Predicting potential release of dissolved organic matter from biochars derived from agricultural residues using fluorescence and ultraviolet absorbance

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Abstract

A combination of fluorescence excitation-emission (EEM) spectrophotometry with parallel factor analysis (PARAFAC) and UV-Visible spectroscopy was used to assess dissolved organic matter (DOM) release by the wheat straw-derived biochar in various environmental conditions. The results showed that the concentration of dissolved organic carbon (DOC) extracted from the biochar ranged from 435 to 5000 mg kg⁻¹, and the high DOM release would occur under the high temperature and alkaline environmental conditions. Four humic acid-like substances (C1, C2, C4 and C5), one fulvic acid-like substance (C3) and one tryptophan-like substance (C6) were identified by PARAFAC model. The distribution of the PARAFAC components in DOM was distinctively different among various extracts, and mainly composed of humic acid-like and fulvic acid-like material (accounting 55-78% and 10-37%). The high temperature enhanced the high release of humic acid-like DOM from the biochar. Humification index (HIX) of DOM released from the biochar in various extracts at the temperature of 20 °C (0.99-1.09) was lower than that at the temperature of 60 °C (1.31-1.92).

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The results obtained in the present study would be beneficial to understand biochar's effects and processes in the possible environmental applications.

Keywords: Biochar; Dissolved organic matter; Fluorescence; Parallel factor analysis; Agricultural residues

1. Introduction

Water scarcity and soil deterioration resulted from rapid population growth, global climate change, and pollution are among the greatest environmental problems worldwide [1,2]. In order to secure water quality and improve soil quality, various technologies including physical, chemical and biological methods have been developed and applied in water and wastewater treatment and soil reclamation in the past decades [3,4]. Depending on its low cost and environmental sustainability, use of biochar has been recognized as the effective, promising and widely used approach, and has been received considerable interest in recent years [5,6].

Biochar, a fine-grained and porous substance, is the carbonaceous product that is obtained by pyrolysis of carbon-rich biomass under oxygen-limited conditions. Biochar has a large surface area (SA), high cation exchange capacity (CEC) and contain ample functional surface groups, e.g. carboxylic, phenolic, hydroxyl, carbonyl, and quinones, which are closely related with the origin of biochar and also significantly depended on pyrolysis conditions [7]. It has been shown that addition of biochar to soil can retain nutrients and sequester carbon and improve crop productivity in agriculture [3, 26]. Other possible potential benefits of biochar as a soil amendment have also been proposed, including mitigation of greenhouse gas emissions and removal of environmental pollutants, increased soil microbial activity [8]. Additionally, biochar application in environmental remediation as an effective adsorbent is reported [9]. A

number of investigations revealed that biochar can be used as a low-cost adsorbent to control pollutant migration in soils due to its strong sorption ability for a variety of pollutants [10,11]. Several studies also demonstrated the large sorption ability of biochar for different kinds of contaminants (such as dyes, pharmaceuticals and heavy metals) from water and wastewater [1,6,12]. These positive effects of biochar on the soil and water ecosystem are directly related to physical and chemical properties of biochar which significantly depends on the biomass source, pyrolysis conditions, and post- and pretreatments [13]. On the other hand, available pyrogenic dissolved organic matter (DOM) released from the biochar is distinctively different structures from organic carbon in the soil and water, and can be the key factor controlling the sorption-desorption and degradation of contaminants [6,14]. In addition, studies employing biochars have suggested that the DOM content of biochars range widely and be controlled by both biomass source and pyrolysis conditions [15], but also determined by the environmental conditions (e.g., temperature and pH) and the biological processes occurring in the water and soil [14]. To understand biochar's effects and processes on the possible environmental consequences that may accompany biochar amendments, extensive studies have been conducted to study the composition and characteristics of DOM released from biochar by, size-exclusion chromatography [16,17,18]. Smith et al. [16] used electrospray ionization fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) as a valuable analytical technique to analyze biochar water extracts at a molecular level, and found that the molecular composition of pinewood-derived biochar water extracts were unique carbohydrate ligneous components and sulfur containing condensed ligneous components. Liu et al. [17] evaluated the release of DOM from thirty-six biochars using ion chromatography and fourier transform infrared spectroscopy (FICR). Their

