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REGULAR ARTICLE

Influence of extractants and filter materials in the extraction of dissolved organic matter (DOM) from subtropical agricultural soil

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ABSTRACT

The dissolved organic matter (DOM) plays an important role in ecological processes in agricultural ecosystems. However, composition of DOM extracted by different methods remains unclear. In this study, pyrolysis–gas-chromatography/mass spectrometry (Py-GCMS) was used to investigate the content and chemical composition of DOM extracted by aqueous K_2SO_4 or H_2O and filter paper (FP) or membrane (FM). The results showed that dissolved organic carbon (DOC) content extracted by K_2SO_4 was 0.83–11.2 times higher than that extracted by H_2O, while it increased by 10.1–75.0% when filtered by FP compared to that filtered by FM. Compared with the H_2O extract, the K_2SO_4 one contained lower proportions of aromatic compounds. The relative proportions of hydrophobic compounds in the K_2SO_4 extract (9.7–89.7%), were higher than in H_2O one (2.6–63.5%), whereas the proportions of hydrophilic compounds exhibited the opposite trend ($p<0.05$). The content and complexity of the DOM extracted by the FP-K_2SO_4 method was higher than that obtained by FM-H_2O one. Taken together, the K_2SO_4 and H_2O extractants affected both the quantity and quality of DOM, whereas the FP and FM filter materials only influenced the DOC content ($p<0.05$). Consequently, effect of the extraction method on DOM properties should be considered when studying DOM composition and corresponding ecological processes.

Keywords: Chemical compound; Dissolved organic matter; Extractant; Filter material

INTRODUCTION

Dissolved organic matter (DOM) is a readily available source of carbon (C), immediate energy, and nutrients for soil microbes (Chantigny et al., 2014). Although DOM only accounts for less than 1% of the total soil organic matter (SOM), its high turnover rate indicates that it plays a key role in physicochemical and biological processes in soil (Boddy et al., 2007). The quantity of DOM reflects the magnitude of the available nutrient pool in soil, as inorganic nutrients (N, P, etc.) are released into soil during the microbial degradation of DOM (McDowell et al., 2003). The chemical composition of DOM greatly influences its ecological processes, such as degradation, adsorption, and migration (Chen and Sparks, 2015; Troyer et al., 2011). For example, low-molecular-weight organic compounds, such as carbohydrates, amino acids, and proteins, are highly degradable and are accompanied by gas emissions and the release of inorganic nutrients (Van Hees et al., 2005). Complex DOM components, such as aromatic and humic-like compounds, are recalcitrant to microbial degradation (Straathof et al., 2014). Hydrophilic DOM compounds, which are less adsorptive and highly mobile, are readily lost from soil, whereas hydrophobic compounds are preferentially adsorbed by soil particles (Jardine et al., 1989). Therefore, DOM composition plays an important role in ecological processes.

The characteristics of DOM are dependent on its extraction method, including extraction time, temperature, water/soil ratio, extractant, and filter material, as examples (Jones and Willett, 2006; Rousk and Jones, 2010). Among these factors, the extractant and filter material are most likely to affect DOM characteristics, such as content and chemical composition (Peltzer et al., 1996; Jones and Willett, 2006). Presently, the classic procedure for DOM extraction is

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based on the use of deionized water and filtration through a 0.45-μm membrane (FM-H_2O method) (Ogura, 1974; Jardine et al., 1989; Feng et al., 2014). Operationally, salt solutions (K_2SO_4 or KCl) and Whatman No. 42 filter paper (2.5 μm) have also been used (FP-K_2SO_4 or FP-KCl) (Jones and Willett, 2006; McDowell et al., 2006). Unfortunately, DOM extracted by different methods may lead to different results. For example, the FM-H_2O-extracted DOC content was higher in paddy soil than in upland soil, whereas the opposite trend was observed in similar soils when the FP-K_2SO_4 method was used (Han et al., 2007; Li et al., 2008). Lu et al. (2004) found that the dynamics of the water-extractable organic-C content was significantly affected by the incorporation and decomposition of photosynthesized rice C in paddy soil, but the salt-extractable organic C content was less affected during rice-plant growth, indicating that extractants can affect the DOC content. To date, considerable attention has been paid to quantifying DOC content and its extraction efficiency. However, the chemical composition of DOM extracted using different methods is largely unknown.

