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ORIGINAL ARTICLE

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Soil quality changes in land degradation as indicated by soil chemical, biochemical and microbiological properties in a karst area of southwest Guizhou, China

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Abstract Not only the nutritional status and biological activity but also the soil ecological functioning or soil health has been impacted profoundly by land degradation in the karst area of southwest China where the karst ecosystems are generally considered as extremely vulnerable to land degradation under intensified land-use changes. The objectives of this study are to elucidate the changes in overall soil quality by a holistic approach of soil nutritional, biological activity, and soil health indicators in the karst area as impacted by intense cultivation and vegetation degradation. Topsoil samples were collected on selected eco-tesserae in a sequence of land degradation in a karst area of southwest Guizhou in 2004. The soil nutrient pools of organic carbon (C_{org}), extractable extracellular carbon (C_{ext}), total soil nitrogen (N_t), alkali-hydrolyzable nitrogen (N_{ah}), total phosphorus (P_t), available phosphorus (P_a) were analyzed by wet soil chemistry. The soil biological properties were studied by means of measurements of microbial biomass carbon (both by fumigation–extraction, FE-C_{mic}, and by calculation from substrate-incubation respiration, SIR-C_{mic}) of respiration [respiration without addition of substrates, basal respiration (BR), and potential respiration (PR) with substrate-incubation] and of soil enzyme activities (invertase, urease, and alkaline phosphatase). Soil health status was assessed by simple indices of C_{mic}/C_{org} and BR/C_{mic} in conjunction with bacterial community structures determined by polymerase chain reaction and denaturing gradient gel electrophoresis. While the nutritional pool parameters, such as C_{org} and C_{ext}, described basically the changes in soil life-supporting capacity with cultivation interference and vegetation declined, those parameters of biological activity such as FE-C_{mic}, SIR, and SIR-C_{mic} as well as bacterial community structures measured by molecular method evidenced well the changes in soil functioning for ecosystem health with the land degradation.

Keywords Karst area · Soil quality · Soil degradation · Soil and ecosystem health · Cultivation · Vegetation decline · Microbiological indicators · Southwest Guizhou

Introduction

The karst area of southwest China covers a land of 550,000 km^2 (Li et al. 2002a). The karst ecosystems with vegetation covers of different types on thin soils by a lithic contact to overlain limestone is considered important in global terrestrial carbon and water cycling in sensitivity to global change (Yuan 1993) and have

been shown to be extremely vulnerable under severe water and soil erosion due to improper human land uses (Yuan 2001; Tuyet 2001; Li et al. 2002b; Wang 2002). Rocky desertification, a land surface process of rapid decline in vegetation cover and truncation of soil body on the sloping lands, has been observed to extend in mountainous karst area of southwest China since 1960s as a result of fast-growing population and intense utilization of land resources in this area (Wang 2002). A land with a total area of 105,000 km^2 has suffered from rocky desertification with severe problems of drought or flooding, shortage in available water as a result of water and soil loss in the karst land in southwest China, dominantly in central and southwestern Guizhou (Academic Divisions of CAS 2003). Thus, serious concerns have risen for public and scientific societies with the extending rocky desertification and the impact on productivity and health of karst ecosystems, and the sustainability of the socio-economical system in the local area. For the last 5 years, national and provincial programs have been developed to protect and/or restore the degraded karst lands in selected sites of Guizhou Province (Yuan 2001; Mei 2003).

Soil degradation in the process of rocky desertification plays a key role in the decline of ecosystem productivity and stability as soil functions of mediating the nutrient and water flow for plant growth, of driving limestone weathering associated with terrestrial C cycling (Pan and Cao 1999), and of supporting the life within the ecosystem would have been reduced to a great deal in the karst area. While many authors have argued that rocky desertification and degradation of karst ecosystems have been related to the constraints of vegetation, geological formations, and the geomorphological status of the karst lands using some bio-geographical and geochemical indices (Wang and Lü 2000; Yu et al. 2002; Li et al. 2005; Liu et al. 2005), the existence of soil cover has been recently recognized as a critical factor in the vulnerability of the karst lands to degradation (Wang et al. 2005). However, soil quality changes associated with the rocky desertification in the karst lands have been poorly understood (Long et al. 2002; Cao et al. 2003).

By taking the example of karst soils from some pilot sites in southwest Guizhou, the purpose of the present study is to: (1) describe the changes in soil nutrient pools, soil microbial, and biochemical properties (microbial biomass, community diversity, and enzyme activity as affected by the desertification process); (2) demonstrate the linkage of pool changes and biological changes to soil functioning and health as an integrated soil quality criteria of the karst ecosystems. The authors aim also at a better understanding of the role of soil quality changes in ecosystem stability involved in vegetation decline or restoration.

