High-heat Effects on the Physical and Chemical Properties of Soil Organic Matter and Its Water-soluble Components in Japan’s Forests: A Comprehensive Approach Using Multiple Analytical Methods

Kazuto SAZAWA,*† Tomoyuki SUGANO,* and Hideki KURAMITZ*†

*Department of Environmental Biology and Chemistry, Graduate School of Science and Engineering, University of Toyama, 3190 Gofuku, Toyama, 930-8555, Japan

† To whom correspondence should be addressed.

E-mail: sazawa@sci.u-toyama.ac.jp; kuramitz@sci.u-toyama.ac.jp
Abstract
Wildfires that expose the soil organic layer to high heat levels can alter soil organic matter (SOM), which includes water-soluble components (WSOM), properties. Various evaluation methods were used to characterize and quantify the effects of high heat levels on SOM and WSOM including ion chromatography, thermogravimetry-differential thermal analysis (TG-DTA), colorimetry, elemental analysis, pyrolysis-gas chromatography-mass spectrometry using tetramethylammonium hydroxide (TMAH-py-GC/MS), total organic carbon (TOC) analysis, three-dimensional excitation-emission matrix (3DEEM) spectroscopy, and high-performance size-exclusion chromatography. In this study, we applied each of these evaluation methods using soil samples that were collected from broadleaf, coniferous, and bamboo forests and peatland in Japan and exposed to different initial high heat levels. Based on the TG-DTA results, the remaining mass in select soil samples markedly decreased when reheated to approximately 200 °C. Comparatively, the TMAH-py-GC/MS results indicated a drastic change in SOM composition and the production of low molecular organic components (< C\textsubscript{10}) at this temperature. The TOC analysis results also indicated a significant increase in WSOM proportion. Colorimetry and elemental analysis results designated that the soil color was dependent upon the initial heating temperature and was related to the H/C and O/C atomic ratios. The results of this study can form the basis for future similar studies for accurately characterizing and quantifying the heat effects on soil, and the effects of increasing wildfires due to climate change.

Keywords: Wildfires, soil organic matter, elemental analysis, TG-DTA, TMAH-py-GC/MS, 3DEEM spectroscopy
Introduction

Soil organic matter (SOM) is the largest carbon reservoir in terrestrial ecosystems.\(^1\) It comprises bulky organic matters (e.g., leaf, branch, and wood debris), degradable organic components (e.g., lipids, carbohydrates, amino acids, and proteins), and humic substances (e.g., fulvic acid, humic acid, and humin). Despite being only a few centimeters to several meters thick on the land surface, the soil organic layer plays a critical role in the carbon cycle of ecosystems. Many factors can affect the quantity and quality of SOM, including temperature, moisture content, vegetation, and microbial communities.

Soil organic matter includes water-soluble components (WSOM) formed by the decomposition of plant debris, microbial substances, and humic substances. The low molecular organic components in WSOM degrade rapidly (in approximately 2–5 d) and contribute to increased plant and microbial activity in a soil environment.\(^2\) Humic substances, such as fulvic and humic acids, constitute a high proportion of the WSOM. And, they play a critical role in facilitating the transportation of metals and nutrients during the carbon cycle.\(^3\) Therefore, although the proportion of water-soluble components in SOM is usually very low, the importance of understanding the chemical properties of WSOM is high.

Wildfires in the world’s forests pose a risk to SOM and present broader environmental challenges. Sizeable fire events have occurred in boreal, Mediterranean, and tropical forest regions around the world. Between 2000 and 2016, the average annual burned area on the earth (as detected by satellite imagery) was estimated to be approximately 340 million hectares. According to previous reports, the global wildfire potential, severity, and season length are predicted to increase under the combination of climate warming and drying.\(^4,5\) *Life on Land*, which is the one of the sustainable development goals (SDGs) adopted by the member states of the United Nations,\(^6\) is strongly focused on protecting forests as a means to strengthen natural resource management and increase land productivity.
Severe wildfires not only burn down a forest’s trees and ground-layer vegetation but also expose its soil organic layer to high heat levels that can alter SOM properties. Previous studies have found that fire events cause the release of large amounts of CO$_2$ into the atmosphere$^{7,8}$ and change the physical, chemical, and biological properties of the soil.$^{1,9-16}$ A comprehensive understanding of wildfires and associated high-heat effects on soil properties is thus important for managing global-scale material circulation and protecting terrestrial ecosystems.

The extent of wildfire effects on the soil environment depends on many factors, including temperature, moisture content, SOM particle size, and organic substance origins. Among these, heating temperature has emerged as an important factor for assessing the effects of wildfires on the soil properties.$^{1,11,12,14}$ Select studies have evaluated the effects of different fire intensities on a soil’s biological and chemical properties using laboratory heating experiments.$^{13,14,17-19}$

