Review

Comprehensive View of Chemical Structure of Soil Organic Matter, a Review

Kosuke Ikeya

Graduate School of Environmental studies, Nagoya University, Furo-cho, Chikusa, Nagoya 464-8601, Japan

Abstract

 The studies on chemical structure of soil organic matter (SOM) are reviewed with mainly focused on those published since around 2000. Here SOM are separated as follows: (1) SOM generated at initial stage in litter layer of soil, (2) bulk SOM and fractionated SOM on the basis of soil particle size or density, (3) fractionated SOM on the basis of the solubility [i.e., humic substances (HS)], (4) dissolved organic matter (DOM), and (5) char. Within those SOM fractions, the chemical structures of (3) HS, especially for humic acids (HAs) and fulvic acids (FAs), have been vigorously examined by non-degradative physical and spectroscopic analyses and chemical/thermal degradations followed by gas chromatography/mass spectrometry. The study results of HAs indicated that, with increasing the degree of humificaiton (or darkness), (i) the proportion of aromatic C and condensed aromatic C in total C increased while that of alkyl C and O-alkyl C decreased, (ii) the yield of lignin-derived phenols and that of aliphatic compounds decreased while the yield of carbon layer planes (or condensed aromatic rings) increased. Similar trend was observed by using a novel technique, Fourier transform ion cyclotron resonance mass spectrometry. The chemical structure of (1) humified-litter, (2) bulk or physically-fractionated SOM, (4) DOM, and (5) char have been examined by mainly solid state ^{13}C NMR. Those SOM except for char were characterized as that each SOM samples had two major signals of relatively resistant alkyl C and decomposable O-alkyl C structure with their different ratios depending on the different degrees of decomposition or humificaiton. Char was characterized with both high aromatic C abundance and great degree of condensation.

Keywords: Soil organic matter (SOM), humic substances (HS), humification, humic acid (HA), fulvic acid (FA), humin, dissolved organic matter (DOM)

Introduction

  Soil organic matter (SOM) includes metabolites of plant and organism, their dead bodies that decomposed to different degrees, and a mixture of those products having different degrees of humification (or darkness), which were generated through biotic/abiotic reactions. Soil organic matter is defined as a mixture of heterogeneous, polydisperse, and high-molecular-weight compounds (or assemblages of physically bonded small compounds) consisting of various kinds of building units. In the decomposed/humified products, the materials that still remained biomolecular structures occur while the other materials having further humification (not remaining their original characteristic) (i.e., humic substances, HS) also

occur. Both the materials have various mixing ratios depending on the environment (Baldock and Skjemstad, 2000). Soil organic matter plays important roles associated with soil fertility in agricultural fields/forest ecosystems and C sequestration in soils. For sustaining and/or improving soil fertility and controlling C sequestration ability in soils, to understand the chemical structure of SOM at molecular level is an essential issue.

  Classically, to investigate the chemical structure of SOM, SOM has been extracted by alkali solution and operationally separated to three fractions so-called humic acids (HAs; soluble in alkali and insoluble in acid solutions), fulvic acids (FAs; soluble in both alkali and acid solutions), and humin (insoluble in alkali and acid solutions). This separation upon the

solubility to aqueous solutions makes a mixture of heterogeneous and polydisperse SOM molecules roughly separated into the three fractions with relatively similar chemical properties, and the properties have been alternatively investigated (MacCarthy, 2001). Within SOM, organic matters dissolved in liquid phase in soil are defined as dissolved organic matter (DOM). Dissolved organic matter flows in soil profiles with interacting to soil matrices. To understand the function of DOM relating with material cycling in soils, the chemical properties have been also investigated.

The SOM molecules are stabilized through their interactions to soil minerals and aggregates, and those molecules receive decomposition and/or alteration. To investigate changes in SOM quality and quantity (or soil elemental dynamics and/or soil formation processes), SOM has been fractionated on the basis of the physical properties, such as particulate size, aggregate size, or density, without chemical modifications since around 1970s (von Lützow et al., 2007). Density fractionation is useful for distinguishing SOM with different degrees of association between minerals and SOM (Wagai et al., 2009). In density fractionation, soil samples are generally fractionated to three fractions (Fig. 1): (a) free low density fraction (f-LF), (b) mineral-

associated low density fraction (m-LF), and (c) high density fraction (HF).

Char (or black carbon, pyrogenic carbon) is regarded as the most chemically stable C form in the environment. In 2000s, char gathered more attentions, because both the sequestrating C ability of char in soils and the potential to sustain and/or improve crop productivity by char amendments was expected. The contribution of char to a constituent of SOM in dark colored soils was suggested (Forbes et al., 2006). Although a general method physically separating char is not likely to be recognized, the chemical structure of chars has been investigated by various techniques (Hammes et al., 2007).

