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Changes in enzyme activities of spruce (*Picea balfouriana*) forest soil as related to burning in the eastern Qinghai-Tibetan Plateau

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Abstract

The effect of forest fire on soil enzyme activity of spruce (*Picea balfouriana*) forest in the eastern Qinghai-Tibetan Plateau was assessed. Six specific enzymes were chosen for investigation: invertase, acid phosphatase, proteinase, catalase, peroxidase and polyphenoloxidase. It was found that the activities of invertase and proteinase were reduced by burning, but the activities of acid phosphatase, polyphenoloxidase and peroxidase increased. Meanwhile, burning significantly ($P < 0.05$) resulted in the decrease of concentrations of available N and K of 0–20 cm depth layer soil, and significantly ($P < 0.05$) decreased concentrations of organic matter content, total N and P, as well as available N, P and K in soil at both 20–40 and 40–60 cm depths except for available P at 20–40 cm soil depth. These results illustrated that burning could influence the enzyme activities and chemical properties of soil not only of upper but also lower soil layers. Correlation analysis indicated that invertase activities in 0–20 cm depth layer soil were significantly positively correlated with organic matter, total N and P, as well as available N and P. Furthermore, all six enzymes studied were sensitive to fire disturbance, and thus could be used as indicators of soil quality. Our study also showed that soil enzyme activities were associated with soil depth, decreasing from top to bottom in both burned and unburned spruce forests. The distribution pattern of soil enzyme activities suggested that the rate of organic matter decomposition and nutrient cycling depended on soil depth, which had important structural and functional characteristics in nutrient cycling dynamics and implications in plantation nutrient management. The finding that burning effects on enzyme activities and soil properties between different soil layers were homogenized was attributed to the 8-years' regeneration of forest after burning.

Keywords: Acid phosphatase; Catalase; Forest fire; Invertase; Peroxidase; Proteinase; Polyphenoloxidase; Spruce (*Picea balfouriana*) forest

1. Introduction

Soil enzymes play an essential role in catalyzing reactions necessary for the decomposition of organic

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matter and nutrient cycling in ecosystems (Taylor et al., 1989; Dilly and Irmler, 1998; Gunadi et al., 1998; Criquet et al., 1999; Johansson et al., 2000), involving a range of plants, microorganisms, animals and their debris (Gramss et al., 1999). Therefore, changes in enzyme could alter the availability of nutrients for plant uptake, and these changes are potentially sensitive indicators of soil quality (Ajwa et al., 1999; Albiach et al., 2000; Aon et al., 2001; Aon and Colaneri, 2001). Soil enzyme activities are greatly affected by soil physicochemical properties and management practices, and often are used as indices of microbial activity and soil fertility (Deng and Tabatai, 1996, 1997; Bandick and Dick, 1999; Kang and Freeman, 1999; Criquet et al., 2000; Alvarez and Guerrero, 2000).

Forest fire is a natural disturbance that can be extremely destructive of vegetation cover and soil, thereby interfering with ecosystem functions, e.g., the maintenance of water supplies, the protection against soil erosion and the accumulation of nutrients (Saá et al., 1993, 1998). In a healthy forest ecosystem, biological decomposition–oxidation processes released soil nutrients at a slow but steady rate, most of the released nutrients are absorbed by plants more or less immediately. By contrast, in a fire-damaged forest excessive soil heating during burning may cause soil enzymes to denature and become inactive, thereby indirectly affecting nutrient cycling. Fire-induced minerlization of the organic components of vegetation, litter and surface soil is very rapid (St. John and Rundel, 1976). Moreover, a portion of the released nutrients may be lost from ecosystem through volatilization or by fly-ash convection during burning or by subsequent leaching into groundwater and runoff. Many studies have reported significant correlations among soil enzyme activities and various soil properties (Kandeler et al., 1999a,b; Kang and Freeman, 1999). Dick et al. (1988) found significant correlation between burning of the residue and acid phosphatase activity in the top 20 cm of soils, but weak correlation with several other soil enzymes. The authors attributed these weak correlations to the fact that the burning effect on microbial populations was confined mostly to the top 2.5 cm of the soil profile.

