Fluorescence regional integration combined with parallel factor analysis to quantify fluorescent spectra for dissolved organic matter released from manure biochars

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Dissolved Organic Matter (DOM) in biochars is important to carbon dynamics and contaminant transport in soils. Fluorescence excitation-emission matrices (EEMs) have been widely used to characterize dissolved organic matter (DOM). In this study, fluorescence regional integration (FRI) and parallel factor analysis (PARAFAC) applied to EEM allows good quantitative assessment of the composition of DOM derived from manure biochars. Manure biochars were produced using four types of manure, chicken, pig, cow, and sheep manure under various pyrolysis temperatures (300–600 °C) and holding times (0–120 min). The results from the determination of dissolved organic carbon (DOC), SUVA254, and humification index (HIX) reflected that high pyrolysis temperature and long holding time led to a significant decrease in DOM quantity, aromaticity and humification. The FRI result showed that the pyrolysis process of DOM released from manure biochars included three changes, aromatic protein-like substance and microbial by-product-like substance generation (300–600 °C), fulvic-acid-like substance decomposition (300–500 °C) and humic acid-like substance decomposition (600 °C). The PARAFAC modeling result showed that the pyrolysis process of DOM released from manure biochars contained two changes: three high molecular-weight humic-like substances decomposition and a low molecular-weight humic-like substance generation. The effect of the holding time on biochar-DOM is more significant at higher pyrolysis temperatures than lower pyrolysis temperatures. The correlation analysis result revealed that the generation of aromatic proteins, microbial by-products and fulvic acid came from the decomposition of humic-like substances including marine humic-like, UVA humic-like, and UVC + UVA humic-like substances. The results obtained in this study would be beneficial to guide the rational production and application of manure biochars.

Introduction

With the development of animal husbandry, the output of livestock manure is increasing. Currently, China’s annual output is approximately 4 billion tons. The chemical oxygen demand of livestock manure is 12.68 million tons, and there are many related pathogenic microorganisms, antibiotics, heavy metals and other pollutants. Therefore, if the excrement of livestock and poultry is left in the environment without treatment, it will present a serious burden to the environment. Manures can be considered as organic fertilizer for soil improvement due to their high content of nutrients, such as carbon, nitrogen, and phosphorus. Composting is a simple way to produce organic fertilizer from livestock and poultry manure.1,2 Composting uses microorganisms to ferment and degrade organic matter, kill pathogenic microorganisms and remove odours. However, composting cannot completely degrade antibiotics in manure,3 and a risk of heavy metal pollution remains.4 Therefore, methods for recycling the excrement of livestock and poultry more efficiently and in an environmentally friendly manner has remained a focus of scientific researchers.

In recent years, biochar has been paid increasing attention for soil amendment or wastewater treatment materials.5,6 Biochar is a form of organic matter with a high carbon content, aromaticity, and stability, which is produced under high temperatures and anaerobic conditions. Pyrolysis of animal manure produces biochar with multiple beneficial uses for improving soil quality and the environment. Compared with biochar from straw and lignin, biochar from livestock manure has a higher pH value and nutrients content,7 which can effectively counteract soil acidification and supplement nutrients to poor soil.
Dissolved Organic Matter (DOM) is a heterogeneous mixture of aromatic and aliphatic organic compounds containing oxygen, nitrogen, and sulphur functional groups. In terms of operation definition, DOM refers to the part of the mixture that can dissolve in water and cannot pass through a 0.45 μm filter membrane. The impact of DOM on ecosystems is very important because it involves multiple processes related to ecosystem balance. DOM is beneficial to the nutrient uptake of plants and promotes the biochemical cycle of carbon. Conversely, the migration or bioconcentration of pollutants can be promoted by DOM due to the strong complexation of DOM with organic pollutants or heavy metals. The environmental behaviour of DOM is closely related to the concentration and chemical composition of DOM. By tracking DOM, one can better predict the impact of DOM on natural processes. To obtain useful information about the production and application of biochar, it is necessary to characterize the DOM released from biochar. However, much plant biochar DOM has already been characterized. The composition of manure-derived biochar has not yet been understood. It is known that there are significant differences between the chemical composition of animal manure DOM and plant DOM. Accordingly, it is necessary to investigate the composition of DOM from manure biochar.

