



Implication of different humic acid fractions in soils under karst rocky desertification

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ABSTRACT

Karst rocky desertification, a kind of intense soil degradation, has become an urgent environmental issue in the karst region of southwest China. It was found that limestone soil, a typical soil of the karst region, is more vulnerable to karst rocky desertification than adjacent other soils although limestone soil has relatively high TOC (total organic carbon) content, but underlying mechanisms remain unclear. To investigate roles of soil properties in karst rocky desertification, three major soils, limestone soil, yellow soil and yellow brown soil, of the karst region of southwest China were sampled and their total extractable humic acid (THA) were fractionated into three sub-fractions, namely, free HA (HAF), encapsulated HA (HAe) and interacted HA (HAi), with a sequential extraction method. These soils and fractionated HA samples were systematically characterized with various analysis methods and discussed. The results showed that limestone soil had higher contents of total organic content (TOC) and THA content, but its THAC/TOC (carbon in THA to TOC) ratio (23.39%) was lower than those of yellow soil (31.44%) and yellow-brown soil (30.74%); C/N ratios and total acid contents of HAe and HAI fractions, which were physically protected by soil minerals/aggregates, were much higher than those of HAF fractions, indicating that HAe and HAI could be more active and degraded easier than HAF once exposed to microbes or/and runoff erosions. Therefore, lower HAF content and significantly lower HAFC/TOC (carbon in HAF to TOC) ratio of limestone soil may underlie the observation that limestone soil showed weak resistance to karst rocky desertification compared to other adjacent soils when the site had been artificial disturbed. We believe that roles of different HA fractions to overall quality and stability of soil deserve more attention.

1. Introduction

Karst landscape accounts for about 12% of the world's total land area (Liu, 2009) and easily occurs karst rocky desertification. Karst rocky desertification is a process of land degradation, represented by serious soil erosion, extensive exposure of bed rock, drastic decrease of soil productivity and appearance of a desert-like landscape (see Fig. 1) (Wang et al., 2004; Yan & Cai, 2015). Karst rocky desertification has occurred in many karst regions all over the world (Jiang et al., 2014), especially, in karst region of southwest China, which is known as the largest karst region in the world with continuous carbonate rock outcrops (Liu, 2009). In addition to the relatively high population density, the rapid economic growth aggravates karst rocky desertification status in southwest China, and karst rocky desertification has become one of the major constraints of the sustainable development in this region (Wang et al., 2004; Yan & Cai, 2015; Zhang et al., 2018).

Fig. 1 shows a comparison of nil rocky desertification and severe

rocky desertification landscapes in the study area. It is generally agreed that karst rocky desertification happens from irrational and intensive land uses in the fragile karst geo-ecological environment (Wang et al., 2004; Xue et al., 2017). The soil loss associated with karst rocky desertification showed a power function relationship with slope gradient/length and runoff depth (Zhang et al., 2018). The geological and geographical conditions are controlling factors which govern the occurrence and degree of karst rocky desertification at karst sites. Prior study also showed that human activities, land use, soil types, environmental geology and topography are inducing factors of karst rocky desertification, and anthropogenic factors (human activities and land use) are the leading factors (Yang et al., 2011). But the role of soil's properties in occurring of karst rocky desertification was seldom investigated. Many types of soils develop in karst region of southwest China, yellow soil, limestone soil and yellow brown soil are three most common ones among them (Office of Soil Survey, 1994), various degrees of karst rocky desertification were observed in adjacent regions of different

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Fig. 1. Landscapes of nil karst rocky desertification and severe karst rocky desertification.

Table 1
Description of sampling sites and selected physical and chemical properties of soils.

	Yellow soil	Limestone soil	Yellow-brown soil
Site	Duxi forest park	Maolan natural reserve	Weining County
Latitude and longitude	26.673°N; 106.684°E	25.307°N; 107.936°E	26.645°N; 104.706°E
MAT (°C)	15	18	11
MAP (mm)	1200	1321	962
Topography	Hill-top	Hill-foot	Hillside terrace
Elevation (m)	1310	765	2200
Vegetative cover	Coniferous forest with shrub	Mixed forest of pine and broadleaf trees with thick shrub	Meadow with sparse shrub
pH (H ₂ O)	4.3	6.4	5.2
Sand (%)	13.4	24.8	24.2
Silt (%)	17.7	52.4	35.6
Clay (%)	68.9	22.8	40.2
CaO (g kg ⁻¹)	2.2	21.6	5.0
CEC (mmol kg ⁻¹)	174.5	209.9	173.5

Note: MAT: annual mean temperature; MAP: annual mean precipitation.

Table 2
Yields of HA fractions and carbon contributions of HA fractions to TOC.

Soil	TOC [%]	C/N	Yields ^b [g kg ⁻¹]				HAC/TOC ^c			
			Haf	HAe	HAi	THA ^a	Haf	HAe	HAi	THA
Y	2.01	15.02	7.20	3.55	1.08	11.83	18.70	9.66	3.08	31.44
L	3.12	10.81	6.06	5.97	2.52	14.55	9.29	9.70	4.40	23.39
B	2.31	10.31	9.53	3.04	0.81	13.38	21.30	7.56	1.88	30.74

^a Total extractable HA.

