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Production and characterization of humic-like substances from pyrolyzed plant materials by chemical oxidation

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Abstract

The accumulation of refractory organic carbon (C), such as highly-humified humic substances, in soil can be of benefit in terms of global warming and soil fertility. To produce organic materials that are similar to refractory humic acids (HAs), wood obtained from a giant dogwood (GDW) a broadleaf tree and maize stalks were pulverized and pyrolyzed at various conditions (225–450ºC for 1 or 2 h), and then oxidized with ruthenium tertoxide (RuO_4), nitric acid (HNO_3), or hydrogen peroxide (H_2O_2). For both plant materials, the maximum yield of dark colored HA-like substances (599 and 539 mg g^{-1} on a C basis), corresponding to Type A HAs, was obtained by pyrolysis at 400°C for 1 h (GDW400/1 and Maize400/1) followed by HNO_3 oxidation. An X-ray diffraction 11 band profile analysis showed that the sizes of the C layer planes (two-dimensional hexagonal lattices in a graphite-like structure) in GDW400/1 and Maize400/1 were distributed from 0.48 to 1.68 nm, equivalent to condensed polyaromatic nuclei containing 4–37 rings. Although the HNO_3 oxidation decreased C layer plane content, the mean C layer plane size was increased from 0.83–1.05 nm to 1.06–1.37 nm. The C layer plane contents in the GDW400/1 or Maize400/1 samples that had been oxidized with HNO_3 were similar to those in the corresponding HA fractions. The mean size of the C layer planes in the HA fraction (1.04 nm for both samples) was smaller than that for a sample of Maize400/1 oxidized with HNO_3 (1.37 nm), but larger than the reported values for soil HAs, suggesting their recalcitrance in soil. The yield and quality of the HA-like substances produced by HNO_3 oxidation did not differ with the plant type used, when a sufficiently high temperature (400ºC) was used for the charring.

Introduction

Char is produced by the incomplete combustion of vegetation or fossil fuels and is ubiquitous in all atmospheric, aquatic, and terrestrial environments (Kuhlbusch, 1998; Masiello and Druffel, 2003; Schmidt and Noack, 2000). In the case of soil, char C has been estimated to account for <1–45% of the total carbon (C) (Schmidt et al., 1999; Skjemstad et al., 2002; Shindo et al., 2004), although those values are largely based on the indirect determination of black carbon in the sample. Charred C also accounts for up to 35% of the C in humic acids (HAs), which are dark-colored amorphous soil organic matter that is soluble in an alkali and insoluble in acid, in Andosols (Shindo et al., 2004). Soil char C is mainly

produced as the result of fires (Bird and Cali, 1998; Dai et al., 2005), and artificial char, so-called biochar, which is applied to agricultural fields with the intent to improve soil fertility (Glaser et al., 2002) or the sequestration of C in soil to mitigate the increasing rate of atmospheric C (Lehmann, 2007) represents another source.

Oxidation, which occurs during the weathering of char, results in the formation of carboxyl groups on the edges of condensed aromatic nuclei (Cheng et al., 2008). An increase in carboxyl group content and fragmentation may encourage the interaction of char with soil particles, e.g., through the formation of coordinate bonding with a polyvalent cation functioning as a bridge. Humic acids can also be produced during these processes (Cohen-Ofri et al.,

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2006). Because of the slow rate of oxidation of whole char under ambient conditions (Nguyen et al., 2009), a chemical treatment with nitric acid (HNO_3) or hydrogen peroxide (H_2O_2) has been applied in model experiments, in attempts to simulate the conversion of char to HAs (Haumaier and Zech, 1995; Trompowsky et al., 2005). For this purpose, RuO_4, a milder oxidant that causes the degradation of polynuclear aromatic compounds into aromatic acids (Blanc and Albrecht, 1991), represents an alternate potential reagent.

Since the structural properties of soil HAs vary depending on the soil type, land use, and soil age (Kawasaki et al., 2008; Watanabe and Takada, 2006), they are frequently grouped into 4 types, for the sake of convenience, based on the degree of dark color (degree of humification; Kumada, 1987). Type A HAs have the greatest degree of humification, carboxyl group content (Tsutsuki and Kuwatsuka, 1978), and aromaticity (Kawasaki et al., 2008) as well as condensed aromatic structure content (Ikeya et al., 2007, 2011), and, of the 4 types of HAs, is generally considered to be the most recalcitrant in soil (Kumada, 1987). It was previously reported that char isolated from buried Andosols contain a large amount of Type A HAs (Nishimura et al., 2006). Thus, the oxidation of char with the intent of increasing Type A HA-like substances prior to its application to agricultural soils is considered to be an effective strategy for dual purposes of enhancing C sequestration and improving soil fertility.

