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Spatial variability of the molecular composition of humic acids from subtropical forest soils

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Abstract

Purpose Humic acid (HA) plays vital roles in controlling the conservation of soil and water in the forest soils. The structure of HA is closely related to its stability. A clear understanding of the structural difference of HA will facilitate the rational assessment and efficient regulation of forest soils. This study aimed to investigate the structural difference of HA in the forest soils, and the difference in the stability of HA was evaluated according to HA structure.

Materials and methods Soils were collected from different elevations and profile depths of Jiugong Mountain (Hubei, China). Soil HA was then extracted and purified. The structure of HA was analyzed by elemental analysis, fluorescence spectroscopy, and solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR). The stability of soil HA was comprehensively accessed by structural complexity, alkyl C/*O*-alkyl C ratio, and hydrophobicity of HA.

Results and discussion The complexity of HA structure was the greatest in soils in middle elevation (1200 m a.s.l.); it tended to decrease with soil depth in all examined soils. Alkyl C (25.1–40.1%) and *O*-alkyl C (21.2–44.3%) were the main structural fractions in HA samples. The percentage of alkyl C was the greatest in the HA from middle elevation while the *O*-alkyl C proportion was the highest in the HA from lower elevation (600 m a.s.l.). Alkyl C percentage tended to decrease with soil depth while *O*-alkyl C showed an opposite trend. The HA aromaticity increased with soil elevation but decreased with soil depth. The hydrophobicity and alkyl C/*O*-alkyl C ratio of HA was the greatest in middle elevation, and these indexes tended to decrease with soil depth.

Conclusions The HA structure in the subtropical forest soils showed clear spatial variability. The structural features of HA implied that the stability of soil HA was the greatest in the middle elevation and it tended to decrease with soil depth in the examined subtropical forest.

Keywords Humic acid · Structure · Variability · Forest soil · NMR · Stability

1 Introduction

Forest ecosystems are important reservoirs of organic matter. Humic substances are the main parts of soil organic matter. Humic acid (HA) dominates the humic substances and contributes to the stability of soil aggregates and the conservation of soil and water in the forest soils (Sutton and Sposito 2005; D'Orazio and Senesi 2009; He et al. 2013; Zhang et al. 2017). A clear understanding of the state especially the structure and stability of HA in the forest soils is of vital importance in the evaluation and regulation of soil quality in the forest.

The chemical recalcitrance and association with minerals are two common pathways of the stabilization of organic matter in the soil (Mikutta et al. 2006; Kleber et al. 2015; Chen et al. 2020). Alkyl C, *O*-alkyl C, aromatic C, and carbonyl C

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are the main structural fractions of soil organic matter (Ussiri and Johnson 2003; Dou et al. 2008; Chiu and Tian 2011; Duarte et al. 2013; Rodríguez-Murillo et al. 2017; Almendros et al. 2018). In general, compared to alkyl carbon, O-alkyl carbon is less resistant to biodegradation (Chiu and Tian 2011; Duarte et al. 2013). Aromaticity indicates the maturity of HA (Tinoco et al. 2015). The HA hydrophobicity which relates closely to the alkyl C and aromatic C plays important roles in the stabilization and sequestration of soil organic matter (Piccolo et al. 1999; Spaccini et al. 2000, 2002; Song et al. 2018). The structure of HA thus is very important in evaluating its fate and function.

