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Formation and mobility of soil organic carbon in a buried humic horizon of a volcanic ash soil

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Abstract

A buried humic horizon (^{14}C age between 5.4 to 6.8 kyr BP) of a volcanic ash soil in Aomori, Japan, which was collected from the depth between 147 and 187 cm at 5 cm-interval (total 8 sub-horizon samples), was investigated to clarify the degree of received biological transformation and mobility of soil organic carbon (SOC) fractions. The SOC fractions were prepared from each sub-horizon samples by extraction and precipitation procedures with controlling pH of the extracted solution, resulting in humin, humic acid (HA), and four fulvic acid (FA) fractions (two hydrophilic FA fractions: FA_1 and FA_2, and two hydrophobic FA fractions: FA_3 and FA_{IHSS}). The prepared SOC fractions were characterized by ^{14}C age and stable isotopic ratios of ^{13}C ($\delta^{13}C$) and ^{15}N ($\delta^{15}N$). The hydrophilic FA fractions showed the highest $\delta^{13}C$ and ^{15}N values, indicating that these SOC fractions had been most enriched with ^{13}C and ^{15}N by biological metabolic processes. On the other hand, the HA fraction showed the lowest $\delta^{13}C$ and $\delta^{15}N$ values, therefore this fraction would have been less-metabolized, although HA fraction has been regarded as well-processed in general. The ^{14}C age of the HA fraction was almost same as the deposition age of the corresponding sub-horizon, indicating that the C in the HA fraction would have been fixed *in situ* right after photosynthesis by plants at the early stage of soil formation and chemically stabilized at soil surface, by fire event, etc. The average rates of vertical translocation of the SOC fractions were low (humin and HA fractions: < 1 mm per century, FA fractions: 1 to 4 mm per century), implying that the vertical translocation of SOC would not be the main mechanisms for forming thick humic horizons. The present study showed strong evidence of the *in situ* formation of SOC on soil surface, and successive up-building accumulation of soil

particles containing SOC would contribute to the formation of the thick humic horizons.

Key words: fulvic acids; humic acid; soil organic carbon; up-building soil formation; $\delta^{13}C$ and $\delta^{15}N$; ^{14}C age

1. Introduction

Volcanic ash soils or Andosols comprise a relatively small area (0.12 billion hectors) among 14 billion hectors of the global terrestrial area (Dahlgren *et al*., 2004), but they contain several times higher concentration of soil organic carbon (SOC) than adjacent non-Andosols (Eswaran *et al*., 1993). It has been estimated that they stored approximately 75 Pg of SOC among 1500 Pg of global SOC in 0 to 100 cm depth of their soil profiles (Dahlgren *et al*., 2004). Therefore, Andosols have been receiving intensive attention as a carbon sink for atmospheric CO_2, and the genesis and accumulation mechanisms of SOC in Andosols have been studied (e.g., Dahlgren *et al*., 2004, etc.).

A soil horizon under thick tephra deposits is suitable for investigating the genesis and accumulation mechanisms of SOC in natural conditions, because the age of C can be determined with ^{14}C dating technique with higher accuracy than recent C and the covering tephra deposits have preserved past conditions from recent anthropogenic influences and external input of recent carbon. Inoue *et al*. (2011) investigated a 40 cm-thick buried humic horizon of an Andosol near Towada volcano, Aomori, Japan, which is interlayered between Towada-Chuseri pumice (5.39 ± 0.14 kyr BP; Hayakawa, 1983) and Towada-Nambu pumice (8.37 ± 0.17 kyr BP; Hayakawa, 1985). They collected 8 sub-horizon samples from the buried humic horizon at 5 cm-interval and investigated ^{14}C age of particulate SOC contained in each of the sub-horizon sample, and clarified up-building pedogenesis of the soil horizon with a rate of ~30 mm per century. In the present study, we further investigated the same sub-horizon samples to clarify the genesis and mobility of SOC fractions in the buried humic horizon by isolating SOC fractions and investigating ^{14}C age and stable isotopic ratio of ^{13}C (δ^{13}C) and ^{15}N (δ^{15}N).

Historically, SOC has been studied by separating into three fractions: humin, humic acid (HA), and fulvic acid (FA) fractions depending on their solubility against acid and alkaline solutions, although this technique has been critically disputed recently (Conte *et al*., 1997 etc.). Humin fraction is insoluble in both acid and alkaline solutions, HA fraction is alkali-soluble and acid-insoluble, and FA fraction is soluble in both acid and alkaline solutions. Because FA fraction has high solubility against water, it would play an important role in forming soluble complexes with metals and hydrophobic organic chemicals (Iimura *et al*., 2012; Maie *et al*., 2004; Stevenson, 1994). The standard method for preparing the FA fraction has been established by International Humic Substance Society (IHSS, http://www.humic-substances.org/) (hereafter referred as FA_{IHSS}), but this procedure isolates a part of hydrophobic FA fractions and most of hydrophilic FA fractions are typically discarded (Hiradate *et al*., 2006). To recover other FA fractions than FA_{IHSS}, Hiradate *et al*. (2006) proposed applying the crude FA fraction on a column filled with hydrophobic resin, eluting the column with water with controlling the pH of the eluent, and precipitating the eluted FA fraction by partial neutralization for preparing hydrophilic (FA_1 and FA_2) and hydrophobic (FA_3 and FA_{IHSS}) FA fractions, and the present study followed this procedure.

The $\delta^{13}C$ and $\delta^{15}N$ values have been used for estimating the source plants (Hiradate *et al.*, 2004; Katsumi *et al.*, 2015; Yoneyama *et al.*, 2001) and the degree of decomposition and humification of SOC (Kramer *et al.*, 2003; Krull *et al.*, 2002; Marin-Spiotta *et al.*, 2009; Panichini *et al.*, 2012). These isotopic ratios serve as indicators of relative degree of microbial processing, because the decomposition and recycling of organic matter by organisms result in enrichment of heavier ^{13}C and ^{15}N stable isotopes due to their preferential stabilizations (Wada *et al.*, 2013). Therefore, the simultaneous increase of $\delta^{13}C$ and $\delta^{15}N$ values in SOC fractions corresponds to the successive transformation of SOC by biological processes among the fractions. Soil humic substances have been assumed to be transformed from plant residues through degradation and polymerization reactions in soils, which would be closely related with biological and enzymatic reactions. Considering from the differences in molecular weight of SOC fractions, Miltner *et al.* (2012) assumed that FAs would be formed first, followed by HA and finally humin, although this has not yet been confirmed with sufficient evidence. In the present study, we determined the $\delta^{13}C$ and $\delta^{15}N$ values of SOC fractions prepared from the buried humic horizon samples to assess the degree of received biological transformation of the SOC fractions.

