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RESEARCH ARTICLE

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Canonical correlation analysis of soil nutrients, microorganisms and enzyme activities in vegetation restoration areas of degraded and eroded soils in northwestern Hunan Province, China

Abstract With the aid of canonical correlation analysis, the relations among soil nutrients, soil microorganisms, and soil enzyme activities were studied in vegetation restoration areas of degraded and eroded soils in the Nverzhai watershed in northwestern Hunan. The main results were as follows: the key factors in soil nutrients, microorganisms, and enzyme activities were N and P elements, number of bacteria, carbon and nitrogen in soil microbial biomass and the activities of urease, polyphenol oxidase, phosphatase, and invertase. The activities of urease and polyphenol oxidase are related to the inversion of N and P elements that had important impact on the accumulation of carbon and nitrogen in soil microbial biomass. Moreover, the activities of urease, polyphenol oxidase, and phosphatase could promote carbon accumulation in microbial biomass; however, invertase activities inhibited the accumulation of microbial biomass nitrogen. On the other hand, urease activities were beneficial to the N element content in soils but unfavorable for P elements. There is a negative relation between polyphenol oxidase activity and N element content. For every canonical variable group, the tendencies of soil nutrients, microorganisms, and enzyme activities to accumulate in different soil layers in different vegetation restoration communities could offer some scientific basis for the diagnosis of the health of the soil and the site type division in the process of vegetation restoration.

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1 Introduction

The problems of soil degradation are very serious and have resulted in widespread soil erosion in China (Ren and Peng, 2001; Peng, 2003; Qi et al., 2007a). Moreover, it is clear that vegetation restoration is an effective approach for harnessing soil degradation and improving the quality of the soil (Cheng and Li, 2004). Soil nutrients, microorganisms, and enzymes are important components of soil ecosystems, and critical indices for soil evaluation have been the focus for scholars all over the world for many years (Jiao et al., 1997; Gu et al., 2000; Hu et al., 2001; Wang et al., 2001; He et al., 2002; Tan et al., 2003; Gun et al., 2005; Wen et al., 2005). Enrichment, space distribution, and reallocation of soil nutrients have a vital effect on the growth, development, and succession of vegetation. The ecological effects of different types of vegetation are reflected in the differentiation of the characteristics of soil nutrients (Jiao et al., 1997; Wang et al., 2001; Gun et al., 2005; Qi et al., 2007b). From the decomposition of propagating remnants in soil ecosystems, microorganisms have become vital ingredients for nutrient pools, which participate in processes of energy flow and cycles of organic matter (Gu et al., 2000; Hu et al., 2001; Xue et al., 2003; Gong et al., 2005). Soil enzymes are not only concerned with the cycle of organic matter, including biological and chemical processes, but also active pools for plant nourishments, usually called biological activators (He et al., 2002; Fan et al., 2003; Xue et al., 2003). There are many successful examples for applying canonical correlation analysis (CCA) for studying community vegetation patterns and soil enzyme activities and nutrients

(Haanstra, 1985; Doelman, 1986; Li et al., 2003; An et al., 2005). However, it has rarely been applied to studying soil nutrients, microorganisms, and enzyme activities in vegetation restoration in districts where the land has been degraded (Hu et al., 2002; Jiang and Zhou, 2003). To offer some basis for vegetation restoration in soil degradation and erosion in the Nverzhai watershed in Northwestern Hunan, we have studied the relations among soil nutrients, soil microorganisms, and soil enzyme activities of the seven typical vegetation restoration communities.