Sultana et al. (2011) produced char from Japanese cedar (JC; *Cryptomeria japonica*) wood, the major coniferous tree planted in Japan, under various pyrolysis conditions and then oxidized the product with RuO_4, HNO_3, or H_2O_2 to produce a larger amount of darker colored HA-like substances. Type A HA-like substances were obtained only when the pyrolyzed residues of JC wood had been oxidized with HNO_3. A higher pyrolysis temperature (400°C) was more effective than lower temperatures (275 and 300°C) in producing these products with regard to yield and degree of humification. The contents of aromatic C, carboxyl C, and condensed aromatic structures in the HA-like substances produced by HNO_3 oxidation tended to be larger than those in the corresponding fraction obtained after H_2O_2 oxidation. Potential sources of biochar applicable to soil include timber, crop residues, food wastes, etc. (Ryu et al., 2007), and the composition and structure can

generally vary with the plant types such as coniferous trees, broadleaf trees, and grass species. The purpose of the present study was to evaluate the effect of pyrolysis and oxidation on the yield and chemical structure of oxidized chars and their HA fraction from source materials other than JC a coniferous tree (softwood). For this purpose, various heating conditions and three different oxidants, namely, RuO_4, HNO_3, and H_2O_2, were applied to samples obtained from giant dogwood (GDW; *Swida macrophylla*) a general broadleaf tree (hardwood) and maize (*Zea mays*) stalks, as a representative annual crop residue.

Materials and Methods

Pyrolysis of plant materials

A GDW log with the bark removed and maize stalks were used in the study. The total C and N contents in the GDW sample were 462 and 1.5 g kg^{-1}, respectively, and the values for the maize sample were 406 and 17.7 g kg^{-1}, respectively. A 500 mg pulverized sample was spread evenly to a thickness of 5 mm in a porcelain crucible (45×36 mm), which was then covered with a lid to prevent direct access to O_2. The samples were pyrolyzed in triplicate in a muffle furnace under the following conditions: 225, 250, 275, 300, 350, 400, and 450°C for 1 h and 225, 250 and 275°C for 2 h. The pyrolyzed residues were weighed and the total C and N contents were determined using a NC analyzer (Sumigraph, NC-800, Shimadzu, Kyoto, Japan). IR spectra were also collected by the KBr method (FT/IR-7000S, JASCO, Tokyo, Japan). The ratio of sample to KBr was fixed at 1:150.

Oxidation of pyrolyzed plant materials with RuO_4, HNO_3, and H_2O_2

GDW samples that were pyrolyzed at 275°C for 2 h (GDW275/2), 300°C for 1 h (GDW300/1), and 400°C for 1 h (GDW400/1), and maize samples that were pyrolyzed at 225°C for 2 h (Maize225/2), 275°C for 2 h (Maize275/2), and 400°C for 1 h (Maize400/1) were used in the following experiments. These samples were oxidized using three methods generally following the same manners as reported by Sultana et al. (2011). In the case of RuO_4 oxidation, 50 mg of char and 500 mg of sodium periodate ($NaIO_4$; a co-oxidant) were suspended in a mixture consisting of 6.7 mL

chloroform, 6.7 mL acetonitrile, and 10 mL water, and 3.3–6.7 mg of $RuCl_3$ n-hydrate was then added. The suspension was stirred at 25°C for 16 h and dried at 40°C using a rotary evaporator. The duration of the reaction and the amount of $NaIO_4$ applied were determined based on the yield of HA-like substances and their degree of humification in preparative experiments using pyrolyzed maize samples (data not shown). The residue was suspended in water, dialyzed using a seamless cellulose tubing (Visking tubing, Viskase, Darien, USA) against distilled water, and freeze-dried.

In the case of HNO_3 oxidation, a 300 mg sample was suspended in 15 mL of 2.6 M HNO_3 in a 100-mL glass vial (20.5 cm × 25 mmφ), and the tightly capped vial was maintained at 104°C for 4 h in an oil bath. The residue was collected on a glass fiber filter (GB-140, ADVANTEC, Tokyo), repeatedly washed with distilled water, and dried.

In the case of H_2O_2 oxidation, a 300 mg sample was suspended in 2.5 M H_2O_2 (15 mL), gently shaken by hand, covered with aluminum foil, and placed in an incubator at 30°C. The suspension was agitated by hand after 1 week, and the residue was collected after 2 weeks in the same manner as used in the HNO_3 treatment.