Generally, salt solutions afford higher extraction efficiencies compared with H_2O (Rousk and Jones, 2010). On the other hand, solutions filtered by filter material with pore sizes exceeding 0.45 μm could contain very fine particulate organic carbon (Nebbioso and Piccolo, 2013). Here, we hypothesize that the composition of the DOM extracted with a salt solution and FP (pore size > 0.45 μm) is more complex than that extracted with H_2O and a filter membrane (pore size: 0.45 μm). Consequently, the present study is aimed at identifying the effects of the extractant and filter material on the chemical composition of DOM from agricultural soils.

MATERIALS AND METHODS

Soil

To investigate the common characteristics of soil DOM by different extraction methods, six agricultural soils (0–15 cm depth) were collected in March 2014 in subtropical China. Soils were stored at the field moisture content in airtight polypropylene bags and immediately transported to the laboratory. The samples were divided into two parts. One was stored at 4 °C prior to DOC extraction, the other was used for SOC and soil pH analysis after drying under low-light conditions. The main soil properties and climate factors are shown in Table 1.

DOC extraction methods

Field-moist soil was mixed with either distilled H_2O or 0.5 M K_2SO_4 (w/v: 1:2) in a polypropylene bottle and placed on an orbital shaker at 200 rpm for 1 h. The samples were then centrifuged at 12,000 ×g (Hitachi, Himac CR 22GII/Rotor, R20A2) for 10 min at 4 °C to remove suspended solids. The supernatant was filtered either through a 0.45-μm filter membrane with pumping or Whatman No. 42 filter paper (the smallest pore size in commonly used filter papers: 2.5 μm), and a small aliquot of each filtrate was retained for DOC-concentration and E_{254} analysis. The remaining filtrate was freeze-dried at −55 °C for pyrolysis–gas-chromatography/mass spectrometry (Py-GC/MS) analysis.

Analytical methods

The concentration of DOC in each extract was determined using an automated TOC analyser (Teledyne Tekmar Phoenix 8000, Mason, USA). The organic carbon in the soil was measured using the K_2CrO_7-H_2SO_4 oxidation procedure, and the pH was determined with distilled water in a soil: water ratio of 1:2.5 (w/v) using a pH meter. The UV absorbance at 254 nm (E_{254}, Shimadzu UV-2450, Shimadzu Corporation, Kyoto, Japan) was used to estimate its aromaticity (Kalbitz et al., 2003). Prior to any UV measurement, the C concentration of DOM was adjusted into 2 mg L^{-1}.

The freeze-dried filtrates (0.5 mg) were introduced as solids via an injector into the SGE pyrojector and pyrolyzed at 450 °C. The temperature of the column was held at 5 °C for 2 min, then raised at a rate of 5 °C/min to 290 °C and kept at this temperature for 15 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injection mode had a split ratio of approximately 1:5. GC/MS of the pyrolysates was performed on a Hewlett Packard 5890 gas chromatograph equipped with an HP 5970 mass-selective detector (Perkin-Elmer, Shelton, CT, USA). Chromatography was carried out on a fused silica column (30 m × 0.25 mm i.d., 0.25 μm film thickness) coated with DB5MS (modified 5% phenyl, 95% methyl silicone). Mass spectra (1 scan/s) were recorded under electron impact at 70 eV. Compound identification was conducted based on comparisons of mass spectra with the NIST-library (National Institute of Standards and Technology Mass Spectral Library) database, published spectra, and authentic standards (Saiz-Jimenez, 1994; Sihombing et al., 1996; Zhou et al., 2010)., We correlated the pyrolysis products with compound types in the original DOM solutions according to the pyrogram. Products not detected in samples were given a zero value in the statistical treatments. The identified products were categorized into several general chemical classes, such as lipids, aromatics, organic acids, phenols and N-compounds (Lu et al., 2005; Plant et al., 2015). The lipids and aromatics were classified as hydrophobic compounds (Ho-compounds) (Karickhoff et al., 1979; Kalmykova et al., 2014), whereas the organic acids, phenols, and N-compounds were grouped as hydrophilic compounds (Hi-compounds) (Kalbitz et al., 2003; Pullicino and Gigliotti, 2007).