Forest usually shows different elevations with various landscapes, vegetation, and climate. Some studies have reported the influence of forest elevation on the structure of soil HA (Martin et al. 1998; Ussiri and Johnson 2003; Chung et al. 2012; Orazio et al. 2014). Martin et al. (1998) found that humic substances from high elevation, low temperature, and high rainfall sites had less condensed aromatic structures. Higher contents of alkyl C, *O*-alkyl C, and carboxyl C but lower contents of aromatic C in the soil HA from the lakeshore than that from the upper locations were found in subtropical perhumid montane forest (Chung et al. 2012). The structure of HA could be also varied in the profile of forest soils. The aromatic polycondensation and humification degree of soil HA increased from the top to the bottom layer of the soil under *Quercus ilex* L., *Carpinus betulus* L., and *Carpinus orientalis* Miller (Orazio et al. 2014). A slight decrease in the amount of *O*-alkyl C in HA with soil depth was found in two acid forest soils (Lopez et al. 2008), but an increase in the amount of *O*-alkyl C in HA with soil depth was found in the forest soils under pine and oak (Duarte et al. 2013). Zech et al. (1997) proposed that the internal factors such as the state of organic matter and the structure of the microbial community resulted in the difference in the humification at the same site while the external factors such as the temperature, moisture, and soil pH were responsible for the difference in the humification at different sites. Forest elevation and soil depth are the spatial dimensions of forest soils. However, the simultaneous comparison on soil HA structure in these spatial dimensions in forests received very few attentions. The structural complexity, hydrophobicity, and alkyl C/*O*-alkyl C ratio of organic matter were important indexes in assessing the stability of organic matter (Piccolo et al. 1999; Spaccini et al. 2000, 2002; Song et al. 2018; Li et al. 2019). Soucémarianadin et al. (2018) found that labile pool of soil organic matter decreased while stable pool of soil organic matter increased with soil depth. Lyu et al. (2017) showed that soil organic carbon (SOC) in deep layer was more sensitive to land use change than SOC in top layer in subtropical forests. However, the difference in the stability of HA in the forest soils also received very few attentions.

The aims of this study are to analyze the spatial variability of the molecular composition of HA in the subtropical forest soils by comparing the structural differences of HA extracted from soils with different profile depths and elevation gradients in the subtropical forest and to access the differences in the stability of HA samples in forest soils according to their structural characteristics.

2 Materials and methods

2.1 Description of sampling sites

Three representative soils at different elevations of Jiugong Mountain (29°19′–29°26′ N, 114° 27′–114°43′ E), a subtropical forest in southeast of Hubei province, China, were collected in this work. These soils were yellow-red soil (YRS, Ultisols), yellow-brown soil (YBS, Alfisols), and meadow soil (MS, Inceptisols), which were collected at 600, 1200, and 1500 m a.s.l., respectively. Soil types and vegetation vary with elevation and the general characteristics of the sites had been described in Jiang et al. (2017). Different layers of each soil profile (0–60 cm) were collected. The soil samples were air-dried and passed through 2 mm sieve for the extraction of HAs.

2.2 Extraction and purification of HA

The total humic extract (THE) was extracted from each horizon of the soils with 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ following a modified method (Xu et al. 2017). The THE was separated into HA and fulvic acid (FA) by precipitation with HCl until the pH was 1.0. The organic carbon in the THE, HA, and FA was determined as described in Nelson and Sommers (1996). The purification of HA involved its successive re-dissolution-precipitation, then the precipitates were treated with 0.1 M HCl/0.3 M HF and dialyzed until the salts were completely removed. Finally, the HA samples were freeze-dried.

2.3 Element composition and structure of HA

The contents of C, H, N, and S in the examined HA were determined with an Elemental Analyser (Vario EL). Ash content was measured after heating 50 mg of HA at 550 °C for 5 h. The O content was calculated as the difference in the sum of element contents, and data obtained were corrected for moisture and ash contents (Traversa et al. 2014).

Fluorescence spectra were recorded on a Jasco FP-6500 luminescence spectrophotometer with aqueous solutions of HA at a concentration of 50 mg C L^{-1}, after overnight equilibration and adjusted to pH 8.0 with NaOH. Emission-excitation matrix (EEM) fluorescence spectra were collected according to the method proposed by Zhang et al. (2015).

Solid-state ^{13}C NMR spectra of the examined HA were recorded on a Bruker Avance-400 WB spectrometer equipped with a standard 4 mm probe head, using a magnetic field of 9.39 T at 297 K. The dried and finely powdered samples were packed in the ZrO_2 rotor closed with Kel-F cap at a spinning rate of 5 kHz. Transients were recorded with a contact time of 2 ms. The length of the proton 90° pulse was 4 μs. A total of 10,000 scans were recorded with 6-s recycle delay for each sample. Chemical shifts were quoted in parts per million from external adamantane (Martin-Neto et al. 2010).

