Relevance of dissolved organic matter generated from green manuring of Chinese milk vetch in relation to water-soluble cadmium

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Abstract

Dissolved organic matter (DOM) can become a carrier of soil contaminants. Therefore, an understanding of the evolution and characteristics of DOM produced by Chinese milk vetch during green manuring is crucial. In this study, DOM solutions from 28 days' manuring with three different organic materials were characterized using three-dimensional fluorescence excitation–emission matrix (3D-EEM) with parallel factor (PARAFAC) analysis, and ultraviolet–visible spectroscopy. With the green manuring milk vetch at flowering period (MVFP), the DOC and water-soluble cadmium (WS-Cd) in soil solution reached 1875 mg/l and 2.64 µg/l, respectively, on day 6 after manuring. The PARAFAC analysis modeled three components: protein-like (tryptophan) and two humic-like components (humic acid and fulvic acid); DOM produced by MVFP was primarily protein-like during the early stage of decomposition. The aromaticity and molecular weight of DOM in the MVFP treatment was lower than in the other treatments, which could promote the release of soil particle-adsorbed Cd to soil solution. Principal components analysis showed that aromaticity was the main factor affecting Cd solubility, and the negative linear correlation of aromaticity with WS-Cd reached 0.4827. The results of this study supported the idea that manuring with MVFP might accelerate Cd infiltration to deep soil with water under gravity.

Keywords Dissolved organic matter · Chinese milk vetch · Water-soluble cadmium · Fluorescence spectrum · Parallel factor analysis

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Abbreviations

CK	Control treatment in which no organic material is added to the pot
MVFP	Milk vetch at flowering period
MVWP	Milk vetch at withering period
RS	Rice straw

Introduction

Dissolved organic matter (DOM), which is a small and reactive fraction of total organic matter occurring from both terrestrial and aquatic environments, is widespread in natural environments (Henderson et al. 2009; Kellerman et al. 2015). During the last decade, research interest in DOM has been growing (Kellerman et al. 2015). DOM, which can pass through a 0.45-µm pore-size filter, is a complex and

heterogeneous mixture with a huge diversity of organic components. DOM is an active material that plays an important role in soil ecosystems, because it is associated with nutrient retention and release, carbon cycling, microbial activity, and soil physiochemical properties. It is generally accepted that the biomass-derived organic compounds in nature, including lignin, cellulose, semicellulose, lipid, and protein, are precursors of DOM (Benner et al. 1987; Leenheer et al. 2003).

Recent studies showed that functional groups, including –COOH, –OH, and C=O, and heterocyclic compounds containing N or S in DOM molecules can interact with soil contaminants by coordination, chelation, or complexation (Kalbitz and Wennrich 1998). DOM can become a carrier of heavy metals, herbicides, pesticides, persistent organic pollutants (POPs), and polycyclic aromatic hydrocarbons (PAHs) (Bauer and Blodau 2006; Kalbitz et al. 2000; Ravichandran 2004; Yang et al. 2017).

Milk vetch (*Astragalus sinicus* L.), which is used as a green manure, is a biennial herb plant of *Astragalus* Linn., Leguminosae, and has been widely planted in South China for centuries. In recent years, with the widespread use of chemical fertilizers, the planting area of milk vetch has gradually decreased, while the drawbacks of excessive fertilizer application have gradually appeared. In the context of China's efforts to control the amount of chemical fertilizer used in agriculture, milk vetch, which has the potential to improve the fertility and structure of soil, enrich microbial flora, reduce the amount of chemical fertilizer, and increase the yield of rice, has gained research attention again.

The carbon to nitrogen ratio (C/N) of milk vetch is about 10–20:1, which is facilitated by biological nitrogen fixation. Green manuring of milk vetch can therefore result in enhanced microbial activity in soil. Strong microbial activity can cause rapid degradation of milk vetch and promote DOM production. Previous studies showed that DOM has effects on the precipitation–dissolution equilibria of heavy metals under natural conditions (Harter and Naidu 1995). It reduces the adsorption of heavy metals in soil mainly through two aspects: (1) The interaction of DOM and free heavy metal ions in soil solution could form a DOM–heavy metal complex; (2) DOM could be preferentially adsorbed by soil and compete with heavy metals for the effective adsorption sites in soil (Giusquiani et al. 1998).

The production of DOM during milk vetch manuring is very complex. It is necessary to reveal the DOM characteristics and the relationship between DOM and heavy metal mobility in the soil, especially in the context of growing concern around heavy metal pollution. Therefore, the aims of this study were (1) to investigate the dynamic changes in DOM concentration after green manuring of milk vetch, (2) to detect the concentration of cadmium (Cd) in soil solution, (3) to compare the ultraviolet (UV) and fluorescence characteristics of DOM produced from different organic materials, and (4) to reveal the possible relationships between the characteristics of DOM constituents and water-soluble Cd (WS-Cd).

Materials and methods

Soil

Soil was collected from a pedon in a fallow rice paddy around a smelting plant with serious Cd pollution, located in Guixi County, Jiangxi Province (28° 18′ 29″ N, 117° 10′ 33″ E). The soil sample was classified as Gleyi-Stagnic Anthrosol according to the Chinese soil system. Adequate amounts of soil were collected from the topsoil layer (0–20 cm) and then transported to the greenhouse for the pot experiment.

Well-established methods were used to characterize the soil in terms of pH (water extraction), organic matter (OM), total nitrogen (TN), total phosphorus (TP), alkali-hydrolyzable N (AN), available P (AP), available K (AK), cation exchange capacity (CEC), dissolved organic C (DOC), and dissolved organic N (DON) (Wu et al. 2010). The soil content of six heavy metals (mercury, Cd, lead, arsenic, chromium, and copper) was measured by mixed acid digestion in a reflux condenser system (ISO 11466-1995). The basic physicochemical characteristics of the soil are listed in Table 1.

Pot experiment

The pot experiment was carried out at the Jiangxi Academy of Agricultural Sciences, Nanchang, Jiangxi Province (28° 33′ 40″ N, 115° 56′ 33″ E).

Milk vetch was carefully collected containing the aboveground parts, roots, and root tumors in the flowering period (April 1, 2016) and withering period (April 25, 2016); rice straw was collected on November 15, 2015, containing only the aboveground parts after threshing. The organic materials of rice straw (RS) and milk vetch in the flowering period (MVFP) and withering period (MVWP) were washed with tap water, cut into 5-cm segments, and stored in plastic bags at − 18 °C.