The Qinghai-Tibetan Plateau is one of the special geographical territories in the world with the specific vegetation zonations. In the easternmost region, i.e., Hengduan Mountains, closed forests usually dominated the high mountains or gentle plateau surfaces, where one of the biggest forest regions in China can be found. Subalpine coniferous forests, dominated by spruce and fir were thought of as primary vegetation caused by vertical changes of climatic conditions (Wu, 2000). Besides the climatic determinants, however, some disturbances such as fire was also considered as an active agent in shaping forest distribution pattern (Wu and Liu, 1998; Winkler, 2000) and which had done enormous damage to forests in the eastern Qinghai-Tibetan Plateau. It was estimated that the area inflicted comprised at least 755,000 ha (6.3% of the whole forest area) with an annual average of 1240 fires in west Sichuan (Yang, 1987). Fires in the subalpine spruce–fir forest belt are generally less frequent, but when occurring they tend to be very intense and destroy large areas. Due to the steep terrain in this region it is very difficult to fight fires (Yang and Li, 1992). Therefore, fire including natural and human ignition, was thought to be one of the most important factors affecting both the pattern and process of forest ecosystems in these remote and harsh high mountains (Wu and Liu, 1998; Winkler, 1998, 2000). So far little is known about the effects of burning on these ecosystems, especially on soil enzyme activities of forest soils, although there is a general awareness of the importance of forest fires and soil enzymes.

A better understanding of effect of forest fire on soil enzyme activities would undoubtedly allow greater understanding of such disturbance on soil community functions of a subalpine coniferous forest ecosystem. Because some soil enzymes respond to sudden disturbances of the soil system, they can aid in developing sustainable ecosystem management practices (Aon et al., 2001; Aon and Colaneri, 2001). The hypothesis proposed in this article therefore is that soil enzyme activities not only could be affected by forest fire but also are related to soil chemical properties of forests, which finally could be used as an indicator to assess the regeneration and/or restoration of forest function.

Criteria for choosing enzyme assays were based on previous experience with their sensitivities to field management, importance in nutrient cycling and organic matter decomposition, as well as simplicity of assaying (i.e., potential to be adopted by commercial labs for routine soil testing) (Guan, 1986). Invertase was chosen for its critical role in releasing low molecular weight sugars that are important as energy sources for microorganisms. Proteinase was included because of its role in releasing inorganic N in the N cycle. Soil phosphatases were intimately involved in the biochemical mineralization of P (McGill and Cole, 1981) because they release inorganic P in the P cycle.

2. Materials and methods

2.1. *Site and treatments*

The study areas are located at Zamtang County of western Sichuan, China (32°21′N, 100°44′E). The mean annual temperature was 5 °C. The mean annual precipitation was 650 mm while the mean annual evaporation was 1200 mm. The mean annual solar radiation amount was as high as 52×10^8 J m^{-2}. Generally, most of rainfall occurs in spring and summer (May–October). The study area was originally dominated by spruce (*Picea balfouriana*) forest, but in some places this was destroyed by fire in 1994. The old-growth spruce forest canopy was uniform with the coverage between 60 and 80%, and its height 30 m or more. Composition of the shrub layer was variable among the different micro-habitats. Normally some broad-leaved evergreen species, such as *Rhododendron yunnanensis*, occur consistently, forming an understorey which may also include *Spiraea alpina*, *Ribes meyeri*, *Sorbus koehneana* and *Prunus pilosiuscula*.

The experiment was conducted at an elevation of 3.450 m a.s.l., about 300 m below the timberline on the burned and unburned patches with typical subalpine brown soil. The experimental plots randomly distributed were 10 m × 10 m with four replications in both a burned zone and an old spruce zone. The controls (unburned zone) closed to the burned zone were old-growth spruce forest with trees more than 400 years old. Their slope angle were 28° with aspect of SW62°. AT the burned sites, the vegetation coverage was less than 10%, and only exist some shrub and herbage although 8 years have passed since the burn.

2.2. *Soil*

Soil samples were collected and pooled in September 2002 with a hand auger from a depth of 0–20, 20–40 and 40–60 cm at 10 locations of each plot. 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 days at room temperature, sieved to pass a 2-mm screen, mixed and sub-sampled for soil enzyme assay and determination of available N, P and K. The other set of sub-samples were ground to pass through a 0.25-mm sieve for the determination of the organic matter content, total N and 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, P and K, respectively.

