

Characteristics and distributions of humic acids in two soil profiles of the southwest China Karst area

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Abstract Characteristics and distributions of humic acid (HA) and soil organic matter (SOM) in a yellow soil profile and a limestone soil profile of the southwest China Karst area were systematically investigated to reveal their evolutions in different soils of the study area. The results showed that characteristics and distribution of SOM along the two soil profiles were notably different. Total organic carbon (TOC) contents of soil samples decreased just slightly along the limestone soil profile but sharply along the yellow soil profile. TOCs of the limestone soils were significantly higher than those of the corresponding yellow soils, and C/N ratios of SOMs showed a similar variation trend to that of TOCs, indicating that SOM can be better conserved in the limestone soil than in the yellow soil. The soil humic acids were exhaustively extracted and further fractionated according to their apparent molecular weights using ultrafiltration techniques to explore underlying conservation mechanisms. The result showed that C/N ratios of HAs from different limestone soil layers were relatively stable and that large molecular HA fractions predominated the bulk HA of the top soil, indicating that HA in the limestone profile was protected while bio and chemical degradations were retarded. Combined with organic elements contents and mineral contents of two soils, we

concluded that high calcium contents in limestone soils may play a key role in SOM conservation by forming complexation compounds with HAs or/and enclosing SOMs with hypergene CaCO₃ precipitation.

Keywords Soil · Soil organic matter · Humic acid · Characteristics · Distribution · Southwest China Karst area

1 Introduction

The Karst area in southwestern China is the largest Karst area with continuous carbonate rock outcrops in the world (Wang 2003). Soil deterioration has been becoming an urgent issue in this area since the increase of human activities, such as farming, mining, and urbanization. The soil deterioration not only constrains sustainable developments but also profoundly shapes geochemical cycles of this area, especially carbon cycle, and it has drawn increased research attention from diverse disciplines. The soil deterioration of the Karst area of southwestern China could be characterized as destroying the soil structure and soil aggregates and decreasing the soil's water-holding capacity and soil fertility (Zhao et al. 2008), all of which are closely related to soil organic matter (SOM). Therefore, a systematic understanding of SOM in typical soils of this area is of important theoretic and practical significance.

SOM is a joint name for all organic substances in soil, and humic substance is the major part of SOM (60–90 wt%) (Dou 2010). In some circumstances, as well as in this study, SOM is referred to as the humic substance of soil. It is known that SOM is a key component of soil and that it controls the structure of soil, influences infiltration and storage of soil water, and supplies trace nutrients for plant growth (Detwiler 1986; Stevenson 1994). However,

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previous studies regarding soils in the Karst area mainly focused on inorganic constituents (Zhu and Li 2002; Wang et al. 2006); few have discussed the origin and quantity of SOM under different land cover or land use conditions (Liu et al. 2005; Liu et al. 2008; Tu et al. 2008). To our knowledge, there are no study reporting characteristics, distribution and evolution of SOM along natural soil profiles in the Karst area. The previous studies show that SOM contents of limestone soils in different Karst areas had clear negative correlations with the stony desertification levels of the areas, as SOM contents decrease dramatically as the stony desertification levels increase (Li 2011). TOC contents in natural soils of the Karst area are relatively high (Liu et al. 2009), and one possible reason for this is that Karst soils normally contain higher amounts of calcium and magnesium ions, which could form stable humus complexes, and thus preserve SOM (Baldock and Skjemstad 2000; Liu et al. 2009). However, the intrinsic mechanisms are either unknown or lack supporting data.

SOM originates from biotic and abiotic transformations of plant, microbial and animal residues in soil (Aiken et al. 1985; Hayes et al. 1989). Because of its high complexity and heterogeneity, SOM can operationally divide into three sub-components: (1) Fulvic acid (FA), which is soluble in aqueous solution at any pH, (2) Humic acid (HA), which is soluble in aqueous solution of $\text{pH} > 2$ but forms a precipitate in acidic solution ($\text{pH} < 2$) and (3) Humin (HM), which is insoluble in aqueous solution at any pH (Stevenson 1994). In the three fractions of SOM, HA, comparatively, is a better indicator for the SOM development, not only because HA is the principal component of SOM (Hayes et al. 1989; Stevenson 1994; Dou 2010) but also because FA is too active and mobile and usually shows little variation along soil profiles (Agnelli et al. 2002) while HM is too inert for ongoing soil environment changes. As an operationally defined SOM fraction, HA is still a very complex mixture of different organic acids and has a wide range of molecular weight (MW) distribution (Swift and Posner 1972; Piccolo and Mirabella 1987; Stevenson 1994), which may mask the occurrence and chemistry changes of HA at different conditions. Prior studies have demonstrated that HA could be further fractionated into relatively simpler fractions using ultrafiltration methods according to their nominal molecular weights. For example, Tonelli et al. and Francioso et al. employed ultrafiltration to separate dissolved organic matter and HA into different fractions and investigated the chemical properties of each fraction (Tonelli et al. 1997; Francioso et al. 2002). Li et al. used sequence extraction and ultrafiltration methods to investigate different fractions of the bulk HA from Pahokee peat, and the results indicated that each batch of extracted HA fraction was composed of two major subunits of aliphatic and aromatic, and that ultrafiltration