In addition to the high C content, the thick humic horizon is also the distinct feature of Andosols. Although Inoue *et al.* (2011) have confirmed the up-building formation of the thick buried humic horizon of the Andosol from Aomori, Japan, the accumulation process of the SOM in the horizon has been still unclear whether the SOM had accumulated *in situ* with the deposition of volcanic ash or a part of SOM had been transferred from upper layer. To answer this question, we tried to clarify the mobility of the SOC fractions isolated from the buried humic horizon from the Andosol profile, i.e., the isolated SOC fractions from 8 sub-horizon samples were analyzed with ^{14}C dating technique, and mean vertical translocation rate was estimated for each SOC fraction.

Therefore, the aims of the present study were to clarify the degree of received biological transformation and mobility of SOC fractions in a thick buried humic horizon of a soil derived from volcanic ash in Aomori, Japan, by investigating $\delta^{13}C$ and $\delta^{15}N$ values and ^{14}C dating. Solid-state ^{13}C nuclear magnetic resonance (NMR) spectra were also measured for selected SOC fractions to interpret the formation processes and mobility of them.

2. Materials and Methods

2.1. Study site and soil samples

The soil samples used in the present study were the same as used in Inoue *et al.* (2011), which had been collected from a buried humic horizon derived from a volcanic ash of Towada volcano, Aomori Prefecture, Japan. The buried humic horizon had occurred between 147 and 187 cm depth, which had been packed by overlaid Towada-Chuseri pumice (5.39 ± 0.14 kyr BP; Hayakawa, 1983) and underlaid Towada-Nambu pumice (8.37 ± 0.17 kyr BP; Hayakawa, 1985) (Fig. 1). Eight sub-horizon samples had been collected at 5 cm-interval within the 40 cm-thick of the buried humic horizon. The sub-horizon samples had been air-dried, sieved through a 2-mm mesh, and subjected to further analysis.

2.2. Preparation of SOC fraction

Twenty gram-portion of the powdered sub-horizon sample was extracted with 160 mL of 0.1 M NaOH in the presence of 3% NaCl overnight. The supernatant was obtained by centrifugation (2380 × *g*, 15 min). This extraction procedure was repeated three times, and the supernatant obtained from each extraction was mixed all together. The residue was suspended in a small amount of distilled water, neutralized with 6 M HCl, washed with distilled water at least three times, and freeze-dried (humin fraction).

The mixed supernatant was acidified to pH 1.0 with 4 M HCl and allowed to stand overnight. The precipitated fraction was separated by centrifugation (2380 × *g*, 15 min; crude HA). The supernatant fraction was filtered through a 0.2 μm-pore cellulose acetate membrane filter (47 mm ϕ, Toyo Roshi Kaisha Ltd, Japan) (crude FA).

The crude HA was dissolved in a small amount of alkaline solution of pH 13.0 by adding 4 M NaOH and centrifuged to remove small soil minerals. Then the supernatant was acidified to pH 1.0 by adding 4 M HCl and allowed to stand overnight, and again it was centrifuged to obtain supernatant and precipitate. The supernatant was collected and added to the crude FA. The dissolution-precipitation cycle was repeated five times until final supernatant becomes light yellow in color. The precipitate was re-dissolved in a small amount of NaOH solution and centrifuge (2380 × *g*) for 2 h to remove small soil minerals. Finally, the supernatant was acidified to pH 1.0 by 4 M HCl, washed with distilled water, and freeze-dried (HA fraction).

The crude FA was loaded to a glass column (20 mm of internal diameter, 300 mm of length) filled with approximately 25 mL of Amberlite XAD-8 resin (Rohm and Haas, Philadelphia, PA, USA) which had been washed sequentially with 0.1 M NaOH, 0.1 M HCl, and distilled water. The eluted non-adsorbed fraction was collected and partially neutralized to pH 5.0 with 4 M NaOH. The precipitate was collected by centrifugation (2380 × *g*, 15 min) (FA_1 fraction). The column was eluted with 400% of column volume (100 mL) of 0.1 M HCl, and the eluted fraction was precipitated by adjusting the pH of the solution to 5.0 with 4 M NaOH and collected by centrifugation (2380 × *g*, 15 min) (FA_2 fraction). The column was eluted with 400% of column volume (100 mL) of distilled water, and the eluted fraction was precipitated by adjusting the pH of the solution to 5.0 with 4 M NaOH. The precipitate was collected by centrifugation (2380 × *g*, 15 min) (FA_3 fraction). Finally, the column was eluted with 50 mL of 0.1 M NaOH, and the eluted fraction was passed through an Amberlite IR 120 resin (H^+ form) (FA_{IHSS} fraction). All FA_1, FA_2, FA_3, and FA_{IHSS} fractions were freeze-dried (Hiradate *et al*., 2007).

2.3. Elemental analysis of C and N in SOC fraction

The total C and N contents of each SOC fraction were measured using an elemental analyzer (Vario PYRO cube, Elementar, Germany).

The $\delta^{13}C$ and $\delta^{15}N$ values of each SOC fraction were analyzed using an isotope ratio mass spectrometer (IsoPrime100, Isoprime Ltd., UK) connected with an elemental analyzer (Vario PYRO cube), with an analytical uncertainty of < 0.1 ‰ (1SD).

2.4. ^{14}C dating

The ^{14}C age of the SOC fraction was determined by an accelerator mass spectrometer at Tono Geoscience Center, Japan Atomic Energy Agency (Saito-Kokubu *et al*., 2015). The SOC sample was wrapped in a tin capsule and combusted at 920°C in an elemental analyzer (vario MICRO cube, Elementar). The resulting gas was passed through a reduction column and a separation column to purify CO_2 from the gas. The purified CO_2 was introduced into an automated graphitization equipment (AGE3, Ion plus AG, Switzerland) in which CO_2 was converted to graphite by reducing CO_2 with hydrogen gas at 580°C for 2h in the presence of iron powder as catalyst (Wacker *et al*., 2010). Radiocarbon ^{14}C was then measured on graphite targets. The ^{14}C data was reported as conventional ^{14}C age (yr BP) with an analytical uncertainty of ^{14}C age less than ± 280 yr BP.

