Relationship between ^{14}C age and structural property of humic acids

Akira Watanabe[A], Sho-Ichiro Ochi[A], Chizuru Kato[A], Kosuke Ikeya[A] and Toshio Nakamura[B]

[A]Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, Japan, Email akiraw@agr.nagoya-u.ac.jp
[B]Center for Chronological Research, Nagoya University, Chikusa, Nagoya, Japan.

Abstract
To understand the relationship between residence time and structural property of soil humic acids (HAs), a HA sample obtained from the A horizon of a Haplic Andosol at a depth of 35-40 cm (247 ± 33 years before present (yBP)) was fractionated to 8 sub-fractions having different degrees of humification, and their ^{14}C age, molecular size distribution (high performance size exclusion chromatography (HPSEC)), C composition (ramp cross polarization/magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectroscopy), and N composition (X-ray photoelectron spectroscopy (XPS)) were measured. Condensed aromatic components were investigated by ruthenium tetroxide oxidation (RTO) method and based on 11 band profile analysis in X-ray diffraction (XRD) spectra. With increasing ^{14}C age, aromatic C and N contents as well as molecular size became larger. RTO yielded degradation products derived from naphthalene to coronene nuclei, and XRD analyses suggested the presence of carbon-layer planes corresponding to 4-37 ring condensed aromatic structures in the HAs. Contents of condensed aromatic components having 5-37 rings increased with increasing ^{14}C age, suggesting their contribution to the longer residence time of darker-coloured HAs with larger aromaticity.

Key Words
[14]C age, condensed aromatic structure, humification, humic acids, X-ray diffraction profile.

Introduction
Humic acids are synthesized secondarily from biomass constituents or their metabolites biochemically and/or chemically in the environment. It is considered that initial HAs lose relatively easier decomposable components while condensation-polymerization and oxidation proceed, which result in more refractory structure. Partial degradation and oxidation of charred plant materials might be another production mechanism of refractory HAs, although their contribution to the whole HAs is unknown. Concentration of aromatic C with increasing soil age was suggested by the comparison of ^{13}C NMR spectra of HAs obtained from the surface and several buried A horizons in a soil profile (Watanabe and Takada 2006). The presence of various condensed aromatic components in HAs was suggested by Kramer *et al.* (2004) using Fourier transform-ion cyclotron resonance mass spectrometry. Although condensed aromatic components may be more recalcitrant than benzene derivatives, it is difficult to estimate their content and structure using ^{13}C NMR. The purpose of the present study is to find components or structural moieties that contribute to the longer residence time of refractory HAs. For this purpose, HAs prepared from a soil layer were fractionated into sub-fractions with different degrees of humification, and the relationship between ^{14}C age and structural property of the sub-fractions was analyzed.

Methods
Preparation of sub-fractions of humic acids
Soil sample used was collected from the A horizon of a Haplic Andosol at a depth of 35-40 cm (247 ± 33 yBP) in Miyazaki, Japan. After plant residues were removed using $ZnCl_2$ solution (density, 1.6), HAs were prepared according to the NAGOYA method (Kuwatsuka *et al.* 1992). The HA sample was fractionated by successive precipitation method with 0.01 M NaOH─acetone solutions. Seven fractions precipitated at acetone mixing ratios of 20% (designated 20P), 30% (30P), ..., and 80% (80P), and a fraction soluble at the mixing ratio of 80% (80S) were obtained as powder samples after removal of acetone and sodium ion.

[14]C age of humic acid sub-fractions
Each sample of the sub-fractions was combusted with CuO, and CO_2 produced was purified and then transformed into graphite by heating with reduced iron under H_2 atmosphere. [14]C concentration in graphite samples was determined using an accelerator mass spectroscopy system, and ^{14}C age was calculated.

Structural analysis of humic acid sub-fractions

Degree of humification of the sub-fractions was evaluated using two variables of A_{600}/C and $\log(A_{400}/A_{600})$, in which A_{600}, A_{400}, and C are absorbances at 400 and 600 nm and C concentration of HAs dissolved in 0.1 M NaOH. Molecular size distribution was measured by HPSEC, and weight average molecular weight (M_w) was calculated from the calibration curve made with several sodium polystyrene sulfonate samples. Composition of C functional groups was estimated from ramp CPMAS ^{13}C NMR spectra (Dria *et al.* 2002). Composition of N functional groups was estimated using XPS (Abe and Watanabe 2004). RTO followed by gas chromatography (GC) was conducted for 4 sub-fractions according to Ikeya *et al.* (2007). Briefly, HAs were reacted with $RuCl_3 \cdot nH_2O$ and $NaIO_4$ at 25°C, and water-soluble products were methylated. Methyl esters of benzenepolycarboxylic acids (BPCAs) with 2 to 6 carboxyl groups derived from naphthalene to coronene structures were determined by GC using internal standard method. XRD spectra were recorded in the scan range of $2\theta = 5\text{-}100°$. A silicon folder was used. The 11 band profile was analyzed to estimate the composition of carbon-layer planes on a weight basis. A mixture of two series of carbon-layer plane models starting from benzene/coronene and pyrene (Fujimoto 2003) was adopted for calculating the theoretical scattering intensities. Total amount of carbon-layer planes in arbitrary units (A.U.) per mg of sample was evaluated from the intensity of 11 band (base-line method).