Fluorescence excitation-emission matrices (EEMs) spectroscopy is widely used to identify DOM in natural or engineering systems. The rich fluorescence information of EEMs can provide key composition information related to DOM. Fluorescence Regional Integration (FRI) was proposed to obtain quantification information of DOM EEMs. It was developed on the basis of the traditional peak method. According to the research purpose, the EEMs spectrum was artificially divided into different regions, and the change of fluorescent substances was quantitatively characterized by calculating the volume percentage of the given region. FRI can comprehensively analyse the information from the total fluorescence spectrum, and comparing the results of different studies is simple owing to the standardized division and nomenclature. The disadvantage of FRI is that it cannot solve the problem of overlapping fluorescence peaks. Parallel factor analysis (PARAFAC) can be used to deconvolute complex EEMs into the dominant fluorescent DOM components, which can realize chemometric separation of complex fluorescence spectra and give quantitative changes. It has been widely used to characterize DOM in natural ecosystem and laboratory samples. However, there is no uniform standard for the definition and name of fluorescent components in the PARAFAC modelling method, which makes it difficult to compare the results of different studies.

Therefore, this study intends to combine FRI and PARAFAC to characterize DOM released from manure derived biochar to get the quantitative information better. The main objective of this study is to explore the effect mechanism of various pyrolysis temperatures and holding times on the composition of the DOM of manure-derived biochar.

Materials and methods
Biochar production
Seven kinds of manure were selected as raw materials of biochar, which include two chicken manures, one pig manure, two cow manures, and two sheep manures. These manures were acquired from several farms in Meizhou City, Guangdong Province, China. Raw manures were air-dried naturally followed by drying at 105 °C in an oven. The dried manures were crushed and passed through a 10-mesh sieve. The prepared manures were pyrolyzed in a tubular furnace (OTL1200, Nanjing University Instrument Factory, China) with a heating rate of 10 °C min⁻¹ and a nitrogen concentration of 500 cm³ min⁻¹. 112 biochar samples were prepared at four pyrolysis temperatures (300 °C, 400 °C, 500 °C, 600 °C) and four holding times (0 min, 30 min, 60 min, 120 min). The biochars were stored in a vacuum dryer for subsequent determination.

DOC and UV-vis analyses
The DOM of 7 raw manures and 112 biochars were extracted by ultra-pure water with the water-solid ratio of 100 : 1 v/m. The mixtures were shaken in a water bath at 25 °C for 24 hours and then filtered with a 0.45 μm filter membrane. The DOM samples were analysed for dissolved organic carbon (DOC) using a TOC/TN analyser (TOC-VCPH, Shimadzu, Japan). UV-visible absorbance spectra on each DOM sample were collected using a UV-vis spectrometer (UV2000, Shimadzu, Japan), within 1 cm quartz cuvettes, with a wavelength of 200 to 600 nm, and Millipore water as the blank. The specific UV absorbance at 254 nm (SUVA254), as an indicator of aromaticity, was determined by dividing the UV absorbance measured at 254 nm by the sample’s DOC concentration.

EEMs analysis
The DOM sample was diluted to 10 mg L⁻¹ for the fluorescence determination. If the DOC concentration of DOM sample was lower than 10 mg L⁻¹, the original solution was determined for the fluorescence determination. The fluorescence EEMs spectra of 119 DOM samples were determined using a fluorescence spectrophotometer (LS55, Perkin Elmer, USA). The scanning range of excitation spectrum and emission spectrum is 200–500 nm and 300–600 nm, respectively.

The excitation wavelengths and emission wavelengths were incrementally increased at 10 nm and 0.5 nm steps, respectively. The pH of all DOM samples was adjusted to 7 by adding 0.1 M HCl or NaOH.

The data of the EEM spectra were analysed by FRI which delineated EEM into five excitation-emission regions based on the fluorescence of model compounds. Fluorescence intensity was integrated beneath each of the five EEM regions. The percent fluorescence response (P₁,n) were calculated for reference. The data of the EEM spectra were analysed by PARAFAC modelling. The excitation wavelengths were restricted in the range of 250–500 nm for PARAFAC analysis. The PARAFAC modelling was conducted with MATLAB 2016a software with...
DOM Fluor toolbox (V1.7). The models were based on the fluorescence intensity and proportional distribution of the four PARAFAC components.

