Dynamics and Spectroscopic Characteristics of Dissolved Organic Matter in Paddy Soils.

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Doctoral Dissertation

Dynamics and Spectroscopic Characteristics of Dissolved Organic Matter in Paddy Soils.

（水田における溶存有機物の分光学的特性

と動態に関する研究）

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Content

Chapter 1 General Introduction

1.1 The role and functions of Dissolved organic matter (DOM)

Dissolved organic matter (DOM) is the most labile fraction of soil organic matter (SOM) in the terrestrial ecosystems. Generally, DOM is the complex fractions that exist in the soil solution, consisting of molecularly complex compounds with different structures and qualities (Baldock and Nelson, 2000; Kalbitz et al., 2000). DOM can be divided into humic substances (HS) and non- humic substances (NHS) in terms of their solubility in the water. HS is the most recalcitrant and hydrophobic fraction while NHS is the easy degradable and hydrophilic fraction (Thurman and Malcolm, 1981). The formation of HS is subject to the condensation of decomposition of plant litter and other organic resources (Kotzé et al., 2016).

DOM only occupy about 0.05% of soil (Zsolnay, 1996). However, it involves in different biochemical and physical processes in the soil (Chantigny, 2003; Lal, 2004; Marinari et al., 2010). It is the direct nutrition source for microorganism and affects the process of organic matter decomposition in soil (Ghani et al.2003). HS, also known as hydrophobic acid fraction (Thurman and Malcolm, 1981), can form complexes with metals as a result of the abundant carboxyl and hydroxyl functional groups (Catrouillet et al. 2014). Those complexes influence metal behavior and then further affect the uptake of nutrients which may have impact on the plant growth in arable soils (Chen, 1996; Garcia-Mina et al.,2004).

DOM showed different characteristics in different ecosystems. Climate and hydrology conditions are important factors affect soil DOM properties in natural ecosystems (Kalbitz et al., 2000). However, in anthropogenic ecosystems, Land management and vegetation of soils may

make up a large proportion in influencing DOM quality and quantity (Chantigny, 2003). Some studies have investigated the variation of DOM dynamics in arable soils in different seasons or for short-term periods. Katoh et al. (2004) investigated the fluxes of DOM in paddy soils and found they were larger in summer than in autumn. Said-Pullicino et al. (2016) and He et al. (2017) observed the different DOM concentrations in wet season and dry seasons of paddy soils. Embacher et al. (2008) found the DOM concentrations was higher under chemical plus farmyard manure input than chemical fertilization within a 3-year period in a sugar beet field. However, knowledge of DOM dynamics in paddy soil is still scarce. It is particularly noted that there are few studies investigating the DOM dynamics over long period of time (> decade). DOM concentrations probably respond differently to long-term and short-term cropping systems and fertilization treatments, but the differences have not been investigated.

1.2 Characteristics of paddy soil

Rice (Oryza sativa) is one of the important crops for feeding half of the world's population (IRRI,2006) and occupies a large proportion of global arable land. Nearly 90% of rice is produced and consumed in Asia (FAO, 1998). According to the online repository of ricepedia, the rice yield of Japan was increasing while the production area and total production amount were decreasing from 1961 to 2012 (URL: http://ricepedia.org/japan),   and the yield of rice per unit area gradually increased. Japanese Rice production is characterized by widespread range of latitudes from north to South. Rice is grown once a year in the northern region with lower annual average temperature and it can be grown twice a year in the southern area with higher annual average temperature.

Rice production require flood irrigation which last from transplanting to harvesting, which results in the development of pedogenetic horizons of topsoil (Kögel-Knabner et al., 2010). The water table level before and after irrigation determines the creation of different paddy soil profile patterns. After flooding, the oxygen is depleted, the reducing environment result in mottled greyish-blue or brown color on the soil profile because of Fe^{2+} (Figure 1.1). Japanese pedologist classified those kinds of typical paddy soil groups as Grey lowland soil and Gley soil. Paddy field is a special aquatic–terrestrial agroecosystem characterized by large DOM concentrations and fluxes (Said-Pullicino et al., 2016; Kögel-Knabner et al., 2010). Flooding and drainage regimes create anaerobic and aerobic conditions. The frequent changes between oxidation and reduction conditions induce a biogeochemical process with redox cycling and special microbial metabolism (Kögel-Knabner et al., 2010), which further affect soil organic carbon (SOC) decomposition rate and DOM dynamics in paddy fields.

Land uses and management practices alter the chemical properties of arable soils. In paddy soil, the management practices include periodical submergence and drainage, ploughing and pudding, liming and fertilization. The repeated submerged and drained practices create anoxic and oxic conditions which are different suitable environments for anaerobic and aerobic microorganism, promoting the microbial decomposition processes of organic (Fierer and Schimel, 2002). As the soil labile organic C fractions, DOM usually respond more quickly to soil management than total C (Ghani et al., 2003). However, the characteristics of DOM in different cropping systems still need to be further investigated.

1.3 Methods to determine DOM quantity and quality

1.3.1 Extraction methods of DOM

It is not possible to obtain the *in situ* DOM solution of the paddy soils of the past decades. The water extractable organic matter of preserved soil samples can be used to represent DOM (Asakawa et al., 2006). The Extractable yields of DOM or HS are varied by the soil to solution ratio, extractant and some other operational practices and conditions (Asakawa et al., 2006). However, Guigue et al., (2014) investigated the different extraction methods: hot-water-extraction, cold-water-shaking-extraction and leaching-extraction, and the differences were not significant in the chemical properties of DOM, except for EC. In terms of shaking and soaking, Asakawa et al., (2006) reported that shaking extraction might induce the plant residue cell lysis which might increase the NHS proportion of DOM. Thus, soaking may be a better method to extract arable soil samples full of invisible plant residues.

Development of methods that can quality and quantity HS fractions of DOM solution have been time-consuming task for researchers. HS quantitative methods were developed using DAX-8 resin absorption, which made it possible to determine HS concentrations and its proportion in DOM solution with small volumes of soil samples (Tsuda et al., 2012). Considering of hard degradable property of HS, determination of HS proportion in DOM can help to evaluate the capability of soil C sequestration of arable soils.

1.3.2 Spectroscopic methods to analyze DOM solution

UV-vis and fluorescence spectroscopy have been used to determine the quantity of DOM in past research. Some fractions of DOM absorb particular regions of UV and visible light

(Borisover et al., 2012). UV-vis and fluorescence spectroscopy offer rapid, sensitive and costless methods to characterize DOM of natural water samples and some extracted soil samples (Borisover et al., 2012; Ohno et al., 2006; Sharma et al., 2017, Sun et al., 2017). In addition, parallel factor analysis (PARAFAC) combined three-dimensional excitation-emission matrices (EEM) which obtained from fluorescence scan has shown its great value in decomposing fluorescence information that hidden in EEM landscapes of DOM (Ohno et al., 2006; Murphy et al., 2013). Calculated from EEMs data, some fluorescent indices such as HIX, BIX and FI evaluates different properties of DOM, e.g. aromaticity, autochthonous or terrestrial origin. Unlike UV and fluorescence spectroscopy, the utilization of ^{1}H Nuclear magnetic resonance (NMR) spectroscopy give a full sight into structure and composition of DOM (Lam and Simpson, 2007). A method called W5-WATERGATE (Liu et al., 1998) improved by Lam and Simpson (2007) successfully provided unsurpassed water suppression and was compatible with all nature water samples tested.

1.4 Aims, approach and structure of this thesis

DOM plays important roles in soil biochemical processes, serving as a sink for carbon cycle in ecosystems. It is an urgent task for us to evaluate how agricultural management effect the quality and quantity of DOM in paddy soils to help develop agricultural management practices to maintain soil carbon and fertility. Thus, in this thesis, soil samples were selected from different areas in Japan, some of which were paddy soils, the others were greenhouse soils.

We aimed to elucidate those aims in the following chapters:

Chapter 2 – to investigate the total carbon and DOM concentrations and dynamics of long-

term paddy field (more than 40 years) under different fertilization treatments in single and double cropping systems;

Chapter 3 – to evaluate the spectroscopic characteristics of DOM of the samples used in Chapter 2 with UV-vis, fluorescence and ^{1}H NMR spectroscopy;

Chapter 4 – to compare the differences of dynamics and spectroscopic characteristics of DOM between paddy soils and greenhouse soils under different fertilization treatments;

Chapter 5 – general discussion and conclusion.

Figure 1.1 Soil profile of grey lowland soil, a typical type of paddy soil.

Chapter 2 Long-term dynamics of total carbon and dissolved organic matter in paddy soil under different fertilizations and cropping systems

2.1 Background and Objective

The effect of fertilization on DOM may show differences in different time scales. As it was mentioned in Chapter 1, data of most studies on paddy soils were obtained by collecting instant water samples (Abe et al., 2011; Said-Pullicino et al., 2016; Singh et al., 2014;) or setting pipes (Katoh et al. 2004) or lysimeter, which means the observation of DOM dynamics can only be limited within a very short period. Thus, the utilization of reserved soil samples is the effective way to understand the dynamics of DOM. The analysed reserved soil samples in the past research were all the upland soils, and there is no research on the paddy soil.

It is a vital task to determine total carbon (TC), DOM, HS contents of different paddy soils under different agricultural practices and in different cropping systems in a long-term experimental period. TC and DOM can be determined by specific instruments. HS fraction in DOM can be absorbed by DAX-8 resin because the acrylic ester (the base material of the resin) and the hydrophobic fraction of HS can absorb to each other. The DAX-8 resin isolation HS quantitative method developed by Tsuda et al. (2012) has been used to analyze different aquatic samples of Japan and some natural water samples in the world (Kida et al., 2015; Sato et al., 2018; Tsuda et al., 2016). And the HS concentration absorbed by DAX-8 did not response to changes in salinity (Kida et al., 2016). In this chapter it was investigated how the conversion of cropping systems and fertilizations affected dynamics of TC contents, DOM and HS concentrations.