Up to date, structural characterization/analysis of SOM has been mainly conducted to the three fractions chemically separated, and the number of the accumulated studies is in the order: HAs > FAs >> humin (Kawahigashi et al., 2013). The reasons are likely that (i) HAs are easy to be prepared and recovered than FAs and (ii) humin is insoluble and can be interfered by minerals for analysis. As shown in the classical authorized books (e.g., Kumada, 1987; Stevenson, 1994), information on the chemical structures could be summarized to the bulk (or average) and componental aspects. Analytical methods to obtain bulk information includes elemental analysis, qualitative assignment or quantitative estimate of the functional groups by infrared absorptions, X-ray diffraction (XRD), and liquid-state nuclear magnetic resonance (NMR) spectroscopy. On the other hand, those to obtain componental aspects include chemical or thermal degradations into building units or fragments followed by gas chromatography/mass spectrometry (GC/MS) since around 1970s. Most of the methods are applicable to HAs and FAs (soluble fractions) but
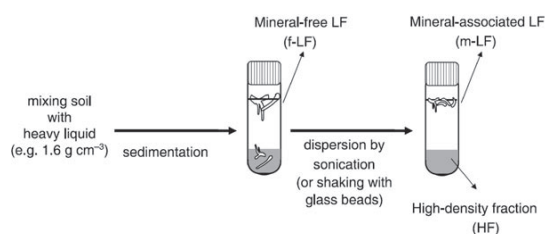

Figure 1. Schematic diagram of the density separation proposed by Golchin et al. (Wagai, 2009).

Table 1 Major soil organic matter (SOM) fractions and applied/applicable (O) or not applicable (N) major analytical techniques to them.

SOM fraction	$^{13}C/^{15}N$ NMR	HPSEC	THM-GC/MS	FTICR-MS
SOM at initial decomposed stage	O	N	O	N
Bulk SOM or fractionated SOM by particle size or density	O	N	N	N
Humic acids, Fulvic acids	O	O	O	O
Humin	O	N	N	N
Dissolved organic matter	O	O	O	O
Char	O	N	N	N

not to humin and still useful up to date (Table 1).

Since around 1980s, with developing and improving analytical techniques in the analytical chemistry field, various powerful techniques have been applied to SOM and/or HS, one of which is solid state NMR. Solid state ^{13}C/^{15}N NMR made it possible to characterize/estimate C/N functional groups in insoluble samples such as humin and bulk SOM (Simpson et al., 2011) (Table 1). Recently, some pulse sequences which detect specific functional groups have been applied to SOM (Mao et al., 2011). Another powerful technique is Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), which examines chemical composition by exactly determining thousands of molecular formulas at one step (Sleighter and Hatcher, 2011). This method is basically applicable to solubilized samples like DOM (Table 1).

In this review, the studies on chemical structures of SOM are introduced with focused on those published after around 2000s. They are separated as follows: (1) SOM generated at initial stage in litter layer of soil, (2) bulk SOM and SOM fractionated physically on the basis of soil particle density, (3) SOM fractionated chemically on the basis of the solubility, (4) DOM, and (5) char. The subject coming up for the studies on chemical structure of SOM is suggested as conclusions.

1. Soil organic matter generated at initial stage

Ono et al. (2009) incubated two species litters (beech and oak) in a litterbag on the forest floors of heterogeneously distributed Cambisols and Andosols, and evaluated the decomposition process by solid state ^{13}C CPMAS NMR. Initially-humified organic matter on the forest floor (L and F horizons) and SOM in the surface soils were also analyzed. In the ^{13}C NMR spectra of the litters (Fig. 2), the signals of O-alkyl C (72 and 104 ppm) that could be ascribed to cellulose structure decreased with the progress of humification. The C compositions in beech and oak litters after 3 years of humification were similar to that of F horizon (Fig. 3). The C functional groups in
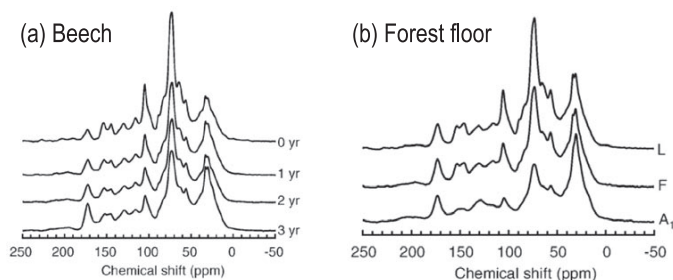

Figure 2. ^{13}C CPMAS NMR spectra of HF-treated samples of humified beech litters (a) and organic matters in L, F, and A$_1$ horizons (b) (Ono et al., 2009).
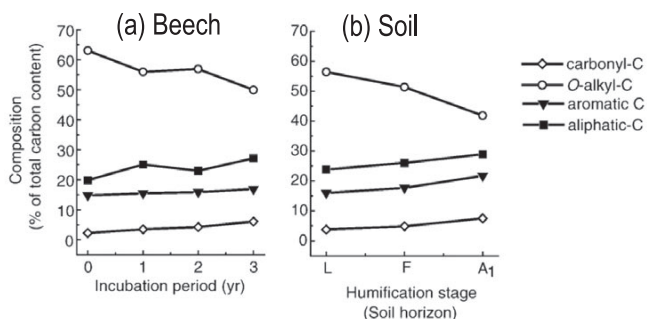

Figure 3. Carbon composition determined by ^{13}C CPMAS NMR for humified beech for 3 years (a) and L, F, and A$_1$ horizons (b) (Ono et al., 2009).