2.6. Solid-state ^{13}C NMR measurement

A powdered sample of SOC fraction (ca. 20 mg) was tightly packed into a high-speed spinning NMR tube (made of zirconia, JEOL, Tokyo, Japan) and introduced into a FT NMR system (ECAII-600, JEOL). Solid-state cross polarization magic angle spinning (CPMAS) ^{13}C NMR signals were recorded at 150.9 MHz, with a contact time of 1 ms, an observation band of 90.6 kHz, 1,024 observation points (resolution; 88 Hz), an acquisition time of 11.3 ms, a pulse interval of 3 s, 3,000 - 77,000 scans (analytical time; 2.5 - 75 h), and 15 kHz of magic angle spinning, with ramp technique. A broadening factor of 200 Hz was used in the Fourier transformation procedure. Chemical shifts were quoted with respect to tetramethylsilane (0 ppm) but were determined by referring to an external standard of adamantane (29.50 ppm). The chemical shift regions 0-45, 45-110, 110-165, and 165-190 ppm were assigned to alkyl C, *O*-alkyl C, aromatic C, and carboxyl C, respectively (Hiradate *et al*., 2007).

2.7. Determination of rate of vertical translocation of SOC fraction

The rate of vertical translocation of each SOC fraction was calculated by following equation:

$$\text{Rate of vertical translocation} = \frac{\text{(current depth of SOC fraction- depth of original deposition)}}{^{14}\text{C age of SOC fraction}} \qquad (1)$$

The depth of original deposition has been reported to be closely related with the ^{14}C age of deposition of the corresponding buried sub-horizon, as follows (Inoue *et al.*, 2011):

[depth of original deposition] = 0.0263 [^{14}C age of deposition] + 5.646 (2)

The equation (2) was used to calculate the depth of original deposition for each SOC fraction in equation (1) by substituting [^{14}C age of deposition] with the [^{14}C age of SOC fraction] in the present study.

3. Results and Discussion

3.1. C and N distributions in SOC fractions

In the present study, almost all of the C contained in the original sub-horizon samples were quantitatively recovered in the SOC fractions (total recovered C: 103 ± 9%, Table 1). Among them, 85% of the total SOC were recovered as humin fraction, 5% as HA fraction, 6% as FA_1 fraction, 1% as FA_2 fraction, 2% as FA_3 fraction, and 4% as FA_{IHSS} fraction. The total recovered N was lower than the total recovered C and 89 ± 6%, probably because N containing compounds were more soluble in water and/or easily hydrolyzed into more soluble forms, resulting in the loss in the precipitation procedure.

Major portions of the C and N in the sub-horizon samples were present in the humin fractions, although it has been reported that C is dominant in hydrophilic plus hydrophobic FA fractions in the surface horizons of alluvial soil, calcareous soil, and volcanic ash soil (Hiradate *et al.*, 2007; 2006). The reason of the small proportion of the FA fractions in the present study would be because the FA fractions would had been microbially decomposed and disappeared after burial, resulting in the dominance of C in the humin fraction.

3.2. Degree of received biological transformation of SOC fractions

The δ^{13}C value is affected by both the vegetation (i.e., around -27 ‰ for C_3 plants and around -13 ‰ for C_4 plants: Hiradate *et al.*, 2004; Krull *et al.*, 2002; Yoneyama *et al.*, 2001) and the degree of received biological transformation (Katsumi *et al.*, 2015; Wada *et al.*, 2013). The δ^{15}N value is known to increase with received biological transformation (Wada *et al.*, 2013). In the present study, both the δ^{13}C and δ^{15}N values were investigated to access the degree of received biological transformation of the SOC fractions.

Averaged δ^{13}C and δ^{15}N values of the SOC fractions were listed in Table 2. The δ^{13}C and δ^{15}N values were strongly dependent on the SOC fractions; relatively high values were observed in hydrophilic FA fractions and the lowest values in HA fractions. In addition, the δ^{13}C values of the SOC fractions were related with the δ^{15}N values (Fig. 2). The slope of the relationship between the δ^{13}C and δ^{15}N values in the present study was 1.60. Wada *et al.* (2013) reported that the enrichment of δ^{13}C and δ^{15}N in organic matter occurs during the decomposition and recycling of plant residues with the slope of 1.61, which is almost the same value obtained in the present study. Therefore, the changes in the δ^{13}C and δ^{15}N values would be strongly affected by biological metabolisms, and the degree of received biological transformation of the SOC fractions prepared in the present study would be in the following order: FA_1 and FA_2 fractions > FA_3, FA_{IHSS} and humin fractions > HA fraction.

The C/N ratios of the SOC fractions also supported the above discussion on the degree of received biological transformation. The C/N ratio has been reported to decrease with biological transformation (Mcgill and Cole, 1981), and the highest C/N ratio was recorded for HA fractions, followed by humin and hydrophobic FA (FA_3 and FA_{IHSS}) fractions and hydrophilic FA (FA_1 and FA_2) fractions (Table 3).

It has long been considered that HA fraction is formed at the final stage of SOC formation, but this would not be applicable to the SOC of the present study. It was also clarified that the hydrophilic FA_1 and FA_2 fractions were the most metabolized SOC fractions. The averaged $\delta^{13}C$ values of FA_1 and FA_2 fractions were 2.9 and 2.6 ‰ higher than that of HA fraction, and those $\delta^{15}N$ values were 4.6 and 3.0 ‰ higher than that of HA fraction, respectively. Based on the result of trophic fractionation ratios (Kramer *et al.*, 2003 and Wynm, 2007), the FA_1 and FA_2 fractions would be biologically metabolized more 2 to 3 times than the HA fraction on average basis. The increases in the averaged $\delta^{13}C$ values from HA fraction for FA_3 and FA_{IHSS} fractions were 1.0 and 0.5 ‰, respectively, and the increases in the averaged $\delta^{15}N$ values were 2.5 and 2.1 ‰, respectively. Therefore, the hydrophobic FA_3 and FA_{IHSS} fractions would also be more biologically metabolized than HA fraction, but the average number of metabolic turnover would be less than that of the hydrophilic FA fractions.

If these SOC fractions had been sequentially formed one after another by only biological metabolism, it would be reasonable to conclude that the HA and hydrophilic FA fractions would be the initial and final product, respectively. However, from the view point of chemical composition of the SOC fractions (Table 4 and Fig. 3), the HA fractions would not be the first product from plants by the biological metabolisms, because the chemical structure of the HA fractions was quite different from plant materials: the HA fraction was quite rich in aromatic C, although plants are composed primarily of *O*-alkyl C (cellulose-rich structure). Therefore, it would be reasonable to postulate that the HA fraction had been formed by carbonization (charred process) under the influence of high temperature at the early stage of SOM genesis and preserved in the buried horizon for a long time because of the high stability of the aromatic C.