Materials and methods

Site description

The research area is in the karst area around Huajiang Gorge, Beipanjiang, the upper Pearl River reach, with elevation in the range of 1,100–1,500 m in the Central Southwest Guizhou, China (25°23′–25°38′N, 105°38′–107°54′E) (Fig. 1). A subtropical mountainous monsoon climate dominates in the area with mean annual rainfall at 1,200 mm and mean annual air temperature at 14.3°C. Rainy season is from May to late August with over 70% of the total rainfall, and dry season during autumn and winter. Bedrock of limestone lies horizontally with vertical fissures well developed. The original soils belong to mollic Inceptisols with a sharp lithic contact mostly within 50–60 cm depth in the profiles. Vegetation cover has been largely destroyed and topsoil has been truncated as a result of intensified cultivation of the sloping karst lands under fast-growing population in this area since 1960s (Wang 2002). In some remote sites (like the top of a hill), shrubs and high grass could be preserved so that a sequence of vegetation decline and soil degradation with different thickness of topsoil could be recognized for study. With the vegetation cover succession from native forest, shrubs, grass, thin grass, and eventually to bared land, soil covers can be usually observed in a sequence with thick topsoil, normal topsoil, thin topsoil, neglectable topsoil, and eventually to no topsoil, accordingly (Wang 2003; Wang et al. 2003). As it can be still found in LiBo, Guizhou, the native karst forest is dominated by *Platycarya longipes* and *Cyclobalanopsis glauca* (Zhu et al. 1995). The shrub is dominated by *Pyracantha foruneana*, *Rose cymosa*, *Itea ilicifolia*, and *Rubus* sp. and grass dominated by *Miscanthus floridulus*, *Heteropogon contortus*, *Cynodon dactylon*, and *Cyperus* sp. (Liu et al. 2005), respectively.

Soil sampling

In this study, six eco-tesserae were chosen in the area, namely: soil under native forest (LS1), shrub (LS2), natural grass (LS3), thin grass (LS4), cropland of corn under conventional management for 19 years (LS5), and severely degraded soil (LS6, abandoned after continuous corn cultivations for more than 20 years), respectively. Three composite soil samples from top layer (0–15 cm) were randomly collected in each eco-tessera in April 2004. The samples were stored in hermetic stainless steel cans while shipping to lab and then kept at 4°C prior to the performance of soil analyses. The site description and soil occurrence is presented in Table 1.

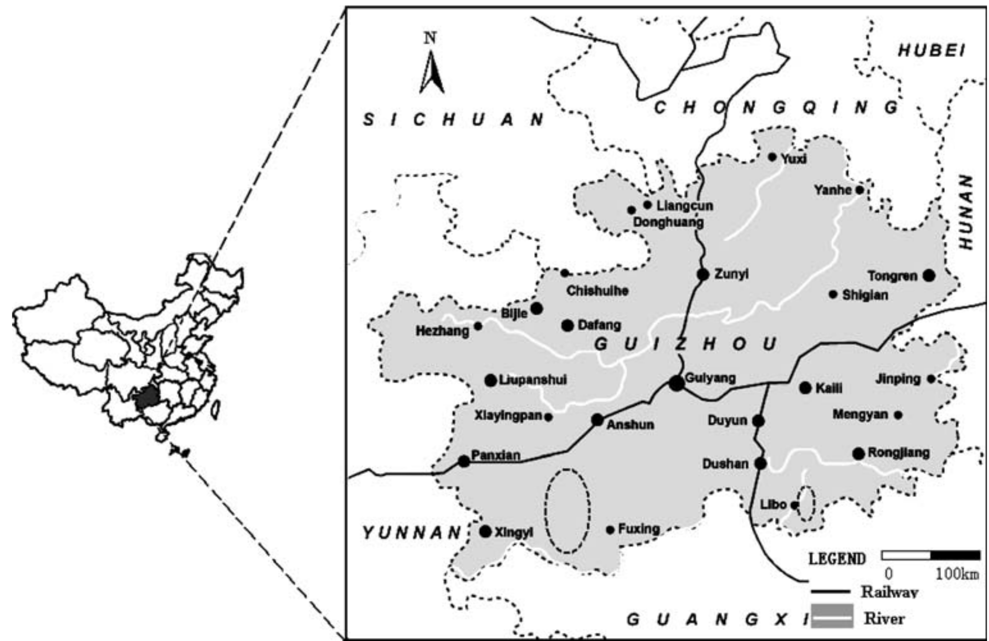

Fig. 1 Sketch map of China and the location of study area inside the *dashed circles*

Table 1 Site description of the studied eco-tesserae and the soils

Eco-tesserae, soil	GPS location	Vegetation, percentage of cover	Soil thickness and coverage rate
Native forest, LS1	N25°23′26″, E107°53′56″	Karst forest, 100%	> 35 cm and > 60%
Shrub, LS2	N25°38′50″, E105°44′14″	Shrub (3–5 m), 80%	25–60 cm and > 40%
Natural grass, LS3	N25°23′27″, E107°53′58″	High grass (80–100 cm), 100%	< 25 cm and > 70%
Thin grass, LS4	N25°38′49″, E105°44′9″	Thin and short grass (< 20 cm), 25%	20–35 cm and < 40%
Corn field, LS5	N25°24′30″, E107°54′9″	Corn, bare soil	35–60 cm and > 85%
Abandoned field, LS6	N25°38′53″, E105°44′16″	Thin and short grass, > 35%	10–30 cm and < 40%

Soil analyses

Chemical analysis

The analysis of basic properties of the studied soils was done following the procedures described by Lu (2000). Soil pH was measured in 1:1 (w/w) soil CO_2-free distilled water suspension. Soil organic carbon (C_{org}) was determined by oxidation with $K_2Cr_2O_7$ in a heated oil bath. Total nitrogen (N_t) was measured with the semi-micro Kjeldahl method and hydrolyzable nitrogen (N_{ah}) by means of the alkali distillation method. Total phosphorus (P_t) and available phosphorus (P_a) was digested with mixture of perchloric and sulfuric acids, and sodium bicarbonate extraction, respectively, and determined with colorimetry of vanadomolybdophosphate according to the protocol described by Bao (2000).