Table 1: Description of locations and basic properties

Soil number	1	2	3	4	5	6
Location	N24°57′41.2″, E108°3′1.3″	N29°30′8.9″, E112°46′27.2″	N29°15′49.7″, E111°31′57.5″	N24°57′38.4″, E108°0′52.0″	N29°29′0.5″, E112°45′58.5″	N29°14′58.3″, E111°32′22.8″
Soil type	Limestone	Red soil	Red soil	Limestone	Red soil	Red soil
Soil parent	Dolomite	Quaternary red clay	Quaternary red clay	Dolomite	Quaternary red clay	Quaternary red clay
Water management	Precipitation	Precipitation+irrigation	Precipitation+irrigation	Precipitation	precipitation+irrigation	precipitation+irrigation
Fertilizer	Chemical fertilizer+manure	Chemical fertilizer+straw	Chemical fertilizer+straw	Chemical fertilizer+manure	Chemical fertilizer+straw	Chemical fertilizer+straw
Plantation	Maize+sweet potato	Cotton+wheat	Cotton+oilseed rape	rice	rice	rice
Annual mean temperature	19.9	16.9	16.5	19.9	16.9	16.5
Altitude (m)	516	51	99	489	32	88
pH	7.26	8.00	4.93	7.66	7.58	4.87
SOC(g/kg)	12.8	14.8	11.5	32.8	19.9	21.7
DOC(mg/kg)	10.8	16.7	15.7	26.6	16.4	47.8
DOC/ SOC (%)	0.08	0.11	0.13	0.08	0.08	0.22
E_{254}	0.28	0.15	0.14	0.05	0.28	0.15

Abbreviations: m, meters; SOC (g/kg), soil organic carbon; DOC (mg/kg soil), dissolved organic carbon; E_{254}, UV absorbance of 2 mg/L DOC at 254 nm. The equation used to calculate SOC was: $C \times 5/V_0 \times (V_0 - V) \times 10^{-3} \times 3 \times 1.1 \times 10^3 / m \times k$, where C is the concentration of potassium dichromate (0.8000 mol/L), '5' (mL) is the volume of the potassium dichromate solution, V_0 is the volume of the ferrous sulfate solution (mL) used to titrate the blank control, V is the volume of the ferrous sulfate solution (mL) used to titrate the sample, '10^{-3}' converts mL to L, '3' is a quarter of the molar mass of the carbon atom, '1.1' is the oxidation correction coefficient, m is the mass of fresh soil (g), and k is the water coefficient. The equation used to calculate DOC was: $C \times V \times 10^{-3}/(m \times 10^{-3}) \times k$, where C is the concentration of DOC determined by the automated TOC analyser (mg/L), V is the volume of extracted DOC solution (mL), '10^{-3}' converts mL to L and g to kg, m is the mass of fresh soil (g), and k is the water coefficient. The E_{254} value was obtained at 254 nm with an ultraviolet spectrophotometer after the concentration (C) of DOM was adjusted into 2 mg/L.

Statistics

The significance of the differences among the four extraction methods was analysed using *t*-tests of paired samples at a significance level of $p < 0.05$ in the SPSS Statistics 16.0 statistical software package (IBM, USA). The figures were created using Origin 8.0.

RESULTS

DOC content and aromaticity

The DOC contents of the treated samples were in the order: FP-K_2SO_4 > FM-K_2SO_4 > FP-H_2O > FM-H_2O ($p < 0.05$). Among extractions that used the same filter material (FM or FP), the DOC contents extracted by K_2SO_4 were 1.17–11.2 and 0.83–6.83 times higher than those extracted by H_2O, respectively. Among extractions that used the same extractant (H_2O or K_2SO_4), the DOC contents of the FP filtrates increased by 12.7–75% and 10.1–61.5% compared with those of the FM filtrates, respectively (Table 2). This indicates that the DOC content is mostly influenced by the extractant, and to a lesser extent by the filter material.

E_{254} values correlate positively with the proportion of aromatic compounds in a sample. For the same filtration material, the E_{254} values for the K_2SO_4 extracts are significantly lower than those of the H_2O extracts (Table 2), indicating that the K_2SO_4 extracts contain a lower proportion of aromatic compounds. For the same extractant, there was no significant difference in the E_{254} value between the FM and FP filtrates.

Hydrophilic and hydrophobic components in DOC extractions

Hi-compounds account for 36.5–97.4% and 10.3–90.3% of the H_2O and K_2SO_4 extracts, whereas Ho-compounds account for 2.6–63.5% and 9.7–89.7%, respectively. The Hi- and Ho-compounds extracted by the different methods consist mainly of organic acids and lipids, respectively, with both of these components accounting for 75% to 100% of the total DOM (Table 3).

