



Dynamics, biodegradability, and microbial community shift of water-extractable organic matter in rice–wheat cropping soil under different fertilization treatments[☆]

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ABSTRACT

Although fertilization plays an important role in determining the contents of soil dissolved organic matters or water-extractable organic matter (DOM, WEOM), knowledge regarding the dynamics, biodegradability, and microbial community shifts of WEOM in response to different fertilization treatments is very limited, particularly in rice–wheat cropping soil. Thus, in the present study, we performed biodegradation experiments using WEOM extracted from samples of soil that had been subjected to four different fertilization treatments: unfertilized control (CK), chemical fertilizer (CF), 50% chemical fertilizer plus pig manure (PMCF), and 100% chemical fertilizer plus rice straw (SRCF). UV spectrum and fluorescence 3D excitation–emission matrix analyses applied to investigate the chemical composition of WEOM revealed that all examined WEOMs were derived from microbial activity and the dominant portion comprised humic acid-like compounds. After the incubation, 31.17, 31.63, 43.47, and 33.01% of soil WEOM from CK, CF, PMCF, and SRCF treatments, respectively, were biodegraded. PMCF-derived WEOM had the highest biodegradation rate. High-throughput sequencing analyses performed to determine the microbial community before and after the incubation indicated that *Sphingomonas*, *Bacillus*, and *Flavisolibacter* were the predominant bacterial genera in the original inoculum derived from the four fertilization treatments. Following biodegradation, we observed that the dominant bacteria differed according to fertilization treatments: *Curvibacter* (43.25%) and *Sphingobium* (10.47%) for CK, *Curvibacter* (29.68%) and *Caulobacter* (20.00%) for CF, *Azospirillum* (23.68%) and *Caulobacter* (13.29%) for PMCF, and *Ralstonia* (51.75%) for SRCF. Canonical correspondence analysis revealed that, shifts in the microbial community were closely correlated with pH and specific UV absorbance at 254 nm. We speculated that the inherent traits of different WEOM and the properties of soil solutions under different fertilization treatments shaped the soil microbial community structure, thereby influencing the biodegradation of WEOM.

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1. Introduction

Soil dissolved organic matter (DOM) is defined as the soluble organic matter fraction that can pass through a 0.45-μm filter

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(Bolan et al., 2011). Although DOM comprises only a small percentage of the soil organic matter, as the most active and mobile fraction, it has a substantial influence on the biogeochemical processes of terrestrial and aquatic environments. DOM plays a vital role not only in the dynamic cycling of nutrients and interaction with contaminants [metal (loid)s and organics], but also has a considerable influence on soil microbial structure and function. Moreover, it makes a significant contribution to the global carbon

cycle (CO_2) and release of greenhouse gases (N_2O) (Siemens et al., 2003). Accordingly, DOM can be used as a sensitive indicator to monitor shifts in ecological processes (Bolan et al., 2011).

The properties and amounts of DOM in soils can be affected by a variety of factors, including edaphic factors, vegetation type, environmental factors, and land management practices, among the latter of which, fertilizer treatments (inorganic or organic) have a significant influence on the DOM of arable soils. In this regard, it has been reported that organic amendments tend to increase DOM in soil, whereas the effects of inorganic fertilization on DOM tend to be dependent on the type and the amount of fertilizer applied (Bolan et al., 2003; Brown et al., 2008; Embacher et al., 2008). Therefore, there is a need for further studies to investigate the influence of different fertilizers on DOM from various arable soils. To this end, in the present study, we focused on the effects of three commonly used fertilization regimes, namely, the application of chemical fertilizer, chemical fertilizer plus rice straw, and chemical fertilizer plus pig manure.

As important soil amendments that affect the characteristics and amounts of DOM in soil as well as soil properties, fertilization treatments play a significant role in regulating the dynamics of DOM (Bolan et al., 2011). A further factor that makes an important contribution to DOM dynamics is biodegradation, which is controlled by intrinsic DOM properties and soil characteristics such as nutrient availability and microbial community structure (Marschnera and Kalbitz., 2003). Accordingly, DOM biodegradability is assumed to be closely related to the nature of fertilizer treatments, although little is currently known regarding the effects of fertilization on the rate of DOM degradation. Previously, detailed investigations have been performed to examine the biodegradation of DOM in natural soils (Kalbitz et al., 2003; Marschnera and Kalbitz., 2003), and incubation studies have indicated that 10–44% of DOM in soil solution can be biodegraded (Embacher et al., 2008). However, considerably less attention has been devoted to assessing the effects of different fertilization regimes on DOM biodegradability. To date, only Xu et al., (2018a) have reported the biodegradation rate of DOM from an Ultisol under four different fertilization treatments, and these authors found that DOM from soil subjected to mineral chemical fertilization (NPK) plus straw and animal manure treatments had higher biodegradability than that from control and NPK treatments. Consequently, there is a need for further data to enhance our current understanding of the influence of fertilization on DOM biodegradability, particularly with respect to soils under rice–wheat cropping. Furthermore, although some previous studies have examined the role played by the soil microbial community in DOM degradation (Bolan et al., 2011; Marschnera and Kalbitz., 2003; Wu et al., 2018), to the best of our knowledge, there have been no studies to date that have examined how the structure of the soil microbial community shifts in response to DOM under different fertilization treatments, and which dominant bacteria are associated with degradation of the recalcitrant carbon of different DOM sources.

Hence, the primary purpose of the present study was to determine the dynamics and biodegradability of water-extractable organic matter (WEOM) in rice–wheat cropping soil under different fertilization treatments, as well as the associated shifts in soil microbial community structure and function. WEOM properties and biodegradation were monitored on the basis of DOC concentrations and spectral absorbance and fluorescence excitation–emission matrix (EEM) measurements. In conjunction, the bacterial community structure was assessed with high throughput sequencing. In addition, we also monitored CO_2 and N_2O emissions to assess the release of greenhouse gases during DOM biodegradation. The findings of this study might strengthen our understanding of the effects of fertilization treatments on

biogeochemical processes in terrestrial and aquatic environments in terms of DOM decomposition and the associated bacterial community.

2. Materials and methods

2.1. Site description and soil sampling

Soil samples were collected from Jintan City, Jiangsu Province, China ($31^\circ39'\text{N}$, $119^\circ28'\text{E}$, 3 m a.s.l.), where different fertilization regimes have been applied in succession since 2010. The soil type is classified as a clay loamy Fe-leachic-gleyic-stagnic Anthrosol, which is cultivated under a long-term winter wheat/summer rice double-cropping system. Soil sampling in the present study was performed in June 2015, with samples being collected from soils subjected to four different fertilizer treatments: an unfertilized control (CK), chemical fertilizer (CF), 50% chemical fertilizer plus pig manure (PMCF), and 100% chemical fertilizer plus rice straw (SRCF). Detailed information of the sampling site and soil physicochemical properties have been reported previously by Hui et al. (2017). Soil samples were sieved (5 mm), and after the removal of visible roots, were air dried for further analyses. Air drying results in higher yields of WEOM due to aggregate disruption and lysis of microbial biomass (Akagi et al., 2007).

2.2. Preparation of WEOM solutions and microbial inocula

Soil WEOM was extracted with 10 mM CaCl_2 using a soil to water ratio of 1:1. Suspensions of the four soil samples under different fertilization were shaken at room temperature for 30 min, then centrifuged at 4500 rpm for 10 min, and finally filtered through the 0.45- μm polycarbonate filter (Embacher et al., 2008). For higher homogeneity of solutions, three replicate extraction from a single soil type were performed and the resulting suspensions were mixed.

