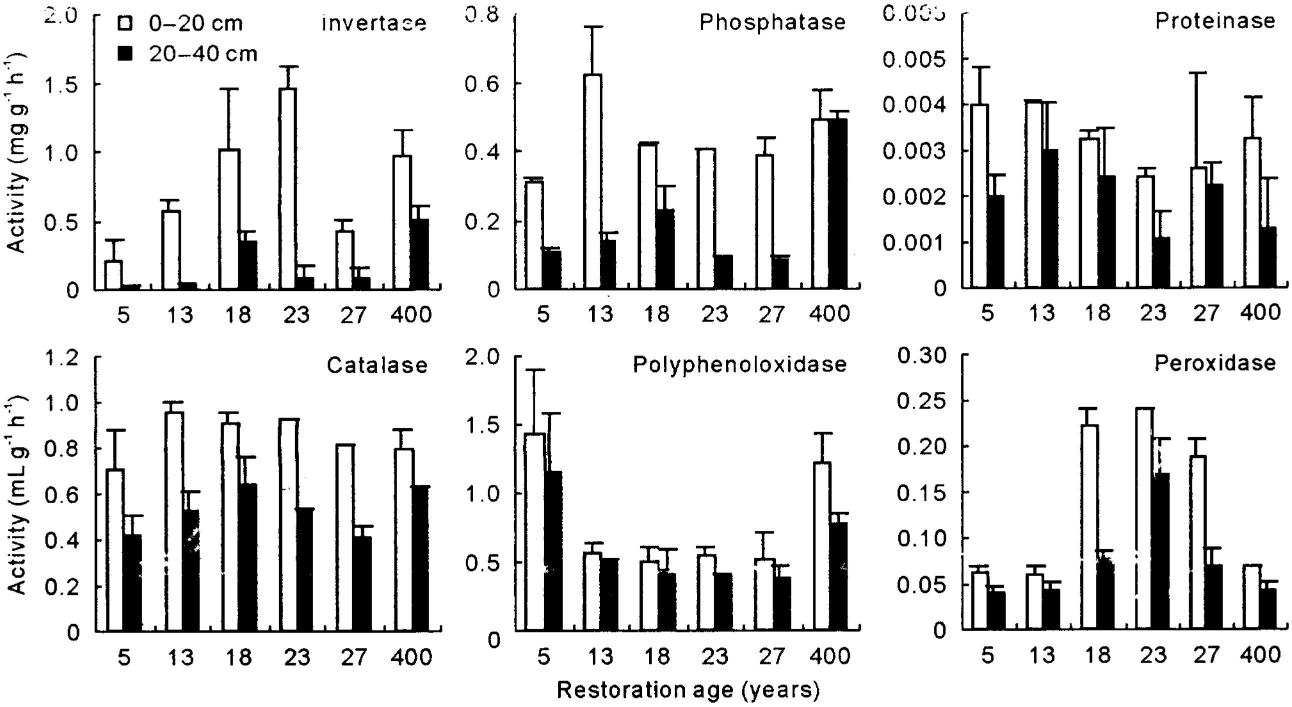

Fig. 1 Changes in activities of 6 enzymes in spruce forests of 5 different restored-aged stands and an old-growth forest.

found in the 5-year-old spruce forests ($P < 0.01$, $n = 4$) among restored spruce forests, which increased by 17% compared with the old growth spruce forest.

Compared with the old growth spruce forest, for the 20–40 cm soil, the invertase activities in spruce forests with restoration histories of 5, 13, 23, and 27 years were significantly decreased by 97%, 90%, 84% and 85% ($P < 0.01$, $n = 4$) respectively. The acid phosphatase activities of 5-, 13-, 18-, 23- and 27-year-old spruce forests decreased by 78%, 71%, 54%, 81% and 84% ($P < 0.01$, $n = 4$), respectively. The polyphenoloxidase activities had 35%, 44%, 48% and 51% ($P < 0.05$, $n = 4$) reductions in the 13-, 18-, 23- and 27-year-old spruce forests, respectively, while that increased by 48% ($P < 0.05$, $n = 4$) for the 5-year-old spruce forest. The peroxidase activities in soils of 18-, 23- and 27-yuar-old spruce forests increased by 80% ($P < 0.01$, $n = 4$), 299% ($P < 0.01$, $n = 4$) and 62% ($P < 0.05$, $n = 4$), respectively.