Biochemical analysis

Microbial biomass and soil respiration: Microbial biomass carbon was determined by chloroform fumigation–extraction method described by Vance et al. (1987). While the carbon extracted from unfumigated samples is generally considered as a measure of extractable extracellular carbon (C_{ext}) (Hofman et al. 2003), microbial biomass carbon (FE-C_{mic}) is obtained from the difference between the fumigated and unfumigated samples. Basal respiration (BR) and potential respiration (PR) were measured using pre-incubation of soil samples. BR was measured as CO_2 evolution without addition of any substrate after 24-h incubation in closed jars, while PR (i.e., substrate-induced respiration) was determined by the same protocol as BR but with actual addition of glucose to the soil. Substrate-induced respiration microbial biomass carbon (SIR-C_{mic}) was calculated from the PR value using the conversion factor 40.04 mg C corresponding to 1 ml^{-1} CO_2 (Dilly and Munch 1998; Hofman et al. 2003). Respiration was measured as CO_2 production with Agilent 4890D gas chromatography equipped with a stainless steel column (Porapak Q) (80/100 mesh) and a flame-ionization detector. Wherein, column, injector, and detector temperatures were 35, 130, and 250°C, respectively. The microbial metabolic

quotient (qCO_2) was calculated by dividing BR (mg CO_2-C kg^{-1} dry soil h^{-1}) by microbial biomass carbon (gFE-C_{mic} kg^{-1} dry soil) (Dilly and Munch 1998). The ratio of microbial biomass carbon to total organic carbon (FE-C_{mic}-in-C_{org}) was also calculated.

Soil enzyme activity: Invertase, urease, and alkaline phosphatase activities were measured according to the methods recommended by Guan (1986), which was done by incubation and measurement of end products. The procedure for urease activity was as follows: treat 5 g fresh soil in a volumetric flask with 1 ml toluene for 15 min. Add 5 ml 10% urea solution and 10 ml phosphatic buffer solution and incubate at 38°C for 24 h, and determine the amount of ammonium in the solution by colorimetry. The procedure for alkaline phosphatase activity determination was: treat 5 g fresh soil in a volumetric flask with 5 drops of toluene and 20 ml 0.5% disodium phenyl phosphate and incubate at 37°C for 2 h. Filter and measure the amount of phenol by colorimetry. And the procedure of invertase was as follows: Add 15 ml 8% sucrose, 10 ml citrate buffer solution, and 5 drops of toluene to 5 g fresh soil in a 50-ml glass bottle and incubate at 37°C for 24 h. Determine the sucrose remaining by colorimetry. For all the enzyme activity measurements, controls without addition of reactants were done with each batch of sample incubation.

DNA extraction and polymerase chain reaction and denaturing gradient gel electrophoresis analysis

DNA of soils was extracted using a fast DNA spin kit (BIO101) as described in manufacturer's instructions (Qbiogene Inc., Carlsbad, CA, USA). The primers used for amplifying a region of 16S rDNA of bacteria were PRBA338F-GC and PRUN518R (Invitrogen Inc., Carlsbad, CA, USA) (Nakatsu et al. 2000). The reaction was carried out with a PTC-225 thermocycler (MJ Research Inc., Waltham, MA, USA) under the following conditions: performance under 95°C for 5 min; 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 30 s, and extension at 72°C for 1 min; and, a single final extension at 72°C for 6 min.

The polymerase chain reaction (PCR) products were resolved on 8% (w/v) polyacrylamide gels in 0.5 TAE buffer using denaturing gradient ranging from 30 to 60% (where 100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was carried out at 70 V for 12 h. Gels were stained with ethidium bromide and visualized on a UV transilluminator and photographed (Bio-Rad, Hercules, CA, USA).

The profiles of denaturing gradient gel electrophoresis (DGGE) were analyzed using the Quantity One software (Bio-Rad, Hercules, CA, USA) to calculate bacterial community similarity values and generate similarity dendrograms (Dice coefficient of similarity) via an unweighted pair group method using arithmetic averages clustering method. Microbial species richness and diversity indices were determined with the methods used by Eichner et al. (1999) and Hedrick et al. (2000).

Statistics

The difference in biochemical and microbiological properties of the soils from different eco-tesserae was tested by a one-way ANOVA while cluster analysis was employed to group the similar tested soils with SPSS 11.0 for windows (LEAD Technologies, Inc., Chicago, IL, USA).