^b Amount of HA fraction obtained from soil.

^c Carbon contribution of HA fraction to TOC.

dominated soils, usually, limestone soil region shows more severe karst rocky desertification than adjacent regions of other soils (Zheng & Wang, 2002). The properties of limestone soil, yellow soil and yellow brown soil are different (see Tables 1 & 2). Limestone soil is an azonal soil with minimal pedogenic development, the most significant feature of limestone soil is that its parent material is exclusively carbonate rock

and it usually produces CO₂ bubbles when treated by acid. Yellow soil and yellow brown soil are zonal soils, they can develop on a wide variety of pedogenic materials. Yellow soil is extensive in the study area and occurs at medium elevations (400–1500 m), yellow brown soil usually occurs at high elevations (1100–2700 m) (Office of Soil Survey, 1994). The adjacent regions of different dominated soils in the study area showed different karst rocky desertification status implies that characteristics of soil may also play roles in karst rocky desertification. However, the intrinsic mechanisms are seldom investigated and remain unclear.

The soil organic matters (SOMs), a practically defined concept of all non-living organic substances in soil, is an important proxy of soil properties, SOMs binds primary particles of soil into aggregates and plays an important role in stabilizing the physical structure of soil (Fageria, 2012; Wang et al., 2015). It had been recognized that the SOMs content correlates negatively with the erodibility of soil (Obalum et al., 2017). As has been expected, a positive linear correlation between the proportion of macro-aggregates and the content of aggregate-associated SOC in the karst region of southwest China was reported (Liu et al., 2017). The interplay between aggregation and formation of mineral-associated SOC is crucial to understand the changes in amounts and stability of SOC during soil erosion (Wang et al., 2014). Fulvic acid (FA), humic acid (HA) and humin (HM) are major components of SOMs, their physicochemical characteristics as well as their mutual mass proportions determine the overall characteristics and behaviors of SOMs. For example, the HA/FA ratio was frequently used as the quality criterion of SOMs, the higher is the HA/FA ratio the higher is the quality of SOMs (Horacek et al., 2017). However, the higher vulnerability of limestone soils to karst rocky desertification than adjacent other soils in the study area seems inconsistent with above ideas, since both quantity and quality of SOMs are high in limestone soils. For example, it was found that limestone soils had higher TOC contents and HA/FA ratios compared to adjacent other soils of the study area (Liu, 2009; Ma et al., 2016; Office of Soil Survey, 1994; Wang et al., 2018), therefore, a comprehensive and systematic in-depth study on SOMs of different soils in karst regions are very necessary for understanding karst rocky desertification better. Different SOMs components may behave differently and contribute differently to the overall soil properties, compared to HM and FA, HA has a moderate reactivity and plays an important role in overall behaviors of soil (Traversa et al., 2014). Some researchers proposed that HA correlated with the stability of soil

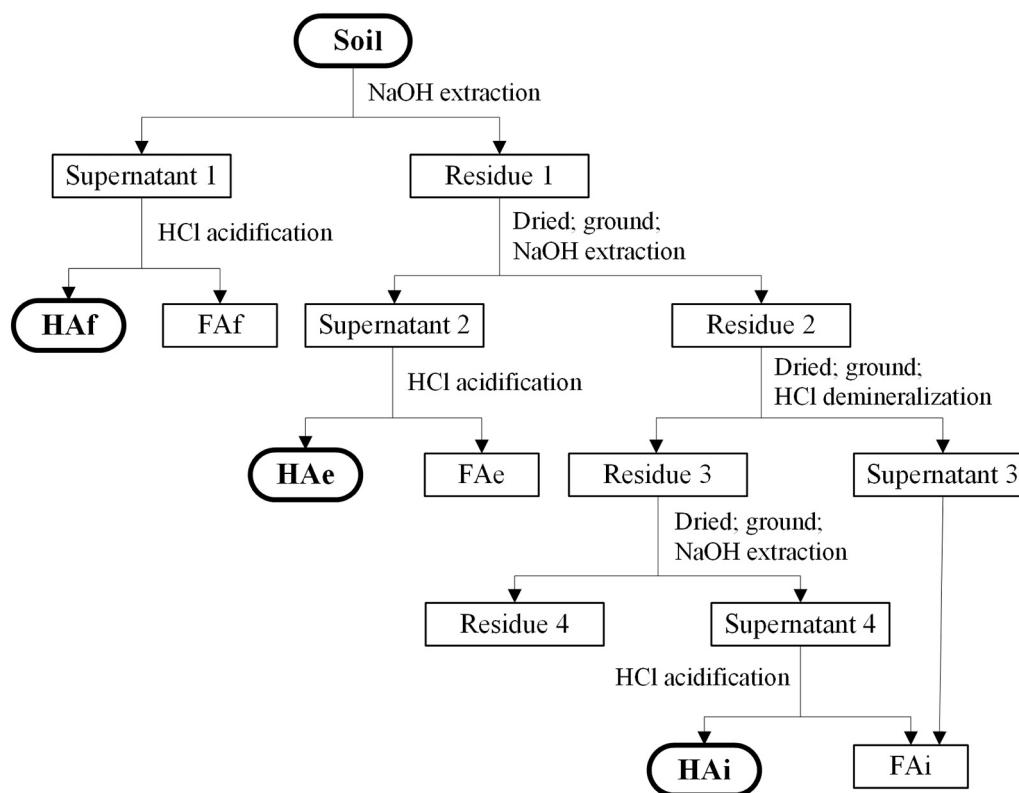


Fig. 2. Flowchart of the sequential HA extraction experiment.

