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RESEARCH ARTICLE

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Seasonal response of soil enzyme activity to thinning intensity of aerial seeded *Pinus tabulaeformis* stands

Abstract Aerial seeding is one of the most important vegetation restoration patterns in remote hilly areas, and studies concerning soil quality and its management have practical value. In 2000, a study of the effect of thinning intensities at five different treatments levels, 0 (CK), 30% (slight thinning), 48.75% (medium thinning), 53.75% (intense thinning) and 65.6% (super intense thinning) on soil enzyme activity was carried out on 9-year-old aerial seeded Chinese pine (*Pinus tabulaeformis* Carr.) stands with an initial density of 8000 trees/hm^2, in the Wangjiapu Aerail Seeding Center, Yanqing County, Beijing. Five years later, the activities of five kinds of soil enzymes, soil urease, alkaline phosphatase, inertase, catalase and polyphenol oxidase in the first 20 cm of soil layer were compared during four seasons. Relationships among soil enzymes and soil physiochemical properties were also analyzed to examine the possibility of using soil enzymes to evaluate thinning intensities. The results showed that the maximum enzyme activities of catalase and polyphenol oxidase occurred in June, those of soil urease and alkaline phosphatase occurred in October, and soil invetase had its maximum in April. In addition, the five soil enzymes were affected differently by thinning intensities. Soil catalase, urease and invertase showed the highest response to a slight thinning, followed by medium thinning, which is the opposite experienced with polyphenol oxidase and alkaline phosphatase. There are statistically significant and positive relationships between soil enzymes and organic matter and available K. It should be noted that soil water was a limiting factor to soil enzyme activity. Compared with soil physicochemical characteristics, soil enzymes were more sensitive to levels of thinning intensities. Among the enzymes, soil alkaline phosphatase and catalase could be regarded as indicators to assess soil quality. It is concluded that a suitable thinning intensity benefits the development of undergrowth and soil enzymes. Generally, when the stand with initial density of 8000 trees/hm^2 grows up to nine years old, the most suitable thinning intensity should be about 50%.

Keywords *Pinus tabulaeformis*, aerial seeding stands, thinning intensity, soil enzyme, soil nutrient

1 Introduction

As one of the properties of forest soil organisms, the activity of forest soil enzymes is, in general, affected by ecological factors such as soil physiochemical compounds, vegetation type and biodiversity (Guan, 1986; Yang et al., 1995; Yang and Wang, 2004). The forest soil enzyme system is widely regarded as the bioactive substance of ecological progress, similar to the material cycle and energy flows in the forest system (Zhou, 1987; Yang and Wang, 2002). Soil enzymes are viewed as a more sensitive and reliable bioactive index than soil microflora, community composition or soil respiration (Guan, 1980). In addition, soil enzyme activity can be used as a sensitive index for both soil under biological stress and the early stage of biological soil restoration (Dick and Tabatabai, 1993; Garcia and Hermandez, 1997). Thus, it is important to compare and examine the seasonal dynamics of soil enzymes affected by thinning intensities for forest management as well as for understanding the progress of ecological interaction between soil enzymes and physiochemical properties.

Afforestation by aerial seeding is an effective method for large areas in remote hills. Stands originating from aerial seeding are a near-natural system formed by manmade progressive succession (Committee of the Forty Years of Afforestation Stands Sown by Plane in China, 1998). The community formation of aerial seeded Chinese

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pine is a progression of seed invasion, competition and colonization into shrub and herb communities, so that it is different from communities of Chinese pine plantations (Chen and Lu, 1997; Li et al., 2006a). Its population distribution is severely clustered (Liu et al., 1983; Liu et al., 1991), and in the aerial seeded Chinese pine areas, the density of forests rapidly become high, and the forests closed quickly. Soil degradation of coniferous stands caused by high density has attracted much attention (Sun and Bao, 2005). Therefore, an appropriate thinning regime should be a key measure for the ecological benefit of the aerial seeded stands. Only a limited number of documents have dealt with the effect of density on tree growth (Yang 1996; Mo et al., 2003; Liu et al., 2003; Guo et al., 2004). In addition, thinning intensities mentioned in these documents were adopted not for their ecological benefits, but for maximum timber production in these ecologically fragile zones. The objectives of our study were: i) to evaluate the impact of thinning treatments on soil enzyme activities, and ii) to select an optimum thinning intensity for a 9-year-old aerial seeded Chinese pine stand.