In the present study, the degree of received biological transformation of the SOC fractions was clarified for a buried humic horizon of an Andosol. It would be important to further clarify whether this finding can be applicable to the other soils. In addition, it would be notable that the chemical fractionation procedure was able to isolated SOC fractions which have different history of genesis and biological transformation. The isolation procedure used in the present study would be applicable to the other soils for the study of the SOC formation and genesis.

3.3. Vertical translocation of SOC fractions

The ^{14}C ages of the SOC fractions prepared from the buried humic sub-horizons in the present study were in the range between 4.6 and 6.6 kyr BP (Fig. 4). Because the buried horizon was overlaid by Towada-Chuseri pumice (5.39 ± 0.14 kyr BP; Hayakawa, 1983), the SOC fractions younger than 5.39 kyr BP would be affected by SOC supplied through the overlying Towada-Chuseri pumice. Such SOC fractions were as follows: FA$_1$ fractions from 1st and 3rd sub-horizons, FA$_2$ fractions from 1st, 3rd, and 5th sub-horizons (no ^{14}C age data for 2nd and 4th sub-horizons), FA$_3$ fractions from 1st to 3rd sub-horizons, and FA$_{IHSS}$ fractions from 2nd sub-horizon. The FA fractions would have higher rate of vertical translocation in the soil profile than the HA and humin fractions.

The ^{14}C ages of the SOC fractions prepared in the present study were plotted against the sampling depths and compared with the deposition ages of corresponding buried humic sub-horizons (Inoue *et al.*, 2011) (Fig. 4). It has been clarified that the sub-horizons had been formed by up-building pedogenesis with a constant rate of 30 mm per century (Inoue *et al*., 2011). Therefore, the difference in soil depth between the SOC fraction and the corresponding original deposition position at same ^{14}C age can be regarded as averaged vertical translocation distance during the mean resident time (^{14}C age). The calculated average rates of vertical translocation for humin and HA fractions in the buried humic layer were both < 1 mm per century, while those for FA_1 to FA_3 fractions and for FA_{IHSS} fraction were 2 to 4 and 1 mm per century, respectively (Table 5). The low rates of the vertical translocation of the SOC fractions in the present study imply that they would be poorly translocated downwards and the thick humic horizon would not be formed by the vertical translocation of SOC.

The ^{14}C ages of the HA fractions were almost the same as those of the deposition ages for all the sub-horizons, indicating that the C in the HA fraction would have been fixed right after photosynthesis by plants at the early stage of soil formation. Kristiansen *et al*. (2003) have studied the ^{14}C age of SOC fractions in prehistorical burial mounds and observed the oldest ages for HA fraction. Similarly, Tonneijck *et al*. (2006) also reported the oldest age for HA fractions in Andosols from Northern Ecuador. These studies were consistent with the present study.

The ^{14}C age of the humin fraction was also close to the deposition age of the corresponding sub-horizon, although it fluctuated in some cases. Humin fraction is the most insoluble SOC fraction against aqueous solution, and it may contain non-humic substances, such as plant residues which could give younger ^{14}C age. Therefore, thorough purification procedure should be applied for preparing sample to determine the ^{14}C age of well humified humin fraction. Although the humin fractions in the present study were prepared with conventional method, they were old comparable with the deposition age of the sub-horizon, meaning that they would not contain significant amount of non-humic substances probably because of the microbial decomposition of non-humic substances during the long burial time of > 5000 years. It was also clarified that the humin fractions were poorly translocated downwards in the Andosol profile.

All the FA fractions were younger than the humin and HA fractions and the deposition ages in the corresponding sub-horizon samples. The FA fractions would easily capture metabolites from plant materials, which would be supplied by leaching through the soil profile (Pessenda *et al*., 2001; Tonneijck *et al*., 2006). Kristiansen *et al*. (2003) also defined FA as a broad group of organic acid which is mobile in soils. Although the FA fractions were the most mobile SOC fractions prepared in the present study, their rates of vertical translocation were not high enough to form the thick humic horizon storing significant amount of SOC in several millennia order.

3.4. Chemical compositional feature of SOC fractions and their relationships with the behavior in soils

The solubility of SOC fractions in water is primarily important to understand and estimate their downward movements in soils. It has been clarified that hydrophilic SOC molecules rich in *O*-alkyl C tend to remain dissolved, while hydrophobic SOC molecules rich in aromatic and aliphatic C are preferentially retained in soils (Anderson *et al*., 1999; Kaiser *et al*., 2004; 2002, Nilsson *et al*., 2001). It would be important to confirm the chemical compositional feature of the SOC fractions to interpret their behaviors in soils.

In the present study, we analyzed the chemical compositional feature of representative SOC fractions with solid-state CPMAS ^{13}C NMR. The SOC fractions were prepared based on chemical procedure, therefore the chemical compositional feature of the resulted SOC fractions prepared by the same procedure should be similar among the different sub-horizon samples. In fact, the standard deviation values of C distribution (Table 4) were all small, and the ^{13}C NMR spectra of HA and FA fractions in the present study (Fig. 3) were very close to those of corresponding fractions prepared with the same procedure from a different soil sample in the previous study (Hiradate *et al.*, 2007).

The ^{13}C NMR spectra of the isolated SOC fractions (Fig. 3) indicated that the FA_1 fraction was characterized by high proportion of hydrophilic *O*-alkyl C (70 ± 5 %) and low proportion of aromatic C (3 ± 1 %) (Table 4), indicating that the FA_1 fraction was rich in sugar moieties and peptide-like structures which could be easily biologically metabolized. The hydrophilic nature of the FA_1 fraction would also be responsible for the high solubility in water and relatively high mobility in the soil horizon.

In the FA_{IHSS} fraction, the higher proportion of aromatic C (20 ± 3 %) and alkyl C (44 ± 6 %) would lead to lower rate of metabolic turnover and lower mobility in the soil horizon than FA_1. For the FA_2 and FA_3 fractions, their chemical compositional features were intermediate between those of FA_1 and FA_{IHSS}, and they were in good agreement with their behavior in the soil horizon.

In the HA fraction, aromatic C (52 ± 2 %) was very rich, resulting in high aromaticity (0.56 ± 0.03) (Table 4). Such chemical compositional feature of the HA fraction in the present study was consistent with that of well-humified (A-type) HA fractions reported by Maie *et al.* (2002) and Watanabe and Fujitake (2008). Watanabe and Fujitake (2008) suggested that aromatic C contained in HA fraction would cause slow degradation of the HA molecules. Tonneijck *et al.* (2006) also reported that the HA fraction can be retained on the soil minerals of Andosols by forming stable complexes and becomes less mobile downwards. The high proportion of aromatic C of the HA fraction in the present study would lead to low solubility in water and low mobility in soil profiles, therefore such molecules would not take part in dissolved organic C transported into underlying soil layers.