For the preparation of inocula, microorganisms in the air-dried soil samples were reactivated by rewetting and preincubating soil for 2 weeks at 20°C . Thereafter, the soils were shaken for 24 h with 4 mM CaCl_2 solution (soil/water ratio = 1:5), left to stand for 30 min, and then filtered through a 4.5- μm polycarbonate filter (Kalbitz et al., 2003; Wang et al., 2008). A 2-mL aliquot of each inoculum was added to an incubation flask at an inoculation ratio of 1:100 (inoculum: WEOM). To maintain conditions as similar as possible to the in situ progression of biodegradation, the four types of inocula were added to solutions of the corresponding source WEOM, using an inoculum concentration of approximately 10^5 cells mL^{-1} .

2.3. Biodegradation experiments

Following the method described by McDowell et al. (2006), we performed a 42-day incubation, with no nutrients added, to determine the biodegradation of WEOM, coupled with repeated analysis of CO_2 production. For each of the four WEOM samples, 200-mL solutions were added in triplicate into 500-mL incubation flasks. After sealing, the flasks were incubated in the dark at 25°C , and gently oscillated by hand daily to prevent the establishment of biofilms. Subsamples (10 mL) from triplicate flasks were removed on days 1, 3, 7, 10, 14, 21, 28, 35, and 42 and filtered through a 0.45- μm syringe filter for further analyses, prior to which, the gas in the headspace was extracted with an injection syringe and the flasks were aerated for 10 min on a clean bench. As a control to assess viability of the inocula, we used 20 mg C L^{-1} of glucose solution (with addition of NH_4NO_3 , K_2HPO_4) to substitute WEOM. Ultrapure water containing inoculum was used as a further control to

measure the carbon dynamic of the added inoculum.

2.4. Chemical analyses and CO₂ and N₂O emission measurements

The pH values of WEOM were determined by pH meter (METTLER TOLEDO FE20, Switzerland). The DOC and total soluble nitrogen (TSN) contents of samples were measured using a JENA MULTI N/C®3100 Total Organic Carbon and Total Nitrogen Analyzer. NH₄⁺ concentration was determined following the indophenol blue colorimetric method (Zhan et al., 2010). Dual-wavelength ultraviolet spectrophotometry was used to measure the NO₃⁻ content (Norman et al., 1985). Water-extractable organic nitrogen (WEON) was determined according to the report of Murphy et al. (2000), by subtracting the mineral nitrogen content from the TSN content. CO₂ and N₂O concentrations in the headspace of each incubation flask were measured using a gas chromatograph (HP7890A; Agilent Technologies, CA, USA). The production of CO₂ is typically used as one of the measures of DOC degradability (McDowell et al., 2006). The production of N₂O is an indicator of nitrogen cycle activity during the microbial degradation of DOM.

2.5. Spectral measurements

Optical methods were used to examine changes in the quality of WEOM. UV absorbance at 280 nm (UV₂₈₀), measured using a UV-1810 spectrophotometer (Yipu, Shanghai, China), was used to determine the aromaticity of WEOM (Chin et al., 1994) and absorbance at 254 nm (UV₂₅₄) was used as a parameter of the relative contents of aromatic structures (Saadi et al., 2006). Specific UV absorbance (SUVA) values were obtained as the UV absorption at 280 nm or 254 nm divided by WEOC concentration (L mg⁻¹ m⁻¹). The absorbance ratio of 240 nm–420 nm (A₂₄₀/A₄₂₀) represents the ultraviolet and visible light absorption capacity of WEOM, respectively, and is negatively related to the degree of polymerization of the aromatic carbon frame of WEOM. The absorbance ratio of 250 nm–365 nm (A₂₅₀/A₃₆₅) reflects the molecular weight distribution of WEOM, with a higher A₂₅₀/A₃₆₅ ratio being indicative of a lower molecular weight (Xi et al., 2018; Yang et al., 2013).

Fluorescence three-dimensional (3-D) excitation–emission matrix (EEM) analyses were applied to investigate the chemical composition of WEOM using an F-4500 fluorescence spectrophotometer (Hitachi Inc., Japan). The fluorescence spectra were collected for excitation wavelengths from 220 to 500 nm at 5-nm increments and for emission wavelengths from 280 to 550 nm at 2-nm increments. Three-dimensional maps were generated using SigmaPlot 10.0. software. A 10 mM CaCl₂ solution served as the reference for all measurements. Three fluorescence-related indices, namely, fluorescence index (f_{450/500}), fluorescence intensity (FI), and humification index (HIX) were also determined. Detailed information regarding the definition of these indices has been presented previously by Liu et al. (2014).

2.6. DNA extraction and high-throughput sequencing

Soil DNA was extracted from 0.5 g of homogenized soil using a FastDNA spin kit (MP Biomedicals, CA, USA). WEOM solution was collected after incubation for 42 d and centrifuged at 6000 rpm for 5 min to obtain the bacterial cells. Compared with the general soil bacterial community, these bacteria are assumed to play a significant role in WEOM biodegradation. DNA was extracted from the bacterial cells using an EasyPure Genomic DNA Kit (TransGen, Beijing, China). DNA quality was assessed by bacterial 16S rRNA PCR, whereas DNA concentrations were measured using a K5600 Micro-Spectrophotometer (Kaiao, Beijing, China). Bacterial 16S rRNA (the V3–V4 region) was amplified using the specific primers

341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGAC-TACNNGGTATCTAAT-3′). Sequencing was performed using an IonS5™XL sequencer at Novogene Bioinformatics Technology Co., Ltd., Beijing, China. The reads were processed using Cutadapt (V1.9.1, Martin, 2011); Operational Taxonomic Units (OTUs) were assigned at the 97% sequence similarity level using Uparse (V7.0.1001) and Mothur and SILVA methods and SSUrRNA database were used for species annotation analysis (Quast et al., 2013). The bacterial sequences (raw data) obtained in this study have been deposited in the NCBI Sequence Read Archive (SRA) databases with the accession number: PRJNA504563.

2.7. Statistical analysis

Statistical analyses were conducted using SPSS (version 16.0) software. The differences of physicochemical properties of WEOM and bacterial diversity indexes under different fertilization treatments were determined by analysis of variance (ANOVA). *P*-values of <0.05 were considered statistically significant. Correlation coefficients (Spearman) were used to analyze the relationships between biodegradable WEOC and different spectral properties. Values for the observed operational taxonomic units (OTU) and Chao1, Shannon, Simpson, and Good-coverage indices were determined using Qiime V1.9.1. Canonical correspondence analysis (CCA) was conducted to determine those factors closely related to the shifts in microbial community structure. Other figures were plotted using SigmaPlot software (version 14.0).

3. Results and discussion

3.1. Content of WEOM

The values presented for soil organic carbon (SOC) and total nitrogen (TN) contents in this study were obtained in our previous study (Hui et al., 2017), both of which decreased in the order SRCF > PMCF > CF > CK (Table 1). Comparably, the content of water-extractable organic carbon (WEOC) followed the same sequence as SOC, with values of 33.03, 47.35, 64.80, and 69.64 mg kg⁻¹ for the CK, CF, PMCF, and SRCF treatments, respectively. The concentrations of water-extractable organic nitrogen (WEON) were considerably lower than WEOC, and followed the same sequence as TN. The extractability of soil organic matter is considered to be an indicator of the proportion of labile WEOC and WEON (Sun et al., 2015). As shown in Table 1, the extractability of soluble organic matter (expressed as WEOC/SOC and WEON/TN) from PMCF- and SRCF-treated soil was significantly higher than that of CK and CF samples (*P* < 0.05). However, no difference was observed between CK, CF, and PMCF and SRCF. These results indicated that the application of PMCF and SRCF increased the production of WEOM. The application of pig manure and rice straw enhances the input of organic carbon and available nutrients, and also accelerates microbial decomposition (Malhi et al., 2011; Xu et al., 2018b).