Results

Soil nutrient pools

The results of soil basic properties tested are presented in Table 2. As influenced by the soil origin of limestone, most of the tested soils have pH values around seven except for LS3 and LS5 with slightly lower pH. The soil organic carbon (C_{org}) content of the topsoil samples decreased in the following order: LS1 and LS2 > LS3 > LS4 > LS5 and LS6 while soil total N in the following order: LS1 > LS2 > LS3 > LS6 > LS5 > LS4, possibly due to N fertilization history in the soils of LS5 and LS6. A significant difference in extractable extracellular carbon (C_{ext}), a labile pool of SOC, was observed between the soils under different vegetation covers. However, the variability of the C_{ext} content with vegetation cover seemed lower than that of C_{org} content despite a similar variation tendency. The total and available pools of the major soil nutrients also followed a similar trend as C_{org} except for the soil under cultivation (LS5). Due to fertilization, soil LS5 possessed a bigger pool of available phosphorus than the soil under thin grass. While generally considered as in a potentially degradation state (Wang 2003; Wang et al. 2003), the soil under shrub tesserae maintained almost the same amount of SOC and the major nutrients as under the native forest, even higher than that under the natural grass.

The variation of SOC and the major nutrients with the vegetation cover illustrated a significant pool reduction of soil nutrients due to the land degradation. The exhaustion of C_{org} and the available nutrients will dramatically depress the biomass production and, thus, affect the soil biological activities.

Microbial and biological properties

The results of soil biological analysis determination are listed in Table 3. Soil microbial respiration has been

Table 2 Soil basic properties and nutrient pools of the six studied soils under different vegetation covers

Soil	pH (H_2O)	C_{org} (g kg^{-1})	C_{ext} (mg kg^{-1})	N_t (g kg^{-1})	N_{ah} (mg kg^{-1})	P_t (g kg^{-1})	P_a (mg kg^{-1})
LS1	7.3 ± 0.5b	83 ± 3a	91 ± 2a	3.7 ± 0.1a	313 ± 15a	1.2 ± 0.1b	17 ± 1a
LS2	7.5 ± 0.6a	80 ± 1a	90 ± 3a	3.5 ± 0.1b	278 ± 10b	1.9 ± 0.03a	19 ± 1a
LS3	6.4 ± 0.2c	55 ± 2b	80 ± 4b	1.4 ± 0.1c	206 ± 18c	0.5 ± 0.05d	14 ± 0.5b
LS4	7.3 ± 0.2ab	27 ± 1.5c	73 ± 2b	0.7 ± 0.02f	97 ± 2d	0.8 ± 0.02c	5 ± 1d
LS5	6.3 ± 0.2c	21 ± 1d	37 ± 1d	0.9 ± 0.1e	103 ± 4d	0.5 ± 0.02d	9 ± 1c
LS6	7.4 ± 0.5ab	21 ± 0.5d	47 ± 2c	1.2 ± 0.05d	97 ± 4d	0.8 ± 0.02c	3 ± 0.5e

Different letters in a single column indicate significant ($p < 0.05$) difference between the soils. Means of three replicates ± standard error

considered as a basic index for soil microbial activity (Kennedy and Papaendick 1995). The soils under native forest and natural grass vegetations (LS1 and LS3) maintained significantly higher BR and PR than those under degraded vegetation and cultivation. Microbial biomass of the soils determined by fumigation was in a decreasing order as follows: LS1 > LS2 > LS3 > LS4 > LS5 > LS6, in a similar tendency as that of C_{org} for these soils. However, the high value of PR of the LS3 evidenced a higher soil microbial C under natural grassland. The other biological and biochemical parameters also changed in such a trend in accordance to their degrees of land degradation. The soil under shrub tessereae had significantly lower values of biological and biochemical parameters than under the native forest and under the natural grass. Nevertheless, much wider variation of the FE-C, activities of soil urease and alkaline phosphatase with the vegetation degradation status was observed than that of the BR and SIR-MIC, indicating a much more prompt response of the microbial functioning behavior to soil degradation. While insignificant soil enzyme activity survived in the soils under cultivation despite of fertilization, the soil under natural grass maintained much higher enzyme activity and substrate-induced respiration biomass.

As qCO_2 was expressed of the CO_2 respired divided by the microbial biomass carbon and thus links directly to both the size and activity of soil microbial population, it has been commonly used as a key parameter for soil quality in conjunction with C_{mic}/C_{org} (Kennedy and Papaendick 1995; Anderson 2003). The qCO_2 values of the soils under degraded vegetation and cultivation were much significantly higher than those under natural forest and shrub (LS1 and LS2) with the difference much greater compared to the difference in C_{mic}/C_{org} (Fig. 2). This could indicate a vulnerability of the microbial community in response to soil degradation in these karst lands and the diversity changes with the domination of the microbes with higher C resource utilization.

Cluster analysis of six soils based on chemical and biochemical properties was plotted in Fig. 3. Accordingly, the studied six soils can be basically grouped into two major categories: LS4, LS5, and LS6 as a group of degraded soils and LS1, LS2, and LS3 as a group of soil under natural and non-degraded forest or grassland vegetation. Therefore, vegetation degradation has resulted in significant decline of soil quality with which the degraded soils shifted to a single group with similar assemblage of soil chemical and biochemical characteristics.