3 Results

3.1 Content of humic substances in the forest soils

The amounts of HA, FA, and THE in the examined soils are shown in Table 1. It was observed that the content of HA in YRS, YBS, and MS decreased from 1.55, 9.67, and 9.19 g kg^{-1} in the top layer to 0.27, 1.56, and 2.90 g kg^{-1} in the deep layer, suggesting that more HA were accumulated in the soil in top layer and higher elevation, similar trends were found in the amount of FA and THE. Proportions of HA in SOC decreased from 14.2%, 24.4%, and 26.6% in the surface layer to 6.2%, 13.2%, and 21.0% along the soil profile of YRS, YBS, and MS, respectively. Trends in proportions of FA in SOC were quite opposite to that of HA in our study, showing a tendency to decrease with the increase in altitude but an increase with the increase of soil depth. The ratio of HA to FA showed that FA was the dominant fraction of THE in the soils in lower altitudes; however, HA was the dominant fraction of THE in the soils in higher elevation especially at upper layer.

3.2 Elemental composition of HA in the forest soils

The elemental composition and atomic ratios of the HA are reported in Table 2. It was found that C and O were the dominant elements in the examined HAs. In YRS and YBS, the C content of the HA decreased with soil depth while the O content increased with soil depth. Moreover, YBS showed the highest amount of C in the HA but the lowest content of O in the HA among the examined soil types. The amounts of H and N in the HA were similar (5–6%) and no obvious difference was observed among the samples. The C/N ratio of the HA decreased with soil depth and the H/C values of all examined HA were higher than 1.0. The O/C ratio of the HA increased with soil depth, and YRS showed the highest O/C ratio in the HA among the examined soil types (Fig. 1), these results implied a greater presence of oxygen-containing groups in the HA in deep layer of soils, especially in YRS.

3.3 Structure of HA in the forest soils

The structural complexity of HA from the examined soils was investigated by fluorescence emission-excitation matrices (EEMs). The EEM spectra of HA are shown in Fig. 2. The HA showed the fluorophores at excitation-emission

Table 1 The amount of humic acid (HA), fulvic acid (FA), total humic extract (THE), and total soil organic carbon (SOC) in the yellow-red soil (YRS), yellow-brown soil (YBS), and meadow soil (MS) with different depths

Soil type/elevation	Profile depth (cm)	HA (g kg^{-1})	FA (g kg^{-1})	HA (g (100 g SOC)$^{-1}$)	FA (g (100 g SOC)$^{-1}$)	HA/FA[a]	THE[b] (g kg^{-1})	SOC[c] (g kg^{-1})
YRS (600 m a.s.l.)	0–15	1.55	4.07	14.2	37.3	0.38	5.62	10.92
	15–30	0.58	2.95	8.6	43.6	0.20	3.53	6.77
	30–45	0.31	2.58	5.9	48.9	0.12	2.89	5.28
	45–60	0.27	1.66	6.2	38.1	0.16	1.93	4.36
YBS (1200 m a.s.l.)	0–15	9.67	10.40	24.4	26.3	0.93	20.07	39.56
	15–30	3.19	7.09	14.1	31.3	0.45	10.28	22.65
	30–45	1.82	5.08	13.2	37.0	0.36	6.91	13.75
	45–60	1.56	3.25	14.7	30.6	0.48	4.82	10.62
MS (1500 m a.s.l.)	0–10	9.19	8.16	26.6	23.6	1.13	17.34	34.61
	10–20	12.22	9.30	29.8	22.7	1.31	21.52	40.99
	20–30	7.28	5.56	25.5	19.5	1.31	12.85	28.56
	30–60	2.90	4.17	21.0	30.3	0.70	7.07	13.78

[a] HA/FA refers to the ratio of humic acid to fulvic acid

[b] THE refers to total humic extract and it is the sum of humic acid and fulvic acid

[c] The contents of SOC were cited from our previous study (Jiang et al. 2017)

Table 2 Elemental composition and atomic ratios of the humic acid (HA) in the yellow-red soil (YRS), yellow-brown soil (YBS), and meadow soil (MS) with different depth

