



Characterization of the chemical composition of soil humic acids using Fourier transform ion cyclotron resonance mass spectrometry

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Abstract

The composition of humic acids (HAs) with varying degrees of humification isolated from 10 common Japanese soils was characterized using negative ion mode electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry at 12 T. In particular, attention was paid to polynuclear aromatic components, which are more associated with the refractory nature of HAs and their resistance to biodegradation in soil than single C ring aromatic entities, such as lignin-like components, and aliphatic functionalities. Thousands of peaks were observed in the m/z range of 200–700, and molecular formulas were assigned to 817–2457 peaks in each sample. The molecular formulas having H/C and O/C ratios similar to those of lipid, protein, and other aliphatics with low double bond equivalents (DBE) of 0–7 were generally observed across the m/z range of 200–700. Although there were a number of molecular formulas having H/C and O/C values similar to those of lignin across the wide m/z range in the HAs with a low degree of humification, most lignin-like molecular formulas in the larger m/z range (450–650) or irrespective of m/z were lacking in the HAs with middle and high degrees of humification, respectively. These observations suggest a longer residence time for lignin monomers/dimers (and their derivatives; m/z 200–400) than larger lignin oligomers (m/z 450–650) in HA structural domains. The number of molecular formulas having H/C and O/C values similar to condensed aromatics increased with increasing degree of humification. The m/z and DBE values of condensed aromatic-like molecular formulas in the HAs with a lower degree of humification were <500 and 10–25, respectively, whilst the ranges expanded to 600 and 30–33, respectively, in the highly-humified black HAs. Kendrick mass defect analysis using a carboxyl group as the characteristic functional group found that 31, 73, and 39 molecular formulas had chain-type, net-type, and biphenyl-type condensed aromatic acids, respectively, as possible structures. Summed peak magnitudes of the condensed aromatic-like molecular formulas, in particular those with higher DBE values (>17) expected to have at least 5 rings, increased with increasing degree of humification and aromatic C content (evaluated by solid-state ^{13}C nuclear magnetic resonance spectroscopy), suggesting their contribution to the intrinsic recalcitrance of HAs in soil.

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1. INTRODUCTION

Soil organic matter (SOM) is derived mainly from plant residues and dead cells of microorganisms. Some SOM components can be assigned to known biomaterials, while others have lost their initial structure through biotic and abiotic reactions. Such materials characterized by brown

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or black color are called humic substances. According to the protocol of classical chemical fractionation, humic substances that are extractable with an alkaline solution and precipitate by acidifying the extract are classified as humic acids (HAs; MacCarthy, 2001). A wide range of turnover times of humic substances contribute to regulation of the global C balance, which may depend both on the strength of their adsorption to soil components (Torn et al., 1997; Baldock and Skjemstad, 2000; Mikutta et al., 2006) and inherent structural resistance to microbial attack (Krull et al., 2003; Wattel-Koekkoek et al., 2003). According to the property of solubility, HAs are composed of molecules rich in both acidic functional groups, mainly carboxyl and phenolic groups, and hydrophobic moieties such as alkyl chains and aromatic cores. The intrinsic persistence of HAs in soils may be determined by the construction units and their combinations and arrangements, and those with higher persistence tend to have a higher degree of structural modification (humification) towards the darker color (Kumada, 1987; Watanabe and Takada, 2006).