Generally, black carbon (char) can be produced by the incomplete combustion (charring) of woody residues. In terms of spectroscopic properties, charring results in loss of the O-alkyl and di-O-alkyl structures that dominate wood and a large increase in aromatic C.$^{11-14}$ On the other hand, the reports on the changes in properties of WSOM caused by the wildfires and laboratory heating are still scarce. Our previous study using peat soil collected from Indonesia revealed that the denaturation and leaching of SOM were caused by heat levels exceeding the ignition temperature.$^{20}$ Additional laboratory studies have been performed with a focus on SOM in forest and peat soils using samples from historic wildfire hotspots (e.g., Alaskan boreal forests, Spanish forests, and Indonesian tropical peatlands), which have been identified as significant sources of CO$_2$ emission.$^{7,8,13}$ In addition to these historic hotspots, wildfire frequencies in humid, subtropical climate regions, which include the forest areas in Japan, are predicted to increase during the 21st century.$^4$ Because previous research has been limited in geographic scope (focusing on historic wildfire hotspots), little is known about high-heat effects on soil properties in Japan.$^{17-19}$
To address this dearth of knowledge, the objective of this study was to apply various evaluation methods for determining high-heat effects on the physical and chemical properties of SOM and its WSOM using soil samples from Japan’s forests. Soil samples were collected from broadleaf, coniferous, and bamboo forests and peatland in Japan and exposed to different high heat levels (100–500 °C). Properties of the thermally treated soil samples were characterized using ion chromatography, thermogravimetry-differential thermal analysis (TG-DTA), colorimetry, elemental analysis, and pyrolysis-gas chromatography-mass spectrometry using tetramethylammonium hydroxide (TMAH-py-GC/MS). In addition, properties of the WSOM extracted from the soil samples were characterized using total organic carbon (TOC) analysis, three-dimensional excitation-emission matrix (3DEEM) spectroscopy, and high-performance size-exclusion chromatography (HPSEC). In each case, the results for the thermally treated soil samples (at heat levels of 100–500 °C) were compared with the results for a corresponding untreated soil sample that had not been initially exposed to heat.

**Experimental**

*Collection of soil samples*

Soil samples to support this study were collected in broadleaf (*Fagus crenata* and *Quercus serrata*), coniferous (*Abies mariesii*), and dwarf bamboo (*Sasa kurilensis*) forests near Toga, Ioritani, Ranjyo, and Kureha in Japan’s Toyama Prefecture. In addition, peat soil samples were collected in a moor near Amou in Japan’s Gifu Prefecture. Figure S1 shows the location of the sampling sites and corresponding thermal analysis results. At each site, the top layer of debris was removed and the soils were sampled at a depth of 0–10 cm. The soil samples were then transported to the laboratory, air dried, and sieved with a 2 mm mesh in preparation for analysis.
**Treatment of soil samples**

A portion of the prepared soil samples (about 5 g) in porcelain crucibles with lids were thermally treated at different temperatures (100, 200, 300, 400, and 500 °C) for 30 min using a muffle furnace (KDF007Ex, Denken, Tokyo, Japan). The heating rate was 3 °C min⁻¹. After cooling, the thermally treated samples were dried under reduced pressure. Select soil samples were designated as controls and were thus not subjected to thermal treatment. WSOM was extracted by shaking a solution of 1:10 sample/distilled water (w/w) at 190 rpm and 25 °C for 24 h in darkness. The solution was then filtered through a membrane with a 0.45 μm pore diameter (mixed cellulose ester, ADVANTEC).

**Analysis of soil samples**

Changing of properties of soil samples by the thermal treatment was investigated based on the results of ion chromatography, TG-DTA, elemental analysis, colorimetry, and TMAH-py-GC/MS, according to the procedures as below. In each case, the results for the thermally treated soil samples were compared with the results for an untreated soil sample that had not been initially exposed to heat.

The pH (H₂O), pH (KCl) and electrical conductivity (EC) of the thermally treated and untreated soil samples were measured in slurries comprising 1:10 sample/distilled water or 1 M KCl.

The concentrations of K⁺ and NH₄⁺ in the slurries were determined using an Advanced Compact Ion Chromatography (IC) 761 system (Metrohm Lt., Herisau, Switzerland) following filtration through a 0.45 μm pore diameter membrane filter (mixed cellulose ester, ADVANTEC, Tokyo, Japan). Concentrations of NO₃⁻ and SO₄²⁻ in the slurries were similarly determined using an Advanced Compact IC 861 system (Metrohm Lt., Herisau, Switzerland).
The combustion characteristics of broadleaf forest and peat soils were determined using TG-DTA (Thermo plus TG 8120, Rigaku, Tokyo, Japan). Soil samples weighing approximately 10 mg were reheated from 30 to 550 °C at a rate of 3 °C min\(^{-1}\). Alumina was used as the reference standard. Select soil samples were combusted at 600 °C for 2 h to determine the loss on ignition (LOI).

The Comission Internationale d’Eclaireage (CIE) value of \(L^*\) is the luminance or lightness component, \(+a^*\) and \(-a^*\) indicate red and green colors, and \(+b^*\) and \(-b^*\) indicate yellow and blue colors, respectively.\(^{18}\) The CIE-Lab color values are derived from the CIE-XYZ color values. A colorimeter (Minolta Spectrophotometer CR-400, Minolta Camera, Japan) was used to determine the soil color (hereafter \(\Delta L^*, \Delta a^*, \text{ and } \Delta b^*\), respectively) against a standard white plate (\(Y = 92.5, x = 0.3162, y = 0.3323\)) and D65 illumination was used as a light source. Prior to analysis, the colorimeter was calibrated using a standard white plate.

The C, H, and N concentrations in the thermally treated and untreated soil samples were determined using a Micro Corder JM-10 CHN analyzer (J-Science Lab Co. Ltd., Japan) at Hokkaido University’s Creative Research Institution-Global Facility Center. The concentration of O in the soil samples was determined by subtracting the total concentrations of C, H, N, and ash elements from 100.