Results and discussion

DOC, SUVA_{254}, and HIX

**DOC.** The effect of pyrolysis temperature and holding time on DOC concentration was shown in Fig. 1. The DOC concentration of seven raw manures differed within the range of 4.57–18.70 g C kg\(^{-1}\) of manure. The average DOC concentration was 10.08 g C kg\(^{-1}\) of manure. The biochar-DOC concentrations were below the average manure-DOC concentration. ANOVA analysis showed that there was a significant effect of pyrolysis temperature and holding time on the DOC concentration of biochar (\(p < 0.05\)). When the holding time was 0 min, the effect of pyrolysis temperature on the DOC concentration was small. When the holding time was more than 30 min, DOC concentration decreased significantly with pyrolysis temperature increasing. This indicated that the decomposition of manure DOM required a specific period under any pyrolysis temperature. When the pyrolysis temperature was higher than 400 °C, the DOC concentration changed a little between 30–120 min holding time, which showed that the DOM component decomposed quickly and achieved stability at 600 °C. The DOC concentration of DOM released from high temperature biochar was lower than the DOM released from low temperature biochar, which has been reported in many studies.\(^{13,20,21}\)

**SUVA\(_{254}\).** The effect of pyrolysis temperature and holding time on DOM SUVA\(_{254}\) was shown in Fig. 2. SUVA\(_{254}\) has a significant positive correlation with the aromatic carbon in DOM, which is used to indicate the aromaticity of DOM.\(^{22,23}\) The SUVA\(_{254}\) value of DOM released from raw manure was between 4.75–7.18 L mg\(^{-1}\) m\(^{−1}\). This indicated that the manure DOM had medium aromaticity and medium hydrophobicity.\(^{24}\) The SUVA\(_{254}\) value of biochar-DOM was lower than that of manure-DOM, which may be due to the decomposition of the aromatic components in manure-DOM in the pyrolysis process to produce simple, non-aromatic substances. It is worthy of note that the change of the SUVA\(_{254}\) value under pyrolysis condition was not entirely consistent with the change of DOC concentration. The decrease of DOC concentration at 500 °C was clear, but the change of the SUVA\(_{254}\) value at 500 °C was not significant. This suggested that the decrease of DOC concentration at 500 °C was predominantly due to the decomposition of non-aromatic components, while at 600 °C it was predominantly due to the decomposition of aromatic components.

**HIX.** The effect of pyrolysis temperature and holding time on DOM. HIX was shown in Fig. 3. The ratio of fluorescence intensity in the range of an excitation wavelength of 254 nm, an emission wavelength between 435–480 and an emission wavelength 300–345 nm was used to reflect the humification index (HIX) of the DOM.\(^{25}\) The HIX value of DOM was 7.47–25.87 and the average value was 15.39, which demonstrates that the manure was not fresh, because the HIX value of fresh manure is...
less than 5. The HIX values in soil and sediment are 10–30 and 6–20, respectively. This demonstrated that the humification degree of the raw manure used in this study was close to that of soil and sediment. The HIX value of biochar-DOM was lower than that of raw manure, indicating that the humification degree of biochar DOM was lower than the raw manure. The effect of holding time on the HIX value was little when the pyrolysis temperature was in the range of 300 °C to 500 °C. When the pyrolysis temperature reached at 600 °C, the HIX value decreased obviously with the holding time increasing.

According to the changes of DOC, SUVA₂₅₄ and HIX values, the DOC concentration, aromaticity, and humification of biochar were lower than that of manure. Under a pyrolysis temperature of 300–400 °C, the concentration, aromaticity, and humification of biochar-DOM did not significantly change. Under a pyrolysis temperature of 500 °C, the DOC concentration decreased significantly, the SUVA₂₅₄ value changed slightly, and the HIX value increased significantly. This indicated that the pyrolysis process at 500 °C involved the decomposition of non-aromatic substances and an increase of humic substances. When the pyrolysis temperature was 600 °C, the decrease of the three parameters suggested the decomposition of all DOM components.