2.3. *Enzyme assays*

Soil enzyme activities were assayed in triplicate air-dried pooled samples of each plot (four plots in both burned and unburned zone, respectively), as described by Guan (1986). Moisture content was determined from loss in weight after drying at 105 °C for 48 h. For measuring invertase activity, 5 g of air-dried soil was incubated for 24 h at 37 °C with 15 mL 8% sucrose, 5 mL phosphate buffer at pH 5.5 and 0.1 mL toluene. The glucose released by invertase reacted with 3-5-dinitrosalicylic acid and 3-amino-nitrosalicylic acid, then was measured at 508 nm (UV 330, Unicam UV–vis). Results were expressed as mg glucose released g^{-1} h^{-1}. For measurement of acid phosphatase activity, 5 g of air-dried soil was incubated for 2 h at 37 °C with 20 mL disodium benzene phosphate dissolved in acetate buffer at pH 5 and 0.1 mL toluene. The phenol released by phosphatase reacted with 0.25 mL ammonium chloride-ammonium hydroxide buffer at pH 9.8, 0.5 mL 4-aminophenazon and 0.5 mL potassium ferricyanide, then measured at 510 nm (UV 330, Unicam UV–vis). Results were expressed as mg P_2O_5 g^{-1} h^{-1}. For measuring proteinase activity 5 g of air dried soil was incubated for 24 h at 30 °C with 20 mL 1% casein and 1 mL toluene. Then 2 mL 0.05 mol L^{-1} sulfuric acid and 12 mL 20% sodium sulfate were added. The aminoacetic acid released by proteinase reacted with 1 mL 2% ninhydrin then measured at 500 nm (UV 330, Unicam UV–vis). Results were expressed as mg NH_2–N g^{-1} h^{-1}.

For measuring catalase activity, 2 g air-dried soil with 40 mL distilled water and 5 mL, 0.3% H_2O_2, was shaken for 20 min (shaking velocity was 150 n min^{-1}), titrated filtrate with 0.025 mol L^{-1} $KMnO_4$. Results were expressed as mL 0.025 mol L^{-1} $KMnO_4$ g^{-1} h^{-1}. For measuring polyphenoloxidase activity 5 g of air-dried soil was incubated for 2 min in water bath at 30 °C, with 10 mL distilled water, 6 mL 0.1% ascorbic acid and 10 mL 0.02 mol L^{-1} catechol. Then added 3 mL 10% phosphoric acid and tittered filtrate with 0.005 mol L^{-1} iodine. Results were expressed as mL 0.005 mol L^{-1} I_2 g^{-1} h^{-1}. For the measurement of peroxidase, the same method was adopted.

2.4. Statistical analysis

Two independent samples nonparametric tests were performed using SPSS computer language program to assess the effects of burning on the activities of soil enzymes and chemical properties. One-way ANOVA tests were performed to assess the differences of soil enzyme activities among the different soil depths. Bivariate correlations (Pearson, two-tailed) were used to analyze correlation among soil enzyme activities and soil chemical properties.

3. Results

3.1. Enzyme activities

Changes in enzyme activities differed greatly between burned and unburned treatments (Fig. 1). Relative to the unburned mature spruce forests, the activities of invertase decreased 27 and 48% ($P < 0.05$, $n = 4$) and 54% in the 0–20, 20–40 and 40–60 cm soil depth layers, respectively. Unlike invertase activity, acid phosphatase activity in burned treatment increased 16 and 25% in 0–20 and 20–40 cm depth layer, respectively, but reduced 54% ($P < 0.05$, $n = 4$) in 40–60 cm depth layer relative to the unburned treatment. Similar to invertase, proteinase activity was lower in the burned than in the unburned treatment, decreasing 16, 38 and 39% in the soil at 0–20, 20–40 and 40–60 cm depths, respectively. Compared to unbumed forests, catalase activity also decreased 22% ($P < 0.05$, $n = 4$) and 15% in burned treatment in the depth of 20–40 and 40–60 cm layer soils, respectively, except for 0–20 cm (up to 6%). Changes of peroxidase activity in the burned treatment were similar to those of polyphenoloxidase. The former increased 155, 80 and 116%, respectively in the soil from 0–20, 20–40 and 40–60 cm depths compared to the unburned control, but the latter increased 107% ($P < 0.05$, $n = 4$), 41% and decreased 3%.