Bacterial genetic communities

Genetic fingerprinting by DGGE of bacterial 16S rDNA amplified fragments showed a few strong dominating bands (Fig. 4). Together with these strong signals, a great number of fainter well-resolved bands appeared in the profiles, which were all considered when analyzed by

Table 3 Biological and biochemical properties of the studied soils. Means of three replicates ± standard error

Soils	BR (CO_2 mg kg^{-1} d^{-1})	PR (CO_2 mg kg^{-1} d^{-1})	SIR-C_{mic} (mg kg^{-1})	FE-C_{mic} (mg kg^{-1})	Invertase-glucose (μg g^{-1} h^{-1})	Urease-N (μg g^{-1} h^{-1})	APP-phenol (μg g^{-1} h^{-1})
LS1	96 ± 3b	204 ± 7a	1,298 ± 13b	1,103 ± 20a	1,608 ± 110ab	34 ± 4a	482 ± 12b
LS2	48 ± 2c	128 ± 3b	815 ± 12c	812 ± 64b	1,335 ± 240bc	19 ± 2b	281 ± 10c
LS3	119 ± 5a	207 ± 6a	1,321 ± 24a	615 ± 53c	1,667 ± 222a	22 ± 1b	774 ± 13a
LS4	40 ± 1d	94 ± 3c	597 ± 15d	238 ± 16d	1,837 ± 217a	9 ± 1c	257 ± 12d
LS5	43 ± 2cd	75 ± 2d	480 ± 13e	189 ± 18de	486 ± 72d	7 ± 1cd	238 ± 8d
LS6	31 ± 1e	62 ± 3e	395 ± 11f	166 ± 26e	1,095 ± 154c	6 ± 1d	84 ± 10e

APP alkaline phosphatase
Different letters in a single column indicate significant difference between the soils ($p < 0.05$)

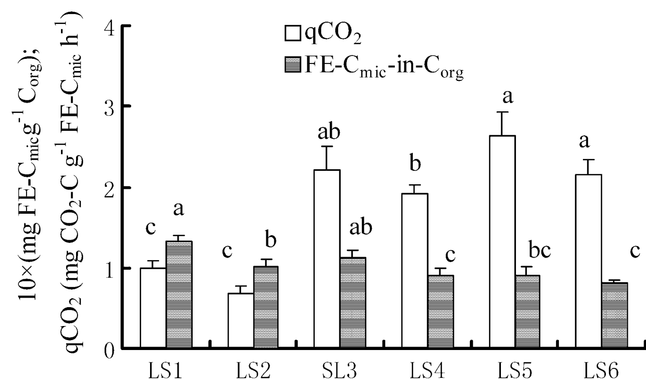

Fig. 2 Metabolic quotient (qCO_2) and ratio of FE-C_{mic} to C_{org} of the studied soils. Different letters on the top of same color columns indicate significant difference ($p < 0.05$)

the Bio-Rad Quantity One software. However, the number of the bands and their dominance varied greatly with vegetation degradation. Apparently, under the degraded vegetation, several bands disappeared as compared to those under natural vegetation cover. DGGE profiles revealed that some bacteria species have vanished due to vegetation decline. This is mutually supported by the above findings of lower microbial carbon with high carbon resource utilization under degraded vegetation cover.

The similarity dendrogram retrieved from DGGE profile by Bio-Rad Quantity One software is shown in Fig. 5. Bacterial community structures were more similar under native forest and non-degraded shrub (LS1 and LS2), while grasslands (LS3 and LS4) maintained a community structure similar to each other. However, LS5 and LS6 had their own distinct bacterial community structures compared to the others, which resulted extinction of some species due to land degradation.

In Table 4 are listed the species richness (S) and Shannon's diversity (H') retrieved from bands on the DGGE profiles for the different soils (Fig. 3) by Bio-Rad Quantity One software analysis. LS1 under native forest harbored most species and highest diversity of bacteria while LS6 maintained lowest species and diversity of bacteria. Change of bacterial genetic diversity and richness with vegetation degradation followed a similar trend as that of nutrient and biochemical parameters as mentioned above.