2 Study area

The study area is located in the Wangjiapu Aerail Seeding Center, Yanqing County, Beijing (40°16′N, 115°40′E). This area is a typically hill and gully loess region and ranges in elevation from 600 to 1200 m. The site is characterized by a warm temperate continental monsoon climate with a mean annual temperature of 8.8°C and a fluctuating annual precipitation between 450 and 550 mm. The main soil type is leached cinnamon soil developed on weathered sandstone. The original vegetation is typical of the flora of northern China. The dominant tree species are *P. tabulaeformis*, *Quercus wutaishanica* Mayr and *Platycladus orientalis* L. (Hou, 1982).

The investigated pine stands originated from seed sown from a plane in 1990. A 600 hm^2 stand was established, of which a stand of 400 hm^2 survived and formed a forest. In the spring of 2000, a thinning trial was examined to determine short-term responses of soil enzymes to varying levels of thinning. The 9-year-old Chinese pine was thinned to 5600, 4100, 3700 and 1830 trees/hm^2. Control plots were left for comparison. These four thinning regimes and the control were replicated three times in a randomized complete block design. Each of the 20 plots was 0.03 hm^2 (20 m × 15 m). After thinning, measures were immediately taken to close the hillsides to facilitate undergrowth developing. The sites were 630–645 m above sea level, north facing slopes of 30.0–37.2° and at least a 40 cm deep soil. In early March 2005, data concerning tree characteristics were collected and are presented in Table 1.

3 Materials and methods

3.1 Soil sampling

In each plot, 16 soil cores were taken randomly to a 20 cm depth with a 5-cm diameter auger. These soil samples were then composted, resulting in one soil sample for each plot and a total of 15 samples, or one for each of the 15 plots. The sampling depth was chosen based on studies showing that microbial biomass and activity are greatest within 20 cm of the soil surface (Mergel et al., 2000; Boyle et al., 2005), a depth wherein we expected the most significant treatment differences. Sampling occurred in early April, June, August and October 2005. This yielded a total of 60 samples.

3.2 Soil characteristics

All soil samples were brought to the laboratory under refrigeration at 4°C for less than seven days before processing. Soil moisture content was determined by a gravimeter at 105°C for each sample. The remaining soils were air dried, crushed, and sieved through a 2-mm screen for soil chemical and enzyme analysis. Soil chemical properties were measured according to methods of Bao (2005) and pH by the potentiometric method. Total N was measured by the Se-$CuSO_4$-H_2SO_4 digestion method. Organic matter content was determined by oxidation-reduction titration with digestion in a furnance. Available P was measured with NH_4F-HCl extraction method and available K with ammonium acetate-frame photometry. Soil enzymes were measured using the following methods described by Guan (1980). Catalase activity was measured by permanganate titration. We added 40 mL of distilled water and 5 mL of 3% hydrogen peroxide solution to 5 g of soil and then incubated this mixture for 24 h at 37°C. Polyphenol oxidase activity was determined by iodometric titration. We added 10 mL of substrate (1% pyrogallol) to 1 g of soil and then incubated