aggregates more significantly than other fractions of SOMs (Piccolo & Mbagwu, 1990; Tejada & Gonzalez, 2008; Tejada et al., 2006), therefore, HA was suggested as an adequate proxy for the quality of SOMs (Buurman et al., 2009).

The overarching objective of this study is to characterize HA fractions extracted from target soils of the study area and to investigate the relation of characteristics of HA with soil behaviors under karst rocky desertification. The International Humic Substances Society (IHSS) recommended extraction method and extensive extraction methods (Song et al., 2002; Xing et al., 2005) were often applied to obtain HA samples from soil/sediment, however, these methods usually obtain the bulk HA sample which may show integrated characteristics of different HA fractions (Di et al., 2016). HA is still a complex organic mixture and defined practically as the SOMs fraction which can be dissolved in base solutions and precipitated in acidic solutions (Aiken et al., 1985; Hayes & Swift, 1978; Stevenson, 1994). The bulk HA sample can be fractionated into sub-fractions according to their physicochemical characteristics and interactions with soil matrix. We assumed that different HA fractions function differently during erosion or/and degradation processes, and the vulnerability of soil to karst rocky desertification may correlate with soil's constituents of HA fractions. To test this assumption, we developed a sequential extraction method to extract three HA fractions from major soils in the karst region of southwest China, and these sequentially extracted HA fractions were systematically characterized and contrasted to investigate the intrinsic correlations of characteristics of different HA fractions and vulnerability of soil to karst rocky desertification. The findings will be helpful to inform policies for sustainable land development management and soil conservation in karst areas.

2. Materials and methods

2.1. Soil sampling, chemical and physical characterization

Yellow soil (Y), yellow-brown soil (B) and limestone soil (L) are major soils in karst region of southwest China and were collected from little disturbed natural sites of Guizhou, the center area of karst region of southwest China. The descriptions of sampling sites and selected properties of soils were list in Table 1. In terms of USDA soil classification, yellow soil and yellow-brown soil are Alfisols and limestone soil is Inceptisols. The soil samples were collected from the surface layer (0–12 cm) of each site after removal of litters, the collected soils were air dried, gently crashed and carefully picked out visible plant debris and stones, sieved through a mesh (< 2 mm) and then stored in glass jars for later use.

Soil pH was determined using a combined glass electrode in deionized water (the ratio of soil (g) to H₂O (mL) was 1:5). Particle-size distributions of soils were determined followed the hydrometer method (ASTM D422-63), percentages of sand, silt and clay in soils were calculated on the basis of hydrometer readings at 40 s and 2 h. Cation exchange capacity (CEC) was determined following a standard method of USEPA (EPA Method 9081). The CaO contents of soils were determined by a polarized wave-length dispersive X-ray fluorescence spectrometer (Axios PW4400) after 0.7 g of each dried soil sample was ground to < 200 mesh and fused with 7 g Li₂B₄O₇ into glass disc. The total organic carbon (TOC) and nitrogen (N) contents of original soil samples were determined by combustion method. In brief, a certain amount of original soil sample was weighed and treated by over dosage of HCl (the ratio of soil (g) to HCl solution (mL) is 1:30) to remove carbonates, the treated soil was collected by centrifugation, washed with deionized water to neutral pH, freeze-dried and weighed again, 10 mg of the treated soil was wrapped with tinfoil and then measured in an elemental analyzer (Vario El III, Germany). The TOC and N contents of soil were reported based on the original soil mass.

2.2. Sequential extraction of HA fractions

Three HA fractions were sequentially extracted from soil samples following the scheme showed in Fig. 2. Briefly, the first HA fraction, named as free HA (Haf), was extracted directly from the original soil sample. The second HA fraction, named as encapsulated HA (HAe), was extracted from the ground soil residue after the extraction steps of Haf. The third HA fraction, named as interacted HA (HAi), was extracted from the partially demineralized soil residue after the extraction steps of HAe.