the litters were decomposable in the order: O-alkyl C > aromatic C > alkyl C > carbonyl C. Therefore, from the initial stage of litter decomposition to the generation stage of SOM humified to greater degrees in L < F < A$_1$ horizons, O-alkyl C components like cellulose structures decomposed more strongly and faster than the other C species. Lorenz et al. (2000) incubated needle leaf litters from black spruce and Norway spruce, respectively, in litterbags at black spruce sites (Canada) and Norway spruce sites (Germany) for 10–12 months and characterized their decompositions by ^{13}C CPMAS NMR (Fig. 4). The intact litters had high proportions of O-alky C in total C (54–61%; 50–112 ppm). During 10–12 months decomposition, alkyl to O-alkyl C ratios increased from 0.58 to 0.64–0.70 (black spruce) and 0.41 to 0.49 –0.64 (Norway spruce), mainly due to a relative increase in alkyl C not to decrease in O-alkyl C. In the organic layers that would contain further decomposed/humified matters, both the proportions of O-alky C and alkyl C in the two needle leaf litters tended to decrease, which was not observed in broad leaf litters by Ono et al. (2009).
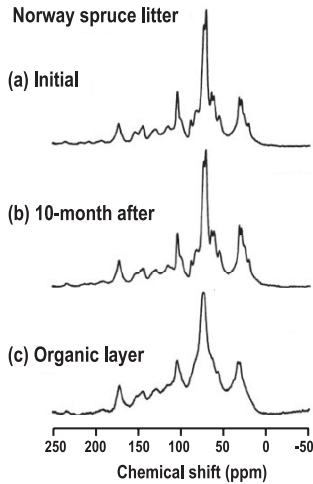
Figure 4. ^{13}C CPMAS NMR spectra of Norway spruce litter incubated at Schluchsee (Germany): initial (a), 10-month decomposed (b), and organic layer (c) (partly revised from Lorenz et al., 2000).

2. Bulk SOM and fractionated SOM by particle size and density

Smernik and Oades (2001) applied solid state ^{13}C direct polarization (or Bloch decay) magic angle spinning (DPMAS) and DP coupled with dipolar dephasing (DPDD) NMR to eight SOM (<53 μm) samples prepared from soils including Phaeozem (i.e., Chernozem), Vertisol, and Luvisol (Fig. 5). They determined relative contributions of overlapping
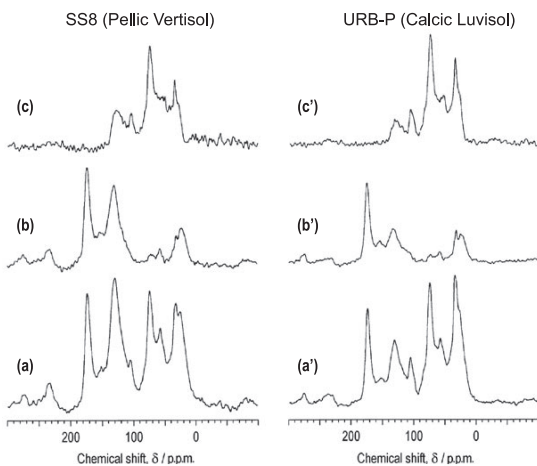

Figure 5. ^{13}C NMR spectra of a Vertisol (left) and a Luvisol (right) whole soil (<53 μm) fractions. (a, a') zero dipolar dephased (i.e., DPMAS); (b, b') 45 μs dipolar dephased (DPDD); (c, c') non-methyl protonated carbon subspectra (partly revised from Smernik and Oades, 2001).

resonances such as C-substituted and H-substituted aromatics (i.e., aromatic C–C/C–H ratio) in 110–145 ppm, where the ratio was estimated as 62–72/28–38% (three Vertisols), 60/40% (one Phaeozem), 53–60/40–47% (two Luvisols). Fang et al. (2010) examined chemical characteristics in bulk SOM and their clay size fractions of four Mollisols by using solid state ^{13}C DPMAS and DPDD NMR (Fig. 6). The proportion of aromatic C–C in total C in both bulk SOM and their clay size fraction was estimated as 27–36%, and the proportion of aromatic C–C in aromatic C–C/C–H was as 69–78%.

Marín-Spiotta et al. (2008) used a density fractionation for soils in the subtropical wet forest zone in southern Puerto Rico, separated into three fractions: a free light fraction (i.e., f-LF; Fig. 1), an occluded LF (i.e., m-LF), and a heavy fraction (i.e., HF). They applied ^{13}C CPMAS NMR to the f-LF and m-LF fractions and the leaf litter on the site. The f-LF and m-LF fractions had higher contributions from alkyl C (29 ppm attributed to long-chain aliphatics) and from *O*-alkyl C (56/150 ppm and 73 ppm attributed to lignin and plant cellulose, respectively) than the leaf litter (Fig. 7). They indicated that the proportion of *O*-alkyl C decreased in the order: leaf litter > f-LF > m-LF while that of alkyl C had the opposite trend, and also that the alkyl C to *O*-alkyl C ratio was higher in m-LF (0.56) than in f-LF (0.37). The similar trends were reported in the review by Wagai (2009). Compared to m-LF and
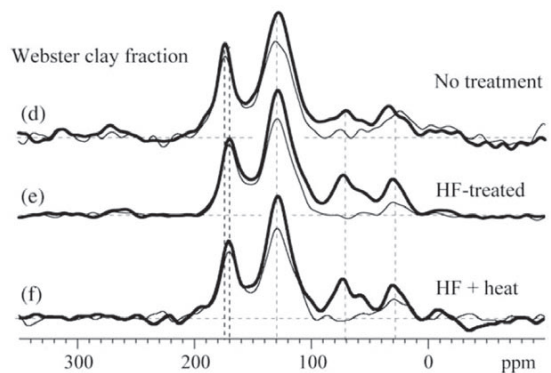