The pH values of WEOM under different fertilization treatments are listed in Table 1, which shows that, with the exception of the pH of SRCF (5.35), the WEOM pH values under different treatments were approximately 6. The lower pH value recorded in the SRCF treatment can be attributed to the production of organic acids in response to the addition of rice straw (Sharma et al., 1989). Similar results were observed in soil pH which was determined in our previous study (Hui et al., 2017).

Given the higher soil contents of WEOC and WEON in response to the PMCF and SRCF treatments, the cumulative CO₂ and N₂O emissions of these materials from PMCF- and SRCF-treated soils during incubation were markedly (*P* < 0.05) higher than the emissions from the CK and CF soils (Fig. S1). However, the N₂O emission

Table 1

Mean values of pH, soil organic carbon (SOC), total nitrogen (TN), WEOC, WEON, biodegradable WEOC (BWEOC), and the extractability of soluble organic matters (expressed as WEOC/SOC and WEON/TN) under different fertilization treatments.

Treatments	pH (CaCl ₂)	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	WEOC (mg kg ⁻¹)	WEON (mg kg ⁻¹)	BWEOC %	WEOC/SOC %	WEON/TN %
CK	6.14 ± 0.08 ^b	14.34 ± 1.46 ^a	1.74 ± 0.13 ^a	33.03 ± 1.85 ^a	18.86 ± 2.22 ^a	31.17 ± 0.004 ^a	0.23 ± 0.03 ^a	1.08 ± 0.08 ^a
CF	6.17 ± 0.04 ^b	18.80 ± 1.92 ^b	1.93 ± 0.12 ^{ab}	47.35 ± 2.06 ^b	21.07 ± 2.11 ^a	31.63 ± 0.02 ^a	0.25 ± 0.03 ^a	1.09 ± 0.07 ^a
PMCF	6.13 ± 0.07 ^b	22.11 ± 1.23 ^c	2.10 ± 0.19 ^b	64.80 ± 0.51 ^c	26.47 ± 0.82 ^b	43.47 ± 0.02 ^b	0.29 ± 0.02 ^b	1.23 ± 0.08 ^b
SRCF	5.35 ± 0.15 ^a	23.29 ± 2.69 ^c	2.12 ± 0.29 ^b	69.64 ± 1.02 ^d	26.65 ± 1.22 ^b	33.01 ± 0.01 ^a	0.30 ± 0.04 ^b	1.24 ± 0.16 ^b

Each value gives the mean of 3 repetition.

Different superscript lowercase letters within the same column indicate significant differences ($P < 0.05$).

of WEOM derived from SRCF-treated soil was significantly ($P < 0.05$) lower than that derived from PMCF-treated soil, and can probably be attributed to suppressive effect of low pH on N₂O emission (Russenes et al., 2016). To sum up, biodegradation of WEOM from soil amended with pig manure and rice straw produced larger amounts of greenhouse gases than that derived from unfertilized soil and chemical fertilizer-treated soil samples.

3.2. Spectroscopic properties

As indicators of aromaticity (Heinz et al., 2015; Kalbitz et al., 2003), we measured SUVA₂₅₄ and SUVA₂₈₀, the values of which

both decreased in the sequence SRCF > PMCF > CK > CF, indicating that SRCF has the highest aromaticity (Table S1). This pattern could be explained by the presence of rice straw in SRCF, the decomposition of which yields numerous aromatic components, notably lignin (Chen et al., 2010). Compared with no fertilizer application, the combination of organic amendments and inorganic fertilizers enhanced the aromaticity of WEOC, which is in accordance with the findings of Zhou et al. (2010) that chemical fertilizer plus pig manure and chemical fertilizer plus crop straw increase the content of aromatic-C in particulate organic matter. Over the course of the 42-day incubation, significant ($P < 0.05$) increases in the values of SUVA₂₅₄ and SUVA₂₈₀ were observed in all treatments (Fig. 1c and

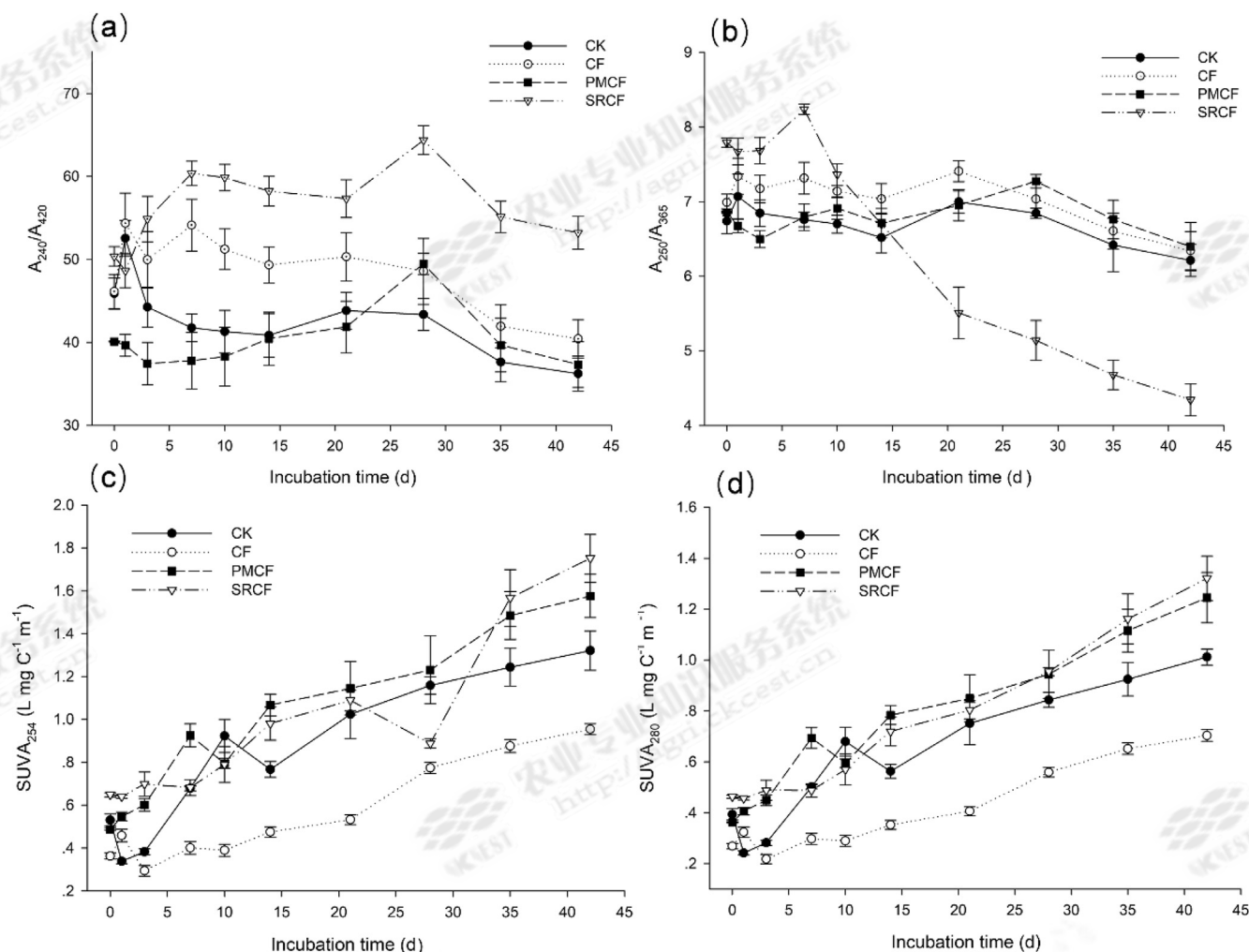


Fig. 1. Dynamics of the spectroscopic characteristics of water-extractable organic matter (WEOM) during a 42-day incubation: (a) A_{240}/A_{420} ; (b) A_{250}/A_{365} ; (c) Specific UV absorbance at 254 nm (SUVA₂₅₄); (d) SUVA₂₈₀. Error bars represent the standard deviation of three replicates.

d), which can be explained by the fact that recalcitrant compounds such as aromatics accumulated during the degradation of organic matter (Kalbitz et al., 2003).