2.2 Materials and methods

2.2.1 Research site and soil sampling

Soil samples were collected from the research site of Mie prefecture agricultural research institute in Mie, Japan (34° 38'N, 136° 29'E), where a paddy field experiment was lasted for over 40 years. According to the record of a nearby area (Kayumi, 25 km away from the research site) provided by Japan Meteorological Agency: mean annual temperature is 14.5°C, and mean annual precipitation is 2058.5 mm for the years 1981–2010 (https://www.data.jma.go.jp/obd/stats/etrn/view/nml_amd_ym.php?prec_no=53&block_no=0510&year=2018&month=&day=&view=; accessed on October 18, 2019). The soil is classified as Gray Lowland soil according to the classification of cultivated soils in Japan which can be classified as Gleyic Fluvisols by World Reference Base for Soil Resources (WRB, 2006) (Obara et al., 2015).

The experiment started from 1976 and continues until now. Samples were collected in 1976 and every year since 1979. Four kinds of fertilization treatment practices were applied (Table 2-1). The first one was chemical fertilizer **(CHE)** which was nitrogen, phosphorus and potassium inputs. The second one was chemical fertilizer and rice straw input **(CHE+ORG)**. The third one was chemical fertilizer without phosphorus input and cattle manure compost input **(MAN)**. The fourth one was named as integrated fertilization **(INT)** which combined chemical fertilizer, rice straw and soil amendment materials (fused phosphate and calcium silicate). For the CHE+ORG and INT treatments, rice straw was added 330 kg 10a^{-1} before 1998 and 600 kg 10a^{-1} after 1998 (Table 2-1). According to the TC and TN contents of rice straw measured in 2015, those values

roughly correspond to inputs (per 10a) of 112 kg C and 1.65 kg N before 1998 and 204 kg C and 3 kg N after 1999, respectively. Cattle manure compost was applied 1000 kg $10a^{-1}$ wet weight in the MAN treatment after harvesting before 1991 and two weeks before transplanting after 1991 (Table 2-1). Those values corresponded to 93–180 Kg C $10a^{-1}$ and 5.6–6.9 Kg N $10a^{-1}$ estimated by the TC and TN content of compost measured from 1993 to 2015.

Throughout the experiment, 1991 was the boundary when cropping system was changed from a rice-wheat/barley double cropping system to a rice single cropping system. In all the treatments, crop straw was removed but stubble was remained in the field through incorporation into the soil after harvesting. In 1979, the soil sample was collected in November, and from 1980 to 1991, soil samples were collected in May (or June) and October, while after 1991, samples were collected only once in September or October. For this study, we only used the samples collected in autumn (September to November). All samples were collected from the Ap horizon at 0–10 cm after crop harvesting. Soil samples were selected for analysis in this study from the following years: 1976, 1980, 1984, 1991, 1995, 2001, 2006, 2010 and 2015.

2.2.2 Soil chemical properties

The soil samples were removed of crop residues, air-dried, sieved to < 2 mm and analyzed for bulk chemical properties (Table 2-2). Soil total carbon and total nitrogen were determined by a CHNS/O PE-2400 analyzer (PerkinElmer, USA) using high temperature combustion method. The methods to determine other properties are also listed here, but they will not be discussed in this dissertation. The results were shown in Table 2-2.

I. Soil samples were extracted at a soil-to-liquid ratio of 1:2.5 with a glass electrode pH meter (F-

52, Horiba, Japan).

II. EC was determined by a soil: solvent ratio of 1:5 using an EC meter (CM-30V, TOA corp., Japan).

III. Exchangeable cation K^+ was extracted and determined by Inductively coupled plasma atomic emission spectrometer ICP-AES (SPS 3100, Hitachi, Japan).

IV. Available nitrogen was determined by incubation method (Bundy and Meisinger, 1994). Soil was adjusted its maximum water holding capacity to 60% and incubated in a UM glass sample bottle at 30℃ for 4 weeks. Available Nitrogen was the difference of the amount of inorganic nitrogen before and after incubation using an ion chromatograph (IC2001, TOSCH crop., Japan).

V. Exchangeable P was determined by the ascorbic acid method (Murphy and Riley, 1962). Soil was extracted by water at a soil: liquid ratio of 1:40. Then suspension was filtered and measured absorbance using a UV-visible spectrophotometer (V-630, JASCO, Japan). The working standard solutions were also measured absorbance which were used for making calibration curve. The P concentration of soil solution corresponded to the value on the calibration curve.

2.2.3 DOM extraction and measurement

Soil samples were soaked in ultrapure water (18.2 MΩ; ADVANTEC, Japan) with a soil:solvent ratio of 1:5 at 5°C dark condition for 24 h (Asakawa et al, 2006). After centrifugation, the extracts were vacuum filtrated through 0.3 µm glass fiber filters (ADVANTEC GF-75, Japan). Pre-measurement showed the concentration of original DOM solution was too high for determination. Thus, the original solution was 10-fold diluted. 20 mL of diluted DOM solution were mixed with 0.2 g (dry weight) DAX-8 resin (Supelco, Bellefonte, PA, USA), and acidified

with 1M H_2SO_4 (special grade, Wako, Japan) to adjust pH to <2. Then the mixture was kept

shaking for 24 h at 5°C dark condition. DAX-8 resin absorbed all of HS so that what was left in

the solution was non-HS (Tsuda et al., 2012). DOM solution and non-HS solution were measured

for dissolved organic carbon (DOC) concentration using TOC-V_{CPH} analyzer (Shimadzu, Kyoto,

Japan). HS concentrations were calculated as the difference between DOM and non-HS

concentrations. Organic C contamination from resin was determined with ultrapure water as a

blank and used for correction (< 0.1 mg C L^{-1}). The blank determination was conducted in

quintuple and HS adsorption were conducted in triplicate. The precision of the replicate

measurements was 1.8% for the average coefficient of variation of all the measurements, and the

average values were used for data analysis. All glassware was acid-washed and pre-combusted

(465°C, 4 h) to avoid contamination.

2.2.4 Statistics

Due to the abnormal distribution of data, the nonparametric analysis of Kruskal–Wallis test

by ranks ($p < 0.05$) was used and the Dunn's test with Bonferroni correction was used for post-hoc

testing. All calculation was carried out in SPSS 19.0 (IBM Corp, Armork, NY, USA). The figures

were made by Origin 2018 (OriginLab Corporation, Northampton, MA, USA).

2.3 Results

2.3.1 Dynamics of TC, DOM and HS in double and single cropping systems.

Generally, TC showed an increasing trend in double cropping system (1976-1991) and

decreasing trend in single cropping system (1991-2015), so as DOM and HS concentrations

(Figure 2.1). The original TC content was 1.73% in 1976, while in 1991 it increased to 2.06%, 2.33%, 2.66% and 2% (CHE, CHE+ORG, MAN and INT treatment, respectively), then decreased to 1.65%, 2%, 2.24% and 1.58% respectively. For the DOM concentration, it was 341 mg C Kg^{-1} in 1976, then increased to 675 mg C Kg^{-1}, 680 mg C Kg^{-1}, 760 mg C Kg^{-1} and 517 mg C Kg^{-1}(CHE, CHE+ORG, MAN and INT treatment, respectively) in 1991, then decreased to 261 mg C Kg^{-1}, 279 mg C Kg^{-1}, 331 mg C Kg^{-1}, 243 mg C Kg^{-1} respectively which were all less than the initial value of 1976. The initial HS concentration in 1976 was 97 mg C Kg^{-1}, while in 1991 it increased to 133 mg C Kg^{-1},129 mg C Kg^{-1},163 mg C Kg^{-1} and 150 mg C Kg^{-1}, and decreased to 64 mg C Kg^{-1}, 66 mg C Kg^{-1}, 78 mg C Kg^{-1}, and 57 mg C Kg^{-1}. The results reveal that TC, DOM and HS content of paddy soils were significantly affected by the change of cropping systems.

2.3.2 TC, DOM and HS under different fertilization treatments

The Kruskal Wallis test by ranks showed that the differences of the TC content under different fertilization treatments were not significant (P = 0.002), while DOM, HS concentrations and HS/DOM ratio did not show significant differences (P = 0.062, P = 0.222, P = 0.100, P = 0.204, respectively; Table 2.3). The post-hoc results of Kruskal–Wallis test by ranks showed that CHE vs. MAN (P = 0.04) and MAN vs. INT (P = 0.016) were significantly different (Table 2.3). The boxplots of different fertilization treatments (Figure 2.2) showed similar results. The interquartile range box of boxplot represents the middle 50% of data. The interquartile range boxes of TC under different treatments (Figure 2.2a) show dissimilarity except the INT and CHE treatments. However, the interquartile range boxes of DOM, HS/DOM and DOM/TC under CHE,

CHE+ORG and MAN treatments (Figure 2.2b) share the similar range of data except the INT

treatment, and the interquartile range boxes of HS share similar range of data. The results reveal

that DOM and HS concentrations of paddy soils were not affected by fertilization, but TC content

was significantly affected by fertilization.

2.4 Discussion

2.4.1 Effect of cropping systems and fertilization on DOM and TC dynamics

TC contents of paddy soils showed different trends in two kinds of cropping systems:

increasing trend in double cropping systems and decreasing trend in single cropping system, so

as the DOM, HS, HS/DOM and DOM/TC ratio. The changes of TC contents between systems

results from the balance of the C inputs and C losses. In this study the vegetation of field was

changed from wheat/barley in double cropping system to fallow in single cropping system from

November to April. Two seasons' incorporation of crop stubble in double cropping system left

more crop residues into soil than in single cropping system, which made the C input larger than

C losses. However, the C input of one season's incorporation of stubble in single cropping system

did not make up the C loss. Soil organic C was reported higher in rice-upland double cropping

systems than single rice cropping systems under chemical fertilization treatment (Huang et al.,

2012; Pan et al., 2009). Cha-un et al (2017) compared the soil C budgets of double and single rice

cropping system and found the single cropping system always had negative soil C budget and this

rice-fallow field management showed a large reduction of SOC which indicated the net losses of

C. The consistency of the results between this research and past research revealed that the

conversion of cropping systems from double cropping to single cropping reversed the increasing trend of soil TC towards increasing trend which might further had an impact on DOM concentration.