2 Study area

The Nverzhai watershed (29°30′N, 110°10′E) is located in Niangxi Village, Chengguan Town, Cili County, belonging to Zhangjiajie City, Hunan Province. The mainly native rock is shale and sandstone, and the soil is a yellow mountain soil. This area belongs to the middle of a semitropical mountainous and seasonally wet temperate zone, flush with energy and precipitation. The average annual amount of sunlight is 1440 h, the average temperature is 16°C, the annual rainfall is 1400 mm, and the frost-free period ranges between 216 and 269 d. The elevation varies from 210 to 917 m, and the area of the watershed is 2.81 km^2. In its complex landform, the Nverzhai watershed has a high density of ravines (2.6 km/km^2), and the drop of the main (28.4 ‰) is due to serious soil erosion and degradation. To improve this condition, rehabilitation of the vegetation, integrated with the establishment of plantations and the program "Closing Hillsides to Facilitate Afforestation" has been in place since 1993. There are now some typical vegetation restoration patterns, such as natural *Pinus massoniana* forests, plantations of *Cunninghamia lanceolata*, *Eucommia ulmoides*, and *Vernicia fordii*, as well as a secondary *Machilus pingii* forest, *Phyllostachys edulis* and *C. lanceolata* mixed forests, and waste shrubs (Qi et al., 2007b). The main shrub species are *Loropetalum chinensis*, *Boehmeria nivea*, *Rubus palmatus*, *Mallotus apelta*, *Acer oblongum*, and *Camellia oleifera*. The main herbaceous species are *Dicranopteris linearis*, *Imperata* spp., *Dianthus caryophyllus*, *Arthraxon hispidus*, *Houttuynia cordatu*, *Ophiopogon japonicusc*, *Senecio scandens*, *Cyperus rotundus*, *Microlepia marginata*, *Cyclosorus acuminatus,* and *Metathelypteris singalanensis*. Outside layer plants are *Dalbergia hancei*, *Lygodium japonicum*, *Thladiantha nudiflora*, *Smilax china*, *Cocculus trilobus*, and *Akebia trifoliate*.

3 Methods

3.1 Plot design and soil sample collection

To contrast with two samples in the waste shrub (CK) community, three plots (20 m × 30 m) were designed in six forest communities, that is, in the natural *P. massoniana* forest (C_1), the *C. lanceolata* plantation (C_2), the *E. ulmoides* plantation (C_3), the *V. fordii* plantation (C_4), the secondary *M. pingii* forest (C_5), and the *P. edulis* and *C. lanceolata* mixed forest (C_6). Soil samples were collected on July 13, 2006. Amounts of 1 kg soil were collected and mixed catercorner with the 0- to 20-cm and 20- to 40-cm soil layers from each plot. Each soil sample was divided into two parts: one was kept at 4°C for analyzing the number of soil microbes and biomass; the other was used to determine soil nutrients and enzyme activities.

3.2 Determination of soil nutrients, microorganisms and enzyme activities

All indices of soil nutrient, microorganism, and enzyme activity were determined with three replications (Nanjing Soil Institute of Chinese Academy of Sciences, 1978). The following methods for each index were used: potassium dichromate for determining the activities of organic matter; diffused absorption (Se powder-$CuSO_4$-H_2SO_4 assimilation) for total nitrogen; Mo-Sb colorimetry for total phosphor; alkaline hydrolysis diffusion for available nitrogen; double acid extraction for available phosphor; flare photometer for available kalium; kalium-imine colorimetry for available B; sulfur-potassium cyanate colorimetry for Mo; atomic absorption spectrophonetry for Zn, Fe, Cu, and Mn; a plate smear method for number of microbes by using fresh soil sample and media of beef extract-peptone, Martin and improved No. 1 Gao for numbers of bacteria, fungi and actinomyciae; chloroform fumigation for microbe biomass C/N; sodium phenolate colorimetry for urease; 3,5-dinitrosalicylic acid colorimetry for sucrase; 2,3,5-triphenylte trazolium chloride (TTC) colorimetry for dehydrogenase; $KMnO_4$ titration for catalase; pyrogallol colorimetry for polyphenol oxidase; ninhydrin colorimetry for proteinase; and di-sodium phenyl phosphate colorimetry for phosphatase.