In both the burned or unburned forests, the vertical distribution trend was similar of six chosen enzymes. The greatest soil enzyme activity occurred in the top 20 cm soil, while the lowest activity was in the soil at 40–60 cm depth.

Two independent samples nonparatnetric tests showed that burning significantly ($P < 0.05$) decreased invertase activities and catalase activities in the soil at 20–40 cm soil layer, but increased the activities of polyphenoloxidase in the top 20 cm and 40–60 cm soil layer and increased catalase activities in the top 20 cm.

3.2. Soil chemical properties

Changes in soil chemical properties also differed greatly between burned and unburned treatments (Fig. 2). Compared to the mature spruce forests (unburned controls), organic matter content, concentrations of total N and P, and available N, P and K were all lower in the burned treatment except for available P at 20–40 cm soil depth, in soil at 0–20 cm depth, organic matter content, and concentrations of total N and P, and available N, P and K in burned treatments decreased 17, 11, 11, 16% ($P < 0.05$, $n = 4$), 52% and 61% ($P < 0.05$, $n = 4$), respectively relative to unburned treatments. In soil at 20–40 cm depth, organic matter content, and concentrations of total N and P, available N and K in burned treatments significantly decreased 52% ($P < 0.05$, $n = 4$), 52% ($P < 0.05$, $n = 4$), 48% ($P < 0.05$, $n = 4$), 41% ($P < 0.05$, $n = 4$) and 46% ($P < 0.05$, $n = 4$), respectively except for available P (up to 30%). Similar to chemical properties of the soil at 0–20 cm depth, organic matter content, concentrations of total N and P, available N, P and K of 40–60 cm layer soils in burned treatments decreased, 66% ($P < 0.05$, $n = 4$),

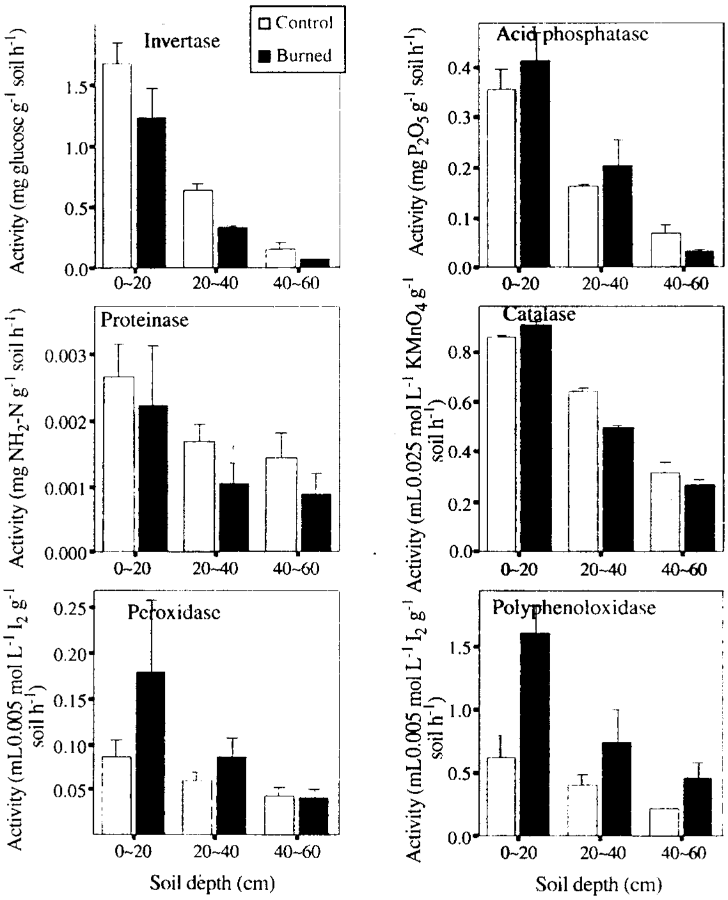

Fig. 1. Changes in soil enzyme activity at three depths after burning. Error bars represented standard deviation, multiplier was 1.0.

