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Ian A. Navarrete[1–3]
Kiyoshi Tsutsuki[1]

[1]Department of Environmental Science, Ateneo de Manila University, Quezon City, Philippines
[2]Laboratory of Environmental Soil Science, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Japan
[3]Soil Science of Tropical and Subtropical Ecosystems, Georg-August University of Goettingen, Goettingen, Germany

Research Article

Chemical and Spectroscopic Properties of Soil Hydrophilic Fulvic Acid Purified by Tangential Flow Ultrafiltration

There is a growing recognition that unless soil hydrophilic fulvic acid (HiFA) is studied together with soil hydrophobic FA (HoFA), we will not fully understand the characteristics of the FA fraction in soils. The aim of this study was to characterize soil HiFA, which were extracted from soil and purified by tangential flow ultrafiltration (TFU), by means of elemental (C, H, O, N, S) and isotope (^{13}C, ^{15}N) analyses, Fourier transform IR (FT-IR) and fluorescence spectroscopy and neutral sugars analysis. Results revealed marked differences between HiFA and HoFA in terms of chemical (elemental, isotopic and neutral sugar compositions) and spectroscopic properties (FT-IR, fluorescence spectra). The HiFA carbon accounted for 46 to 80% of the total FA carbon and thus, an important constituent of the FA fraction. On average, neutral sugars contents in HiFA is twofold larger than in HoFA and are mostly dominated by rhamnose, galactose, and fucose, while in HoFA, mannose was the most abundant neutral sugars, indicating that not only quantity, but also quality of neutral sugars varies between HiFA and HoFA. To further enhance the understanding of the characteristics and composition of soil HiFA including its turnover rate, fate and chemical transformation in soils, it is indispensable to isolate and purify HiFA. As such, purification of soil HiFA by TFU is satisfactory for such purpose.

Keywords: Elemental analysis; Fluorescence spectroscopy; FT-IR; Isotope (^{13}C, ^{15}N) analysis; Neutral sugars

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1 Introduction

The soil fulvic acid (FA) fraction that can be extracted using the International Humic Substances Society method [1] is a complex mixture of organic compounds that can be operationally fractionated into two portions, *i.e.*, humic (HS) and non-humic substances (NHS). HS in the FA fraction is polymeric yellowish brown to yellow colored organic materials with unknown molecular structure, while NHS include carbohydrates, peptides, and lipids that exhibit minor chemical structure changes as decomposition progresses [2]. Thus, any analytical methods that fractionate FA into operationally defined fractions are important in understanding the functions of FA from general biochemical and physicochemical perspectives. Accordingly, it is difficult to differentiate between HS and NHS in the FA fraction [3] and, thus requires the fractionation of the organic compounds using hydrophobic adsorbents including XAD-8 (or DAX-8) and polyvinylpyrrolidone (PVP) resins [1]. The yellowish brown to yellow colored organic materials that are adsorbed onto hydrophobic resins, which tends to emphasize hydrophobic features of the FA fraction, is termed "hydrophobic FA" (HoFA) or the so-called "generic FA" [2], while the non-adsorbed solution that passed through hydrophobic resins is termed "hydrophilic FA" (HiFA). While soil HoFA is widely studied [3–5], soil HiFA is typically discarded [1, 3, 6] and, thus large uncertainty remains in our understanding of soil HiFA including its sources, turnover rate and complexation with metals and hydrophobic organic chemicals, *e.g.*, [6]. Kuwatsuka et al. [3] argued that if both HiFA and HoFA are studied together, the fractionation of FA is critical in elucidating the full characteristics of the total FA fraction. Despite the perceived view that the dynamics of HiFA regulate the fate of plant nutrients and movement and transport of metals and hydrophobic organic chemicals in soils, HiFA have been largely overlooked in biogeochemical studies.