Fig. 4 Percentage distribution of biochar-DOM fractions from FRI analysis: (a) schematic diagram of fluorescence area division; (b) the percentage of fluorescence response of aromatic protein I; (c) the percentage of fluorescence response of aromatic protein II; (d) the percentage of fluorescence response of fulvic acid like substances; (e) the percentage of fluorescence response of microbial by-product–like substances; (f) the percentage of fluorescence response of humic acid-like substances.
The whole EEMs spectrum was divided into five regions by the FRI method in accordance with the literature. The five regions were assigned as region I-aromatic protein I, region II-aromatic protein II, region III-fulvic acid-like, region IV-microbial by-product-like, and region V-humic acid-like (Fig. 4a). The
percentage of fluorescence response in a specific region \( (P_i) \) was calculated as the ratio of the EEM volume in the specific region and the EEM volume in the entire EEM (Fig. 4b-f).  

As shown in Fig. 4b, c, and e, the \( P_{II} \), \( P_{III} \), and \( P_{IV} \) of DOM released from any pyrolysis conditions was higher than that of raw manure-DOM, which indicated that aromatic protein substances and microbial by-product substance were generated during the pyrolysis process, and particularly at 600 °C. The \( P_{III} \) of DOM released from biochars produced at 300–500 °C (including 600 °C = 0 min) was lower than that of raw manure DOM. The \( P_{III} \) of DOM released from biochars produced at 600 °C was higher than that of raw manure DOM. This indicated that the fulvic acid-like substance decomposed at lower pyrolysis temperatures and was produced at high pyrolysis temperatures. The \( P_{V} \) of biochar-DOM was higher than that of raw manure-DOM when the pyrolysis temperature was between 300–400 and was lower at 600 °C. This indicates that the humic acid-like substance was generated at lower temperatures and decomposed at high pyrolysis temperatures. It has been found that there was a significant relationship between molecular weight and its fluorescence characteristics.  

The molecular weight of protein-like substance was usually less than 0.5 kDa, and the peak molecular weight of fulvic acid and microbial by-product was 0.5–1 kDa, while the molecular weight of humic acid was usually greater than 1 kDa. Therefore, the pyrolysis process at lower pyrolysis temperature was accompanied with the decomposition of medium molecular weight into lower molecular weight and the formation of higher molecular weight.

In summary, at 300–500 °C, the fulvic acid-like substance was the main decomposed component. Aromatic protein-like substance, microbial by-product-like substance, and humic acid-like substance were produced. At 600 °C, humic acid-like substance was the main decomposed component which generated the aromatic protein-like substance, the microbial by-product-like substance, and the fulvic acid-like substance.

**PARAFAC analysis**

In this study, 7 fluorescence EEM spectra of raw manure-DOM, and 119 fluorescence EEM spectra of manure biochar-DOM were acquired. The excitation ranges were restricted to 250–500 nm to avoid the deteriorating signal/noise ratio in the low excitation wavelength region for PARAFAC analysis.

Four fluorescent components (C1–C4) were identified by PARAFAC modelling, and the EEM contours of each individual component were illustrated in Fig. 5. As reported in the literature, the C1 component \( (\lambda_{Ex}/\lambda_{Em} = 310/404 \text{ nm}) \) was categorized as UVA marine humic-like,  

C31, and terrestrial humic-like (665–1000 Da),  

microbial humic-like,  

and humic-like highly processed terrigenous.  

C32 The C2 component \( (\lambda_{Ex}/\lambda_{Em} = 350/436 \text{ nm}) \) was assigned a microbial reduced quinone-like component,  

C34 UVA humic acid-like,  

C33 microbial humic-like, and humic-like less processed terrigenous.  

C34 The C3 component \( (\lambda_{Ex}/\lambda_{Em} = 340/383 \text{ nm}) \) was ascribed microbially-processed substance.  

C32 The C4 component \( (\lambda_{Ex}/\lambda_{Em} = 260, 400/490 \text{ nm}) \) was categorized as UVC + UVA humic-like,  

C33 UVA humic-like, terrestrial reduced quinone-like component,  

C31 and humic-like > 1000 Da. Therefore, according to the literature the C1–C4 components were assigned as: C1-marine humic-like, C2-UVA humic-like, C3-microbial by-product, C4-UVC + UVA humic-like.