3.3 Canonical correlation analysis (CCA)

Canonical correlation analysis (CCA) is a kind of multivariate analytical method for studying the correlation between two or more groups of variables. We selected the following 12 soil nutrient indices: organic matter (X_1), total nitrogen (X_2), total phosphor (X_3), available nitrogen (X_4), available phosphor (X_5), available kalium (X_6), available B (X_7), available Mo (X_8), available Zn (X_9), available Fe (X_{10}), available Cu (X_{11}), and available Mn (X_{12}) and five indices of soil microorganisms: number of bacteria (Y_1), number of fungi (Y_2), number of actinomyciae (Y_3), carbon in microbial biomass (Y_4), and microbial biomass nitrogen (Y_5). As well, we opted for the following seven indices of soil enzyme activity: urease (Z_1), sucrase (Z_2), dehydrogenase (Z_3), catalase (Z_4), polyphenol oxidase (Z_5), proteinase (Z_6), and phosphatase (Z_7). We constructed $X_{(12\times42)}$, $Y_{(5\times42)}$, and $Z_{(7\times42)}$ matrices of soil nutrient, microorganism, and enzyme activity indices, respectively, Then, we established U, V, and W linear combinations of canonical vectors for nutrients, microorganisms, and enzyme activities.

$$U = a_1X_1 + a_2X_2 + \cdots + a_pX_p \quad (1)$$

$$V = b_1Y_1 + b_2Y_2 + \cdots + b_qY_q \quad (2)$$

$$W = c_1Z_1 + c_2Z_2 + \cdots + c_rZ_r \quad (3)$$

where, a_1, a_2, …, a_{12}, b_1, b_2, …, b_5, and c_1, c_2, …, c_7 are coefficients to be estimated, from the largest canonical correlation coefficient among the vectors U, V, and W.

3.4 Statistical methods

CCA and cluster analysis were carried out with Data Processing System (DPS) software.

4 Results and analysis

4.1 CCA of soil nutrients and soil microorganisms

According to Wilk's λ and Chi-square tests for canonical correlation coefficients (Table 1), one pair of canonical variables (U_1, V_1) of soil nutrients and microorganisms was selected, with a canonical correlation coefficient of 0.8855 and a probability of 0.0003.

$$U_1 = -0.0380X_1 + 0.2280X_2 - 0.2670X_3 - 0.3068X_4 + 0.7264X_5 - 0.0417X_6 + 0.1552X_7 + 0.1353X_8 + 0.3449X_9 - 0.4154X_{10} - 0.0961X_{11} + 0.2787X_{12}$$

$$V_1 = -0.6078Y_1 + 0.1368Y_2 + 0.2309Y_3 + 1.2511Y_4 - 0.5156Y_5$$

From the coefficients of U_1 and V_1, we see that the dominant factors were available phosphor (X_5) in the nutrient index and carbon in the microbial biomass (Y_4) in microorganism index, which shows that the transformation of soil phosphor has a great bearing on the accumulation of carbon in the microbial biomass. There are clearly positive effects.

With canonical redundancy analysis, 23.96% of the variance of soil nutrients and 17.85% of the variance of soil microorganisms could be explained by the canonical variable U_1, and 22.76% of the variance of organisms and 18.79% of the variance of soil nutrients could be explained by the canonical variable V_1. Applying on-the-plot survey data in the calculation of the equations of U_1 and V_1 and using a minimum distance method in stepwise cluster analysis, the ordination of the canonical variables of soil nutrients and microorganisms is drawn as Fig. 1, which clustered into categories I, II, and III.

Type I: 0- to 20-cm soil layers of C_3, C_4, C_5, and C_7.

Type II: 0- to 20-cm soil layers of C_1 and C_2; 20- to 40-cm soil layers of C_3, C_4, and C_5.

Type III: 20- to 40-cm soil layers of C_1, C_2, and C_7; the 0- to 40-cm soil layer of C_6.

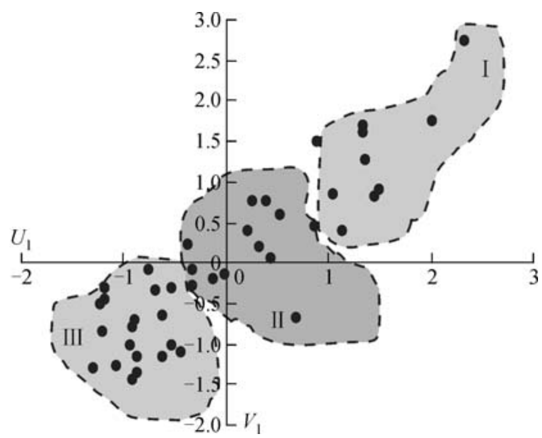

Fig. 1 Ordination and cluster of canonical variables of soil nutrients and microorganisms

4.2 CCA of soil nutrients and soil enzyme activities

Given Wilk's λ and Chi-square tests of canonical correlation coefficients (Table 1), two pairs of canonical variables (U_1, W_1) and (U_2, W_2) of soil nutrients and enzyme activities were selected. The canonical correlation coefficients were 0.8604 and 0.8284, with probabilities of 0.0004 and 0.0166, respectively.