In general, less altered components in HAs that have lighter color, such as carbohydrates (Tate et al., 1990; Allard, 2006), proteinaceous materials (Abe and Watanabe, 2004; Maie et al., 2006), aliphatic acids, and lignin phenols (Ikeya et al., 2004; Deport et al., 2006; Cerli et al., 2008), are relatively mobile. On the contrary, intensely modified (i.e., humification has progressed), more complex and chaotic structured HA molecules are recalcitrant in soil (Hernández and Almendros, 2012). During progression of humification, aromatic C and free radicals (like quinone/semi-quinone structures) are concentrated with increasing degrees of dark color (Watanabe et al., 2005). However, the structure of the aromatic C is likely more important than the amount of aromatic C to the reactivity of HAs. More complicatedly, some extrinsic factors (such as interactions with soil particles, occlusion in micro-aggregates, entering into pores or interlayers in amorphous oxides and crystalline clay minerals) may affect the degradability of HAs in various states of humification (von Lutzow et al., 2006). Furthermore, partially-degraded and oxidized charred materials having amorphous C layer planes can be incorporated in this humus fraction (Shindo et al., 1986; Forbes et al., 2006; Solomon et al., 2007). Simultaneous increases in aromatic C or condensed aromatic structures with quinone/semi-quinone groups and acidic functional groups, such as carboxyl groups, may be a result of these various processes. Although the evaluation of the degree of humification based on the degree of dark color is primitive, it is useful as an index expressing the extent of the structural modifications that the HA pool has experienced in soil. Transition of the chemical structures of HAs expressed by that of dark color is likely related to their reactivity, and consequently, to the cycles of C and other elements, as is shown in the parallel increase and decrease in aromatic and aliphatic components, respectively, with increasing soil age (up to 10,000 yBP) (Watanabe and Takada, 2006). Iimura et al. (2013) showed an increase in aliphatics and a decrease in the degree of humification of HAs with time after land use change from grassland to forest. To estimate the stability/reactivity of

HAs and (bio)geochemical cycles of C and other elements constituting them, it is necessary to elucidate the chemical composition of HAs.

The occurrence of various condensed aromatic components in HAs has been estimated based on analyses using reductive cleavage (Stevenson, 1989), ruthenium tetroxide oxidation (RTO; Ikeya et al., 2007; Polvillo et al., 2009), Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS; Kramer et al., 2004), and X-ray diffraction (XRD; Ikeya et al., 2011). Smaller condensed aromatic compounds having 2–5 rings are synthesized by soil microorganisms (Kumada and Hurst, 1967; Fujitake et al., 1998; Zancada et al., 2003), which are potential contributors of condensed aromatic structures in HAs. A possible origin of larger condensed aromatic nuclei in HAs is charred plant materials that are widely distributed in soil, although the production of condensed aromatic nuclei from biomaterials without experiencing pyrogenic reactions has also been suggested (Glaser and Knorr, 2008; Chen et al., 2014).

Fourier transform ion cyclotron resonance mass spectrometry allows for accurate mass to charge (m/z) measurement and subsequent molecular formula assignment to the thousands of peaks detected in the mass spectrum of a complex mixture, due to the ultrahigh resolving powers of the instrument (>400,000 on average for 12 T instruments; Sleighter and Hatcher, 2011). While FTICR-MS has been applied to dissolved organic matter (DOM; e.g., Kim et al., 2003; Stenson et al., 2003; Dittmar and Koch, 2006; Sleighter and Hatcher, 2007), the application of this technique to soil organic matter is limited in number, and typically the composition of molecular formulas assigned was investigated mainly by comparing their elemental composition with those of biomolecules on a van Krevelen diagram (Ohno et al., 2010, 2014; Ikeya et al., 2012). According to Ohno et al. (2010), the majority of components in sodium hydroxide-extractable HAs plotted in the H/C–O/C region similar to that of lipids and the boundary between lignin and condensed aromatics, while the H/C and O/C space similar to those of lignin were lacking in the HAs extracted with sodium hydroxide after soil was treated with a hydrochloric acid solution (presumably associated with calcium). One of the benefits of FTICR-MS analysis is that the functional groups substituted on condensed aromatic rings can be estimated. Kendrick mass defect (KMD) analysis that is used in FTICR-MS datasets can separate components having the same skeletal formulas with various numbers of a particular functional group (Stenson et al., 2003; Kramer et al., 2004). Kramer et al. (2004) tentatively assigned 84 peaks from a highly aromatic HA sample to linearly fused and net-like condensed aromatic acids, as well as various biphenyl type aromatic acids, using KMD analysis. Dittmar and Koch (2006) showed condensed aromatic compounds having 6–7 rings with 2–3 carboxyl groups and other functional groups (including methyl, ethylene, and hydroxyl) as potential structures for 33 molecular formulas with low H/C (<0.9) and O/C (0.25) ratios in marine DOM samples.

Characterizing soil HAs with varying degrees of humification in terms of individual components, emphasizing the

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