The TMAH-py-GC/MS technique provides useful information on detailed structural characterization of SOM.\(^{22-24}\) TMAH can be used to cleave ester and ether-linkages via thermochemolysis, with the formation of methyl esters of phenols and carboxylic acids.\(^{25}\) Therefore, TMAH-py-GC/MS was applied to the soil samples before and after thermal treatment to take the detail information of their structure. Soil samples weighing approximately 2 mg from the broadleaf forest (B-I) and peatland were placed on a pyrofoil (F590, Japan Analytical Industry Co., Ltd., Japan). A 25 µL portion of TMAH (40 mg mL\(^{-1}\) in methanol-acetone solution) (Sigma-Aldrich) and a 10 µL portion of nonadecanoic acid (0.06 mg mL\(^{-1}\) in acetone solution, internal
standard) (Sigma-Aldrich) were added to the soil in the pyrofoil. The pyrofoils were placed in a JHP-5 Curie point pyrolyzer (Japan Analytical Industry, Tokyo, Japan) that was connected to a Shimadzu GC/MS QP 2010 system (Kyoto, Japan). A DB-5 column was used for separating the pyrolysates (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, USA). The National Institute of Standards and Technology (NIST) Mass Spectra Library was used to identify the pyrolysate compound peaks based on a minimum similarity of 80%. For each soil sample, we calculated the relative peak areas by divided each area by the corresponding internal standard value (35.5 min retention time). These individual compounds were grouped and coded as follows: L represents lignin-derived compounds; O represents other aromatic compounds; Cel represents cellulose-derived compounds; F < 20 and F > 20 represent short- (< C_{20}) and long-chain (> C_{20}) fatty acid methyl esters (FAME), respectively; FD represents fatty acid dimethyl esters; and U < 10 and U > 10 represent unidentified low (< C_{10}) and high molecular (> C_{10}) organic components, respectively.

**Analysis of water-soluble components**

TOC analysis, 3DEEM spectroscopy, and HPSEC were employed to characterize the WSOM fractions from the thermally treated and untreated soil samples.

The dissolved organic carbon (DOC) concentrations were directly measured using a TOC analyzer (TOC-5000A, Shimadzu, Kyoto, Japan).

The 3DEEM spectrum was measured using a fluorescence spectrophotometer (Mode LS-55, PerkinElmer, CA, USA) with a scanning wave range of 200–600 nm for both excitation (Ex.) and emission (Em.). The relative fluorescence intensity (RFI) was calibrated and expressed in quinine sulfate units (QSU): 1 QSU = 1 μg L^{-1} of quinine sulfate monohydrate in a solution of 0.05 M H_{2}SO_{4} at Ex./Em. = 355/450 nm.
HPSEC was applied to estimate the molecular weight of the WSOM, a 20 μL aliquot of WSOM was injected into a Jasco PU-2080 plus Intelligent HPLC pump system equipped with a UV-2075 UV/vis detector (Japan Spectroscopic Co., Tokyo, Japan). The mobile phase consisted of a mixture of 0.01 M phosphate buffer (pH 7.0) and acetonitrile (75/25 = v/v), and the flow rate was set at 0.75 mL·min⁻¹. A TSK-Gel α-M column (Tosoh, 7.8 mm inner diameter × 300 mm) was used as the solid phase. The UV absorption of WSOM was measured at 260 nm. Reference substances for construction of the molecular weight calibration curves included polystyrene sulfonate sodium salts with molecular weights of 0.21, 4.3, 6.8, 17, 32, 77, 150 and 2,600 kDa (Fluka, Buchs, Switzerland). The number average molecular weights (\(M_n\)), weight average molecular weights (\(M_w\)) and degree of dispersion (\(M_w/M_n\)) of the water-soluble components in each thermally treated and untreated soil sample were estimated.

**Results and discussion**

*The properties of each soil sample prior to any thermal treatment*

Table S1 shows the pH, EC, DOC concentration, LOI, color (using \(L^*a^*b^*\) coordinates), and elemental compositions (C, H, N, and O concentrations). In our previous study, EC and LOI values were significantly higher for soils from the coniferous forests than from the broadleaf forests. In this study, however, the same trend was not observed. Instead, pH and EC values differed depending on sampling site. The LOI values were initially highest in the soil samples from the coniferous forests (C-I and C-II, respectively) and the broadleaf forests (B-I and B-III, respectively). The DOC concentrations were initially highest in B-I and C-II and comparable to the concentrations found in peat. Soil samples from the peatland had the highest initial EC and LOI values, as well as the highest initial concentrations of C, H, N, and O.
The pH and electrical conductivity results

Figure 1 (a) and (b) show the pH (H$_2$O) and EC, respectively, for each of the thermally treated and untreated soil samples in slurries. For temperatures $>$ 200 °C, the pH (H$_2$O) increased as the heating temperature increased consistently across all soil samples. The C-I and C-III had the highest pH values. When heated to 500 °C, each of the soil samples from C-I, C-II, and C-III, irrespective of sampling site, reached a neutral or slightly alkaline pH (6.93–8.15).

The EC of the soil samples heated to $\geq$ 200 °C was 1.9–6.5 times higher than the corresponding untreated soil samples. The C-I had the highest increase in EC following thermal treatment.

These findings are consistent with our previous field studies, which found that tropical peat and broadleaf forest soils had higher pH and EC values when exposed to high heat levels. This phenomenon is due to the exchangeable cations released by the combusting SOM and the H$^+$ and Al$^{3+}$ ions adsorbed on the negative charge of the soil colloids.

Ion chromatography results

Figure 1(c–f) shows the NO$_3^-$, NH$_4^+$, K$^+$, and SO$_4^{2-}$ concentrations, respectively, for each of the thermally treated and untreated soil samples in slurries. The NO$_3^-$ concentrations were initially highest in the C-I, B-IV, and the bamboo forest, but, in each case, these concentrations decreased following thermal treatment.