The invertase activities in 0–20 cm soils of spruce forests with restoration histories of 13, 18, 23, 27 years and over 400 years old-growth spruce forests were significantly higher than those in 20–40 cm ($P < 0.01$, $n = 4$). The acid phosphatase activities in 0–20 cm soils of 5-, 13, 18-, 23- and 27-year-old spruce forests were higher than those in 20–40 cm soils ($P < 0.01$, $n = 4$). The catalase activities of all treatments in 0–20 cm soil were higher than those in 20–40 cm soils ($P < 0.01$, $n = 4$). The same results were found for the peroxidase activities of 18-, 23-, and 27-year-old spruce forest ($P < 0.01$, $n = 4$) and over 400 years old-growth spruce forest ($P < 0.05$, $n = 4$). Thus, the greatest soil enzyme activities were found in the 0–20 cm soil and decreased with depths (Fig. 1)

Soil OM, N and P

Analysis of the data across all treatments showed that organic matter, total N (except for the 23-year-old forest), and total P in the 0–20 cm soil of the different restored spruce forests were significantly different ($P < 0.01$, $n = 4$) from the old growth forest. Hydrolysable N (except for the 13-year-old forest) and available P ($P < 0.01$, $n = 4$) in the 20–40 cm soil were significantly lower ($P < 0.01$, $n = 4$) in the restored spruce forests than in the old growth forests. And available P in soils of 5-, 13-, 18- and 23-year-old spruce forests increased gradually, but declined after a stand age of 23 years (Fig. 2).

Organic matter, total N (except for over 400-year-old spruce forest), hydrolysable N (except for over

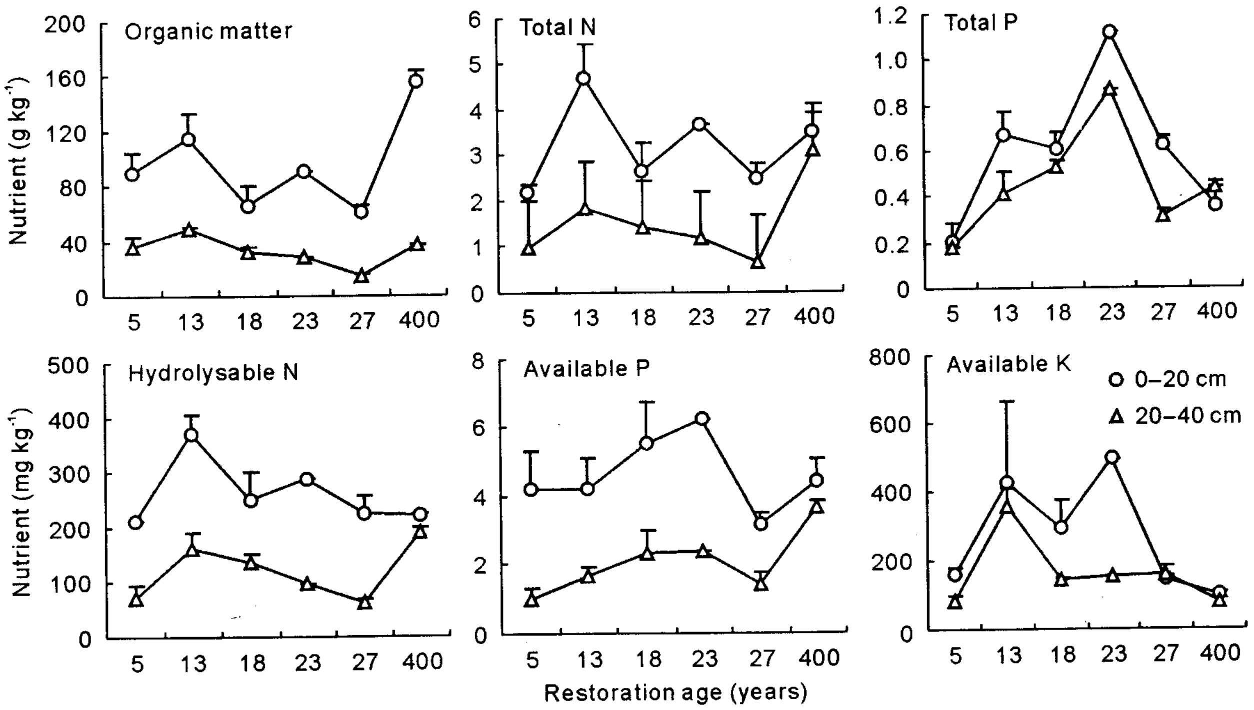

Fig. 2 Changes in soil organic matter, total N, total P, hydrolysable N, available P, and available K for 5 restored stands and an old growth forest.