Soil type/elevation	Profile depth (cm)	C (%)	H (%)	N (%)	O (%)	S (%)	C/N	H/C	O/C
YRS (600 m a.s.l.)	0–15	47.2	5.8	5.2	40.9	0.9	10.6	1.5	0.7
	15–30	45.1	6.0	5.2	42.5	1.2	10.1	1.6	0.7
	30–45	43.5	6.1	5.3	43.7	1.4	9.6	1.7	0.8
	45–60	40.6	6.2	4.6	47.1	1.5	10.3	1.8	0.9
YBS (1200 m a.s.l.)	0–15	54.8	5.9	4.6	34.3	0.4	13.9	1.3	0.5
	15–30	50.8	5.9	5.3	37.5	0.5	11.2	1.4	0.6
	30–45	47.1	5.8	5.3	41.2	0.6	10.4	1.5	0.7
	45–60	46.1	5.9	5.4	42.0	0.6	10.0	1.5	0.7
MS (1500 m a.s.l.)	0–10	48.8	5.5	4.5	40.6	0.6	12.7	1.3	0.6
	10–20	48.8	5.2	4.4	41.0	0.6	12.9	1.3	0.6
	20–30	48.2	5.5	5.1	40.4	0.8	11.0	1.4	0.6
	30–60	45.9	6.3	5.6	41.2	1.0	9.6	1.6	0.7

wavelength pairs of about 455–475$_{ex}$/520–540$_{em}$ and 465–475$_{ex}$/590–595$_{em}$. These peaks implied the occurrence of complex structures such as the greatly conjugated quinones and/or phenols with a certain polycondensation degree in the macromolecules (D'Orazio et al. 2014; Rodríguez et al. 2014; Traversa et al. 2014). The peak around 455–475$_{ex}$/520–540$_{em}$ suggested the presence of molecules with highly condensed structures (Rodríguez et al. 2014). The fluorescence intensity of peaks related closely to the structural complexity of HA. Lower fluorescence intensity of peaks implied higher structural complexity (D'Orazio et al. 2014; Rodríguez et al. 2014: Traversa et al. 2014). The fluorescence intensity of HA tended to increase with soil depth in three soil profiles, implying more complex structures with high polycondensation degree in the HA in top soil than in deep soil. The fluorescence intensity of HA in three soils followed the sequence YBS < MS < YRS, revealing the most complex structure of HA from the soils in the middle elevation (1200 m a.s.l.).

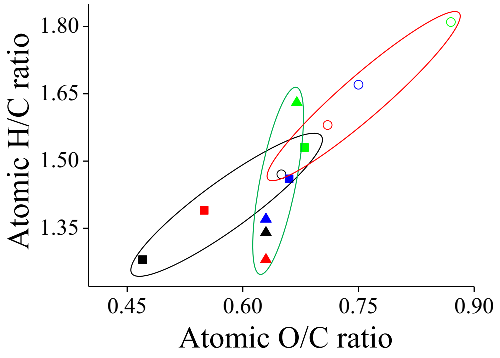

Fig. 1 The Van Krevelen diagram (Van Krevelen 1950) of humic acid in the yellow-red soil (YRS), yellow-brown soil (YBS), and meadow soil (MS) with different depths (Circle, square, and triangle stand for samples from YRS, YBS, and MS, respectively. Black, red, blue, and green stand for the soil layer 0–15, 15–30, 30–45, and 45–60 in YRS and YBS and 0–10, 10–20, 20–30, and 30–60 in MS)

The specific structure of HA from the examined soils was investigated by ^{13}C CP/MAS NMR spectroscopy. The ^{13}C CP/MAS NMR spectra of the HA from the examined soils are shown in Fig. 3. The major C functional groups exhibited by the spectra signals can be divided into alkyl C (0–45 ppm), *O*-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carbonyl C (160–220 ppm). The distribution of the above C functional groups in the examined HAs is shown in Table 3. The alkyl C and *O*-alkyl C were the dominant C fractions in the examined HAs, accounting for 25.1–40.1% and 21.2–44.3%, respectively. The proportions of alkyl C and *O*-alkyl C were similar in the HA from MS. The percentage of alkyl C was the greatest in the HA from YBS and it tended to decrease with soil depth. The proportion of *O*-alkyl C was the highest in the HA from YRS and it tended to increase with soil depth. The alkyl C/*O*-alkyl C ratio of the HA from YBS and YRS then tended to decrease with soil depth, and it was the highest in the HA from YBS among the examined soils. The proportion of aromatic C was pronounced in the HA from MS and it decreased with soil depth in YBS and YRS. The aromaticity rates of HA from YRS, YBS, and MS were 7.4–20.4%, 18.0–25.1%, 14.5–27.0%, respectively. The peaks around 153 and 130 ppm, which contributed most to the aromaticity on the NMR spectra of HA varied with altitude and soil depth. These two signals in NMR spectra were quite strong in top layers and gradually decayed with the increase of soil depth, and even vanished in the bottom of the soils. The alkyl C and aromatic C are the hydrophobic fractions of soil organic carbon (Piccolo et al. 1999; Spaccini et al. 2000, 2002; Song et al. 2018). The hydrophobicity of HA was highest in YBS and lowest in