Comparatively, the NH$_4^+$ concentrations increased substantially across all soil samples when heated to 200 °C, and then gradually decreased as heating temperatures increased. Wildfires have been found to increase the concentrations of NO$_3^-$–N and NH$_4^+$–N in the soil. In generally, ammonium is directly produced by the combustion of organic N, while nitrate forms from ammonium some weeks or months after fire. Prieto-Fernandez et al. also found increased NH$_4^+$–
N and NO$_3^-$–N concentrations at the soil’s subsurface (5–10 cm depth) one month after a fire event.$^{29}$

A similar trend was observed for the K$^+$ concentrations in the thermally treated samples. The K$^+$ concentrations increased substantially across all soil samples when heated to 200 °C, and then generally decreased as heating temperatures increased. This common trend suggested that the cations (NH$_4^+$ and K$^+$) bound to soil colloids were released by heating at 200 °C.

Conversely, the SO$_4^{2-}$ concentrations exhibited minimal variation as the heating temperature increased, with one notable exception. The SO$_4^{2-}$ concentrations in the C-I showed a marked increase as the heating temperature increased. Previous studies have suggested that an increased concentration of SO$_4^{2-}$ in the soil is attributable to the oxidation of S in the organic material and the release of sequestered SO$_4^{2-}$ from plant litter tissue.$^{30-33}$

The collective ion chromatography results indicated that a heating temperature of 200 °C is most significant when considering the effects of high heat on the ionic components in the soil. Some differences were observed, however, among the different soil types.

*Thermogravimetry and differential thermal analysis results*

Figure 2 shows the results of the TG-DTA for thermally treated and untreated soil samples from the B-I and the peatland. The distinct TG and DTA curves show the mass loss and the heat release rate, respectively, for the soil samples during the pyrolysis process. The ignition temperature ($T_v$), combustible gas release temperature (peak A), carbide combustion temperature (peak B), and mass loss at 550 °C were determined from the TG-DTA results and are summarized in Table S2.

For the broadleaf forest soil samples, the DTA curves indicated that the combustible gas release temperature (peak A) shifted right (towards higher temperatures) relative to the untreated
soil samples when heated to temperatures ≥ 300 °C. In addition, the carbide combustion temperature (peak B) decreased for samples heated to temperatures ≥ 400 °C.

For the peatland soil samples, the combustible gas release temperature (peak A) was detected using the DTA curves at 302 °C for samples heated to temperatures ≤ 300 °C; for samples heated to temperatures > 300 °C, no peak was detected. Similarly, the carbide combustion temperature (peak B) was detected at 389 °C; higher peak values were observed for samples heated to temperatures ≥ 300 °C. These results are consistent with previous study findings using tropical peat collected from Indonesia.20

The combustible gas release temperature (peak A) has been attributed to the combustion of polysaccharides, decarboxylation of acidic groups, and dehydration of hydroxylate aliphatic structures.34,35 Comparatively, the carbide combustion temperature (peak B) is related to the combustion of aliphatic structures derived from plant decompositions and aromatic structures.

For the soil samples from both the B-I and the peatland, the TG curves indicated that the mass loss decreased (remaining mass increased) as the heating temperature increased. Soil samples heated to temperatures ≥ 300 °C had less mass loss than soil samples heated to temperatures < 300 °C. During the pyrolysis process, the remaining mass in the soil samples markedly decreased when reheated to approximately 200 °C. Temperatures > 200 °C likely destroy the soil aggregates. Josa et al. found that aggregate size distributions and stability in Spanish soil were decreased when heated to a temperature of 200 °C.36 This temperature was previously shown to affect the cation (NH₄⁺ and K⁺) concentrations in this study’s thermally treated soil samples.

The collective TG-DTA results indicated that the denaturation of organic components begins at temperatures of 200–300 °C. In this phase, flame-retardant SOM was produced.
Colorimetry and elemental analysis results

In our previous research using Indonesian peat, we found that the Δa* and Δb* values substantially decreased when samples were heated above the ignition temperature of volatile matter and exhibited linear negative correlations with the atomic ratios of H/C and O/C. This method proved useful for easily evaluating the dehydration and decarboxylation of soil caused by heating and was thus applied to this study’s soil samples from Japan’s forests.

Figure 3 shows the colorimetry results for the thermally treated and untreated soil samples, relative to the red-green (Δa*) and yellow-blue (Δb*) hue-chroma component standards. Table S3 details these same results. For heating temperatures ≤ 400 °C, the Δa* and Δb* values across all soil samples decreased as heating temperatures increased. Most notably, the hue components in the soil samples heated to temperatures of 300 and 400 °C changed from a red–yellow color to a blue–green color following thermal treatment.

Based on the elemental analysis results, Table 1 summarizes the H/C and O/C atomic ratios for the thermally treated and untreated soil samples. Across most of the soil samples, the H/C and O/C atomic ratios decreased as heating temperatures increased to 200 °C, decreased again as heating temperatures increased to 300 °C, but increased as heating temperatures increased to 400 °C. Exceptions included the B-II, which showed increased H/C and O/C atomic ratios at 300 °C; and soil samples from the peatland, which showed decreased H/C and O/C atomic ratios at 400 °C. In addition, the H/C and O/C atomic ratios in soil samples heated to temperatures ≤ 300 °C exhibited a positive linear correlation with the ΔL*, Δa* and Δb* values (H/C vs. ΔL*: \( r^2 = 0.42, p < 0.0001 \); H/C vs. Δa*: \( r^2 = 0.35, p < 0.0001 \); H/C vs. Δb*: \( r^2 = 0.52, p < 0.0001 \); O/C vs. ΔL*: \( r^2 = 0.37, p < 0.0001 \); O/C vs. Δa*: \( r^2 = 0.27, p < 0.0001 \); O/C vs. Δb*: \( r^2 = 0.43, p < 0.0001 \)).