TC also showed significant difference under different fertilization treatments, but DOM and HS concentrations, HS/DOM and DOM/TC ratio did not show significant differences. On the one hand, fertilization treatments did not directly increase DOM, but they did affect DOM via their effects on microbial activity (Embacher et al., 2008; Sun et al., 2017). On the other hand, chemical fertilizer did not last long enough to significantly alter DOM concentration in the surface soil (Xu et al. 2013) and the DOM concentration was also observed an increasing and then sharply decreasing trend within the same season after the poultry manure application in a corn field (Singh et al.,2014). Thus, the large variation of DOM over time irrespective of the fertilization treatments imply that DOM dynamics was responded more strongly to the agricultural practices than to the fertilization treatments.

2.4.2 Effect of cropping systems and fertilization on labile C pool

Soil TC pool comprises labile and stable C pools. Labile C pool is important indicator of soil quality (Zhang et al., 2006) which can be readily used by microorganism (Huang and Schoenau, 1996). DOM represent the majority of soil labile C pool. Its proportion in TC (DOM/TC) indicates the bioavailability of TC pool. Labile C mainly derives from plant litter and root exudates (Wang and Wang, 2011). There were More crop residues input in the double cropping system than in the single cropping system. DOM/TC ratio showed a similar trend with DOM, suggesting that both DOM and labile C pool were more susceptible to the change of cropping systems than to the

change of fertilization.

Generally, DOM in paddy soil controlled by irrigation, fertilization and cultivation practices. In this research, the change of cropping systems was the more powerful method to increase DOM concentrations than fertilization in paddy field. However, within the same cropping system, manure compost input can be an effective way to increase SOC. Because in most of the sampling years, MAN treatments always showed higher DOM concentrations than other treatments.

2.5 Conclusion

A long-term experiment last for over 40 years was conducted in the paddy field in the research field of Mie Prefecture, Japan. With the stubble incorporation into field, double cropping system can significantly improve TC content, DOM and HS concentration of paddy field. However, change of fertilization did not show significantly effect on DOM and HS concentration. The effect of fertilization on DOM concentrations was diminished soon after the application of fertilization. Thus, the DOM in paddy soil was more susceptible to the change of cropping system than fertilization.

Table 2.1

Basal and additional fertilizers, organic materials, and soil amendments added to the field under different treatments each year.

Treatment	Basal fertilizer (kg 10a^{-1})			Topdressing (kg 10a^{-1})		Organic material (kg 10a^{-1})						Soil amendment (kg 10a^{-1})	
						Rice straw[‡]			Cattle manure compost[§]				
	N	P$_2$O$_5$	K$_2$O	N	K$_2$O	TC	TN	C/N	TC	TN	C/N	P$_2$O$_5$	CaSiO$_2$
Chemical fertilizer (CHE)	25	38	29	40	40	-	-	-	-	-	-	-	-
Chemical fertilizer + rice straw (CHE+ORG)	25	38	29	40	40	112 (204)	1.65 (3)	68	-	-	-	-	-
Cattle manure (MAN)	-	-	-	40	40	-	-	-	93–180	5.6–6.9	13–30	-	-
Integrated fertilization (INT)	25	38	29	40	40	112 (204)	1.65 (3)	68	-	-	-	400	2000

‡ Rice straw was added 330 kg 10a^{-1} before 1998 and 600 kg 10a^{-1} after 1999.

§ Cattle manure compost input was 1000 kg 10a^{-1} (wet weight).

Table 2.2 Chemical properties of the paddy soil samples

		pH (H$_2$O)	EC (mS cm^{-1})	TC (%)	TN[§] (%)	C/N	Exchangeable cation K$^+$ (cmol c kg^{-1})	Available nitrogen (mg 100 g^{-1})	P$_2$O$_5$ (mg 100 g^{-1})
1976		-[†]	-	1.73	-	-	-	-	-
1980	CHE	6.0	-	1.94	0.15	12.93	0.2	12.8	29.8
	CHE + ORG	5.6	-	1.97	0.12	16.41	0.2	12.8	22.3
	MAN	5.9	-	2.02	0.15	13.47	0.2	14.4	22.9
	INT	6.5	-	1.86	0.14	13.28	0.3	12.6	41.2
1984	CHE	5.9	-	1.70	0.12	14.17	0.2	6.7	29.2
	CHE + ORG	5.9	-	1.88	0.13	14.46	0.3	6.1	23.5
	MAN	5.7	-	1.95	0.14	13.92	0.2	7.1	23.5
	INT	7.0	0.05	1.77	0.13	13.62	0.2	6.1	48.1
1991	CHE	5.6	0.05	2.06	0.16	12.875	0.4	11.6	18.0
	CHE + ORG	5.4	0.05	2.33	0.17	13.70	0.6	13.9	18.0
	MAN	5.4	0.08	2.66	0.19	14	0.9	13.8	24.8
	INT	6.3	0.14	2.00	0.15	13.33	0.6	11.9	27.5
1995	CHE	5.6	0.17	1.95	0.15	13	0.2	13.3	18.0
	CHE + ORG	5.3	0.15	2.24	0.17	13.18	0.3	17.3	14.7
	MAN	5.6	0.15	2.56	0.20	12.8	0.4	22.2	18.1
	INT	6.1	0.10	2.12	0.16	13.25	0.3	13.7	26.6
2001	CHE	5.9	0.09	1.79	0.15	11.93	0.2	11.4	18.3
	CHE + ORG	5.8	0.12	1.87	0.15	12.47	0.3	11.5	16.3
	MAN	5.8	0.11	2.05	0.16	12.81	0.3	12.6	19.3
	INT	6.7	0.10	1.83	0.14	13.07	0.3	11.4	28.7
2006	CHE	5.9	0.11	1.74	0.15	11.6	0.2	11.8	21.2
	CHE + ORG	5.7	0.13	1.96	0.17	11.53	0.3	12.5	18.5
	MAN	6.0	0.11	2.28	0.18	12.67	0.3	12.8	22.8
	INT	6.6	0.08	1.86	0.16	11.625	0.2	12.8	34.5
2010	CHE	5.9	0.09	1.92	-	-	-	-	-
	CHE + ORG	5.7	0.12	2.08	-	-	-	-	-
	MAN	6.1	0.09	2.34	-	-	-	-	-
	INT	6.7	-	1.98	-	-	-	-	-
2015	CHE	-	-	1.65	-	-	-	-	-
	CHE + ORG	-	-	2.00	-	-	-	-	-
	MAN	-	-	2.24	-	-	-	-	-
	INT	-	-	1.58	-	-	-	-	-

[†] Not analyzed

Figure 2.1 The dynamics of (a) total carbon (TC, %), (b) dissolved organic matter (DOM) concentration (mg C Kg^{-1} soil), (c) humic substances concentration (HS, mg C Kg^{-1} soil), (d) HS/DOM ratio (%) and (e) DOM/TC (%)of the paddy soil samples. (CHE: chemical fertilization; CHE+ORG: CHE + organic fertilization; MAN: cattle manure fertilization; INT: CHE + ORG + soil amendment materials (fused phosphate and calcium silicate)