The lack of knowledge and research interest on HiFA can be attributed to the complexity in its purification [6], because FA is soluble under alkaline and acidic conditions [1, 2] leading to the difficulty in dehydration and demineralization processes [6]. In addition, soil HiFA obtained as solutions is contaminated by salts, which is extremely difficult to remove in solutions. To address this problem, Hiradate et al. [6] proposed a method of purifying soil HiFA by adsorption and precipitation. While their method is suitable when a weak ligand such as sodium hydroxide is used in extracting

Correspondence: Dr. I. A. Navarrete, Department of Environmental Science, School of Science and Engineering, Ateneo de Manila University, Loyola Heights, Quezon City 1108, Philippines
E-mail: inavarrete@ateneo.edu

Abbreviations: EEM, excitation-emission matrix; FA, fulvic acid; FT-IR, Fourier transform infrared; HiFA, hydrophilic FA; HoFA, hydrophobic FA; HS, humic substance; NHS, non-humic substance; PVP, polyvinylpyrrolidone; TFU, tangential flow ultrafiltration

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Soil Air Water

Table 3. Elemental and isotopic characteristics of hydrophilic FA and hydrophobic FA

	Weight % on ash-free basis						Atomic ratio				‰	
Soil	C	H	N	S	O	Ash	H/C	O/C	N/C	C/N	$\delta^{13}C$	$\delta^{15}N$
Hydrophillic FA												
Andosol	41.4	6.10	4.25	1.54	46.7	2.40	1.75	0.85	0.09	11	−21.9	6.4
Acrisol	38.7	6.14	4.75	2.01	48.4	3.07	1.89	0.94	0.11	10	−17.2	6.7
Phaeozem	40.6	6.12	3.07	1.71	48.5	4.20	1.80	0.90	0.06	15	−17.9	7.4
Cambisol	36.6	5.68	3.00	4.65	50.0	9.54	1.85	1.03	0.07	14	−23.7	9.1
Average	39.3	6.01[b]	3.77[b]	2.48	48.4	4.80	1.82[b]	0.93	0.08[b]	12[a]	−20.2	7.4[b]
Hydrophobic FA												
Andosol	43.0	4.04	1.75	1.27	50.0	1.52	1.12	0.87	0.03	29	−24.6	3.0
Acrisol	42.9	3.71	1.67	1.57	50.2	2.24	1.03	0.88	0.03	30	−22.7	2.8
Phaeozem	45.0	4.13	2.37	1.19	47.3	1.22	1.09	0.79	0.05	22	−21.9	4.1
Cambisol	38.9	4.30	1.88	5.83	49.1	3.70	1.32	0.95	0.04	24	−27.3	3.1
Average	42.4	4.05[a]	1.92[a]	2.46	49.1	2.17	1.14[a]	0.87	0.04[a]	26[b]	−24.1	3.3[a]

Averages for hydrophilic FA and hydrophobic FA in a column not sharing a common letter are statistically different at $p < 0.05$

detected for elemental C, S, and O contents in HiFA and in HoFA (Tab. 3). The H/C and N/C ratios were larger ($p < 0.05$) in HiFA than in HoFA, whereas O/C ratios did not differ between HiFA and HoFA. The H/C ratios of HoFA ranged from 1.03 to 1.32 (on average: 1.14), whereas the H/C ratios of HiFA ranged from 1.75 to 1.89 (on average: 1.82) and is larger than the H/C ratios of cellulose [19]. High H/C ratios of HiFA in this study support the contention of Chen et al. [20] that H/C ratios of >1.3 suggest the presence of polysaccharide compounds and this view is well supported by the FT-IR spectral data. Higher ($p < 0.05$) H/C ratios in HiFA than in HoFA suggest a more aliphatic character of HiFA [21]. Since oxygen in HiFA and in HoFA is similar, more aliphatic compounds in HiFA suggest lower aromaticity [16]. Taken together, these results indicate that hydrophobic organic contaminants have less efficient binding with HiFA [22], while HoFA may interact preferentially with hydrophobic pollutants [23].

When H/C versus O/C and H/C versus N/C ratios were plotted (Fig. 2), the distribution of HoFA and HiFA samples together with HiFA from aquatic HoFA, *i.e.*, [24], were clearly separated in a vertical direction. This result suggests that H/C ratios are good predictors of the differences in elemental composition between HiFA and HoFA. Interestingly, soil HiFA and aquatic HoFA were grouped together, suggesting similarities in their elemental compositions. The TFU has resulted in a lower ash content in HiFA (Tab. 3), suggesting that TFU was effective in reducing inorganic impurities in the samples, which is another advantage of the TFU method. In general, HiFA had a higher ash content than HoFA, attributed to a higher content of inorganic components including metal cations in solution and differences in the isolation and purification procedures (see Section 2).