Preparation of HA-like substances from pyrolyzed plant materials and determination of the degree of humification

Pyrolyzed residues of plant materials or those oxidized subsequent to the pyrolysis were placed in a centrifuge tube, and 0.1 M NaOH was added at a rate of 10 mL mg^{-1}. After purging the air from inside the tube, including that in the suspension, with a stream of N_2, the tube was capped tightly and shaken for 24 h at 25°C. The extract was collected by centrifugation (20,000×g for 15 min), and the HA fraction, after acidification to pH 1 and allowing it to stand overnight, was recovered as precipitate. The HA fraction was redissolved in 0.1 M NaOH, and the absorbance at 400 and 600 nm was measured immediately (UV-2400, Shimadzu). Another aliquot was diluted 6 fold with 0.066 M KH_2PO_4 (pH <6), and the C concentration was determined using a dissolved C analyzer (TOC-V$_{CPH}$, Shimadzu) after bubbling with N_2 to remove CO_2. Two variables that have been used for evaluating the degree of humification of soil HAs, $\log(A_{400}/A_{600})$, the ratio of absorbance at 400 nm to that at 600 nm in the

logarithmic scale, and A_{600}/C, absorbance at 600 nm per mg C mL^{-1}, were obtained (Ikeya and Watanabe, 2003). A smaller $\log(A_{400}/A_{600})$ and/or a larger A_{600}/C indicate a higher degree of humification. Powdered samples of HA-like substances were obtained from GDW400/1 and Maize400/1 after oxidation with HNO_3 by re-precipitating, dialyzing against distilled water using Visking tubing, and freeze-drying.

Measurement of X-ray diffraction profile

GDW400/1, Maize400/1, and the oxidized residues of GDW400/1 and Maize400/1 after the HNO_3 treatment as well as their HA fractions were examined. Since the XRD profile of Maize400/1 included several sharp peaks probably derived from metal oxides, which interfered with the analysis, the sample was washed with 1 M HCl by gently shaking at room temperature overnight before the measurement. A 24.2-mg sample was placed in a silicon holder (Overseas X-Ray Service, Saitama, Japan), and the XRD profile was measured using an X-ray diffractometer, XRD6100 (Shimadzu), under the following conditions: target, cupper Kα; wavelength, 0.154 nm; tube voltage/current, 40 kV/30 mA; scan mode, step scanning at 0.1°; and scan range, 2θ = 5–100° (counting time, 6 s) and 60–100° (counting time, 12 s). The 11 band profile was corrected for the absorption factor of C atom (Fujimoto and Shiraishi, 2004) and analyzed using a Carbon Analyzer DiHiGa Series 2007 (Ryoka System, Tokyo). A mixture of two series of C layer plane models starting from benzene/coronene and pyrene was adopted to calculate their theoretical scattering intensities (Fujimoto, 2003). The composition of the C layer planes on a weight basis was obtained by fitting the theoretical XRD profile to the observed value. The total amount of C layer planes in arbitrary units (A.U.) per mg of sample was evaluated from the theoretically calculated 11 band profile (base-line method) and expressed as relative values when the 11 band area of the GDW400/1 was regarded as 100 A.U. mg^{-1}. Experimental error was less than 6 A.U. mg^{-1}.

Results

Pyrolysis of plant materials

Figure 1 provides information on the amount and C and N contents of the GDW and maize residues after pyrolysis under various conditions, except for
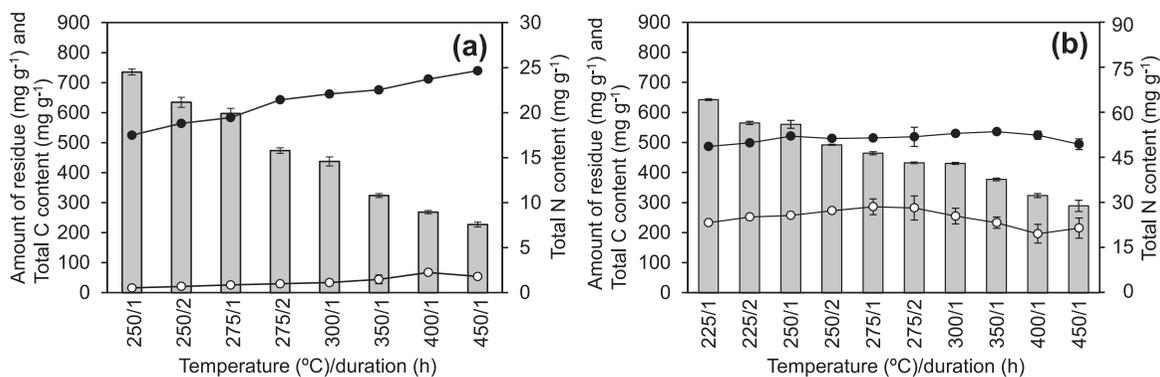
Figure 1. Amount and C and N contents of giant dogwood (a) or maize (b) residues after pyrolysis at various heating conditions. Bars, amount of residue; closed circles, C content; open circles, N content. Vertical lines indicate standard deviations ($n = 3$).

