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Dynamics of Soil Microbial Properties following Land Utilization Types in a Karst Region, Southwest China*

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Land-use change can have great influences on soil conditions and microbes are likely respond to these changes. However, such responses are poorly characterized as few studies have examined how changes in soil microbes do, or do not, correlate with environmental factors across land-use types. Soil microbial, conventional, and mineral properties and vegetation were investigated and analyzed under farmland, grassland, brush, plantation forest, secondary forest, and primary forest in the karst region of southwest China. Soil main microbial populations varied among land-use types, total populations were large in the primary forest and farmland, and low in the plantation forest. The three forests had a higher proportion of bacteria, and other types had a higher proportion of actinomycetes, while all the types had a low proportion of fungi. Soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were highest in primary forest. Only MBC and microbial populations had a perfect fractal relationship. MBC had closest relationships with Shannon index in tree layer and TN, Fe_2O_3, and CaO. Soil microbial biomass was high, while microbial status was perfect in the primary forest. Microorganisms were significantly correlated with vegetation, soil nutrients, and minerals following land utilization types in the karst region of China.

Key words: Soil microbe, Environmental factor, Land utilization type, Karst region, China.

Soil microbes are widely recognized as an integral component of terrestrial ecosystems, and as early indicators to evaluate changes in soil quality due to their high sensitivity to land use change (Rietz and Haynes, 2003; Yuan *et al*., 2007). Soil microbes are driving many fundamental nutrient cycling processes, soil structural dynamics, degradation of pollutants, stabilization of soil aggregates various other services (Powlson *et al*., 1987) and respond quickly to environmental perturbations due to their short generation time and their intimate relation with their surroundings, attributed to their higher surface to volume ratio. Meanwhile, soil microbes promote energy flow and substances recycles through decomposing animals and vegetation, and then profoundly change vegetation pattern at the early succession stage and the later succession processes (Kardol *et al*., 2007). In turn, plant species composition and community structure influence soil microbial properties on account of microbes feeding on plant

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secretes and remains of dead plants and animals (Hooper *et al*., 2000). It is documented that the relationships between soil microbes, plant, and soil nutrients were close (Peng *et al*., 2011; Du *et al*., 2011).

Depression between karst hills is a typical landscape with an area of 12.7 × 10^4 km^2, mostly located in Guangxi, in southwest China. Karst poverty was serious owing to composite degradation state similar to desertification and acute conflict between human being and land resulting from low soil fertility and severe soil erosion (Peng *et al*., 2008). In order to control expansion of rocky desertification, many researches on soil microbial properties under different environmental conditions have been documented in karst areas. Spatial pattern of soil microbes varied in different ecosystems (Liu *et al*., 2010; Zhang *et al*., 2012). Measurements of returning land from farming to forestry or grass meliorated the soil microbial properties, among which natural restoration had the best effects (Lu *et al*., 2012; Long *et al*., 2004). However, studies on soil microbial characteristics under different land utilization types in karst region were little.

In the study, two representative community types were respectively chosen in the six land use types (farmland, grasses, shrub, plantation forest, secondary forest, and primary forest), in the karst region in southwest of China. The major aims of the study were to investigate (1) fractal features between soil microbial populations and soil microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) and (2) relationships between soil microbial properties and soil conventional nutrients, mineral nutrients, and vegetation. This would provide theoretical basis for vegetation recovery and ecological restoration in the area even in the whole karst region.

MATERIALS AND METHODS

Description of the experimental site

The experimental site is located in Huanjiang Maonanzu Autonomous County, northwest Guangxi Province (107°51´ to 108°43´E, 24°44´ to 25°33´N), China, with a peak elevation of 1028.0 m. The location has a subtropical monsoon climate, with an annual average temperature of 19.3 °C and annual average precipitation of 1389 mm, approximately 70% of which occurs from April to September. The frost-free period lasts for about 290 days and the average annual duration of bright sunlight is around 1450 h. The average annual evaporation is 1571 mm and the average relative humidity is 70%. Huanjiang became one of the counties with rocky desertification comprehensive management in China, due to thin soil layer, bare rocks, and steep slopes resulting in serious loss of soil and water and rocky desertification. The soil was chromic or brown rendzinas derived from carbonate rocks. The land utilization types in the studied area were farmland, grassland, shrub, plantation, secondary forest and primary forest, with distinct vegetation communities.