For the FP filtrates, the relative proportion of Ho-compounds is higher and that of the Hi-compounds is lower in the K_2SO_4 extracts compared to the H_2O extracts ($p < 0.05$). However, for the FM filtrates, there are no significant changes in the relative proportions of these components between the K_2SO_4 and H_2O extracts. These results indicate that the DOM composition, in response to the extraction method, is also affected by the filter material type, although the difference in the DOM composition between the FM and FP filtrates is not significant using the same extractant. The proportion of Ho-compounds in the DOM extracted by the FP-K_2SO_4 method is significantly higher than that in FM-H_2O, whereas the Hi-compounds exhibit the opposite trend ($p < 0.05$).

Table 2: DOC content and UV absorbance

Soil number	DOC content (mg/kg)						E_{254}					
	1	2	3	4	5	6	1	2	3	4	5	6
FM-H_2O	13.2	17.9	9.9	48.7	20.6	20.6d	0.33	0.13	0.15	0.05	0.08	0.19a
FM-K_2SO_4	28.6	67.4	120.3	172.3	63.0	87.1b	0.04	0.07	0.03	0.02	0.04	0.03b
FP-H_2O	23.12	24.4	16.9	54.9	27.4	27.3c	0.29	0.21	0.19	0.06	0.11	0.27a
FP-K_2SO_4	42.2	92.5	132.4	219.2	74.9	140.7a	0.03	0.07	0.03	0.03	0.06	0.03b

The different superscript letters indicate significant differences at the $P<0.05$ level among four extraction methods. Abbreviations: FM-H_2O; Filter membrane-H_2O. FM-K_2SO_4; Filter membrane-K_2SO_4. FP-H_2O; Filter paper-H_2O. FP-K_2SO_4.; Filter paper-K_2SO_4

Table 3: Relative proportion of DOC compounds obtained by different extraction and filtration methods (%)

Compound type		Ho-compounds	Lipids	Aromatics	Hi-compounds	Organic acids	Phenols	*N*-compounds
FM-H_2O	1	51.9	47.8	4.1	48.1	48.1	0	0
	2	2.6	2.6	0	97.4	97.4	0	0
	3	34.3	30.7	3.6	65.7	62.3	2.1	1.3
	4	15.7	11.6	4.1	84.3	63.8	2.5	18.0
	5	39.1	37.2	1.9	60.9	46.7	0.2	14.0
	6	35.8b	31.3b	4.5	64.2a	62.4a	0.9	0.9
FM-K_2SO_4	1	89.7	89.7	0	10.3	10.3	0	0
	2	9.7	9.7	0	90.3	72.9	0	17.4
	3	21.6	21.6	0	78.4	78.4	0	0
	4	13.8	13.8	0	86.2	83.5	0	2.7
	5	61.6	61.6	0	38.4	38.4	0	0
	6	56.4ab	56.4ab	0	43.6ab	36.0ab	0	7.6
FP-H_2O	1	63.5	61.3	2.2	36.5	34.3	2.2	0
	2	14.6	14.6	0	85.4	85.4	0	0
	3	30.8	26.9	3.9	69.2	65.2	0	4.0
	4	24.4	18.0	6.4	75.6	74.1	0	1.5
	5	33.9	28.3	5.6	66.1	65.0	1.1	0
	6	11.4b	11.4b	0	88.6a	75.1a	0	13.5
FP-K_2SO_4	1	85.2	85.2	0	14.8	14.8	0	0
	2	55.5	55.5	0	44.5	44.5	0	0
	3	53.1	53.1	0	46.9	46.9	0	0
	4	44.1	44.1	0	55.9	48.5	0	7.4
	5	43.4	43.4	0	56.6	47.7	0	8.9
	6	63.1a	63.1a	0	36.9b	36.9b	0	0

The different superscript letters indicate significant differences at the $P<0.05$ level among four extraction methods. Chemical compounds such as lipids and aromatics were classified as Ho-compounds, whereas organic acids, phenols, and *N*-compounds were grouped as Hi-compounds. Abbreviations: FM-H_2O; Filter membrane-H_2O. FM-K_2SO_4; Filter membrane-K_2SO_4. FP-H_2O; Filter paper-H_2O. FP-K_2SO_4; Filter paper-K_2SO_4. Hi-compound; hydrophilic compound. Ho-compound; hydrophobic compound