At the beginning of the incubation period, values of A_{240}/A_{420} followed the sequence $SRCF > CK = CF > PMCF$ (Fig. 1a). However, over the course of the incubation period, the A_{240}/A_{420} values of different fertilization treatments tended to decrease and, with the exception of SRCF soil, showed no significance differences at the end of the incubation. The results indicate a higher degree of polymerization of benzene ring carbon in CK-, CF- and PMCF-derived WEOM after the 42-day incubation. A_{250}/A_{365} values have previously been found to be negatively correlated with molecular weight (Xi et al., 2018). In the present study, we observed a slight decrease in the A_{250}/A_{365} ratio in CK, CF, and PMCF samples, whereas a more substantial decrease was recorded in the SRCF samples, indicating that the molecular weight of SRCF-derived WEOM progressively increases during the biodegradation process. This pattern can probably be explained by the fact that small-sized colloidal organic matter aggregate into large-sized colloids at lower pH (acidic conditions) (Xu et al., 2018a).

Three-dimensional-EEM fluorescence spectra maps for the four WEOM samples (before biodegradation) show the presence of just two identifiable peaks (peak A and peak B; Fig. 2). This is in accordance with the findings of D'Orazio et al. (2014) and Saadi et al. (2006), who identified two peaks as humic-like and fulvic-

like fluorescence, thereby indicating that humic acid-like compounds comprise the predominant portion of the compounds in WEOM derived from all treatments, which has also previously been reported in an Ultisol (Xu et al., 2018a). FI values are closely related to the structure of WEOM and higher FI values are attributed to the enrichment of simple components of small molecular weight, such as hydroxyls, methoxyls, and amino groups, whereas lower FI values could be ascribed to the accumulation of complex components with higher molecular weight, such as carboxylic groups (D'Orazio et al., 2014; Xi et al., 2018). The FI values (peak A + peak B) of WEOM derived from CK, CF, PMCF, and SRCF treatments were 642, 635, 1053, and 1194, respectively, indicating that the molecular structure of WEOM from CK and CF treatments were more complex than that from PMCF- and SRCF-derived WEOM, which is consistent with the findings of Xu et al., (2018a) that the molecular structure of DOM from CK and NPK treatments was more complex than that from NPK plus straw and animal manure treatments. The $f_{450/500}$ ratio is an important index that can be used to discriminate between edaphic and microbially derived sources. Values of approximately 1.4–1.5 indicate edaphic sources, whereas those of approximately 1.7–2.2 are indicative of microbially derived sources (Li et al., 2014; Wei et al., 2014). The values for the $f_{450/500}$ of WEOM-derived from four fertilizer treatments in the present study ranged from 1.806 to 1.979 (Table S1), thereby indicating that, in each case, the WEOM was derived from microbial sources. HIX

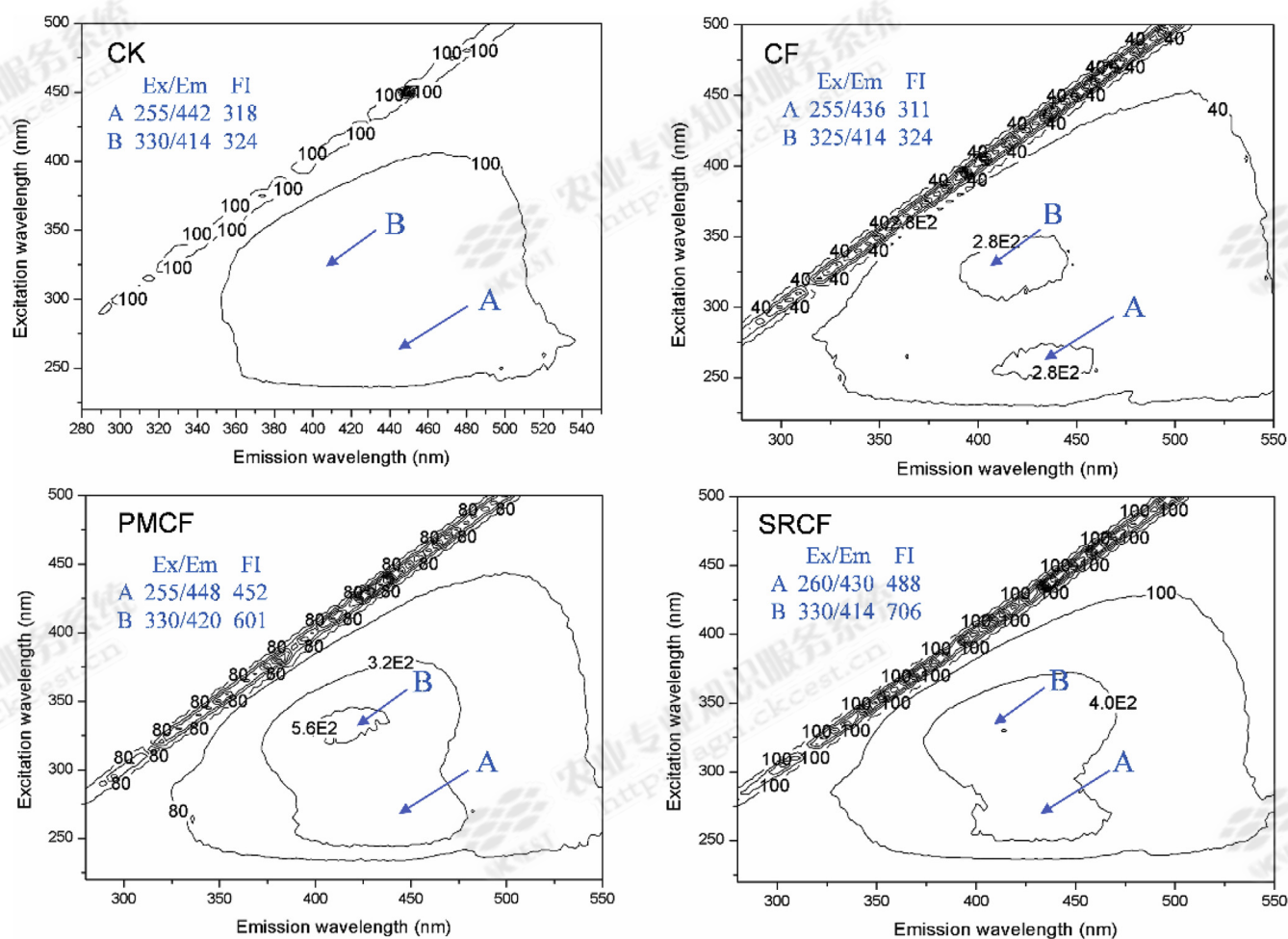


Fig. 2. Fluorescence excitation–emission matrices (EEMs) of WEOM from examined soil samples under different fertilization regimes. Contour lines are based on WEOM-normalized fluorescence intensity values. A and B represent two major fluorescence peaks identified as humic-like and fulvic-like constituents.

values are correlated with the degree of condensation or conjugation, reflecting soil humification, and are affected by aromaticity (indicated by $SUVA_{254}$ and $SUVA_{280}$) and complexity (represented by FI) (Sun et al., 2013). Higher HIX values are indicative of WEOM in a more condensed or humified state (Embacher et al., 2008). As shown in Table S1, HIX values followed the sequence CK > CF > SRCF > PMCF. The HIX values of DOM originating from animal manure and plant residues are generally lower than those from soil, which probably explains our finding that WEOM from the CK treatment had the highest HIX value, whereas that from the PMCF treatment had the lowest humus level (Table S1).