The relative distribution of the four PARAFAC components in each DOM sample was calculated in accordance with the literature (Fig. 5).  

The average proportional distribution of component1 (C1%), C2 (C2%), C3 (C3%), and C4 (C4%) in DOM released from raw manure was 38.0%, 34.0%, 5.52%, and 22.4%, respectively. It should be noted that a high proportion of marine humic-like substance was found in manure-DOM. This may be due to the existence of some similar components in the marine DOM and manure-DOM. The composition of marine DOM includes mainly protein and polysaccharide substances, metabolized by phytoplankton, which also exist in manure-DOM.

As shown in Fig. 5, the effect of pyrolysis temperature and holding time on C1% and C2% was similar. When the

| Table 1 | Pearson correlation among SUVA254 and fluorescence related indices of DOM released from the biochar produced under different pyrolysis conditions (n = 112)a,b |
|-----------------|--------------|-------|-------|-------|-------|-------|-------|-------|-------|
| SUVA254 | HIX | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 |
| SUVA254 | 1.000 | 0.268 | -0.525 | -0.791 | -0.661 | -0.634 | 0.320 | 0.654 | 0.749 | -0.244 | 0.702 |
| HIX | 0.268 | 1.000 | 0.648 | 0.873 | 0.855 | 0.708 | -0.873 | -0.959 | -0.801 | -0.868 | 0.039 | 0.740 |
| P1 | -0.525 | 0.648 | 1.000 | 0.873 | 0.855 | 0.708 | -0.873 | -0.959 | -0.801 | -0.868 | 0.039 | 0.740 |
| P2 | -0.791 | 0.873 | 0.873 | 1.000 | 0.866 | 0.810 | -0.959 | -0.801 | -0.868 | 0.039 | 0.740 |
| P3 | -0.661 | 0.855 | 0.866 | 0.866 | 1.000 | 0.535 | -0.787 | -0.550 | -0.641 | -0.058 | 0.628 |
| P4 | -0.634 | -0.194 | 0.708 | 0.810 | 0.535 | 1.000 | -0.935 | -0.777 | -0.884 | -0.136 | 0.470 |
| P5 | 0.235 | 0.312 | 0.783 | 0.810 | 0.535 | 1.000 | 0.386 | 0.907 | 0.099 | 0.619 |
| C1 | 0.654 | 0.161 | -0.598 | -0.801 | -0.550 | -0.777 | 0.806 | 1.000 | 0.913 | -0.167 | 0.602 |
| C2 | 0.235 | 0.266 | -0.695 | -0.866 | -0.641 | -0.884 | 0.907 | 1.000 | -0.048 | 0.649 |
| C3 | -0.288 | 0.363 | -0.358 | -0.039 | -0.058 | -0.136 | 0.999 | -0.167 | -0.048 | 1.000 | 0.561 |
| C4 | 0.304 | 0.149 | -0.426 | -0.740 | -0.628 | -0.470 | 0.619 | 0.669 | 0.640 | -0.561 | 1.000 |

a Green color: positive correlation is significant at the 0.001 level.  
b Yellow color: negative correlation is significant at the 0.001 level.
pyrolysis temperature was 300–400 °C, the C1% and C2% of biochar-DOM were generally higher than that of raw manure-DOM, which indicated that the low-temperature pyrolysis increased the proportion of marine humic-like and UVA humic-like components. When the pyrolysis temperature was 500–600 °C, the C1% and C2% of biochar DOM were basically lower than that of raw manure-DOM which indicated that marine humic-like and UVA humic-like components could not resist high-temperature pyrolysis. The C3% of DOM released from biochars produced at any pyrolysis conditions was higher than that of raw manure-DOM. This indicated that the microbial by-product could be generated under different pyrolysis conditions. The optimum condition for C3 production was 600 °C for 60 min. The C4% of DOM released from biochars produced at all pyrolysis conditions was lower than that of raw manure-DOM. The decrease of C4% at 300 °C at 0 min indicated that the UVC + UVA humic-like substance decomposed easily, even at low-temperatures with a short holding time. The longer emission wavelength of the C4 component indicated that it contained higher molecular weight and more hydrophobic components. The molecular weight of C4 is usually greater than 1000 Da, which is generally defined as reducing quinone like components. The decomposition of C4 and the generation of C3 indicated that the high molecular weight DOM has been decomposed into low molecular weight substance.