results showed that the C in the organic acids (OAs) represented 1 to 60% of the total dissolved organic (DOC) released and hydroxyl, aliphatic, and quinone were the predominant functional groups of all biochars [17]. In addition, Liquid Chromatography – Organic Carbon Detection (LC–OCD) as an automated size-exclusion form of chromatography has been used to characterize thermogenic humic acids, and the result indicated that LC–OCD had the capacity to provide a fingerprint of water-extractable organic carbon [18]. In recent years, fluorescence excitation-emission (EEM) spectrophotometry and parallel factor analysis (PARAFAC) have also emerged as a rapid and common technique for analyzing the composition of DOM, and offered the specific fluorescence information present in the sample [19]. Moreover, EEM-PARAFAC analysis has been successfully utilised as quantitative and qualitative measures of DOM across a range of natural and engineered systems including, lakes and rivers, wastewater, drinking water and marine systems [20,21,22]. However, a limited number of studies have reported the use of fluorescence analysis for the assessment of DOM released by the biochar potentially used as a soil amendment or applied in water and wastewater treatment processes. The major hypothesis of this study is that the quantity and quality of DOM released from biochars will change in various environmental conditions, and fluorescence EEM spectroscopy and PARAFAC analysis can be used to characterize and predict the DOM release from biochars.

Therefore, the aim of this study was to quantify the DOM release by the wheat straw-derived biochar extracted in various environmental conditions, and chemical characteristics of DOM released from the biochar were examined using UV-Visible spectroscopy. Furthermore, by combining with EEM-PARAFAC analysis, the components of DOM released by the biochar were identified, and the distribution of

the identified EEM-PARAFAC components was predicted for a better understanding of biochar's DOM release properties.

2. Material and Methods

2.1 Source and characterization of the biochar

The biochar used in this experiment was produced from a typical agricultural residue (wheat straw). The raw wheat straw was rinsed with tap water and air-dried at 35 °C before it was ground to <2 mm prior to pyrolysis. The raw material was pyrolyzed in a modified muffle furnace system under N₂ atmosphere with the temperature being raised to 500 °C at a rate of 10 °C/min and maintained at the peak temperature for 4 h. The biochar samples were then ground and sieved to <74 µm (200 mesh). Finally, the biochars were stored in the desiccator for later use. The used biochar was highly alkaline with the pH value of 10.3 and its specific surface area was 4.49 m² g⁻¹. The contents of C, H, O and N of the biochar were 53.9%, 4.1%, 27.2% and 1.1%, respectively, while the contents of major inorganic elements were K (1.0%), Si (0.6%), Ca (0.3 %), Mg (0.1%) and Na (0.02%). In addition, the molar H/C ratio, molar O/C ratio and polarity index [(O + N)/C] were 0.51, 0.41 and 0.48, respectively.

2.2 Batch extraction experiments

No standard DOM extraction methodology has been developed for biochars. Therefore, batch experiments were performed to investigate the DOM released by the wheat straw-derived biochar extracted in various environmental conditions (temperature and pH), which was used to estimate amount and characteristics of DOM likely to be released by biochar in the natural environment. The obtained biochar was dried at 60 °C for 2 h before the extraction experiment began. Each biochar sample was extracted in various extraction solutions made by adding 0.1 mol/L HCl

(pH 1.0), 0.1 mol/L NaOH (pH 13.0), 0.1 mol/L NaCl (pH 7.0) and deionized water (pH 5.5-7.0) independently. In addition, the effect of temperature on DOM release of biochar samples was assessed at 20 and 60 °C. All extraction experiments with five replicates were conducted with a solid liquid ratio of 1:10. About 1.0 g of each biochar sample was added to 20 mL of extraction fluid in 50 mL plastic centrifuge tubes and placed horizontally on a mechanical platform shaker (150 rpm) in the dark for 2 h. The suspensions were centrifugated at 16000 × g for 10 min, and then the supernatants were filtered through a 0.45 µm pore size membrane. Finally, the filtrate was used for the later DOM assays.

2.3 Analytical methods

The amount of DOM extracted from the biochar was calculated from the concentration of dissolved organic carbon (DOC) of the batch extraction samples, which was determined by the total organic carbon analyzer (TOC-L CPN, Shimadzu, Japan). In addition, in order to determine chemical characteristics of DOM released from the biochar, the UV absorption spectra was analyzed on a UV-Visible spectrophotometer (UV Probe-1780, Shimadzu, Japan) by using Milli-Q water to make base line, scanning 400-250 nm by 1 nm.