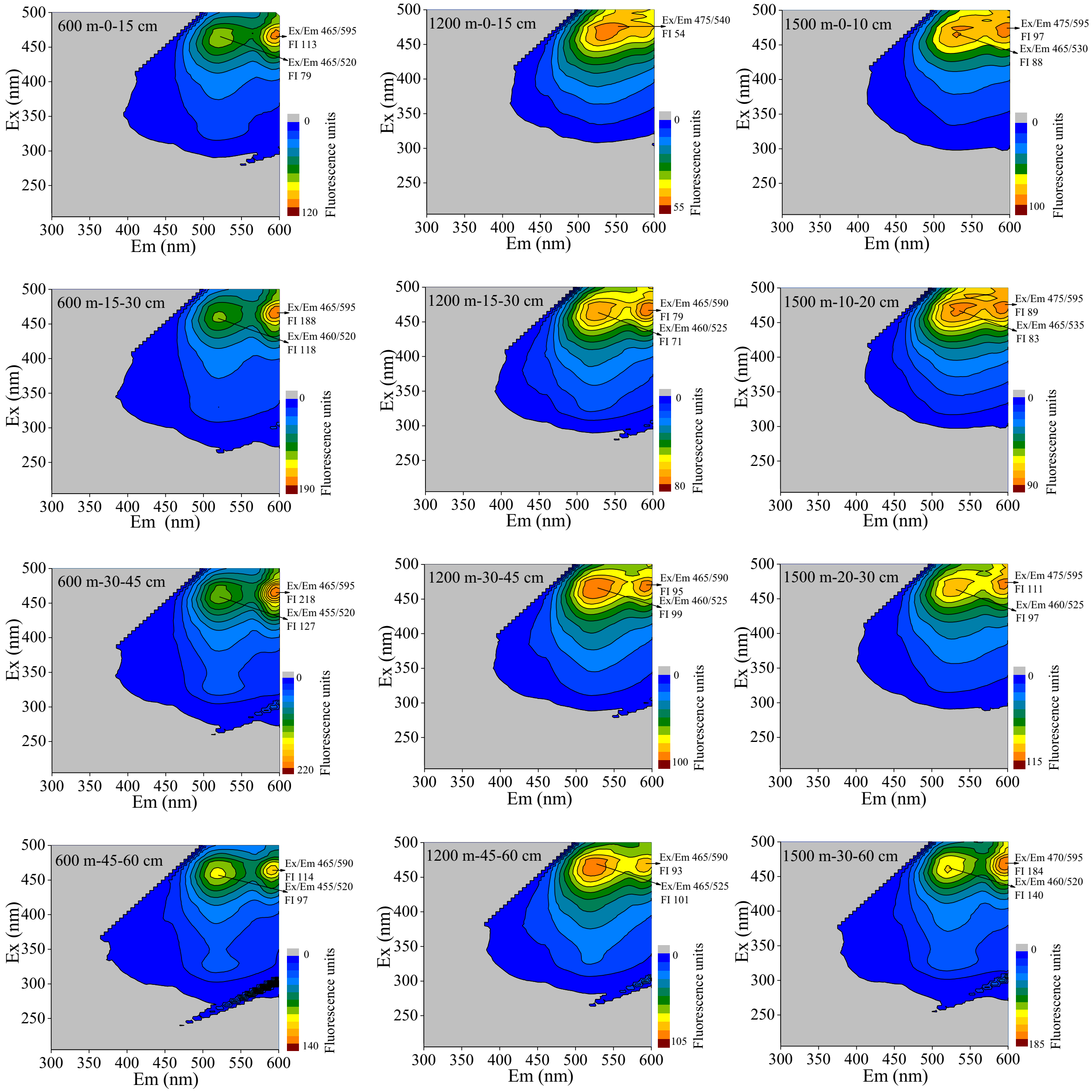

Fig. 2 Fluorescence emission-excitation matrices (EEMs) of the humic acid in the yellow-red soil (YRS), yellow-brown soil (YBS), and meadow soil (MS) with different depths