400-year-old spruce forest) and available P (except for over 400-year-old spruce forest) in the 0–20 cm soils were higher ($P < 0.01$, $n = 4$) than those in the 20–40 cm soils (Fig. 2).

As shown in Table II, in the 0–20 cm soil, invertase activity was significantly ($P < 0.01$) correlated with catalase, peroxidase, total P and available P. Also acid phosphatase was found highly significantly correlated with catalase ($P < 0.01$), organic matter ($P < 0.05$), total N ($P < 0.01$) and hydrolysable N ($P < 0.01$). Proteinase and polyphenoloxidase activities were negatively correlated with peroxidase ($P < 0.01$). In addition, catalase activity was significantly correlated with total N ($P < 0.05$), total P ($P < 0.01$), hydrolysable N ($P < 0.01$), and available P ($P < 0.05$). Polyphenoloxidase was correlated with total P ($P < 0.01$) and hydrolysable N ($P < 0.05$). And peroxidase was found to have a highly significant correlation with organic matter and total P ($P < 0.01$).

TABLE II

Correlation coefficients (r) among the 6 enzyme activities and 5 chemical properties in the 0–20 cm forest soil ($n = 24$)

Variable	APHO	PROT	CATA	POLY	PERO	OM	TN	TP	HN	Available P
Invertase	0.17	−0.28	0.57**	−0.22	0.56**	0.17	0.38	0.65**	0.30	0.53**
Acid phosphatase (APHO)		0.36	0.54**	−0.27	−0.32	0.49*	0.79**	0.19	0.73**	0.03
Proteinase (PROT)			−0.17	0.08	−0.53**	0.21	0.13	−0.35	0.25	−0.17
Catalase (CATA)				−0.30	0.31	−0.10	0.50*	0.54**	0.66**	0.41*
Polyphenoloxidase (POLY)					−0.60**	0.35	−0.32	−0.74**	−0.43*	−0.14
Peroxidase (PERO)						−0.62**	−0.21	0.70**	−0.07	0.36
Organic matter (OM)							0.61**	−0.19	0.22	−0.04
Total N (TN)								0.48*	0.87**	0.16
Total P (TP)									0.52**	0.46*
Hydrolysable N (HN)										0.12

*, **Significant at 0.05 and 0.01 probability levels, respectively.

DISCUSSION

Soil enzyme activity changes and the restoration process

The decrease in soil enzyme activities of spruce forests with different restoration ages relative to an old growth spruce forest could have been due to the fact that a wide variety of microorganisms and plants were necessary to produce the soil enzymes (Guan, 1986); however, when forests were cut this destroyed not only trees but also microorganisms in the soil. Eivazi and Bayan (1996) revealed similar results in their studies where long-term forest burning always reduced activities of all studied enzymes including acid phosphatase, α-and β-glucosidase, arylsulfatase, and urease.

In ecosystems, invertase played a critical role in releasing low molecular weight sugars that were important as energy sources for microorganisms. Also proteinase was important in nutrient cycling because it released inorganic N in the N cycle; meanwhile phosphatase was believed to play a pivotal role in phosphorus cycles because of its role in releasing inorganic P in the P cycle. Thus, both proteinase and phosphatase regenerated inorganic nutrients from organic materials, which has been reported as the rate-limiting step in the cycling process (Guan, 1986). Therefore, their activity would be a good indicator of organic C, N, and P mineralization potential and biological activity of the soil.