technique can effectively fractionate the heterogeneous bulk HA into relatively homogeneous fractions and help explain the evolution of HA in peat samples (Li et al. 2003, 2004). Unfortunately, their results were limited in relation to the evolution of HA since all HA samples were obtained from a single batch of peat sample, and therefore lacked comparison with HA samples of different ages under similar biogeochemical conditions.

The characterization study of HA samples from different layers of a soil profile can extend the understanding of their evolution and reveal their response to biogeochemical environment changes of soil. A systematic study of HA along the soil profiles in the southwest China Karst area can improve understandings about evolution and behaviors of SOM in this special area and contribute to estimating the accurate model of the regional carbon cycle and budget. To this end, the aims of this study were threefold: (1) to characterize SOM and HA in two natural soils, yellow soil and limestone soil, the most common soils in the southwest China Karst area; (2) to distinguish geochemical variations of SOM and HA along the profiles of these two soils; and (3) to discuss evolutions of SOMs in Karst soils.

2 Materials and methods

2.1 Soil Sampling and preparation

Two typical Karst soils, limestone soil (LS) and yellow soil (YS), were collected from Guizhou, southwest China. The LS samples were taken from the site (25.309°N; 107.947°E) in the Maolan state forest park, southeast Guizhou, where the average annual temperature is about 18 °C, the average annual precipitation is about 1370 mm, and the outcrop source rock is dark limestone rock. The YS samples were taken from the site (26.674°N; 106.686°E) in the Duxi forest park, center Guizhou, where the average annual temperature is about 15 °C, the average annual precipitation is about 1117 mm, and the outcrop source rock of the soil is light grey dolomitic limestone rock. The main vegetation at both sites is mainly *Pinus yunnanensis* with small amount of bush. At each site, the litter layer was removed by hand, and soil samples were collected by a stainless steel shovel. The sampling section was 50 cm by 50 cm, and three layers of soil, named as top layer soil (0–12 cm), middle layer soil (12–24 cm) and bottom layer soil (24–36 cm), were taken along the soil profiles. A total of more than 50 kg of fresh soil was collected for each soil layer. The samples were air dried and gently crushed to pass 2 mm sieves. Visible debris of roots and stones was picked out by hand in the laboratory and then stored for later uses. The top layer, the middle layer and the bottom

layer of the limestone soil and the yellow soil samples were named as LS1, LS2, LS3, YS1, YS2 and YS3, respectively.

2.2 Extraction of humic acids

Humic acids were extracted from soil samples using a modified IHSS standard procedure detailed elsewhere (Ma and Xiao 2011). To briefly describe this procedure, the limestone soil sample (5 kg) was treated with 0.1 M HCl solution at a soil weight to solution volume ratio of 1:4 in a polypropylene bottle to remove potential carbonates, and after the slurry had been stirred under protection of N₂ gas for 4 h, the upper solution was syphoned out and centrifuged. Then, the solid residues were put back into the polypropylene bottle and the supernatants were collected for other uses. The soil slurry was then adjusted with 1 M NaOH solution to neutral, and the ratio of soil weight to solution volume was restored to 1:4 by adding 0.5 M NaOH solution. After 4 h agitation and 2 h settlement under protection of N₂ gas, the upper solution was syphoned out and centrifuged at 3500 rpm (2632 g) and as quickly as possible, the residues were returned to the bottle and were repeatedly extracted until the color of the upper solution became transparent to slightly yellowish (usually more than 10 times). Under protection of N₂ gas, all supernatants were collected and acidified to pH 1 with 6 M HCl, stored stillly overnight in darkness to allow them to fully precipitate and then were centrifuged at 3500 rpm (2632 g) to collect the precipitate, which we called raw HA. Under N₂ gas protection, the raw HA was fully dissolved in 0.1 M KOH solution, and an appropriate amount of analytical reagent KCl was added to attain an aqueous K⁺ concentration of 0.3 M. The solution was centrifuged at 10,000 rpm (9060 g) for 10 min to remove residual clay minerals, the supernatant was then acidified to pH 1 with 6 M HCl and left to sit overnight in darkness. After centrifugation at 3500 rpm (2632 g) for 10 min, the obtained precipitate was treated successively by 0.1 M HCl and 0.1 M HCl/0.3 M HF solutions to further remove residual minerals, dialyzed against distilled water to desalt, and then freeze-dried to obtain humic acids, which we named as the bulk HA (BHA). The BHA was transferred into N₂ gas flushed brown bottles and stored at room temperature for later uses. The extraction procedures for yellow soil samples were the same as those used with the limestone soil samples, except that the first acidification step was cancelled since little carbonate exists in yellow soil samples.