the GDW that was heated at 225°C for 1 or 2 h which did not show blackish color. The amount of residue decreased with increasing temperature and increasing duration of pyrolysis, from 735 to 227 mg g^{-1} for GDW and from 645 to 290 mg g^{-1} for maize. The C and N contents in the pyrolyzed GDW residues ranged between 525–740 mg g^{-1} and 0.5–2.2 mg g^{-1}, respectively. The C content of the GDW sample increased gradually with increasing temperature up to 450°C. The N content also increased up to a treatment temperature of 400°C and then decreased. The C and N contents in the pyrolyzed residues of maize ranged between 490–540 and 20–29 mg g^{-1}, respectively. Different from GDW, the C and N contents in maize tended to increase with increasing temperature up to 350°C and 275°C, respectively, and then decreased. As such, the C content was larger in the GDW samples than in maize when the pyrolysis conditions were same and the difference was larger at a higher temperature.

Figure 2 shows IR spectra of the raw and pyrolyzed materials of GDW. The spectral features of GDW250/1 were similar to that for the raw material, suggesting that the structure of the organic constituents were altered less. In the IR spectrum of GDW275/2, the absorption due to

polysaccharides at around 1050 cm^{-1} (alcoholic C–O) was nearly absent, while the absorptions at 1620 (conjugated C=C/C=O), 1720 (carboxyl C=O), and
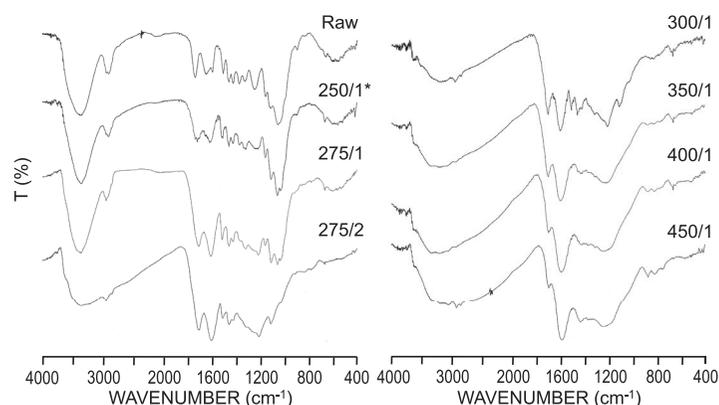
Figure 2. IR spectra of raw material and pyrolyzed residues derived from giant dogwood.
* Pyrolysis temperature (°C)/duration (h).
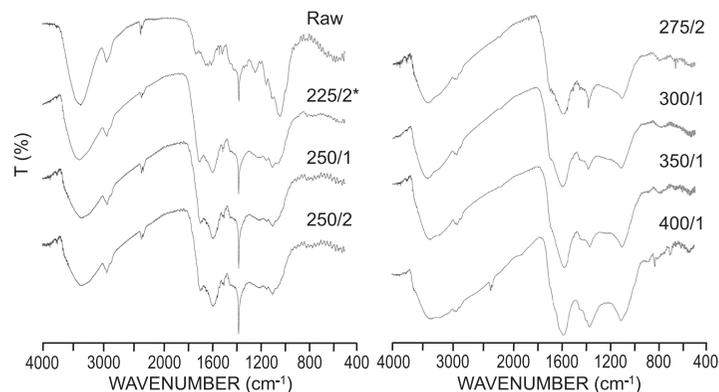
Figure 3. IR spectra of raw material and pyrolyzed residues of maize.
* Pyrolysis temperature (°C)/duration (h).

around 3100 (aromatic C–H) cm^{-1} were increased. The spectrum of GDW300/1 was similar to that of GDW275/2, and, for samples treated at higher temperatures, the sharp peaks at 1113, 1457, and 1510 cm^{-1} disappeared and the absorption at 1720 cm^{-1} was decreased.

The IR spectra of the maize samples (Fig. 3) showed that polysaccharide components were lost in the Maize225/2 sample, as indicated by the smaller absorptions at 1000–1100 cm^{-1} and those at around 3400 cm^{-1} (O–H) compared with the raw material and Maize225/1 (data not shown). On the contrary, the absorptions corresponding to aromatic structures at 1620 and around 3100 cm^{-1} increased with increasing pyrolysis temperature. The absorption at 1720 cm^{-1} increased at lower heating temperatures and then decreased in proceeding from Maize275/2 toward Maize400/1.

