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Roles of composts in soil based on the assessment of humification degree of fulvic acids

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ABSTRACT

This study was conducted to assess the humification degree in fulvic acids (FA) from different composts, and to reveal their roles after soil amending based on their excitation-emission matrices (EEM) of the fluorescence spectra and projection pursuit regression (PPR) analysis. Two peaks were detected in EEM spectra of FA from all composts, and three components were identified by the parallel factor analysis (PARAFAC) model. The assessment of the humification degree of FA using the ratios between the values of the percent fluorescence response in the visible and ultraviolet regions ($P_{I,n}/P_{II,n}$) generally agreed with that using the distributions of FA components (C1, C2 and C3). The PPR considering the parameters ($P_{I,n}/P_{II,n}$, C1, C2 and C3) further ranked the composts with similar of FA, and the FA humification degree decreased in the order: GW, TSW, LW and SW > CM, MSW, and PM > MC and KW. The results showed that the compositions of FA were similar to each other in composts from different materials, but there were distinct differences in the humification degree of FA owing to the different distributions of each component in composts. Therefore, based on the redistribution of components, a method for regulating the humification degree of FA during composting was presented. Furthermore, the suitable soil application of composts with different humification degrees was also demonstrated.

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1. Introduction

Composting is defined as an economical and environment-friendly biological process of aerobic thermophilic microbial degradation of wastes by populations of indigenous microorganisms, which can convert organic wastes into a soil amendment rich in humic substances and nutrients, mitigate groundwater contamination and reduce air pollution and greenhouse gas (GHG) emissions (He et al., 2011a; Yan et al., 2016; Zhang et al., 2016). Organic matter is partially transformed into more stable and complex macromolecules such as humic substances in the biological process of composting (Lashermes et al., 2012). Understanding the characteristic of the humic substances formed during composting is relevant because they constitute a stable fraction of carbon, thus regulating the carbon cycle and the release of nutrients, such as nitrogen, phosphorous and sulfur. After soil application, they play an important role in global carbon cycling and in the regulation of the mobility and fate of environmental contaminants and also have many positive benefits in creating a suitable medium for plant growth (Christl et al., 2005; Harrison, 2008).

Over the years, a number of hypotheses have been presented on how humic matter is formed. Though there is not enough evidence to support the hypothesis that the de novo formation of humic polymers is quantitatively relevant for humus formation (Schmidt et al., 2011), the view relied on alkali and acid extraction methods from previous soil chemists has dominated for years, that is, fulvic acids (FA; soluble at all pH values) were a relatively more lively group constituent in humic substances when compared with humic acids (HA; soluble in alkaline media and insoluble in acidic media) (Ryan and Weber, 1982; Bai et al., 2008). Various parameters have been proposed to study the degree of humification such as humification index (HI), a ratio of peak A (fulvic acid-like substances) to peak C (humic acid-like) in Excitation emission matrix (EEM) fluorescence spectroscopy; fluorescence index (FI), the ratio of emission intensity at 450 nm and 500 nm at 370 nm excitation (McKnight et al., 2001; Ohno, 2002). Since composting implies the formation of some humic-like substances by microorganisms whose metabolism

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predominantly occurs in the water-soluble phase (Said-Pullicino et al., 2007; Bernal et al., 2009), a study of the degree of humification occurring in the water-extract FA may be useful for assessing compost. However, most reports focused on the characteristics of dissolved organic matter (DOM) during composting, and few studies have examined the humification degree in humic substances of different composts.

FA contain characteristic structures such as aromatic rings with three to five substituents (mainly hydroxyl, methoxyl or aliphatic hydrocarbon groups with some aldehyde and keto functional groups also attached to some of the aromatic nuclei), aromatic–aliphatic ethers, carboxylic groups, sugars and amino acids. Traditionally, the fractions of fulvic acids were characterized by size exclusion chromatography, elemental analysis (C, H, N, S), as well as spectroscopic techniques, including ultraviolet visible (UV-VIS), Cross-Polarization Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance (CP-MAS 13C NMR), Fourier transform infrared spectroscopy (FT-IR), and fluorescence spectroscopy (Christl et al., 2005). Specifically, fluorescence spectroscopy is used as a non-destructive, simple, non-separative and accurate tool to quantify the humification and maturity during composting (Hur et al., 2009; Fernandez-Romero et al., 2016). The fluorescence characteristics of DOM have been extensively investigated owing to the technological advances in fluorescence spectroscopy, especially the development excitation – emission (EEMs) (Coble, 1996). Application of EEM as a tool for assessment of compost products is well documented, owing to its high sensitivity and simplicity (Yu et al., 2010a; Wu et al., 2012). The parameters used to assess the humification degree of humic substances include an intensity ratio between the fluorescence peaks at different wavelengths (Zsolnay et al., 1999), an area ratio between different fluorescence spectra regions in traditional fluorescence spectra (Kalbitz et al., 1999), and the percentage of fluorescence response ($P_{i,n}$) calculated by fluorescence regional integration (FRI) in an excitation – emission matrix (EEM) fluorescence spectra (Chen et al., 2003). They also include the distribution of components identified by EEM fluorescence spectra combined with parallel factor analysis (EEM-PARAFAC) (Yu et al., 2010b; Markechova et al., 2014). Apart from the analysis methods of FRI and EEM-PARAFAC in fluorescence spectra, there was also a useful method based on second derivative synchronous fluorescence to characterize DOM (Yu et al., 2013).