The fluorescence EEM and PARAFAC analyses were also conducted on all extracts. All samples were adjusted to a pH 7 before analysis, and the fluorescence EEM spectra were scanned by a fluorescence spectrophotometer (F97 Pro, Lengguang Tech., China). All the cuvettes were rinsed by 5% HNO₃ solution before analysis. Excitation and emission wavelength ranges were set from 200 to 550 nm and 250-600 nm respectively. Excitation and emission scanning intervals were set at 5 nm and 2 nm respectively. Scan speed was set at 1200 nm min⁻¹, and excitation and emission slit

widths were fixed at 10 nm. Photomultiplier detector voltage was fixed at 640 V.

PARAFAC was used to model the dataset of fluorescence EEMs. It statistically decomposes the complex mixture of DOM fluorophores into independent components about their spectral shapes or numbers, thus allowing the estimation of the true underlying EEM spectra between the samples. The approach of PARAFAC modeling to EEMs has been described in detail in previous studies [21,23]. In this study, DOM Fluor v.1.7 Toolbox for MATLAB was used to fit the PARAFAC model over a data set comprising all extraction samples. The correct number of components was identified by several methods of split half analysis, residual analysis, and visual inspection [23]. PARAFAC modeling was conducted with non-negativity constraint. No outliers were identified during the analysis.

2.4 Statistical analysis

MATLAB R2009a (Mathworks, USA) was used to perform the PARAFAC modeling task [23]. The indicator of specific UV absorbance (SUVA) was determined by dividing the absorbance a_{254} by the corresponding DOC concentration [21]. Slope ratio (S_R) of the shorter UV wavelength region (175-195 nm) to that of the longer UV wavelength region (350-400 nm) was determined [24]. Additionally, humification index (HIX) was calculated using the area under the Em spectra 435-480 nm divided by the peak area 300-345 nm + 435-480 nm, at Ex 254 nm [21]. Statistical analyses (mean value and standard deviations) were conducted via SPSS 13.0 software (SPSS Inc., Chicago, USA). The data were analyzed by a two-way analysis of variance (ANOVA), and the LSD-t test was used for significant difference testing. Significance levels were reported as non-significant ($p > 0.05$), significant ($p < 0.05$).

3. Results and Discussion

3.1 Variations and characteristics of DOM released from the biochar

Figure 1 presents the quantity of DOM released from the biochar in various extracts (T1: deionized water; T2: 0.1 mol/L HCl; T3: 0.1 mol/L NaOH; T4: 0.1 mol/L NaCl) at different temperatures (20 °C and 60 °C). The values of DOC extracted from the biochar were obviously variable, ranging from 435 to 5000 mg kg⁻¹. DOC of extraction samples were significantly influenced by different treatments, and the value of DOC from alkaline extractant was significantly higher than that from deionized water following by acidic extractant and salt extractant. This result may be due to large number of surface oxygen-containing functional groups (such as aliphatic ethers, carboxyl and alcohols) in biochars. Moreover, it can be concluded that DOM released from the biochar might primarily be attributed to the acid-rich portion of the biochar, while water-extractable DOM was relatively small. The results in this study also suggest that most of the DOM from the biochar was only released when the pH in the water and soil increased. This is in agreement with previous studies which found that amounts of DOC extracted from the biochar were strongly correlated with the chemical properties of acid functional group density and volatile matter content [15]. These biochar parameters might be inter-correlated and inversely related to micropore surface area, indicating that the material that fills biochar's micropores carried its acidity and also, apparently, contains the portion of organic matter in biochar most susceptible to leaching [25]. Additionally, Figure 1 shows a clear influence of different temperatures: all extractants extracted more DOC at temperatures of 60 °C than that at temperatures of 20 °C, which indicated that thermal conditions might result in a higher release of DOM in this experiment, causing

higher DOM solubility and the desorption of higher fixed carbon content from the biochar [22]. Two-way ANOVA also showed temperature and extraction independently had significant effects on the concentration of DOC released from the biochar (Table 1), and interactions were detected between two factors (temperature and extraction). Overall, from our experiment, temperature and pH of the extraction had an important effect on DOM release from the biochar. A statistically significant interaction between temperature and pH of the extraction on DOM content, which suggests the high release of DOM from the biochar will occur under the high temperature and alkaline environmental conditions when the biochar is potentially used as a soil amendment or applied in water and wastewater treatment processes. However, there would be potentially a possibility that DOM released from the biochar could change with repeated extraction experiments. On the other hand, DOM released from the biochar might vary depending on the extraction methods and extracting duration.

