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EFFECTS OF VEGETATION RESTORATION IN PURPLISH SOIL HILLY LAND ON SOIL MICROBIAL CHARACTERISTICS AND ENZYME ACTIVITIES

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Abstract

Spatial sequence, instead of time sequence, is used to analyse the effect of vegetation restoration in purplish soil hilly land of Hengyang of Hunan Province on soil microbial characteristics and enzyme activities and analyse their relationship. Results show (1) with the progress of vegetation restoration, soil dissolved carbon, alkali-hydrolysable nitrogen and rapidly available phosphorus, microorganism quantity, carbon-nitrogen content, respiration rate and activities of urease, invertase and catalase are significantly increased ($p < 0.05$). (2) Compared with the early stage (I), soil respiration rates in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) increase significantly, particularly in the middle and early stage (II) ($p < 0.05$). Moreover, compared with the initial stage (I), the differences in soil microbial biomass carbon and nitrogen ratio and metabolic entropy in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) are not significant ($p > 0.05$). (3) Correlation analysis shows that soluble carbon, alkali-hydrolysable nitrogen and rapidly available phosphorus are pairwise correlated. These soil indicators have good correlation with microbial biomass carbon and nitrogen content as well as urease, invertase and catalase activities ($p < 0.001$). Findings show that vegetation restoration can significantly improve soil biological properties.

Introduction

Microorganisms and enzymes are important promoters of soil nutrient cycling and transformation. All biochemical reactions in soil can be completed with the participation of microorganisms and enzymes (López *et al.* 2012). Microbial indicators and soil enzyme activities are sensitive to the change of soil microorganisms (Varma and Oelmüller 2007). Therefore, the study on changes in soil microbial characteristics and enzyme activities in the process of vegetation restoration, as well as the relationship between soil physical-chemical properties and microbial characteristics and enzyme activities, has great significance to vegetation restoration.

Purplish soil hilly land of Hengyang of Hunan Province, with an area of 1.625×10^5 hm^2, represents the ecological disaster prone areas in Hunan. In this area, vegetation is sparse; soil erosion and seasonal drought are also severe (Liu and Yang 2014). To improve the ecological environment of this area, a number of research on soil physical and chemical properties and soil moisture have been conducted (Yang *et al.* 2010,Yang *et al.* 2012). However, the research on changes in soil microbial characteristics and enzyme activities in vegetation restoration are rare. This work aims at studying the change in soil microbial characteristics and enzyme activity by using spatial sequence instead of time sequence (Kent and Coker 1992) and determine the relation of change in soil microbial characteristics and enzyme activity with soil physical and chemical properties. This study provides the scientific basis for vegetation restoration in this region.

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Materials and Methods

Hengyang purplish soil hilly land area is located in south central Hunan, which is located in the middle reaches of Xiangjiang (geographical coordinate: 110°32′ 16″ - 113°16′32″ E, 26°07′05″-27°28′24″ N). Hengyang have the hillock as the main geomorphological type which is distributed in the middle of 60 - 200 m above sea level. This region has a subtropical monsoon humid climate, with an annual average temperature of 18°C, extreme maximum temperature of 40.5°C, extreme minimum temperature of –7.9°C, average annual rainfall of 1325 mm and annual average evaporation of 1426.5 mm. The average relative humidity is 80%, and the annual frost free period is 286 days (Yang *et al.* 2014).

In August 2011, the sample plots in the middle and lower slopes along the contour were selected. The slopes (degrees and directions), altitudes, bare rock ratio and other ecological factors of the sample plots are almost the same. Plant communities were ranked in an increasing trend according to succession sequence as below: the early stage (i) (*Setaria viridi* community), the middle and early stage (ii) (*S. viridi* community), the middle and late stage (iii) (*Robinia pseudoacacia* + *S. viridi* community) and the late stage (iv) (*Liquidamdar formosana* + *Vitex negundo* var. *cannabifolia* − *Robinia pseudoacacia*). A 20 m × 20 m plot was set in each sample for vegetation survey and soil physical-chemical experiment. Meanwhile, five 1 m × 1 m quadrats were set in four corners and centre of each plot. Ten soil samples (0 - 20 cm), with 'S' or 'Quincunx' shape, were taken from each small quadrant and then mixed into a mixture sample. After removing visible stones, large roots (root diameter ≥ 1 mm) and residual roots in the soil, the soil was divided into two parts. One part of soil was dried in the shade for the determination of soil physical and chemical properties; another part was placed in a refrigerator at 4°C after being screened (2 mm) for the determination of soil microbial characteristics and enzyme activity. The findings are shown in Table 1.

Table 1. The basic condition of sampling sites.