In summary, the pyrolysis temperature and holding time have a significant effect on the composition of DOM released from manure-derived biochars. The high-weight-molecular humic-like substance (C4) decomposed, and the low-weight-molecular microbial by-products (C3) were generated in the entire pyrolysis temperature range. The medium-weight-molecular humic-like substance (C1 and C2) was generated at low-temperature and decomposed at high temperature.

**Relationship between DOM composition indices**

Correlation analysis of SUVA254, HIX, P_i% and C_i% was employed to elucidate the interactions between different DOM composition indices (Table 1). As shown in Table 1, SUVA254 had a positive correlation with P_i%, C1%, C2% and C4% (p < 0.001). It indicated that humic like substances owned higher aromaticity. HIX had a positive correlation with P_i% and C3% (p < 0.001), and had a negative correlation with P1%, P2% and P3% (p < 0.001). It indicated that the decrease of HIX with pyrolysis temperature increasing was due to the decomposition of humic-like substances and the production of fresh fluorescences including aromatic proteins and microbial by-product substance. P1%, P2%, P3% and P4% had negative correlation with C1%, C2% and C3%, which indicated that the generation of aromatic proteins, microbial by-product and fulvic acid are come from the decomposition of humic –like substances including marine humic-like, UVA humic like, UVC + UVA humic-like substances. The Pearson correlation result indicated that the FRI method and PARAFAC modelling both could elucidate the effect of pyrolysis temperature and holding time on biochar-DOM composition.

### Table 2 Exponential regression analysis of DOM composition parameters (i.e., SUVA254, P_i%, C_i% and DOC of different biochar samples (n = 112))

| Parameter | Fitting equation | R² |
|-----------|------------------|----|
| SUVA254   | $Y = 4.4e^{0.5x}$ | 0.589** |
| P_i       | $Y = 52.1e^{-2.2x}$ | 0.239** |
| P_II      | $Y = 194.9e^{-2.7x}$ | 0.614** |
| P_III     | $Y = 399.3e^{-2.7x}$ | 0.324** |
| P_IV      | $Y = 220515.7e^{-16.3x}$ | 0.500** |
| P_V       | $Y = 0.5e^{0.03x}$ | 0.557** |
| C1%       | $Y = 1.7e^{0.4x}$ | 0.267** |
| C2%       | $Y = 0.41e^{5.4x}$ | 0.649** |
| C3%       | $Y = 149.4e^{-6.3x}$ | 0.540** |
| C4%       | $Y = 6.7e^{11.8x}$ | 0.251** |

*Statistically significant at the probability level of 0.01 (F-test).

The DOC concentration of biochar-DOM are often detected by TOC analyser. Since the change of DOC concentration under pyrolysis condition are due to the change of DOM components, the DOM composition indices were selected to predict the DOC concentration of biochar-DOM. We predicted the DOC concentration based on the exponential regression analysis between DOC and SUVA254, P_i% and C_i% (Table 2). As shown in Table 2, the DOC concentration could be fitted well with SUVA254, P_i% (except P1%) and C_i% (except C3%). The good agreement between DOC concentration and DOM composition indices demonstrated that UV-vis spectroscopy and fluorescence spectroscopy all could be used to estimate the DOC concentration of biochar-DOM.

### Conclusions

The EEMs combined with FRI calculation and PARAFAC modelling showed the characterisation of DOM derived from manure biochars depends on the pyrolysis temperature and holding time. At 300 to 500 °C, fulvic-acid-like substance was the main decomposed component, while humic acid-like substance was the main decomposed component at 600 °C. In the pyrolysis temperature of 300–600 °C, the generation of aromatic proteins, microbial by-product and fulvic acid are generated from the decomposition of humic-like substances including marine humic-like, UVA humic like, UVC + UVA humic-like substances in the pyrolysis process of manure biochars. The results in this study suggested that the integration using of FRI and PARAFAC for manure biochars characterisation better understanding of biochar nature and functionality.

### Conflicts of interest

There are no conflicts to declare.

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