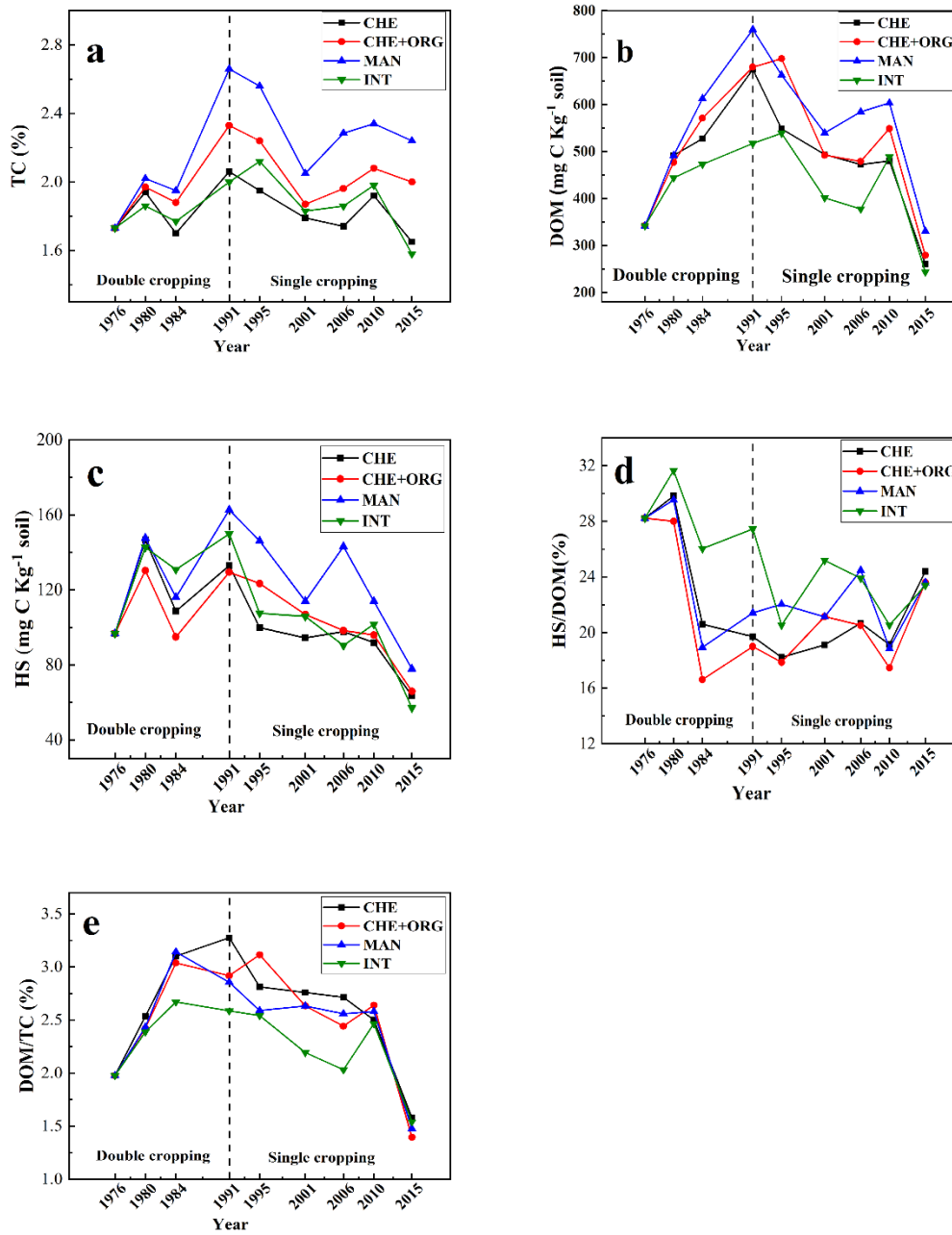Figure 2.2 Boxplots for different fertilization treatments of (a) total carbon (TC, %), (b) dissolved organic matter (DOM) concentration (mg C Kg^{-1} soil), (c) humic substances (HS, mg C Kg^{-1} soil), (d) HS/DOM ratio (%), (e) DOM/TC ratio (%) of the paddy soil samples. (CHE: chemical fertilization; CHE+ORG: CHE + organic fertilization; MAN: cattle manure compost fertilization; INT: CHE + ORG + soil amendment materials (fused phosphate and calcium silicate)
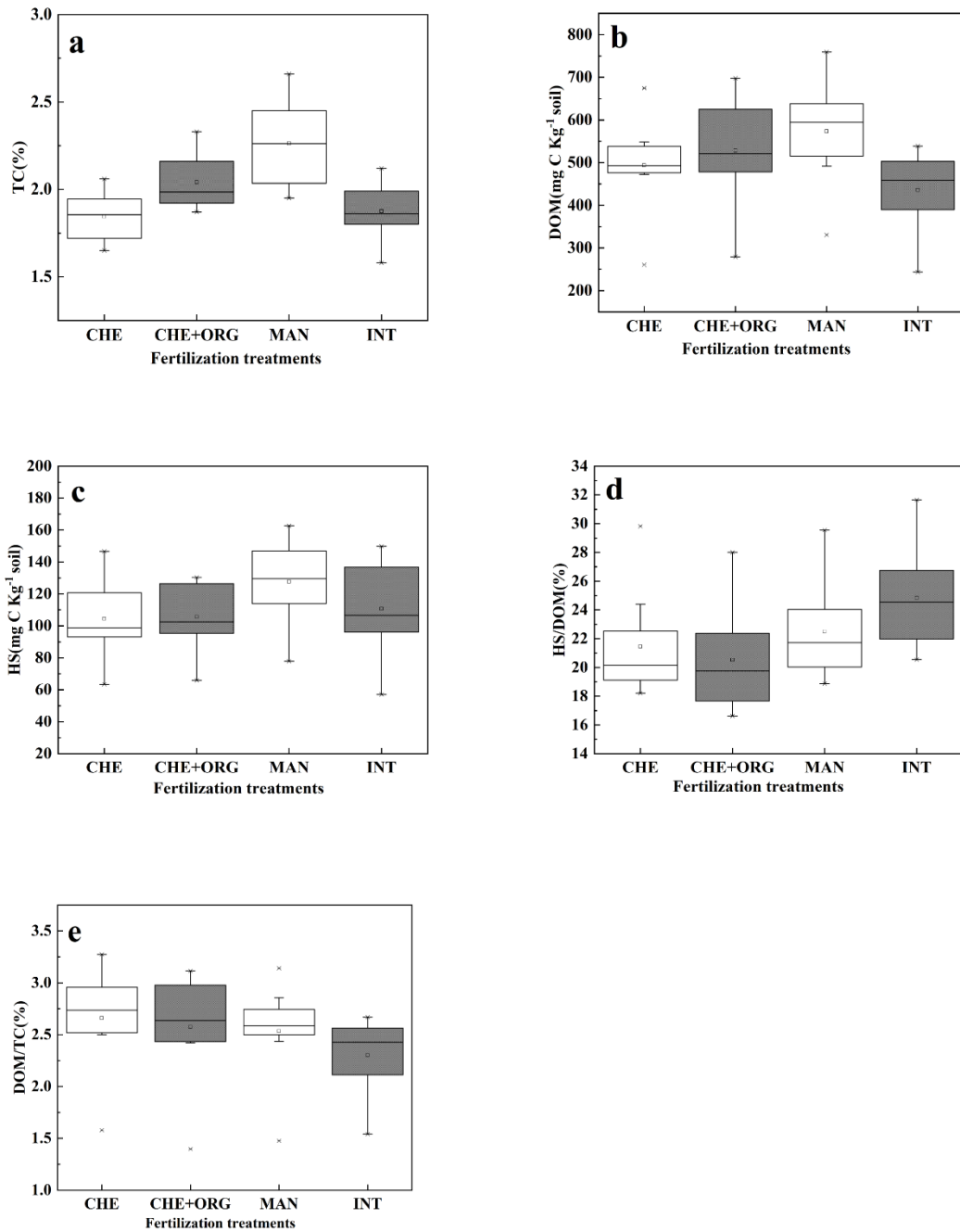
Table 2.3 Kruskal–Wallis test by ranks of the effect of independent factor "Fertilization treatment" on soil TC (%), DOM/TC (%), DOM, HS, HS/DOM (%).

	TC	DOM/ TC	DOM	HS	HS/ DOM
P value	0.002*	0.204	0.062	0.222	0.100
Pairwise comparison by Dunn's test with Bonferroni Correction					
CHE vs. CHE+ORG	NS	NS	NS	NS	NS
CHE vs. MAN	0.04	NS	NS	NS	NS
CHE vs. INT	NS	NS	NS	NS	NS
CHE+ORG vs. MAN	NS	NS	NS	NS	NS
CHE+ORG vs. INT	NS	NS	NS	NS	NS
MAN vs.INT	0.016*	NS	NS	NS	NS

TC=total carbon; DOM= dissolved organic matter concentration; HS= water extractable humic substance concentration.

* Significantly different at the 0.05 level.

"NS" Not significantly different at the 0.05 level.

Table 2.4

Spearman's rank correlation coefficients (r) between different pairs of parameters (TC, DOM/TC, DOM, HS and HS/DOM).

	TC	DOM/TC	DOM	HS	HS/DOM
TC	1.00				
DOM/TC	0.17	1.00			
DOM	0.65**	0.81**	1.00		
HS	0.46**	0.36*	0.55**	1.00	
HS/DOM	-0.21	-0.62*	-0.56**	0.32	1.00

* Significant at P < 0.05.

** Significant at P < 0.01.

Chapter 3 Spectroscopic characteristics of dissolved organic matter in paddy soil under different fertilizations and cropping systems

3.1 Introduction

Different kinds of agricultural management influences quantity and quality of arable soils and further affect the agricultural productivity (Chantigny et al., 2003). Chapter 2 already described the investigation of how the change of cropping system and fertilizations affected the dynamics of TC contents, DOM and HS concentrations in paddy soils. Nevertheless, the spectroscopic characteristic of DOM may be affected by agricultural practices such as cropping system and fertilization, which is also needed to be evaluated. It may provide a new sight into DOM in paddy soils and help stakeholders make appropriate agricultural strategies.

UV-vis and fluorescence spectroscopy have been applied in past research to study the spectroscopic characteristics of DOM in natural water samples. The technique of PARAFAC combined with EEMs measured by fluorescence spectroscopy has been improved for the past few decades, which widely used in aquatic ecosystems such as ocean, lake, river, wet land and so on. There were only few studies conducted in agricultural ecosystems. Lam and Simpson (2007) reported a new approach (W5-WATERGATE) to obtain the spectrum of DOM using ^{1}H NMR spectroscopy, which has been only applied on natural water samples. The information about the spectroscopic characteristics of DOM in paddy soil is still scarce. This Chapter investigated the spectroscopic properties of DOM in paddy soil in two kinds of cropping systems under different fertilization treatments.

3.2 Soil samples and Methods

Research site was the same as Chapter 2 mentioned and so as the soil samples. The DOM extraction procedure was also the same as described in Chapter 2.

3.2.1 UV-vis spectrophotometry

The absorbance value measured at specific wavelengths reflect different properties of DOM solution, such as aromaticity, molecular size and humification degree (Jaffrain et al., 2007; Kalbitz et al., 2000). The absorbance at the wavelength of 254nm divided by the DOM concentration of the corresponding solution (SUVA$_{254}$) has been widely used to evaluate the aromaticity of DOM (Weishaar et al., 2003). Original DOM solution was determined the absorbance from 200 to 550 nm by a UV-visible spectrophotometer (Jasco V-630, JASCO, Japan) The equation for calculation is:

$$SUVA_{254} = A_{254} / C_{DOM}$$

where A$_{254}$ represents the absorbance (m^{-1}) at 254nm and C$_{DOM}$ represent the DOM concentration (mg C L^{-1}) of solution (Weishaar et al., 2003).

3.2.3 Fluorescence spectroscopy

A Hitachi F-2700 spectrophotometer (Hitachi, Tokyo, Japan) was used to determine the fluorescence excitation-emission matrices (EEMs). Before measurement, original DOM solution was diluted by ultrapure water or 0.1M NaOH to decrease inner filter effect (IFE) and increase the number of measured samples. According to Ohno and Bro (2006), the absorbance of DOM solution should be less than 0.1 to minimize IFE. The alkali solution was covered to

prevent photodegradation of fluorescent components. The setting measurement parameters are listed here:

Excitation wavelengths and interval: 240–450 nm; 5nm

Emission wavelengths and interval: 300–550 nm; 2nm

Scanning speed :1500 nm/min

Response time: 0.04s

The ultrapure water and NaOH blank solution were prepared for blank determination on every measurement day.

The correction procedure for IFE was to correct the EEM data by the corresponding absorbance:

$I_{corr} = I_{obs} \times 10^{0.5 \times (Aex+Aem)}$

Where I_{corr} is the corrected fluorescence intensity, I_{obs} is the original fluorescence intensity, and A_{ex} and A_{em} represent the UV absorbance at the corresponding excitation and emission wavelengths, respectively (Lakowicz, 2006). Then the subsequent EEM was subtracted the corresponding blank EEM to reduce the effect of Raman and Rayleigh scatting. Then, the resulting EEM was normalized with the Raman peak area of blank EEM (Emission wavelength from 370nm to 428 nm at excitation wavelength of 350 nm) (Lawaetz and Stedmon, 2009).