Figure 6. ^{13}C DPMAS NMR spectra of Webster whole-soil and Webster clay fraction: (d) no chemical treatments, (e) HF treatment at ambient temperature, (f) heating at 60 °C during HF treatment. Thick lines are from all types of carbons, thin lines are from all quaternary carbons and CH[3] groups (Fang et al., 2010).
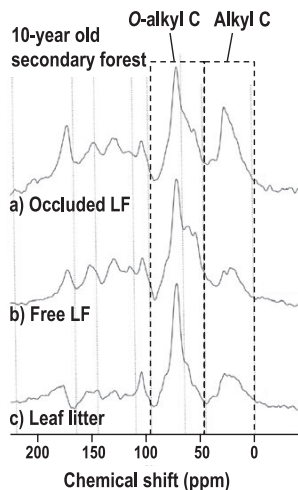
Figure 7. Representative ^{13}C CPMAS NMR spectra of occluded light fraction (i.e., m-LF in Fig. 1) (a), free light fraction (i.e, f-LF) (b), and leaf litter (c) from a 10-year old secondary forest (partly revised from Marín-Spiotta et al., 2008).

Figure 8. Correlation between the proportion of each C species in total C estimated by ^{13}C CPMAS NMR and A_{600}/C (Ikeya et al., 2004). Asterisks (* and ***) indicate statistical significance (P <0.05 and 0.005, respectively) for linear correlations.

f-LF fractions, it seems that HF fractions have been less examined by ^{13}C NMR. Wagai et al. (2008) applied ^{13}C CPMAS NMR to SOM fractions prepared from soils under rainforests at Mt. Kinabalu (Borneo) and showed that the proportion of aromatic C decreased in the order, f-LF > m-LF > HF, while that of alkyl C and O-alkyl C did not have any trend.

3. Fractionated SOM on the basis of solubility to aqueous solution

Humic acids (HAs)

Tate et al. (1990) estimated C composition in various soil HAs by using solid state ^{13}C CPMAS NMR, indicated that, with increasing the degree of darkness, the proportion of aromatic C in total C increased while that of alkyl C and O-alky C decreased. Similar trends were observed by Maie et al. (2002) and Ikeya et al. (2004) (Fig. 8). Abe and Watanabe (2004) and Maie et al. (2006) applied X-ray photoelectron spectroscopy and/or ^{15}N CPMAS NMR to various soil HAs, indicated that the proportion of aromatic N in total N increased while that of amide/peptide decreased with increasing the degree of darkness. Those data help us outline an average chemical structure of HAs.

"Thermally-assisted hydrolysis and methylation (THM)" or "thermochemolysis" followed by GC/MS analysis is one of the most powerful techniques to characterize or determine a wide range of components
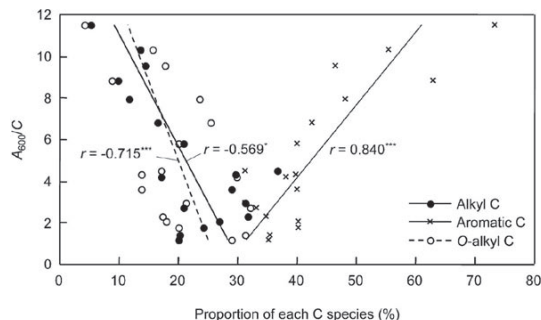
in SOM and HS. Ikeya et al. (2004) applied THM-GC/MS to various soil HAs. The yield of aliphatic compounds including mono/di carboxylic acids (0.4–4.2 mg g^{-1}) and that of lignin-derived phenols (0.2–30.6 mg g^{-1}) was significantly smaller in the HAs with greater degree of humificaiton (Fig. 9). These results suggested that other aromatic components than phenol single ring in a highly-humified HAs were not detected in this analysis but could largely contribute to their high aromatic C content and dark color. Although the other compounds such as monosaccharides, amino acids, and their derivatives were rather less detected by the method, such compounds in soil HAs had been released by acid hydrolysis since around 1970s (Stevenson, 1994), and those contents were significantly larger in the HAs with lower degree of humification (as shown by Tsutsuki and Kuwatsuka in 1970s).