65% ($P < 0.05$, $n = 4$), 60% ($P < 0.05$, $n = 4$), 64% ($P < 0.05$, $n = 4$), 64% ($P < 0.05$, $n = 4$) and 51%<$P < 0.05$, $n = 4$), respectively, in comparison to unburned treatments.

Two independent samples nonparametric tests showed that burning significantly ($P < 0.05$) reduced concentrations of available N and K in soil at 0–20 cm depth, and significantly ($P < 0.05$) decreased concentrations of organic matter content, total N and P, as well as available N, P and K in soil at both 20–40 and 40–60 cm depths except for available P at 20–40 cm soil depth.

4. Discussion

Compared with the unburned treatments, the burning of coniferous forests reduced the activities of invertase and proteinase in the soil, but increased the activities of acid posphatase, polyphenoloxidase and peroxidse. The relatively low activities of invertase, proteinase and catalase in the burned treatment could have resulted from the fact that these enzymes were produced by a wide group of microorganisms and plants, but the forest floor vegetation was destroyed by burning and burned ecosystems' vegetation had hardly recovered although 8 yesrs have passed since the burn. This result was consistent with those of Eivazi and Bayan (1996) who studied the effects of long-term prescribed burning on enzyme activities in a forest ecosystem and found that β-glucosidase activity was greatly reduced by burning, mainly due to the destruction of forest floor vegetation. The lack of statistical significance between treatments could have been attributed to the large variability between replicate plots. The significant

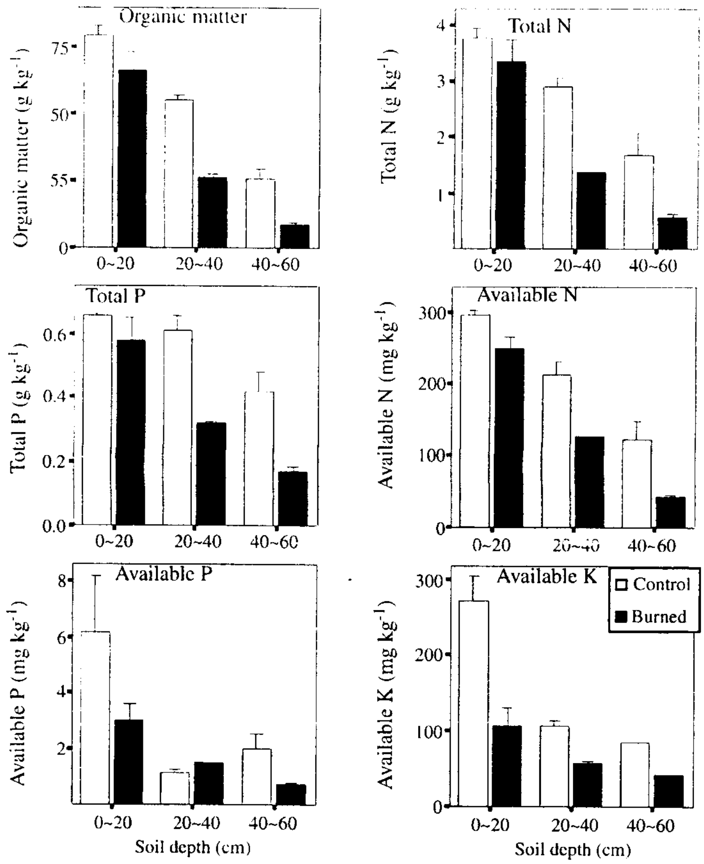

Fig. 2. Changes in chemical properties of soil at three depths after burning. Error bars represented standard deviation, multiplier was 1.0.