Table 1 General conditions of sample plots of aerial seeded 15-year-old *P. tabulaeformis* stands

	density/trees·hm^{-2}	mean height/m	DBH/cm	dominant species
CK (control)	8000	2.79	3.12	*Spiraea pubescens–Achnatherum pekinense*
I (slight)	5600	3.12	4.32	*Vitex negundo–Viola yedoensis*
II (medium)	4100	3.32	4.55	*Spiraea pubescens–Calamagrostis arundinacea*
III (intense)	3700	3.47	4.90	*Vitex negundo–Carex lanceolata*
IV (super intense)	1830	2.84	4.14	*Ostryopsis davidiana–Arundinella hirta*

this mixture for 24 h at 37°C. Invertase activity was determined by sodium thiosulfate titration. We added 10 mL of 20% cane sugar and a 10 mL phosphate buffer (pH 5.5) to 10 g of soil and then incubated this mixture for 24 h at 38°C. Urease activity was measured by an indophenol blue colourimetric method. We added 10 mL of 10% urea and 20 mL citric acid buffer (pH 6.7) to 10 g of soil and then incubated this mixture for 24 h at 38°C. Alkaline phosphatase activity was measured by 10 mL of 25 mg/mL substrate (p-nitrophenyl phosphate), and 10 mL borate buffer (pH 9) were added to 10 g of soil and then incubated for 24 h at 38°C.

3.3 Data analysis

Data collected from the field survey and laboratory analysis were processed and analyzed by Microsoft Excel (XP) and SPSS 11.5.

4 Results and analysis

4.1 Seasonal response of soil enzyme activity to thinning

As seen in Figs. 1a and 1b, the maximum activities of both catalase and polyphenol oxidase were evident in June, while the minimum activities of catalase and polyphenol oxidase were observed in April and October. Difference in catalase activities among the five treatments was most significant in October, while this was not significant in April, June and August. Responses of polyphenol oxidase activity were more sensitive to thinning than catalase. During the growing season, the maximum activity of catalase was in the slight thinning intensity, while that of polyphenol oxidase was in medium thinning intensity, indicating that the two kinds of oxidoreductase enzymes responded to thinning differently. It should be noted that the activity of both enzymes was lower at the super