This study showed that forest felling, which was one of main forest disturbances on the eastern Qinghai-Tibetan Plateau, has influenced soil enzyme activities, but some enzymes, such as invertase and peroxidase activities usually increased up to the 23 year point as the stands aged (Fig. 1). This suggested that forest felling influenced carbon, nitrogen, and phosphorus cycling in the forest ecosystem, but could be gradually restored with indigenous species. Studies on the changes of biological and physicochemical properties of soils during restoration processes have shown the same results (Bao *et al.*, 2002; Pang *et al.*, 2002; Xu and Xu, 2003; Zhang *et al.*, 2003). The results of this study and the referenced studies were attributed to understanding the restoration of ecological functions after the rehabilitation of vegetation cover.

Unlike other soil enzymes, the catalase activities in all restored forests were close to those of the old growth spruce forest. Catalase used a two-electron transfer mechanism to split hydrogen peroxide into molecular oxygen and water, and protected cells from damage caused by reactive oxygen species (Guwy *et al.*, 1999). It was also found that polyphenoloxidase activities were greater in the early restoration stages.

Greater soil enzyme activities in surface soils than at lower depths

This work indicated that soil enzyme activities decreased with depth (Fig. 1). This result was in agreement with that of Chen (2003) who found that phosphatase activity was the highest in the A horizon and the least in the C horizon. The soil enzyme activity distribution pattern suggested that the transfer rate of decomposed organic matter and nutrient cycling depended on soil depth. Therefore, this pattern had important structural and functional characteristics in nutrient cycling dynamics and implications for plantation nutrient management. Because of increased soil enzyme activities in surface soil horizons, organic matter transformation was faster than in deeper soil horizons. The rapid release of inorganic nutrients in the soil was important for forest restoration. This meant that in the early years of forest restoration, application of organic fertilizer to surface soils might be more effective than at depth. This conclusion was consistent with previous studies too (Aon and Colaneri, 2001; Taylor *et al.*, 2002).

Correlation between soil enzymes and physicochemical properties

Some soil enzyme activities of spruce forests in the eastern Qinghai-Tibetan Plateau were correlated with organic matter, total N and P, hydrolysable N and available P (Table II). This suggested that soil enzymes were crucial for soil fertility formation, and they could not only provide available nutrients for plant uptake, but also accumulate organic matter.

Soil quality indicators

Soil enzyme activity differed, depending on the stand ages and therefore appeared to be useful for monitoring changes in soils over time. Peroxidase and invertase were relatively sensitive to changes and had a stable trend in changes in the ecosystem. This result was consistent with that of Badiane *et al.* (2001) who found that β-glucosidase and amylase activities were significantly higher in the oldest natural fallows, and they were immediately responsive to soil disturbances. So, many soil enzymes could be used as indicators to evaluate the stability and sustainability of restored forests.

There has been a growing recognition of the need to develop sensitive indicators for soil quality that reflected the effects of management and could assist managers in promoting long-term sustainable terrestrial ecosystems. It would be difficult to establish a single biological or chemical measurement that could adequately reflect soil quality without taking into consideration the factors affecting the formation of a given soil. However, soil enzyme activity should be considered as one of these important indices. Indeed, this study indicated that enzyme activities were not only sensitive to ecosystem management practices, but also correlated with soil physicochemical properties. Therefore, temporal changes in enzyme activities should be included as one of the indicators when evaluating sustainable forest management practices.

CONCLUSIONS

Overall, enzyme activities agreed with the three hypotheses: soil enzyme activities a) generally increased with the restoration process; b) were greater in surface soils than at lower depths; and c) the various soil enzymes and selected soil physicochemical properties were significantly correlated.

It was also pointed out that early indicators of ecosystem stress could function as "sensors" whose timely perturbations might foretell ecosystem degradation. Thus, compared to classical and slowly changing soil properties, some enzymes could be used as soil quality indicators during an ecological restoration process.

Although enzymes undoubtedly could perform functions critical in soil nutrient cycling, the role these assays could play in determining "soil health" is less clear. They may be most useful for monitoring trends (positive or negative) in a soil over time. However, no apparent pattern that could be applied to all enzyme activities was discernable.

Therefore, further research is needed to confirm the role of enzyme activities in nutrient cycling for Qinghai-Tibetan Plateau ecosystems. In addition, the relevance of these surface soil changes to the long-term sustainability of this ecosystem needs further evaluation.

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