A total of six BHA samples, named as LSBHA1, LSBHA2, LSBHA3, YSBHA1, YSBHA2, and YSBHA3, were obtained from top layer, middle layer and bottom layer of the limestone soil and the yellow soil profiles, respectively.

2.3 Fractionation of humic acids by ultrafiltration

The bulk humic acids of the top layer yellow soil (YSBHA1) were separated into 8 fractions of different nominal molecular weights by a mini-Pellicon tangential flow ultrafiltration device from Millipore (Billerica, MA 01821, USA) using seven cassettes equipped with Biomax membranes, whose nominal molecular weight cut off are 5, 10, 30, 50, 100, 300 and 500 kD. Under N₂ gas protection, a total of 40 g YSBHA1 solid sample were dissolved in 8 L background alkaline solution (pH 11) containing 10⁻³ M NaOH and 100 mg/L NaN₃ to prepare the initial YSBHA1 solution (5000 mg/L); the initial YSBHA1 solution was then ultrafiltered following a combined diafiltration and concentration mode detailed elsewhere (Kilduff and Weber 1992; Li et al. 2004) with a flow rate of 200 ml/min under N₂ gas protection to obtain different HA fractions. The procedures of the combined diafiltration and concentration ultrafiltration mode are briefly described here: the 8 L initial LSBHA1 solution in the source tank was first ultrafiltered by the 500 kD molecular cut off membrane to generate 24 l of filtrate (MW <500 kD) by continuously adding in the background alkaline solution to keep the solution volume in the source tank unchanging (diafiltration mode). Next, the background alkaline solution ceased to be added, and continued ultrafiltration occurred until the solution in the source tank was reduced to 2 L (concentration mode). The 2 L solution in the source tank was then moved out, acidified to pH 1 by 6 M HCl, stored overnight in darkness for precipitation, centrifuged at 1000 rpm (9060 g) to remove supernatant, and then freeze-dried to collect HA solid. The obtained solid was dialyzed against distilled water until no reaction occurred with AgNO₃ detection and then freeze-dried to obtain the HA fraction of MW >500 kD, which was named YSHA1-F1. The 30 L HA solution collected after LSHA1-F1 was operated under concentration mode to concentrate its volume from 30 to 2 L, then operated under diafiltration mode with an additional 2 L of the background alkaline solution. The 2 L of solution in the source tank was treated way as the acquisition of LSHA1-F1 in order to obtain the HA fraction of 300 kD < MW <500 kD, which was named YSHA1-F2. The leftover 30 L HA solution was operated repeatedly to obtain the HA fractions of 100 kD < MW <300 kD, 50 kD < MW <100 kD, 30 kD < MW <50 kD, 10 kD < MW <30 kD and 5 kD < MW <10 kD, which were named as YSHA1-F3, YSHA1-F4, YSHA1-F5, YSHA1-F6, YSHA1-F7, respectively. The last 30 L solution was concentrated to 2 L by rotatory evaporation and operated accordingly to obtain the HA fraction of MW <5 kD, which was named YSHA1-F8. A total of 8 HA fractions were fractionated from YSBHA1: YSHA1-F1, YSHA1-F2, YSHA1-F3, YSHA1-F4, YSHA1-F5, YSHA1-F6, YSHA1-

F7 and YSHA1-F8. The bulk humic acids of the top layer limestone soil (LSBHA1) was operated using the same procedures except that we had to stop further separation after obtaining LSHA1-F5 (MW <50 kD) since there might be not enough HA in the solution for further separation since the color of HA solution had become very light. Therefore, a total of 5 HA fractions were fractionated from LSBHA1: LSHA1-F1 (MW >500 kD), LSHA1-F2 (300 kD < MW <500 kD), LSHA1-F3 (100 kD < MW <300 kD) LSHA1-F4 (50 kD < MW <100 kD) and LSHA1-F5 (MW <50 kD).

2.4 Elemental composition of soils, BHAs and fractionated HAs

The elemental compositions (C, H, N and O) of all HA and soil samples were determined by a Vario El-III Elemental Analyzer (Elementar, Germany) following a standard high-temperature combustion procedure (Nelson and Sommers 1982). In brief, the air-dried bulk soil sample (10 g) was ground using an agate mortar and pestle to pass through a stainless steel sieve of 100 mesh (Fisher Scientific, USA). Then, the ground sample was treated with 0.5 N HCl solution at room temperature for at least 3 h to dissolve carbonate minerals, next, the solid was centrifuged and then rinsed with Mill-Q water until neutral. The solid was then freeze-dried and a portion of it was used for analyzing elements contents. The mineral compositions of the soil samples were analyzed using the standard wet chemical method described elsewhere (Пономарёв А. 1959). The E4/E6 values of HA solutions (100 mg/L) at pH 7 were measured by a UV–Visible spectroscopy (AquaMate Plus, Thermo Scientific, USA).

