

Effects of ecological succession on chemical characteristics of humic and fulvic acids in a Japanese volcanic ash soil.

Yasuo Iimura^A, Mitsuru Hirota^B, Kenji Tamura^B, Teruo Higashi^B, Nobuhide Fujitake^A

^AGraduate School of Agricultural Science, Kobe University, Kobe, Japan, Email fujitake@kobe-u.ac.jp

^BGraduate School of Life and Environmental Science, University of Tsukuba, Tsukuba, Japan.

Abstract

To obtain further details regarding effects of ecological succession on chemical characteristics of humic and fulvic acids in a Japanese volcanic ash soil, humus quantitative analysis, HPSEC, and liquid-state ¹³C NMR analysis were performed with surface mineral soils (0–20 cm). Grassland at site 1 has been maintained by mowing, while the maintenance of sites 2 (pine forest) and 3 (broad-leaved forest) was discontinued approximately 30 and more than 100 years ago, respectively. The concentration of humic acids decreased from 60.4 to 47.4 C g/kg in only 30 years (site 1 vs. site 2), and then decreased from 47.4 to 30.2 C g/kg after 100 years (site 2 vs. site 3). Simultaneously, the concentration of fulvic acids decreased only slightly throughout the series (site 1 to site 3). Physicochemical properties appeared to be affected by succession only in humic acids. In particular, the proportion of aryl C moieties in the humic acid of site 2 decreased from 49.6 to 31.4%, a level similar to that of site 3. The findings of this study clearly demonstrate that humic acids, but not fulvic acids, significantly changed with decreasing aryl C content, over the first 30 years and then remained fairly constant.

Key Words

Secondary succession; Black soil; Stability of soil organic matter; Carbon sequestration.

Introduction

Japanese volcanic ash soils have very thick and dark-colored A horizons with large amounts of humic acids (Wada 1986) which are characterized by their highly aromatic structures and stabilities such as black carbon (Shindo and Honma 2001). Nevertheless, the disappearance of the melanic epipedon with a decreasing aromatic C and increasing alkyl C proportion of humic acids was observed in ecological succession over only 20–30 years in the grassland/forest ecotone of volcanic ash soil in Japan (Golchin *et al.* 1997). However, there is no clear information on how such quantitative changes in each carbon species affect chemical properties of humic substances. To obtain clearer information about this, we therefore aimed: (1) to quantify the time series variation of humic and fulvic acid, dominant portions of soil organic matter; (2) to determine the chemical properties of humic and fulvic acids quantitatively. We investigated the concentration of humic and fulvic acids extracted from volcanic ash soil at the same study site as that of Golchin *et al.* (1997). To obtain the quantitative variations in each carbon species and their effects on the chemical properties of humic and fulvic acids, we performed liquid-state ¹³C nuclear magnetic resonance (¹³C NMR) spectroscopy with the humus quantitative analysis. We also determined the relative molecular weight of each humic and fulvic acids by high-performance size exclusion chromatography (HPSEC).

Materials and Methods

Study area

The study area is situated in the campus of the Sugadaira Montane Research Center (SMRC) of Tsukuba University, Nagano Prefecture, Japan (36°30'N, 128°20'E), at 1320 m above sea level. The mean annual temperature is 6.5°C and the mean annual precipitation is 1190 mm. The initial soil is derived from volcanic ash, classified as Typic Melanudand (USDA Soil Taxonomy). The study sites were managed as grassland for several hundred years. At the site 1, regular mowing has been practiced to maintain the grassland (site area: 6 ha); however, at the site 2, mowing of the grasslands ceased approximately 30 years ago (site area: 8.5 ha), and at the site 3, grassland was invaded by forest at least more than 100 years ago (site area: 14 ha). The dominant vegetative cover was Japanese pampas grass, *Miscanthus sinensis*, at the site 1, *Pinus densiflora* with an understory of *Sasa* spp. at the site 2 and *Quercus crispula* with an understory of *Sasa* spp. at the site 3. Location and present site sketch of the study area are shown in Figure 1.