In a previous study (Sultana et al., 2011), three JC samples pyrolyzed at 275°C for 2 h, at 300°C for 1 h, and at 400°C for 1 h were used for the subsequent chemical oxidation, based on the yield and IR spectra of the pyrolyzed residues. In the present study, three GDW samples produced by the same pyrolysis conditions as the JC samples were examined. For the maize samples, Maize225/2 was selected as well as Maize275/2 and Maize400/1 because the yield was larger (567 mg g^{-1}) and the IR spectra indicated that a significant structural difference from the raw material existed, which was not found for the woody samples.

Oxidation of pyrolyzed plant materials

The recovery (percent residue) of the pyrolyzed plant materials after three oxidation treatments on a weight basis is presented in Table 1. H_2O_2 oxidation was the mildest, and 92–96% and 63–78% were recovered from the GDW and maize samples, respectively. The recovery after RuO_4 oxidation, 80–94% for the GDW samples and 30–73% for the maize samples, did not differ significantly from that for the H_2O_2 oxidation of the samples, except for Maize225/2 (30%), while the recovery after HNO_3 oxidation varied substantially among the samples, 11–92%. GDW400/1 was the most resistant to HNO_3 oxidation, followed by Maize400/1.

Table 1 Recovery of the pyrolyzed giant dogwood (GDW) and maize samples after chemical oxidation.

Sample	RuO_4 oxidation (%)	HNO_3 oxidation (%)	H_2O_2 oxidation (%)
GDW275/2	81 a	12 c	94 a
GDW300/1	80 a	25 b	92 a
GDW400/1	94 a	92 a	96 a
Maize225/2	30 c	11 d	63 b
Maize275/2	73 ab	16 d	64 ab
Maize400/1	72 ab	71 ab	78 a

Values followed by different character differ at P <0.01 within the same source material.

The recoveries for GDW400/1 and Maize400/1 after HNO_3 oxidation, 92% and 71%, were similar to those after oxidation with RuO_4 or H_2O_2.

The yields of HA-like substances from the pyrolyzed materials with and without subsequent chemical oxidation are compared in Fig. 4, taking the mass loss during the oxidation treatment into consideration (on a pyrolyzed material basis). Oxidation with RuO_4 resulted in no positive effect on the yield of HA-like substances, except for Maize275/2. The use of HNO_3 as an oxidant resulted in a dramatic increase in the yield of HA-like substances from Maize400/1 (599 mg C g^{-1} C) and GDW400/1 (539 mg C g^{-1} C). In the H_2O_2 treatment, the maximum yield of HA-like substances from the maize and GDW samples was found for Maize275/2 and GDW275/2 (598 and 652 mg C g^{-1} C), respectively. It is clear that the pyrolysis conditions for producing the largest yield of HA-like substances differed for HNO_3 and H_2O_2 as oxidants.

Figure 5 shows the $\log(A_{400}/A_{600})$–A_{600}/C diagram of the HA-like substances. The yield of HA-like substances from the unoxidized GDW400/1 was too small to permit the degree of humification to be
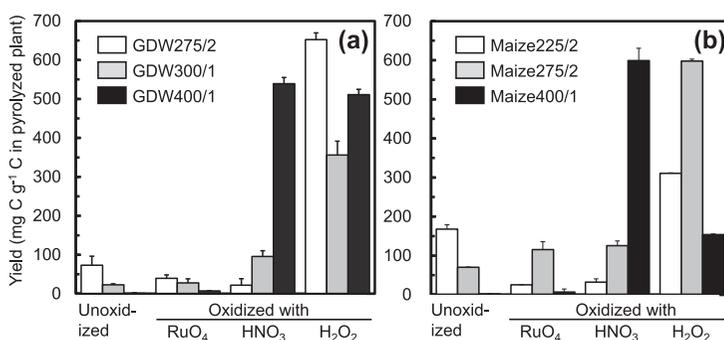
Figure 4. Yield of HA-like substances from pyrolyzed residues of giant dogwood (a) and maize (b) with or without subsequent chemical oxidation. Vertical lines indicate standard deviation (n = 3).
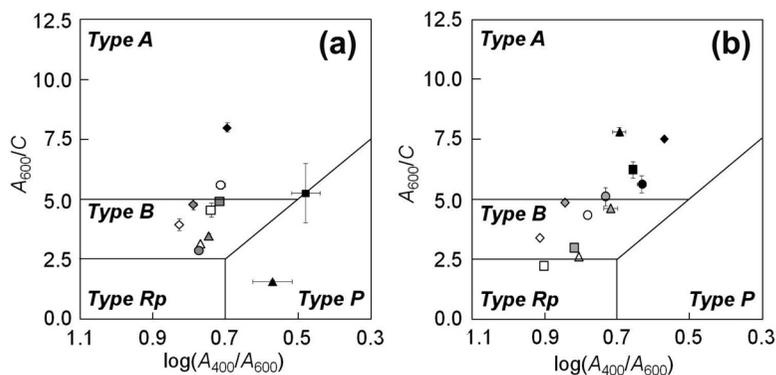
Figure 5. log(A_{400}/A_{600})–A_{600}/C diagram of HA-like substances obtained from giant dogwood (a) and maize (b) after pyrolysis (circles) and the subsequent oxidation with RuO$_4$ (triangles), HNO$_3$ (diamonds), or H$_2$O$_2$ (squares). White symbols, Maize225/2 and GDW275/2; gray symbols, Maize275/2 and GDW300/1; black symbols, Maize400/1 and GDW400/1. Vertical and horizontal lines indicate standard deviation (n = 3).