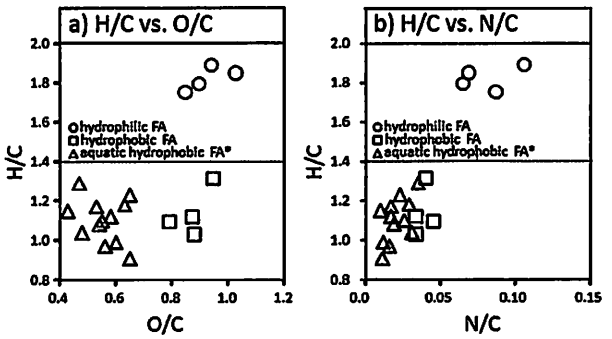

Figure 2. Relationships between (a) H/C vs. O/C and (b) H/C vs. N/C of hydrophilic FA and hydrophobic FA.
*Aquatic HoFA data were adapted from Tsuda et al. [24].

3.3 Isotopic contents

Natural ^{13}C abundance in HiFA and HoFA fluctuated across different soils (Tab. 3). This fluctuation reveals differences in $\delta^{13}C$ values among organic materials as a result of the differences in vegetation, C_3 and C_4 plants [11] and thus, the diagenesis and source of the FA fractions. Although the present vegetation of the study area is dominated by trees, we are not certain if that is the case in the past land use. $\delta^{13}C$ values in HoFA, −22 to −25‰, was less negative than those of HiFA, −17 to −24‰ and this trend of more negative $\delta^{13}C$ values in HoFA is consistent with the data reported in the literature, *i.e.*, [25]. $\delta^{13}C$ values of HoFA were less than HiFA, indicating that HoFA is more oxidized than HiFA and the oxidized nature of HoFA is in agreement with the elemental composition (Tab. 3). The less negative $\delta^{13}C$ values of HiFA compared with HoFA is the result of large amounts of neutral sugars and likely to peptides/proteinaceous material since hemicelluloses, cellulose and amino acids from plant cells tend to have higher $\delta^{13}C$ values than whole plant cells [26]. Furthermore, carbohydrates and amino acids synthesized by microorganisms tend to be enriched in $\delta^{13}C$ than the original substrate [27]. Taken together, these results indicate that HoFA is more altered and enriched in strongly degraded plant materials or enriched with microbial-derived compounds than HiFA. Since both HiFA and HoFA also derive from recent photosynthates including fresh and older plant materials, root exudates and secondary microbial metabolites [18], it is difficult to ascertain the possible diagenesis of HiFA and HoFA based from $\delta^{13}C$ values alone.

$\delta^{15}N$ values are higher ($p < 0.05$) in HiFA than in HoFA, suggesting faster rates of N cycling [28] in HiFA. When comparing the oxidized versus reduced forms of nitrogen between HoFA and HiFA, the latter is enriched with the oxidized form of nitrogen, probably from the conversion of NH_4–NO_3^- because of the enriched ^{15}N in the samples. Enrichment of ^{15}N in HiFA is the result of the ongoing decomposition of organic matter [29] since HiFA are held by weaker bonds, while HoFA, which is bound via the ligand exchange, are retained in mineral soil for a longer period [30]. Consistent with the results of

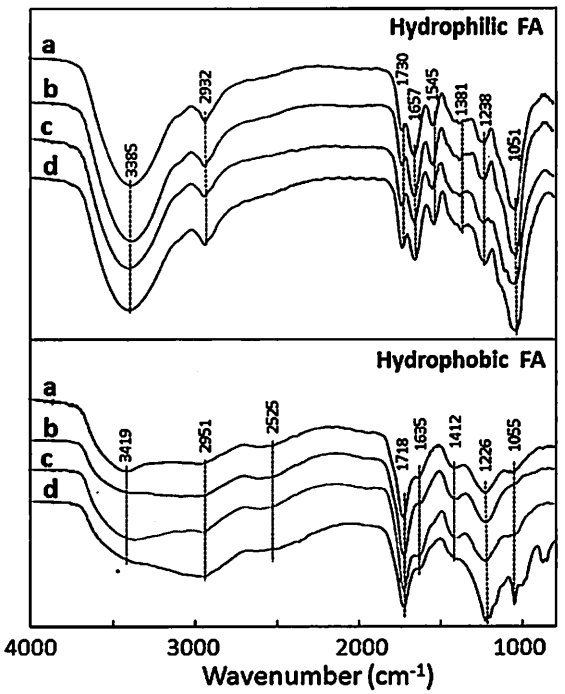

Figure 3. FT-IR spectra of hydrophilic FA and hydrophobic FA, (a) Andosol, (b) Acrisol, (c) Phaeozem, and (d) Cambisol.