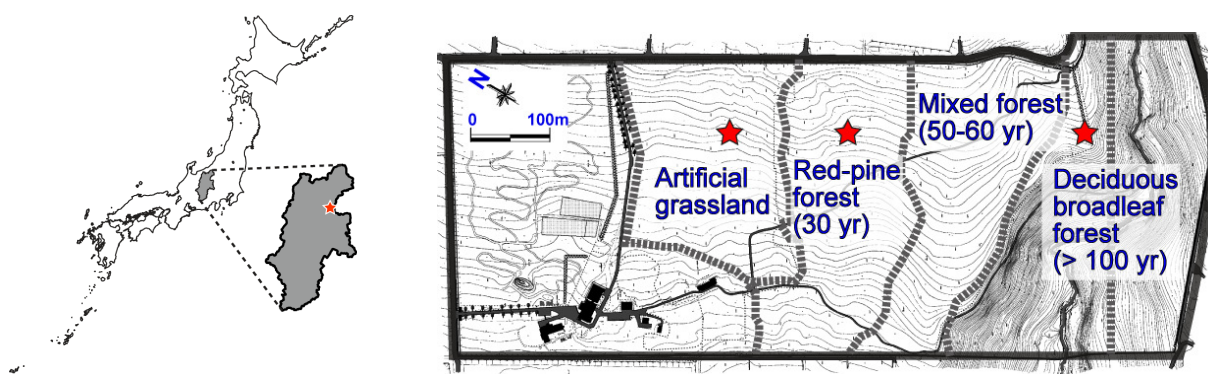


Figure 1. Location and site sketch of the study area.

Preparation of humic and fulvic acids

Soil samples were collected from 0–20 cm surface mineral soil. The amounts of humic and fulvic acids were determined according to the method described by Ikeya and Watanabe (2003). For chemical characteristics, humic and fulvic acids were isolated using standard methods of the international humic substances society (IHSS) with some modifications (Swift 1996; Fujitake and Kawahigashi 1999).

Analysis of humic and fulvic acids

The relative molecular weight of humic and fulvic acids was estimated according to the method of Asakawa *et al.* (2008). Liquid-state ^{13}C NMR spectra were obtained using a Bruker Avance 500 spectrometer with 5 mm diameter sample tubes. Approximately 30–50 mg of the sample was dissolved in 0.4 mL of 0.5 mol/L NaOD in D_2O . Chemical shifts were referenced to sodium 3-trimethylsilylpropionate-2,2,3,3- D_4 . To obtain quantitative conditions for the integration of the ^{13}C NMR spectra, ^{13}C signals were proton-decoupled using the inverse-gated decoupling technique as follows: spectrometer frequency, 125.76 MHz; pulse width, 45° ; acquisition time, 0.839 s. A total repetition time of 2.5 s was used to permit complete relaxation of all the spins. To improve the signal-to-noise ratio, a line broadening of 50 Hz was used. Scans numbering 10,000 to 20,000 were accumulated. Resonance areas were calculated using electronic integration. To obtain quantitative information, the spectra were divided into the following six regions (Fujitake and Kawahigashi 1999): alkyl C (5–50 ppm), O-alkyl C (50–110 ppm), aryl C (110–145 ppm), O-aryl C (145–165 ppm), carboxylic C (165–190 ppm) and carbonyl C (190–220 ppm). Aromaticity proposed by Hatcher *et al.* (1981) was calculated by expressing the aryl and O-aryl C (110–165 ppm) as a percentage of the alkyl, O-alkyl, aryl and O-aryl C (5–165 ppm).

Results and Discussion

Concentration of humic and fulvic acids

The concentration of humic acids in each surface horizon decreased with succession from 60.4 to 30.2 C g/kg throughout the series. The concentration of fulvic acids also decreased from 21.5 to 16.2 C g/kg, but seemed almost unchanged with succession compared to humic acid concentrations. The distribution of humic and fulvic acids is shown in Figure 2. The size of pie charts indicates the relative amount of humic and fulvic acids.