As described above, the chemical compositional feature of the SOC fractions prepared in the present study reasonably explained their chemical and biological behaviors in the soil horizon. The hydrophilic FA fraction was the most mobile fraction, followed by the hydrophobic FA fraction, and the HA fraction, although all of the mobility would not be high enough to explain the formation of the 40 cm-thick of the humic horizon in several millennia. Successive up-building accumulation of soil particles containing SOC would contribute to the formation of the thick humic horizons.

3.5. Genesis of HA fractions in the present study

The genesis of HA fraction has long been discussed especially focusing on the origin of the aromatic C. As sources of the aromatic C, monocyclic benzene rings (e.g., lignins and tannins: Stevenson, 1994, etc.) and polycyclic benzene rings (e.g., charred plant materials with graphite-like structure: Golchin *et al.*, 1994; Shindo *et al.*, 2004, etc.) have been proposed. The latter source would reasonably explain the low $\delta^{13}C$ and $\delta^{15}N$ values of the HA fractions of the present study, because charring reaction does not require biological processes. The charring reaction would also explain the rapid formation of the HA fraction right after photosynthesis. The HA fractions in the present study could be derived through charcoal or charred plant fragments which had been formed by fire event, etc., at the early stage of soil formation.

4. Conclusion

In the present study, SOC fractions were prepared from eight sub-horizons of a buried humic horizon of an Andosol by employing chemical fractionation procedures, resulting in humin, HA, FA_1, FA_2, FA_3, and FA_{IHSS} fractions. It was clarified that the SOC fractions had received different degree of biological transformation, and the mobility of them in the soil profile were different depending on their chemical compositional features. The FA_1 fraction was the most severely biologically transformed SOC fraction among the prepared fractions in the present study, and the mobility of FA_1 to FA_3 in the soil profile was the highest and 2 to 4 mm per century. The HA fraction had received less biological transformation, and its mobility in the soil profile was limited to < 1 mm per century. Judging from the information on the genesis and mobility, the HA fraction was considered to be formed at the early stage of soil formation and chemically stabilized in the soil horizon. Fire event could be a possible formation mechanism responsible for the high aromaticity of the HA fraction. For FA_2, FA_3, and FA_{IHSS} fractions, the degree of biological processes received was intermediate between the HA and FA_1 fractions. The mobility of the prepared SOC fractions was well-explained by the chemical compositional feature determined by solid-state CPMAS ^{13}C NMR spectra. Because of the low mobility of all of the SOC fractions, the vertical translocation of SOC would not be the main mechanisms for forming the 40 cm-thick humic horizons, and successive up-building accumulation of soil particles containing SOC would contribute to the formation of the thick humic horizons.

Declarations of interest: none

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Figure captions

Fig. 1. Location of sampling site illustrated on isopach map of To-Cu and To-Nb tephras based on Machida and Arai (2003) (a) and sampling position in the soil profile (b). To-a: Towada-a, To-b: Towada-b, To-Cu: Towada-Chuseri, To-Nb: Towada-Nambu. The deposition ages of To-a* and To-b** are calculated ^{14}C ages and cited from Machida *et al.* (1981) and Oike and Shoji (1974), respectively. The deposition ages of To-Cu*** and To-Nb*** are non-calculated ^{14}C ages and cited from Hayakawa (1983;1985).

Fig. 2. Relationship between $\delta^{13}C$ and $\delta^{15}N$ values of humin, humic acid (HA), hydrophilic fulvic acid (FA_1 and FA_2), and hydrophobic fulvic acid (FA_3 and FA_{IHSS}) fractions prepared from eight sub-horizon samples from a buried humic horizon occurred between 147 and 187 cm depth of an Andosol near Towada volcano. The $\delta^{13}C$ and $\delta^{15}N$ values of FA_2 (152 - 157 cm and 162 - 167 cm depths) and FA_{IHSS} (172 - 177 cm depth) were not detected due to low yield.

Fig. 3. Solid-state cross polarization magic angle spinning ^{13}C nuclear magnetic resonance spectra of hydrophilic fulvic acid (FA_1 and FA_2), hydrophobic fulvic acid (FA_3 and FA_{IHSS}), and humic acid (HA) fractions prepared from a sub-horizon sample between 162 and 167 cm depth from a buried humic horizon occurred between 147 and 187 cm depth of an Andosol near Towada volcano.

Fig. 4. Comparison between deposition age of sub-horizon (●: Inoue *et al.*, 2011) and ^{14}C age (○) of (a) humin, (b) humic acid (HA), (c and d) hydrophilic fulvic acid (c:

FA_1, d: FA_2), and (e and f) hydrophobic fulvic acid (e: FA_3, f: FA_{IHSS}) fractions prepared from eight sub-horizon samples from a buried humic horizon occurred between 147 and 187 cm depth of an Andosol near Towada volcano. Error bars indicate the uncertainty (2σ) of the corresponding ^{14}C age determination. The ^{14}C age of FA_2 from the 2nd and 4th sub-horizons (152 - 157 cm and 162 - 167 cm depths) and FA_{IHSS} from the 6th sub-horizon (172 - 177 cm depth) was not detected due to low yield.