Maize225/2 (Fig. 5b). A similar trend was also observed after oxidation with the three reagents. The HA-like substances in five maize samples, four from Maize400/1 and one from Maize275/2, can be classified as Type A, and those in the Maize400/1 that had been oxidized with HNO$_3$ or RuO$_4$ showed the highest degree of humification.

The results obtained indicate that the combination of pyrolysis at 400°C for 1 h and oxidation with HNO$_3$ resulted in the largest yield of refractory HAs from both GDW and maize.

determined. The highest degree of humification was obtained for the HNO$_3$ oxidation of GDW400/1 (Fig. 5a). The HA-like substances in the HNO$_3$-oxidized GDW400/1 and those in the unoxidized GDW275/2 were classified as Type A in the Kumada classification (Kumada, 1987; Ikeya and Watanabe, 2003), and those in the H$_2$O$_2$-oxidized GDW400/1 were intermediate Types A and P, since they appeared on the border in the plot diagram.

The degree of humification of the HA-like substances in the unoxidized maize samples was higher in the order Maize400/1, Maize275/2, and

Size distribution of C layer planes in the pyrolyzed and oxidized plant materials and their humic acid fractions

Figure 6 shows the XRD profiles of GDW400/1, GDW400/1 oxidized with HNO$_3$, and HA-like substances in the HNO$_3$-oxidized GDW400/1. The same series of XRD profiles for Maize400/1 are shown in Fig. 7. The XRD profiles were similar among the samples and showed three peaks or shoulders at 2θ = 24° or 25° (002 band), around 40° (10 band), and around 80° (11 band). The theoretically calculated 11 bands are superimposed
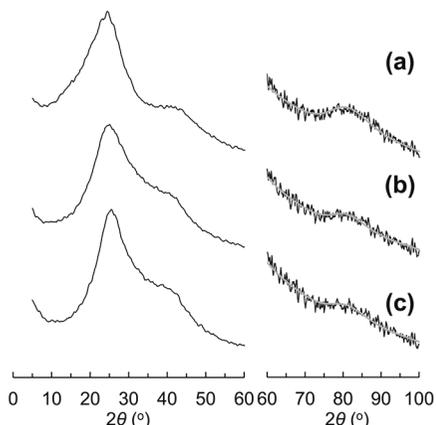
Figure 6. X-ray diffraction profiles of GDW400/1 (a), GDW400/1 oxidized with HNO$_3$ (b), and HA-like substances in GDW400/1 oxidized with HNO$_3$ (c).
Vertical scales in the profiles between θ = 60 and 100° (right ones) were expanded (×6). Gray lines indicate theoretical profiles.
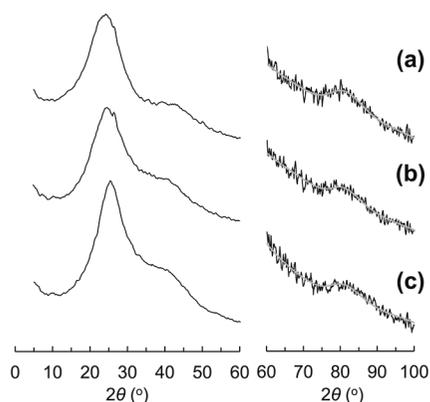
Figure 7. X-ray diffraction profiles of Maize400/1 (a), Maize400/1 oxidized with HNO$_3$ (b), and HA-like substances in Maize400/1 oxidized with HNO$_3$ (c).
Vertical scales in the profiles between θ = 60 and 100° (right ones) were expanded (×6). Gray lines indicate theoretical profiles.