In order to assess the fluorescent qualities of DOM, some fluorescence indices were used calculated from EEM data. Different fluorescence indices were calculated as follows:

ⅰ. HIX = ($\sum I_{435-480}$)/ ($\sum I_{300-345}$ + $\sum I_{435-480}$)   at ex 254nm   (Ohno,2002)

ⅱ. FI= I_{470}/I_{520} at ex 370nm   (Cory and McKnight, 2005)

ⅲ. BIX= I_{380}/I_{430} at ex 310nm   (Huguet et al., 2009)

"I" is the fluorescence intensity, thus, the HIX is the spectrum area of emission wavelength of 435–480 nm divided by the area of 300–345 nm plus 435–480 nm, at excitation wavelength of 254nm; FI is the ratio of fluorescence intensity of emission wavelength of 470nm to 520nm, at excitation wavelength of 370nm; BIX is the ratio of fluorescence intensity of emission wavelength 380nm to 430nm at the excitation wavelength of 310nm.

66 samples were carried out PARAFAC modeling using MATLAB (version 2017b, MathWorks, Natik, MA, USA) by the drEEM 0.3.0 and N-way toolbox (Murphy et al. 2013). Due to the large range of fluorescent intensities of the DOM samples, the DOMfour toolbox was not successfully validated before the utilization of drEEM toolbox. A demand provided by drEEM can normalize the EEM data to take all fluorescence intensities into consideration (Murphy et al., 2013). 3- and 5- component models were all validated with more than 60% core consistencies which were sufficiently high (Bro and Kiers, 2003). However, by comparing the residuals of 5-component model, there was not enough peak information in 3-component model. Thus, it was considered that 5-component model was the most proper model (Figure 3.2). All half splits were also validated (Figure 3.3).

To compare fluorescence components between studies, Murphy et al., (2014) created an open access online database called OpenFluor. The five components C1 to C5 were compared with the components reported in past studies in OpenFlour. Because direct conversion from the fluorescence intensities to contents is not feasible, the fluorescence intensity maxima (F_{max}) was used as the substitute for relative concentration of each samples (Murphy et al. 2013). The proportion of F_{max} of each component, $F_{max}/\sum F_{max}$ (denoted as C_p), represent relative abundance of each fluorescence component.

3.2.4 ^{1}H NMR spectroscopy

The five soil samples of 1976, 1991 CHE, 1991 MAN, 2015 CHE and 2015 MAN were selected for the measurement of ^{1}H NMR spectroscopy. About 2 mL DOM solution was evaporated with rotary evaporator (EYELA, N-N series), until about 200 μL solution was left in the evaporation flask (Maruemu Corp. Japan). Then the solution was transferred into a 5 mm NMR tube (PS-001, SHIGEMI). 10μL 0.002% 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (TMSNa; 98 atom % D, Euriso-Top, France), 10μL 10M NaOD (99.9 atom % D, Sigma-Aldrich, USA), and about 300-500μL D_2O (99.9 atom %C, Aldrich, USA) were added into NMR tube (5mm, Shigemi). NMR tube was cleaned with acetone. Measurements were conducted on an NMR spectrophotometer (Bruker Corporation, Billerica, MA, USA) with a 5 mm TBI probe. Spectra were externally calibrated to the trimethylsilyl resonance (0 ppm) of TMS. According to Kawahigashi et al. (1996), the obtained ^{1}H NMR spectrum was divided into 5 chemical shift regions. The region of 0.5-0.9 ppm were the saturated aliphatic protons attached to the terminal methyl groups (H_γ). The region from 0.9 ppm to 1.6 ppm were protons on the position of methylene β attached to olefins or aromatic rings (H_β). The region of 1.6-3.0 ppm were protons on methyl and methylene α attached directly to the aromatic or carbonyl carbons, ester groups, and olefins (H_α). H_{Al} represent the alkyl protons which were the combination of H_α, H_β and H_γ. The region of 3.0-4.3 ppm were protons on carbons attached to methoxyl groups (H_{C-O}). The region of 6.0-9.0 ppm were the protons attached to carbons of heteroaromatic and aromatic rings (H_{Ar}). The proportion of each functional group was calculated by the proportion of each region which hereafter refers to H_{Ar}, H_{C-O} and H_{Al}.

3.2.5 Statistics

The statistics methods were the same as in chapter 2. The Kruskal–Wallis test by ranks (p < 0.05) and the Dunn's test with Bonferroni correction was applied to evaluate the differences between fertilization treatments. The method of Spearman's rank correlation coefficients was applied to investigate the relation between optical indices.

3.3 Results

3.3.1 SUVA$_{254}$

Except under MAN treatment, SUVA$_{254}$ decreased until 1995, and then fluctuant increased in single cropping system (Figure 3.1). SUVA$_{254}$ did not show significant differences under different fertilization treatments (P = 0.124; Table 3.1).   SUVA$_{254}$ showed a significant correction with HS/DOM ratio (r = 0.68, P < 0.05; Table 3.2)

3.3.2 Fluorescence indices

HIX is the index which can help to evaluate the humification degree of DOM samples. A higher HIX is related to the more condensed structure of SOC (Zsolnay et al., 1999). Generally, HIX gradually decreased from 1976 to 2015 (Figure 3.1b), and different fertilizer treatments showed slightly different fluctuations. Different fertilization treatments showed a significant effect on HIX (P = 0.006, Table 3.1) in this study. Thus, regardless of the agricultural systems, condensation and humification degree of DOM reduced in paddy soil.

FI and BIX showed an increasing and a fluctuant decreasing trend in two different cropping

systems respectively except under INT treatment. BIX and FI were significantly responsive to the fertilization (P = 0.009, P = 0.006, table 3.1). In addition, the multiple comparison result and Fig.3.1c and d revealed that the BIX and FI under INT treatment were significantly lower than other treatments.

3.3.3 PARAFAC modeling

A 5-compoent model was obtained from the PARAFAC analysis (Figure 3.2). Each split-half analysis was also validated (Figure 3.3). Compared with the past data in OpenFlour, C1, C2 and C3 were assigned as humic-like components, while C4 and C5 were assigned as protein-like components. C1 showed two excitation peak positions at < 250nm and 305 nm and one emission peak at 424 nm, and it was reported as fulvic-like component by Ohno and Bro (2006) and Yamashita et al. (2011). Compared with C1, C2 was a component with two excitation maxima at < 250nm and 375nm and one emission maxima at 480nm which was red shifted. The red-shifted emission wavelength peak suggested that C2 was more aromatic and condensed than C1 (Yamashita et al., 2011). The molecular size of C1 and C2 was reported to be small to medium by Ishii and Boyer (2012). C3 was reported as a microbial humic-like component by Osburn et al. (2016) with excitation and emission peaks at 315 nm and 384 nm. C3 was usually found in agricultural wastewater and manure (Osburn et al. 2016; Singh et al. 2014; Chen et al. 2018). C4 with excitation and emission maxima of 275nm and 316nm respectively was assigned as tyrosine-like component (Ohno and Bro 2006; Zhang et al. 2011). C5 (EX/EM 290nm/360nm) was assigned to Tryptophan-like component that has been reported to exist in agricultural soils (Ohno and Bro 2006; Singh et al. 2014).

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Overall distribution of C1 occupied the largest proportion (about 45%), and the other components shared proportions ranging from 6% to 21% as the order: C4 > C2 > C3 > C5 (Figure 3.4). The component proportions showed different trends over time (Figure 3.5): C_p1 was decreasing in double cropping system and tended to be stable in single cropping system; C_p2 stayed constant until 2006, then started to increase under some fertilization treatments; and C_p3 showed fluctuating and increasing trends; and C_p4 showed an obvious increasing trends. C_p5 had a decreasing trend. C_p1 and C_p4 significantly responsive to different fertilization treatments (Table 3.2).

3.3.4 Fractional groups proportion

The proportions of each functional group of samples collected from fields under CHE and MAN treatments were shown in Figure 3.6. The proportions of H_{Ar} were less than 5%. 10 times of the proportions of H_{Ar} were shown in Figure 3.6 in order to compare their trends. The trends of proportions of H_{Ar} were decreased and then increased under CHE and MAN treatments in both double and single cropping systems. However, H_{C-O} and H_{Al} showed different trends in two kinds of cropping systems or under different treatments. H_{C-O} decreased from 1976 to 2015 under CHE treatment, while it increased before 1991 and then decreased after 1991 under MAN treatment. H_{Al} showed the opposite trends to H_{C-O} under two treatments.

3.4 Discussion

A high value of $SUVA_{254}$ represents high proportion of aromatic carbon of DOM. $SUVA_{254}$ showed different trends in the two cropping systems. It is considered that double cropping

system is a more intensive kind of land use than single cropping system. Past research reported that highly intensive land use would increase the proportion of condensed aromatic carbon (Arshad et al., 2010; Xu et al. 2013). Tillage such as plowing, rotary and residue incorporation intensified soil disturbance to bring more oxygen and then increase decomposition and humification of DOM (Bolan et al., 2011). Nevertheless, the trends of $SUVA_{254}$ did not in line with the past research. Further investigation is needed to explore why the aromaticity of DOM showed different trends in different kinds of agricultural ecosystems.

$SUVA_{254}$ under different fertilization treatments did not show statistical differences ($P = 0.124$; Table 3.1). There was a positive correlation between $SUVA_{254}$ and HS/DOM ratio ($r = 0.68$, Table 3.2), which suggested that HS comprised most of aromatic substances of DOM.