effect of burning on activities of invertase and proteinase may suggest that these enzymes can be used as an indicator of soil disturbance. In our study, the higher activities of acid phosphatase, polyphenoloxidase and peroxidase in the burned coniferous forest compared to the unburned controls indicated that burning, although it might result in lower inorganic N, P and K concentrations, provided essential nutrients or conditions suitable for the production of these enzymes. The increased activity of catalase in the top 20 cm soil of the burned forest may be due to the changing forms of organic compounds caused by burning, which catalyzes the oxidation and hydrolysis of organic matter. Boerner et al. (2000) assessed the effect of prescribed fire on soil enzyme activities in oak-hickory (*Quercus-Carya*) forests in southern Ohio, USA, and also found that acid phosphatase activity was generally reduced by burning and decreased with increasing long-term soil water potential. By contrast, β-glucosidase differed little, while chitinase activity increased after fire, in proportion to fire intensity, and phenol oxidase activity was highly variable and did not correlate well with fire.

There was no apparent pattern that could be applied to all enzyme activities. Highly significant correlations occurred between inveretase and organic matter, total N and P, plus available N and P (Tables 1–3). Correlation coefficients between invertase and organic matter, total N and P, as well as available N, P and K showed that chemical properties were positively correlated with invertase activity, possibly because of the lower soil nutrient levels caused burning and therefore, the lower production of invertase enzyme. Polyphenoloxidase activity was also responsible for nutrient release from nitrogenous compounds and organic matter (Tables 1 and 2), but contrary to invertase activity, that of polyphenoloxidase was

Table 1
Correlation coefficients (r-value) between enzyme activities and chemical properties of soil at 0–20 cm depth

Variable	B	C	D	E	F	OM	TN	TP	N	P	K
A	0.62	0.14	0.03	−0.75*	0.66	0.98**	0.96**	0.78*	0.93**	0.83*	0.46
B		−0.13	0.75*	−0.04	−0.41	0.57	0.74*	0.49	0.33	0.48	−0.28
C			−0.02	−0.26	0.43	0.12	0.09	0.07	0.15	0.13	0.12
D				0.43	0.02	0.01	0.24	0.11	−0.27	−0.08	−0.64
E					0.59	−0.77*	−0.64	−0.65	−0.87*	−0.58	−0.77*
F						−0.73*	−0.71*	−0.72*	−0.72*	−0.47	−0.50
OM							0.97**	0.84**	0.96**	0.80*	0.54
TN								0.84**	0.87**	0.76*	0.37
TP									0.82*	0.34	0.67
N										0.74*	0.74*
P											0.16

$n = 8$. A: Invertase; B: phosphatase; C: proteinase; D: catalase; E: polyphenoloxidase; F: peroxidase; OM: organic matter; TN: total N; TP: total P; N: available N; P: available P; K: available K.

* Indicates significance of treatment at $P < 0.05$ level.

** Indicates significance of treatment at $P < 0.01$ level.

always greater in soil of the burned forest than in the unburned controls (Fig. 1). This may have resulted from burning and 8 years regeneration after burning increasing some early successional plants and microorganisms, which further led to the high production of polyphenoloxidase. Such results suggest that the production of these enzymes was greatly affected by soil chemical properties, although some enzymes were produced by plants, mocroorganisms and animals (Gramss et al., 1999).

The decrease in chemical concentrations in soil from the burned forest could be attributed to fire-induced minerlization of the organic components on vegetated ground. Moreover, a portion of the released nutrients may be lost from the ecosystem through volatilization, or by fly-ash convection during burning, or by subsequent leaching into groundwater and runoff. After burning, organic matter would decline in both quantity and quality because there would be less new material entering the decomposition chain, and more of what was left would be resistant to decomposition, this would inevitably lead to a different distribution of microorganisms and differences in the abundance of both total and inorganic nutrients. These results are consistent with studies of tallgrass prairie soils by Ajwa et al. (1999) who found that inorganic N concentrations were significantly lower in soils of burned sites than with unburned treatments, and these concentrations were significantly different among the sampling dates. Studies on the effects of burning on N and C budgets in the Konza prairie showed an increase (14%) in inorganic N after the first-year burning and a decrease (8%) after repeated burning (Ojima et al., 1990). These studies indicated the increased mineralization of C, N and P due to higher soil temperatures after the removal of plant detritus following burning.