3 Results and discussion

3.1 The properties of the soil profile samples

The basic physicochemical properties of the studied soils are showed in Table 1. The pH values are 4.62, 4.65 and 4.90 for top, middle and bottom layers of YS, respectively,

and are 6.69, 6.62 and 6.62 for the three layer samples of LS, suggesting that YS is an acidic soil and LS is a neutral soil. The measured total organic carbon (TOC) contents and total nitrogen (TN) contents decrease sharply along the YS profile from top to bottom; the TOC content is 2.58 wt% in the top layer and is 0.43 wt% in the bottom layer of the YS profile, and the TN content is 0.17 wt% in the top layer and is 0.06 wt% in the bottom layer of the YS profile. This change is also expressed visually by color changing along the profile, as the soil color is blackish-brown at the top layer and changes gradually to yellowish-brown at the bottom layer. The C/N ratio decreases from 17.7 to 8.4, indicating the soil organic matters in the lower YS layer are relatively rich in nitrogen content. Similar changes were also observed in the LS profile; TOC and TN contents decreased along the LS profile but not as notably as that in the YS profile. The TOC content is 3.17 wt% in the top layer and is 2.09 wt% in the bottom layer of the LS profile, the TN content is 0.31 wt% in the top layer and is 0.22 wt% in the bottom layer of the LS profile, accordingly, and the colors of all three LS layers are blackish-gray. The minerals' contents show the typical natures of the two soils. Compared to those of LS samples, YS samples have much lower calcium contents, relatively higher iron and aluminum contents, and relatively lower silica contents, consistent with their different pedogenic rocks and environments. Additionally, TOC and TN contents of LS samples are higher than those of corresponding YS samples, C and N contents decline slightly along the LS profile but drop sharply along the YS profile, indicating that the stability of soil organic matter in LS is relatively higher than in YS at the study area.

3.2 The properties of BHAs extracted from two soil profiles

The elemental compositions, ash contents, and other features of BHAs extracted from the YS profile and the LS profile samples are summarized in Table 2. In both profiles, C contents of BHAs decrease along profiles from top to bottom, C contents of YSBHAs decrease from 55.71 to

Table 1 The basic physicochemical properties of the soil samples

Sample	Ph (H ₂ O)	Element composition (wt%)			C/N ^a	Inorganic composition (wt%)			
		TOC	TN	H		SiO ₂	Al ₂ O ₃	Fe ₃ O ₄	CaO
YS1	4.62	2.58	0.17	0.95	17.70	38.69	23.92	12.14	0.22
YS2	4.65	0.77	0.09	0.90	9.98	39.91	24.26	12.10	0.27
YS3	4.90	0.43	0.06	0.85	8.36	42.53	24.65	11.92	0.33
LS1	6.69	3.17	0.31	0.76	11.93	59.10	14.16	6.00	2.16
LS2	6.62	2.46	0.25	0.69	11.48	60.30	14.41	5.80	2.25
LS3	6.62	2.09	0.22	0.71	11.08	61.56	14.65	6.20	2.37

^a Atomic ratio

51.69 wt%, and C contents of LSBHAs decrease from 49.88 to 46.16 wt%. O contents of BHAs increase along profiles from top to bottom, that is, increasing from 34.47 to 35.63 wt% in the YS profile and increasing from 41.24 to 45.30 wt% in the LS profile. The contents of N and H of two BHAs show different changes in profiles; in the YS profile, the N and H contents of YSBHAs increase respectively from 3.92 to 5.66 wt% and from 5.48 to 5.91 wt%, while in the LS profile, they decrease respectively from 6.02 to 5.73 wt% and from 6.20 to 6.04 wt%. The changes of organic elements (C, N, H and O) in YSBHAs along the YS profile are consistent with those reported in the literature, e.g. C/N ratios decreased along the soil profiles, (Agnelli et al. 2002; Abakumov et al. 2010), while the changes of organic elements in LSBHAs along the LS profile, especially C/N ratios remained nearly unchanged along the profile, and were quite different from those in the YS profile and others reported in the literature. It should be noted that the ash contents of BHAs from two soil profiles differed remarkably. Although the extraction procedure and burning condition were nearly identical, ash contents of LSBHAs were much higher than those of YSBHAs, and especially, the ash content of LSBHA2 was as high as 12.30 wt%. One possible explanation for phenomenon is that contents of HA-metal chelate complexes in LSBHAs are higher than in YSBHAs, which is supported by higher contents of residue metals in LSBHAs than YSBHAs (data not presented). However, the reason for LSBHAs containing higher residual metals than YSBHAs after the same extraction and purification procedure is still not clear.