In detail, the original soil sample (100 g) was immersed in NaOH solution (400 mL, 0.5 mol L^{-1}) under N_2 ambience and gently shaken for 4 h. The supernatant was separated after centrifugation (3500 rpm, 10 min) and a suitable amount of NaOH solution was refilled, this step was repeated several times until the supernatant became transparent and nearly colorless. All collected supernatants were combined and acidified with HCl (6 mol L^{-1}) to $\text{pH} = 1.0$, stood overnight under N_2 ambience, centrifuged, and the precipitation was collected as the raw Haf sample. After extraction of Haf, the soil residue was deionized, dried and carefully ground using an agate mortar to break down large soil aggregates/particles. The extraction and collection steps of HAe were the same to those of Haf, supernatants were combined, acidified, and the precipitation was collected as the raw HAe sample. The soil residue after the extraction of HAe was deionized, dried again, and treated with over dosage of HCl (200 mL, 6 mol L^{-1}) to remove certain soil minerals like carbonates. The supernatant was decanted after centrifugation (3500 rpm, 10 min), and the residue was extracted again with NaOH solution, the extraction and collection steps of HAI were the same to those of Haf and HAe, supernatants were combined, acidified, and the precipitation was collected as the raw HAI sample.

The purification procedures of Haf, HAe and HAI samples followed exactly the procedure detailed elsewhere (Ma et al., 2016). Briefly, the HA sample was dissolved completely by KOH solution (0.1 mol L^{-1}) in a centrifuge bottle to reach an approximate concentration of 10 g L^{-1} . A certain amount of KCl solid was allowed to make the K^+ concentration about 0.3 mol L^{-1} , the solution was shaken and centrifuged to collect supernatant, the supernatant was then acidized to $\text{pH} = 1.0$ with HCl solution (6 mol L^{-1}), and the precipitate was collected by centrifugation. The obtained precipitate was demineralized twice by HCl/HF solution ($0.1/0.3 \text{ mol L}^{-1}$) for 24 h at room temperature, and then dialyzed against deionized water using a dialysis tube (3500 Da) until no reaction with AgNO_3 (0.1 mol L^{-1}). The precipitate was freeze-dried, gently ground and sieved (100 mesh) before it was marked as the final HA sample.

2.3. Characterizations of HA samples

The element compositions (including C, H, N) of HA samples were measured by combustion method in the same elemental analyzer and were reported after normalization by ash contents, the content of oxygen in HA sample was estimated by subtraction method. The ash content of HA sample was determined by burning the HA sample at 750°C in a muffle furnace for 4 h.

The HA samples were further characterized by Fourier transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy and potentiometric titration. The FTIR spectra of HA samples were recorded on a Bruker VERTEX 70 FTIR Spectrometer (Bruker Corporation, Germany) with attenuation total reflection technique, the scanning range was $4000\text{--}400 \text{ cm}^{-1}$. The method was detailed elsewhere (Ma et al., 2014). The UV-Vis spectra of HA samples were obtained by a Cary 300 UV-Vis spectrophotometer (Agilent Technologies, USA) with a scanning range of $800\text{--}200 \text{ nm}$. Briefly, the solid HA sample (5.0 mg) was dissolved in a NaHCO_3 solution (0.05 mol L^{-1} , 100 mL), then an aliquot solution was transferred into a quartz cuvette (1 cm path length) for measurement, the E_4/E_6 ratio refers to the ratio of absorbance at 465 and 665 nm. The contents of acidic groups in HA

samples were determined by a T50 titrator (Mettler Toledo, USA) equipped with a Mettler DG-111-SC pH electrode. The acid groups of HA samples were determined followed the potentiometric titration method described by Fernandez et al. (Fernandez et al., 2007). Briefly, the HA sample (50 mg) was dissolved thoroughly in 10 mL of the mixed solution (NaOH (0.126 mol L^{-1}) and NaNO_3 (1 mol L^{-1})). The solution was diluted carefully with deionized water to the volume of slightly smaller than 100 mL, the pH was adjusted to 3 with HCl (1 mol L^{-1}), and then the solution volume was adjusted to exact 100 mL by deionized water. The solution was titrated by the NaOH solution (0.126 mol L^{-1}) with an increment of $10 \mu\text{L}$ until $\text{pH} = 11$. The acidic groups (carboxyl and phenolic hydroxyl groups) were quantified following the method by McCallum and Midgley (McCallum & Midgley, 1975).

3. Results

Three different HA fractions, Haf, HAe and HAI, were obtained by the sequentially extraction method. Practically, three HA fractions were obtained according to their different binding conditions with soil matrix. The Haf fraction was free from protections of soil aggregates and was dissolved easily in base solution. The HAe fraction was protected or encapsulated by large soil aggregates and can be extracted when soil aggregates were broken down. The HAI fraction was either encapsulated in fine soil aggregates or interacted firmly with soil minerals, such as carbonates, oxides and clays, and can be extracted only when these soil minerals were spoiled. The amounts and physico-chemical characteristics of these three HA fractions in soil could be linked to the properties of bulk soil and the vulnerability of soil to karst rocky desertification.