HIX, FI and BIX were significantly affected by fertilization treatments ($P = 0.006$, 0.009 and 0.006, respectively, Table 3.2). HIX represent the humification degree of DOM (Zsolnay et al. 1999). Different kinds of fertilizations bring different kinds of organic materials into soil, which changes the condensation and decomposition rate of DOM. Thus, fertilization influenced the decomposition and humification degree of DOM in paddy soil. FI and BIX are fluorescent indices which involve with microbial activity. High FI reveals that DOM has a close link with microbial origin (Cory and McKnight 2005). High BIX also indicates a high contribution of fresh DOM derived from microbial activity (Huguet et al., 2009). FI and BIX were positively correlated with WEOM/TC ($r = 0.70$ and 0.52, respectively, Table 3.2) and negatively correlated with HS/DOM ($r = -0.71$ and -0.66, respectively, Table 3.2), suggesting that microbial derived DOM is the main source of labile C pool. Negative relationship between HS/WEOM and WEOM/TC ($r = -0.62$) strongly supports it. More microbial-derived DOM likely reduced the

proportion of HS in DOM in paddy soil.

Different fertilization significantly affected the proportion of tyrosine-like component (C_p4). C_p4 was higher under MAN treatment than other treatments (Table 3.3). Protein-like components such as tyrosine-like component are semi or semi-labile fractions of DOM derived from microbial activity (Yamashita et al., 2011). The manure fertilization could input more labile C fractions into field than chemical fertilization (Liu et al. 2013; Yan et al. 2007). Ohno and bro (2006) also compared manure-, soil- and crop- extracted DOM, and they found there was higher protein-like component proportion in manure extracted DOM. Thus, manure compost fertilization might lead to more protein-like proportion in DOM in paddy soil. All in all, both the increase of TC and C_p4 with manure compost input suggested that manure compost fertilization not only improve SOC storage but also provide more labile C into paddy soil.

The proportions of functional groups H_{C-O} and H_{Al} obtained from the ^{1}H NMR spectrum showed different trends in two kinds of cropping systems and under CHE and MAN treatments. However, H_{Ar} showed a decreasing and then increasing trend in double and single cropping systems which was similar to the trend of $SUVA_{254}$. The proportion of H_{Ar} were similar under CHE and MAN treatments. Thus, the change of cropping system and fertilizations affected the distribution of functional groups.

3.5 Conclusion

We examined the spectroscopic characteristic of paddy soil DOM in two different cropping systems and under different fertilization treatments. $SUVA_{254}$ and fluorescence indices (FI and BIX) showed different trends in double and single cropping systems.   Fluorescent parameters

(HIX, BIX and FI) and the fluorescent components (C1 and C4) were significantly affected by fertilization treatments. The proportion of functional groups was subject to the change of cropping systems and fertilizer input. Thus, spectroscopic characteristics of DOM in paddy soils were subjected to the change of cropping systems and fertilizer input. Compared with the quantitative method of HS and DOM used in Chapter 2, the optical indices are more easier and time saving to determine the composition and humification degree of DOM in paddy soils.
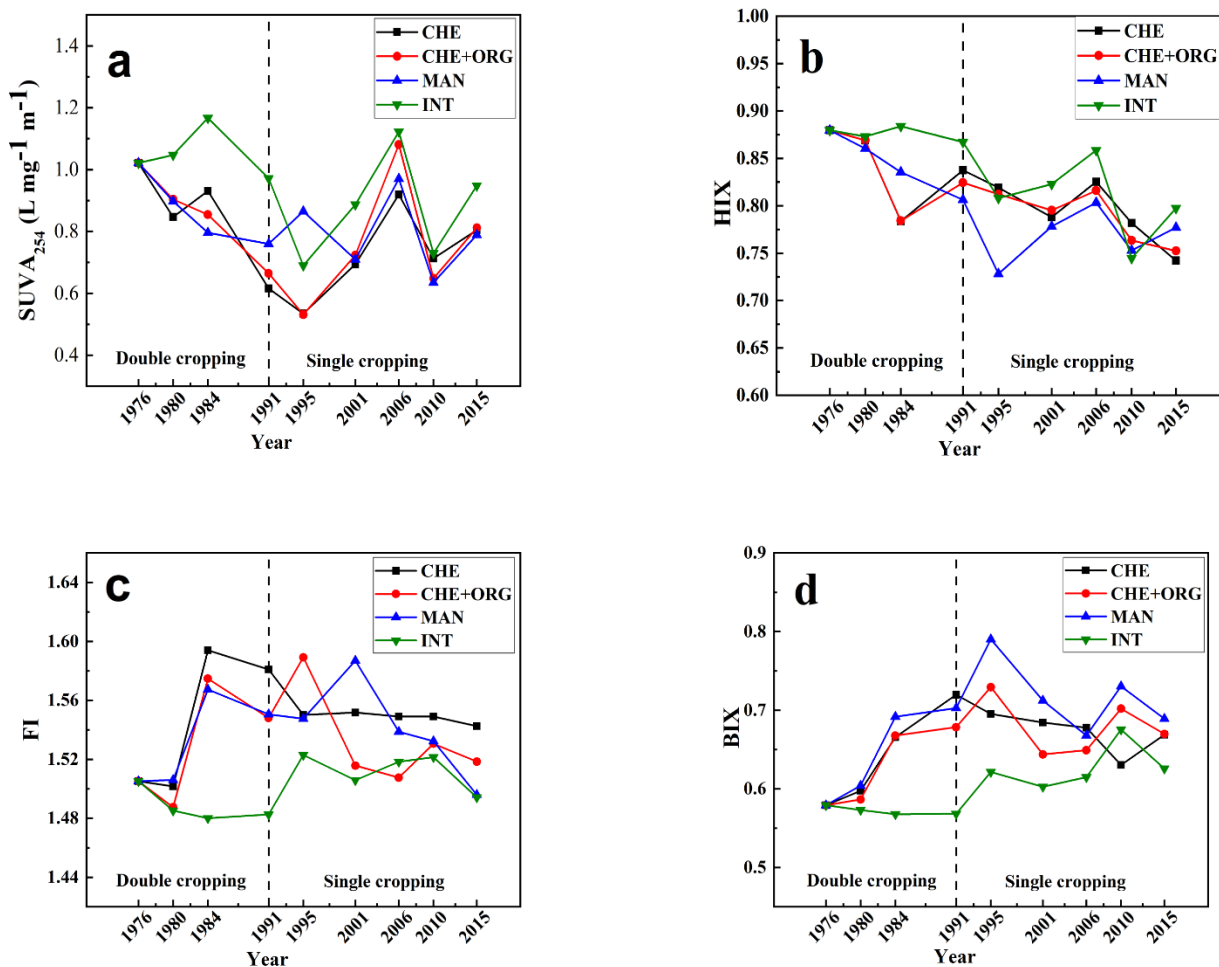
Figure 3.1 Variation of different spectroscopic indices (a, $SUVA_{254}$; b, HIX; c, FI; d, BIX) under different treatments. (CHE: chemical fertilization; CHE+ORG: CHE + organic fertilization; MAN: cattle manure fertilization; INT: CHE + ORG + soil amendment materials (fused phosphate and calcium silicate)

Table 3.1. Kruskal–Wallis test by ranks of the effect of independent factor "Fertilization treatment" on optical indices of $SUVA_{254}$, HIX, FI, and BIX.

	$SUVA_{254}$	HIX	FI	BIX
P value	0.124	0.006*	0.009*	0.006*
Pairwise comparison by Dunn's test with Bonferroni Correction				
CHE vs. CHE+ORG	NS	NS	NS	NS
CHE vs. MAN	NS	NS	NS	NS
CHE vs. INT	NS	NS	0.007*	NS
CHE+ORG vs. MAN	NS	NS	NS	NS
CHE+ORG vs. INT	NS	NS	NS	NS
MAN vs.INT	NS	0.005*	NS	0.005*

$SUVA_{254}$ =specific UV absorbance at 254 nm; HIX= humification index; FI = fluorescence index; BIX= biological or autochthonous index.

* Significantly different at the 0.05 level.

"NS" Not significantly different at the 0.05 level

Table 3.2　Spearman's rank correlation coefficients (r) between different pairs of soil parameters.

	TC	DOM/TC	DOM	HS	HS/DOM	SUVA$_{254}$	HIX	FI	BIX
TC	1.00								
DOM/TC	0.17	1.00							
DOM	0.65**	0.81**	1.00						
HS	0.46**	0.36*	0.55**	1.00					
HS/DOM	-0.21	-0.62*	-0.56**	0.32	1.00				
SUVA$_{254}$	-0.45*	-0.41*	-0.54**	0.09	0.68**	1.00			
HIX	-0.57**	-0.11	-0.39*	0.12	0.43*	0.52*	1.00		
FI	0.17	0.70**	0.63**	-0.06	-0.71**	-0.56**	-0.43*	1.00	
BIX	0.45**	0.52**	0.60**	-0.051	-0.66**	-0.62**	-0.72**	0.78**	1.00

* Significant at P < 0.05.

** Significant at P < 0.01.

Table 3.3 Kruskal-Walls Test by ranks of the effect of independent factor "Fertilization treatment" on F_{max} proportion in $\sum F_{max}$ (C_p).

	C_p1	C_p2	C_p3	C_p4	C_p5
P	0.008*	0.059	0.061	0.028*	0.532
Pairwise comparison by Dunn's test with Bonferroni Correction					
CHE vs. CHE+ORG	NS	NS	NS	NS	NS
CHE vs. MAN	NS	NS	NS	NS	NS
CHE vs. INT	NS	NS	NS	NS	NS
CHE+ORG vs. MAN	NS	NS	NS	NS	NS
CHE+ORG vs. INT	NS	NS	NS	NS	NS
MAN vs.INT	0.004*	NS	NS	0.028*	NS

C_p1–C_p5: the proportion of F_{max} of each fluorescence component.

* Significantly different at the 0.05 level.