The structure and composition of soil WEOM is potentially determined by the deposition of the recalcitrant fraction and the dynamic balance between the input and the output of the labile fraction (Xi et al., 2018). Animal manure and rice straw in organic fertilizer can directly contribute to the input of labile organic matter into soil, which can explain the considerably lower molecular complexity and humification of WEOM derived from PMCF- and SRCF-treated soils compared with that derived from the CK

treatment. However, consistent with the hypothesis of Xu et al., (2018a) that mineral fertilization could maintain or increase the molecular complexity of soil DOM, CF treatment maintained the molecular complexity of the WEOM in the present study, and decreased the aromaticity and humification, probably because chemical fertilizers promote crop growth and microbial activity.

3.3. Biodegradability of WEOM

In the 42-day incubation, glucose solution instead of WEOM served as the control to examine inoculum viability. As shown in Fig. 3b, glucose was almost complete mineralized within 2 weeks, and no temporal course (which typically represents the lag phase of DOM degradation) of CO_2 emission was observed in the examined samples, indicating that the density and activity of microbial inoculants were adequate in all incubations (Kalbitz et al., 2003). At the end of the 42-day incubation, we found that 31.17, 31.63, 43.47, and 33.01% of soil WEOM from the CK, CF, PMCF, and SRCF treatments, respectively, had been biodegraded

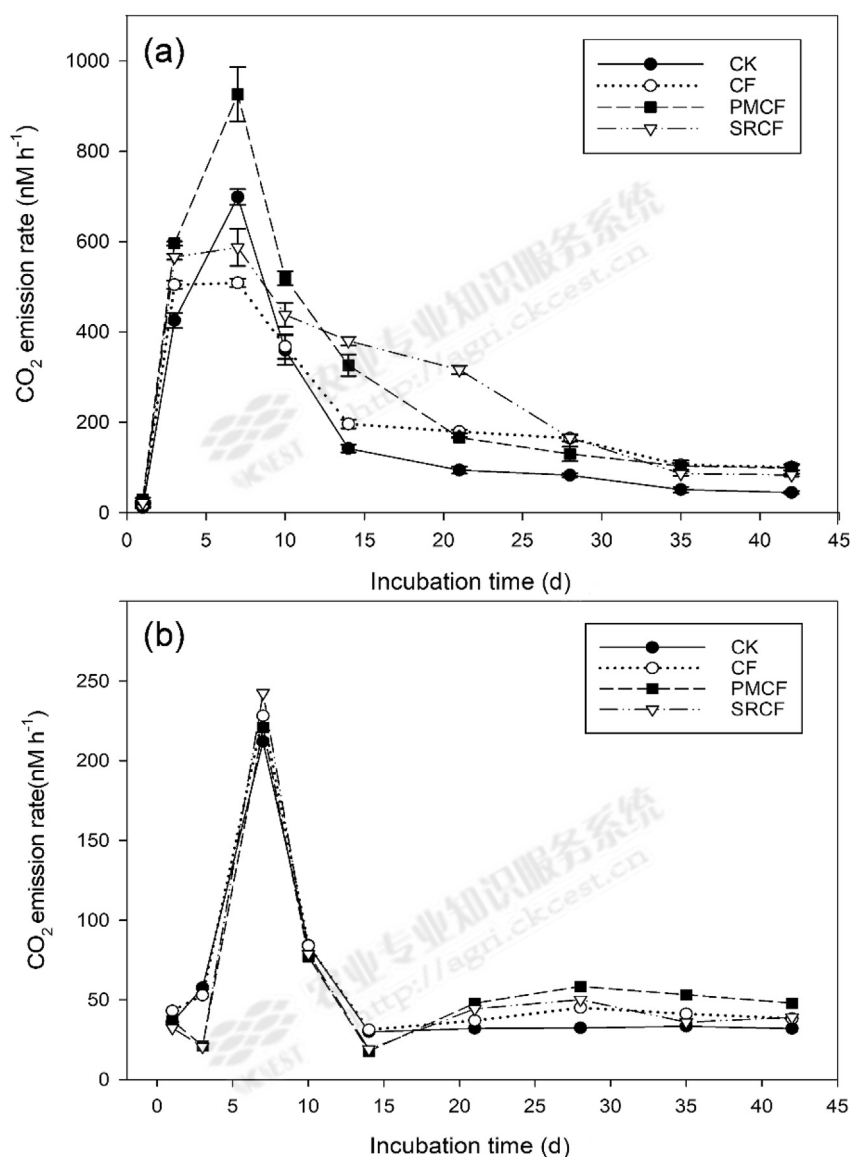


Fig. 3. CO_2 emission rates of (a) four WEOM derived from soil subjected to different fertilization treatments and (b) glucose solution during incubation. Results are presented as the mean values of three repetition and error bars indicate the standard deviation (SD).

Table 2
Estimates of bacterial diversity before (inoculum) and after biodegradation. B. CK, B. CF, B. PMCF, and B. SRCF represent the samples after biodegradation. Each value gives the mean of 3 repetition. Different superscript lowercase letters within the same column indicate significant differences ($P < 0.05$).

Treatments	observed_species	shannon	simpson	chao1	goods_coverage
CK	1642 ± 81 ^b	7.83 ± 0.10 ^b	0.98 ± 0.00 ^b	1744.7 ± 138.5 ^a	0.995 ± 0.002
CF	1632 ± 36 ^b	7.39 ± 0.05 ^b	0.97 ± 0.00 ^{ab}	1835.1 ± 139.1 ^a	0.992 ± 0.001
PMCF	1675 ± 44 ^b	7.61 ± 0.11 ^b	0.98 ± 0.00 ^b	1820.9 ± 79.5 ^a	0.993 ± 0.001
SRCF	1393 ± 31 ^a	6.94 ± 0.04 ^a	0.97 ± 0.00 ^{ab}	1594.1 ± 142.5 ^a	0.993 ± 0.002
B. CK	236 ± 19 ^{ab}	4.18 ± 0.33 ^b	0.88 ± 0.03 ^b	286.9 ± 4.3 ^a	0.998 ± 0.000
B. CF	249 ± 2 ^{ab}	4.54 ± 0.16 ^c	0.92 ± 0.01 ^c	306.5 ± 21.2 ^{ab}	0.998 ± 0.000
B. PMCF	301 ± 5 ^b	5.43 ± 0.05 ^d	0.95 ± 0.00 ^d	393.5 ± 6.2 ^c	0.998 ± 0.000
B. SRCF	228 ± 12 ^a	3.39 ± 0.14 ^a	0.72 ± 0.03 ^a	322.1 ± 19.2 ^b	0.998 ± 0.000