Distribution of DOM components across the molecular weight range

The distributions of DOM components extracted by H_2O and K_2SO_4 using the same filter material were different (Fig. 1, Fig. 2). In the same FM or FP filtrate, DOM components less than 100 Da in size were only found in the H_2O extract (Fig. 1a, c), which accounted for 0.24% or 0.68% of the Hi-compounds (Fig. 2-a, c), and 0.21% or 1.28% of the Ho-compounds in the DOM composition (Fig. 2-e, g), respectively; DOM components larger than 400 Da in size were only found in the K_2SO_4 extract (Fig. 1-b, d), which was composed of 10.5% or 16.4% Ho-compounds in FM or FP filtrate (Fig. 2-f, h), respectively. This indicates that the composition of the DOM extracted by K_2SO_4 was more complex than that extracted by H_2O.

DISCUSSION

Generally, H_2O extracts are composed of dissolved and weakly adsorbed compounds (Joo et al., 2008). The results from this study show that the K_2SO_4 extracts contain more DOC than the H_2O extracts. As K_2SO_4 is a salt, its ions can assist in the dissolution of chemical compounds initially adsorbed onto soil particles through ion-exchange interactions (Jones and Willett, 2006). Thereby, in addition to soluble and weakly adsorbed compounds, the K_2SO_4 extracts also contain adsorptive compounds. Compared with H_2O, the K_2SO_4 extracts contained larger hydrophobic fractions (Table 3, Fig. 2-a, b, c, d) that are readily adsorbed on soil particles, which reveals the higher DOC-extraction capacity of K_2SO_4 over H_2O. The hydrophobic fractions in the K_2SO_4 extracts are composed of aliphatic constituents

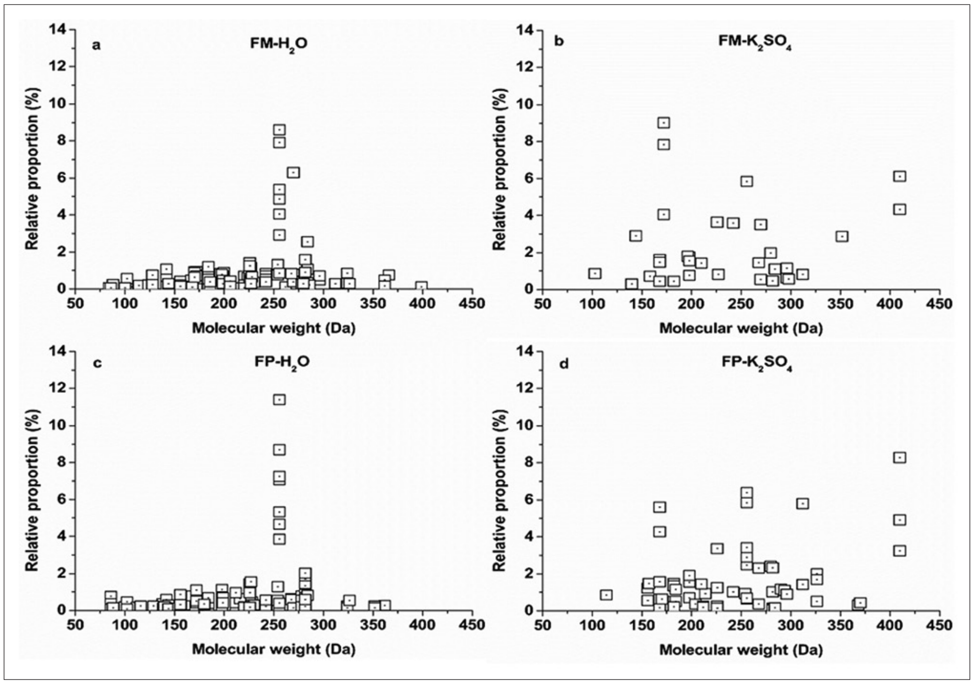

Fig 1. Relative proportion of DOC compounds of different molecular weight ranges. The data portray the mixed results of all experimental soils. Abbreviations: FM-H_2O; Filter membrane-H_2O. FM-K_2SO_4; Filter membrane-K_2SO_4. FP-H_2O; Filter paper-H_2O. FP-K_2SO_4; Filter paper-K_2SO_4. According to the relative proportion of chemical composition from six DOM samples and their DOC contents, we mixed the chemical composition of DOM together to evaluate molecular weight distributions of DOM components. The relative proportion of each component was calculated as p × c/(c1+c2+c3+c4+c5+c6) × 100%. p: the relative proportion of each component. c: the soil DOC content. c1-c6: The DOC content from six soil DOM samples, respectively.

rather than aromatic compounds (Table 3), which may be related to the rich aliphatic content of the organic materials in farmland soils, such as straw and cellulose (Putun et al., 2004; Wu, 2013).