The additional analyses of $SUVA_{254}$ index and S_R were used to further study the chemical characteristics of DOM released from the biochar by UV-visible spectrophotometry (Figure 2). The average $SUVA_{254}$ of DOM released from the biochar in various extracts at temperatures of 20 °C was 4.7-78.9 L mg C⁻¹m⁻¹, an indication of higher aromaticity [21]. By contrast, the average $SUVA_{254}$ of released DOM at temperatures of 60 °C ranged from 0.2 L mg C⁻¹m⁻¹ to 4.7 L mg C⁻¹m⁻¹, indicating a lower aromaticity [21]. Moreover, the levels of $SUVA_{254}$ of released DOM were significantly variable in different extracts in this study. The S_R parameter of DOM released from the biochar in various extracts at temperatures of 20 °C ranged in 1.8 and 2.2, while the S_R value of released DOM at temperatures of 60 °C was observed to be 3.0-4.4. The S_R parameter was proposed as a proxy for DOM molecular weight,

i.e., high and low S_R values represent low and high molecular weight DOM [24]. Overall, the levels of $SUVA_{254}$ of released DOM were higher but the S_R value was lower in all extracts at temperatures of 20 °C, meaning that extraction samples had the characteristic of higher molecular weight and degree of humification than that at temperatures of 60 °C. According to previous studies, smaller aliphatic DOM molecules were more strongly sorbed to biochar than larger aromatic ones [26], and DOM could contain aromatic and aliphatic moieties along with hydrophilic and hydrophobic regions. Therefore, the change of environmental conditions (such as temperature and pH) would affect the desorption processes of DOM occurring in micropores and the characteristics of DOM released by biochar [14,27].

3.2 EEM–PARAFAC components of DOM released from the biochar

Similar PARAFAC components have been observed and described throughout the previous literature of DOM fluorescence, and a number of matching peaks were found from a review of literature and the OpenFluor database of PARAFAC aquatic fluorescence components [20, 21]. The components of DOM released by the biochar in different extraction solutions and temperatures in this study were identified based on the fluorescence EEM and PARAFAC analyses. As shown in Figure 3 and Figure 4, six fluorescent components (C1-C6) were identified by PARAFAC analyses based on the EEMs of all extraction samples in the present study, and contour plots of individual components from the 6-component PARAFAC model are also presented in Figure S1. The origins and properties of corresponding components are shown in Table 2. As seen in Table 2, the identified components comprised four humic acid-like substances (Component 1, Component 2, Component 4 and Component 5), fulvic acid-like substances (Component 3) and tryptophan-like substances (Component 6). The C1

component was characterized by the peak at 340 nm excitation with 426 nm emission wavelengths. This component was UVC/UVA humic acid-like, and was in agreement with the spectral features of PARAFAC components of terrestrial humic-like materials [28,29,30,31]. The C2 component was characterized by the peak at 395 nm excitation with 474 nm emission wavelengths, which was defined as a humic acid-like compound with high-molecular-weight [30,32]. It was similar to a humic acid-like structure of the biochar-borne dissolved organic carbon observed by Uchimiya et al. [22]. The EEM spectral characteristic of the C3 component was characterized by the peak at 465 nm excitation with 518 nm emission wavelengths. It was confirmed as an fulvic acid-like compound, which was also described as reduced humic-like compound correlated with anomeric, acetal and ketal C [32,33]. The C4 component was composed of two peaks with excitation maxima at 380 nm and 518 nm with emission at 590 nm. This component has also not been traditionally defined, but partially resembled a reported humic acid -like compound [32,34]. Two excitation maxima at 300 nm and 378 nm, at 490 nm emission were evident in the C5 component. These fluorescence characteristics could be categorized as UVA humic acid -like compound with low-molecular-weight [31,32]. The C6 component was characterized by the peak at 200 nm excitation with 294 nm emission wavelengths, which was confirmed as a tryptophan-like compound, which represented aromatic protein containing tryptophan and tyrosine as described in other previous reports [28,32,35].