Experimental design and investigation

After an overall investigation, two representative community types were respectively chosen under six land utilization types, *i.e.*, slope farmland: cultural history with corn (*Zea mays*) + soybean (*Giycine max*) and sugarcane (*Saccharum sinensis*) + sweet potato(*Ipomoea btatas*); grassland: *Ischaemum indicum* and *Imperata cylindrical*; shrub: *Vitex negundo* and *Rhus chinensis*; plantation forest: *Zenia insignis* and *Toona sinensis*; secondary forest: *Alangium chinense* and *Itoa orientalis*; primary forest: *Biota orientalis* and *Sinosideroxylon pedunculatum*. Plots with 20 m×20 m were established on lower part of slopes with identical or similar slope direction, slope angle, and elevation. Each community type had three plots and there were a total of 36 plots. Each plot was divided into four quadrats of 10 m×10 m and 16 micro-quadrats of 5 m×5 m using the interpolation method. Taken micro-plot as basic unit, all the individual trees with *DBH* ≥ 1 cm were tagged, identified, measured for breast-height diameter, layer coverage, breadth of crown and height. Brush and grass species, population, height, and other growth status were investigated in five micro-quadrats with five points sampling at random. Geographic information of longitude and latitude, and elevation of the center of the plots was obtained from a GPS (E640+ Mobile Mapper).

Soil sampling and chemical analysis

Simultaneously, several soil cores (5 cm diameter) were taken from the marked area of each land utilization type at the 0-20 cm soil depth. These samples were mixed to form a pooled sample and divided in two parts for physicochemical as well as microbiological analyses. Soil samples were processed (sieved through 2 mm to remove roots and gravels) and stored in fridge at a constant temperature of 4 °C for microbial analyses. Air dried soil samples were used for physicochemical analysis.

All the soil properties were determined using three replicated samples. Soil main nutrients included pH values (pH), soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen (AN), available phosphorus (AP), available potassium (AK), SiO_2, Fe_2O_3, CaO, MgO, Al_2O_3, and MnO, were analyzed in the laboratory according to Bao (2000) and Liu (1997).

Soil microbial biomass C, N, and P

Soil MBC, MBN and MBP were estimated with the chloroform fumigation and extraction procedure (Brookes *et al*., 1985; Vance *et al*., 1987). Soil MBC was calculated as MBC = Ec/0.45 (Wu *et al*., 1990), where Ec (organic C extracted from fumigated soil)) – (organic C extracted from non-fumigated soil). Soil MBN was calculated as: MBN = $E_N/0.54$, where E_N = (total N extracted from fumigated soil) – (total N extracted from non-fumigated soil) (Brookes *et al*., 1985; Joergensen and Mueller, 1996). Soil MBP was calculated as MBP = {[(inorganic P (Pi) extracted from fumigated soil)] – (Pi extracted from non-fumigated soil)}/0.40 (Brookes et al., 1982). A correction for incomplete extraction of Pi released by $CHCl_3$ was made by determining the percentage of recovery of a known quantity of Pi spiked to $NaHCO_3$ solution followed by extraction of a non-fumigated soil.

Soil microbial counts

Bacteria, actinomycetes and fungi were measured with standard plate count methods (Lorch *et al*., 1995). Duplicate samples of 10g were extracted with sterile phosphate tamponed water in six steps of dilution, and cultivated on sterile plates. For bacteria, plates with PCA (Plate count Agar) were incubated for 48h at 30 °C. For actinomycetes, plates with actinomycetes agar were incubated for 6 days at 25°C. For fungi, plates with OGYEA (Sabouraud Oxytetracycline Agar Base) were incubated for 6 days at 25°C. values were expressed in CFU (colony forming unit) ml^{-1}.