YRS. A decrease in the hydrophobicity of HA with soil depth was observed in both YRS and YBS, whereas no obvious difference in the HA hydrophobicity was detected in MS except a dramatic reduction in the subsoil. These results suggested that HA from the soils in the top layer and higher elevation was generally more aromatic than that from the soils in the deep layer and lower elevation, the hydrophobicity of HA also tended to decrease with soil depth, but the HA from the soils in the middle elevation (1200 m a.s.l.) was the most hydrophobic.

4 Discussion

4.1 Content of humic substances in the forest soils

Higher contents of HA and FA were found in the soils in higher elevation and top layer than that in lower elevation and deep horizon in this study. Organic matter was the substrate for humification, the amount of organic input was suggested as primary factor controlling the accumulation of humic substances (Abakumov et al. 2013). Grass was the

Table 3 Distribution of C chemical groups in the humic acid in the yellow-red soil (YRS), yellow-brown soil (YBS), and meadow soil (MS) with different depths determined by CP/MAS ^{13}C NMR

Soil type/elevation	Profile depth (cm)	Alkyl 0–45 ppm	*O*-Alkyl 45–110 ppm	Aromatic 110–160 ppm	Carbonyl 160–220 ppm	Aromaticity[a]	Hydrophobicity[b]
YRS (600 m a.s.l.)	0–15	30.7	31.1	15.9	22.3	20.4	0.87
	15–30	25.5	42.6	13.3	18.6	16.3	0.63
	30–45	25.1	44.3	10.7	19.9	13.3	0.56
	45–60	29.3	40.8	5.6	24.3	7.4	0.54
YBS (1200 m a.s.l.)	0–15	40.1	21.2	20.5	18.2	25.1	1.54
	15–30	39.6	22.4	17.9	20.1	22.4	1.36
	30–45	36.1	26.9	17.3	19.7	21.6	1.15
	45–60	37.6	27.4	14.2	20.8	18.0	1.07
MS (1500 m a.s.l.)	0–10	26.9	32.4	20.9	19.8	26.1	0.92
	10–20	29.9	26.9	19.0	24.2	25.1	0.96
	20–30	28.5	29.0	21.3	21.2	27.0	0.99
	30–60	31.8	35.2	11.4	21.5	14.5	0.76

[a] Aromaticity (%) = [aromatic C peak area (110–160 ppm)] 100 [total peak area (0–160 ppm)]

[b] Hydrophobicity = [(0–45 ppm) + (110–160 ppm)] [(45–110 ppm) + (160–220 ppm)]

dominant plant in the region of MS, and pine and bamboo were the dominant vegetation in the region of YBS and YRS, respectively (Jiang et al. 2017). The biomass of grass in aboveground and belowground died every year and provided the soil with abundant sources of humic substances. However, pine and bamboo which were perennial vegetation supplied the organic sources for soil humic substances mainly through defoliation and root residues. Therefore, the organic input was greatest in MS. Higher moisture and lower temperature in the higher elevation than in the lower elevation also facilitated the accumulation of humic substances in MS. Defoliation and root residues contributed together to the organic sources for soil humic substances in the top layer, but the amount of soil humic substances in the deep layer mainly relied on root residues. The organic input in the soils thus was higher in the top layer compared to that in the deep layer.