3.1. The properties of soils and the distributions of different HA fractions in soils

The main properties of soils and extraction amounts of HA fractions were listed in Table 1 and Table 2, respectively. The measured TOC contents of yellow soil, limestone soil and yellow-brown soil were 2.01, 3.12 and 2.31%, respectively, all of which are relatively high compared to previous reports of soils with notable rock desertification in the study area. This is not surprising since the vegetation covers of all sampling sites are quite well. The C/N ratios of three soils were different, reflecting the different vegetation types of sampling sites, e.g. the yellow soil whose vegetation cover dominated by coniferous forest showed the highest C/N ratio (15.02). The total extractable HA (THA) were quite abundant in all three soils, the THA content in limestone soil was 14.55 g kg^{-1} , slightly higher than those of yellow soil (11.83 g kg^{-1}) and yellow-brown soil (13.38 g kg^{-1}). While the THA/TOC ratio (the carbon contribution of THA to TOC) of limestone soil was 23.4 wt%, significantly lower than those of yellow soil (31.4 wt%) and yellow-brown soil (30.7 wt%). The distributions of three HA fractions in three soils were different significantly. Limestone soil showed the lower Haf content (6.06 g kg^{-1}) and much lower HafC/TOC ratio (9.29 wt%) than those of yellow soil (7.20 g kg^{-1} and 18.70 wt%) and yellow brown soil (9.53 g kg^{-1} and 21.30 wt%). On the contrary, limestone soil showed significantly higher HAe and HAI contents, and higher HAeC/TOC and HAIc/TOC ratios compare to those of yellow soil and yellow-brown soil.

3.2. Characteristics of different HA fractions

The three sequentially extracted HA fractions from each soil were systematically inspected to explore the relationships between their chemical characteristics and their occurrence states in soil. Two results were apparent, first, the sequential extraction method did obtain three different HA fractions from the bulk soil and amounts of three HA fractions were comparable; second, the different HA fractions showed

Table 3

The element compositions of different HA fractions (Ash-free).

Soil	Haf (wt%)					HAe (wt%)					HAI (wt%)				
	C	H	O	N	Ash	C	H	O	N	Ash	C	H	O	N	Ash
Y	53.28	5.61	36.70	4.18	2.00	55.74	4.18	36.55	3.48	1.88	59.43	4.45	33.85	2.14	3.57
L	49.52	5.72	38.68	6.53	3.42	52.03	4.56	39.05	4.27	2.53	54.86	3.68	39.33	2.02	0.77
B	52.35	4.89	40.21	4.18	1.36	58.26	4.51	33.81	3.22	1.46	54.88	3.74	39.08	2.22	2.51

Table 4C/N, H/C and O/C atomic ratios and E₄/E₆ ratios of different HA fractions.

Soil	Haf				HAe				HAI			
	C/N	H/C	O/C	E ₄ /E ₆	C/N	H/C	O/C	E ₄ /E ₆	C/N	H/C	O/C	E ₄ /E ₆
Y	14.88	1.26	0.52	5.63	18.65	0.89	0.49	4.24	32.40	0.89	0.43	4.11
L	8.84	1.38	0.60	4.55	14.19	1.04	0.56	4.48	31.71	0.80	0.54	4.09
B	14.58	1.11	0.58	6.70	21.08	0.92	0.44	4.31	28.81	0.81	0.53	3.93

significantly different characteristics. We believe these two results were interrelated and of potential significance on soil quality and evolution.

3.2.1. Elemental compositions of different HA fractions

The element compositions and ash contents of three HA fractions were listed in Table 3, the atomic ratios were listed in Table 4. The C contents of Haf samples (49.52–53.28 wt%) were lower than corresponding those of HAI and HAe samples (52.03–59.43 wt%), the H and N contents of Haf samples were higher than corresponding those of HAe and HAI samples. The C/N atomic ratios of three HA fractions of three soils showed a consistent variation, they were apparently higher in HAe and HAI samples than in Haf samples. The different chemical compositions may represent the different evolution states, or humification degrees, of the three HA fractions, it was suggested that the C/N atomic ratio of SOM reflects the degree of microbial decomposition of the plant-derived organic matter (Stevenson, 1994). The C/N atomic ratios of HAe and HAI were significantly higher than the corresponding Haf fractions which may indicate that Haf fractions have undergone more intensive microbial transformation than HAe and HAI fractions. This result is consistent with findings of previous studies, for example, Amelung and Zech (Amelung & Zech, 1996) found that C/N atomic ratios of the interior SOM of soil aggregates were higher than the surface SOM of soil aggregates and they ascribed it to the lower microbial accessibility of the interior SOM of soil aggregates. Conversely, the sequential extraction strategy itself demonstrated that HAe and HAI fractions were bound more closely with the soil matrix and were separated from accessing of microorganisms, especially HAI. Therefore, we considered that the higher C/N atomic ratios of Haf fractions could imply that they have undergone more biological degradation or transformation.