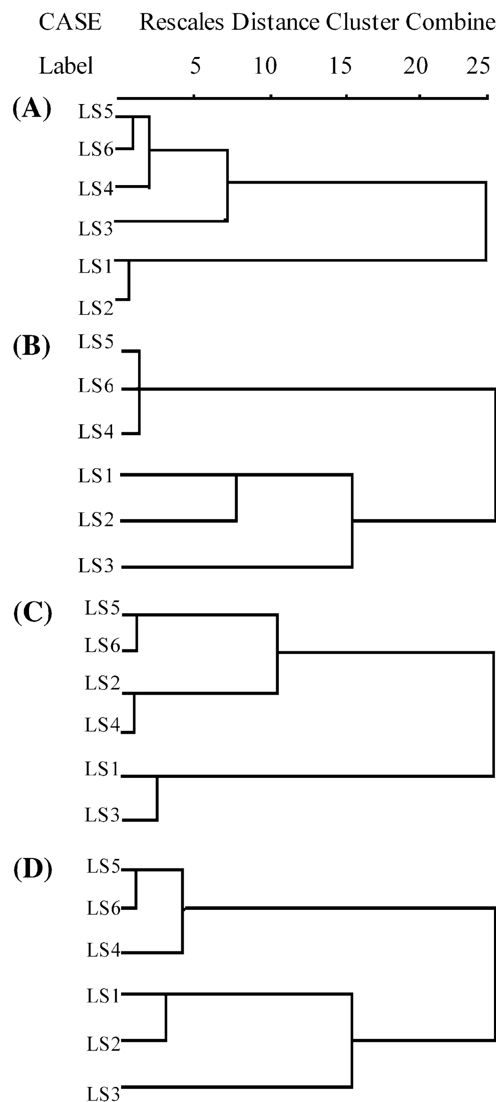

Fig. 3 Cluster analysis of six soils based on chemical and biochemical properties. *Scale* indicates Euclidean distance. **a** Based on C_{org}, C_{ext}, N_t, N_{ah}, P_t, and P_a; **b** based on BR, PR, qCO_2, SIR-C_{mic}, and FE-C_{mic}; **c** based on invertase, urease, and neutral phosphatase; **d** based on all of the chemical and biochemical properties

Discussion

Pool change as soil quality indicator for land degradation

In general, nutrient pools are the key to soil fertility. The effect of cultivation and soil erosion on pool reduction of major nutrients has been widely documented. Both cultivation and erosion diminishes the SOC pool and, in turn, the potential available pool of N and P in natural soils (Saviozzi et al. 1997; Hajahhasi et al. 1997; Lemenih et al. 2005) through disruption of the equilibrium between the competing processes of humus formation and mineralization (Saviozzi et al. 2001). Enhanced C mineralization and loss of soil organic matter (SOM)-associated nutrients could be induced by cultivation and erosion by disaggregation and promoted exposure of microaggregate-protected SOM to microbial decomposition. The data shown in this study were in agreement with the above findings. With respect to the

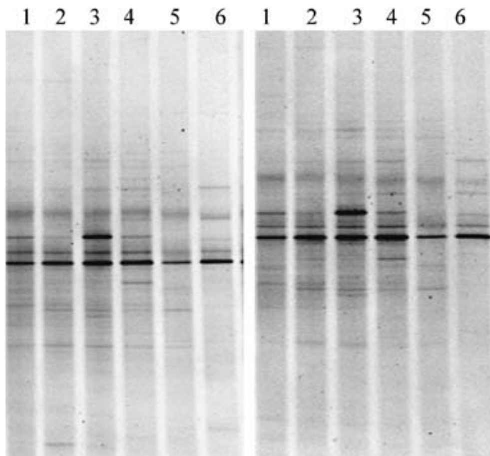

Fig. 4 DGGE fingerprints (negatively converted) of PCR-amplified 16S rDNA extracted indirectly from soil bacterial community. *Lane 1* LS3; *lane 2* LS4; *lane 3* LS1; *lane 4* LS2; *lane 5* LS5; *lane 6* LS6

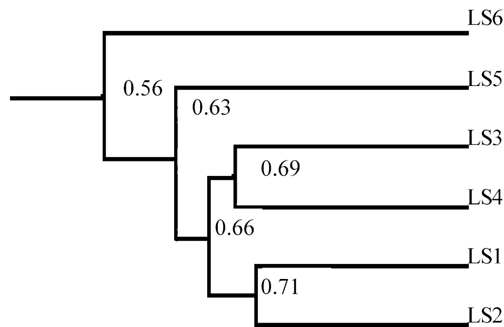

Fig. 5 Cluster analysis (UPGMA, Dice coefficient of similarity) of molecular banding patterns generated by PCR-DGGE from Fig. 4. The similarity dendrogram (scale 0–1) was calculated from two bands of Fig. 4 by Bio-Rad Quantity One software

size of C pool, high level of C_{org} and C_{ext} was found in soils both under native forest (LS1) and shrub (LS2) even if the shrub vegetation was considered in a state of potential degradation (Wang 2003; Wang et al. 2003). However, C_{org} was significantly lower in LS4 under thin grass as vegetation declining led to low input of plant-derived organic matter (Garcia et al. 2002), whereas, high C_{ext} and relatively low C_{org} content were observed in LS3 and LS4 both under grassland vegetation. Normally, changes in land uses could lead to a shift or gradual degradation of vegetation, which in turn could affect both quality and quantity of SOM. The SOM in LS3 and LS4 derived from herbaceous plants may be more labile than that from forest trees, giving rise to a higher ratio of C_{ext} to C_{org}. On these sloping karst soils with lithic contact, once native vegetation was destroyed, soil nutrient pool would be fast reduced by soil loss (Long et al. 2002; Yang and Zhu 1999). Cultivation

Table 4 Species richness (*S*) and Shannon's diversity (*H*′) retrieved from bands on the DGGE profiles (Fig. 3) after Bio-Rad Quantity One software analysis