$\delta^{13}C$, it is difficult to ascertain the diagenesis of the FA fraction based on the $\delta^{15}N$ values alone.

3.4 FT-IR spectra

FT-IR spectra differentiate sharply between HiFA and HoFA (Fig. 3), indicating that the chemical structure and composition of HiFA is different to HoFA. However, no major differences were observed in the positions of the absorption bands in HiFA and HoFA in different soils, suggesting that the chemical structure and composition of HiFA and HoFA do not vary in different soils. Specifically, a broad peak centered at 3385 cm^{-1}, which is assigned to the O–H stretching of various hydroxylated functional groups, is more intense in HiFA than in HoFA. A peak at 1730 cm^{-1} resulted from the C=O stretching of COOH, which is more intense in the HoFA than in the HiFA. A peak at 1657 cm^{-1} due to the C=O stretching of amide groups (amide I band) and C=O and/or C=O of H-bonded conjugated ketones is more intense and sharp in the FT-IR spectra of HiFA but is weak in the FT-IR spectra of HoFA. A peak at 1545 cm^{-1} due to the interaction between N–H bending and C–N stretching of amide II is present in the FT-IR spectra of HiFA but is totally absent in the FT-IR spectra of HoFA. A weak peak at 1395 cm^{-1} due to the O–H deformation and COO- stretching and stretching of phenolic C–O is clearer in the FT-IR spectra in HiFA than in the FT-IR spectra of HoFA. The results further reveal that HiFA is rich of aliphatic compounds as indicated by a strong peak at 2932 cm^{-1} (CH_2 and CH_3 bands) and polysaccharide or polysaccharide-like substances as indicated by a strong peak at 1050 cm^{-1}. The presence of aliphatic and polysaccharide compounds in HiFA is in agreement with the results of the elemental analysis (Tab. 3). In contrast, HoFA is rich in carboxylic groups as evident by strong peaks at 2525, 1718, and 1226 cm^{-1}, respectively [2]. These results suggest that FT-IR spectroscopy aid in differentiating HiFA and HoFA of the FA fraction.

3.5 Fluorescence spectroscopy

The EEMs contour maps shown in Fig. 4 distinguish sharply the spectral patterns of HiFA and HoFA, but not across different soils. A shift towards shorter excitation maximum in HiFA and shift towards longer excitation maximum in HoFA (Tab. 4) indicate differences in the chemical spectra (Fig. 4) and thus, the chemical components of HiFA and HoFA. Larger λ_{em} values of HoFA, which indicate lower emission energy, were probably derived from a more condensed and/or more highly substituted aromatic ring structure [31] and