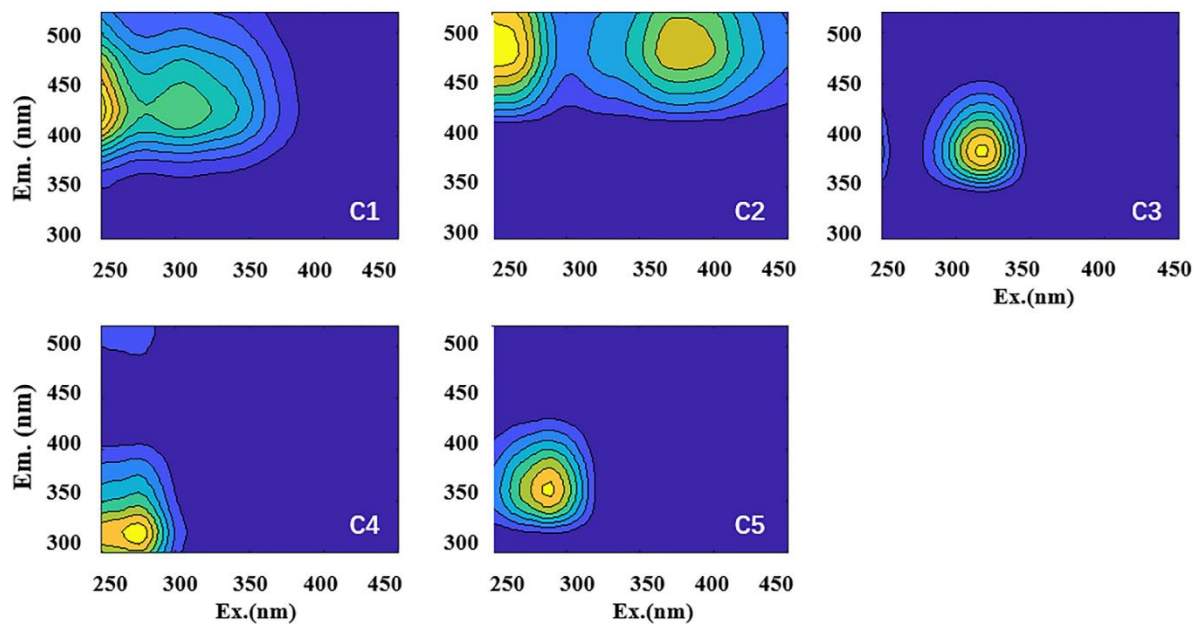"NS" Not significantly different at the 0.05 level.

Figure 3.2 Fingerprints of five-component model decomposed by PARAFAC analysis.
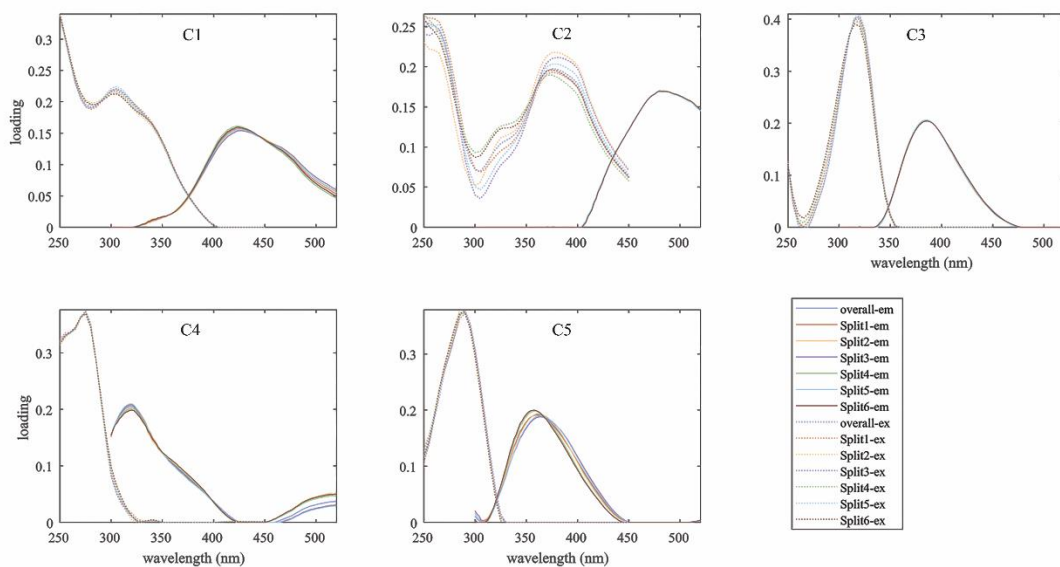
Figure 3.3 Loadings of excitation and emission spectra of the identified PARAFAC components (*overall-ex* and *overall-em*) and the result of the split-half analysis (Split1–6). The dotted and solid curves represent excitation and emission spectra, respectively. C1, humic-like component; C2, humic-like component; C3, microbial humic-like component; C4, tyrosine-like component; and C5, tryptophan-like component.
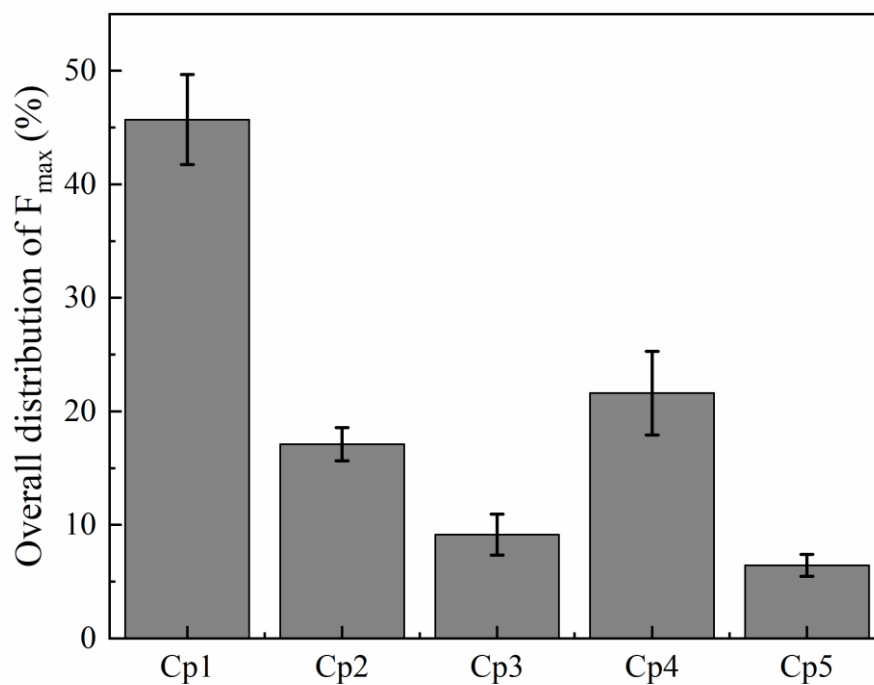
Figure 3.4 The overall distribution of F_{max} proportion of five fluorescence components (C_p1–C_p5: the proportion of five PARAFAC fluorescent components. Error bars show the standard deviations (n = 33)).
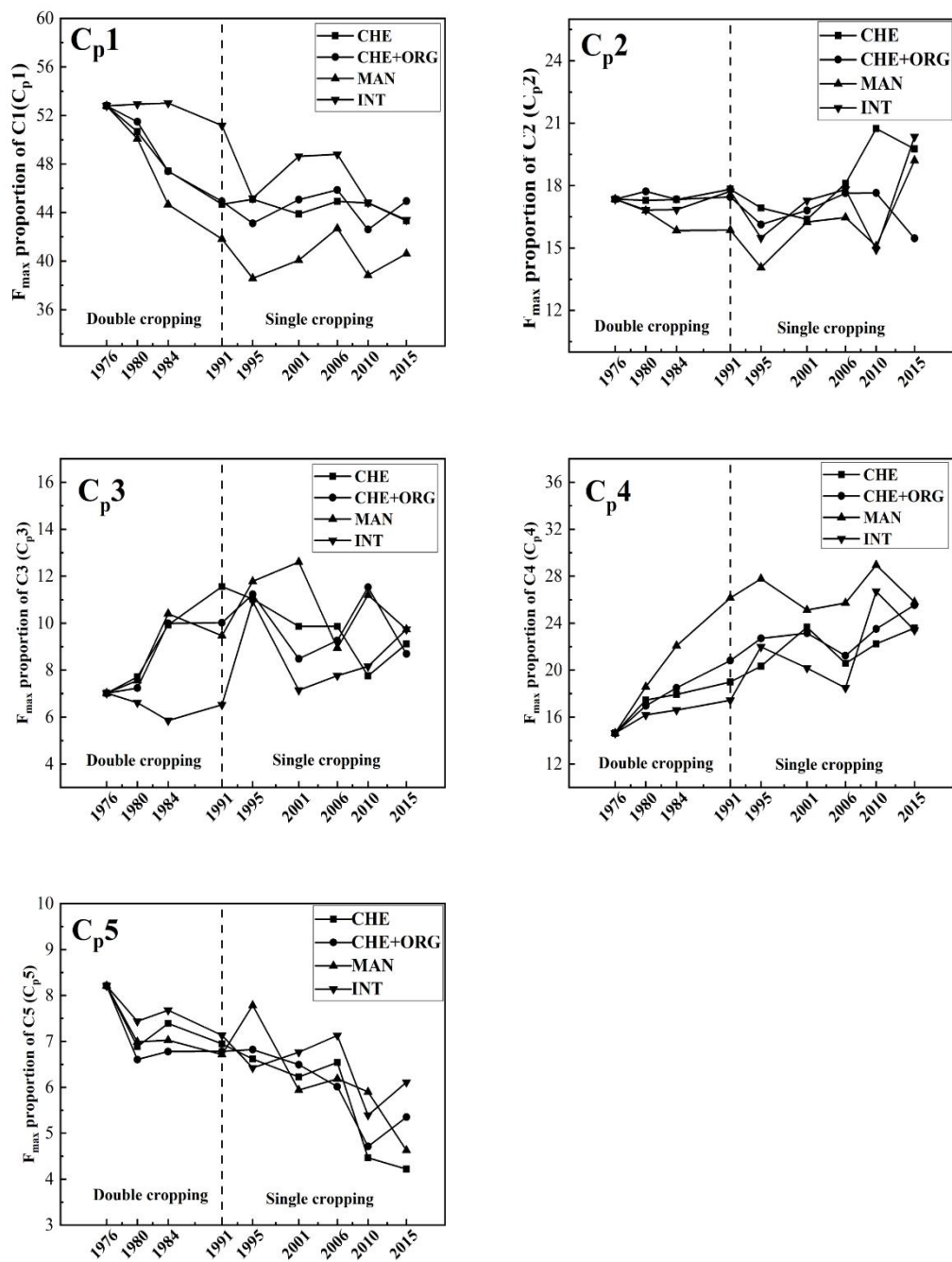
Figure 3.5 The variation of F_{max} proportion of each fluorescence component (C_p1–C_p5) over sampling time under different treatments. (CHE: chemical fertilization; ORG: CHE + organic fertilization; MAN: cattle manure fertilization; INT: CHE + ORG + soil amendment materials (fused phosphate and calcium silicate))
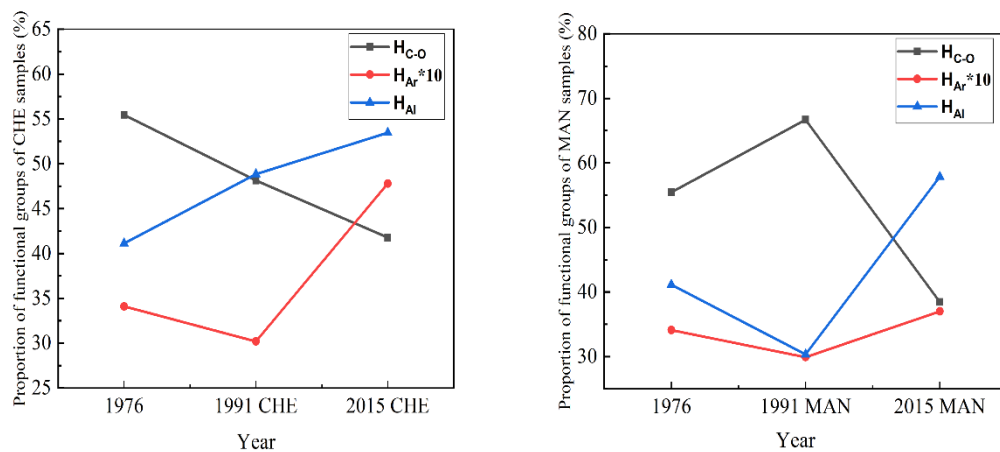
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Figure 3.6 The proportions of fictional group obtained using ^{1}H NMR spectroscopy. (CHE = chemical fertilization treatment, MAN = manure compost fertilization treatment, H_{C-O} = protons attached to carbons on methoxyl groups, H_{Ar} = protons attached to carbons of heteroaromatic and aromatic ring, H_{Al} = alkyl protons.)