Some soil enzyme activities were highly correlated with other soil enzymes and soil chemical properties studied (Tables 1–3). The positive significant correlations among some soil enzymes and chemical properties suggested that burning had similar effects on these variables. Eivazi and Bayan (1996) studied the effects of long-term prescribed burning on enzyme activities in a broadleaved deciduous forest ecosystem and found that long-term burning of the forest floor debris always reduced the activities of all enzymes studied and inorganic N concentrations. They also found that burning treatments were more apparent than seasonal variability.

It was found that burning of spruce forests had diverse effects on soil enzyme activities, and some enzymes are more sensitive indicators of burning in pristine ecosystems than chemical properties. Burning appears to alter the rate of organic matter turnover and therefore, affect microbial biomass and the production of enzymes. Because many soil enzymes are immediately responsive to soil disturbance (burning), catalyzing the oxidation and hydrolysis of organic matter, and tend to stabilize when a particular management system is established, they can be used as indices of environmental stability and soil quality for sustainable management. Therefore, temporal changes in enzyme

Table 2
Correlation coefficients (*r*-value) between enzyme activities and chemical properties of soil at 20–40 cm depth

Variable	B	C	D	E	F	OM	TN	TP	N	P	K
A	−0.40	0.4	0.78*	−0.37	−0.34	0.79	0.78*	0.67	0.59	−0.95**	0.922**
B		−0.20	−0.4	0.30	−0.49	−0.27	−0.33	−0.28	−0.30	0.43	−0.11
C			0.66	−0.65	0.18	0.58	0.59	0.61	0.61	−0.25	0.39
D				−0.52	−0.26	0.98**	0.99*	0.98*	0.96**	−0.62	0.79*
E					−0.49	−0.43	−0.46	−0.44	−0.44	0.36	−0.28
F						−0.41	−0.35	−0.33	−0.27	0.23	−0.57
OM							0.99**	0.98**	0.95**	−0.63	0.85*
TN								0.99**	0.96**	−0.62	0.82*
TP									0.99**	−0.47	0.72**
N										−.39	0.64
P											−0.87*

n = 8. A: Iovertase; B: phosphatase; C: proteinase; D: catalase; E: polyphenoloxidase; F: peroxidase; OM: organic matter; TN: total N; TP: total P; N: available N; P: available P; K: available K.
* Indicates significance of treatment at $P < 0.05$ level.
** Indicates significance of treatment at $P < 0.01$ level.

activities should be considered when disturbance and the sustainability of forest management practices were evaluated.

Our study also indicated that soil enzyme activities were greatest in association with surface soils and decreased with depth in both burned and unburned spruce forests. The result was consistent with those of Chen (2003) who found that phosphatase activity was highest in soil of the A horizon and least in C horizon. The distribution pattern of soil enzyme activities suggested that the rate of organic matter decomposition and nutrient cycling depended on its depth in soil. Therefore, this pattern had important structural and functional characteristics in nutrient cycling dynamics and implications in nutrient management of plantations. Because of increased soil enzyme activities in surface soil horizons, the transformation of organic matter was faster than that in deeper horizons. The rapid release of inorganic nutrients in soil was important for forest regeneration or restoration after burning demonstrated in our study site where forests were burned 8 years ago. Factors contributing to the higher activities in the topsoil may include effects on the rhizosphere which is known to be a zone of increased microbial and enzyme activity (Chen, 2003).

It was observed, however, that burning influenced enzyme activities and chemical properties of soil not only of the upper soil horizons but also lower layers.

Table 3
Condition coefficients (*r*-value) between enzyme activities and chemical properues of soil at 40–60 cm depth

Variable	B	C	D	E	F	OM	TN	TP	N	P	K
A	0.97*	−0.21	0.72*	−0.44	−0.25	0.88**	0.97**	0.92**	0.96**	0.99**	0.56
B		−0.10	0.71	0.56	−0.22	0.95**	0.99**	0.98**	0.98**	0.99**	0.72*
C			−0.04	−0.10	0.43	0.15	−0.02	0.08	−0.02	−0.08	0.43
D				−0.13	−0.17	0.64	0.72*	0.68	0.71	0.74*	0.38
E					−0.24	0.64	−0.55	−.0.65	−0.49	−0.50	−0.71
F						−0.1	−0.20	−0.11	−0.24	−0.23	0.10
OM							0.97**	0.99**	0.97**	0.94**	0.89*
TN								0.99**	0.99**	0.99**	0.75*
TP									0.97**	0.97**	0.83*
N										0.99**	0.75*
P											0.68