(Table 1). These values are consistent with those obtained in numerous laboratory incubation studies, which have indicated that 10%–44% of soil DOM is microbially degradable (Kalbitz et al., 2003; Sachse et al., 2001). Similarly, 23.9%–42.7% of soil DOM from Ultisol subjected to different fertilization treatments had been biodegraded (Xi et al., 2018). Interestingly, the WEOM derived from PMCF-treated soil showed the highest biodegradation rates, followed by that from SRCF soil, whereas WEOM from the CK and CF treatments showed greater resistance to biodegradation (Table 1). The biodegradability of DOM is determined by three main categories of factors: intrinsic DOM characteristics (e.g., molecular structure), soil properties (e.g., pH), and external factors (e.g., temperature) (Marschner and Kalbitz, 2003). In the present study, we maintained external factors at constant levels during the incubation, and the structure of WEOM and soil pH were determined. Significant negative correlations were observed between biodegradable WEOC (BWEOC) and HIX ($R^2 = 0.621$, $P < 0.05$) and between BWEOC and A_{240}/A_{420} ($R^2 = 0.782$, $P < 0.05$). The highest biodegradability recorded for the WEOM derived from PMCF-treated soil can be ascribed to the lowest values of HIX and A_{240}/A_{420} ratio. Consistently, Kalbitz et al. (2003) and Sun et al. (2016) have also reported that structures with a low level of conjugation and condensation are more biodegradable. In addition, with the exception of the WEOM derived from SRCF-treated soil, a significant positive correlation was detected between the biodegradability and FI value of the examined WEOM (data not shown), indicating that a more complex structure is less biodegradable, which explained the lower biodegradability of the WEOM derived from the CK and CF treatments. Even though WEOM from SRCF-treated soil had a lower HIX value and less complex structure, it had a considerably lower biodegradability than that derived from PMCF-treated soil. This could be explained by the notably lower pH of the material during incubation (Fig. S2). As an important parameter of soil property, pH influences microbial activity and the structure of DOM molecules. Low pH results in more condensed larger-sized molecules (Marschner and Kalbitz, 2003; Xu et al., 2018a), which tends to hamper biodegradation. Furthermore, the lower CO₂ emission rate of WEOM from SRCF-treated soil than that of WEOM from PMCF-treated soil (Fig. 3a) during the early period of incubation indicates that microbial activity was suppressed to a certain degree by the low pH.

3.4. Shifts in the bacterial community

Shifts in soil microbial composition are closely related to the turnover of DOM. It has been documented that during the initial period of incubation (within 1.5 d), the microbial community is affected by labile C, whereas in later stages, the microbial composition shifts to become more diverse and compatible with the

degradation of recalcitrant C (Wu et al., 2018). Hence, in the present study, we mainly focused on the changes in microbial community composition occurring during the latter stages of incubation compared with those occurring in the initial inoculum, with the aim of gaining an insight on the various dominant bacterial species responsible for degrading WEOM derived from the different fertilization treatments.

We detected between 1393 ± 32 and 1675 ± 44 OTUs in the inocula extracted from soil samples under the four fertilization treatments. After incubation, the number of observed bacterial species had decreased to within the range of 228 ± 12 to 301 ± 6. This reduction in bacterial richness can probably be explained by the fact that some species were unable to adapt to the incubation conditions and their numbers gradually declined. In addition, concomitant with the depletion of labile carbon in the culture, there was assumed to be an increase in the intensity of competition between bacteria for carbon resources, with most microbes being eliminated and only those bacteria adapted to metabolizing recalcitrant C persisting (Wu et al., 2018). The microbial community in SRCF-treated material harbored the fewest OTUs, both before and after incubation, whereas there was no significant difference ($P > 0.05$) in the number of OTUs detected among other three treatments (Table 2). The values obtained for Shannon and Simpson indices tended to indicate that, with the exception of SRCF, fertilization treatments had little observable effect on bacterial diversity. However, notable difference ($P < 0.05$) was observed at the end of the degradation experiment, with the resultant diversity decreasing in the sequence of: B. PMCF > B. CF > B. CK > B. SRCF (B. CK, B. CF, B. PMCF, and B. SRCF represent the samples after the biodegradation, Table 2). This result indicates that the bacterial community associated with the PMCF treatment probably harbored organisms with a wide range of metabolic potentials to exploit diverse sources of recalcitrant C, which may have contributed to the highest biodegradation of WEOM derived from PMCF-treated soil. The significantly lower bacterial diversity associated with the SRCF treatment (both before and after incubation) can probably be attributed to the low pH value. In this regard, Rousk et al. (2010) and Wu et al. (2017) have previously reported that bacterial diversity is positively correlated to soil pH, with lower diversity being recorded in acidic samples and higher diversity in neutral samples. A similar relationship between pH and bacterial diversity has also been observed in an aquatic environment (Maas et al., 2013).

Phylogenetic classification revealed that Proteobacteria was the most abundant and dominant phylum both before and after the incubation (Fig. S3). After incubation, the proportion of Proteobacteria markedly increased, whereas that of other relatively abundant phyla had changed to some extent. At the generic level (Fig. 4), *Shingomonas*, *Bacillus*, and *Flavisolibacter* were abundant in the

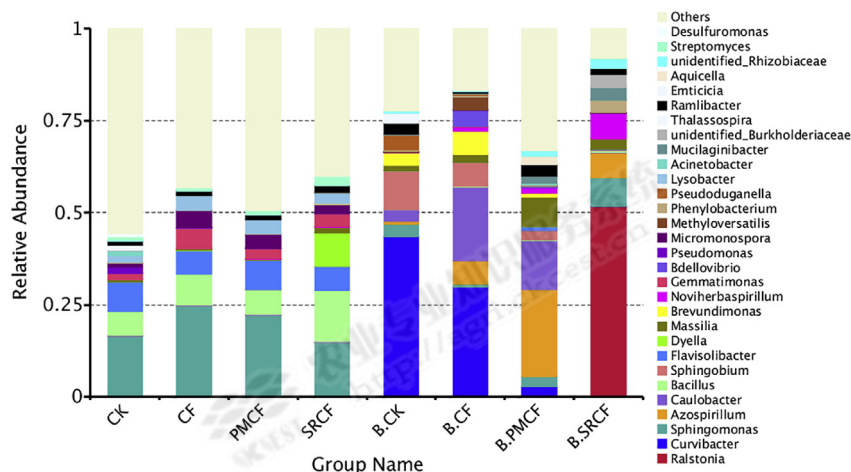


Fig. 4. Relative abundance of the major bacterial genera (top 30) before and after biodegradation. B. CK, B. CF, B. PMCF, and B. SRCF represent the samples after biodegradation.

original inoculum from four fertilization treatments, comprising 14.83–24.74%, 6.39–14.07%, and 6.35–8.16%, respectively. However, their relative abundance decreased after incubation. Interestingly, the genus *Dyella* was considerably more abundant in the SRCF inoculum than in that of the other treatments, with a relative abundance of 9.07%. The occurrence of *Dyella* can probably be explained by the fact that the species in this genus are cellulolytic bacteria (Guo et al., 2016) and have been reported to contribute to lignocellulose decomposition (Desiderato et al., 2018), which probably enabled *Dyella* to dominate the microbial community in the SRCF treatment. In addition, *Streptomyces* and *Bacillus* were also enriched in the SRCF treatment (Fig. S4). After incubation, significant differences were observed in the dominant genera able to utilize recalcitrant C in the WEOM derived from different soil samples. For CK and CF treatments, species of *Curvibacter* (43.25% in CK, and 29.68% in CF) were the most abundant bacteria, followed by *Sphingobium* (10.47% in CK) and *Caulobacter* (20.00% in CF), respectively. In contrast, *Azospirillum* (23.68%) and *Ralstonia* (51.75%) were the dominant genera in the PMCF and SRCF treatments, respectively, with species of *Caulobacter* (13.29%) being the second most dominant bacteria in the PMCF treatment. In a previous study that examined the biodegradation of sediment-derived dissolved DOM, *Azospirillum*, which is known to degrade proteinogenic amino acids, alicyclic, and recalcitrant aromatic compounds, was also enriched at the end of incubation (Wu et al., 2018). *Curvibacter* are typically distributed in oligotrophic natural mineral waters (Lesaulnier et al., 2017), and accordingly, it is assumed that WEOM derived from the CK treatment soil with the lowest nutrient levels, provides a suitable environment for *Curvibacter* growth. However, general soil pathogens *Ralstonia* were substantially enriched during the biodegradation of WEOM derived from the SRCF soil sample. A shift in the microbial community composition (top 35 genera) before and after incubation was also clearly shown in the heatmap (Fig. S4). Except for the dominant bacteria demonstrated in Fig. 4, various genera were enriched under the different fertilization treatments after incubation, namely *Emticicia*, *Pseudoduganella*, and *Ferrovibrio* under CK treatment; *Brevundimonas*, *Methyloversatilis*, and *Bdellovibrio* under CF treatment; *Massilia* and *Aquicella* under PMCF treatment; unidentified_Rhizobiaceae, *Mucilaginibacter*, *Noviherbaspirillum*, *Phenylobacterium*, and unidentified_Burkholderiaceae under SRCF treatment, although the relative abundance of these genera was relatively low. The relative abundance of dominant OTUs and the corresponding number of sequences is demonstrated in Fig. S5.