3.2.2. UV-Vis spectroscopy

It has been suggested that the UV-Vis scanning spectra of different HA samples are often quite similar, lacking intensive peaks and absorbance intensity decreasing continuously along the scanning range (Giovanela et al., 2010). However, this study observed some differences among UV-Vis scanning spectra of different HA fractions (Fig. 3). The UV absorbance intensities of three sequentially extracted HA fractions were obviously different, in a trend of HAe > HAI > Haf, suggesting aromatic degrees of HAe and HAI should be higher than that of Haf (Senesi et al., 1996). The UV-Vis spectra of Haf in three soils showed larger diversity than those of HAe and HAI samples, suggesting that the chemical properties of Haf fractions may differ significantly in different soils, while the chemical properties of HAe and HAI fractions are relatively inert to soil types. A small shoulder peak around 270 nm, observed in all three Haf samples but not in HAe and HAI samples, was

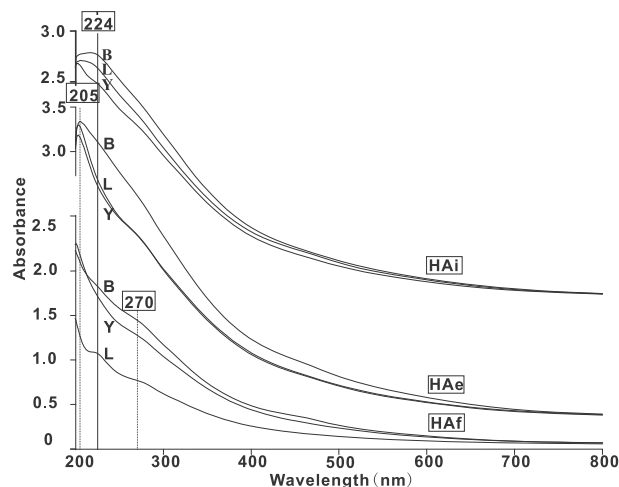


Fig. 3. UV-Vis scanning spectra of different HA fractions from three soils.

attributed to the overlapping absorbance of chromophores present in the humic cores (Giovanela et al., 2010; Peuravuori & Pihlaja, 1997) and/or the absorbance of lignin (Berto et al., 2005; Fookan & Liebezeit, 2000; Giani et al., 2010). This could mean more components other than lignin existing in HAe and HAI samples. Peaks around 205–230 nm were attributed to α - β -unsaturated acids, esters and amides signals (Senesi et al., 1996), they could be recognized easier in spectra of HAe and HAI samples than in Haf samples, suggesting that HAe and HAI fractions may contain more α - β -unsaturated acids, esters and amides groups than Haf fraction, however, a specific conclusion needs more data. The E₄/E₆ ratio, an useful index for humic substances, is mainly but not exclusively governed by the molecular size or the apparent molecular weight of humic substances, the lower is the E₄/E₆ ratio, the higher is the molecular weight (Brunetti et al., 2012; Calderoni & Schnitzer, 1984; Chen et al., 1977; Enev et al., 2014; Giovanela et al., 2010; Schnitzer, 1982; Stevenson, 1994). The E₄/E₆ ratios of the three HA fraction samples were listed in Table 4. Generally, the E₄/E₆ ratios of Haf fractions were higher than those of HAe and HAI fractions, suggesting that Haf has the relatively lower molecular weight than HAe and HAI, and this could be instructive in understanding the different evolutions of the sequentially extracted HA fractions.

3.2.3. FTIR spectroscopy

The FTIR spectra of three HA fractions were compiled and shown in Fig. 4, the meaningful peaks were marked in the figure. In general, FTIR spectra of the nine HA samples were similar in terms of the number and

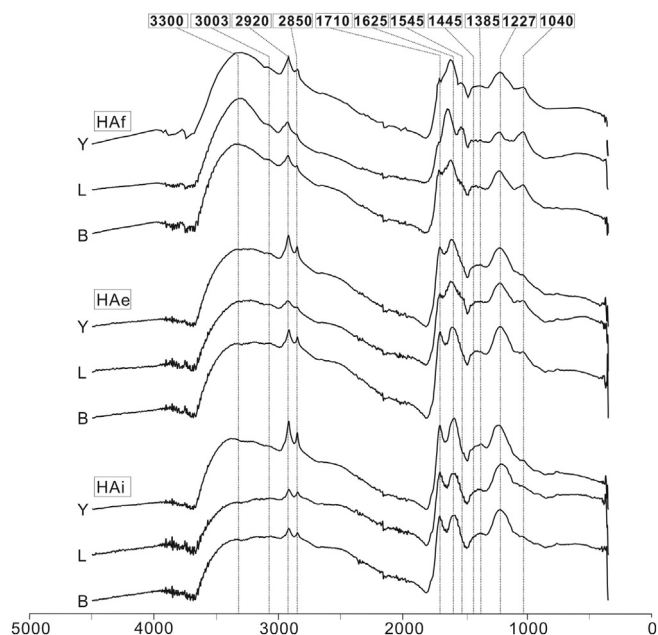


Fig. 4. FTIR spectra of different HA fractions from three soils.

the position of major peaks, however, the intensity of several peaks of the different HA samples showed appreciable differences, especially between different HA fractions. The HAF samples showed significantly higher absorption band at 1040 cm^{-1} than HAE and HAI fraction samples, indicating that HAF fractions had higher carbohydrate contents than HAE and HAI fractions, since the absorption band at 1040 cm^{-1} was found to represent the C–O asymmetric stretch vibration of carbohydrates (Giovanela et al., 2010; Kalbitz et al., 1999; Peschel & Wildt, 1988; Stevenson & Goh, 1971). The absorption band at 1545 cm^{-1} is believed to represent amide groups (Traversa et al., 2014), and were remarkably higher in HAF samples than in HAE and HAI samples, suggesting that HAF samples had higher amide group contents than HAE and HAI samples. This is consistent with the above element analytical result that HAF fractions had lower C/N ratios and the later titration result that HAF fractions had higher total acidities. It further implied that 205–230 nm peaks in UV–Vis spectra of HAE and HAI samples represented α - β -unsaturated acids and esters contents, but not amides in this study.