The first pair canonical variables is:

$$U_1 = -0.0805X_1 - 0.6448X_2 + 0.7630X_3 + 0.3569X_4 - 0.0136X_5 - 0.1941X_6 - 0.4074X_7 - 0.0367X_8 + 0.1150X_9 - 0.2140X_{10} + 0.1577X_{11} + 0.5120X_{12}$$

$$W_1 = -1.4244Z_1 + 0.0999Z_2 + 0.2884Z_3 - 0.3425Z_4 + 0.6965Z_5 + 0.3402Z_6 + 0.2493Z_7$$

and the second pair:

$$U_2 = 0.4684X_1 - 0.6904X_2 + 0.0639X_3 + 0.4902X_4 + 0.1094X_5 + 0.0821X_6 + 0.5436X_7 + 0.0364X_8 + 0.1148X_9 - 0.5375X_{10} - 0.2732X_{11} + 0.0452X_{12}$$

Table 1 Wilk's λ and Chi-square tests of canonical correlation coefficients

ttem	canonical vector no.	canonical correlation coefficients	Wilk's λ	χ^2	df	p
CCA of soil nutrients and soil microorganisms	1	0.8855	0.0376	104.9605	60	0.0003**
	2	0.7819	0.1742	55.9177	44	0.1074
	3	0.6186	0.4482	25.6784	30	0.6914
	4	0.4203	0.7260	10.2459	18	0.9236
	5	0.3439	0.8817	4.0274	8	0.8546
CCA of soil nutrients and soil enzyme activities	1	0.8604	0.0130	134.5572	84	0.0004**
	2	0.8284	0.0502	92.7643	66	0.0166*
	3	0.7706	0.1599	56.8281	50	0.2358
	4	0.5800	0.3936	28.9030	36	0.7935
	5	0.4573	0.5932	16.1904	24	0.8811
	6	0.4267	0.7500	8.9180	14	0.8363
	7	0.2882	0.9170	2.6877	6	0.8469
CCA of soil microorganisms and soil enzyme activities	1	0.8698	0.0478	104.8738	35	0.0001**
	2	0.7342	0.1966	56.1189	24	0.0002**
	3	0.6765	0.4266	29.3946	15	0.0143*
	4	0.4257	0.7865	8.2851	8	0.4061
	5	0.1985	0.9606	1.3872	3	0.7085

Note: * means $p < 0.05$, ** $p < 0.01$.

$$W_2 = -0.7318Z_1 + 0.2649Z_2 + 0.2443Z_3 + 0.1687Z_4 + 1.3810Z_5 + 0.2055Z_6 + 0.0514Z_7$$

From the coefficients of U_1 and W_1, we see that the dominant factors are total nitrogen (X_2) and total phosphor (X_3) in the nutrient index, and urease (Z_1) and polyphenol oxidase (Z_5) in the enzyme activities. These results are similar to those of the second pair of canonical variables (U_2, W_2) where the dominant factors are total nitrogen (X_2), available B (X_7), available Fe (X_{10}) in the nutrient index, and urease (Z_1) and polyphenol oxidase (Z_5) in the enzyme activities. These results conform to the basic regularity of nitrogen catalyzed by urease and carbon catalyzed by polyphenol oxidase. On the other hand, it also shows that urease and polyphenol oxidase are indirectly affected by the transformation of soil nitrogen and phosphor through their effects on some soil biological and chemical processes. The relation of nitrogen and urease was positive and negative with nitrogen and polyphenol oxidase, phosphor and urease.