3.3 The elemental compositions of ultrafiltration fractionated HAs

The YSBHA1, which is the BHA extracted from the top layer of the YS profile, was fractionated into eight fractions of different molecular weights (YSHA1-F1 to YSHA1-F8). The LSBHA1, which is the BHA extracted from the top layer of the LS profile, was fractionated into five fractions

of different molecular weights (LSHA1-F1 to LSHA1-F5), using the tangential flow ultrafiltration method. The major geochemical properties of these samples are summarized in Table 3. The items of YSHA1-cal and LSHA1-cal are the sums of size fractionated humic acids, representing the recovery rates of the BHAs. Both YSHA1-cal and LSHA1-cal weigh more than 95 % mass of YSBHA1 and LSBHA1, respectively, and the calculated elements contents of C, H, N and O of YSHA1-cal and LSHA1-cal are nearly identical to the measurements of YSBHA1 and LSBHA1, respectively, indicating that ultrafiltration procedures employed in this study did not change the properties of the original humic acids.

Table 3 indicates that general molecular weight distributions of YSBHA1 and LSBHA1 samples are similar in general, e.g. the large molecule HA fractions (MW >50 kD) are the major constituents of these BHA samples, and the small molecule HA fractions (MW <50 kD) are the minor constituents of these bulk HA samples. Nonetheless, the size distributions in the two BHAs remain notably different; for example, the sum of large molecular fractions (YSHA1-F1–F4) and the sum of small molecular fractions (YSHA1-F5–F8) occupy more than 70 wt% and about 25 wt% of YSBHA1, respectively, and the sum of large molecular fractions (LSHA1-F1–F4) and the small molecular fraction (LSHA1-F5) occupy more than 91 wt% and about 3.5 wt% of LSBHA1, respectively. The LSBHA1 sample is predominated by large molecule fractions and the small molecule fractions are quite rare, the weight percentage of LSHA1-F5 in the LSBHA1 is as low as about 3.5 wt%, and practically speaking, further fractionations become impossible as the visual color of the LSHA1-F5 solution has become very slight and transparent. This scenario is quite different from that of YSBHA1, in which the percentage of fraction with molecule weight smaller than 50 kD is about 25 wt% and the percentage of the finest fraction (YSHA1-F8) is still more than 8 wt%.

The contents of organic elements (C, N, H and O) in ultrafiltration fractionated HA fractions of the two soil samples show different features. The C content is 48.88

Table 2 Elemental composition, atomic ratio, ash content, and E4/E6 of BHAs from two soil profiles

Sample	Elemental composition (wt%) ^a				Atomic ratio			Ash (wt%)	E4/E6
	C	N	H	O	C/N	H/C	O/C		
YSBHA1	55.71	3.92	5.48	34.47	16.58	1.17	0.46	0.22	8.4
YSBHA 2	53.24	4.93	5.91	35.05	12.60	1.32	0.49	<i>N.D.</i>	9.7
YSBHA 3	51.69	5.66	5.91	35.63	10.65	1.36	0.52	<i>N.D.</i>	11.4
LSBHA1	49.88	6.02	6.20	41.24	9.67	1.48	0.62	1.55	7.3
LSBHA 2	47.50	5.63	6.05	45.23	9.84	1.51	0.71	12.30	7.1
LSBHA 3	46.16	5.73	6.04	45.30	9.40	1.56	0.74	4.49	7.0

^a Corrected to the ash content

Table 3 Molecular size, yield, elemental composition, atomic ratio, ash content and E4/E6 of ultrafiltration fractionated HAs fractions and BHAs from top layers of two soil profiles

Sample	MW (kD)	Mass (wt%)	Element composition (wt%) ^a				Atomic ratio			Ash (wt%)	E4/E6
			C	N	H	O	C/N	H/C	O/C		
YSBHA1	N/A	100	55.71	3.92	5.50	34.47	16.58	1.17	0.46	0.22	8.4
YSHA1-F1	>500	25.81	57.41	4.35	6.40	33.92	15.40	1.33	0.44	1.24	6.0
YSHA1-F2	500-300	6.12	55.55	4.17	5.40	35.93	15.54	1.16	0.49	N.D.	6.2
YSHA1-F3	300-100	21.07	56.85	4.53	5.75	36.36	14.64	1.20	0.48	1.46	8.4
YSHA1-F4	100-50	17.27	55.95	4.12	5.06	37.75	15.84	1.08	0.51	0.98	8.6
YSHA1-F5	50-30	5.56	55.13	3.54	4.69	38.03	18.17	1.01	0.52	1.00	8.8
YSHA1-F6	30-10	3.24	54.92	3.12	4.43	38.40	20.53	0.96	0.52	0.35	8.9
YSHA1-F7	10-5	7.95	54.91	2.89	4.42	39.92	22.16	0.96	0.55	1.24	9.1
YSHA1-F8	<5	8.14	55.50	2.23	4.51	37.22	29.03	0.97	0.50	N.D.	15.4
YSHA1-cal	N/A	95.12	56.31	3.94	5.45	36.46	16.66	1.15	0.49	N/A	N/A
LSBHA1	N/A	100	49.88	6.02	6.20	41.24	9.39	1.45	0.62	1.55	7.3
LSHA1-F1	>500	45.33	48.88	6.74	6.70	42.15	8.46	1.63	0.65	4.79	6.0
LSHA1-F2	500-300	4.88	51.55	6.29	6.01	39.70	9.56	1.40	0.58	0.72	7.3
LSHA1-F3	300-100	32.23	51.27	6.27	5.66	37.86	9.54	1.32	0.55	0.84	8.1
LSHA1-F4	100-50	9.53	53.79	4.75	4.98	38.60	13.21	1.11	0.54	0.38	9.6
LSHA1-F5	<50	3.52	55.42	3.22	4.86	37.05	20.08	1.05	0.50	0.93	10.4
LSHA1-cal	N/A	95.49	50.55	6.23	6.07	40.03	9.47	1.43	0.59	N/A	N/A