CCA was performed to determine the essential biochemical

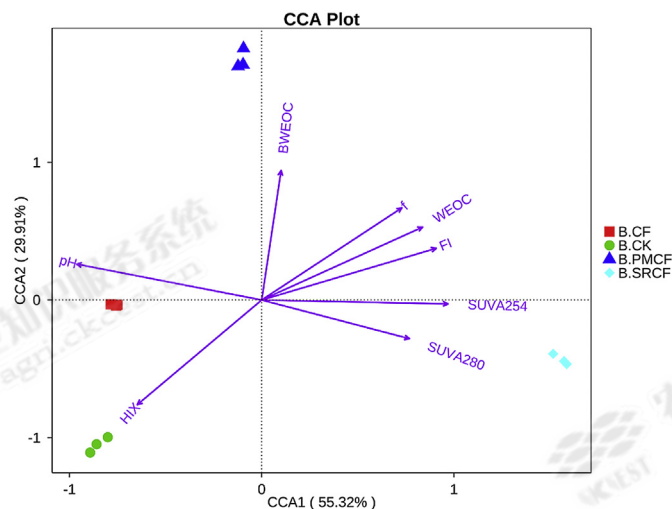


Fig. 5. A canonical correspondence analysis plot showing the relationship between biochemical variables (arrows) and microbial community structure (different symbols). FI represents fluorescence intensity, HIX represents humification index, *f* represents $f_{450/500}$ values, WEOC is the content of water-extractable organic carbon, and BWEOC is the biodegradable WEOC.

factors affecting bacterial community structure (Fig. 5), and clearly indicated that microbial community composition was closely correlated with pH and SUVA254. These findings tend to indicate that the shifts in microbial community composition were predominantly influenced by the intrinsic characters of WEOM, such as aromaticity, as well solution properties, such as pH. These factors have also been reported to be the controlling factors for DOM biodegradability (Marschnera. and Kalbitz., 2003). It has been reported that natural organic matter affects microbial community structure, both in soil (Ding et al., 2015) and groundwater (Wu et al., 2018; Zhang et al., 2015) circumstances. Similarly, WEOM also affects microbial community structure during the process of biodegradation. We speculated that the inherent traits of WEOM and solution properties influence the biodegradation of WEOC, probably by shaping microbial community structure. In summary, the WEOM derived from soil subjected to different fertilizer treatments had different inherent and solution properties, which resulted in differences in microbial composition and biodegradation rate.

4. Conclusion

This study revealed the dynamics, biodegradability, and microbial community shifts of WEOM derived from rice–wheat cropping soil under different fertilization treatments. PMCF- and SRCF-derived WEOM had higher WEOC contents, and accordingly, produced higher cumulative CO₂ and N₂O emissions during biodegradation than did the WEOM derived from CK- and CF-treated soil. Spectral analyses showed that WEOMs from PMCF- and SRCF-treated soil was less complex and had lower humus levels, which resulted in these WEOMs having higher biodegradation rates than WEOM derived from CK- and CF-treated soils. However, the lower pH of SRCF-derived WEOM affected the molecule size of this material and the related microbial activity, which accounted for considerably lower biodegradability of SRCF-derived WEOM compared with that of PMCF. High-throughput sequencing indicated that the dominant bacteria implicated in utilization of recalcitrant C in different WEOM varied with fertilization treatment. This can be attributed to the specific inherent characteristics and solution properties of WEOM derived from soil subjected to the different fertilizer treatments, which in turn shaped the associated microbial communities. The present study might enhance our understanding regarding the effects of fertilization treatments on the biogeochemical processes that occur within DOM, a material that links terrestrial and aquatic environments. However, in situ field trials are necessary to determine the actual changes in DOM and microbial community succession.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2019.03.091>.