3.3 Distribution of identified EEM–PARAFAC components

To better assess the difference and variability of DOM released from the biochar in various extracts at different temperatures, component distribution in different treatments was evaluated in terms of the fluorescence intensities. Figure 5 shows the

relative abundance of the six PARAFAC components in DOM released from the biochar in various extracts under different temperatures. As a whole, the distribution of the six PARAFAC components in DOM was observed to be distinctively different among various extracts in this study. Specifically, the relative abundance of six fluorescent components (C1-C6) of DOM in deionized water at the temperature of 20 °C accounted for 16.6%, 19.5%, 22.8%, 17.8%, 10.8% and 12.5%. The DOM composition from acidic extractant was dominated by humic acid-like material (C2) with 26.8% and fulvic acid-like material (C3) with 37.1%, respectively. However, there was a similar variation in PARAFAC component distribution in alkaline and salt extractant, and humic acid-like material (C1 and C2) and fulvic acid-like material (C3) was the main DOM composition. The relative abundance of humic acid-like components (C1 and C2) was 61% and 66.4% in alkaline and salt extractant respectively, while fulvic acid-like component (C3) was only 14.7% and 21.9% in alkaline and salt extractant respectively. It should be noted that the increased temperature could also affect the relative contribution of PARAFAC components. When temperature was 60 °C, the relative abundance of two components (C1 and C2) of DOM in deionized water significantly increased to 35.8% and 27.3%, representing a higher release of humic acid-like and fulvic acid-like material. However, the relative abundance of other components (C3-C6) decreased, suggesting a lower release of tryptophan-like material. The similar tendency was observed to in PARAFAC component distribution in alkaline and salt extractant when temperature increased. The tryptophan-like material (C6) became the relatively important component. For the PARAFAC component distribution of DOM in acidic extractant, the increased temperature was observed to raise the proportion of C1 component evidently, but reduced the relative abundance of C3 component (fulvic acid-like). Moreover, the

abundance of tryptophan-like component (C6) had a slightly decrease and accounted for 8.3%. In summary, the above results indicated that the different extracting conditions significantly changed the distribution of the different components of DOM released from the biochar, and the high temperature enhanced the high release of humic acid-like DOM from the biochar. This can be explained by the levels of humification index (HIX) [21]. As shown in Figure 6, HIX of DOM released from the biochar in various extracts at the temperature of 20 °C was low with the value ranging between 0.99-1.09. While the value of HIX of DOM extracted at the temperature of 60 °C was higher (1.31-1.92). The results in the present study were also demonstrated previously by Uchimiya et al. [14,22], who employed EEM-PARAFAC technique to quantify the remaining portions of amended biochar, and showed that DOC released by amended biochar during the course of a field trial was mainly fulvic and humic acid fractions which might be beneficial for sorption. Similarly, Jindo et al. [36] investigated the effect of biochar addition (10% v/v) to a manure composting matrix on the chemical composition of humic substances during the composting process, and the results of thermogravimetric and spectroscopic analysis indicated that biochar addition into the composting mixture caused a high C content in the humic moiety and an enhanced recalcitrant and stable chemical structure of humic substances. In particular, the light fraction of humic substances was reinforced by an increase in the functional groups and aromatic character [36]. Zhang et al. [37] investigated the effects of biochar as an amendment to sewage sludge composting dynamics by adding six dosage of biochar, and the results indicated that humic and fulvic acid 35-42% and 24-28% higher in the higher dosage of biochar amended treatments than other treatments. Moreover, it was concluded that higher dosage of biochar could reduce the length of the active phase and enhance the humification [37].

Overall, the present results indicated that EEM-PARAFAC can easily be incorporated as a characterization tool in studying the variation of chemical components in DOM released by the biochar [22, 25]. In addition, it can be used to predict the release and transform of DOM from the biochar in the future applicaion.

4. Conclusions

The DOM release by the wheat straw-derived biochar was significantly influenced by various extracts and temperature, and was increased with the rising of the pH and temperature. Six fluorescent components were found based on the EEM-PARAFAC analyses. Moreover, the distribution of the PARAFAC components was distinctively different among various extracts, and the high temperature enhanced the high release of humic acid-like DOM from the biochar. EEM-PARAFAC analyses combining with UV-Visible spectroscopy might offer a valid alternative to assess the chemical quality of DOM released from biochars in the possible environmental applications.