N/A not available, ND not detected

^a Corrected to the ash content

wt% in the LSHA1-F1 and becomes 55.42 wt% in the LSHA1-F5, contents of N, H, and O are 6.74, 6.70 and 42.15 wt%, respectively, in the LSHA1-F1 and decrease to 3.22, 4.86 and 37.05 wt%, respectively, in the LSHA1-F5. In summary, C contents increase but N, H, and O contents decrease as the apparent molecular weights of HAs in LSBHA1 decrease. The C, N, H, and O contents in the ultrafiltration fractionated fractions of YSBHA1 do not show any consistent change trends. The contents of C, N, and H decrease and contents of O increase as the apparent molecular weight of HAs decrease from YSHA1-F1 to YSHA1-F7, but the smallest molecular fraction, YSHA1-F8, is different. For example, its O content (37.22 wt%) shows a notable decline compared to that of YSHA1-F7 (39.92 wt%), indicating its different evolution.

4 Discussion

Yellow soil and limestone soil are the most common soils in the Karst area of southwest China. Although the regional climate conditions and the main vegetation of the two sampling sites are similar, characteristics and distributions of SOM and extracted HA fractions along YS and LS profiles show significant differences, implying that the

evolutions and preserve mechanisms of SOM in the two soil profiles are different.

The atomic ratios of BHAs (Fig. 1) from the two soil profiles indicate that C/N ratios of BHAs in LS samples are significantly smaller than those in corresponding YS samples, but H/C and O/C ratios of LSBHAs are larger than those in corresponding YSBHAs. The lower C/N ratio indicates that BHAs from limestone soils include more N-containing groups, while the larger ratios of H/C and O/C suggest that BHAs from limestone soils may contain more carbohydrate fractions. The average C/N ratios of three-year old leaf-litters from two study regions are quite similar, as all of them fall in a range of from 17 to 21 with a mean of about 20 (Xiong et al. 2013). Prior study had shown that elemental compositions of soil HA mainly depend on transformations of litters by soil organisms (Howard et al. 1998). If the raw materials of forming soil HAs are mainly litters, the distinctive difference C/N ratios of LSBHAs and YSBHAs may suggest different biogeochemical alterations in the two study sites. The white rot fungi are key soil organisms in the decomposition of lignin into polyphenols, and they favor the neutral soil condition. It was found that the decomposition rate of lignin is remarkably affected by the pH of soil. The pH of soil affects decomposition of organic