References

- Akagi, J., Zsolnay, A., Bastida, F., 2007. Quantity and spectroscopic properties of soil dissolved organic matter (DOM) as a function of soil sample treatments: air-drying and pre-incubation. *Chemosphere* 69, 1040–1046.
- Bolan, N.S., Adriano, D.C., Curtin, D., 2003. Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Adv. Agron.* 78, 215–272.
- Bolan, N.S., Adriano, D.C., Kunhikrishnan, A., James, T., McDowell, R., Senesi, N., 2011. Dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. *Adv. Agron.* 110, 1–75.
- Brown, T.T., Koenig, R.T., Huggins, D.R., Harsh, J.B., Rossi, R.E., 2008. Lime effects on soil acidity, crop yield, and aluminum chemistry in direct-seeded cropping systems. *Soil Fert. Plant Nutr.* 72, 634–640.
- Chen, H.L., Zhou, J.M., Xiao, B.H., 2010. Characterization of dissolved organic matter derived from rice straw at different stages of decay. *J. Soils Sediments* 10, 915–922.
- Chin, Y., Aiken, G.R., J., O.E., 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environ. Sci. Technol.* 28, 1853–1858.
- D'Orazio, V., Traversa, A., Senesi, N., 2014. Forest soil organic carbon dynamics as affected by plant species and their corresponding litters: a fluorescence spectroscopy approach. *Plant Soil* 374, 473–484.
- Desiderato, J.G., Alvarenga, D.O., Constancio, M.T.L., Alves, L.M.C., Varani, A.M., 2018. The genome sequence of *Dyella jiangningensis* FCAV SCS01 from a lignocellulose-decomposing microbial consortium metagenome reveals potential for biotechnological applications. *Genet. Mol. Biol.* 41, 507–513.
- Ding, J., Zhang, Y., Wang, M., Sun, X., Cong, J., Deng, Y., Lu, H., Yuan, T., et al., 2015. Soil organic matter quantity and quality shape microbial community compositions of subtropical broadleaved forests. *Mol. Ecol.* 24, 5175–5185.
- Embacher, A., Zsolnay, A., Gättinger, A., Munch, J.C., 2008. The dynamics of water extractable organic matter (WEOM) in common arable topsoils. II. Influence of mineral and combined mineral and manure fertilization in a Haplic Chernozem. *Geoderma* 148, 63–69.
- Guo, L., Zheng, S., Cao, C., Li, C., 2016. Tillage practices and straw-returning methods affect topsoil bacterial community and organic C under a rice–wheat cropping system in central China. *Sci. Rep.* 6, 33155.
- Heinz, M., Graeber, D., Zak, D., Zwirrmann, E., Gelbrecht, J., Pusch, M.T., 2015. Comparison of organic matter composition in agricultural versus forest affected headwaters with special emphasis on organic nitrogen. *Environ. Sci. Technol.* 49, 2081–2090.
- Hui, C., Guo, X., Sun, P., Lin, H., Zhang, Q., Liang, Y., Zhao, Y., 2017. Depth-specific distribution and diversity of nitrite-dependent anaerobic ammonium and methane-oxidizing bacteria in upland-cropping soil under different fertilizer treatments. *Appl. Soil Ecol.* 113, 117–126.
- Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E., 2003. Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma* 113, 273–291.
- Lesaulnier, C.C., Herbold, C.W., Pelikan, C., Berry, D., Gerard, C., Le Coz, X., et al., 2017. Bottled aqua incognita: microbiota assembly and dissolved organic matter diversity in natural mineral waters. *Microbiome* 5, 126.
- Li, Y., Wang, S., Zhang, L., Zhao, H., Jiao, L., Zhao, Y., et al., 2014. Composition and spectroscopic characteristics of dissolved organic matter extracted from the sediment of Erhai Lake in China. *J. Soils Sediments* 14, 1599–1611.
- Liu, M., Zhang, Z., He, Q., Wang, H., Li, X., Schoer, J., 2014. Exogenous phosphorus inputs alter complexity of soil-dissolved organic carbon in agricultural riparian wetlands. *Chemosphere* 95, 572–580.
- Maas, E.W., Law, C.S., Hall, J.A., Pickmere, S., Currie, K.I., Chang, F.H., et al., 2013. Effect of ocean acidification on bacterial abundance, activity and diversity in the Ross Sea, Antarctica. *Aquat. Microb. Ecol.* 70, 1–15.
- Malhi, S., Nyborg, M., Solberg, E.D., McConkey, B., Dyck, M., Puurveen, D., 2011. Long-term straw management and N fertilizer rate effects on quantity and quality of organic C and N and some chemical properties in two contrasting soils in Western Canada. *Biol. Fert. Soils* 47, 785–800.
- Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability of dissolved organic matter in soils. *Geoderma* 113, 211–235.
- Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J.* 17, 10–12.
- McDowell, W.H., Zsolnay, A., Aitkenhead-Peterson, J.A., Gregorich, E.G., Jones, D.L., demann, D.J., et al., 2006. A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. *Soil Biol. Biochem.* 38, 1933–1942.
- Murphy, D.V., Macdonald, A.J., Stockdale, E.A., Goulding, K.W.T., Fortune, S., Gaunt, J.L., et al., 2000. Soluble organic nitrogen in agricultural soils. *Biol. Fert. Soils* 30, 374–387.
- Norman, R.J., Edberg, J.C., Stucki, J.W., 1985. Determination of nitrate in soil extracts by dual-wavelength ultraviolet spectrophotometry. *Soil Sci. Soc. Am. J.* 49, 1182–1185.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596.
- Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., et al., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* 4, 1340–1351.
- Russenes, A.L., Korsath, A., Bakken, L.R., Dorsch, P., 2016. Spatial variation in soil pH controls off-season N₂O emission in an agricultural soil. *Soil Biol. Biochem.* 99, 36–46.
- Saadi, I., Borisover, M., Armon, R., Laor, Y., 2006. Monitoring of effluent DOM biodegradation using fluorescence, UV and DOC measurements. *Chemosphere* 63, 530–539.
- Sachse, A., Babenzien, D., Ginzl, G., Gelbrecht, J., Steinberg, C.E.W., 2001. Characterization of dissolved organic carbon (DOC) in a dystrophic lake and an adjacent fen. *Biogeochemistry* 54.
- Sharma, P.K., Datta, S.K.D., Redulla, C.A., 1989. Effect of percolation rate on nutrient kinetics and rice yield in tropical rice soils. *Plant Soil* 119, 121–126.
- Siemens, J., Haas, M., Kaupenjohann, M., 2003. Dissolved organic matter induced denitrification in subsoils and aquifers. *Geoderma* 113, 253–271.
- Sun, J., Guo, L., Li, Q., Zhao, Y., Gao, M., She, Z., et al., 2016. Three-dimensional fluorescence excitation-emission matrix (EEM) spectroscopy with regional integration analysis for assessing waste sludge hydrolysis at different pretreated temperatures. *Environ. Sci. Pollut. Res. Int.* 23, 24061–24067.
- Sun, L., Chang, S.X., Feng, Y.S., Dyck, M.F., Puurveen, D., 2015. Nitrogen fertilization and tillage reversal affected water-extractable organic carbon and nitrogen differentially in a Black Chernozem and a Gray Luvisol. *Soil Till. Res.* 146, 253–260.
- Sun, S., Liu, J., Li, Y., Jiang, P., Chang, S.X., 2013. Similar quality and quantity of dissolved organic carbon under different land use systems in two Canadian and Chinese soils. *J. Soils Sediments* 13, 34–42.
- Wang, J., Li, C., Yu, S., Li, S., 2008. The biodegradation of dissolved organic carbon and nitrogen in brown earth with different fertility levels. *Acta Ecol. Sin.* 28,

- 6165–6171.
- Wei, Z., Zhao, X., Zhu, C., Xi, B., Zhao, Y., Yu, X., 2014. Assessment of humification degree of dissolved organic matter from different composts using fluorescence spectroscopy technology. *Chemosphere* 95, 261–267.
- Wu, X., Wu, L., Liu, Y., Zhang, P., Li, Q., Zhou, J., et al., 2018. Microbial interactions with dissolved organic matter drive carbon dynamics and community succession. *Front. Microbiol.* 9, 1234.
- Wu, Y., Zeng, J., Zhu, Q., Zhang, Z., Lin, X., 2017. pH is the primary determinant of the bacterial community structure in agricultural soils impacted by polycyclic aromatic hydrocarbon pollution. *Sci. Rep.* 7, 40093.
- Xi, M., Zi, Y., Wang, Q., Wang, S., Cui, G., Kong, F., 2018. Assessment of the content, structure, and source of soil dissolved organic matter in the coastal wetlands of Jiaozhou Bay, China. *Phys. Chem. Earth* 103, 35–44.
- Xu, H., Lin, H., Jiang, H., Guo, L., 2018b. Dynamic molecular size transformation of aquatic colloidal organic matter as a function of pH and cations. *Water Res.* 144, 543–552.
- Xu, P., Zhu, J., Fu, Q., Chen, J., Hu, H., Huang, Q., 2018a. Structure and biodegradability of dissolved organic matter from Ultisol treated with long-term fertilizations. *J. Soils Sediments* 18, 1865–1872.
- Yang, X., Peng, L., Li, S., Wang, S., 2013. Effect of mangrove leaf litter decomposition on soil dissolved organic matter. *Ecol. Environ. Sci.* 22, 924–930.
- Zhan, X., Liu, C., Fan, H., Lv, X., 2010. Comparison between two N- ammoniacal measurements in water napierian reagent colorimetric method and indophenol-blue colorimetric method. *J. Environ. Sci. Manag.* 35, 132–135.
- Zhang, P., Wu, W.M., Van Nostrand, J.D., Deng, Y., He, Z., Gihring, T., et al., 2015. Dynamic succession of groundwater functional microbial communities in response to emulsified vegetable oil amendment during sustained in situ U(VI) reduction. *Appl. Environ. Microbiol.* 81, 4164–4172.
- Zhou, P., Pan, G.X., Spaccini, R., Piccolo, A., 2010. Molecular changes in particulate organic matter (POM) in a typical Chinese paddy soil under different long-term fertilizer treatments. *Eur. J. Soil Sci.* 61, 231–242.