Table 2 Relative content and size distribution of C layer planes in GDW400/1 and Maize400/1 before and after HNO$_3$ oxidation and HA-like substances (HA fr.) in the GDW400/1 and Maize400/1 oxidized with HNO$_3$.

Sample	Relative C layer plane content (A.U. mg^{-1})	Mean size of C layer planes (nm)	Weight proportion of each size (nm) of C layer plane (%)							
			0.48 (4)[*]	0.72 (7)	0.96 (14)	1.20 (19)	1.44 (30)	1.68 (37)	1.92 (52)	2.16 (61)
GDW400/1	100	0.83	30	28	18	14	7	3	0	0
GDW400/1 oxidized with HNO$_3$	44	1.06	13	20	20	20	14	10	3	0
HA fr. in GDW400/1 oxidized with HNO$_3$	39	1.04	11	19	25	24	13	8	0	0
Maize400/1	81	1.05	15	21	18	17	15	14	0	0
Maize400/1 oxidized with HNO$_3$	50	1.37	0	6	20	24	19	16	9	5
HA fr. in Maize400/1 oxidized with HNO$_3$	48	1.04	0	18	25	14	11	8	0	0

*Number of benzene rings in condensed aromatic structure models corresponding to respective sizes of C layer plane.

on the observed 11 bands. The results of an 11 band profile analysis are presented in Table 2. The relative content of C layer planes based on the 11 band area was the largest in the case of the GDW400/1 sample, followed by Maize400/1. When the C layer plane content in GDW400/1 is regarded as 100 A.U. mg^{-1}, the content in the Maize400/1 sample that was washed with 1 M HCl was 81 A.U. mg^{-1}.

The size of the C layer planes (Table 2) in GDW400/1 and Maize400/1 was distributed from 0.48 nm, corresponding to pyrene, to 1.68 nm, corresponding to a condensed aromatic structure consisting of 37 rings, with mean values of 0.83 and 1.05 nm, respectively. While the HNO$_3$ oxidation reduced the C layer plane content in GDW400/1 and Maize400/1 to 44 and 50 A.U. mg^{-1}, respectively, the mean C layer plane size was increased to 1.06–1.37 nm. The relative content and mean size of C layer planes in the HA-like substances obtained from the HNO$_3$-oxidized GDW400/1, 39 A.U. mg^{-1} and 1.04 nm, were similar to those for the HNO$_3$-oxidized GDW400/1. The values for the HA-like substances in the HNO$_3$-oxidized Maize400/1 were 48 A.U. mg^{-1} and 1.04 nm, respectively.

Discussion

The amounts and IR spectra of the pyrolyzed residues indicated that the charring and combustion of maize proceeded at lower temperatures than those for GDW (Figs. 1–3). However, the Maize225/2 and Maize275/2 samples were sensitive to oxidation with HNO$_3$ and RuO$_4$ (Table 1), and the degree of humification of their HA fractions were decreased by chemical oxidation (Fig. 5b). Before oxidation, the GDW275/2 and Maize275/2 samples contained a small amount (70–73 mg g^{-1} on C basis) of HA-like

substances that were classified as Type A, which may be related to the increased intensities of absorptions due to carboxyl C=O and aromatic C=C and C–H in the IR spectra. Although the H$_2$O$_2$ oxidation left 63–64% of the initial weight both for Maize225/2 and Maize275/2 with increasing the yield of HA-like substances (Fig. 4b), their degree of humification was decreased (Fig. 5b). Similar results were also observed for GDW275/2. Thus, partial charring at temperatures less than 300°C followed by chemical oxidation was not effective in terms of producing materials containing a larger amount of recalcitrant HA-like substances, even though the mass loss of plant materials was smaller.

The pyrolysis of GDW at 275°C for 2 h and that at 300°C for 1 h resulted in similar amounts of residue (Fig. 1a), and the IR spectra of the GDW275/2 and GDW300/1 samples (Fig. 2) were also similar. However, the recovery (Table 1) and the content (Fig. 4a) and degree of humification (Fig. 5a) of the HA fraction after oxidation with HNO$_3$ or H$_2$O$_2$ were different. A similar trend was also observed for JC (Sultana et al., 2011). The degree of humification of the HA-like substances in the GDW300/1 that had been oxidized with HNO$_3$ or H$_2$O$_2$ was larger than that in the corresponding GDW275/2 samples, respectively. However, both the yield and degree of humification of the HA-like substances after HNO$_3$ or H$_2$O$_2$ oxidation from GDW300/1 were smaller than those from GDW400/1.