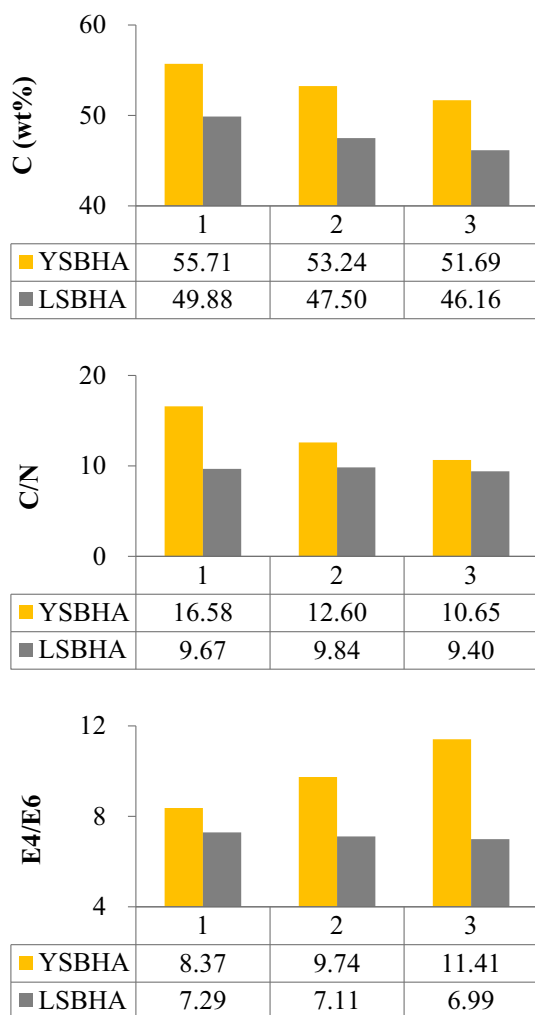


Fig. 1 The atomic ratios of C/N, O/C and H/C in the BHAs

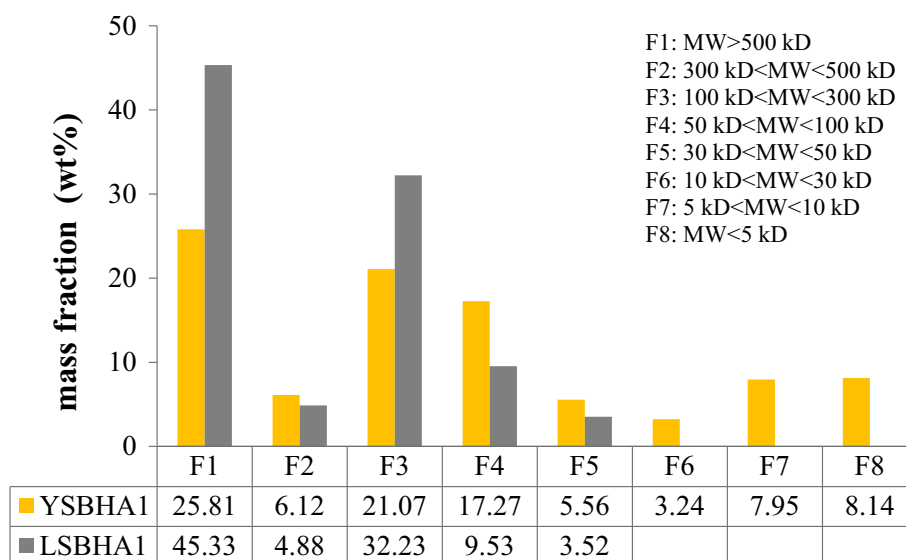
matter by regulating the activity of enzyme, such as horseradish peroxidase (Setti et al. 1998; Ghasempur et al. 2007), and by controlling the growth of soil microorganisms (Pometto and Crawford 1986). Lignin in soil may be oxidized into simple phenols, such as p-coumaryl, coniferyl and sinapyl alcohols, even into CO_2 and H_2O (Freudenberg and Nash 1968), and the simple phenols and other carbohydrates may polycondense with amino acids/peptides and polymerize by peroxidases catalyzed oxidation coupling reaction to form humic acids (Cerli et al. 2008). Polycondensation of oxidized polyphenols with amino acids/peptides could be an important source of nitrogen content of HA (Haider et al. 1965; Howard et al. 1998; Schulten and Schnitzer 1997; Swift and Posner 1972). Therefore, in spite of effects from many other possible influence factors, such as constituents of root-litter and microflora, the higher pH of limestone soil as compared to that in yellow soil should be an important factor underlying the observation that BHAs from

limestone soils have smaller C/N ratios than those from the corresponding yellow soils.

The C/N ratios of BHAs along the limestone soil profile and the yellow soil profile show different scenarios, and they keep unchanged along the limestone soil profile but decrease rapidly along the yellow soil profile. This seems to contradict higher microorganism activities in the limestone soil, since biodegradation normally will diminish the nitrogen content of humic acid (Hempfling et al. 1988). To settle this contradiction, we hypothesize that there exists certain mechanism(s) of protecting humic acids from degradation in the limestone soil profile. The protection mechanisms may arise from two scenarios. The first scenario, soil humic acids may complex with Ca^{2+} ions to form a relatively stable complexation, calcium-humate (Hu et al. 2012; Yan et al. 2012), and the calcium-humate is much less liable to biodegradations than humic acids. The limestone soil contains high contents of calcium (with an average of 2.26 wt% in CaO form), which is about one order magnitude higher than that of the yellow soil (0.27 wt% in average), and the molar ratio of $\text{Si}/(\text{Al} + \text{Fe})$ in LS samples is about 2.8 (Table 1), all of which are in favor of sorbing HA via ‘ Ca^{2+} bridging’ and preserving humic acid (Kloster et al. 2013; Mikutta et al. 2007). The second scenario, insulation of SOM by CaCO_3 in the limestone soil could be another preservation mechanism. Although the studied limestone soils are classified as neutral soils, the relatively strong bubble reaction when the limestone soils were treated with diluted hydrochloride acid solution confirmed the existence of free CaCO_3 . Meanwhile, the limestone soil is usually a thin formation on the carbonate mother rock of the study area, and bicarbonate and calcium ions contents in groundwater/soil water are usually high (Wang 2003). The weather in the study area alternates quickly between wet and dry, so the limestone soil is either soaked by water during rain time or is completely dried during draught time, causing reoccurrence of dissolving and precipitating of CaCO_3 , which makes it possible for SOM to be insulated by hypergene CaCO_3 precipitate. Once SOM is insulated by CaCO_3 precipitate, accessibility of SOM to soil microorganisms and chemical reaction potential of SOM are both reduced, and thus humic acids are prevented from degradation. However, roles of the two mechanisms in preserving SOM in limestone soils cannot be quantitatively distinguished so far.