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References

- [1] Y. Yao, B. Gao, H. Chen, L. Jiang, M. Inyang, A.R. Zimmerman, X. Cao, L. Yang, Y. Xue, H. Li, Adsorption of sulfamethoxazole on biochar and its impact on reclaimed water irrigation, *J. Hazard. Mater.* 2012 (209-210) 408-413.
- [2] H. Wu, J. Zhang, H.H. Ngo, W. Guo, Z. Hu, S. Liang, J. Fan, H. Liu, A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation, *Bioresour. Technol.* 2015 (75C) 594-601.

- [3] A.R. Zimmerman, B. Gao, M.Y. Ahn, Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils, *Soil Biol. Biochem.* 2011 (43) 169-1179.
- [4] H.Wu, J. Fan, J. Zhang, H.H. Ngo, W. Guo, S. Liang, J. Lv, S. Lu, W. Wu, S. Wu, Intensified organics and nitrogen removal in the intermittent-aerated constructed wetland using a novel sludge-ceramsite as substrate, *Bioresour. Technol.* 2016 (210) 101-107.
- [5] M.H. Duku, S. Gu, E.B. Hagan, Biochar production potential in Ghana—A review, *Renew. Sust. Energ. Rev.* 2011 (15) 3539-3551.
- [6] D. Mohan, A. Sarswat, Y.S. Ok, C.U. Pittman Jr, Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent--a critical review, *Bioresour. Technol.* 2014 (160) 191-202.
- [7] A.J. Eykelbosh, M.S. Johnson, E.G. Couto, Biochar decreases dissolved organic carbon but not nitrate leaching in relation to vinasse application in a Brazilian sugarcane soil, *J. Environ. Manage.* 2015 (149) 9-16.
- [8] J. Lehmann, J. Gaunt, M. Rondon, Bio-char sequestration in terrestrial ecosystems – a review, *Mitig. Adapt. Strateg. Glob. Change.* 2006 (11) 403-427.
- [9] M.I. Al-Wabel, A. Al-Omran, A.H. El-Naggar, M. Nadeem, A.R.A. Usman, Pyrolysis temperature induced changes in characteristics and chemical composition of biochar produced from conocarpus wastes, *Bioresour. Technol.* 2013 (131) 374-379.
- [10] G. Cornelissen, O. Gustafsson, T.D. Bucheli, M.T.O. Jonker, A.A. Koelmans, P.C.M. Van Noort, Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation, *Environ. Sci. Technol.* 2005 (39) 6881-6895.
- [11] K. Kameyama, T. Miyamoto, T. Shiono, Y. Shinogi, Influence of sugarcane

- bagasse-derived biochar application on nitrate leaching in calcareous dark red soil, *J. Environ. Qual.* 2012 (41) 1131-1137.
- [12] M. Zhang, B. Gao, S. Varnosfaderani, A. Hebard, Y. Yao, M. Inyang, Preparation and characterization of a novel magnetic biochar for arsenic removal, *Bioresour. Technol.* 2013 (130) 457-462.
- [13] X. Chen, G. Chen, L. Chen, Y. Chen, J. Lehmann, M.B. McBride, A.G. Hay, Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution, *Bioresour. Technol.* 2011 (102) 8877-8884.
- [14] M. Uchimiya, I.M. Lima, K. Thomas Klasson, S. Chang, L.H. Wartelle, J.E. Rodgers, Immobilization of heavy metal ions (CuII, CdII, NiII, and PbII) by broiler litter-derived biochars in water and soil, *J. Agric. Food Chem.* 2010 (58) 5538-5544.
- [15] A. Mukherjee, A.R. Zimmerman, W.G. Harris, Surface chemistry variations among a series of laboratory-produced biochars, *Geoderma*, 2011 (163) 247-255.
- [16] C.R. Smith, R.L. Sleighter, P.G. Hatcher, J.W. Lee, Molecular characterization of inhibiting biochar water-extractable substances using electrospray ionization fourier transform ion cyclotron resonance mass spectrometry, *Environ. Sci. Technol.* 2013 (47) 13294-13302.
- [17] P. Liu, C.J. Ptacek, D.W. Blowes, W.R. Berti, R.C. Landis, Aqueous leaching of organic acids and dissolved organic carbon from various biochars prepared at different temperatures, *J. Environ. Qual.* 2015 (44) 684-695.
- [18] Y. Lin, P. Munroe, S. Joseph, R. Henderson, A. Ziolkowski, Water extractable organic carbon in untreated and chemical treated biochars, *Chemosphere*, 2012 (87) 151-157.
- [19] T. Anumol, M. Sgroi, M. Park, P. Roccaro, S.A. Snyder, Predicting trace organic compound breakthrough in granular activated carbon using fluorescence and UV

absorbance as surrogates, *Water Res.* 2015 (76) 76-87.