GDW400/1 and Maize400/1 were nearly free of HA-like substances (Fig. 4), as evidenced by the decrease in the absorption due to carboxyl C=O in the IR spectra (Figs. 2 and 3). Although the HA-like substances in the Maize400/1 that had been oxidized with RuO$_4$ were classified as Type A (Fig. 5), the yield was quite small. Lastly, the RuO$_4$ treatment did

not greatly increase the amount of HA-like substances produced in any of the samples. The recovery after the RuO_4 oxidation was similar to those reported for coal, 74–87% (Stock and Wang, 1986). The formation of carboxyl groups and/or fragmentation into smaller molecules may have been insufficient to produce a residue that is soluble in an alkaline solution. Oxidation with HNO_3 and H_2O_2 resulted in the degradation of a large portion of GDW400/1 and/or Maize400/1 into HA-like substances. Although the yield of HA-like substances from the oxidized GDW400/1 was similar between the HNO_3 and H_2O_2 treatments, the HA-like substances produced during the H_2O_2 treatment were classified as Type P close to Type A. The HA-like substances produced in the H_2O_2-oxidized Maize400/1 are classified as Type A, but their yield was one-fourth that from the HNO_3-oxidized Maize400/1. As such, the HNO_3 treatment was the most effective oxidant for the degradation of plant materials, including a coniferous (JC; Sultana et al., 2011) and broadleaf (GDW) tree species as well as a grass species (maize), pyrolyzed at charring temperature (400^oC) into recalcitrant HA-like substances. These procedures yielded 144 and 194 mg of C of HA-like substances that can be classified as Type A from 1 g of a raw GDW and maize sample, respectively.

At higher heating temperatures the C content in the pyrolyzed residues was larger in the case of GDW than in maize, while the decrease in the amount of residue tended to be greater for GDW (Fig. 1), subsequently the proportion of C remaining at 400^oC was similar between GDW and maize, i.e., 41–42% of the initial value. According to Nguyen et al. (2010), the C layers in a biochar produced from maize at 350^oC were short and randomly arranged, compared with those produced from wood shavings of oak (Quercus ssp.) that were treated at the same temperature, as evidenced by transmission electron microscopy. However, the XRD 11 band profile analysis in the present study suggests that the development of condensed aromatic structures in the charred maize was comparable to that in the charred GDW (Table 2).

During the HNO_3 oxidation of GDW400/1 and Maize400/1, 38–56% of the C layer planes disappeared (Table 2). However, the mean C layer size was larger after HNO_3 oxidation, indicating that the smaller C layer planes were preferentially

degraded. For example, the pyrene sized (0.48 nm) C layer planes decreased to 0–20% of the initial amount. On the contrary, the sum of the amount of ≥1.44 nm C layer planes, corresponding to condensed aromatic structures consisting of ≥30 rings, taking the mass loss during chemical oxidation into consideration, was almost same before and after the HNO_3 treatment, namely, 10 versus 12 A.U. mg^{-1} for GDW400/1 and 24 versus 25 A.U. mg^{-1} for Maize400/1. The detection of larger C layer planes in the 11 band profile analysis of the HNO_3-oxidized samples, which were not observed in the unoxidized sample, might be the result of the improved resolution of peaks derived from the larger C layer planes due to a large and uneven loss of peaks derived from smaller C layer planes. Since the range and mean size of the C layer planes in the charred plant materials separated from three deep layer soils were 0.96–1.92 nm and 1.26–1.37 nm, respectively (Sultana et al., 2010), it is possible that pyrolysis products having smaller C layer planes may undergo degradation in soil faster than those having larger C layer planes.

The mean size of the C layer planes in the HA-like substances from the HNO_3-oxidized Maize400/1 (1.04 nm) was similar to that in the corresponding fraction from the HNO_3-oxidized GDW400/1. Those values, however, were smaller than the mean size of the C layer planes in the whole sample of HNO_3-oxidized Maize400/1 (Table 2), which may be limited by the solubility of the HA-like substances in water at a high pH. According to Ikeya et al. (2011), the range and mean size of C layer planes in soil HAs with a higher degree of humification (Type A) were 0.48–1.68 and 0.82–0.96 nm, respectively. Thus, the condensed aromatic components in the HA-like substances in the GDW400/1 or Maize400/1 samples that had been oxidized with HNO_3 would also be expected to be recalcitrant in soil.

Conclusions

The pyrolysis of GDW or maize at 400^oC for 1 h, followed by HNO_3 oxidation, resulted in the formation of a material that contained a large amount of Type A HA-like substances. In this context, pyrolysis at a lower temperature or oxidation treatments using H_2O_2 and RuO_4 were much less effective, even if total C loss was smaller. Different plant types, i.e., woody or grass species, were similar

in that both gave the maximum yield and the development of C layer planes of HA-like substances.

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