Fifteen kilograms soil was sieved to < 8 mm and then placed in a pot with 15 kg water; the soil was then soaked thoroughly for 10 days. The experiment was conducted with four treatments (control (CK), MVFP, MVWP, RS) and three replicates of every treatment. Organic materials were supplied to the soil at 0.2% (dry matter) and the details are shown in Table 2. All samples were applied to the pots on June 9, 2016, and the soils were fully mixed.

Sample collection

In this experiment, the Rhizon MOM soil solution sampler (Rhizosphere Research Products B.V., Wageningen,

Table 1 Chemical properties and heavy metal contents of the experimental soil

Item	pH	OM (g/kg)	T-N[a] (g/kg)	T-P (g/kg)	A-N (mg/kg)	A-P (mg/kg)	A-K (mg/kg)	CEC (cmol/kg)	Hg (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	As (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	DOC[b] (mg/kg)
Value	4.76	18.8	1.76	0.541	202	84.4	219.5	10.5	0.158	0.693	37.6	15.3	37.8	61.0	45.4
Chinese standard[c]	–	–	–	–	–	–	–	–	0.500	0.300	80	30.0	250	50.0	–

[a] T-N means total nitrogen, the same for T-P; A-N means available nitrogen, the same for A-P and A-K

[b] Dissolved organic carbon extracted at soil/water 1:2.5 (w/v)

[c] Soil Environmental Quality Standard of China (GB 15618-2018) for acid soils (pH < 5.5)

The Netherlands) was adopted to collect soil solution. The front of this sampler is a 12-cm porous polyester tube, with a diameter of 2.5 mm and a mean pore size of 0.15 μm. The Rhizon MOM sampler was vertically buried in the middle of the pot, and a threaded syringe (30 ml) was connected to the end of the sampler. The soil solution was aspirated with the syringe at 9:00 a.m. on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 16, 19, 22, 25, and 28 after manuring. All samples collected were stored in two 15-ml centrifuge tubes at − 18 °C. After the extraction of the soil solution, 50 ml deionized water was added to each experimental pot.

Characterization of DOM

DOC and DON, representing the DOM in solution, were determined using a multi N/C analyzer (3100, Analytik Jena, Germany), and results were given in milligrams per liter. For each sample, 30 μl sample was processed in triplicate at 850 °C.

Cd content in soil solution was determined with GFAAS (PinAAcle900T, Perkin-Elmer, USA). The matrix modifier was 0.5% $NH_4H_2PO_4$. For Cd determination, 20 μl sample and 5 μl matrix modifier were injected into the graphite tube; the ashing temperature was 500 °C and the atomization temperature was 1500 °C. Samples were analyzed in triplicate.

All samples were filtered through 0.2-μm polysulfone filters before analysis. The concentrations of DOC, DON, and WS-Cd were quantified by the area of the peaks. All results obtained for each sample are the average of three measurements in terms of coefficient of variation ($\leq 5\%$).

Three-dimensional fluorescence excitation–emission matrix (3D-EEM) was performed with scan mode using a Cary Eclipse spectrometer (Varion, USA), with the excitation (Ex) range from 200 to 452 nm and the emission (Em) range from 200 to 550 nm, in increments of 2 nm and 5 nm, respectively. The Ex and Em bandwidths were 5 nm, and the scan speed was 2400 nm/min. The voltage of the photomultiplier tube was set to 700 V for low-level light detection. A Milli-Q water blank of the 3D-EEMs was subtracted to eliminate the Raman scatter peaks of each sample.

The UV spectra were determined with a UV–Vis spectrophotometer (Lambda25, Perkin-Elmer, USA) using a 1-cm quartz colorimetric cuvette, with a scanning range of 200–700 nm at a scanning speed of 240 nm/min; the wavelength at which the lamp switched from tungsten to deuterium was 380 nm. All samples were maintained at room temperature (∼ 20 °C) and in the dark to prevent microbial degradation and photodegradation.

The parallel factor modeling

The parallel factor (PARAFAC) modeling approach has been described in detail (Bro and Kiers 2003; Stedmon and Bro

Table 2 Added amount of exogenous organic materials for different treatments

Treatment	Added organic material	Added proportion (% of soil)	Added weight (fresh sample in g)	Added weight (air-dried sample in g)
CK (control)	No	0	0	0
MVFP	Milk vetch at flowering period	0.2[a]	150	–
MVWP	Milk vetch at withering period	0.2	–	30
RS	Rice straw	0.2	–	30

[a] On dried weight basis with 15 kg soil. The moisture content of the fresh sample is about 80%; the converted air-dried weight of the sample was 30 g

2008), and it was performed with MATLAB 2008 (Mathworks Inc., USA) using the *N-way* Toolbox (Andersson and Bro 2000) and DOMFluor (Stedmon and Bro 2008). Before PARAFAC modeling, some preprocessing steps were used to eliminate the influence of scatter lines. An EEM of deionized H_2O was subtracted from the EEM of each soil solution to remove the lower-intensity Raman scatter effects (Stedmon et al. 2003), and the Rayleigh scatter lines were removed by setting the fluorescence intensity values of these data points as missing. In this study, 48 soil solution samples were measured. The core consistency diagnostic score of each PARAFAC model with one to seven components was fitted to the data. The core consistency close to 100% implies an appropriate model, but, if the consistency score is low (< 60%), it is an indication that the presupposed number of components is invalid (Bro and Kiers 2003). The number of fluorescence components was verified by split-half and residuals of loading analysis (Stedmon and Bro 2008).

DOM optical properties

The DOM content in soil solution affected the fluorescence intensity and UV–Vis absorbance directly, so the intensity or absorbance of different samples cannot be compared directly. Two methods were used to eliminate the influence: intensity/absorbance at a specific wavelength divided by DOC concentration and the ratio of absorbance/absorbance at two different wavelengths.

The fluorescence index (FI) and humification index (HIX) were calculated from EEM data and used to evaluate the sources and the humification degree of DOM. The biological index (BIX) was also used to indicate the presence of the autochthonous biological activity.

FI, the ratio of fluorescence intensity with an emission wavelength at 450 nm and 500 nm, with excitation wavelength at 370 nm (McKnight et al. 2001; Ylla et al. 2012), was mainly used to characterize the source of humus in DOM. A high FI value (~ 1.7–2.0) reflects organic matter with an autochthonous origin, whereas a low FI (~ 1.3–1.4) reflects organic matter with an allochthonous origin (Wickland et al. 2007).

HIX, based on the location of emission spectra, is a ratio of the areas under the emission spectra over 435–480 nm to that over 300–345 nm at excitation of 255 nm (in some studies, 254 nm was used (Zhang et al. 2010, Zsolnay et al. 1999). The HIX value increases with the degree of aromaticity of DOM, because the emission spectrum at excitation 255 nm becomes red-shifted. A high HIX value means a high degree of humification and a complex molecule such as a high molecular weight aromatic (Senesi et al. 1991).