Re-vegetation stages	I	II	III	IV
Slope/(°)/Aspect	21/SW	23/SW	22/SW	23/SW
Altitude/(m)	118	121	115	117
Position	The middle and lower slope	The middle and lower slope	The middle and lower slope	The middle and lower slope
Coverage/(%)	0	25	55	65
Dominant vegetation		*S. viridi; Eleusine indica*	*R. pseudoacacia; Melia azedarach; S. viridi*	*L. formosana; V. negundo;* var. *cannabifolia; Melia azedarach*

I = Initial, II = Middle and early, III = Middle and late, IV = Late. Same as in other Tables.

Chemical properties of soil: Dissolved organic carbon (DOC) (*w*/mg/kg) was extracted by using K_2SO_4 and determined through potassium dichromate digestion method. Alkali-hydrolysed nitrogen (AN) (*w*/mg/kg) was determined through alkali-hydrolysed diffusion absorption method. Available phosphorus (AP) (*w*/mg/kg) was extracted using $NaHCO_3$ and determined through ultraviolet spectrophotometry after molybdenum antimony colour-resistance (Bao 2000).

Microbial characteristics of soil: Microbial biomass carbon (MBC) (*w*/mg/kg) was determined through chloroform fumigation-K_2SO_4 extraction, with *K*-conversion coefficient of 0.38 (Vance *et al.* 1987). Microbial biomass nitrogen (MBN) (*w*/mg/kg) was determined through chloroform

fumigation-K_2SO_4 extraction-nitrogen automatic analyser method, with *K*-conversion coefficient of 0.45 (Sparling *et al.* 1993). Respiration rate (RER) ($w/g \cdot kg^{-1} \cdot d^{-1}$), which refers to respiratory volume of unit organic carbon in unit time, was determined through alkali absorption method. Metabolic entropy (qCO_2) ($w/mg \cdot mg^{-1}$) was determined by the volume of CO_2 generated from unit MBC every day (Zibilsk 1994, Anderson 2003).

Soil enzyme activities: Urease (URE) (*w*/ml/g) was determined by using phynol-sodium hypochlorite colorimetry. Invertase (INV) (*w*/ml/g) was determined by using 3,5-dinitrosalicylic acid (DNS). Catalase (CAT) (*w*/ml/g) was determined through $KMnO_4$ titration. Phosphatase ($w/mg \cdot g^{-1}$) was determined through disodium phenyl phosphate method (acid phosphatase (ACP) buffer was acetate in pH 5.0; alkaline phosphatase (ALP) buffer was borate in pH 9.6) (Guan 1986).

The experimental data were denoted by 'average value of three data ± standard deviation'. Duncan's multiple comparison was used to test the significance of difference (a = 0.05). Pearson analysis was used to make simple correlation analysis between all research indicators.

Results and Discussion

With the progress of vegetation restoration, soil soluble carbon, alkali-hydrolysable nitrogen and rapidly available phosphorus increase significantly ($p < 0.05$). Compared with the early stage (I), the three values in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) are increased by 24.06, 244.38% and 361.93; 35.90, 73.50 and 143.59% and 38.67, 88.32 and 182.48%, respectively.

Table 2. Variations of soil DOC, AN and AP in different re-vegetation stages.

Re-vegetation stage	DOC/ (mg/kg)	AN/ (mg/kg)	AP/ (mg/kg)
I	50.7 ± 6.09 a	117 ± 10.8 a	137 ± 12.1 a
II	62.9 ± 7.00 a	159 ± 12.5 ab	190 ± 20.0 ab
III	174.6 ± 15.54 ab	203 ± 20.4 b	258 ± 23.5 b
IV	234.2 ± 22.76 b	285 ± 30.5 c	387 ± 40.3 c

Different lower case letters in same column mean significant differences among different re-vegetation stages at the 0.05 level. The same in below table. AN: Alkali-hydrolysable nitrogen; AP: Available phosphorus.

Soil MBC content increases significantly ($p < 0.05$) with the progress of vegetation restoration (Table 3). Compared with the early stage (I), soil MBC contents in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) are respectively increased by 71.21%, 94.70% and 156.82%. The MBN content in the late stage (IV) is significantly higher than those in the early stage (I), the middle and early stage (II) and the middle and late stage (III) ($p < 0.05$). The soil MBN contents in the early stage (I), the middle and early stage (II) and the middle and late stage (III) are only 25.09, 35.15 and 43.17% of the soil MBN content in the late stage (IV). The soil MBC–nitrogen ratio in the late stage (IV) is significantly lower than those in the early stage (I) by 64.37%, those in the middle and early stage (II) by 52.69% and those in the middle and late stage (III) by 63.10% ($p < 0.05$). The respiration rates in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) are significantly higher by 122.37, 28.95 and 40.79%, respectively, than that in the early stage (I) ($p < 0.05$). The metabolic entropy in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) is higher than that in the early stage (I), with insignificant difference ($p > 0.05$).