Results

In the fractional precipitation, HAs with higher degrees of humification precipitated at lower acetone concentrations. The ^{14}C age was older in the sub-fractions precipitated at lower acetone concentrations and ranged from 104 to 530 yBP. The ^{14}C age correlated positively with A_{600}/C ($r^2 = 0.93$; $P < 0.005$) and negatively with $\log(A_{400}/A_{600})$ ($r^2 = 0.97$; $P < 0.005$). The relationship between ^{14}C age and A_{600}/C was expressed as follows:

$$^{14}\text{C age} = 29.4\, A_{600}/C + 78.8 \tag{1}$$

Since the sub-fraction samples for structural analysis were prepared separately from the samples used for ^{14}C age analysis, their ^{14}C age was estimated using equation 1.

The M_w value estimated by HPSEC was larger in an older fraction. The proportion of aromatic C in total C (37-51%) showed a positive correlation to ^{14}C age ($r^2 = 0.93$; $P < 0.005$). Positive correlation was also observed between the proportion of aromatic N in total N (7-19%) and ^{14}C age ($r^2 = 0.88$; $P < 0.05$), while the proportion of peptide/amide N in total N decreased with increasing ^{14}C age ($r^2 = 0.92$; $P < 0.05$).
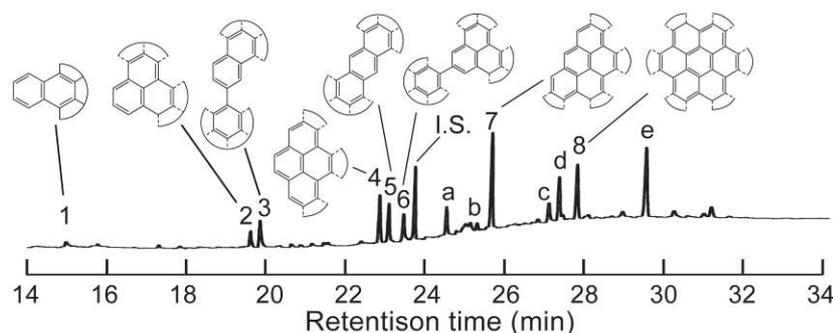

Figure 1. GC chromatogram of degradation products from sub-fraction (50P) of Miyazaki humic acids by RuO₄ oxidation. I.S., Internal standard (nonadecanoic acid methyl ester). Peaks 1, 2-3, 4-6, 7, and 8 were assigned to benzene-di-, tri-, tetra-, penta-, and hexa-carboxylic acids methyl esters. Their representative condensed aromatic nuclei origins are shown above peak Nos. Peaks a-e were assigned to benzenepolycarboxylic acids having other side chains than carboxyl groups.

Table 1. Yields of degradation products derived from condensed aromatic structures from the sub-fractions of Miyazaki humic acids with different ^{14}C age by RuO₄ oxidation (mg/g).

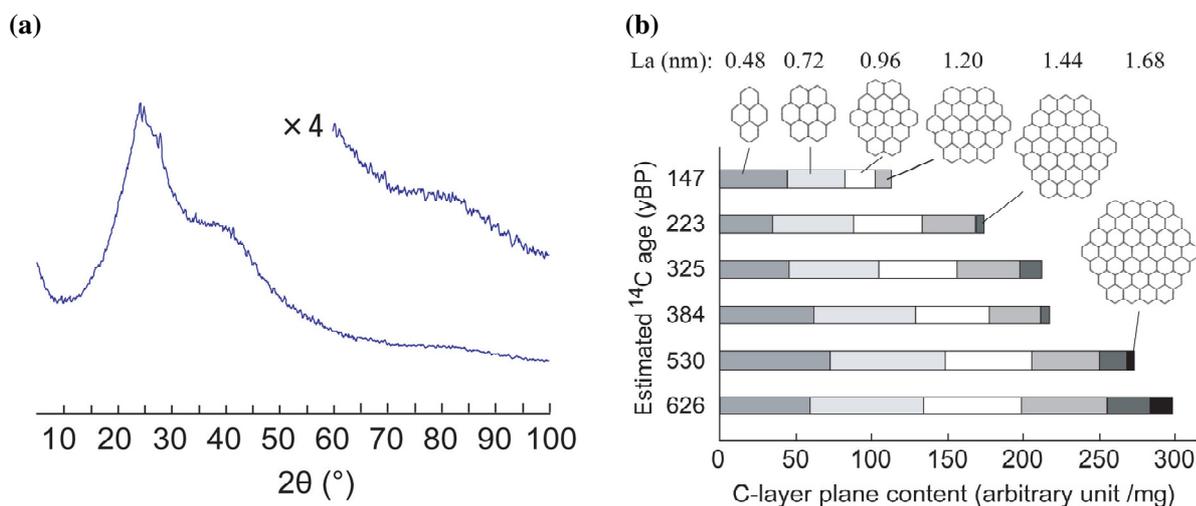| Number of aromatic rings in original structure	Estimated ^{14}C age of sub-fraction (yBP)			
	530	325	223	147
2 (Peaks 1 and 3 in Figure 1)	6.0	3.8	6.4	5.6
3 (Peaks 2, 5, and 6)	15	9.8	15	11
4 (Peak 4)	7.9	5.9	7.0	5.0
5-7 (Peaks 7 and 8)	35	21	17	8.7
Unknown (Peaks a-e)	47	27	18	14
Total	111	68	63	44