BIX was determined by the ratio of fluorescence intensity at 380 nm to 430 nm, both at excitation of 310 nm. A high BIX value (> 1.0) reflects that the DOM was freshly produced by biological or autochthonous microorganisms, while a low BIX value (< 1.0) indicates that the DOM contains little biological material and is mainly allochthonous input (Huguet et al. 2009).

Molar absorptivity at 254 nm ($SUVA_{254nm}$) and 284 nm ($SUVA_{284nm}$) was put forward to represent the aromaticity of DOM (Granskog et al. 2007; Weishaar et al. 2003). The formulae were $SUVA_{254nm} = (UV_{254nm}/DOC) \times 100$ (Xie et al. 2012) and $SUVA_{280nm} = (UV_{280nm}/DOC) \times 100$.

E_{253}/E_{203}, the ratio of absorbance at 253 nm with 203 nm, was applied to describe the average molecular weight of DOM.

Statistical analyses

Data in this experiment were analyzed by Excel and Origin 8.5. 3D-EEM was plotted by SigmaPlot 13.0. Statistical analysis was performed with SPSS (Statistical Program for the Social Sciences) 24.0. The linear model was validated with analysis of variance (ANOVA).

Results and discussion

Changes in DOM concentrations during manuring with different organic materials

The concentration of DOC and DON in soil solution during manuring is shown in Fig. 1a, b, respectively. The DOC concentration in the CK treatment remained at a low level of
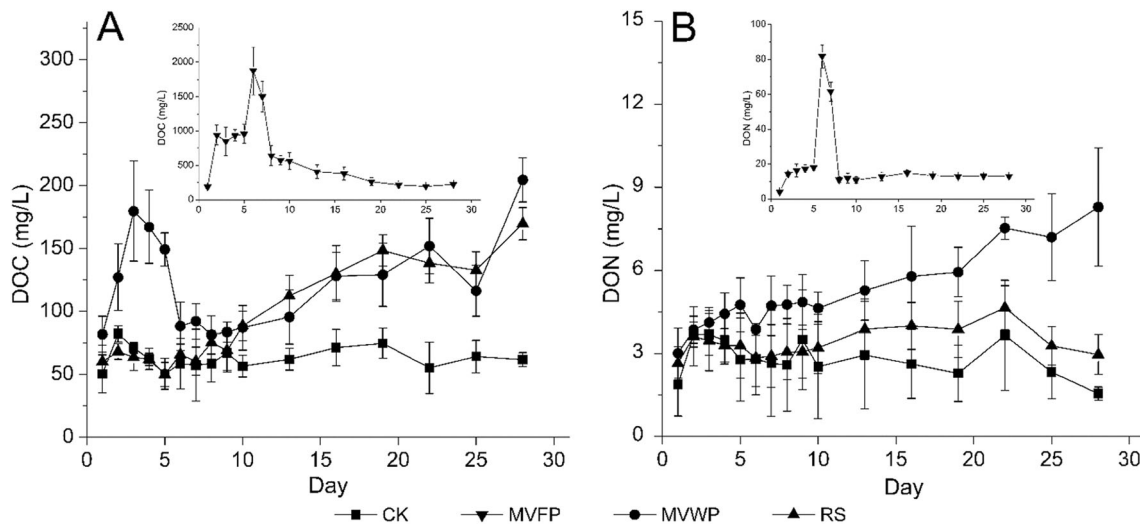
Fig. 1 Dissolved organic carbon (DOC) (**a**) and dissolved organic nitrogen (DON) (**b**) concentration of different treatments in soil solution

about 70 mg/l during the whole monitoring period (Fig. 1a). In the early stage (about the first 10 days), the DOC concentration in the RS treatment was not significantly different from that of the CK treatment. In contrast, the DOC in the MVWP treatment reached a maximum value of 180 mg/l on day 3 and then decreased to a minimum value on day 8. The DOC in the MVWP and RS treatments increased gradually in the later stage of green manuring (10–25 days), and finally reached about 204 and 170 mg/l, respectively. This result might indicate that MVWP is different to RS, and contains easily decomposed organic matter; therefore, it decomposed rapidly and generated DOM during the first 3 days. After about 10 days, the refractory organic components, such as lignin, cellulose, and hemicellulose, both in RS and MVWP, decomposed gradually and generated DOM.

The DOC in the MVFP treatment was significantly different to that of the other treatments. On day 2, after manuring, the DOC concentration in soil solution soared to 941.6 mg/l, and remained stable to day 5; then, it sharply increased to 1874.6 mg/l on day 6, which was ten times more than that in the MVWP and RS treatments. Subsequently, the DOC concentration in MVFP declined to 224 mg/l on day 28, which was not significantly different ($P > 0.05$) from that of the MVWP treatment.

Similar to the trend of DOC concentration in the soil solution, the DON concentration of MVFP increased dramatically to 14.3 mg/l on day 2 after manuring, and then had a significant peak of 81.7 mg/l on day 6. Subsequently, the DON decreased to and remained at 12–14 mg/l in the later stage of manuring. This was significantly higher than those of MVWP and RS.

The DON concentration of the MVWP treatment was significantly different ($P < 0.05$) from that of the CK treatment 13 days after manuring, and was significantly different from the RS treatment ($P < 0.05$) 19 days after manuring. These

results indicated that with the decomposition of leguminous green manure (milk vetch), the N compounds fixed in root nodules were released into the soil solution, so the DON concentration in these treatments was significantly higher than that of the RS treatment.

There were no significant differences between the other two treatments, RS and CK ($P > 0.05$), during the entire monitoring period, which indicates that there was limited decomposition of N compounds from the RS.

Variation in WS-Cd

The WS-Cd content in soil solution of the MVFP treatment showed an obvious increase on day 2, and then remained at relatively stable levels until another increase occurred on day 5, with the maximum concentration of 2.64 μg/l appearing on day 6 (Fig. 2). This trend was very similar to the changes in
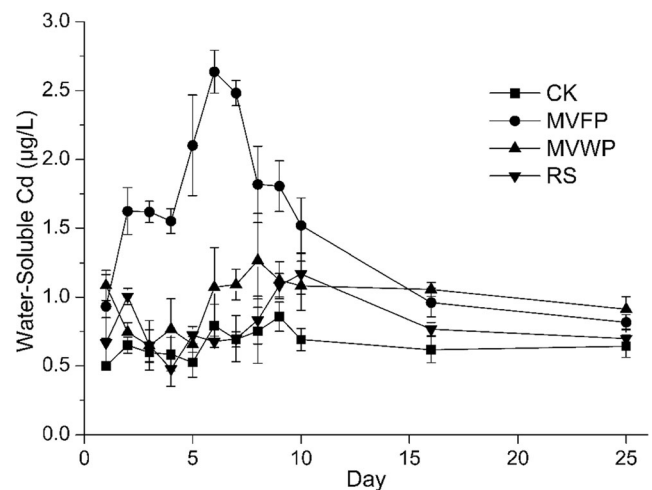
Fig. 2 The water-soluble cadmium (Cd) concentration of different treatments in soil solution

DOC and DON in soil solution. This result indicates that the DOM in soil might affect the solubility and environmental behavior of Cd. There is potential for the activation of Cd by DOM generated from fresh milk vetch, which needs careful consideration.