For heating temperatures > 400 °C, the Δa* and Δb* values substantially increased across all soil samples except those collected from the C-I, the B-II, and the peatland. Similar phenomena
have been reported in soils with a high iron content in which goethite (αFeOOH, with a yellow color) was converted to hematite (αFe₂O₃, with a reddish-brown color) or maghemite (γFe₂O₃, with a red color). Recrystallization of some minerals, such as Fe and Al oxyhydroxides, is known to occur when soils are heated to temperatures > 400 °C.  

**Tetramethylammonium hydroxide pyrolysis-gas chromatography-mass spectrometry results**

Figure S2 shows the TMAH-py-GC/MS results for thermally treated and untreated soil samples from the B-I and the peatland. As observed in the chromatograms, the frequencies and intensities of the pyrolysate compound peaks increased as the heating temperatures increased up to 200 °C. This finding is consistent with the results from the colorimetry and elemental analysis describe above. For heating temperatures ≥ 300 °C, opposite trends were observed. Consistent with previous TG-DTA results, nonpyrolyzable carbon (char and soot) formed during thermal treatment. Table S4 lists the various pyrolysate compounds identified in the chromatograms.

Figure 4 shows the relative proportions of these same pyrolysate compound groups for thermally treated and untreated soil samples from the B-I and the peatland. The untreated peat soil sample contained more aromatic lignin derived compounds and unidentified high molecular (> C₁₀) organic components compared to the untreated B-I (relative peak area of L: B-I = 1.64, Peat = 5.39; U > 10: B-I = 4.7, Peat = 14.9). In addition, slight difference in FAME were observed (relative peak area of F < 20: B-I = 1.08, Peat = 1.74; F > 20: B-I = 1.02, Peat = 4.16). Consistent across both soil types, the soil samples heated to a temperature of 200 °C contained the highest proportions of lignin-derived compounds and short-chain FAME (relative peak area of L: B-I = 5.87, Peat = 14.8; F < 20 B-I = 4.40; Peat = 8.65). Contributing to this finding, the relative peak areas of hexadecanoic acid methyl ester (C₁₆₇, Fig. S2) and 3,4-dimethoxy benzoic acid methyl ester, as known as veratic acid methyl ester (Ver, Fig. S2) significantly increased for the soil samples heated to 200 °C (relative peak area of C₁₆₇: B-I = 2.38, Peat = 3.79; Ver: B-I = 1.64;
Peat = 3.90). At this same temperature, the relative peak areas of the unidentified low molecular organic components (< C_{10}) increased for the soil samples from the B-I and the peatland (relative peak area of U < 10: B-I = 14.6; Peat = 32.7), and the relative peak area of an unidentified pyrolysis product significantly increased after 6.85 min for the soil sample from the peatland (Un, Fig. S2). These results suggest that the SOM compounds were changed drastically at temperature above 200 °C. Comparatively, long-chain FAME peaks were not detected after 35–55 min for the soil samples heated to a temperature of ≥ 300 °C. This finding suggests that the presence or absence of the long-chain FAME peaks may be useful for evaluating the high-heat effects on SOM.

*Total organic carbon results for WSOM*

After characterizing the properties of the original thermally treated and untreated soil samples, the properties of the WSOM extracted from the soil samples were characterized using TOC analysis. Figure 5 shows the DOC concentrations for the WSOM from the thermally treated and untreated soil samples. Consistent across all soil samples, the DOC concentrations of the soil samples heated to 200 °C were higher than the corresponding untreated soil samples. For soil samples heated to temperatures ≥ 300 °C, the DOC concentrations decreased as heating temperatures increased.

This finding, related to the exportation of DOC from the soil, is consistent with our previous findings using tropical peat. These TOC analysis results indicate that, when heated to a temperature of 200 °C, the denaturation of SOM induces the elution of a high concentration of organic carbon components from the soil. These organic carbon components have different physicochemical properties.
Three-dimensional excitation-emission matrix spectroscopy and high-performance size-exclusion chromatography results for WSOM

Figure 6 shows the 3DEEM spectroscopy results for the WSOM from the thermally treated and untreated soil samples from the B-I and the C-III. Figure S3 shows the 3DEEM spectroscopy results for each of the other soil samples. A humic-like peak (peak C, Ex./Em. = 320–360/420–460 nm) was detected in each of the untreated soil samples (Fig. S3).\(^{39}\) A blue shift in the excitation and emission wavelength of peak C was observed following thermal treatment, indicating a low molecular weight and the presence of low humified organic compounds.\(^{40}\) In addition, shorter emission wavelength peaks at 350–375 nm were detected in soil samples heated to a temperature of 300 °C. For soil samples from the C-I, C-III and the B-IV, longer emission wavelength peaks at 500–550 nm were also detected (Fig. 6 and Fig. S3). Previous studies have reported that the longer emission wavelength peaks were derived from a high molecular weight and the presence of aromatic humic acids.\(^{41–43}\)

As compared with the \(M_w\), it is clear that the \(M_n\) values decreased obviously for soil samples heated by 100–200 °C (Table S5). This indicates that the WSOM was partially changed to low-molecular compounds by heating. In addition, the \(M_w\) values decreased for soil samples heated to 300–400 °C.

The results obtained from 3DEEM spectroscopy and HPSEC designated that the main components of SOM such as fulvic and humic acids were denatured by heating above the carbide combustion temperature (peak B in DTA curve). These findings are supporting that the phenomena regarding the increment of DOC concentration in the soil samples heated to 200 °C and the dramatic changes in fluorescence properties of DOC.