Plots	LS1	LS2	LS3	LS4	LS5	LS6
H′	1.37	1.23	1.30	1.20	1.07	0.76
S	22	19	20	16	12	9

with harvest on these soils could also dramatically exhaust the nutrient pool so that nutrient status as indicated by the contents of N_t, N_{ah}, P_t, and P_a of LS5 and LS6 were even lower than in grasslands. Comparatively, native vegetation cover provides re-cycling of soil nutrients by accumulation in topsoil as in the case of LS1, LS3, thus supporting high soil fertility. In a word, cultivation and serious vegetation decline could markedly decrease soil nutrient supply capacity. Therefore, nutrient pool change can be an apparent indication of soil quality change in the process of land degradation rather than that of land potential degradation in the karst area.

Microbial and biological parameters as indicators of karst soil quality

Soil microbial respiration reflected the overall activity of the microbial population. However, BR was strongly influenced by the available carbon resource in soils (Cheng et al. 1996). The soils under well-protected natural vegetation (LS1) maintained high microbial activity with high BR and PR values but lower qCO_2 and higher C_{mic}/C_{org} than those degraded or under cultivation. According to Van Veen et al. (1985), soils with a lower plant cover failed to have a high potential microbial activity because of low labile organic matter input. Similarly, lower C_{ext} of LS5 and LS6 may be attributed to cultivation without residual return as re-input of organic matter, which would cause, in turn, the high utilization of indigenous SOM giving rise to a higher qCO_2 and lower C_{mic}/C_{org}. The depleting effect on SOM was also observed in soils under degraded grassland due to insufficient SOM input in the process of land degradation. Microbial biomass itself has been considered as a more sensitive indicator of SOM quantity and quality to human interference of management and/or pollution than the total organic carbon alone (Hofman et al. 2003; Singh et al. 1989; Powlson 1994; Fritze et al. 1996; Dilly and Munch 1998; Kandeler et al. 1999; Bending et al. 2004). In this study, size and activity of soil microbe were significantly affected by degradation of vegetation cover than SOM and the other nutrient indicators. Generally, decline of vegetation cover results in a lower input of organic matter to soil available to microorganisms and, thus, induce lower microbial biomass and

BR due to shortage of C resource (Powlson et al. 1987). Obviously, the soils LS1 and SL3 under natural vegetations contained high microbial biomass carbon, while soil microbial biomass carbon was relatively low under shrub and thin grass. A great reduction of microbial biomass carbon under LS5 and LS6 can be contributed to depletion of SOM under intensive cultivation by tillage disruption and exhaustion of the nutrients associated with SOM (Table 2). Garcia et al. (2002) also reported that microbial activity (microbial biomass carbon, BR and oxidoreductase and hydrolase activity) decreased while plant cover had been destroyed on limestone soil.

The C_{mic}/C_{org} ratio has been adopted as a critical indicator of soil carbon available to microbial growth and could be used to develop a site-specific baseline value for different soil systems as well as a stability indicator for quick recognition of an environmental change (Anderson 2003). This ratio was significantly high under LS1, while soils (LS4, LS5, and LS6) poor in major nutrients possessed low C_{mic}/C_{org} ratio. In fact, C_{mic}/C_{org} ratio depended on nutrient status and soil management in arable fields (Anderson 2003). It seemed that high soil quality could be characterized by high C_{mic}/C_{org} ratio in the present study. Whereas, this parameter failed to discriminate the effect of cultivation on the soils as LS5 has the same high ratio as LS3. The qCO_2 value reflected the maintenance energy requirement for microorganisms and would increase in a distorted ecosystem in relation to a stable ecosystem (Anderson 2003; Haynes 1999). From Fig. 2, qCO_2 value in LS1 and LS2, the latter generally considered as a potential degradation state, was significantly low, indicating a more or less stable ecosystem under natural vegetation while the value for LS3 (under natural grassland) was in the same order as those obtained for degraded soils LS4, LS5, and LS6. Anderson and Domsch (1990) reported a significantly lower qCO_2 under continuous crop rotation (heterogeneous resource input) than that under continuous monocultures from agricultural plots for 15 years. Bardgett and Shine (1999) also attributed a decrease in qCO_2 to higher plant diversity. Although the studied soil LS3 was under the natural grass vegetation, the high qCO_2 value could be accounted for by the mono-resource of SOM by grass plants. In other words, C_{mic}/C_{org} ratio and qCO_2, generally concerned as indicators of soil quality or ecosystem stability, could not significantly discriminate the different soil quality of the studied soils in the karst soil ecosystem concerned with vegetation decline alone.