After 10 days of manuring, the WS-Cd content showed a gradual decline to a low level, and became similar to that of the MVWP and RS treatments. With the decomposition of the DOM bound to Cd, Cd might be released into soil solution and re-adsorbed by the soil particles. The Cd activation by fresh milk vetch was temporary for about 10 days under the experimental conditions.

D-EEM-PARAFAC components of DOM

For the combined set of soil solutions on days 1, 6, 10, and 25, PARAFAC models with one to seven components were computed. The core consistency diagnostic scores for the one- to seven-component models were 100, 100, 81.1, 66.5, 45.1, 6.77, and 4.41, respectively. The core consistency diagnostic score of the appropriate number of components should be close to 100% for the models, and the lower limit should be above 60%. The three- or four-component models, with core consistency diagnostic scores of 81.1% and 66.5%,

respectively, could be chosen to describe the EEM data. Further research of split-half and residuals of loading analysis showed that the three-component model was the appropriate PARAFAC model to describe the data set.

The excitation (Ex) and emission (Em) characteristics of the DOM components identified in the soil solutions are shown in Fig. 3. Components 1 (C1) and 3 (C3) were humic-like components (Liu et al. 2007; Wu et al. 2012). Depending on the differences in the Ex and Em wavelength regions, C1 and C3 are likely to be HA and FA, respectively. Among them, the peaks of Ex/Em = 232/460 nm and Ex/Em = 328/460 nm for C1 represent terrestrial HA in the short- and long-wavelength regions, respectively, while the peaks of Ex/Em = 232/400 nm and Ex/Em = 320/400 nm for C3 represent terrestrial FA in the short- and long-wavelength regions, respectively (Wu et al. 2012).

Component 2 (C2) had a primary fluorescence peak at an Ex/Em wavelength of 220/305 nm and a secondary peak at 276/305 nm, which were similar to those of a protein-like fluorescent compound at low- and high-excitation regions (Zhang et al. 2010) and confirmed as a tryptophan-like fluorescence peak (Coble 1996). It was reported that C2 was related to the bioavailability and microbial activity of DOM (Fellman et al. 2009).
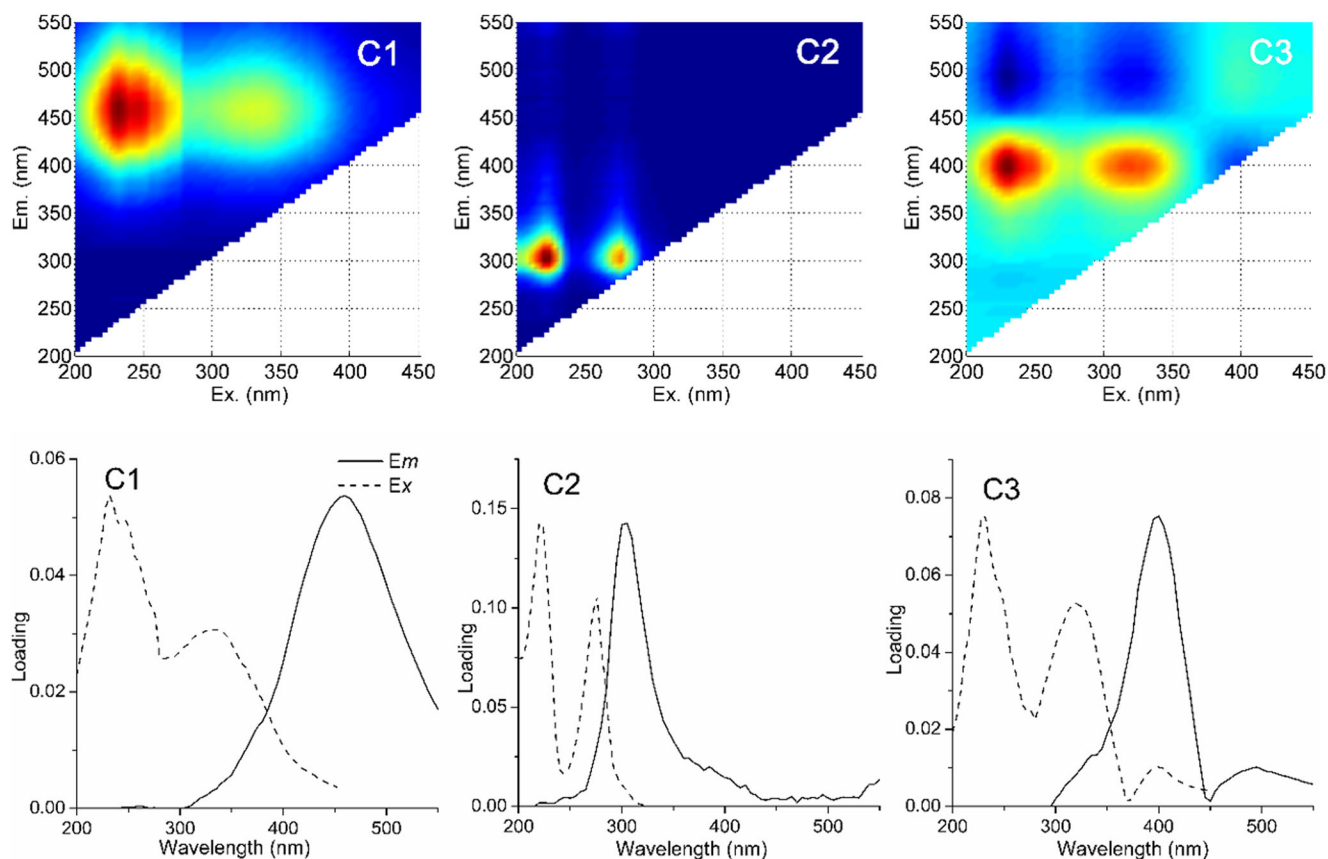
Fig. 3 Excitation–emission matrix (EEM) of three-dimensional fluorescence and loading of three components (C1, C2, and C3) of dissolved organic matter (DOM) during the green manuring

The proportions of these three organic components in soil solution at days 1, 6, 10, and 25 after manuring of exogenous organic matter are shown from left to right, respectively, in Fig. 4. Overall, the proportions of C1 and C3 in the treatments with organic material additions (MVFP, MVWP, and RS) were lower than for that of CK, especially for the MVFP treatment; the proportions of component C2 were significantly higher than that of CK in the treatments with organic material in the later stage of manuring. The proportions of these three components in different treatments gradually converged on day 25.