With canonical redundancy analysis, 12.04% and 19.81% of the variance of soil nutrients and 11.52% and 18.50% of the variance of soil enzyme activities could be explained by the canonical variables U_1 and U_2, respectively. Simultaneously, 15.56% and 26.95% of the variance of soil enzyme activities and 8.91% and 13.60% of the variance of soil nutrients could be explained by the canonical variables, W_1 and W_2, respectively. Applying on-the-plot survey data to the calculation of the equations for U_1, U_2, W_1 and W_2 and using a minimum distance method in stepwise cluster analysis, the ordination of the two pair of canonical variables of soil nutrients and enzyme activities are drawn as Figs. 2 and 3.

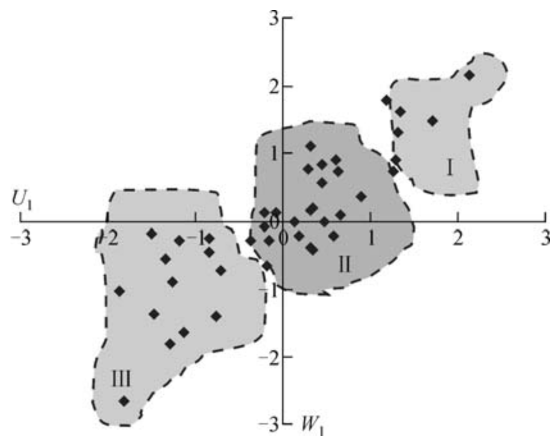

Fig. 2 Ordination and cluster of the first pair canonical variables of soil nutrients and enzyme activities

In Fig. 2, types were clustered into categories I, II, and III.

Type I: 20- to 40-cm soil layers of C_3, C_4, C_5, and C_7.

Type II: 0- to 20-cm soil layers of C_2, C_3, C_4, and C_5.

Type III: the 0- to 20-cm soil layer of C_7; 0- to 40-cm soil layers of C_1 and C_6.

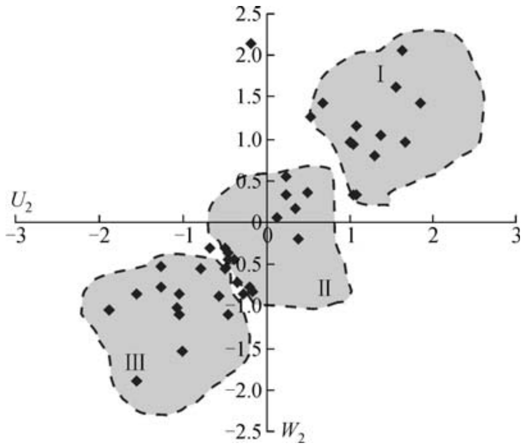

Fig. 3 Ordination and cluster of the second pair canonical variables of soil nutrients and enzyme activities

In Fig. 3, types were clustered into categories I, II, and III.

Type I: 0- to 20-cm soil layers of C_1, C_2, C_3, C_4, C_5, C_6, and C_7.

Type II: 20- to 40-cm soil layers of C_2, C_4, and C_5.

Type III: 20- to 40-cm soil layers of C_1, C_3, C_6, and C_7.

4.3 CCA of soil microorganisms and soil enzyme activities

Given Wilk's λ and Chi-square tests of canonical correlation coefficients (Table 1), three pairs of canonical variables (V_1, W_1), (V_2, W_2), and (V_3, W_3) of soil microorganisms and enzyme activities were selected whose canonical correlation coefficients were 0.8698 (p = 0.0001), 0.7342 (p = 0.0002), and 0.6765 (p = 0.0143).

The first pair canonical variables is:

$$V_1 = -0.6075Y_1 - 0.0749Y_2 - 0.1185Y_3 - 0.8744Y_4 + 0.5313Y_5,$$

$$W_1 = -0.7578Z_1 + 0.0283Z_2 - 0.2989Z_3 - 0.1912Z_4 + 0.2582Z_5 - 0.0208Z_6 - 0.5025Z_7,$$

the second pair:

$$V_2 = 0.0263Y_1 + 0.4773Y_2 - 0.6195Y_3 - 0.5715Y_4 + 1.2316Y_5,$$

$$W_2 = 0.5682Z_1 - 0.1197Z_2 - 0.8688Z_3 - 0.2719Z_4 - 0.2937Z_5 + 0.0762Z_6 + 0.2171Z_7.$$

and the third pair:

$$V_3 = 0.8574Y_1 - 0.4821Y_2 + 0.2652Y_3 - 1.1973Y_4 + 0.5430Y_5,$$

$$W_3 = 1.8621Z_1 - 0.2964Z_2 + 0.4357Z_3 - 0.3016Z_4 - 2.1026Z_5 + 0.1186Z_6 + 0.1680Z_7.$$

From the coefficients of V_1 and W_1, we see that the dominant factors were number of bacteria (Y_1) and carbon in microbial biomass (Y_4) in the microorganism index and urease (Z_1) and phosphatase (Z_7) in the enzyme activities. Especially for the largest positive effect of carbon in microbial biomass and urease, it shows that urease activities promote the accumulation of carbon in microbial biomass. According to the coefficients of V_2 and W_2, microbial biomass nitrogen (Y_5) and dehydrogenase (Z_3) were the dominant factors in the second pair of canonical variables. Moreover, there is a clear negative effect between these factors, which demonstrates that dehydrogenase activities are unfavorable to the accumulation of microbial biomass nitrogen. This is similar to the canonical variables (V_3, W_3) where the dominant factors were carbon in microbial biomass (Y_4) and polyphenol oxidase (Z_5), which shows that polyphenol oxidase activities have a positive effect on the accumulation of carbon in microbial biomass carbon.

With canonical redundancy analysis, 34.52%, 13.98%, and 12.29% of the variance of soil microorganisms and 25.66%, 8.33%, and 2.38% of the variance of soil enzyme activities could be explained by the canonical variables V_1, V_2, and V_3, respectively. At the same time, 33.91%, 15.45%, and 5.19% of the variance of soil enzyme activities and 26.12%, 7.54%, and 5.63% of the variance of soil microorganisms could be explained by the canonical variables W_1, W_2, and W_3. Applying on-the-plot survey data to the calculation of the equations for V_1, V_2, V_3, W_1, W_2, and W_3 and using a minimum distance method in stepwise cluster analysis, the ordination of the three pairs of canonical variables of soil microorganisms and enzyme activities are drawn as Figs. 4–6.

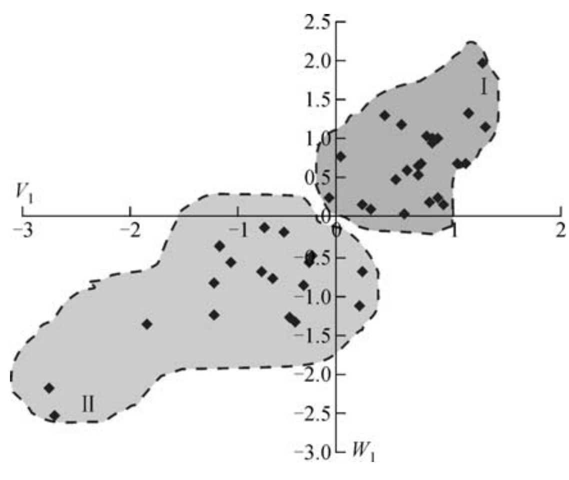

Fig. 4 Ordination and cluster of the first pair canonical variables of soil microorganisms and enzyme activities

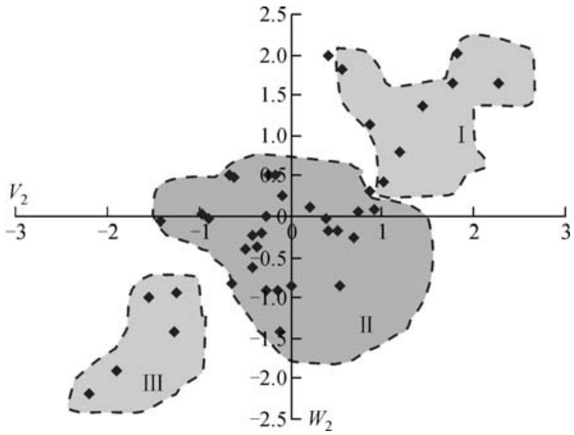

Fig. 5 Ordination and cluster of the second pair canonical variables of soil microorganisms and enzyme activities