Soil enzymes catalyze all biochemical transformations and could indicate the biochemical potential and possible resilience as well as the effect of remediation operation and environmental stress. Measurements of soil enzymatic activities had been extensively conducted to indicate biological activity and soil health (Bergstrom and Monreal 1998; Bandick and Dick 1999; Kandeler et al. 2000; Badiane et al. 2001; De la Paz Jimenez et al. 2002; Gil-Sotres et al. 2005) and to offer information of human interference on biogeochemical cycling of the terrestrial ecosystems (Kandeler et al. 1996; Wick et al. 2000; Hinojosa et al. 2004). Although intervase activity did not differentiate very much between the soils, urease and alkaline phosphatase activities were high for soils LS1, LS3, and LS3 while low for soils LS4, LS5, and LS6, suggesting that cultivation and vegetation decline had depressed these two enzyme activities. Saviozzi et al. (2001) also reported that, compared to undisturbed systems (native grassland and poplar forest), long-term (45 years) corn cultivation at intensive level caused a marked decline in alkaline phosphatase and urease activities due to frequent ploughing. In addition, Garcia et al. (2002) found that the values of phosphatase and urease activities fell in soils with plant cover decreased, since plant cover decline was linked to changes in the N and P availability.

Soils with high quality generally preserved good nutrient supply and bio-physical conditions, and supported large size and high activity of soil microorganisms. Data of soil nutrient pool, biochemical, and biological properties were taken into account concerning the effect of degradation on soil quality in conjunction with soil microbial parameters in this study. A cluster analysis showed clear discrimination of the soils under different vegetation and cultivation (Fig. 3). On the whole, three groups clustered on base of all parameters can represent the different categories of soil quality changes in the karst area.

Moreover, compared to the native forest, most of the microbial and biological parameters under vegetation decline or cultivation decreased more sharply than parameters of soil organic carbon and nutrient pool. In addition, under shrub vegetation considered in a state of potential degradation (Wang 2003; Wang et al. 2003), there were the same high most of parameters of soil organic carbon and nutrient pools as these under native forest, even higher than these under nature grass; however, except FE-C_{mic}, soils under shrub had significantly lower values of most microbial and biological parameters than under native forest and nature grass. Thus, microbial and biological parameters could be considered sensitive to land degradation or vegetation declining than those of nutrient pools.

Bacterial genetic communities as soil quality indicators of the karst soils

Although the process-level measurements (microbial biomass, respiration rates and enzyme activities) provided an important understanding of gross microbial

processes and their potential role in soil health, they could offer little information of qualitative community-level changes of soil microbial population for any given microbial process that could be carried out by diverse taxon of microorganisms. Therefore, quantitative and qualitative changes in the composition of soil microbial community could serve as important and sensitive indicators of both short- and long-term changes in soil health (Hill et al. 2000; Schloter et al. 2003). DGGE profiles in this work suggested that bacterial community structures of the pair soils of LS1 and LS2, and of LS3 and LS4 were similar to each other while the soils of LS5 and LS6 maintained their own distinct bacterial community structure from the other soils, indicating the shift of the bacterial community caused by the cover decline and cultivation. In addition, bacterial species richness and Shannon's diversity was greatly reduced in the soils under cultivation and vegetation decline compared to those under natural vegetation. Since plants could drive the changes in microbial community structure (Marschner et al. 2001; O'Donnell et al. 2001), microbial diversity showed a high association with organic matter content (Zhou et al. 2002; Gomez et al. 2004), dissolved organic carbon content, and C/N ratio (Marschner et al. 2003). Here, soils of LS1 and LS2 under woody trees showed high content of C_{org} and C_{ext}, while LS3 and LS4 under herbaceous plants owned medium content of C_{org} and C_{ext} as well as high C/N ratio (Table 2). Therefore, decline in vegetation cover and intense cultivation may have caused a decrease in soil organic carbon supply and, in turn, shift of bacterial diversity. This could be supported by the findings of Degens et al. (2000) that depleting organic C stock in soils under land-uses change caused decline in the catabolic diversity of soil microbial communities. In a word, bacterial community structures were sensitive in response to vegetation change as well as the soil quality change. Cultivation and vegetation degradation could not only change bacterial community structures but also diminish bacterial species richness and diversity.

Conclusions

Although most of chemical, biochemical and biological parameters reflected changes in soil quality under vegetation decline as well as under cultivation, microbial and biological parameters were more sensitive to land degradation or vegetation declining than parameters of nutrient pools in the karst area. Bacterial community structures determined by molecular method (PCR-DGGE), as a molecular footprint, traced sensitively soil quality and health changes caused by the vegetation decline and cultivation. After all, cluster analysis could be used for grouping the parameters and categorizing soil types with different status of soil quality and health.

The karst ecosystem characterized by its extreme vulnerability in the southwest China was sensitive to land-use changes. Cultivation and vegetation decline exerted a profound influence on soil degradation by depleting C_{org}, C_{ext} and some enzyme activities, and decreasing pool size and activity of microorganisms and bacterial diversities. In addition, soil under shrub may be considered as a potentially degraded soil as soil microbial activity and bacterial diversity was significantly low compared to those under native forest vegetation while high nutrient content was preserved. Therefore, intense cultivation or activities leading to vegetation decline on the sloping lands should be prohibited to avoid soil quality deterioration or even rocky desertification for maintaining the future sustainability in the karst area. However, the concrete effects by the factors influencing vegetation decline and the time course of the land degradation and soil quality and functioning still need further study both by site-specific monitoring as well as data integration.

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