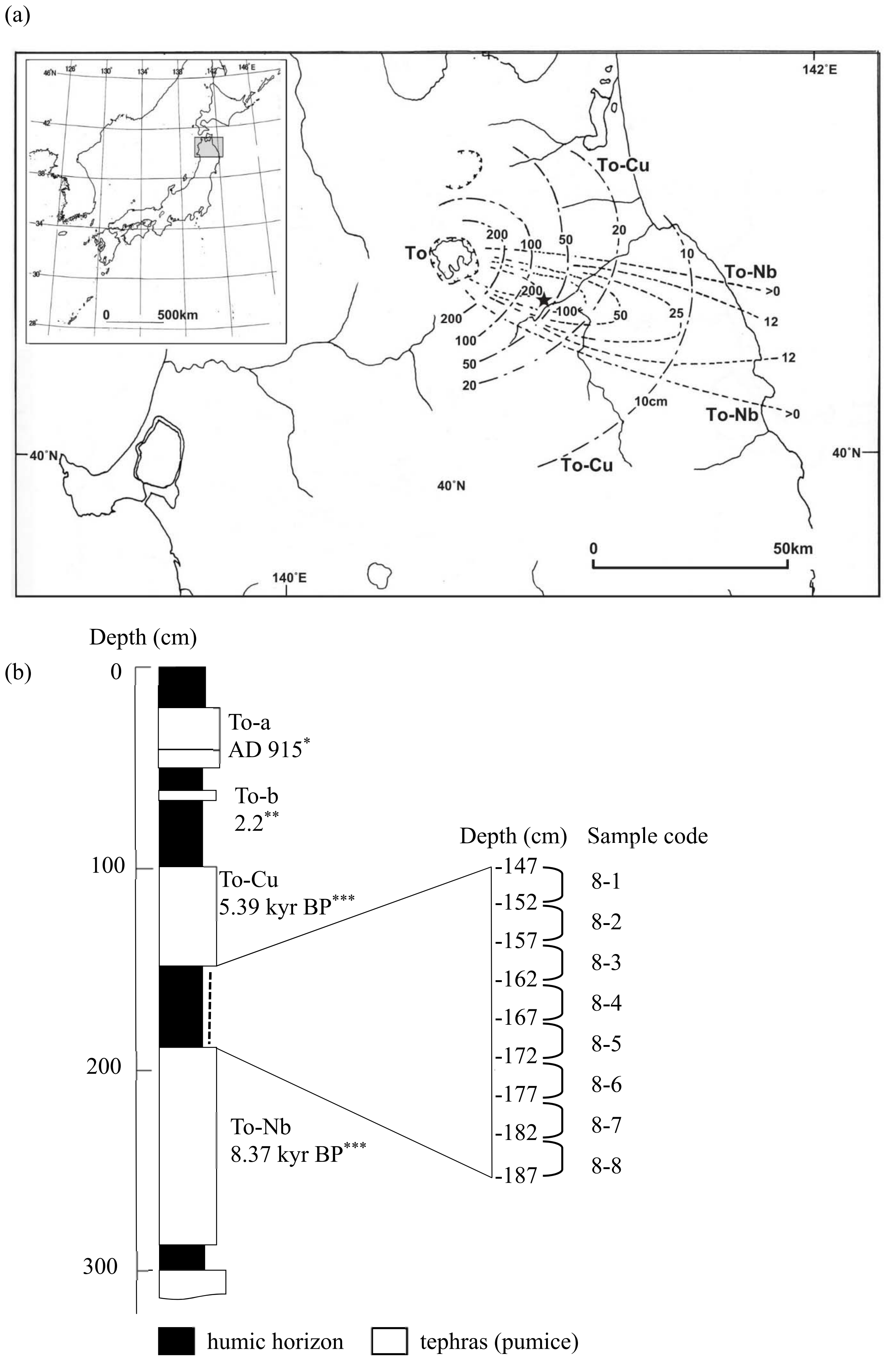

Fig.1. Wijesinghe *et al*., 2019

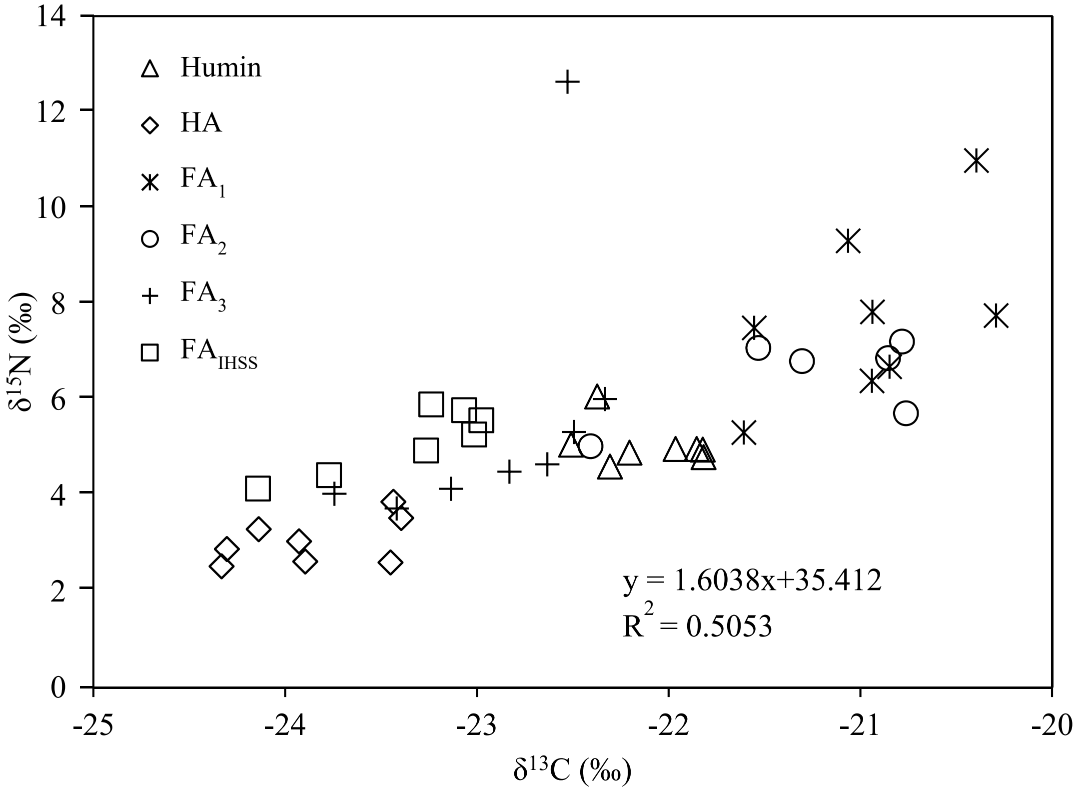

Fig. 2. Wijesinghe *et al.*, 2019

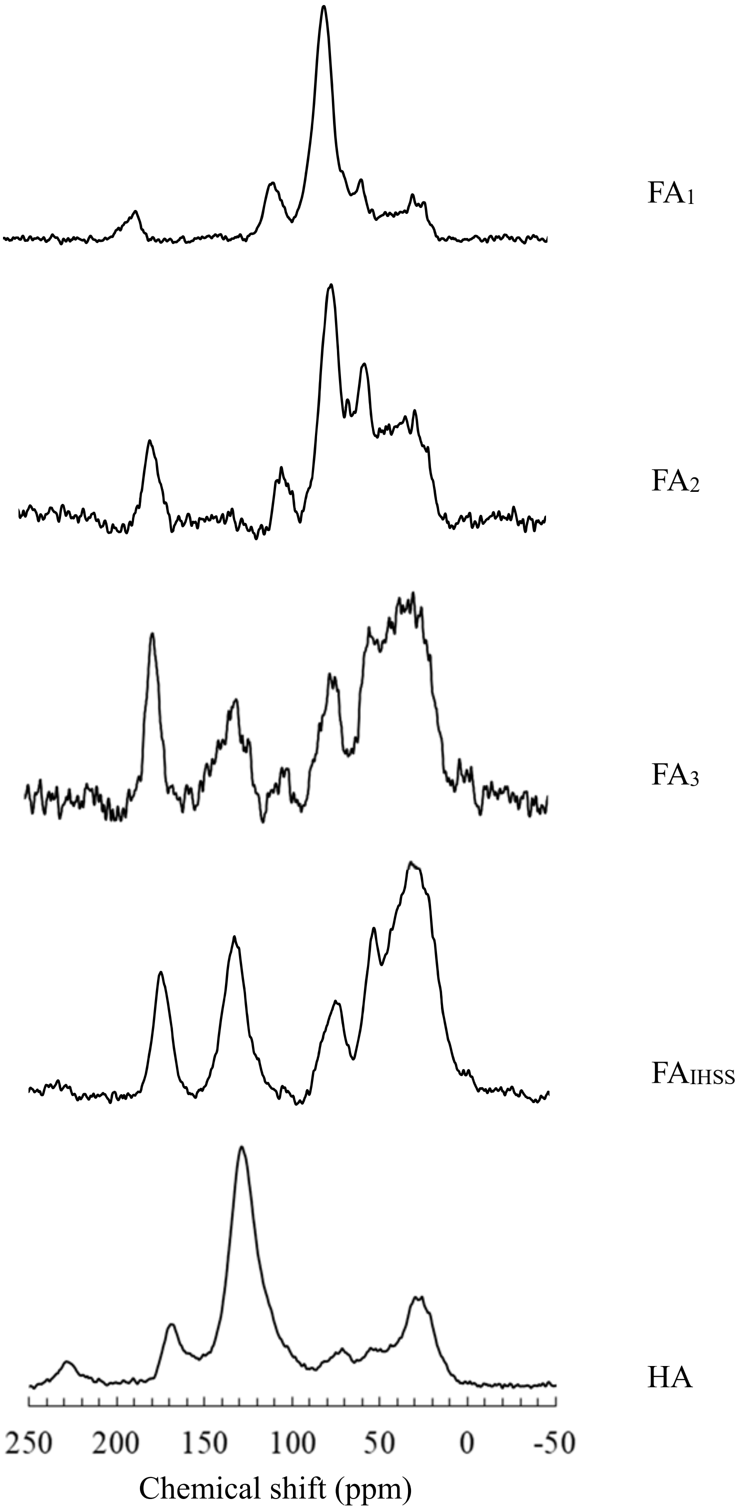

Fig. 3. Wijesinghe *et al*., 2019

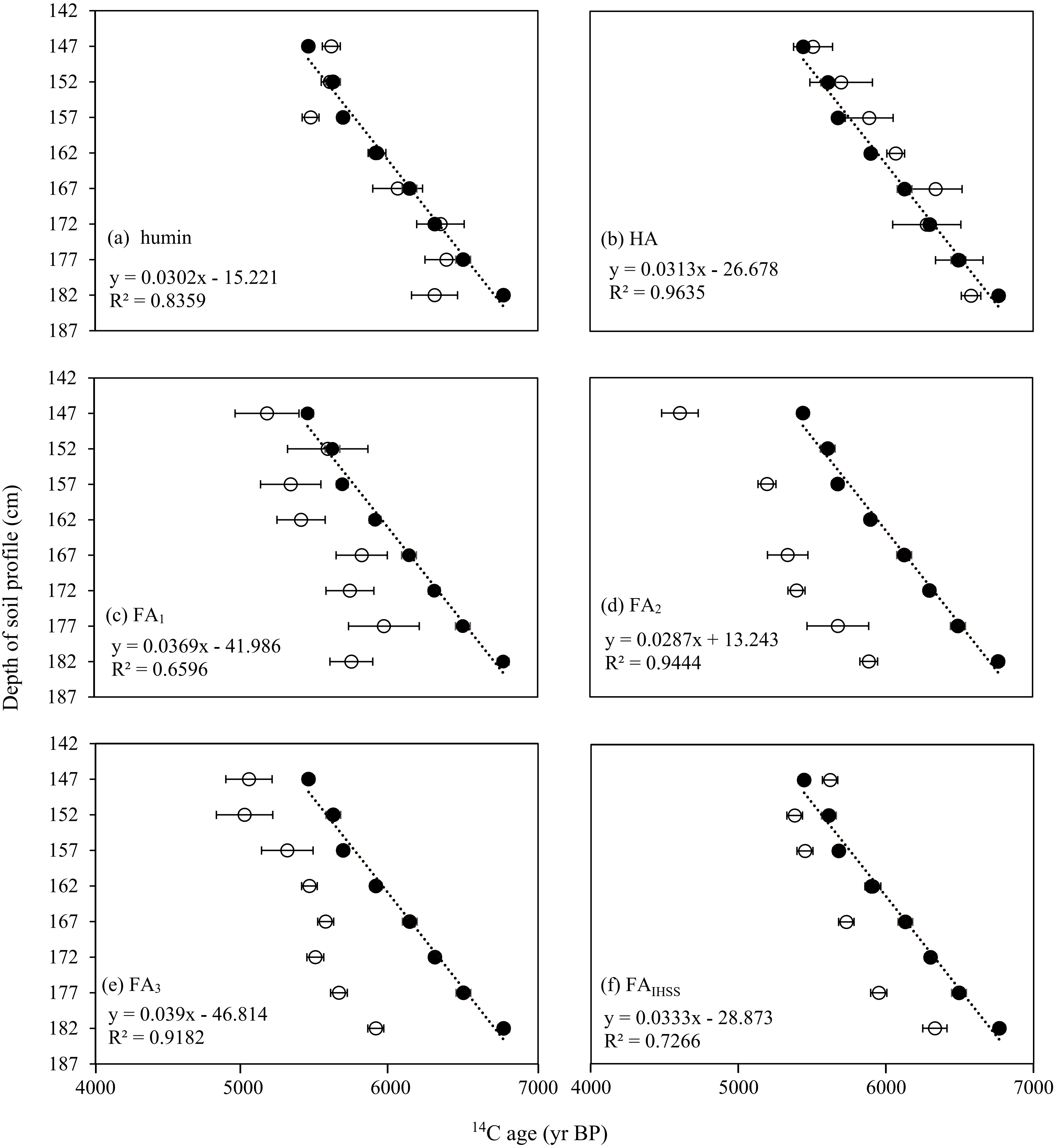

Fig. 4. Wijesinghe *et al*., 2019

Table 1. Averaged C and N recovery of humin, humic acid (HA), hydrophilic fulvic acid (FA_1 and FA_2), and hydrophobic fulvic acid (FA_3 and FA_{IHSS}) fractions prepared from eight sub-horizon samples from a buried humic horizon occurred between 147 and 187 cm depth of an Andosol near Towada volcano.