The peak at 1710 cm^{-1} was attributed to the C=O stretch vibration of various groups, such as carboxyl acid, carboxylate, ester, ketone, and amide (Adani et al., 2007; Peng et al., 2014; Traversa et al., 2014). The 1710 cm^{-1} peaks of HAF samples were remarkably smaller than those of HAE and HAI samples, indicating HAF samples have considerably lower C=O contents. However, the element analytical result showed that O/C ratios of HAF samples were slightly higher than those of HAE and HAI samples, this verified the conclusion in the discussion of the 1040 cm^{-1} adsorption band that HAF samples contain more carbohydrates than HAE and HAI samples. The distinguishable peaks at 1710 and 1625 cm^{-1} for HAE and HAI samples demonstrated that they contain more carboxylic acids than HAF samples. The peaks at 1385 , 1445 , and 2920 cm^{-1} were related with C–H bond signals in methyl or methylene groups, they were smaller in HAF samples indicated that HAF fractions may contain less methyl or methylene groups than HAE and HAI fractions.

3.2.4. The acidic groups

As shown in Table 5, the HAI fraction had the highest carboxylic group contents and the HAF fraction had the lowest carboxylic group contents, which verified the results of FTIR spectra. The lowest phenolic group contents were found in HAI fractions and the highest phenolic

Table 5

The distribution of acidic groups in the different HA fractions ($\text{mmol g}^{-1}\text{C}$).

Soil	HAF			HAe			HAI		
	COOH	ArOH	Total	COOH	ArOH	Total	COOH	ArOH	Total
Y	4.931	1.510	6.441	5.849	1.742	7.591	6.288	1.221	7.509
L	3.729	1.618	5.347	6.181	1.679	7.860	6.737	1.267	8.004
B	5.587	1.690	7.277	6.253	1.533	7.786	7.261	1.267	8.528

group contents were found in HAE fractions (except yellow-brown soil whose HAF fraction had a higher phenolic group content). The total acidic group contents (sum of carboxylic and phenolic groups) of HAI and HAE samples were significantly higher than those of HAF samples. The content of carboxylic and phenolic groups determines the acidity of HA samples, affects interactions of HA and soil minerals, and such plays important roles in evolution of soil HA. We believe that HAE and HAI fractions interacted more closely with soil matrix, their high acidic group contents, especially carboxylic groups, indicated that carboxylic groups are major agents for HA to bind with the soil matrix. The higher carboxylic groups content usually means the higher hydrophilicity and solubility of HA, however, HAE and HAI fractions could not be extracted until soil samples were ground repeatedly and demineralized by the HCl solution, implying that the soil matrix has significant effects on the behavior of HAs and provide more protection to certain HA fractions.

4. Discussion

Rocky desertification, a phenomenon of extreme erosion and degradation of soils, was developed in various degrees at different soil sites in the karst region of southwest China. For example, it was reported that limestone soil dominated location usually showed more severe desertification status compared to adjacent areas of other soils when overlying vegetation was spoiled (Zheng & Wang, 2002), but underlying mechanisms remained unclear.

This study found that TOC and THA contents of limestone soil were significantly higher than those of yellow and yellow-brown soils, which is consistent with previous reports of higher TOC content in limestone soils than in other soils (Liu, 2009; Ma et al., 2016; Office of Soil Survey, 1994; Wang et al., 2018). The notably higher calcium content in limestone soil than in other soils could be one reason for higher TOC and THA contents in limestone soil, since prior studies had found that SOM can be preserved by forming Ca^{2+} -SOM complexes (Baldock et al., 1992; Bimüller et al., 2016; Duhaufour, 1976; Lopez-Sangil & Rovira, 2013; Oades, 1988) and/or by being encapsulated in secondary carbonates (Cunliffe et al., 2016; Lopez-Sangil & Rovira, 2013). Given that limestone soil dominated regions showed more severe rocky desertification than other soils dominated regions, it is reasonable to question the abilities of high TOC and THA contents in stabilizing aggregate structures and improving erosion resistance of limestone soil. Therefore, the role of HA quality in karst rocky desertification should not be ignorable. We propose that the vulnerability of limestone soil to karst rocky desertification may associate with its low THAC/TOC ratio.