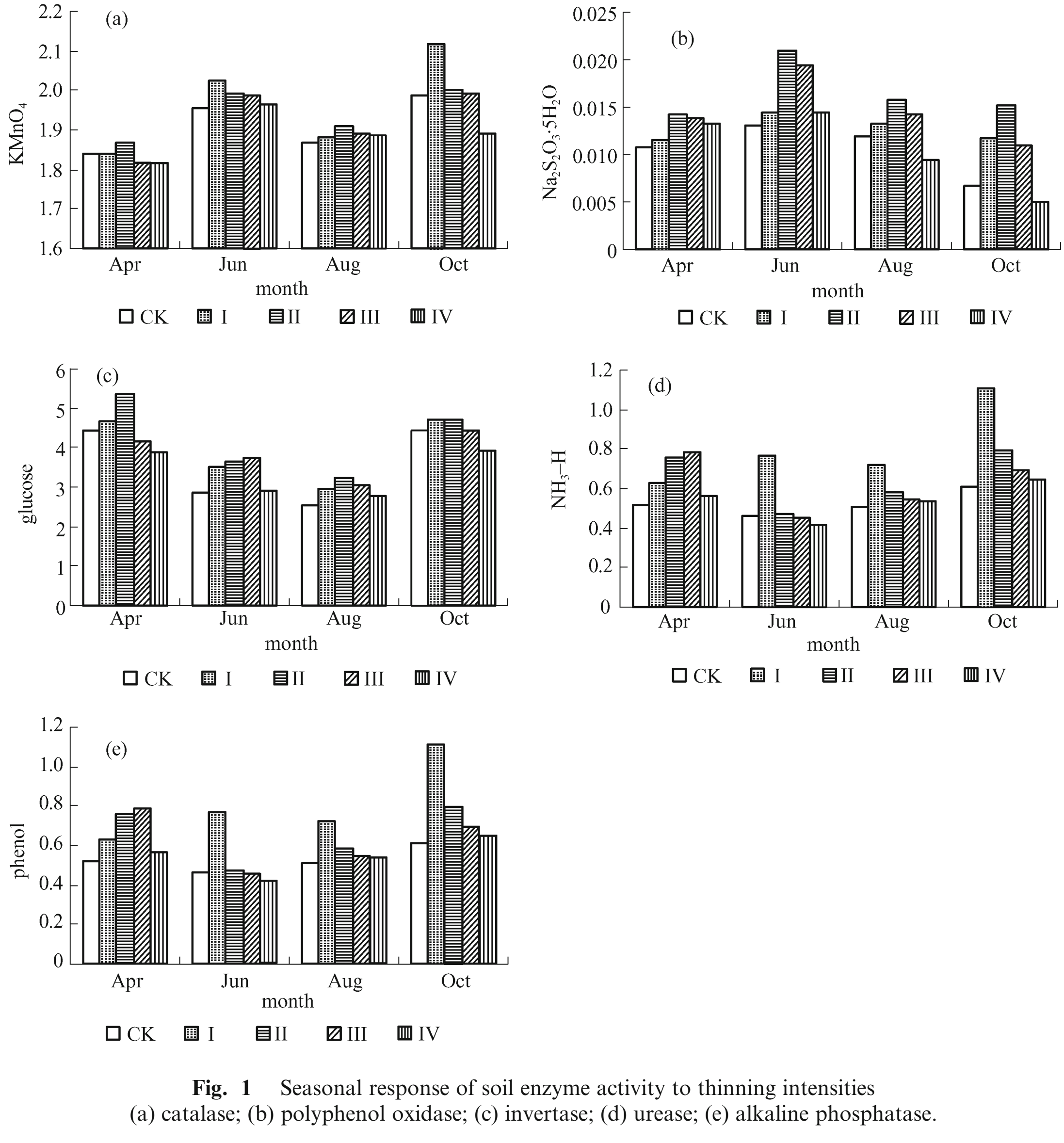

Fig. 1 Seasonal response of soil enzyme activity to thinning intensities
(a) catalase; (b) polyphenol oxidase; (c) invertase; (d) urease; (e) alkaline phosphatase.

thinning intensity than at CK. Yang and Wang (2004) considered that root exudates and the amount and quality of litter were affected by the species and composition of the undergrowth and therefore also by the enzyme activity. Adoption of a moderate thinning intensity was valuable to the structure of aerial seeded stands and species diversity of the undergrowth (Li et al., 2007). As a consequence, the diversities of both root exudates and litter composition were improved so that the essential, abundant and equilibrium nutrients were available for much of the organism populations. This accounts for the maximum activity of catalase at the slight thinning intensity and of polyphenol oxidase at the medium level of thinning intensity, while the undergrowth was dominated by *Ostryopsis davidiana* at the super thinning intensity and its diversity was lower than that for CK (Li et al., 2007). The simple root composition could be responsible for the soil enzyme activity. When thinning, the increase of polyphenol oxidase activity would be beneficial to phenolics inverting to quinones, indicating that the accumulation of phenolics decreased and thus the soil toxins were reduced (Wei et al., 2001).

Invertase, urease and alkaline phosphatase are three kinds of common hydrolases. Figures 1c, 1d and 1e show that the maximum activity of invertase was in April, while that of both urease and alkaline phosphatase occurred in October. The results do not agree with the argument that enzyme activity reached the maximum in months with high temperature (Burger and Kelting, 1999; Criquet et al., 2000). This could be related to the perceived rule that Chinese pine grows rapidly right from the start at age one. On the one hand, the peak of Chinese pine growth appeared between June and August when the trees needed excess nutrients, resulting in the condition that soil nutrients could be depleted or out of balance. Thus, there could have been a lack of urease, alkaline phosphatase and invertase, i.e., a deficiency in organic compounds of C and N. On the other hand, the litter peak production of the pine and its undergrowth was in October and the litter ‘‘ignition agent’’effect could stimulate organism activity in relation to soil urease and alkaline phosphatase, causing the activity of the two kinds of enzymes to improve significantly.