The findings show that the activities of urease and catalase in soil are significantly increased ($p < 0.05$) with the progress of vegetation restoration (Table 4). The activities of urease and catalase in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) are 122.92%, 130.21 and 247.92% and 56.14, 91.23 and 115.79%, respectively, higher than that in the early stage (I). Invertase activities in the middle and late stage (III) and the late stage (IV) are significantly higher than those in the early stage (I) and the middle and early stage (II) ($p < 0.05$), which increased by 44.52 and 95.21%, respectively, for acid phosphatase and 93.58 and 161.47%, respectively, for alkaline phosphatase in all restoration stages. The differences are not significant ($p > 0.05$).

Table 3. Variations of soil microbial properties in different re-vegetation stages.

Re-vegetation stages	MBC/ (mg/kg)	MBC/ (mg/kg)	MBC/ MBN	RER/ ($g.kg^{-1}.d^{-1}$)	Metabolic entropy qCO^2/ (mg/mg)
I	132 ± 22.5 a	14.7 ± 1.09 a	8.98 ± 0.87 ab	0.76 ± 0.08 c	1.24 ± 0.43 a
II	226 ± 34.6 ab	20.6 ± 3.43 a	10.97 ± 1.98 a	1.69 ± 0.12 a	1.75 ± 0.32 a
III	257 ± 38.0 ab	25.3 ± 2.87 a	9.16 ± 2.54 ab	0.98 ± 0.09 ab	1.36 ± 0.65 a
IV	339 ± 41.7 c	58.6 ± 6.54 b	5.78 ± 0.67 b	1.07 ± 0.15 b	1.83 ± 0.79 a

MBC: Microbial biomass carbon, MBN: Microbial biomass nitrogen, RER: Respiration rate.

Table 4. Soil enzyme activities in different re-vegetation stages.

Re-vegetation stages	URE/ (ml/g)	INV/ (ml/g)	CAT/ (ml/g)	ACP/ (mg/g)	ALP/ (mg/g)
I	0.96 ± 0.03 a	1.46 ± 0.04 a	0.57 ± 0.04 a	1.31 ± 0.02 a	0.79 ± 0.03 a
II	2.14 ± 0.01 ab	1.09 ± 0.03 a	0.89 ± 0.03 b	1.33 ± 0.03 a	0.58 ± 0.04 a
III	2.21 ± 0.02 ab	2.11 ± 0.02 b	1.09 ± 0.02 c	1.35 ± 0.01 a	0.67 ± 0.05 a
IV	3.34 ± 0.02 b	2.85 ± 0.03 c	1.23 ± 0.03 c	1.28 ± 0.02 a	0.95 ± 0.04 a

URE: Urease; INV: Invertase; CAT: Catalase; ACP: Acid phosphatise; ALP: Alkaline phosphatise.

Soluble carbon, alkali-hydrolysable nitrogen and rapidly available phosphorus are pairwise correlated (Table 5). These soil indicators have good correlation ($p < 0.001$) with MBC and nitrogen content as well as urease, invertase and catalase activities. The correlation coefficients between urease activity and MBC and nitrogen content are 0.86 and 0.87 ($p < 0.001$), respectively. The correlation coefficient between catalase activity and MBC content is 0.81 ($p < 0.01$), and its correlation coefficients with urease and invertase activities are 0.90 and 0.89 ($p < 0.001$), respectively. The correlation coefficient between MBC - nitrogen ratio and MBN is –0.71 ($p < 0.05$).

During vegetation restoration of Hengyang purplish soil hilly land, the growth of plants increases the vegetation cover and reduces the loss of runoff, sediment and nutrients. Root exudation and litter increase the input of organic carbon, promote the cycling and transformation of carbon and nitrogen and accelerate the growth and reproduction of microorganisms, thus increasing MBC and nitrogen content and the correlative enzyme activities to carbon and nitrogen element. However, the content of rapidly available phosphorus in soil is significantly increased ($p < 0.05$) with the progress of vegetation restoration (Table 2), but the changes in acid (alkaline) phosphatase in all restoration stages are not significant, and the correlation between rapidly available phosphorus and acid (alkaline) phosphatase does not reach the significant level ($p > 0.05$) (Tables 4 and 5). The

Table 5. Correlation of all indicators in soil.