The sequential extraction strategy roughly simulated varied disturbances may exert to natural soil. The first extraction step, in which soil was soaked in base solution with slight stirring, representing the status of slight disturbance; the second extraction step, in which large soil aggregate and partial mineral coating were broken by the operation of gentle grinding, representing the status of moderate disturbance; the third extraction step, in which fine soil aggregate and most mineral coating were broken by manual grinding and/or acid extraction, representing the status of severe disturbance. The different disturbance levels may expose different fractions and amount of SOM to runoffs and microorganisms, by such distinctive erosion and degradation consequences of soil may occur. We believed that HAE and HAI fractions, which were extracted after artificial disturbances, were protected better

by soil matrix and likely experienced less microbial transformation. This point of view can be supported by following characterization results: 1) the corresponding HAE and HAI fractions extracted from three different soils are quite similar in terms of elemental compositions, FTIR spectra and UV–Vis spectra; 2) the C/N ratios of HAE or HAI fractions are significantly higher than corresponding those of HAF fractions, and 3) the molecular weights of HAE and HAI fractions, indicated by the E_4/E_6 ratio, were relatively larger and less varied compared to those of HAF fractions. In addition, HAE and HAI fractions showed higher polarity than HAF fractions, e.g. HAE and HAI fractions contained higher total acidic group contents and carboxylic group contents than corresponding HAF samples did, and contents of phenolic group in HAE fractions were also higher than in HAF fractions (Table 5); the contents of α - β -unsaturated acids in HAE and HAI fractions were higher than those in HAF fractions (Fig. 3); the amounts of C=O groups estimated from FTIR spectra in HAE and HAI fractions were significantly higher than in HAF fraction (Fig. 4). The higher polarity suggests that HAE and HAI fractions should be more soluble and more mobile once being dissociated from soil matrix, the higher C/N ratios suggests that HAE and HAI fractions should be more biodegradable once being dissociated from soil matrix. Therefore, we believed that soils with higher proportions of HAE and HAI fractions may be more susceptible to disturbances, and that this finding can help in understanding differential occurrences of karst rock desertification on different soils in karst area.

Our results showed that the HAF yield of limestone soil was significantly lower, the HAF/TOC ratio of limestone soil was 9.3%, significantly lower than those of yellow soil (18.7%) and yellow-brown soil (21.3%), and also lower than those reported by others (20%) (Grasset & Amblès, 1998a; Grasset & Amblès, 1998b). The lower HAF/TOC ratio of limestone soil resulted in its higher sensitivity to environmental changes and vulnerability to karst rocky desertification implies that chemical properties of SOM and interactions of SOM with soil matrix play important roles in vulnerability of limestone soil to rocky desertification.

The reasons for different distributions of HA fractions in limestone soil from other two soils may be manifold, among which the significantly higher calcium content in limestone soil could be important one. It had been reported that calcium ion can prevent HAs from rapid decomposition through forming certain stable complexes with HAs and/or coating HAs as secondary soil minerals, such as carbonates (Baldock et al., 1992; Duchaufour, 1976; Oades, 1988). The CaO content of limestone soil was 5 to 10 times higher than those of yellow soil and yellow-brown soil (Table 1), and therefore explained the higher yields of HAE and HAI in limestone soil. Calcium is one of easily weathered elements in soil and can be removed from surface soil through many ways, such as being leached by rain (Poss & Saragani, 1992) and assimilated by plants and microorganisms. In karst areas, calcium in limestone soil can be supplemented by carbonate bedrock materials and litters of calciphilous plants under natural vegetation conditions (Bigelow & Canham, 2017; Carnol & Bazgir, 2013; Dijkstra, 2003; Palma et al., 2000), the continual calcium supplements restrain exposure of HAE and HAI to microbes, and rainwater and further maintain the physical structure of soil aggregate. However, if the covering plants are spoiled, the Ca^{2+} supplement from litters will be significantly reduced, and HAE and HAI will be exposed when the content of Ca^{2+} in soil is diminished, given the easier decomposition and leaching properties of HAE and HAI, limestone soil, the HAE and HAI enriched soil, will show a rapid loss of SOM and quick soil degradation. This may explicate observations of that limestone soil in the study area maintains a higher TOC content under natural ecological conditions but tends more vulnerable to karst rocky desertification once it has been artificially disturbed.

5. Conclusion

Karst rocky desertification is an urgent issue in the karst area of southwest China and has been found developing variously in regions of different dominated soils. This study developed a sequential extraction method to extract three different HA fractions according to their interactions with soil matrix from three major soils in the study area, and systematically characterized these soils and their HA fractions to explore determinant factors of soil's vulnerability to rocky desertification. The results showed that limestone soil has notably higher TOC content than yellow and yellow-brown soils, but its THAC/TOC ratio is relatively lower. HAF, the most easily extracted HA fraction and the main cementing agent of soil aggregates, has relatively lower C/N ratio, lower total acidity and stronger resistance to biodegradation and erosion. HAE and HAI, which integrate intimately with soil matrix, have relatively higher C/N ratios, higher total acidity, and are vulnerable to runoff erosion or/and biodegradation when it is exposed. Compared to adjacent other soils, limestone soil has significant lower proportion of HAF and higher proportions of HAE and HAI, which may underlie the observation that limestone soil region usually shows a rapid trend of rocky desertification once the natural vegetation covers are deteriorated.

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