Statistic analysis

The 35 indexes were divided into four groups of variables, among which, soil microbial properties as the first group including MBC, MBN, and MBP, microbial number of bacteria, fungi, and actinomycetes; vegetation indexes as the second group, including richness, Simpson index, Shannon index, Evenness, density, coverage, and average height in tree layer, shrub layer, and grass layer, respectively; soil conventional nutrients as the third group, including pH, SOC, TN, TP, TK, AN, AP, AK; soil mineral nutrients as the fourth group, including SiO_2, Fe_2O_3, CaO, MgO, Al_2O_3, and MnO. Vegetation community diversity indexes were calculated according to (Ma *et al*., 1995) and fractal features according to (He and Zhao, 2003; Wang *et al*., 2008; Wang *et al*., 2010). Data processes, fractal feature calculation, and canonical correlation analysis were conducted in SPSS 16.0. One-way ANOVA analysis was used to determine the effect of a single factor, and tests with $p<0.05$ and $p<0.01$ were considered significant.

RESULTS

Changes in soil microbial populations

Soil microbial populations and composition varied in the land utilization types in the karst region (Table 1). The sum of soil microbial populations increased in the order of slope farmland > primary forest > grassland > secondary forest > shrub > plantation forest, which in slope farmland significantly higher than the other land utilization types, while the other types had no significant differences between each other. This may be due to manmade fertilization in the slope farmland resulted in an increase of microbial populations. Bacteria population was in the sequence of primary forest > slope farmland > secondary forest > shrub > grassland > secondary forest, which in primary forest and slope farmland had no significant differences but significantly higher than the other land utilization types. Actinomycetes population was in the order of slope farmland > primary forest > grassland > shrub > secondary forest > manmade forest, which in slope farmland was significantly higher than the other land utilization types. Bacteria accounted for 11.3%-63.6% of soil microbial population composition in six land utilization types. The ratio of fungi population in the three forest types was largest, which was significantly larger than brush and grassland ($p<0.05$) and significantly

Table 1. Soil Main microbial populations under different land-utilization types*

Types	Bacteria		Fungi		Actinomycetes		Sum (cfu ·g^{-1})
	Quantity(cfu · g^{-1})	%	Quantity (cfu · g^{-1})	%	Quantity (cfu · g^{-1})	%	
Slope farmland	856147.50Aab	11.3Bc	40755.41Aab	0.5Aa	8739897.33Aa	88.2Aa	9636800.25Aa
Grassland	111095.58Ab	41.9Ab	442.97Ab	0.1Aa	226056.50Bb	58.0Bb	337595.03Bb
Shrub	77916.34Ab	41.1Ab	538.71Ab	0.3Aa	105523.41 Bb	58.6Bb	183978.45Bb
Plantation forest	27134.67Ab	60.2Aab	258.07Ab	0.6Aa	14312.83 Bb	39.3Bbc	41705.57Bb
Secondary forest	128991.13Ab	63.6Aa	1157.28Ab	0.6Aa	59260.92 Bb	35.8Bc	189409.33Bb
Primary forest	1949717.15Aa	58.7Aab	71574.13Aa	0.9Aa	1119279.75 Bb	40.5Bbc	3140570.89Bb

*Different capital letters and lowercase letters within a row indicated significant difference at 0.01 and 0.05 levels, respectively.

Table 2. Soil microbial biomass carbon, nitrogen, and phosphorus under different land-utilization types*

Types	MBC (mg·kg^{-1})	MBN (mg·kg^{-1})	MBP (mg·kg^{-1})	MBC/SOC	MBN/TN	MBP/TP	MBC/MBN
Slope farmland	448.76ABb	78.35Bb	13.52Aa	1.68Aa	2.71Aab	1.03Aa	6.44ABa
Grassland	317.51Bb	52.50Bb	13.54Aa	1.07ABabc	2.03Aa	9.63Aa	7.02Aa
Shrub	246.43Bb	136.88Bb	41.18Aa	0.76ABbc	4.83Aab	5.21 Aa	2.77Cb
Plantation forest	239.69Bb	75.55Bb	14.21Aa	1.56Aab	5.03Aab	1.47Aa	3.20BCb
Secondary forest	182.15Bb	180.31ABb	33.98Aa	0.34Bc	3.32Aab	5.86Aa	1.04Cb
Primary forest	946.21Aa	323.31Aa	44.78Aa	1.41ABab	7.23Aa	5.76Aa	2.75Cb