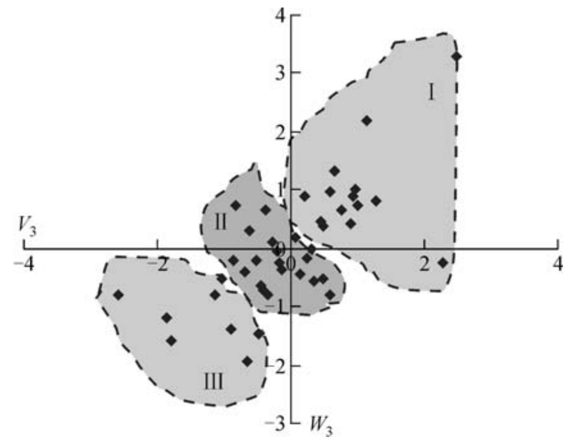

Fig. 6 Ordination and cluster of the third pair canonical variables of soil microorganisms and enzyme activities

In Fig. 4, types were clustered into categories I and II.

Type I: 20- to 40-cm soil layers of seven vegetation communities.

Type II: 0- to 20-cm soil layers of seven vegetation communities.

In Fig. 5, types were clustered into categories I, II, and III.

Type I: 20- to 40-cm soil layers of C_5 and C_6; the 0- to 40-cm soil layer of C_1.

Type II: 20- to 40-cm soil layers of C_2 and C_3; 0- to 20-cm soil layers of C_4, C_5, C_6, and C_7.

Type III: 0- to 20-cm soil layers of C_2 and C_3; 20- to 40-cm soil layers of C_4 and C_7.

In Fig. 6, types were clustered into categories I, II, and III.

Type I: the 0- to 40-cm soil layer of C_6; 20- to 40-cm soil layers of C_1, C_3, C_4, C_5, and C_7.

Type II: 20- to 40-cm soil layers of C_1, C_2, C_3, and C_4.

Type III: 0- to 20-cm soil layers of C_5 and C_7.

5 Conclusions and discussion

The dominant factors were nitrogen and phosphor in the soil nutrient indices and the number of microbes and carbon in microbial biomass/carbon in microorganisms. For enzyme activities, the dominant factors were urease, polyphenol oxidase, and dehydrogenase. Enzyme activities of urease and polyphenol oxidase are concerned with the transformation of soil nitrogen and phosphor, which affects the accumulation of carbon in microbial biomass and nitrogen. Based on their positive or negative effects, we can draw the conclusion that urease, polyphenol oxidase, and phosphatase are effective in the accumulation of carbon in microbial biomass; however, dehydrogenase was unfavorable to the accumulation of microbial biomass nitrogen. Another conclusion is that urease could increase soil nitrogen content and decrease phosphor content. However, there is a negative relation between polyphenol oxidase and nitrogen elements. So in degraded and eroded areas, soil nitrogen and phosphor elements, released from decomposing litter and decaying remnants, depend on microorganism activities. On the other hand, they also control indirectly soil enzyme activities and carbon in microbial biomass carbon/nitrogen accumulation and react to the vegetation restoration process.

The relations among available kalium, number of fungi, number of actinomyciae, sucrase, catalase, proteinase, and other soil biological chemical indices were statistically not significant. These findings are consistent with similar results of catalase (An et al., 2005). However, in *Eucalyptus* plantations soil catalase activities were related to kalium transformation, phosphor fixation and nitrogen conversion (Li et al., 2002). In non-irrigated farmland, soil catalase activities are related not only to organic carbon contents but also to available phosphor (Fan and Hao, 2003). In the Nverzhai watershed, possible reasons for catalase activities not to be seen as clearly related with soil nutrients or microorganisms are that soil erosion resulted in the loss of nutrients, that carbon and nitrogen contents decreased, that microorganism activities were reduced, and that the quality of vegetation restoration and community structures worsened. Therefore, the annual return of litter to the soil and root exudates lessened, which further resulted in lower soil catalase production.

In addition, the results of ordination and clustering of canonical variables of soil nutrients, microorganisms, and enzyme activities in different soil layers in different vegetation restoration communities, show a tendency for clustering of similar soil characteristics that offer a basis for the diagnosis of the health of the soil and for site type division.

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