When thinning, the three kinds of hydrolase activities were increased to some extent. Urease activity reached its peak at the slight thinning intensity, while the activities of invertase and alkaline phosphatase reached their peaks at medium thinning intensity. Urease and phosphatase are specific enzymes. Ammonia, as the product of urease reaction, is one of the nitrogen sources, and alkaline phosphatase is a limiting factor for organic phosphate conversion into inorganic phosphate. As a consequence, thinning can promote N and P efficiency in aerial seeded Chinese pine stands with high density.

4.2 Relations between soil enzyme activity and physiochemical properties

In Table 2, it can be seen that the differences in soil moisture content during the four months were statistically more significant than bulk density, pH and soil nutrients. The mean soil moisture contents under the five treatments were 21.11%, 7.52%, 6.93% and 3.91%, respectively, showing that soil moisture content is highly related to precipitation. The maximum level of total N and available P occurred in August, while both organic matter and available K were maxima in April and October, respectively.

In the five treatments, only the soil moisture content was higher at medium thinning intensity than at CK, indicating

Table 2 Effect of different thinning intensities on soil physiochemical properties

month	treatment	soil moisture content/%	bulk density /g·cm^{-3}	organic matter/g·kg^{-1}	pH	total N/g·kg^{-1}	available P /mg·kg^{-1}	available K /mg·kg^{-1}
April	CK	3.89	1.049	41.744	7.89	1.047	1.234	48.28
	I	3.17	1.068	43.336	6.96	1.560	1.423	69.56
	II	5.81	1.097	51.591	6.35	1.642	1.679	86.28
	III	3.26	1.026	46.088	6.74	1.576	1.362	54.44
	IV	3.42	1.098	42.304	6.76	1.470	1.133	47.08
June	CK	7.13	1.138	26.657	7.29	0.969	1.137	52.31
	I	7.62	1.263	30.954	7.14	1.352	1.186	76.64
	II	8.35	1.175	35.082	7.22	1.261	1.332	75.50
	III	7.28	1.281	34.394	7.18	1.249	1.569	59.94
	IV	7.26	1.119	28.323	7.32	1.073	2.031	52.82
July	CK	23.70	1.105	44.935	7.21	1.248	1.178	59.28
	I	19.31	1.265	47.463	7.02	1.853	1.202	89.88
	II	20.44	1.187	39.209	7.02	1.642	1.378	91.12
	III	20.43	1.191	37.489	6.89	1.352	1.586	73.65
	IV	21.68	1.122	32.495	6.79	1.268	2.422	61.18
October	CK	6.10	1.175	41.623	7.55	1.158	1.208	117.87
	I	4.89	1.235	43.336	6.99	1.193	1.374	156.38
	II	6.45	1.133	42.648	6.82	1.658	1.750	164.95
	III	9.54	1.142	41.616	6.80	1.268	1.444	128.41
	IV	7.70	1.115	39.813	6.88	1.097	1.326	102.52

that the relation between thinning intensity and soil moisture content was not positive. One possibility is that litter decomposes slowly and a massive amount of litter improves its water storage. With an increase of thinning intensity, the correlation between bulk density and pH shows an irregular trend. The maximum amount of organic matter, total N and available K was reached at medium thinning intensity, followed by slight and intense thinning intensities. The amount of organic matter, total N and available K were improved by 26.3%, 26.2% and 25.15% over the CK, showing that available K was less sensitive to thinning than organic matter and total N. Total P content is much greater than available P in cinnamon soil (Guan, 1986). Our results show that amount of available P was improved by 9.01%–45.34% in the stands affected by thinning over the CK stand.