With respect to condensed aromatic structure in soil HAs associated with their chemical stability, Ikeya et al. (2011) analyzed 11 band in XRD profile of various soil HAs to estimate distribution of C layer planes. They indicated the occurrence of the C layer planes consisting of 4–37 rings and also that both the relative content and average size of C layer planes (0.78–0.96 nm) were larger in the HAs with greater degree of darkness (Fig. 10). Katsumi et al. (2015) analyzed 002 band in XRD profile of various soil HAs, indicated that the mean thickness of stacking structure was 1.04–1.84 nm and the stacking number was 3.01–5.40 and that each value positively correlated to both the aromatic C content and the degree of darkness.
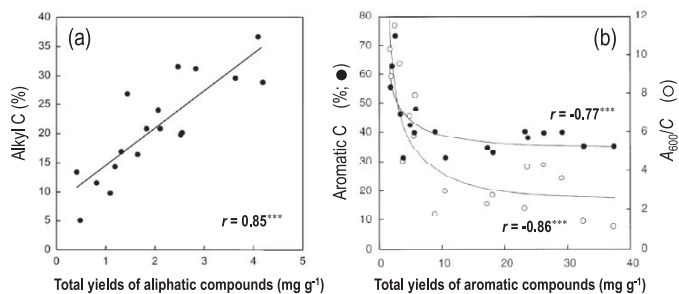
Figure 9. Correlations between: (a) the total yields of aliphatic compounds from humic acids upon thermochemolysis and the proportion of alkyl C in total C (estimated by ^{13}C CPMAS NMR), and (b) the total yields of aromatic compounds from humic acids upon thermochemolysis and the proportion of aromatic C in total C or A_{600}/C (Ikeya et al., 2004). An asterisk (***) indicates statistical significance (P <0.005) to the curve fitting.

Similar trends were obtained in FTICR-MS analysis of various soil HAs, where thousands of molecular formulas were detected with similar relationships between their chemical composition and the degree of darkness (Fig. 11; Ikeya et al., 2015). Fujitake et al. (2012) applied 33 HAs prepared from more than five types of soils to high-performance size exclusion chromatography and determined their weight-average molecular weight calibrating with sodium polystyrene sulfonate. They showed the difference in the average molecular weight in the order: Andosols (7792 Da) = Chernozems (8327 Da) < Cambisols (24054 Da) = Histosols (25543 Da).
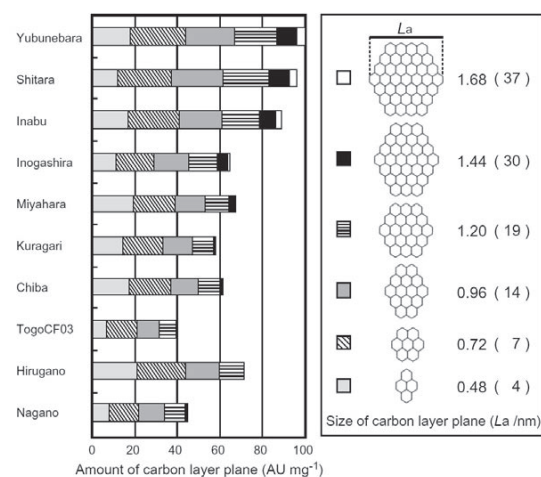

Figure 10. Relative content of the carbon layer planes in soil humic acids (arbitrary units per mg) (Ikeya et al., 2011). Number in parentheses indicates the number of rings in the model structure.

In average structure, the soil HAs with greater degree of darkness, that often seen in black color soils (e.g., Andosols), had relatively small average molecular weight and high aromatic C content. At component level, the aromatic C components were mainly consist of highly condensed aromatic rings with 1 nm average size, where they were highly stacking up to ca. 5, and of less content of small aromatic rings such as phenols. In contrast, the HAs with lower degree of darkness, that occur in less decomposed organic substances just like peat or organic horizons (e.g., Histosols), had large average molecular weight and high alkyl/O-alkyl C content, where biomolecular components (such as aliphatic acids, phenolic rings, monosaccharides, and amino acids/peptides) and those partly altered could be still more remained. The HAs with moderate degree of darkness, that often isolated from forest soils or Cambisols are likely to have medium properties between the HAs with greater and lower degrees of humification.

Fulvic acids (FAs)

Iimura et al. (2012) analyzed 36 FAs recovered as DAX-8 adsorbed fraction from five soil types (Andosols, Cambisols, Chernozems, Podzols and Acrisols) by using liquid state ^{13}C NMR. They indicated that the C composition in soil FAs generally had similar values: aromatic C (29.4 ± 3.3% in average) > O-alkyl C (23.8 ± 3.4%) > carboxyl C (21.9 ± 2.1%) = alkyl C (20.0 ± 3.2%) > carbonyl C (5.2 ± 1.2%) and, generally, the composition was significantly not different among the five soil types. The high abundance of carboxylic C was similarly observed in FAs (DAX-8 adsorbed fraction) from a hardwood forest soil (Ussiri and Johnson, 2003) and from acid forest soils in Galicia (Spain) (López et al., 2008) by ^{13}C CPMAS NMR. The weight-average molecular weight of the 36 FAs were small values (983–1776 Da), which had relatively small difference compared with that in the case of HAs (Fujitake et al., 2012). In the THM-GC/MS analysis of two soil FAs (Andosol and Cambisol; Watanabe et al., 2004), phenol compounds and benzenepolycarboxylic acids (BPCAs) were major compounds yielded, followed by minor aliphatic acids. Similar trends were
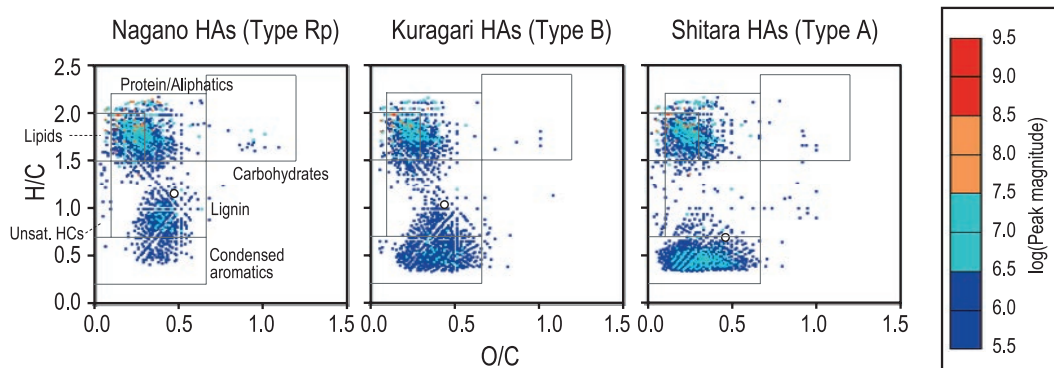
Figure 11. van Krevelen diagrams of the molecular formulas assigned to the FTICR mass spectra of Nagano Type Rp, Kuragari Type B, and Shitara Type A humic acids (Ikeya et al., 2015). Open circles indicate the atomic H/C and O/C values of the bulk humic acids, and boxes define the regions for lipid, protein (including other aliphatics), lignin, condensed aromatics, carbohydrate, and unsaturated hydrocarbons.