Soil temperatures during a wildfire typically range from 200 to 300 °C.\(^{12}\) Therefore, based on the collective 3DEEM spectroscopy and HPSEC results, a wildfire event would likely cause the leaching of the WSOM, particularly those components with a high fluorescence and a low
molecular weight. This phenomenon may subsequently affect the transport of nutrients and the carbon cycle in terrestrial ecosystems.

Conclusions

In this study, we applied various evaluation methods to determine the effects of high heat levels (100–500 °C) on SOM and WSOM using soil samples collected from broadleaf, coniferous, and bamboo forests and peatland in Japan. Each evaluation method applied in this study produced unique and complementary results that provided a more comprehensive understanding of high-heat effects on Japan’s soils. Key findings from this study follow.

Based on the TG-DTA results for B-I and the peatland, the remaining mass in the soil samples markedly decreased when heated to above 200 °C. It was confirmed that the elution of exchangeable cations and low molecular DOC also occurred at this heating temperature by ion chromatography, TOC, and HPSEC. In the natural environment, these phenomena may strongly affect a soil’s nutrient dissolving and carbon cycling abilities in forest areas exposed to wildfires. The results obtained from colorimetry, elemental analysis, DTA, 3DEEM, HPSEC indicated a drastic changes in WSOM components and the production of flame-retardant SOM, when soil samples were heated to 300–400 °C. For soil samples initially heated to a temperature of 500 °C, colorimetry results indicated a marked increase in the red-yellow intensity of the soil samples. This change may be due to the production of Fe oxide, such as hematite (αFe₂O₃) or maghemite (γFe₂O₃).

The results of this study substantially contributed to the state of knowledge in both of these topic areas and demonstrated the unique and complementary results originating from the various evaluation methods used. This knowledge will support future efforts to accurately characterize and quantify the high-heat effects on soil and will more broadly support efforts to characterize and quantify the effects of increased wildfires attributable to climate change.
Acknowledgements

This work was supported by the Japan Society for the Promotion of Science (JSPS) via a Grant-in-Aid for Scientific Research (26740042). We would like to thank Editage (www.editage.com) for English language editing.

Supplementary Information

Location of the sampling sites are shown in Fig. S1. General soil sample properties prior to any thermal treatment are shown in Table S1. The ignition temperature ($T_v$), combustible gas release temperature (peak A), combustion temperature of carbide (peak B) and mass loss at 550 °C of broadleaf forest soil (B-I, III, IV) and peat soil are shown in Table S2. The $\Delta L^*$, $\Delta a^*$ and $\Delta b^*$ values of untreated and thermal treated soils are shown in Table S3. The relative intensities of identified pyrolysate compounds for the thermally treated and untreated soil are shown in Table S4. TMAH-py-GC/MS chromatograms from untreated and thermally treated samples of broadleaf forest soil (B-I) and peat are shown in Fig. S2. 3DEEM fluorescence spectra of water-soluble soil organic matter (WSOM) from untreated and thermally treated samples from broadleaf forest (B-II, III, IV), conifer forest (C-I, II), bamboo and peat soil are shown in Fig. S3. The number-average molecular weight ($M_n$) and weight-average molecular weight ($M_w$) for the water-soluble soil organic matter (WSOM) from untreated and thermal treated soils are shown in Table S5. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References

1. J. A. González-Pérez, F. J. González-Vila, G. Almendros, and H. Knicker, *Environ. Int.*, 2004, 30, 855.
2. K. Kalbitz, S. Solinger, J.-H. Park, B. Michalzik, and E. Matzner, *Soil Sci.*, **2000**, 165, 277.

3. S. McDonald, A. G. Bishop, P. D. Prenzler, and K. Robards, *Anal. Chim. Acta*, **2004**, 527, 105.

4. M. Flannigan, A. S. Cantín, W. J. De Groot, M. Wotton, A. Newbery, and L. M. Gowman, *For. Ecol. Manage.*, **2013**, 294, 54.

5. N. P. Gillett, A. J. Weaver, F. W. Zwiers, and M. D. Flannigan, *Geophys. Res. Lett.*, **2004**, 31, L18211.

6. UN (United Nations), Draft outcome document of the United Nations summit for the adoption of the post-2015 development agenda, **2015**.

7. J. T. Randerson, H. Liu, M. G. Flanner, S. D. Chambers, Y. Jin, P. G. Hess, G. Pfister, M. C. Mack, K. K. Treseder, L. R. Welp, F. S. Chapin, J. W. Harden, M. L. Goulden, E. Lyons, J. C. Neff, E. A. G. Schuur, and C. S. Zender, *Science (80-. ).*, **2006**, 314, 1130.

8. Yustiawati, Y. Kihara, K. Sazawa, H. Kuramitz, M. Kurasaki, T. Saito, T. Hosokawa, M. S. Syawal, L. Wulandari, Hendri I, and S. Tanaka, *Environ. Sci. Pollut. Res.*, **2015**, 22, 2384.