*Different capital letters and lowercase letters within a row indicated significant difference at 0.01 and 0.05 levels, respectively larger than slope farmland ($p<0.01$), while that in brush and grassland was significantly higher than grassland ($p<0.05$). Actinomycetes accounted for 35.8%-88.2% of the sum of soil microbial population, which in the slope farmland was significantly higher than the other land utilization types at $p<0.01$, which in the grassland and shrub was significantly higher than primary forest and secondary forest at $p<0.05$. Fungi accounted only for 0.1%-0.9% of the sum of soil microbial population, and the differences between the land utilization types were not significant.

Variations in soil microbial biomass C, N, and P

Soil MBC, MBN, and MBP varied between the land utilization types in the karst region (Table 2). Soil MBC presented in the order of primary forest > slope farmland > grassland > shrub > plantation forest > secondary forest, among which primary forest was significantly higher than slope farmland at $p<0.05$ and significantly higher than the other land utilization types at $p<0.01$, while the differences between the other types were not significant. Soil MBN was in the sequence of primary forest > secondary forest > shrub > slope farmland > plantation forest > grassland, among which primary forest was significant higher than the other types at p<0.05 and there were no significant differences among other types. Soil MBP presented in the order of primary forest > brush > secondary forest > plantation forest > grassland > slope farmland, with no significant differences among the types. Moreover, the ratios of MBC to SOC (MBC/SOC), MBN to TN (MBN/TN), and MBP to TP (MBP/TP) ranged from 0.34 to 1.68, from 2.03 to 7.23, and from 1.03 to 9.63, respectively. The values of MBC/MBN ranged from 1.04 to 7.02, among which of grassland and slope farmland was largest and significantly higher than other types at $p<0.05$, while other types had no significant differences.

Fractal characteristics of MBC, MBN, MBP and microbial populations

Soil microbial biomass could reflect microbial population quantity which takes part in regulating energy and nutrient cycling and conversion of soil organic matter. From Table 3, MBC had fine fractal relationships with fungi, bacterium, and actinomycetes populations, with correlation coefficients being 0.653, 0.696, and 0.518 at significant level ($p<0.01$), respectively. The values of fractal dimensions, D, were 2.323, 4.424, and 3.511, respectively, which indicated these fractal relationships had statistical meaning. After F test, fractal correlation coefficients between MBN, MBP and bacterium, fungi, actinomycetes populations did not reach significant levels, which suggest no fractal relationships exist among them.

Relationships between soil microorganisms and environment

In the section, the 35 indices examined in the six land-use types within the karst region were divided into four classes. Canonical correlation analysis (CCA) was used to determine the relationships among soil microbial properties and vegetation, soil physicochemical properties, and minerals. The sum of the first, the second and the

Table 3. Fractal characteristic models between soil microbial population and biomass

Models	Correlation coefficients*R*	*F*-test *t*-		test			
				Constant		Slope *k*	
		F	P	t	P	t	P
$\ln Y_C=2.323+0.286\ln X_F$	0.655	25.528	<0.01	3.457	<0.01	5.053	<0.01
$\ln Y_C=4.424+0.183\ln X_B$	0.619	21.077	<0.01	15.228	<0.01	4.591	<0.01
$\ln Y_C=3.511+0.181\ln X_A$	0.579	17.178	<0.01	6.576	<0.01	4.145	<0.01
$\ln Y_N=3.530+0.096\ln X_F$	0.208	1.541	0.223	3.844	<0.01	1.241	0.223
$\ln Y_N=4.426+0.034\ln X_B$	0.109	0.405	0.529	11.402	<0.01	0.637	0.529
$\ln Y_N=4.779-0.010\ln X_A$	-0.030	0.030	0.863	6.910	<0.01	-0.174	0.863
$\ln Y_P=1.997+0.078\ln X_F$	0.147	0.753	0.392	1.871	0.07	0.868	0.392
$\ln Y_P=2.954-0.006\ln X_B$	-0.016	0.008	0.928	6.582	<0.01	-0.091	0.928
$\ln Y_P=3.128-0.018\ln X_A$	-0.047	0.074	0.787	3.938	<0.01	-0.272	0.787