In addition, filter materials also affect the DOC content. FP filtrates were shown to contain higher DOC contents than FM filtrates, due to the larger pore size (2.5 vs. 0.45 mm, respectively). Of the particulate organic carbon, very fine particles of about 0.45 to 6 mm in size constitute the major fraction. Furthermore, most bacteria and phytoplankton are larger than 0.45 mm. Colloidal matter consisting of fine particles in the 0.001–1.0 mm range is regarded as 'subparticulate' (between particulate and dissolved organic matter). Therefore, very fine particulate organic matter, some bacteria and phytoplankton (0.45–2.5 mm), and colloidal matter (0.45–1.0 mm) largely account for the higher levels of organic carbon in the FP filtrates (2.5 mm).

Bioavailability is a vital index with which to evaluate DOC characteristics, and is determined by chemical composition (Marschner and Kalbitz, 2003; Kalbitz et al., 2000). The results show that more hydrophobic compounds are recovered by K_2SO_4 extraction, and components with diameters of 0.45–2.5 mm are contained in the FP filtrates (Table 3). Particulate organic matter is relatively high in inorganic content and is heterogeneous in composition (e.g., containing lignins, lipids, and humic substances) (Sundh, 1992). Hydrophilic compounds are more readily used by microorganisms, whereas hydrophobic compounds are refractory (Lichter et al., 2005; Kalbitz and Kaiser, 2008). Soil colloidal matter is rich in humic acid, which is composed of aromatic compounds, saccharides, and fatty acids (Junet et al., 2013; Chen and Sparks, 2015). Finally, cell walls are the primary organelles of bacteria, and contain peptidoglycans, teichoic acid, lipopolysaccharides, and so on (Cummins and Harris, 1956). These high-molecular-weight chemical compounds are complex and refractory to microbial degradation (Repeta et al., 2002). This clearly demonstrates that the K_2SO_4-FP method increases the complexity of the extracted DOM as compared with the H_2O-FM method. Consequently, research based on DOM materials extracted by different methods may lead to different results, because various DOM components play different roles in ecological processes. For example, during the microbial degradation of DOM, components rich in N and P (i.e., amino acids and nucleotides) are transformed into inorganic nutrients (i.e., ammonium, nitrate, phosphate) (Moschonas et al., 2017; Brailsford et al., 2017). Low-molecular weight organic acids can improve the transport of metal ions in soil through the formation of metal-organic acid complexes (Wu et al., 2010). Complex compounds generally contribute to SOM sequestration,