However, in the later stage after manuring, there was a significant difference in the relative proportions of each DOM component in the different treatments. The proportions of C1 and C3 in the different treatments increased in the order MVFP < MVWP < RS, while the proportion of C2 followed the opposite trend.

The content of C2 in the MVFP and MVWP treatments first increased and then decreased (Table 3); the change in concentration of C2 in the RS treatment was slower than that of the other two treatments. The content of C2 in the MVFP and MVWP treatments increased from 102.1 and 28.6 mg/l on day 1 to 1709.6 and 43.4 mg/l, respectively, on day 6. By day 25, the contents of C2 were 38.36 and 15.23 mg/l, respectively. In comparison, the content of C2 in the RS treatment increased significantly after day 10, and the content of C2 in the CK treatment remained stable.

During the whole manuring process, there was a gradual increase in the content of C1 and C3 components (Table 4). The C1 and C3 contents in the MVFP, MVWP, and RS treatments on day 25 were 1.59 to 2.49 times those on day 1.

UV-absorption and fluorescence characteristic parameters of DOM

The UV-absorption and fluorescence parameters revealed the changes in DOM components in the soil after adding organic materials. Combining the characteristics of these two methods, we obtained the overall changes in the aromaticity, molecular weight, and humification degree of DOM, so as to

Table 3 Concentrations of parallel factor (PARAFAC) analysis components of dissolved organic matter (DOM) in soil solution on different days after manuring treatments of milk vetch in the flowering period (MVFP), milk vetch in the withering period (MVWP), rice straw (RS), and control (CK)

Treatment	Day after manuring	Component concentration (mg/l)		
		C1	C2	C3
MVFP	1	40.43	102.11	46.12
	6	48.52	1709.64	116.44
	10	65.30	402.38	96.35
	25	65.54	38.36	91.88
MVWP	1	29.03	28.60	24.19
	6	22.96	43.36	21.91
	10	26.14	30.76	30.09
	25	46.23	15.23	54.87
RS	1	19.16	16.60	24.35
	6	23.10	20.28	22.40
	10	31.67	21.15	35.60
	25	47.78	25.44	59.52
CK	1	18.99	10.43	21.02
	6	22.87	13.53	22.17
	10	21.88	11.85	22.77
	25	28.74	11.05	34.44

understand the mechanisms affecting WS-Cd content in soil solution.

Aromaticity of DOM molecule

The $SUVA_{254nm}$ and $SUVA_{280nm}$ values of DOM over time are shown in Fig. 5a, b. The $SUVA_{254nm}$ and $SUVA_{280nm}$ values of CK were always higher than those of the treatments with organic material, which means that the application of organic materials reduced the aromaticity of DOM in soil. Moreover, the $SUVA_{254nm}$ and $SUVA_{280nm}$ values of MVFP were far lower than those of the other treatments in the early stage of manuring, and showed the lowest values of 0.052 and 0.044, respectively, on day 5.
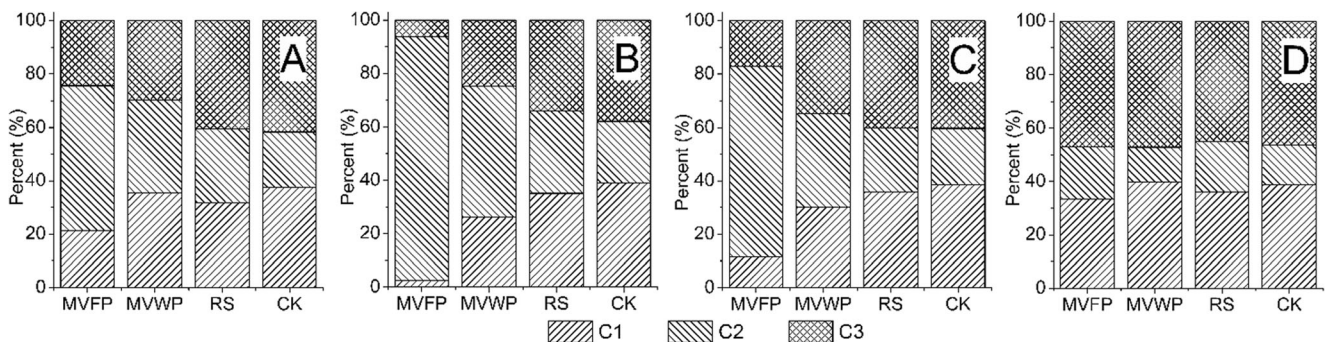
Fig. 4 The proportions of the three components in dissolved organic matter (DOM) on day 1 (D1) (**a**), day 6 (D6) (**b**), day 10 (D10) (**c**), and day 25 (D25) (**d**) after manuring

Table 4 Correlation analysis of ultraviolet (UV) absorption values; fluorescence spectrum parameters; proportions of dissolved organic matter components; and concentrations of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and water-soluble cadmium (WS-Cd)

	$SUVA_{254nm}$	$SUVA_{280nm}$	E_{250}/E_{365}	FI	HIX	BIX	%C1	%C2	%C3	DOC	DON	WS-Cd
$SUVA_{254nm}$	1											
$SUVA_{280nm}$	0.998**	1										
E_{250}/E_{365}	−0.501**	−0.519**	1									
FI	0.416**	0.417**	−0.334*	1								
HIX	0.608**	0.599**	−0.121	0.528**	1							
BIX	−0.352*	−0.348*	0.236	−0.359*	−0.546**	1						
%C1	−0.805**	−0.803**	−0.443**	0.509**	0.831**	−0.566**	1					
%C2	−0.739**	−0.739**	0.356*	−0.503**	−0.899**	0.568**	−0.964**	1				
%C3	−0.635**	−0.635**	−0.257	0.516**	0.906**	−0.534**	0.872**	−0.971**	1			
DOC	−0.672**	−0.672**	0.574**	−0.621**	−0.560**	0.558**	−0.819**	0.788**	−0.711**	1		
DON	−0.591**	−0.588**	0.537**	−0.611**	−0.483**	0.590**	−0.740**	0.709**	−0.638**	0.975**	1	
WS-Cd	−0.646**	−0.642**	0.422**	−0.511**	−0.652**	0.580**	−0.828**	0.834**	−0.787**	0.890**	0.861**	1