Y_C: Soil MBC; Y_N: Soil MBN; Y_P: Soil MBP. X_B: Bacteria population; X_F: Fungi population; X_A: actinomycetes population.

third largest eigenvalues had a proportion about 75% out of the sum of all eigenvalues, which indicated that they could represent most information in this problem. The eigenvectors associated soil microorganisms and vegetation were marked on the Fig.1. The first eigenvalue $\lambda_1 = 9.110$ (data not shown). Its square root $\rho_1 = 0.976$ ($P<0.01$) is the correlation coefficient between the first pair of canonical variables u_1 and v_1. It can be found that the weight of MBC and MBP were most important in u_1, while Shannon index in tree layer in v_1. This indicated soil MBC and MBP had the largest positive effects on the Shannon index in the tree layer. The second eigenvalue $\lambda_2 = 4.297$ (data not shown), whose square root $\rho_2 = 0.876$ ($P<0.01$). The weights of each variables showed that MBC and quantity of fungi were highly negatively correlated with evenness in grass layer.

The eigenvectors associated soil microorganisms and physicochemical properties were marked on the Fig.2. The eigenvalues $\lambda_1 = 5.442$, $\lambda_2 = 3.515$, and $\lambda_3 = 1.438$ (data not shown), whose square root $\rho_1 = 0.952$, $\rho_2 = 0.905$, $\rho_3 = 0.777$ ($P<0.01$), respectively. The weights of the first pair of canonical variables revealed that quantity of fungi were positively correlated with AN. In the second pair of canonical variables, MBC and quantity of fungi were highly correlated with SOC and TN. In the third pair of canonical variables, quantity of actinomycetes was negatively correlated with SOC and positively correlated with TN.

The eigenvectors associated soil microorganisms and total minerals were marked on