[20] N.P. Sanchez, A.T. Skeriotis, C.M. Miller, Assessment of dissolved organic matter fluorescence PARAFAC components before and after coagulation-filtration in a full scale water treatment plant, *Water Res.* 2013 (47) 1679-1690.

[21] Y. Wang, D. Zhang, Z. Shen, C. Feng, J. Chen, Revealing sources and distribution changes of dissolved organic matter (DOM) in pore water of sediment from the Yangtze estuary, *PLoS One*. 2013 (80) e76633.

[22] M. Uchimiya, Z. Liu, K. Sistani, Field-scale fluorescence fingerprinting of biochar-borne dissolved organic carbon, *J. Environ. Manage.* 2016 (169) 184-190.

[23] C.A. Stedmon, R. Bro, Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial, *Limnol. Oceanogr.* 2008 (6) 572-579.

[24] Y. Yamashita, N. Maie, H. Briceno, R. Jaffe, Optical characterization of dissolved organic matter in tropical rivers of the Guayana Shield, Venezuela, *J. Geophys. Res.* 2010 (115) 214-221.

[25] A. Mukherjee, A.R. Zimmerman, Organic carbon and nutrient release from a range of laboratory-produced biochars and biochar–soil mixtures. *Geoderma*, 2013 (193) 122-130.

[26] A. Smebye, V. Alling, R.D. Vogt, T.C. Gadmar, J. Mulder, G. Cornelissen, S.E. Hale, Biochar amendment to soil changes dissolved organic matter content and composition, *Chemosphere*. 2016 (142) 100-105.

[27] M. Keiluweit, P.S. Nico, M.G. Johnson, M. Kleber, Dynamic molecular structure of plant biomass-derived black carbon (biochar), *Environ. Sci. Technol.* 2010 (44) 1247-1253.

[28] H. Xu, H. Cai, G. Yu, H. Jiang, Insights into extracellular polymeric substances of cyanobacterium *Microcystis aeruginosa* using fractionation procedure and parallel

factor analysis, *Water Res.* 2013 (47) 2005-2014.

[29] A. M. McIntyre, C. Guéguen, Binding interactions of algal-derived dissolved organic matter with metal ions, *Chemosphere*, 2013 (90) 620-626.

[30] S.K. Ishii, T.H. Boyer, Behavior of reoccurring PARAFAC components in fluorescent dissolved organic matter in natural and engineered systems: a critical review, *Environ. Sci. Technol.* 2012 (46) 2006-2017.

[31] J.B. Fellman, E. Hood, R.G. Spencer, Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review, *Limnol. Oceanogr.* 2010 (55) 2452-2462.

[32] W. Chen, P. Westerhoff, J. A. Leenheer, K. Booksh, Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter, *Environ. Sci. Technol.* 2003 (37) 5701-5710.

[33] C.J. Williams, Y. Yamashita, H.F. Wilson, R. Jaffé, M.A. Xenopoulos, Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems, *Limnol. Oceanogr.* 2010 (55) 1159-1171.

[34] C. Santín, Y. Yamashita, X.L. Otero, M. A. Alvarez, R. Jaffé, Characterizing humic substances from estuarine soils and sediments by excitation-emission matrix spectroscopy and parallel factor analysis, *Biogeochemistry*, 2009 (96) 131-147.

[35] F. Lu, C.H. Chang, D.J. Lee, P.J. He, L.M. Shao, A. Su, Dissolved organic matter with multi-peak fluorophores in landfill leachate, *Chemosphere*, 2009 (74) 575-582.

[36] K. Jindo, T. Sonoki, K. Matsumoto, L. Canellas, A. Roig, M.A. Sanchez-Monedero, Influence of biochar addition on the humic substances of composting manures, *Waste Manag.* 2016 (49) 545-52.

Figure Captions:

Figure 1 DOC released from the biochar in various extracts (T1: deionized water; T2: 0.1 mol/L HCl; T3: 0.1 mol/L NaOH; T4: 0.1 mol/L NaCl) at different temperatures (20 °C and 60 °C). All values are given as mean $\pm$ s.d. from five replicates. Different letters indicate significant differences between different extraction treatments ($P < 0.05$). Asterisks indicate statistical differences between different temperature treatments ($p < 0.05$).