Note: %C1, %C2, and %C3 are the percentages of the three components of dissolved organic matter obtained by the parallel factor modeling the three-dimensional fluorescence excitation–emission matrix

*Significant correlation at the 0.05 level (two-tailed); **significant correlation at the 0.01 level (two-tailed)
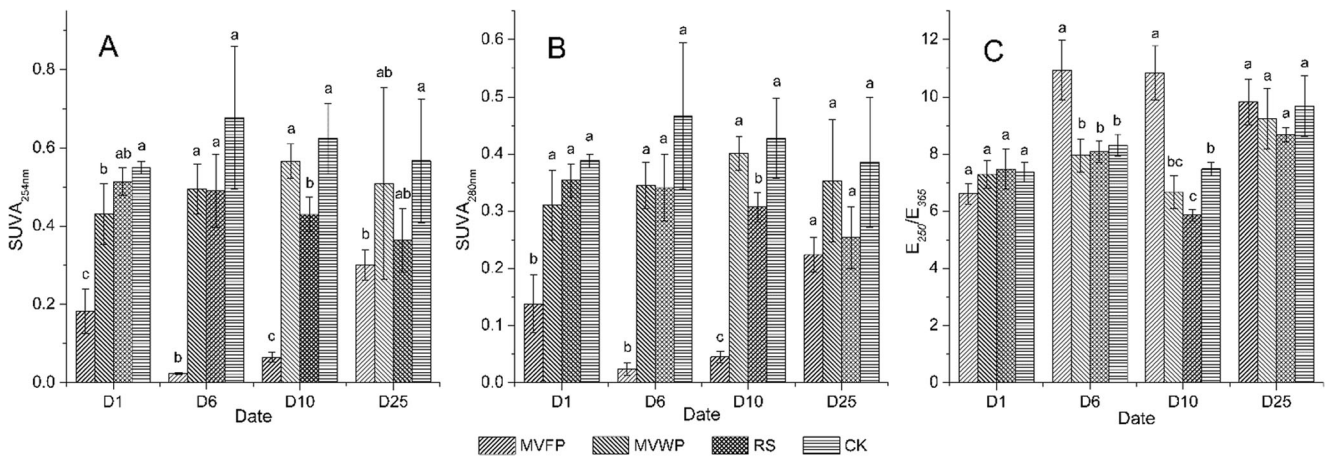
Fig. 5 The molar absorptivity at 254 nm (SUVA254nm) (**a**) and 284 nm (SUVA284nm) (**b**) and the ratio of absorbance at 253 nm with 203 nm (E_{250}/E_{365}) (**c**) on different days after manuring. Note: Different letters on the same group of bar plots indicate significant differences between treatments ($P < 0.05$).

The variations in the three fluorescence indices with treatments and sampling dates are shown in Fig. 6b. The HIX ranged from 0.252 to 10.64; the lowest and highest values of this index in all treatments occurred on days 5 and 26, respectively, after manuring. From day 1 to day 6 and further to day 25, the HIX of the MVFP treatment decreased from 2.442 to 0.252 and further to 6.699, lower than for all the other treatments ($P < 0.05$). Simple compounds, like proteins, decomposed into soil solution in the early stage, resulting in the decline in DOM aromaticity; then, the HIX increased with the humification of DOM in the later stage. This phenomenon of first decreasing and then increasing was consistent with the values of SUVA$_{254nm}$ and SUVA$_{280nm}$ (Fig. 5). A significant and positive linear relationship was found between SUVA$_{254nm}$ and HIX ($R^2 = 0.4021$, $P < 0.01$), and between SUVA$_{280nm}$ and HIX ($R^2 = 0.3949$, $P < 0.01$).

It was confirmed that the aromatic compounds in DOM containing unsaturated carbon–carbon bonds have strong UV absorption at 254 nm; a larger value of absorbance at 254 nm under the same DOC concentration indicated a higher aromaticity of DOM (Dilling and Kaiser 2002). Chin et al. (1994) studied the organic matter of water, which showed that the absorbance of organic matter at 280 nm also provided some information about the DOM humification degree and molecular weight. A lower aromaticity showed a higher system energy, lower stability, and higher chemical activity of DOM molecules, which could increase the interaction between DOM and Cd.

Average molecular weight of DOM

The UV absorbance value was directly affected by the DOC concentration in soil solution, but the influence of concentration could be excluded by using the ratio of absorbance at two specific wavelengths. De Haan and De Boer (1987) considered that the ratio of absorbance at 250 nm and 365 nm (E_{250}/
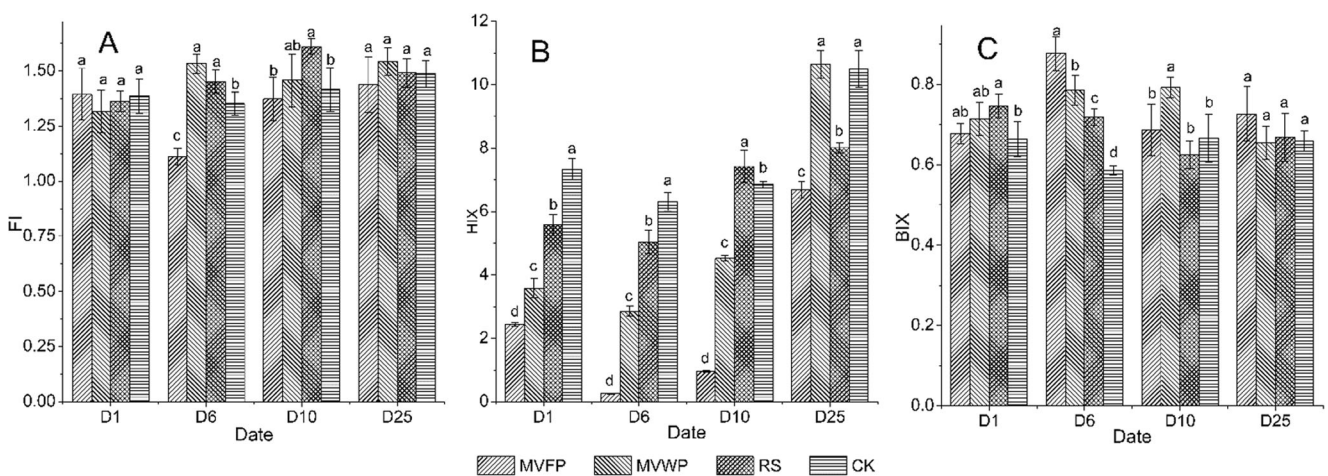
Fig. 6 The values of fluorescence index (FI) (**a**), humification index (HIX) (**b**), and biological index (BIX) (**c**) on different days after manuring. Note: Different letters on the same group of bar plots indicate significant differences between treatments ($P < 0.05$).