(a)

(b)

Figure 2. X-ray diffraction profile of sub-fraction 50P (a) and relative content of various sizes of carbon-layer planes in the sub-fractions of Miyazaki humic acids (b). L_a indicates the size of carbon-layer plane.

Figure 1 shows an example of the GC chromatogram of RTO products. In RTO, condensed aromatic components are degraded mainly into BPCAs. In Table1, the estimated ^{14}C age of each fraction is shown instead of fraction name. Sum of the yield of BPCAs was largest and smallest in the oldest and youngest fractions, respectively. Difference among the fractions was more conspicuous in the yield of BPCAs derived from 5- to 7-ring condensed aromatic structures than in those from 2- to 4-ring condensed aromatic structures (Table 1). Figure 2a shows the representaive XRD profile of Miyazaki HAs (50P). The 002 band ($2\theta = 24°$) was most prominent, followed by 01 band at around $2\theta = 40°$. The 11 band with maximum at around $2\theta = 80°$ was weak but clearly observed in all the fractions. The 11 band analysis indicated the occurrence of multiple sizes of carbon-layer planes from 0.48 to1.20 nm, corresponding to condensed aromatic structures consisting of 4 to 19 rings, in the youngest fraction (Figure 2b). Larger carbon-layer planes up to 1.68 nm corrsponding to 37-ring condensed aromatic structures were observed in the older fractions. Total amount of condensed aromatic structures based on the intensity of 11 band was larger in the older samples and positively correlated to the proportion of aomatic C in total C ($r^2 = 0.985$).

Conclusion

Sub-fractions of HAs having different ^{14}C age in a soil layer showed different structural properties. Aromatic C and N became more abundant with increasing ^{14}C age. RTO and XRD analyses showed that the contents of condensed aromatic components that have 5-37 rings were greater in the older fractions. These results were not contradictory to the larger M_w in older than younger fractions. It was therefore concluded that aromatic components with a larger degree of condensation contributed to the longer residence time of highly-aromatic and darker-coloured HAs.

References

Abe T, Watanabe A (2004) X-ray photoelectron spectroscopy of nitrogen functional groups in soil humic acids. *Soil Science* **169,** 35-43.

Doria KJ, Sachleben JR, Hatcher PG (2002) Solid-state carbon-13 nuclear magnetic resonance of humic acids at high magnetic field strengths. *Journal of Environmental Quality* **31,** 393-401.

Fujimoto H (2003) Theoretical X-ray scattering intensity of carbons with turbostratic stacking and AB stacking structures. *Carbon* **41**, 1585-1592.

Ikeya K, Ishida Y, Ohtani H, Yamamoto S, Watanabe A (2007) Analysis of polynuclear aromatic and aliphatic components in soil humic acids using ruthenium tetroxide oxidation. *European Journal of Soil Science* **58,** 1050-1061.

Kramer RW, Kujawinski EB, Hatcher PG (2004) Identification of black carbon derived structures in a volcanic ash soil humic acid by Fourier transform ion cyclotron resonance mass spectrometry. *Environmental Science and Technology* **38**, 3387-3395.

Kuwatsuka S, Watanabe A, Itoh K, Arai S (1992) Comparison of two methods of preparation of humic and fulvic acids, IHSS method and NAGOYA method. *Soil Science and Plant Nutrition* **38,** 23-30.

Watanabe A, Takada H (2006) Structural stability and natural ^{13}C abundance of humic acids in buried volcanic ash soils. *Soil Science and Plant Nutrition* **52,** 145-153.