EEM is a powerful tool for determining DOM substances (Yu et al., 2010a). EEM offers several major advantages over single-scan methodologies such as providing new information regarding the fluorescence DOM composition of a sample (Coble et al., 1993). However, to conquer the difficulty in identifying individual fluorescence components in sample, simple and multivariate data analysis technique PARAFAC have been used (Stedmon et al., 2003). PARAFAC analysis can decompose fluorescence EEMs into independent groups of fluorescence components and provide a unique solution to the FA EEM dataset, and it is regarded as an important analytical tool to characterize the FA in composting (He et al., 2013). The use of fluorescence spectral techniques to characterize the same sample can generate a large number of fluorescence parameters, and the humification degree of FA could be affected by a combination of multiple fluorescence parameters, not necessarily by each measure individually. Therefore, an assessment method utilizing multiple fluorescence parameters would be necessary. Projection pursuit regression (PPR) is a nonlinear multivariate regression procedure proposed by Friedman and Stuetzle (1981). Its basic idea is to project high dimension to low dimensional space, and it tries to find the intrinsic structural information hidden in the high dimensional data. At present, it has been applied successfully to tackle some chemical problems (Ghasemi and Zolfonoun, 2013).

The objectives of this study were (1) to obtain FA fluorescence characteristics from different composts; (2) to select fluorescence parameters, which were suitable for estimating the humification degree in DOM; (3) to assess the humification degree of FA from different composts using EEM-PARAFAC and PPR; (4) to suggest suitable application methods of composts with different humification degrees.

2. Materials and methods

2.1. Sample collection and storage

Nine trapezoidal piles of Green waste (GW), Kitchen waste (KW), Tomato stem waste (TSW), Chicken manure (CM), Straw waste (SW), Litter waste (LW), Municipal solid waste (MSW), Pig (Swine) manure (PM) and a co-compost (MC) mixed with biogas residue. All the composts were prepared by Shanghai Songjiang Composting Plant, and each composting pile contained approximately 2 t of raw material (1.5 m high with a 2×3 m base). The C/N was adjusted about 25 for each material by mixing sawdust at the initial stage of composting. The composting time was approximately 45 days according to the composting period of the plant. When the composting finished, approximately 3 kg of samples were collected from several sites of the compost pile and stored at 4 °C for the analysis of FA. The samples for analysis were homogenized using the methods of coning and quartering (Bernabé et al., 2011). And all the samples for analyzing FA were carried out by triplicate. Details of the composting properties are described in Table S1.

2.2. Extraction of fulvic acid

Compost samples (10 g) were mixed with 0.1 M ($NaOH + Na_4P_2O_7$) with a solid to water ratio of 1:10 (w/v). The mixtures were shaken for 24 h with a rotational speed of 150 rpm at room temperature and centrifuged at 10,000 rpm for 15 min. The obtained supernatant was filtered through a 0.45 μm membrane filter and then acidified to a pH of 1 with 6 M HCl. The mixtures were allowed to stand for 24 h at 4 °C and again centrifuged at 10,000 rpm for 15 min. The obtained supernatant was passed through an XAD-8 macroporous resin column and an H^+-saturated cation exchange resin for further FA purification (He et al., 2011b). The stationary phases used for the FA purification were XAD-8 resin and 732 cation exchange resin, which have been described by He et al. (2011b). The mean final concentrations of FA in different composts were 116.04 ± 2.05 mg/L (GW), 148.04 ± 3.21 mg/L (KW), 168.51 ± 3.13 mg/L (TSW), 188.00 ± 4.30 mg/L (CM), 82.60 ± 1.51 mg/L (SW), 77.55 ± 1.95 mg/L (LW), 183.32 ± 2.54 mg/L (MSW), 156.13 ± 3.75 mg/L (PM) and 60.54 ± 0.85 mg/L (MC), respectively.

2.3. Fluorescence spectroscopy analysis

Fluorescence spectroscopy was recorded using a Hitachi F-7000 fluorescence spectrophotometer (Hitachi High Technologies, Japan) in a 1 cm clear quartz cuvette at room temperature (20 ± 2 °C). The emission wavelength was recorded from 250 to 600 for 2 nm increments and excitation wavelength was recorded from 200 to 550 for 10 nm increments. The concentration of FA was diluted to the same level of 10 mg/L before the measurement to decrease the influence of varying FA concentrations among the compost samples in contribution to fluorescence intensities (Yu et al., 2009). The EEM spectra were recorded for the excitation spectra from 200 to 550 nm at intervals of 10 nm while the emission spectra ranged between 250 and 600 nm, with data saved for every 2 nm increments. The scan speed was set at 1200 nm min^{-1}. The fluorescence intensities were converted to Roman Unit (R.U.). Meanwhile, Milli-Q water blank EEMs were deducted from the

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