E_{365}) could be adopted to characterize the DOM molecular weight. There was a negative correlation between aromaticity, molecular weight, and the value of E_{250}/E_{365} (Peuravuori and Pihlaja 1997).

The values of E_{250}/E_{365} for the soil solution on different sampling dates are shown in Fig. 5c. The E_{250}/E_{365} of MVFP was significantly greater than that of the other treatments on days 5–10 after manuring, with values of 10.58 and 10.84, respectively. Generally, the E_{250}/E_{365} value was negatively correlated with the molecular weight of DOM (Helms et al. 2008; Huguet et al. 2009; Wang et al. 2009), which indicated that the molecular weight of DOM produced by MVFP was significantly lower than that of the other treatments. The values of E_{250}/E_{365} on day 5 after manuring were in the order of MVFP > MVWP > RS > CK, which indicated that the order of average molecular weight from high to low was CK < RS < MVWP < MVFP. Low molecular weight DOM was dominated by protein-like components, while high molecular weight DOM was dominated by humic-like components. This result was consistent with those of Fig. 5, confirming the variation of each component during the decomposition process.

Previous studies showed that DOM of low molecular weight and with hydrophilic components was not easily adsorbed by soil particles. This indicated the high mobility and strong ability to form heavy metal complexes of these DOM components (Antoniadis and Alloway 2002; Schmitt et al. 2003).

DOM sources

A high BIX value (> 1.0) reflects that DOM was freshly produced by biological or autochthonous microbial input (Huguet et al. 2009). The BIX values in all treatments during the green manuring period were < 1.0, and varied within the range of 0.586–0.877 (Fig. 6c), suggesting that the organic matter was mainly from external input, such as decomposition of plant residues. The BIX values first increased and then decreased during the manuring period in MVFP and MVWP treatments, and the maximum values were 0.877 and 0.792 on days 6 and 10, respectively. However, the BIX values in the RS treatment decreased during the manuring process, with values of 0.747 and 0.719 on days 1 and 6, respectively. It was noteworthy that the BIX value of the CK treatment was around 0.6 and decreased to < 0.6 on day 6.

FI was used as an indicator of the relative contributions of DOM derived from terrestrial versus microbial origins. The FI values in all treatments were within the range of 1.111 to 1.610 with a mean value of 1.42 (Fig. 6a). This result indicated that the DOM in soil solution mainly came from the decomposition of the added organic material (MV and RS). The FI value of the MVFP treatment decreased to 1.111 on day 6 and then increased to 1.37–1.43, indicating that the composition of fresh milk vetch generated much more DOM than the other

organic matter treatments, especially in the early stage of green manuring. Moreover, the decomposition of fresh organic matter had almost been completed at a later stage, and the gradually slower decomposition made the FI value return to normal levels (~ 1.5).

The BIX and FI values indicated the microbial activity and the DOM source in soil solution, and the results were consistent with each other. The low FI value clearly demonstrated that the DOM mainly came from the decomposition of exogenous organic matter, but not from microbial metabolism, because the BIX value was low.

## Relationship between DOM and WS-Cd content

The results of correlation analysis of UV and fluorescence parameters; the concentrations of DOC, DON, and WS-Cd; and the proportion of each component are shown in Table 4.

There was no significant correlation between the absorbance value of any single wavelength and the DOM content in soil solution. Therefore, the molar absorptivity of DOM ($SUVA_{254nm}$ and $SUVA_{280nm}$) was used for further analysis.

The $SUVA_{254nm}$ and $SUVA_{280nm}$ parameters of DOM in soil solution showed a significant positive correlation with each other, and the correlation coefficient reached 0.998 ($P < 0.01$); these results confirmed the presence of benzene compounds in the DOM. Phenylamine has two extremely strong absorption peaks near 254 nm and 280 nm. Simultaneously, $SUVA_{254nm}$ and $SUVA_{280nm}$ were negatively correlated with concentrations of DOC, DON, and WS-Cd and the proportions of these components, which illustrated that the aromaticity of DOM produced by exogenous organic matter was lower than that of the original soil, leading to an increase in the WS-Cd concentration.

E_{250}/E_{365} was negatively correlated with $SUVA_{254nm}$ and $SUVA_{280nm}$, with correlation coefficients of − 0.501 and − 0.519 ($P < 0.01$), respectively. Humification of DOM resulted in an increasing of average molecular weight, which significantly reduced the aromaticity of DOM (Peuravuori and Pihlaja 1997).

There was a clear correlation between the proportion of each component and the WS-Cd concentration in this study ($P < 0.01$), with all correlation coefficients > 0.70. Furthermore, the proportions of C1 and C3 were negatively correlated with the proportion of C2, with correlation coefficients of − 0.964 and − 0.971, respectively. The proportions of C1 and C3 were also negatively correlated with the WS-Cd concentration. This indicated that the increase or decrease of FA and HA contents in soil solution was completely synchronized, but this trend was the opposite to that of the protein-like material. The WS-Cd concentration was highly consistent with the variation of the protein-like component. The FI and BIX values confirmed that C2 was produced from the exogenous organic material (i.e., the green manuring of milk vetch).
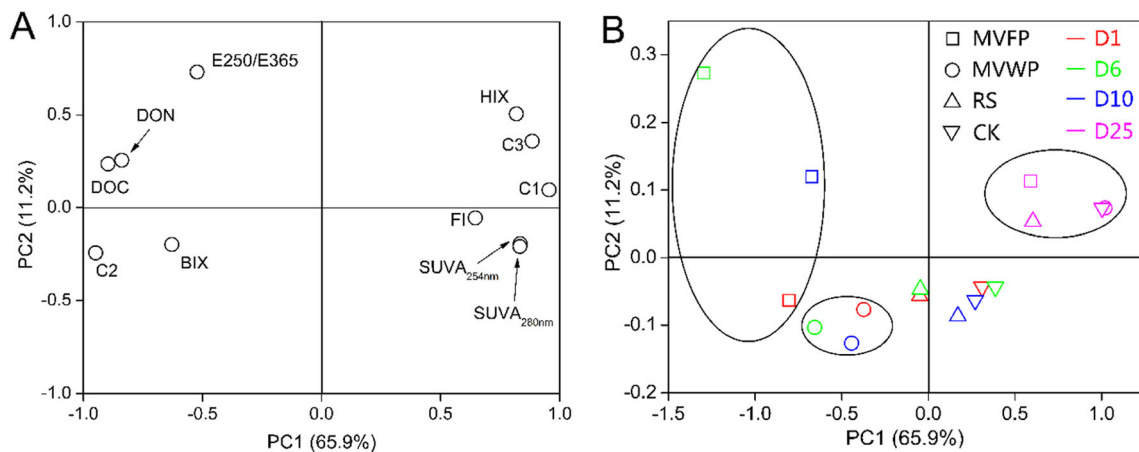
Fig. 7 Loading plots (**a**) and score plots (**b**) for principal components 1 and 2 (PC1 and PC2, respectively) of dissolved organic matter (DOM) fractions from green manuring