4.2 Structural differences of HA in the forest soils

The alkyl C/*O*-alkyl C ratio could be used to evaluate the degree of organic matter decomposition (Baldock et al. 1997). Our results showed that the alkyl C/*O*-alkyl C ratio of the HA from YBS and YRS tended to decrease with soil depth. This phenomenon was different from the finding of Ussiri and Johnson (2003). But Duarte et al. (2013) also showed an increase in the amount of *O*-alkyl C in HA with the depth of soil under pine. The alkyl C is recalcitrant C fraction while the *O*-alkyl C is comparatively less resistant to microbial decomposition (Chiu and Tian 2011). Our previous study showed that the activity of β-glucosidase and sucrase which were responsible for the degradation of carbohydrates also decreased with soil depth (Jiang et al. 2017); *O*-alkyl C thus probably was preserved in the deep layer because of weak bio-attack. Besides, the microbial metabolism of carbohydrates in the top layer led to the accumulation of alkyl C (Chiu and Tian 2011). In this study, the NMR peaks at both 30 ppm and 32 ppm can be found in the NMR spectra of HA in the top layer; however, a decrease or even disappearance of peak at 32 ppm was found in the NMR spectra of HA in the deep layer (Fig. 3). The amorphous polymethylenic $(CH_2)_n$ chains which showed a peak at around 30 ppm had both trans and gauche conformation and high molecular mobility, the crystalline polymethylenic C which showed peak at around 32 ppm were all-trans in conformation and more resistant to degradation than amorphous moiety (Kögel-Knabner et al. 1992; Dodla et al. 2011; Duarte et al. 2013). The NMR peaks at both 30 ppm and 32 ppm thus implied that the recalcitrance of alkyl C was higher in the HA in top layer than that in deep layer. The decrease of alkyl C/*O*-alkyl C ratio in the HA with soil depth in this study therefore was mainly due to the biodegradation of the easily decomposable carbohydrates which were the main component of the *O*-alkyl C and the preservation of the recalcitrant alkyl C fraction in the top layers (Kögel-Knabner 1993; Hayes 1998; Chung et al. 2012). Surface layer also had priority over deep layer in transporting oxygen into soil, and aerobic conditions usually facilitated the intensity of humification (DiDonato et al. 2016; Xu et al. 2017). Moreover, the decrease of alkyl C/*O*-alkyl C ratio in the HA with soil depth also could be contributed to other factors such as the depth of the water table, or the in situ production of uronic acids among the components of the root exudates.

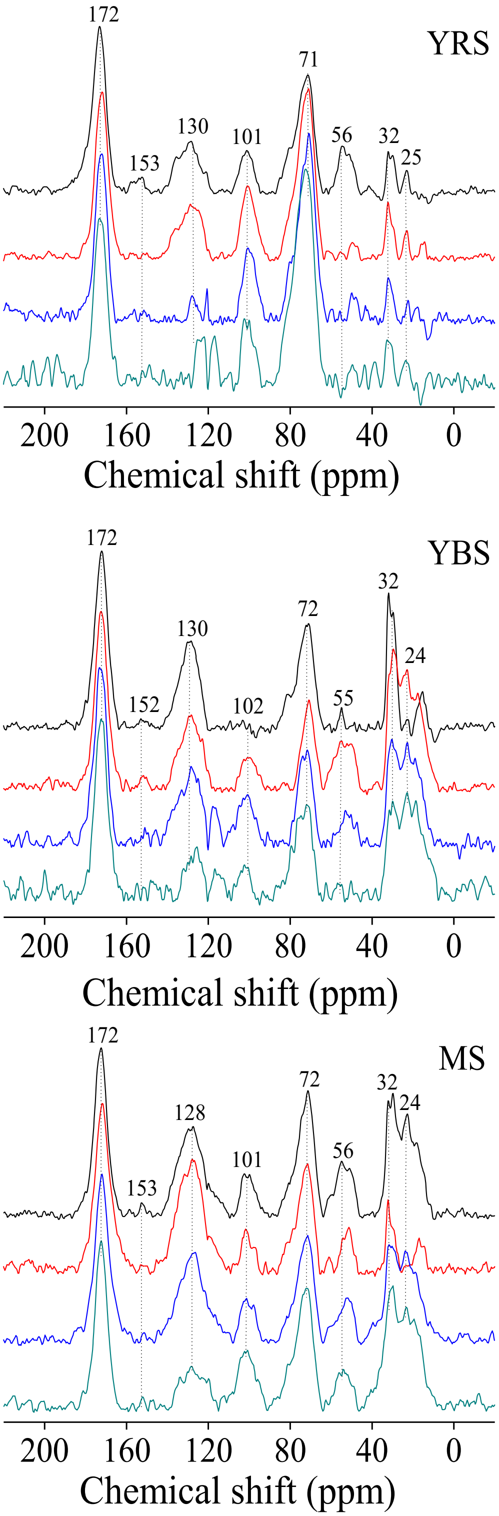

Fig. 3 ^{13}C NMR spectra of the humic acid in the yellow-red soil (YRS), yellow-brown soil (YBS), and meadow soil (MS) with different depths (The first, second, third, and fourth line from upper to bottom respectively stands for the soil layer 0–15, 15–30, 30–45, and 45–60 in YRS and YBS and 0–10, 10–20, 20–30, and 30–60 in MS)