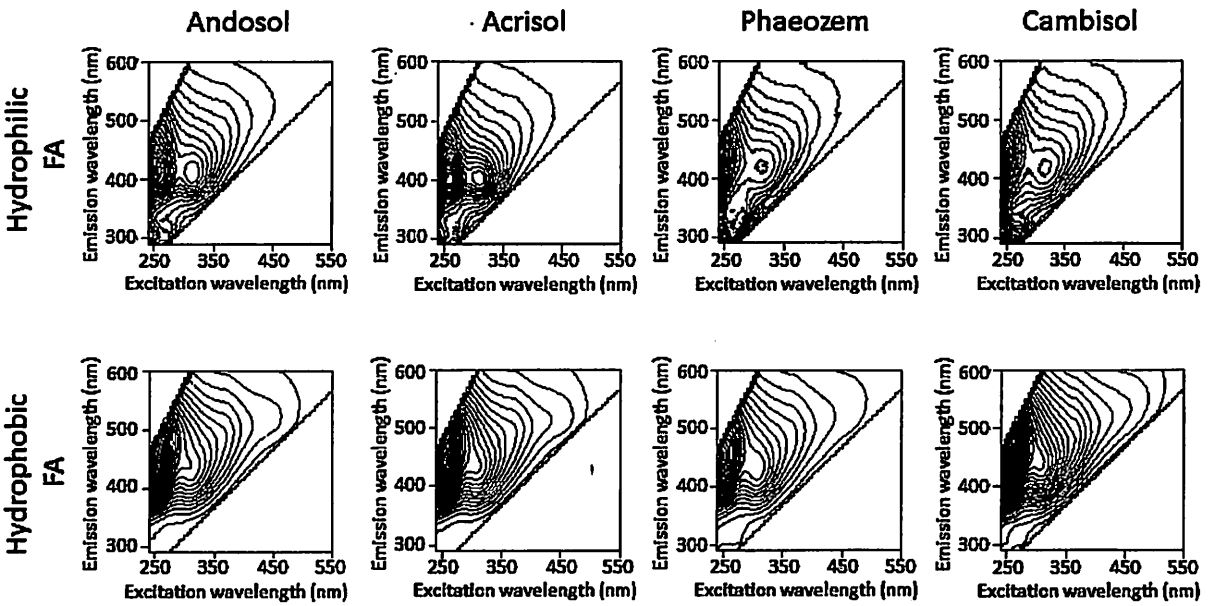

Figure 4. Fluorescence EMMs contour maps of hydrophilic FA (top) and hydrophobic FA (bottom) in different soils.

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Table 4. Fluorescence index (FI) and EEM peaks of hydrophilic FA and hydrophobic FA

Soil	Fulvic acid fraction	$EEM_{\lambda pair\ maxima}$	FI[a]
Andosol	Hydrophilic	240_{ex}*/404_{em} 315_{ex}*/412_{em}	1.37
	Hydrophobic	240_{ex}*/442_{em} 305_{ex}*/438_{em}	1.11
Acrisol	Hydrophilic	240_{ex}*/402_{em} 310_{ex}*/402_{em}	1.53
	Hydrophobic	240_{ex}*/434_{em} 305_{ex}*/434_{em}	1.11
Phaeozem	Hydrophilic	240_{ex}*/426_{em} 315_{ex}*/426_{em}	1.40
	Hydrophobic	240_{ex}*/436_{em} 305_{ex}*/434_{em}	1.11
Cambisol	Hydrophilic	240_{ex}*/420_{em} 310_{ex}*/424_{em}	1.46
	Hydrophobic	240_{ex}*/438_{em} 305_{ex}*/442_{em}	1.14

*Since the observations extended only to 240 nm excitation, the actual position of the peak is unknown, for clarity in this paper, 240 nm was used.
[a] FI: fluorescence index (ex = 370 nm; em = 470/520 nm).

suggest an increased probability of electron transitions between the single state and ground state that are occurring in organic molecules as a result of a greater molecular complexity [32, 33]. The relatively smaller λ_{em} values in HiFA indicate the presence of a simple structural component, a small degree of aromatic substitution and polycondensation, low levels of conjugated chromophores, and the presence of electron-donating substituents such as carboxyl groups [33, 34]. These results suggest that the degree of condensation of aromatic ring systems is less in HiFA than in HoFA. For example, Hiradate et al. [6] reported that HiFA is mainly composed of hydrophilic C including *O*-alkyl C and carboxylic C, suggesting that HiFA were rich in uronic acid-like/or peptide-like structures, while HoFA was rich in hydrophobic C including aromatic C and aliphatic C, and poor in hydrophilic *O*-alkyl C. The EEMs spectra of HiFA and HoFA are different, thus fluorescence spectroscopy can be a sensitive technique for characterizing and differentiation of HiFA and HoFA in the FA fraction.

The FI values (Tab. 4) were relatively higher in HiFA than in HoFA, which indicate differences in the sources of the FA fractions [12]. According to McKnight et al. [12], lower FI values in HoFA suggest that its source is of plant origin, whereas the relatively higher FI values in HiFA suggest microbial origin.

3.6 Amount and composition of neutral sugars

On average, total neutral sugars in HoFA were smaller in Andosol and larger in Phaeozem, while in HiFA, total neutral sugars were larger in Acrisol and smaller in Andosol (Tab. 5). Specifically, neutral sugars in HiFA are two-fold larger ($p < 0.05$) than in HoFA. Among the eight neutral sugars, glucose, which is considered of plant origin since glucose resynthesis by microorganisms seldom occurs in soils [35], was larger than other neutral sugars. Glucose and galactose were higher ($p < 0.05$) in HiFA than in HoFA and this trend is consistent with the data presented in the literature, *e.g.*, [36]. A high correlation ($p < 0.05$) between the amounts of glucose and total neutral sugars in both HiFA and HoFA suggests that accumulation of organic carbon in soils is influenced by organic compounds of plant origin. The HiFA are dominated ($p < 0.05$) by rhamnose, galactose, and fucose which are partially derived from microbial origin [35]. On the other hand, HoFA is dominated by mannose which originates from microbial origin [13, 35] and ribose had the smallest amount in HoFA and HiFA. These results suggest that not only the quantity, but also the quality of neutral sugars varies between HiFA and HoFA.