The WS-Cd content was positively correlated with the DOC and DON contents and the proportion of C2, but was negatively correlated with $SUVA_{254nm}$, $SUVA_{280nm}$, HIX, and the proportions of C1 and C3. This phenomenon occurred because the addition of high chelating activity and low aromaticity of the protein-like materials in soil solution provided a large number of active functional groups, creating conditions suitable for Cd chelation.

Principal components analysis (PCA) was conducted to examine the relationships among the UV absorbance and fluorescence parameters and DOM components of each treatment (Fig. 7a). Two principal components (PCs) were identified, explaining 77.1% of the total variance in the data set; PC1 explained 65.9% of the variance and PC2 explained 11.2%. Loadings and scores of PCA are summarized in Fig. 7b, where the scores for different treatments and sampling dates are marked with different labels and colors.

The variance in PC1 was mostly explained by the fluorescence parameter HIX, the proportions of C3 and C1, and the UV-absorption parameters $SUVA_{280nm}$ and $SUVA_{254nm}$; meanwhile, PC1 was inversely proportional to the proportion of C2 (Fig. 7a). Therefore, this PC was closely related to the aromaticity and humification of DOM. The variance in PC2 was mostly explained by the UV-absorption parameters of E_{250}/E_{365}, and the concentrations of DOC and DON, which indicated that the PC2 was related to the degree of DOM molecular weight and content.

The DOM profiles were clustered into four groups in the PCA score plot, as expected. Groups 1 and 2 were the MVFP and MVWP treatments in the early stage, group 3 was the RS and CK treatments in the early stage, and group 4 contained all the treatments in the late stage of green manuring. Among them, the scores of group 1 (MVFP D1, D6, D10) on PC1 were −0.803, −1.297, and −0.673, respectively. These results
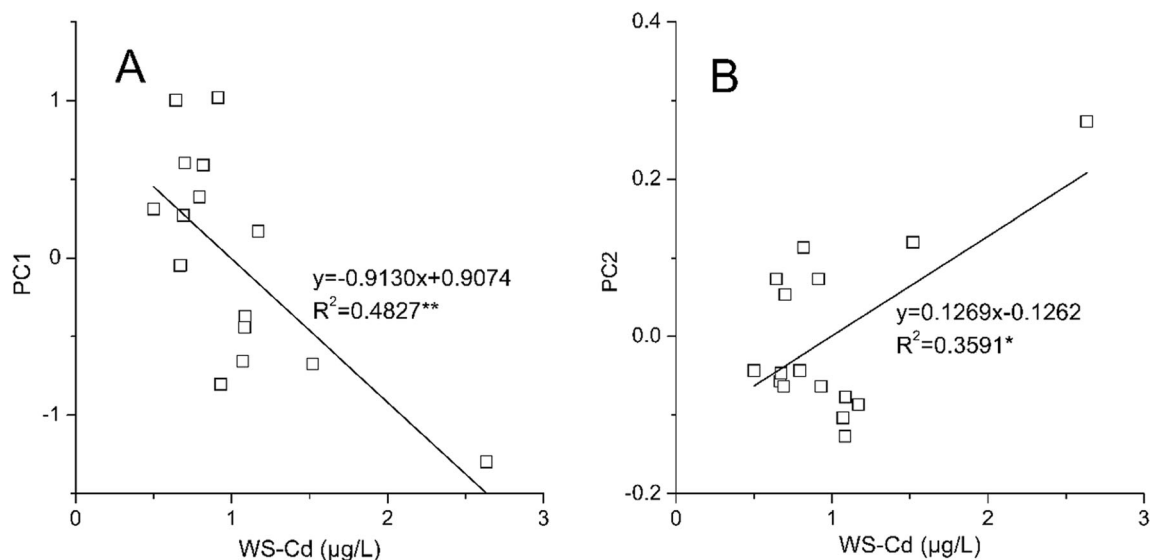
Fig. 8 Scatter plot of water-soluble cadmium (WS-Cd) and principal components 1 (PC1) (**a**) and 2 (PC2) (**b**) in the milk vetch in withering period (MVWP) treatment. * Significant correlation at the 0.05 level (two-tailed), ** significant correlation at the 0.01 level (two-tailed)

verified that the DOM showed different characteristics on the different days and treatments. The aromaticity of MVFP and MVWP in the early stage was lower than that of RS and CK, and it confirmed that the aromaticity of DOM in soil solution had the most significant effect on WS-Cd. Each PC generated from the PCA was used to draw a scatter plot with WS-Cd content (Fig. 8a, b). Correlation analysis showed that there was a negative correlation between PC1 and WS-Cd concentration, and a positive correlation between PC2 and WS-Cd concentration, with coefficients of determination of 0.4827 and 0.3591 ($n = 16$), respectively. This might be explained by the relationship between aromaticity and WS-Cd content, that is, the WS-Cd content increased with low-aromaticity DOM. The aromaticity of DOM plays a more important role in promoting Cd dissolution than molecular weight.

Conclusion

Through the analysis of DOM produced during manuring of milk vetch, rice straw, and the WS-Cd content, it was found that a large amount of DOM was produced during the MVFP treatment, which resulted in more Cd being dissolved into soil solution in the form of WS-Cd when compared with the other treatments. This was persistent for about 10 days after manuring.

3D-EEM fluorescence combined with PARAFAC analysis showed the existence of protein-like material, which was produced by the decomposition of milk vetch, and then humification increased the humic-like components. The UV absorbance and fluorescence characteristic parameters confirmed that the aromaticity and average molecular weight of DOM produced by MVFP were lower than those of the other treatments. The DOM produced by milk vetch promoted the dissolution of Cd probably because of the lower aromaticity of DOM molecules and the large amount of protein-like component.

All these results confirmed that the DOM generated by fresh milk vetch had a high chemical activity, and indicated that the application of milk vetch to contaminated farmland should be conducted at least 10 days before the next crop.

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