Greater aromaticity of HA was observed in the soils in higher elevation and top layer than that in lower elevation and deep horizon in this study. Aromaticity indicated the maturity of HA (Tinoco et al. 2015). The decrease in the aromaticity of the HA with soil depth in our study was opposite to some previous literatures (Traversa et al. 2014). The difference in the humification at the same site was mainly ascribed to the difference in the internal factors such as the state of organic matter and the structure of the microbial community (Zech et al. 1997). The pH of the top soil was lower than that of the deep soil in this study (Jiang et al. 2017), which benefited the insolubility of HA and the persistence of condensed aromatic structure in the top soil. Our previous study showed that the activity of polyphenol oxidase was also higher in the soils in deep layer than that in top layer (Jiang et al. 2017). Polyphenol oxidase played important roles in catalyzing the oxidation of recalcitrant aromatic compounds (Floch et al. 2007). Higher activity of polyphenol oxidase in the deep soils also did not benefit the accumulation of aromatic compounds. The greatest aromaticity of the HA among the examined soils was found in MS. The difference in the humification at different sites could be mainly ascribed to the difference in the external factors such as the temperature, moisture, and soil pH (Zech et al. 1997). The lower temperature and greater moisture in higher elevation of the current study reduced the intensity of lignin degradation (Traversa et al. 2014). However, MS soil showed the lowest pH among the examined soils (Jiang et al. 2017); low pH favored the storage of organic carbon because it can reduce the biodegradation of organic carbon in soil. Olk et al. (1995) and Abakumov et al. (2013) showed that aged HA had greater aromaticity than younger HA. Greater aromaticity of HA in YBS than in YRS was found (Table 3); it could be due to the higher content of condensed guaiacyl-type lignins in pine than in defoliation vegetation such as bamboo (Fernández-Getino et al. 2010). Conifers have guaiacyl-type lignin, which is more resistant to degradation due to having a more condensed three-dimensional structure with a greater number of bonds between its structural units. On the other hand, bamboo is a perennial grass having lignin with a higher number of syringyl and *p*-hydroxyphenyl units, making lignin less condensed and more easily degradable.

4.3 Influence of HA structures on the stability of soil organic matter

The hydrophobicity of HA in our study decreased with soil depth. The alkyl C and aromatic C are the hydrophobic fractions of SOC; these hydrophobic organic carbon are also recalcitrant fraction of organic carbon (Piccolo et al. 1999; Spaccini et al. 2000, 2002; Song et al. 2018; Li et al. 2019). The hydrophobicity, as well as the alkyl C/*O*-alkyl C ratio and structural complexity of HA, implied that the stability of HA was greatest in the soil of middle elevation (1200 m a.s.l.) and tended to decrease with soil depth in the examined forest.

Moreover, hydrophilic fractions of HA could be sequestrated within hydrophobic domains, which can protect labile organic carbon against further microbial degradation (Piccolo et al. 1999; Spaccini et al. 2000, 2002; Song et al. 2018). In this study, the change of HA hydrophobicity along soil depth was in consistent with the content of SOC, which may imply that the hydrophobicity of HA was also an important intrinsic factor in stabilizing soil organic matter.

5 Conclusions

More complex structure was found in the HA in top soil than in deep soil, and soil HA from the middle elevation (1200 m a.s.l.) showed the most complex structure. Alkyl C and *O*-alkyl C were the main structural fraction in all HA samples. The percentage of alkyl C was the greatest in soil HA from middle elevation (1200 m a.s.l.) and it tended to decrease with soil depth. The proportion of *O*-alkyl C was the highest in soil HA from lower elevation and it tended to increase with soil depth. The aromaticity of HA increased with elevation but decreased with soil depth. The hydrophobicity of HA also tended to decrease with soil depth, but soil HA from the middle elevation (1200 m a.s.l.) was the most hydrophobic. The structural features of HA implied that the stability of HA was the greatest in the soils of middle elevation (1200 m a.s.l.) and tended to decrease with soil depth in the examined subtropical forest.

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