A number of studies have proven that the relation between soil nutrient content and soil enzymes is positive, while few documents dealt with the relation between soil nutrient content and soil enzyme activity when stands were thinned. As shown in Table 3, the four enzymes, except polyphenol oxidase, were related to soil moisture content. Soil moisture content was positively related with alkaline phosphatase and negatively with urease, invertase and catalase, showing that the impact of soil moisture content on soil enzymes was passive. Thinning could have minimal effect on litter decomposition and a massive litter layer keeps more rain in the summer, while limited precipitation in the spring in Beijing keeps the soil moisture content merely at 3.17%–5.81%. Excessive or insufficient soil moisture was detrimental to soil enzymes. Less bulk density means more porosity, more air and water permeability and more soil enzyme activity, indicating that the relation among bulk density and urease, alkaline phosphatase, catalase and polyphenol oxidase was positive. Catalase was positively related with pH, corresponding to the *Cupressus funebris* Endl., mixed stands of *Populus* × *xiaozhuanica* W. Y. Hsu et Liang cv. Balizhuangyang and *Robinia pseudoacacia* L. The positive correlations between urease, alkaline phosphatase, invertase and organic matter prove that organic matter has an effect on soil enzymes (Guan, 1986; Zhou, 1987). Soil available K was positively related with urease, alkaline phosphatase, invertase and catalase.

4.3 Analysis of soil quality indicators

A soil information system of twelve indicators such as soil enzymes, soil moisture content, bulk density, pH and soil nutrient was adopted by using principal component analysis (PCA) to evaluate the relation between soil enzymes and soil nutrients and to construct indicator systems. As shown in Table 4, the variation explained by components 1, 2, 3, 4 and 5 was 33.03%, 19.42%, 16.57%, 10.50% and 9.95%, respectively. The cumulative explained variation of the five components was 89.47% and can be used to reflect the relationship among soil indicators. Eigenvectors from PCA of soil quality are shown in Table 5. Component 1 is heavily weighted by soil alkaline phosphatase, total N and organic matter. As a consequence, alkaline phosphatase, total N and organic matter can be regarded as the main indicator system of the soil of aerial seeded Chinese pine stands. Component 2 includes catalase, available K and bulk density. Component 3 consists of soil water-related information. Components 4 and 5 include polyphenol oxidase and available P, respectively. Therefore, it is possible to assess soil fertility for cinnamon soil by soil enzymes.

5 Conclusions and discussion

Aerial seeded Chinese pine communities occasionally replace shrub and herb communities. This progression promotes succession and consumes greater amounts of soil nutrients synchronously (Li et al., 2006b). On the one hand, aerial seeded Chinese pine stands with high density are intensely competitive. On the other hand, light

Table 3 Relationships among soil enzyme activities, soil nutrients and pH values

	soil moisture water	bulk density	organic matter	pH	total N	available P	available K
urease	−0.244	0.163	0.510*	−0.417	0.371	−0.118	0.695**
alkaline phosphatase	0.213	0.159	0.670**	−0.488*	0.667**	0.006	0.702**
invertase	−0.707**	−0.234	0.563**	−0.239	0.195	−0.095	0.499*
catalase	−0.145	0.646**	−0.367	0.176	−0.312	0.049	0.621**
ployphenol oxidase	−0.000	0.336	−0.188	−0.059	0.281	0.036	−0.187

Note: * means significant at $\alpha = 0.05$, ** significant at $\alpha = 0.01$

Table 4 Principal component analysis of soil information system

item	component 1	component 2	component 3	component 4	component 5
eigenvalues	3.96	2.33	1.99	1.26	1.19
contribution ratio of variance/%	33.03	19.42	16.57	10.50	9.95
cumulative contribution ratio/%	33.03	52.45	69.02	75.52	89.47