Besides physicochemical and biogeochemical conditions of soil matrix, characteristics and formation-degradation reactions of humic acids in soil also play important roles in their distribution and evolution along the soil profile. The molecular weight is one of the most important characteristics of soil humic acids. Prior studies have revealed that the E4/E6 ratio of humic substance is substantially related with its molecular weight: the smaller the

Fig. 2 The distributions of HAs with different molecular sizes in YSBHA1 and LSBHA1



E4/E6 ratio the higher the molecular weight (Chen et al. 1977). The E4/E6 ratios for LSBHA1 and YSBHA1 are 7.3 and 8.4, respectively, indicating that the average molecular weight of LSBHA1 is relatively larger than that of YSBHA1. This could be related with the different humification stages of two BHAs; however, the underlying reasons for this phenomenon need further discussing. The BHAs from top layers of limestone soil and yellow soil profiles were further classified into different fractions according to their apparent molecular weights, which were determined by cut-off sizes of ultrafiltration membranes. The distributions of different apparent molecular weight HAs in the two BHA samples show notable differences (Fig. 2). In YSBHA1, contents of small molecular HA fractions are significantly less than those of large molecular HA fractions, but their existence is not ignorable. For example, YSHA1-F8 occupies more than 8 wt% of the total mass of YSBHA1, and this distribution pattern is very similar to that of the peat humic acid reported by Li et al. (2004). LSBHA1, on the other hand, are predominated by large molecular HA fractions and small molecular fractions are almost ignorable. As we have described above, litter material inputs are quite similar at the two sample sites, and the apparent molecular size distributions of two BHAs could be another piece of supporting data that degradation or fragmentation processes for large HAs turning into small HAs were restrained in the limestone soil.

The characteristics and distributions of soil humic acids in soils of Karst areas have important implications in understanding soil development and degeneration along with the change of ecosystems in Karst areas. Yellow soil, which is usually thick and develops A-B-C layers, is the most distributed zonal soil of the southwest China Karst area. The elemental characteristics of yellow soil humic

acid and their variations along the profile are comparable to those reported for the Pahokee peat humic acid (Li et al. 2004) and other soil profiles (Tonelli et al. 1997; Francioso et al. 2002), indicating that the evolution of its humic acids is mainly controlled by water-temperature and microbiological conditions. However, limestone soil, the typical azonal soil of the southwest China Karst area, and its elemental characteristics and distributions of its humic acid along the profile are significantly affected by calcium ion as well as other mineral compositions. On one hand, this preserves humic acid from chemical and biological degradations when the soil and its natural vegetation cover are not severely disturbed, resulting in relatively higher TOC contents in limestone soils. On the other hand, limestone soil tends to be rapidly eroded when limestone soil is tilled or vegetation cover is destroyed since either calcium-humate complexation or carbonate insulation has reduced the ability of humic substance to bind soil minerals and to form large and stable aggregates. Therefore, in the limestone soil region it is easier for stony desertification to occur.

5 Conclusion

This study investigated and compared the characteristics and distributions of SOM and HA fractions along two vertical profiles of yellow soil and limestone soil in the Karst area of southwest China. Although the main vegetation of the two study sites are similar, contents of SOM and HAs in soil samples, elemental compositions of SOM and HAs, and their variation trends along the profiles showed notable differences. The TOC contents decreased sharply along the yellow soil profile from top to bottom but

only decreased slightly along the limestone soil profile, and the TOC content in each layer of the limestone soil profile was higher than the corresponding one of the yellow soil profile. The BHAs isolated from the limestone soil profile have larger apparent molecular size and contains less C and more N, H, and O than those isolated from the yellow soil profile. Unlike the BHA from the top yellow soil layer, the BHA from the top limestone soil layer is predominated by large HA molecule fractions (>100 kD), and small HA molecule fractions (<50 kD) are ignorable. All of this evidence suggested that calcium-humate complexation and calcium carbonate insulation could be the mechanisms for protecting HA from degradation in the noninterference limestone soil site. However, the contribution of each mechanism needs further investigation.

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