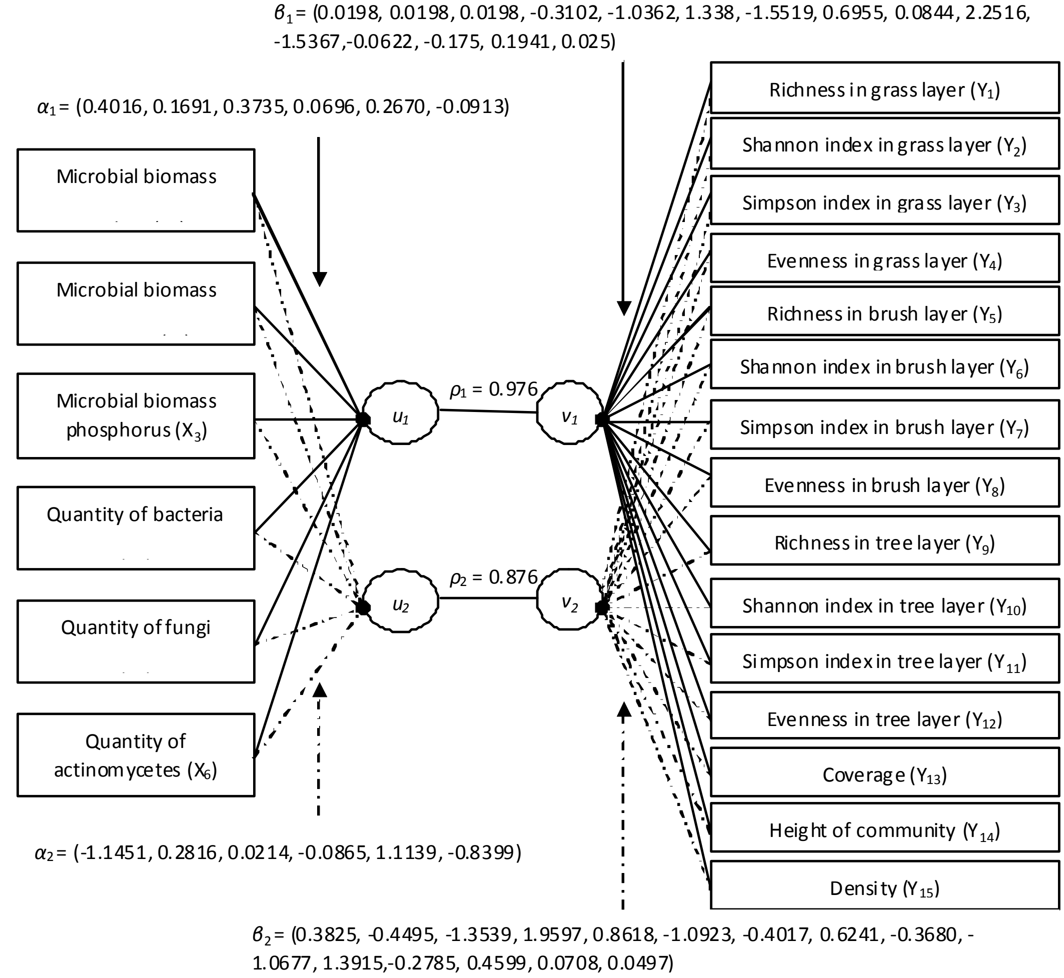

Fig. 1. CCA analysis result of soil microorganism and vegetation

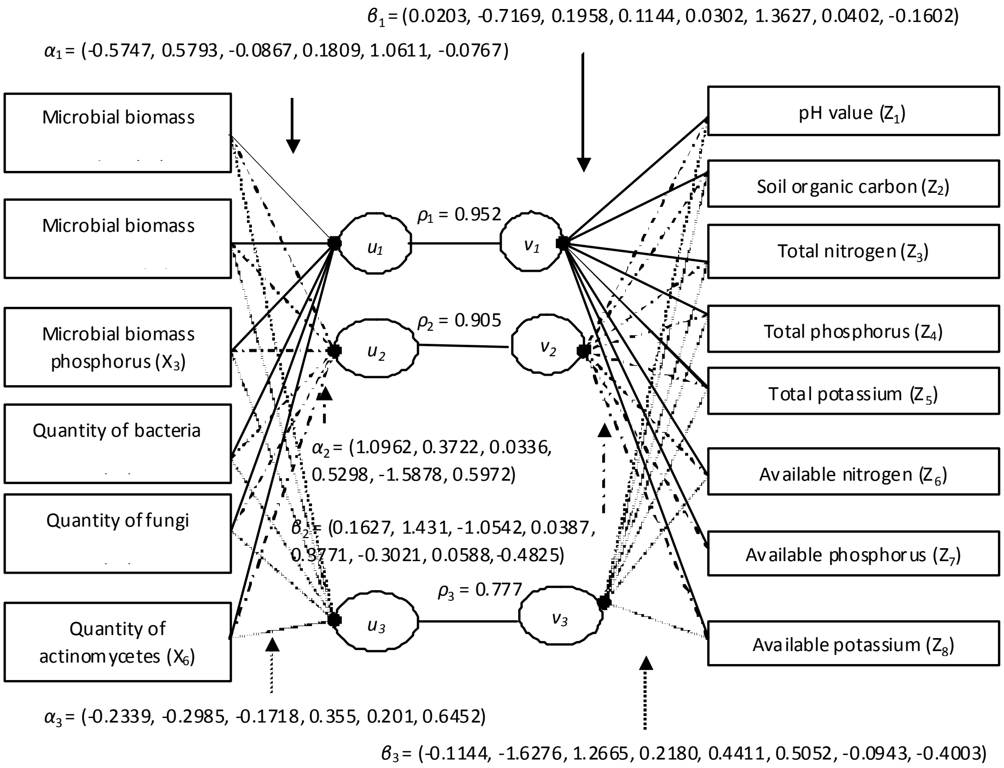

Fig. 2. CCA analysis result of soil microorganism and physicochemical properties

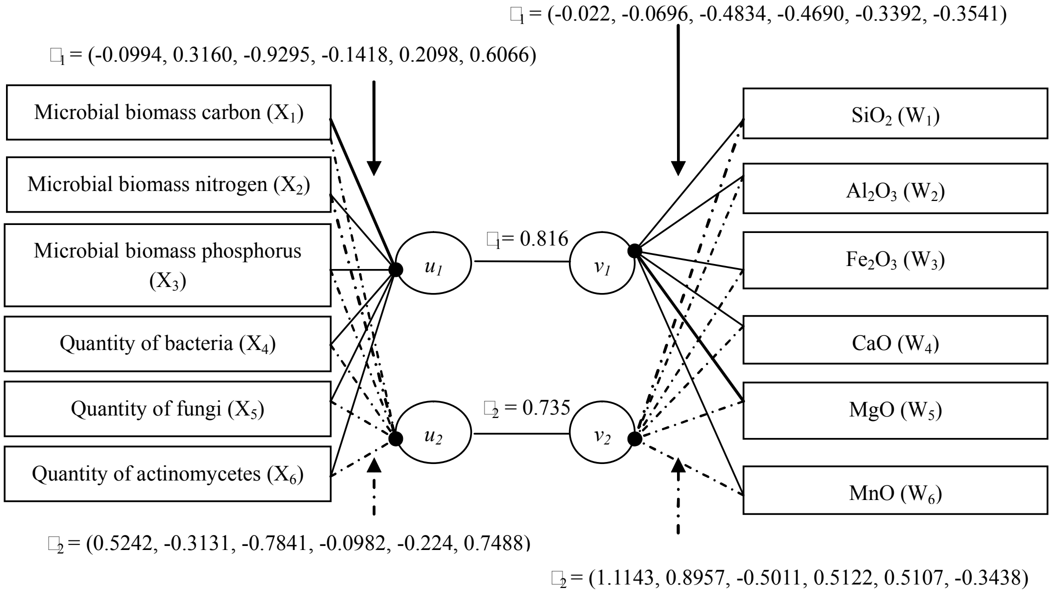

Fig. 3. CCA analysis result of soil microorganism and soil total minerals

the Fig.3. The eigenvalues $\lambda_1 = 3.999$, and $\lambda_2 = 3.368$ (data not shown), whose square root $\rho_1 = 0.816$, and $\rho_2 = 0.7355$ ($P<0.01$), respectively. The weights of the first pair of canonical variables indicated that MBP was positively related to Fe_2O_3 and CaO. In the second pair of canonical variables, MBP and quantity of actinomycetes were closely related to SiO_2 and Al_2O_3.

DISCUSSION

Soil microbial properties under different land utilization types in the karst region

Soil microbial populations, distribution, and composition, to a large extent, influence and even determine the biological activity, decomposition of organic matter, humus synthesis, soil aggregate formation, and soil nutrients conversion (Harris, 2003). Soil microbial population was large in the karst region, among which, the sum, bacterium and actinomycetes population were largest in the primary forest and lowest in the plantation forest. Commonly, bacteria predominated in soil microbial population composition. However, soil microbial population composition varied in the karst region. In the slope farmland, grassland, and brush, actinomycetes accounted for larger proportions, maybe owing to its fecundity, competitive power and consumption on soil nutrients stronger than other populations. Bacteria population was larger in the three forests, and fungi accounted for less than 1%. This was agreed with Song *et al*. (2006).