Figure 2 Specific UV absorbance (SUVA₂₅₄) and slope ratio (S_R) from UV absorbance of DOM released from the biochar in various extracts (T1: deionized water; T2: 0.1 mol/L HCl; T3: 0.1 mol/L NaOH; T4: 0.1 mol/L NaCl) at different temperatures (20 °C and 60 °C). All values are given as mean $\pm$ s.d. from five replicates. Different letters indicate significant differences between different extraction treatments ($P < 0.05$). Asterisks indicate statistical differences between different temperature treatments ($p < 0.05$).

Figure 3 EEM fluorescence spectra of DOM released from the biochar in different extracts (a: deionized water; b: 0.1 mol/L HCl; c: 0.1 mol/L NaOH; d: 0.1 mol/L NaCl) at 20 °C. Each EEMs was generated from five samples.

Figure 4 EEM fluorescence spectra of DOM released from the biochar in different extracts (a: deionized water; b: 0.1 mol/L HCl; c: 0.1 mol/L NaOH; d: 0.1 mol/L NaCl) at 60 °C. Each EEMs was generated from five samples.

Figure 5 Relative distribution of EEM-PARAFAC analysis components in DOM released from the biochar in various extracts (T1: deionized water; T2: 0.1 mol/L HCl; T3: 0.1 mol/L NaOH; T4: 0.1 mol/L NaCl) at different temperatures (20 °C and 60 °C). All values are given from five replicates.

Figure 6 Humification index (HIX) based on EEM-PARAFAC analysis of DOM

released from the biochar in various extracts (T1: deionized water; T2: 0.1 mol/L HCl; T3: 0.1 mol/L NaOH; T4: 0.1 mol/L NaCl) at different temperatures (20 °C and 60 °C). All values are given from five replicates. Different letters indicate significant differences between different extraction treatments ($P < 0.05$). Asterisks indicate statistical differences between different temperature treatments ($p < 0.05$).

Table 1 Summary of two-way ANOVA on the interactions between temperature (20 and 60 °C) and extractant (T1: deionized water; T2: 0.1 mol/L HCl; T3: 0.1 mol/L NaOH; T4: 0.1 mol/L NaCl) on the DOC released from the biochar.

Source of variation	DF	<i>F</i>	<i>P</i>
Temperature	1	130.148	< 0.001
Extractant	3	307.069	< 0.001
Temperature × Extractant	3	32.788	< 0.001

Table 2 Maxima locations and characteristics of the six components identified by fluorescence-PARAFAC analysis in this study and their comparison with previously identified components.

This study		Previous studies		References
Component	Ex/Em (nm)	Compound	Description and assignment	
C1	340/426	Humic acid-like	UVC+UVA humic acid-like	[23, 29, 30, 31]
C2	395/474	Humic acid-like	High-molecular-weight humic acid-like	[30, 32]
C3	465/518	Fulvic acid-like	Reduced humic-like, correlated with anomeric, acetal and ketal C	[32, 33]
C4	380 (515)/ 590	Humic acid-like	Unknown	[32, 34]
C5	300/378 (490)	Humic acid-like	UVA humic acid-like, low molecular weight humic acid	[31,32]
C6	200/294	Tryptophan-like	Aromatic protein containing tryptophan and tyrosine	[23, 32, 35]

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Figure 2 Specific UV absorbance ($SUVA_{254}$) and slope ratio (S_R) from UV absorbance of DOM released from the biochar in various extracts (T1: deionized water; T2: 0.1 mol/L HCl; T3: 0.1 mol/L NaOH; T4: 0.1 mol/L NaCl) at different temperatures (20 °C and 60 °C). All values are given as mean $\pm$ s.d. from five replicates. Different letters indicate significant differences between different extraction treatments ($P < 0.05$). Asterisks indicate statistical differences between different temperature treatments ($p < 0.05$).

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Figure 6 Humification index (HIX) based on EEM-PARAFAC analysis of DOM released from the biochar in various extracts (T1: deionized water; T2: 0.1 mol/L HCl; T3: 0.1 mol/L NaOH; T4: 0.1 mol/L NaCl) at different temperatures (20 °C and 60 °C).

All values are given from five replicates. Different letters indicate significant differences between different extraction treatments ($P < 0.05$). Asterisks indicate statistical differences between different temperature treatments ($p < 0.05$).