observed in the chemical composition upon FTICR-MS analysis of five soil FAs (Ikeya et al., 2012, personal data), where indicated that aromatic C components in the FAs were consist of lignin-like phenols dominantly and of smaller condensed aromatic components (2–7 rings) compared with those in soil HAs. Monosaccharides were also released from two soil FAs by acid hydrolysis (Watanabe et al., 2004), which and their derivatives could contribute to *O*-alkyl C and alkyl C signals on their NMR spectra.

In average structure, soil FAs had relatively similar C composition irrespective of soil types and were characterized with high carboxyl C content (ca. 20%) compared with soil HAs. At component level, aromatic C structure in soil FAs had lower degree of condensation (than that in soil HAs) and was dominantly composed of single rings (i.e., phenols and BPCAs) and of minor smaller condensed aromatic components.

Humin

The number of studies for humin is less compared with that for the soluble fractions (HAs and FAs; Kawahigashi, 2013). Simpson and Johnson (2006) prepared humin fractions by alkali-extraction and hydrogen-fluoride (HF) treatment from six soils including two brown/black Chernozemic soils and Phaookee peat soil and they applied ^{13}C CPMAS NMR to the bulk soils and their humin fractions (Fig. 12). They semi-quantitatively estimated that

the peat soil and the peat humin have similar composition of C functional groups while the brown/black Chernozemic humins had higher proportions of alkyl C (29–37%) than the corresponding bulk soils (17–23%).

Simpson and his co-workers tried to recover HS from extraction residue as humin and characterized the humin. For example, Song et al. (2011) recovered 70–80% of the residue after extracting HAs and FAs as humin for two soils in Ireland and Brazil by using dimethylsulfoxide/6% sulfuric acid as extract. While ^{13}C CPMAS and CPDD NMR spectra of the two HAs showed dominant or moderate signals in aromatic C and carboxyl C regions (Fig. 13a, e), those of the corresponding DMSO-soluble humin showed strong peaks of both aliphatic hydrocarbon (33 ppm) and carboxyl groups (173 ppm). They were also characterized with hydrocarbon- (104 ppm), peptide- (73 ppm), and lignin- (56 and 150 ppm) derived
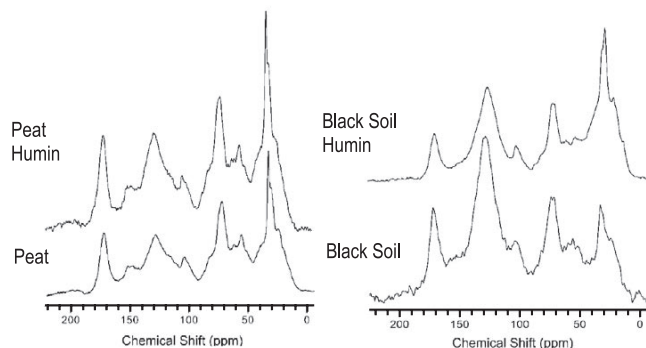

Figure 12. ^{13}C CPMAS NMR spectra of the peat and black Chernozemic soil samples and their corresponding humin samples (Simpson and Johnson, 2006).

components (Fig. 13c, g). Although the characteristic of the humin seems to be different among the two soils (e.g., one had dominant aromatic C signal while another one did not have that), alkyl C components, assignable to methylene chain, are commonly likely to be dominant in the humin. They considered that the major components of humin are hydrophobic biomolecules, such as long-chain fatty acids, waxes, and cuticular materials, which are likely to be adsorbed on soil clays and be protected from solubilization. Although molecular components in humin seemed to be less investigated, similar kinds of components detected in HAs and FAs are considered to occur as building units of humin.