Some neutral sugars that are associated with the soil mineral matrix (*i.e.*, bound to iron and aluminum oxides) are resistant to biodegradation as documented in Andosol [6, 14, 36, 37] and Acrisol [14]. Although the amount of mannose was not statistically different between HiFA and HoFA, the slightly higher mannose in HoFA suggest that mannose is relatively stable against microbial decomposition. Across soil types, the amount of mannose is smaller in Andosol than in Cambisol, Acrisol, and Phaeozem and this trend is consistent for both HiFA and HoFA. Since HiFA have more neutral sugars than HoFA, it plays a significant role in aggregate formation, nutrient cycling, metal-complexation and serves as an energy source for microbes and, as such, an indicator of soil quality [14, 35].

3.7 Concluding remarks

From the results of the present study, the chemical and spectroscopic characteristics of HiFA and HoFA are distinctly

Table 5. Yield of neutral sugars ($g\,C\,kg\,C^{-1}$) and their molar compositions (%)

Soil	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	Total
Hydrophilic FA									
Andosol	29.1 (10.2)	28.2 (9.9)	3.2 (1.1)	19.0 (6.7)	18.8 (6.6)	4.3 (1.5)	31.7 (11.1)	151.5 (53.0)	206
Acrisol	81.5 (10.0)	47.2 (5.8)	12.6 (1.5)	59.8 (7.3)	61.9 (7.6)	22.5 (2.7)	80.5 (9.8)	452.5 (55.3)	617
Phaeozem	62.4 (16.4)	29.8 (7.8)	15.3 (4.0)	23.9 (6.3)	9.0 (2.4)	10.9 (2.9)	20.7 (5.5)	208.3 (54.8)	249
Cambisol	35.6 (9.5)	14.8 (4.0)	7.0 (1.9)	24.1 (6.5)	17.4 (4.7)	6.3 (1.7)	31.5 (8.4)	236.5 (63.4)	292
Average	52.2 (11.5)[b]	30.0 (6.9)[b]	9.5 (2.1)	31.7 (6.7)[b]	26.8 (5.3)	11.0 (8.7)[b]	41.1 (8.7)[b]	262.2 (56.6)[b]	341[b]
Hydrophobic FA									
Andosol	0.7 (1.5)	0.7 (1.5)	0.2 (0.5)	6.0 (12.4)	3.1 (6.3)	10.4 (21.4)	1.4 (2.8)	26.1 (53.7)	41
Acrisol	2.8 (2.1)	2.0 (1.5)	3.6 (2.8)	3.0 (2.3)	11.6 (9.0)	24.1 (18.6)	8.6 (6.7)	73.9 (57.0)	118
Phaeozem	9.9 (5.1)	8.4 (4.3)	11.3 (5.8)	10.2 (5.2)	26.4 (13.6)	36.9 (19.0)	21.6 (11.1)	69.8 (35.9)	155
Cambisol	8.6 (6.4)	7.4 (5.6)	9.6 (7.2)	8.8 (6.6)	20.9 (15.7)	25.5 (19.1)	17.5 (13.2)	34.9 (26.2)	99
Average	5.5 (3.8)[a]	4.6 (3.2)[a]	6.2 (4.1)	7.0 (6.6)[a]	15.5 (11.2)	24.2 (19.5)	12.3 (8.5)[a]	51.2 (43.2)[a]	103[a]

Values in parentheses denote molar percentage in total sugar.
Averages for hydrophilic FA and hydrophobic FA in a column not sharing a common letter are statistically different at $p < 0.05$.

different, particularly in terms of the content of carbon, elemental and isotopic analysis, neutral sugars compositions and FT-IR and fluorescence spectra. Within soil types, any differences in the FT-IR and fluorescence spectra suggest any differences in the sources of the FA fractions. The soil carbohydrate-C content in HiFA as proportion of the total soil FA carbon was very high, suggesting that it is an important constituent of the FA fraction. The results of this study suggested that to better understand the characteristics and composition of HiFA, including its turnover rate, fate and chemical transformation in soils, it is indispensable to isolate and purify HiFA. As such, purification of soil HiFA by TFU would be satisfactory for such purpose.

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