Soil microbial biomass is an ideal biological index of soil quality (Arunachalam and Pandey, 2003). Soil MBC, MBN, and MBP were important indexes of soil biological fertility, while vegetation types were the important factors to soil microbial activities (Zhu *et al*., 2006). Contents of MBC, MBN, and MBP in the karst region was close to or even past those in the subtropical paddy field (Liu *et al*., 2003), especially highest in the primary forest. This indicated that vegetation recovery highly influenced soil microbial biomass in the karst fragile ecosystems, and also reflected that soil microbial biomass was an indicator in monitoring soil quality. Under this circumstance of no external disturbances, soil microbial biomass can not fully reflect microbial activity, structure, and function (Vance *et al*., 1987). Therefore, from the microbial view, besides the absolute amount of microbial biomass, the proportion of MBC, MBN, and MBP respective to SOC, TN, and TP should be taken into account for revealing soil microbial quality variation under different utilization types. The ratios of MBC to SOM in the surface layer in dry land ranged from 0.5% to 4%, and those of MBN to TN from 2% to 6%, and MBP to TP from 1% to 5% (Wu, 1994). In comparison, both the ratios of MBC to SOC and MBP to TP were low in the surface soil in karst region, resulting from the contents of SOC, MBC, TP, and MBP declining synchronously. Whether the ratios of MBC to MBN were constant, scholars had various viewpoints. In the study, the ratios of MBC to MBN in slope farmland and grassland were similar to 6.7 (Anderson and Domsch, 1980) and 6.2 in red soil (Chen and He, 1998), when that in the brush, plantation, and secondary forest was obviously lower. This suggest that the karst area lacks soil, but around bared rocks could increase weathering litter input to soil resulting in higher contents of soil organic carbon and nitrogen and longer periods of microbial metabolism function. Therefore, in order to maintain nitrogen resources and other nutrients for vegetation growth, microbial activities should be improved to keep high substance metabolism ability.

Fractal characteristics between soil microbial biomass and microbial populations

Since fractal theory has been introduced into ecology, it is widely adopted in the studies on distribution pattern of vegetation population, soil aggregates, and other fields (Wang *et al*., 2008; Dang *et al*., 2007). In the study, soil MBC, bacteria, fungi, and actinomycetes populations had highly significant fractal features and the accuracy of fractal model and fractal dimensions (D) reached a significant level. Fractal dimensions (D) represent a variable spatial distribution dimension feature, whose larger values indicated the variable accumulated more in space and distributed larger spaces (Lu *et al*., 2012). In the study area, accumulation of soil MBC differed from the spatial pattern of soil microbial populations, related most to bacteria ($D= 4.424$), following by actinomycetes ($D=3.511$) and fungi ($D=2.323$). This may be resulted from differences in soil parental material, main vegetation types, and human activities. The fractal relationships between MBN, MBP and the three microbial populations did not reach significant levels, and the causes need further researches. Not so in models of returning farmland to forests or grassland in the karst region, the relationships between MBC and bacteria, and actinomycetes, and between MBN and fungi showed distinct fractal features (Lu *et al*., 2012). Therefore, variation in soil MBC could be analyzed to predict dynamic changes in microbial populations under different land utilization types, and to effectively warn on soil health variation.

Relationships between soil microbes and environment

Under human disturbances, climax ecosystems in ttthe karst region, i.e azonal evergreen and deciduous broad-leaved mixed forest, degenerated at various degrees, even rocky desertification at some zones (Song *et al*., 2008). In recent years, karst degraded ecosystems have been recovered gradually along the full operation of projects of grain for green, natural forest protection, and rocky desertification management, and implement of kinds of environmental protection policy (Zeng *et al*., 2007). The relationships between soil microobes, nutrients, minerals, and vegetation were always dynamic in the karst region. Secondary minerals generated during carbonate rocks weathering processes continuously release various mineral nutrients and result in enhancement in soil fertility and soil microbial properties, and then together regulate vegetation species composition, vegetation types, and growth and development status. The vegetation biomass accumulation and litter in return improve soil fertility and microbial properties constantly (Peng *et al*., 2010; Han *et al*., 2012). Therefore, soil microbes, vegetation, soil main nutrients, and minerals mutually interaction and together develop in succession. Results of canonical correlation analysis showed all the accumulative contribution percentage of the former three eigenvalues exceeded 75%, which indicated soil microbes had distinct canonical correlation with vegetation, soil main nutrients, and soil minerals, especially closely correlated with soil main nutrients. Soil MBC had the largest load capacity in soil microbial biomass, Shannon index in tree layer, available nitrogen, Fe_2O_3, and CaO. Thus, the relationship between soil MBC and vegetation diversity, soil nitrogen and mineral nutrients should be wholly considered in ecological restoration of fragile ecosystems in the karst region. Increasing vegetation diversity in tree layer, soil main nutrients, and minerals such as Fe_2O_3 and CaO to improve soil microbial active environment to increase soil microbial biomass and populations, and then to facilitate healthy development of the whole ecosystems.

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