As summary, humin fractions could be generally characterized with higher alkyl C abundances
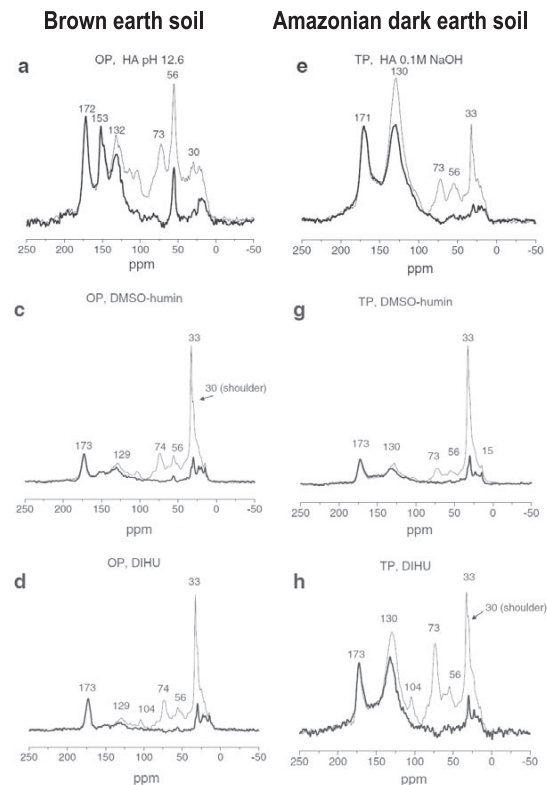
compared with the corresponding HAs, FAs, and bulk soils while aromatic C and alkyl C contents in humin were likely to be variable.

4. Dissolved organic matter (DOM) in soil

Sanderman et al. (2008) compared chemical properties of litter/throughfall and SOM/DOM at different depths for two soils in California (U.S.A.) by using ^{13}C CPMAS NMR: a Typic Haplustolls under grassland and an Inceptisol under Redwood and Douglas fir. The NMR spectra of the DOM samples at each depths in the Inceptisol had the dominance of O-alkyl C and showed a strong peak at alkyl C region (0–45 ppm) ascribed to aliphatic chain structure and a strong peak at carbonyl C (165–210 ppm) ascribed to major functional groups in organic acids compared with those of corresponding SOM samples (Fig. 14). They showed that alkyl C to O-alkyl C ratio tended to decrease with increasing depth in the order: litter = Oe < A1 < AB horizon for SOM, and throughfall = 0 cm < 7 cm < 15 cm depth for DOM, and the similar result was obtained from the Typic Haplustolls samples. Similar trends were observed for water-extractable HS isolated from litter and soil horizons in a Dystric Cambisol in Japan (Asakawa et al., 2007). Kaiser et al. (2001) analyzed

Figure 13. Variable amplitude CP-TOSS (thin line) and corresponding CPDD (thick line) ^{13}C NMR spectra of isolates from a Brown earth soil: (a) HAs isolated at pH 12.6 after sequential extractions at pH 7 and at pH 10.6, (c) humin isolated with DMSO+6%(v/v) H$_2$SO$_4$, and (d) DMSO/H$_2$SO$_4$ insoluble humin. Those from an Amazonian dark earth soil: (e) HAs isolated in 0.1 M NaOH, (g) humin isolated with DMSO+6% (v/v) H$_2$SO$_4$, and (h) DMSO insoluble humin (Song et al., 2011).
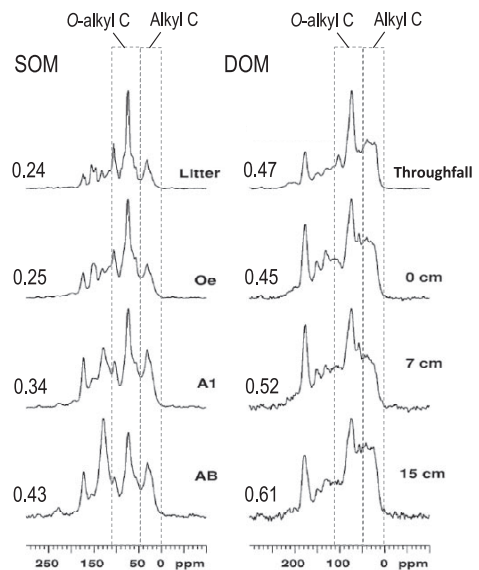

Figure 14. ^{13}C CPMAS NMR spectra of soil and macropore DOM collected at same depths at an Inceptisol under Redwood and Douglas fir (Sanderman et al., 2008). The values on the spectra are alkyl C to O-alkyl C ratio.

DOM samples of forest floor seepage water in two forest soils under Scots pine and European beech in Bavaria (Germany) for a 27-month period (1997–1999). They also indicated the dominance of *O*-alkyl C (33–57%) in the two DOM samples by using liquid state ^{13}C NMR.

At the molecular components, sum of hydrolysable neutral and acidic carbohydrates (10–66 mg g^{-1}; acid hydrolysis), sum of lignin-derived phenols (6–24 mg g^{-1}; CuO oxidaiton), and sum of hydrolysable amino sugars (1–54 mg g^{-1}; acid hydrolysis) were determined in combined three-month DOM samples of the two forest floors (Kaiser et al., 2001). The C–O of carbohydrates, methoxyl groups of lignin-phenols, and C–O/N of amino sugars (and possibly their derivatives) could contribute to strong *O*-alkyl C signals. D'Andrilli et al. (2010) characterized DOM samples from fen and bog porewaters in a peatland soil of Minnesota (U.S.A.) by using FTICR-MS. Most of molecular formulas in the samples were assigned to lignin-phenols (blue plots in Fig. 15), and minor ones were to condensed aromatic compounds (red plots).