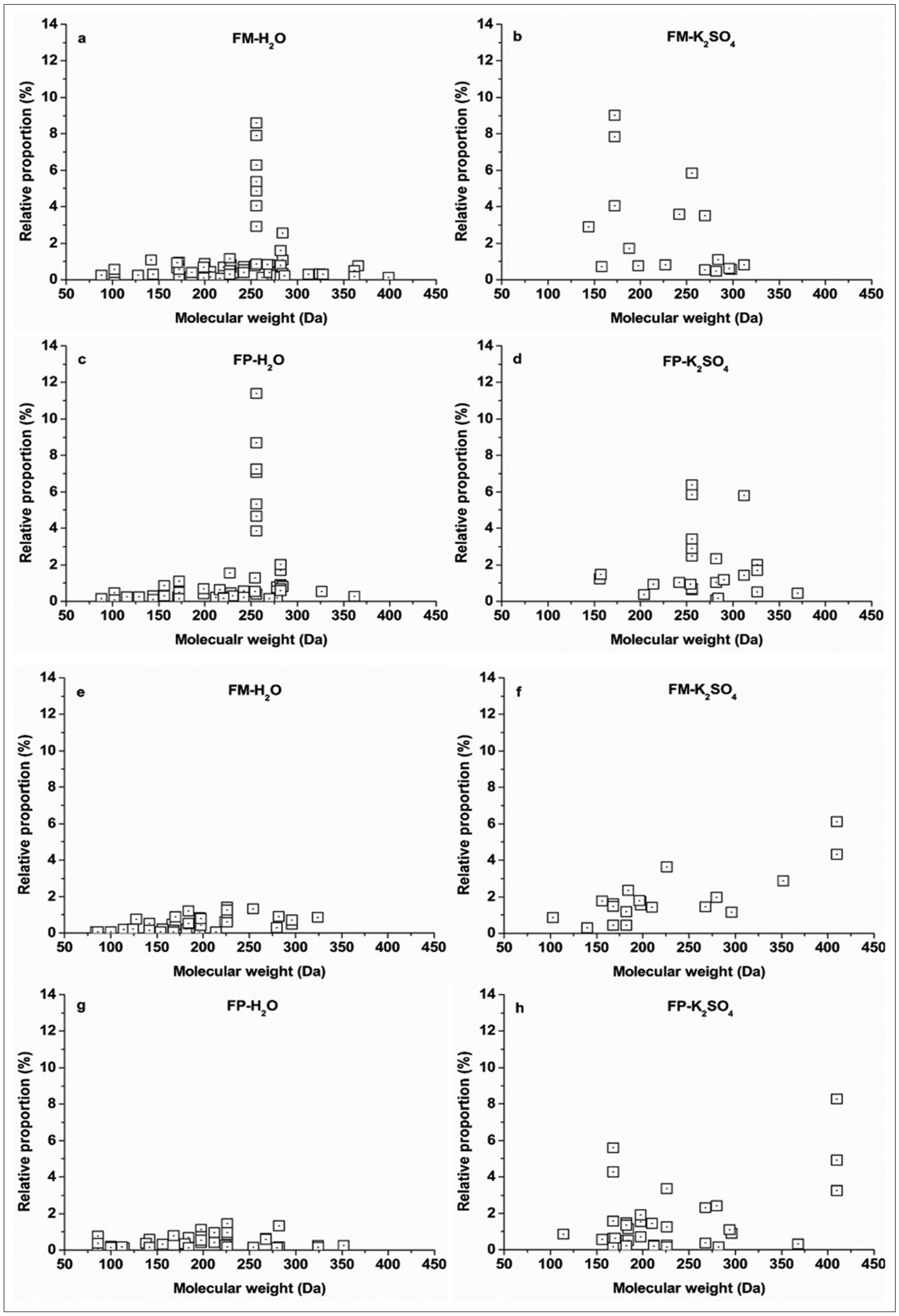

Fig 2. Relative proportion of hydrophilic (Fig. 2-a, b, c, d) and hydrophobic (Fig. 2-e, f, g, h) fractions at different molecular weights for different extraction methods. The data represent the mixed results for all experimental soils. Abbreviations: FM-H_2O; Filter membrane-H_2O. FM-K_2SO_4; Filter membrane-K_2SO_4. FP-H_2O; Filter paper-H_2O. FP-K_2SO_4; Filter paper-K_2SO_4.

as they are less used by microorganisms (Nebbioso and Piccolo, 2013; Cotrufo et al., 2013).

Pyrolysis-GC/MS is a powerful tool for the determination of organic matter composition, which can be used for the detailed separation, identification, and relative quantification of individual DOM components. Through the controlled thermal degradation of DOM during py-GCMS analysis, the original structures of the DOM components can be determined from the pyrolysis products in the pyrogram (Schulten and Gleixner, 1999; Leenheer and Croue, 2003; Greenwood et al., 2012). However, the molecular-weight distributions of DOM components obtained through different extraction methods needs to be investigated.

CONCLUSION

Due to their different pore sizes, filter materials significantly influenced DOC content ($p < 0.05$), but not DOM composition ($p > 0.05$). Due to differences in extraction abilities, extractants can significantly influence the quantity and quality of DOM. This study demonstrated that, compared with the FM-H_2O method, DOM extracted by the FP-K_2SO_4 method was characteristically higher in content and complexity. In view of this, the effect of the extraction method on DOM properties should be noted when studying composition and ecological processes of DOM.

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Author's contributions

X. D. Zheng and C. M. Liang: statistical analysis, wrote the article. X. B. Chen and Y. J. Hu: contributed to writing of discussions. J. S. Wu and Y. R. Su: study design, supervision of study.

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