n = 8. A: Invertase; B: phosphatase; C: proteinase; D: catalase; E: polypheaoloxidase; F: peroxidase; OM: organic matter; TN: total N; TP: total P; N: Available N; P: available P; K: available K.
* Indicates significance of treatment at $P < 0.05$ level.
** Indicates significance of treatment at $P < 0.01$ level.

These results, to some extend, differed from those of Saá et al. (1998) who found the enzymes in the top 5 cm of soil were much more severely affected by fire than deeper layers, which caused oxidation of organic P and a marked decrease in the content of residual P. This phenomenon most probably results from 8-years' regeneration of forest after burning, which could serve to homogenize burning effects on enzyme activities and soil properties between different soil layers.

Overall, it was revealed that the trends in enzyme activities 8-years following burning were not always consistent with our predictions. In comparison with unburned spruce forests, the difference of soil enzyme activities was not always significant and soil enzyme activities were not always less in the soils of burned forests than in unburned forests, although the soil chemicals tested were higher in the soil of unburned mature forests than in burned forests. Therefore, although soil enzymes were found to discriminate between burned and unburned forests and were relatively stable and sensitive to the changes of ecosystem, they were thought to be useful indicators for monitoring changes in disturbance ecosystem (Badiane et al., 2001). It is still necessary, however, to combine enzyme activities and soil chemical properties to evaluate the burned and the regenerated forests.

There is a growing recognition for the need to develop sensitive indicators of ecosystem health. Water and air quality standards are established, but several factors make soil quality parameters that reflect management and assist managers in promoting long-term sustainability of terrestrial ecosystems more difficult to define, measure, and regulate because soils are inherently variable. Thus, soil is a vital natural resource, currently without comprehensive standards of quality, and 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 formation of a given soil. Indeed, enzyme activities have been shown by our and other studies, to be sensitive to disturbance, and soil enzyme activities tend to stabilize after establishing a particular management system (Albiach et al., 2000; Marcote et al., 2001; Aon et al., 2001; Chen, 2003; Zhang et al., 2004). Therefore, soil enzyme activity is an important indicator of ecosystem health, and temporal dynamics in enzyme activities should be used to evaluate the degree of disturbance of a forest ecosystem.

5. Conclusion

Understanding ecosystem functions on a firm scientific basis includes knowledge of the underlying biological and biochemical processes (including response to all kinds of disturbance). Soil is an important component of most terrestrial ecosystems. Soils, through their physical structure, physico-chemical properties and biological activities, are driving variables, which control the structure and function of the ecosystem they support. The soil of course does not exist in isolation but rather is a part of the ecosystem, which also includes micro-organisms, plants, animals and dead material, all of which interact. Therefore, any disturbance or management that humans impose on one part of the system may be reflected in changes of function or process of another part. As we generally take this for granted in "normal" ecosystems, it should be no surprise that we need to manipulate soil processes during restoration work following disturbance. Such knowledge is required to provide strategies and approaches for forest resource managers and policy makers, to promote long-term ecosystem sustainability. Early indicators of ecosystem stress may function as a "sensor" whose perturbation may warn of ecosystem degradation as compared to other classical and slowly changing soil properties. Thus some enzymes can be used as soil quality indicators during the regeneration or restoration process.

Although soil enzymes undoubtedly perform functions critical to the cycling of nutrients, the role these assays can play in determining the "soil" health is less clear. They may be most useful for monitoring temporal trends (positive or negative) in soil. This would eliminate problems such as seasonal changes and inherent differences on activities to be early indicators of management-induced changes in the soil. There was no apparent pattern that could be applied to all enzyme activities. Further research is needed to confirm this hypothesis, and the response of enzyme activity to disturbance. Effects of disturbances on soil enzymes also need further evaluation in the specific Qinghai-Tibetan Plateau ecosystems, which could provide rational management measures to promote their long-term sustainability.

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