9. T. Hernández, C. García, and I. Reinhardt, *Biol. Fertil. Soils*, **1997**, 25, 109.

10. L. F. Debano, *J. Hydrol.*, **2000**, 231–232, 195.

11. G. Certini, *Oecologia*, **2005**, 143, 1.

12. H. Knicker, *Biogeochemistry*, **2007**, 85, 91.

13. J. Mataix-Solera, A. Cerdà, V. Arcenegui, A. Jordán, and L. M. Zavala, *Earth-Science Rev.*, **2011**, 109, 44.

14. G. Almendros and F. González-Vila, *Spanish J. Soil Sci.*, **2012**, 2.

15. J. Rodríguez, J. A. González-Pérez, A. Turmero, M. Hernández, A. S. Ball, F. J. González-Vila, and M. Enriqueta Arias, *Catena*, **2017**, 158, 82.
16. J. Rodríguez, J. A. González-Pérez, A. Turmero, M. Hernández, A. S. Ball, F. J.
González-Vila, and M. E. Arias, *Sci. Total Environ.*, 2018, 634, 650.
17. N. Katsumi, K. Yonebayashi, and M. Okazaki, *Sci. Total Environ.*, 2016, 541, 23.
18. K. Sazawa, T. Wakimoto, N. Hata, S. Taguchi, S. Tanaka, M. Tafu, and H. Kuramitz,
*Anal. Methods*, 2013, 5, 2660.
19. K. Sazawa, H. Yoshida, K. Okusu, N. Hata, and H. Kuramitz, *Environ. Sci. Pollut. Res.*, 2018.
20. K. Sazawa, T. Wakimoto, M. Fukushima, Y. Yustiawati, M. S. Syawal, N. Hata, S.
Taguchi, S. Tanaka, D. Tanaka, and H. Kuramitz, *ACS Earth Sp. Chem.*, 2018, 2, 692.
21. K. Sazawa, M. Tachi, T. Wakimoto, T. Kawakami, N. Hata, S. Taguchi, and H.
Kuramitz, *Int. J. Environ. Res. Public Health*, 2011, 8.
22. N. Yamauchi, W. Toyodome, K. Umeda, N. Nishida, and T. Murae, *Anal. Sci.*, 2004, 20,
1453.
23. H. Iwai and M. Yamamoto, *Anal. Sci.*, 2019, 35, 665.
24. H. Iwai, *Anal. Sci.*, 2017, 33, 1231.
25. S. Amir, M. Hafidi, L. Lemee, J. R. Bailly, G. Merlina, M. Kaemmerer, J. C. Revel, and
A. Ambles, *J. Anal. Appl. Pyrolysis*, 2006, 77, 149.
26. H. Yoshida, K. Sazawa, N. Wada, N. Hata, K. Marumo, M. Fukushima, and H.
Kuramitz, *Catena*, 2018, 171, 22.
27. M. Fukushima, M. Yamamoto, T. Komai, and K. Yamamoto, *J. Anal. Appl. Pyrolysis*,
2009, 86, 200.
28. J. M. Arocena and C. Opio, *Geoderma*, 2003, 113, 1.
29. A. Prieto-Fernandez, M. C. Villar, M. Carballas, and T. Carballas, *Soil Biol. Biochem.*, 1993, 25, 1657.
30. R. R. Blank, F. Allen, and J. A. Young, *Soil Sci. Soc. Am. J.*, 1994, 58, 564.
31. J. Chorover, P. M. Vitousek, D. A. Everson, A. M. Esperanza, and D. Turner,

_Biogeochemistry, 1994_, 26, 115.

32. R. Blank and D. Zamudio, _Int. J. Wildl. Fire_, 1998, 8, 79.

33. J. D. Murphy, D. W. Johnson, W. W. Miller, R. F. Walker, E. F. Carroll, and R. R.

Blank, _J. Environ. Qual._, 2006, 35, 479.

34. O. Francioso, D. Montecchio, P. Gioacchini, and C. Ciavatta, _Appl. Geochemistry_, 2005,

20, 537.

35. D. Montecchio, O. Francioso, P. Carletti, D. Pizzeghello, S. Chersich, F. Previtali, and S.

Nardi, _J. Therm. Anal. Calorim._, 2006, 83, 393.

36. Josa, R., Arias, X., Solé, A., “Soil erosion and degradation as a consequence of forest

fires”, ed. Sala, M., Rubio, J.L., Geoforma E ed., 1994, Logroño, 29.

37. Mullins, C.E., _J. Soil Sci._, 1977, 28, 233.

38. Sertsu, S. M., Sanchez, P. A., _Soil Sci. Soc. Am. J._, 1978, 42, 940.

39. P. G. Coble, C. E. Del Castillo, and B. Avril, _Deep. Res. Part II Top. Stud. Oceanogr._,

1998, 45, 2195.

40. T. Ohno, A. Amirbahman, and R. Bro, _Environ. Sci. Technol._, 2008, 42, 186.

41. D. M. McKnight, E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, D. T. Andersen,

E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Anderson, _Limnol.

Oceanogr._, 2001, 46, 38.

42. C. A. Stedmon, S. Markager, and R. Bro, _Mar. Chem._, 2003, 82, 239.

43. P. Kowalczyk, M. J. Durako, H. Young, A. E. Kahn, W. J. Cooper, and M. Gonsior,

_Mar. Chem._, 2009, 113, 182.
Table 1  Hydrogen/carbon (H/C) and oxygen/carbon (O/C) atomic ratios for the thermally treated and untreated soil samples (n.d. = no data).