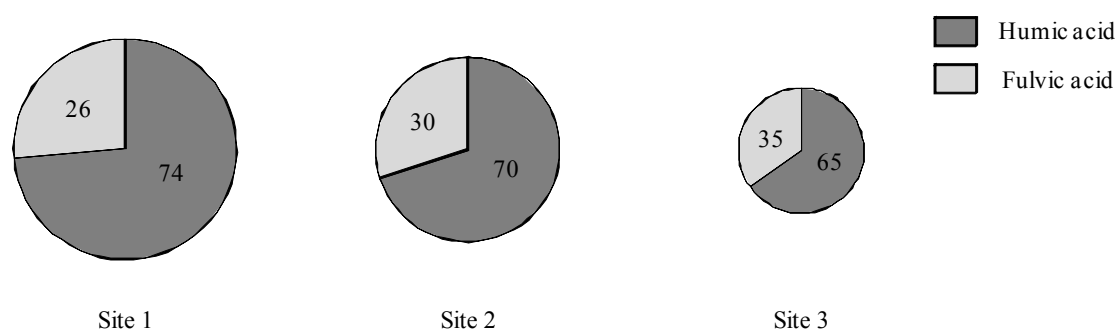


Figure 2. Distribution of humic and fulvic acid of each site.

Table 2. Distribution of carbon species in all humic and fulvic acids determined using liquid-state ^{13}C nuclear magnetic resonance spectroscopy.

	Carbonyl (220-190)	Carboxyl (190-165)	O-Aryl (165-145)	Aryl (145-110)	O-Alkyl (110-45)	Alkyl (45-10)	Aromaticity
Humic acids							
site 1	5.30	19.2	6.90	49.6	13.0	6.00	0.72
site 2	1.36	17.3	6.46	31.4	26.3	17.2	0.42
site 3	2.90	17.9	6.80	35.0	17.9	19.4	0.48
Fulvic acids							
site 1	5.15	18.8	5.54	23.8	29.3	17.4	0.34
site 2	5.62	19.1	6.72	21.1	25.5	21.9	0.31
site 3	5.37	23.3	6.74	21.6	20.5	22.5	0.33

Characteristics of humic and fulvic acids

The weight average molecular weight (M_w) and peak average molecular weight (M_p) of humic acids clearly increased with succession from 5.4 to 12.9 kDa and from 2.9 to 3.6 kDa, respectively. However, the number average molecular weight (M_n) of each humic acid did not parallel the M_w and M_p . In contrast, the M_w and M_p of each fulvic acid did not appear to be correlated with succession. The ^{13}C NMR spectra of all samples showed almost the same peaks in the general chemical shift regions, but the magnitude of the peaks varied especially in humic acids. The distributions of the carbon species of all humic and fulvic acids are shown in Table 2. The proportion of aryl C notably decreased (from 49.6 to 31.4) while O-alkyl C and alkyl C proportion increased (from 13.0 to 26.3 and from 6.0 to 17.2, respectively) from site 1 to 2. There was only a slight difference not only in the aryl C proportion but also aromaticity of humic acids between sites 2 and 3 (from 31.4 to 35.0 and from 0.42 to 0.48, respectively). The changes in the composition of fulvic acids were extremely small compared to humic acids.

Conclusion

In the present study, we obtained important insights into the changes in terms of quantity and quality of humic substances in surface mineral soils (0–20 cm) in the grassland/forest ecotone of volcanic ash soil in Japan, by applying humus quantitative analysis, HPSEC analysis, and liquid-state ^{13}C NMR spectroscopy. The findings of this study clearly demonstrated that the chemical properties of humic acids, but not fulvic acids, significantly changed with decreasing aryl C content during the first 30 years of succession (site 1 vs. site 2) and then showed a continuous, though less dramatic, decrease after 100 years (site 2 vs. site 3).

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