SOC fraction	C recovery (%)	N recovery (%)
Humin	85.4 ± 11.1	74.1 ± 7.2
HA	4.9 ± 1.2	3.3 ± 1.2
FA_1	5.7 ± 0.8	5.6 ± 0.9
FA_2	1.1 ± 1.1	1.3 ± 1.3
FA_3	1.9 ± 0.8	2.3 ± 1.5
FA_{IHSS}	3.7 ± 2.0	2.0 ± 1.5
mean ± SD	102.7 ± 8.8	88.5 ± 5.9

Table 2. Averaged $\delta^{13}C$ and $\delta^{15}N$ values of humin, humic acid (HA), hydrophilic fulvic acid (FA_1 and FA_2), and hydrophobic fulvic acid (FA_3 and FA_{IHSS}) fractions prepared from eight sub-horizon samples* from a buried humic horizon occurred between 147 and 187 cm depth of an Andosol near Towada volcano.

SOC fraction	$\delta^{13}C$‰	$\delta^{15}N$‰
humin	-22.1 ± 0.3	5.0 ± 0.4
HA	-23.9 ± 0.4	3.1 ± 0.5
FA_1	-21.0 ± 0.5	7.7 ± 1.8
FA_2	-21.3 ± 0.6	6.1 ± 1.0
FA_3	-22.9 ± 0.5	5.6 ± 2.7
FA_{IHSS}	-23.4 ± 0.4	5.2 ± 0.7

*: The $\delta^{13}C$ and $\delta^{15}N$ values of FA_2 (152 - 157 cm and 162 - 167 cm depths) and FA_{IHSS} (172 - 177 cm depth) were not detected due to low yield.

Table 3. The C/N ratio of humin, humic acid (HA), hydrophilic fulvic acid (FA_1 and FA_2), and hydrophobic fulvic acid (FA_3 and FA_{IHSS}) fractions prepared from eight sub-horizon samples from a buried humic horizon occurred between 147 and 187 cm depth of an Andosol near Towada volcano.

Sample code	Sampling depth (cm)	C/N					
		humin	HA	FA_1	FA_2	FA_3	FA_{IHSS}
8-1	147-152	14.3	18.9	12.8	9.4	10.1	13.9
8-2	152-157	16.4	18.9	13.6	-*	12.4	14.9
8-3	157-162	14.4	18.0	12.0	9.0	13.5	14.7
8-4	162-167	14.7	18.2	13.1	-*	13.3	15.7
8-5	167-172	15.0	17.2	13.3	10.2	13.9	16.2
8-6	172-177	14.9	17.4	14.4	9.4	14.6	-*
8-7	177-182	14.5	17.5	13.7	10.0	14.4	16.7
8-8	182-187	14.7	17.7	11.7	12.6	14.4	17.4
mean ± SD		14.9 ± 0.7	18.0 ± 0.6	13.1 ± 0.9	10.1 ± 1.3	13.3 ± 1.5	15.7 ± 1.2

-*: The C/N ratio of FA_2 (152 - 157 cm and 162 - 167 cm depths) and FA_{IHSS} (172 - 177 cm depth) was not detected due to low yield.

Table 4. Distribution of carbon species and aromaticity values of humin, humic acid (HA), hydrophilic fulvic acid (FA_1 and FA_2), and hydrophobic fulvic acid (FA_3 and FA_{IHSS}) fractions prepared from four sub-horizon samples (147 - 152, 162 - 167, 172 - 177, and 182 - 187 cm depths) from a buried humic horizon occurred between 147 and 187 cm depth of an Andosol near Towada volcano.

Fine fractionated organic C	C species (%)				Aromaticity[a]
	Carboxyl C (165 - 190 ppm)	Aromatic C (110 - 165 ppm)	*O*-alkyl C (45 - 110 ppm)	Alkyl C (0 - 45 ppm)	
humin[*]	9.9	19.5	20.7	41.5	0.24
HA	5.2 ± 1.0	52.2 ± 2.0	20.7 ± 1.4	21.0 ± 1.4	0.56 ± 0.03
FA_1	7.4 ± 3.0	3.1 ± 1.0	70.0 ± 4.9	18.2 ± 1.9	0.04 ± 0.01
FA_2	10.1 ± 1.3	4.2 ± 1.4	56.3 ± 2.1	27.2 ± 0.8	0.05 ± 0.02
FA_3[*]	11.8	14.0	32.0	41.6	0.16
FA_{IHSS}	10.2 ± 3.7	20.0 ± 3.3	25.4 ± 2.0	44.0 ± 5.7	0.23 ± 0.04

[a]: aromatic C/ (aromatic C+ *O*-alkyl C+ alkyl C).

[*]: The data was from one sub-horizon sample occurred between 162 and 167 cm depth.

Table 5. The rate of vertical translocation of humin, humic acid (HA), hydrophilic fulvic acid (FA$_1$ and FA$_2$), and hydrophobic fulvic acid (FA$_3$ and FA$_{\mathrm{IHSS}}$) fractions prepared from eight sub-horizon samples from a buried humic horizon occurred between 147 and 187 cm depth of an Andosol near Towada volcano.

Sampling depth (cm)	Rate of vertical translocation (mm/century)[a]					
	humin	HA	FA$_1$	FA$_2$	FA$_3$	FA$_{\mathrm{IHSS}}$
147-152	0.0	0.0	**	**	**	0.0
152-157	0.0	0.0	0.0	-*	**	**
157-162	1.4	0.0	**	**	**	1.5
162-167	0.2	0.0	2.7	-*	2.4	0.2
167-172	0.4	0.0	1.5	**	2.7	1.9
172-177	0.0	0.2	2.7	4.5	4.0	-*
177-182	0.6	0.1	2.5	3.9	4.0	2.5
182-187	1.7	0.5	4.4	3.6	3.6	1.6
average	0.5	0.1	2.3	3.8	3.3	1.3

The minimum rate of vertical translocation was assumed to be zero.

[a]: (current depth of SOC fraction – depth of original deposition)/^{14}C age of SOC fraction.

*: The rates of vertical translocation of FA$_2$ (152 - 157 cm and 162 - 167 cm depths) and FA$_{\mathrm{IHSS}}$ (172 - 177 cm depth) were not calculated due to low yield.

**: SOC fraction younger than the overlaid Towada-Chuseri pumice (5.39 ± 0.14 kyr BP) was not considered in the calculation.