|       | Untreated | 100 °C | 200 °C | 300 °C | 400 °C | 500 °C |
|-------|-----------|--------|--------|--------|--------|--------|
| B-I   |           |        |        |        |        |        |
| H/C   | 2.04      | 1.99   | 1.70   | 1.22   | 1.25   | 1.38   |
| O/C   | 0.92      | 0.85   | 0.67   | 0.58   | 0.69   | 0.67   |
| C-I   |           |        |        |        |        |        |
| H/C   | 1.92      | 1.93   | 1.62   | 1.13   | 1.16   | 1.16   |
| O/C   | 0.92      | 0.93   | 0.70   | 0.60   | 0.61   | 0.63   |
| B-II  |           |        |        |        |        |        |
| H/C   | 1.83      | 2.03   | 1.68   | 2.62   | 1.82   | 3.64   |
| O/C   | 0.84      | 0.90   | 0.66   | 1.30   | 0.89   | 1.57   |
| C-II  |           |        |        |        |        |        |
| H/C   | 2.05      | 2.00   | 1.79   | 1.14   | 1.39   | 1.87   |
| O/C   | 0.95      | 0.84   | 0.74   | 0.54   | 0.70   | 0.86   |
| B-III |           |        |        |        |        |        |
| H/C   | 3.86      | 4.12   | 3.10   | 1.79   | 6.65   | n.d.   |
| O/C   | 1.84      | 1.85   | 1.31   | 0.75   | 3.27   | n.d.   |
| C-III |           |        |        |        |        |        |
| H/C   | 2.96      | 2.92   | 2.87   | 2.57   | 5.12   | 9.45   |
| O/C   | 1.43      | 1.33   | 1.26   | 1.24   | 2.57   | 4.49   |
| B-IV  |           |        |        |        |        |        |
| H/C   | 2.03      | 2.12   | 2.07   | 1.76   | 2.34   | 3.72   |
| O/C   | 0.94      | 0.95   | 0.91   | 0.83   | 1.17   | 1.64   |
| Bamboo|           |        |        |        |        |        |
| H/C   | 2.35      | 2.08   | 1.88   | 1.47   | 1.64   | 2.49   |
| O/C   | 1.02      | 0.91   | 0.87   | 0.68   | 0.79   | 1.07   |
| Peat  |           |        |        |        |        |        |
| H/C   | 1.61      | 1.56   | 1.16   | 0.96   | 0.79   | 0.76   |
| O/C   | 0.58      | 0.55   | 0.38   | 0.36   | 0.38   | 0.39   |
Figure Captions

Fig. 1  The pH (H₂O), electrical conductivity (EC), and ion chromatography results for the thermally treated and untreated soil samples in slurries: (a) pH (H₂O), (b) EC, (c) NO₃⁻, (d) NH₄⁺, (e) K⁺, and (f) SO₄²⁻ concentration.

Fig. 2  Thermogravimetry (TG) and differential thermal analysis (DTA) results for the thermally treated and untreated soil samples from the broadleaf forest near Toga (B-I) and the peatland near Amou, where Tv is the ignition temperature of the volatile matter, Peak A is the combustible gas release temperature, and Peak B is the carbide combustion temperature.

Fig. 3  Colorimetry results for the thermally treated and untreated soil samples, relative to the red-green (Δa*) and yellow-blue (Δb*) hue-chroma component standards.

Fig. 4  Relative proportions of pyrolysate compound groups for the thermally treated and untreated soil samples from the broadleaf forest near Toga (B-I) and the peatland near Amou, where L represents lignin-derived compounds; O represents other aromatic compounds; Cel represents cellulose-derived compounds; F < 20 and F > 20 represent short- (< C₂₀) and long-chain (> C₂₀) fatty acid methyl esters (FAME), respectively; FD represents fatty acid dimethyl esters; and U < 10 and U > 10 represent unidentified low (< C₁₀) and high molecular (> C₁₀) organic components, respectively.

Fig. 5  Dissolved organic carbon concentrations for the water-soluble soil organic matter (WSOM) from the thermally treated and untreated soil samples.
Fig. 6  Three-dimensional excitation-emission matrix (3DEEM) fluorescence spectra for the water-soluble soil organic matter (WSOM) from the thermally treated and untreated soil samples from the broadleaf forest near Toga (B-I) and coniferous forest near Ranjyo (C-III).
Fig. 1  The pH (H$_2$O), electrical conductivity (EC), and ion chromatography results for the thermally treated and untreated soil samples in slurries: (a) pH (H$_2$O), (b) EC, (c) NO$_3$-, (d) NH$_4$+, (e) K$^+$, and (f) SO$_4^{2-}$ concentration.
Fig. 2  TG and DTA results for the thermally treated and untreated soil samples from the broadleaf forest near Toga (B-I) and the peatland near Amou, where $T_v$ is the ignition temperature of the volatile matter, Peak A is the combustible gas release temperature, and Peak B is the carbide combustion temperature.
Fig. 3  Colorimetry results for the thermally treated and untreated soil samples, relative to the red-green ($\Delta a^*$) and yellow-blue ($\Delta b^*$) hue-chroma component standards.
Fig. 4  Relative proportions of pyrolysate compound groups for the thermally treated and untreated soil samples from the broadleaf forest near Toga (B-I) and the peatland near Amou, where L represents lignin-derived compounds; O represents other aromatic compounds; Cel represents cellulose-derived compounds; F < 20 and F > 20 represent short- (< C\textsubscript{20}) and long-chain (> C\textsubscript{20}) fatty acid methyl esters (FAME), respectively; FD represents fatty acid dimethyl esters; and U < 10 and U > 10 represent unidentified low (< C\textsubscript{10}) and high molecular (> C\textsubscript{10}) organic components, respectively.
Fig. 5 Dissolved organic carbon concentrations for the water-soluble soil organic matter (WSOM) from the thermally treated and untreated soil samples.
Fig. 6  Three-dimensional excitation-emission matrix (3DEEM) fluorescence spectra for the water-soluble soil organic matter (WSOM) from the thermally treated and untreated soil samples from the broadleaf forest near Toga (B-I) and coniferous forest near Ranjyo (C-III).
Graphical Index