As summary, chemical structure of DOM was characterized with a dominance of *O*-alkyl C, which could be contributed from lignin-derived phenols, carbohydrates, and amino sugars.

5. Char

Char is known to have a high aromaticity. For example, Kaal et al. (2008) investigated the chemical properties of macroscopic (>2 mm) charcoals isolated from five different depths in a Haplic Umbrisol (humic/alumic) in Campo Lameiro (Spain). The charcoals had the high proportion of aromatic C in total C (70–79%; 110–165 ppm), by using ^{13}C DPMAS NMR, followed by carboxyl C (8–14%) (Fig. 16). Although they conducted pyrolysis- or THM-GC/MS analysis of the charcoals, condensed aromatic components were not detected, except for benzene and its derivatives with large proportions (54–93%) that were considered to be derived from the charcoals. Mao et al. (2012) examined char residue in two soils (dark earth soil in Amazon and Mollisol in Iowa, U.S.A.) by using ^{13}C DPMAS and DPDD NMR, estimated the proportion of aromatic C in total C as 66% and 52%, respectively, followed by that of carboxyl C (15 and 16%). They also estimated the

proportion of aromatic C–C in total C as 41% and 37%, respectively. Sultana et al. (2010) analyzed charred plant materials isolated from three buried Andosols in Japan by using ^{13}C ramp CPMAS NMR
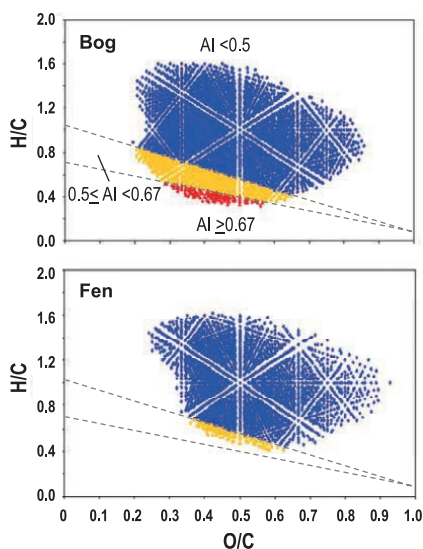

Figure 15. Van Krevelen diagrams of the molecular formulas assigned from DOM samples (a bog at 0.17 m and a fen at 0.20 m) by FTICR-MS. Plots were separated with dotted lines with aromaticity index (AI): AI <0.5 (blue), 0.5≤ AI <0.67 (yellow), and AI ≥0.67 (red) (partly revised from D'Andrilli et al., 2010). AI is calculated as follow: AI = (1+C–O–1/2H)/C–O. AI is higher in the higher degree of condensation.
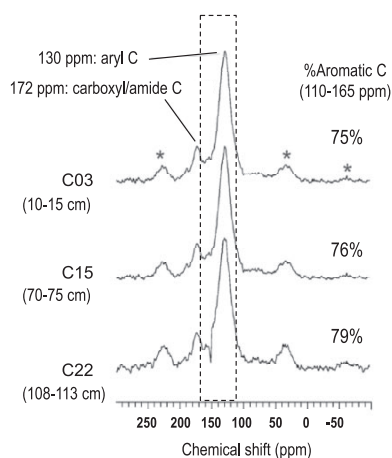

Figure 16. Representative of ^{13}C NMR spectra of charcoals collected from different depths in a Haplic Umbrisol: C03, 10–15 cm; C15, 70–75 cm; C22, 108–113 cm. Spinning side bands of aryl C are indicated by asterisks (Kaal et al., 2008). Asterisks (*) indicate spinning side bands.

and XRD 11 band analysis. They indicated that the proportion of aromatic C was 61–74% and the average size of C layer plane in the materials was 1.26 –1.37 nm, where their size was larger than that in soil HAs (0.78–0.96 nm; Ikeya et al., 2011).

Therefore, no surprisingly, char is generally characterized with the largest proportion of aromatic C followed by large carboxyl C and with developed C layer plane structure.

Conclusions

The most vigorously examined SOM fraction on chemical structure is operationally separated fractions by solubility, i.e., HAs followed by FAs. The details of chemical compositions in HAs and FAs can be more clearly characterized or estimated in both specific C functional groups and composition of components at molecular level since around 2000s than those before as described above. Major SOM fractions defined in this article and major analytical techniques that applied/applicable or not applicable to examine chemical structures of each SOM fraction are summarized in Table 1. Undoubtedly, the applicability of those techniques to samples depends on the solubility of each SOM fraction to solvent. In principle, it is difficult to apply HPSEC, THM-GC/MS, and FTICR-MS to humin, bulk/physically fractionated SOM, and char samples without solubilizing. In several studies, THM-GC/MS analysis was conducted for alkali extracts from bulk SOM samples with regarding the extract as representative of the bulk SOM. At present, solid state NMR is only the method with the highest applicability to all SOM fractions. To more progress the analysis of the chemical structure of insoluble SOM samples, effective separation methods of organic matters from soil minerals in humin and bulk/physically fractionated SOM and effective solubilizing methods of char are strongly expected to be developed.

Acknowledgments

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