Table 5 Principal component eigenvectors of soil information system

factor	*X*1	*X*2	*X*3	*X*4	*X*5	*X*6	*X*7	*X*8	*X*9	*X*10	*X*11	*X*12
1	0.882	0.874	0.825	−0.677	0.633	−0.234	0.440	0.005	0.162	0.400	−0.030	−0.060
2	0.324	−0.127	−0.203	0.080	0.500	0.942	0.751	0.750	0.002	0.124	0.083	0.010
3	−0.044	−0.103	0.227	−0.065	0.358	0.136	0.225	−0.383	−0.948	0.851	−0.002	−0.118
4	−0.236	0.350	−0.211	−0.115	−0.183	0.105	−0.291	0.353	−0.076	−0.125	0.957	−0.020
5	0.024	0.029	−0.243	−0.523	−0.027	0.057	0.068	−0.176	0.119	−0.017	0.016	0.945

Note: *X*1–*X*12 represent alkaline phosphatase, total N, organic C, pH, urease, catalase, available K, bulk density, moisture content, invertase, polyphenol oxidase and available P, respectively.

and organisms for pure coniferous stands are short with a high canopy cover (Zhou et al., 2002; Li et al., 2004), litter decomposes and nutrients return slowly. Since they consume a lot of nutrients and returning nutrients in the form of litter, aerial seeded Chinese pine stands causes soil infertility. As a result, it is very important to do thinning effectively when these pine stands have a dense canopy. Our study shows that the adoption of a suitable level of thinning intensity can increase the activity of soil enzymes. When the stands were treated with medium thinning intensity, soil enzyme activities reached their highest level corresponding to the highest soil physiochemical properties. It can be concluded that a reasonable thinning intensity should be about 50% when the stand with an initial density of 8000 trees/hm^2 grows to age nine. Moreover, aerial seeded Chinese pine stands have a dense canopy and are commonly found in remote hilly regions at high altitude and with poor accessibility. Therefore, an appropriately heavy thinning intensity should be adopted to reduce thinning frequencies by prolonging the thinning intervals. Density of the stands in the experiment can decrease to about 3700 trees/hm^2. In practice, extreme thinning intensity should be applied with caution because soil enzyme activities were not much higher in the stands with 1830 trees/hm^2 than in the CK stands.

In the soil indicator system, physiochemical properties, soil enzymes alkaline phosphatase and catalase, and soil moisture content accounted for almost 90% of the total variation in the system. As a consequence, soil enzymes could be regarded as sensitive indicators to evaluate management of the forest ecosystem. It should be noted that the five kinds of enzymes responded differently to the thinning intensities during the four seasons. The activities of catalase and polyphenol oxidase reached their peaks in June, that of invertase appeared in April, and both urease and alkaline phosphatase peaked in October. The five kinds of enzyme peaks did not appear in the heat of August and the results did not totally agree with the point of view that the peak of soil enzyme activity should appear in the hot season (Burger and Kelting, 1999; Criquet et al., 2000). This could be attributed to the growth and litter decomposition of this species. In addition, enzymes responded to thinning intensity differently. Catalase and urease activity reached their maximum at the slight thinning intensity. Invertase, polyphenol oxidase and alkaline phosphatase reached their maxima at the medium level of thinning intensity. Activity of the five enzymes was lower at the extreme thinning intensity than in CK plots, as a proper thinning intensity could optimize the forest structure and enhance its species diversity. Differences in root exudates, quality and amount of litter caused by the species and composition of the undergrowth could explain the discrepancy of soil enzyme activities. Similar to season (Aon and Colaneri, 2001; Xiong et al., 2004), forest type (Zhao and Wang, 1995; Hu et al., 2002; Xiong et al., 2004; Li et al., 2007), afforestation pattern (Li et al., 2006c), succession stage (He et al., 2005) and soil type (Zhou, 1987), thinning intensity can be considered as one of the factors that affect soil enzymes. Thus, to evaluate soil enzyme activity, it is important not only to apply a single enzyme, but both soil enzymes particularly suited to heterogeneous conditions and enzymes that are closely and widely related to soil fertility (Zhou, 1987).

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