Item	DOC	AN	AP	MBC	MBN	MBC/MBN	RER	qCO_2	URE	INV	CAT	ACP
AN	0.91***											
AP	0.92***	0.94***										
MBC	0.86***	0.91***	0,90***									
MBN	0.85***	0.88***	0.89***	0.60								
MBC/MBN	–0.52	–0.48	–0.49	–0.21	–0.71*							
RER	0.23	0.08	0.10	0.21	–0.08	0.32						
qCO_2	0.19	0.41	0.35	0.19	0.39	–0.43	0.45					
URE	0.79**	0.90***	0.91***	0.86***	0.87***	–0.42	0.34	0.46				
INV	0.87***	0.84***	0.89***	0.60	0.78	–0.56	–0.36	0.29	0.69*			
CAT	0.85***	0.86***	0.90***	0.81**	0.73	–0.44	0.25	0.45	0.90***	0.89***		
ACP	–0.31	–0.15	–0.18	–0.20	–0.19	–0.22	0.05	0.26	–0.09	–0.18	0.00	
ALP	0.43	0.35	0.30	0.34	0.51	–0.35	–0.27	–0.36	0.27	0.34	0.08	–0.35

DOC: Soluble carbon; AN: Alkali-hydrolysable nitrogen; AP: Rapidly available phosphorus; MBC: Microbial biomass carbon; MBN: Microbial biomass nitrogen; MBC/MBN: Microbial biomass carbon–nitrogen ratio; RER: Respiration rate; $qCO2$: Metabolic entropy; URE: Urease; INV: Invertase; CAT: Catalase; ACP: Acid phosphatase; ALP: Alkaline phosphatase. *Mean correlation at 0.05 level; **Mean correlation at 0.01 level and ***Mean correlation at 0.001 level.

activity of phosphatase is not only affected by rapidly available phosphorus but also closely related to the humus content of the soil and microbial population, which can decompose phosphorus compound (Kautz *et al.* 2004, Kaur *et al.* 2005). Therefore, here the differences in the humus content of the soil and microbial population decomposing phosphorus compounds in all restoration stages may be the reason for the insignificant difference in phosphatase activity.

With the progress of vegetation restoration, respiration rate of soil is significantly increased, especially in the middle and early stage (II), which had the highest respiration rate ($p < 0.05$) (Table 3). Moreover, the ability and strength of soil microorganisms to decompose mineral organic matter are enhanced (Joergensen *et al.* 1995). Even in different restoration stages, the difference in metabolic entropy is insignificant ($p > 0.05$) (Table 3). However, with the progress of restoration, metabolic entropy shows the increasing trend, indicating that the progress of restoration helps young active microbial population in soil to increase (Vance *et al.* 1987). Different MBC and nitrogen ratios reflect the strong changes in community structure of soil bacteria and fungi. The carbon-nitrogen ratio of bacteria is commonly 3.5, and the carbon-nitrogen ratio of fungi is about 10 -15 (Paul and Clark 1996). In the experiment, the changes in soil MBC-nitrogen ratio in different restoration stages are not obvious. The carbon-nitrogen ratio in the late stage (IV) is lower, which may be attributed to the decrease in fungal population with the increase of bacterial population. More changes in the characteristics of microbial population will be further studied. In this paper, the MBC-nitrogen content and enzyme activity increase significantly ($p < 0.05$) with the progress of vegetation restoration (Table 3). MBC-nitrogen content and soil enzyme activity are important biological features and symbol of soil quality improvement. Therefore, during vegetation restoration, the study on soil microbial characteristics should be enhanced to better guide the sustainable development of agriculture and forestry in this area.

In brief, with the progress of vegetation restoration, soluble carbon, alkali-hydrolysable nitrogen, rapidly available phosphorus, MBC-nitrogen content and respiration rate are significantly increased ($p < 0.05$), and the activities of urease, invertase and catalase are also increased significantly ($p < 0.05$). Among which, the above indicators and activities in the late stage (IV) are the highest. However, the difference in acid (alkaline) phosphatase between different restoration stages is not significant ($p > 0.05$). Compared with the early stage (I), soil respiration rates in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) are obviously increased. Particularly, soil respiration rate in the middle and early stage (II) is the highest ($p < 0.05$). Moreover, compared with the early stage (I), soil MBC-nitrogen ratios and metabolic entropy in the middle and early stage (II), the middle and late stage (III) and the late stage (IV) are insignificantly different ($p > 0.05$). Besides, the correlation analysis shows that soluble carbon, alkali-hydrolysable nitrogen and rapidly available phosphorus are pairwise correlated. They have good correlation with MBC and nitrogen content as well as urease, invertase and catalase activity ($p < 0.001$).

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