Chapter 4 Short-term dynamics and spectroscopic characteristics of dissolved organic matter in paddy soil: a comparison with greenhouse soil

4.1 Introduction

The long-term ($\geq$ 40 years) dynamics and spectroscopic characteristics of DOM in paddy soil have been discussed in Chapter 2 and Chapter 3, of which the samples were collected in the same research site in Mie. However, the dynamics and characteristics of DOM may show different trends and patterns in short-term period (< 10 years) in other places. Thus, the paddy soil samples in single rice cropping system in Osaka, were analyzed using the same methods in Chapter 2 and Chapter 3. In order to compare with paddy soils, greenhouse soils reserved in this institute were also analyzed.

The area of greenhouse production has been widely increased recent years. Temperature, humidity and soil conditions can be monitored and controlled in greenhouse. Farmers usually grow cash crops 4 or 5 times per year, which results in high crop productivity and commercial benefits. There are some studies conducted to investigate the SOC content or fractions in greenhouse cropping system. For instance, Qiu et al. (2010) found the TC stock decreased after the conventional cereal production system changed to a greenhouse vegetable system. Lou et al., (2011) reported a significantly increasing in TC content under manure treatments but an insignificant change in TC under chemical fertilization over 20 years period. Wang et al. (2011) compared data from lots of greenhouse vegetable or convention cropping systems and found that C stock was higher in greenhouse vegetable cropping systems than conventional cropping systems.

Both paddy and greenhouse fields are agricultural ecosystems with high C stock. Paddy fields are open fields characterized by flooding irrigation, while greenhouse fields are closed fields characterized by controllable agricultural management with intensive cultivation and fertilization. Two kinds of management practices might lead to different kinds of C storage patterns. Thus, it is an important task to explore how C was stored in closed and open agricultural ecosystems, which may help to make appropriate strategies to maintain C storage. Soil samples were collected from both greenhouse soil and paddy soil in Osaka prefecture to compare their differences in both DOM and HS concentrations, and the spectroscopic characteristics of DOM.

4.2 Materials and methods

4.2.1   Sampling site

Paddy soil samples were collected from the Research Institute of Environment, Agriculture, and Fisheries, Osaka, Japan (34° 31'N, 135° 36'E) where conducted single rice cropping system. Greenhouse soil samples were collected from farmers' fields located in Kumatori, Senshu, Osaka, Japan (34° 23'N, 135° 21'E). Their locations were showed in Figure 4.1. Mean annual temperature is 15.9°C, and mean annual precipitation is 1187 mm from 1981-2010 (Sakai city which is near both experiment sites, accessed from http://www.data.jma.go.jp/obd/stats/etrn/view/nml_amd_ym.php?prec_no=62&block_no=1062&year=&month=&day=&view=). The temperature sometimes exceeds 35°C in summer, resulting from relatively low precipitation and radiation of heat from the city area (heat island effect). Soil was classified as Typic Epiaquepts (Soil Survey Staff, 1999).   The   experiment

was conducted since 2005. There were three kinds of fertilization treatments: PD-NF, no fertilization input; PD-CHE, chemical fertilizer input; PD- MAN, cattle manure compost input. The input amounts of fertilization treatments were described in Table 4.1. It was time consuming to analysis all samples from 2008 until now. Thus, samples collected in the following years were selected for analysis: 2009, 2011, 2014, 2016 and 2018.

Greenhouse soil was also classified as Typic Epiaquepts (Soil Survey Staff, 1999). The crops in greenhouse are crown daisy (*coronarium. Syn, Leucanthemum coronarium*) planted 4-5 times per year, and taro (*Colocasia esculenta Schott*) planted every 2 years. Thus, it can be considered as a rotation cropping system. Soil samples were collected since 2003 until now. There were three kinds of fertilization treatments conducted: GH-CHE, chemical fertilization; GH-RS, rice straw input; GH-MAN, manure compost input. The fertilization information was descried in Table 4.1. The production area of manure compost was changed from 2016, and the TC content compost was improved in order to increase productivity. Because the farmers needed to make sure their incoming exceeds the expenses, it was impossible to conduct the no fertilization treatment. Thus, the fertilization treatments were different in paddy and greenhouse fields. Soil samples in the following years were selected: 2012,2013, 2015, 2016,2017 and 2018.

5 replicated samples were collected at each site to a depth of 15 cm of Ap horizon and mixed after transporting back to laboratory. All samples were air dried, removed crop residues and crashed to <2mm for analysis. Chemical analysis method was the same as described in Chapter 2. Chemical properties of samples are shown in Table 4.2

In order to increase samples number to conduct PARAFAC modeling, some soil samples were added into the modeling from Mie prefecture, Japan. Those samples were collected every

five years since 1979 to 1994 from 10 different locations in Mie. All fields were conducted single rice cropping. Fertilization information was provided in Table 4.3.

4.2.2   DOM extraction, UV-vis and fluorescent measurement and PARAFAC.

DOM extraction procedure was the same as described in Chapter 2. The DOM solution was measured the UV absorbance and EEMs using the methods described in Chapter 3. PARAFAC modeling was conducted with 54 EEMs. A model with only three components was validated and the components compared with past modeling data in OpenFlour. The three components hereafter refer to C1 to C3. The fingerprint of each fluorescence component was shown in Figure 4.2. C1 was a component with the excitation wavelength maximum at 310nm and the emission wavelength maxima at 412nm. It was reported by Yamashita et al (2011) as a humic-acid type component. C2 was a humic-like component with the peak position at 355nm/ 480nm (EX/EM). C1 and C2 were reported as humic-like components by Romero et al. (2017) as medium and large size humic-like component linked with plant materials resistant to microbial decomposition (Cory and McKnight, 2005). C3 was a tyrosine-like component with excitation wavelength peak at 275nm and emission wavelength peak at 318nm reported by Yamashita et al. (2010). The maximum fluorescent intensity (F_{max}) of each component was then normalized by the DOM concentration of samples, which were C1/DOM, C2/DOM and C3/DOM.

4.2.3 ^{1}H NMR spectroscopy

The experiment procedure was the same as described in Chapter 3.

4.2.4 Data analysis

Because some data was not normally distributed, Kruskal–Wallis test by ranks was applied to evaluate the effect of fertilization treatments on parameters. All calculation was carried out in SPSS 19.0 (IBM Corp, Armork, NY, USA). The dataset of DOM, HS, HS/DOM (%), TC (%), HIX, BIX, FI, H_{C-O}, H_{alkyl}, H_{Ar}, C1/DOM, C2/DOM, and C3/DOM was conducted principle component analysis (PCA) to compare the differences of characteristics of DOM between paddy and greenhouse soils, using the R 3.5.1 (R Core Team, Vienna, Austria).

4.3 Results

4.3.1 Dynamics of TC contents and DOM concentration in paddy and greenhouse soils

The dynamics of TC contents, DOM and HS concentrations over the sampling time under three different treatments were shown in Figure 4.3. The trends of TC content under RS and CHE treatments were stable in the range of 1.5% to 1.7% and 1.4% to 1.5%, respectively, while TC content under MAN showed a slightly decreasing trend within the range of 2.0% to 2.4% in greenhouse soils. In the paddy soils, the TC contents trends also kept stable under NF and CHE treatments in the range of 1% to 1.4% and 1% to 1.35%, respectively, while it decreased from 2.14% in 2012 to 1.54% in 2016 and then increased to 2.99% in 2018 under MAN treatment. The TC contents under three fertilization treatments in greenhouse cropping system was in the order: MAN > RS > CHE, while in paddy soil the TC contents were in the order: MAN > RS = CHE. DOM concentrations decreased over the sampling year in both paddy and greenhouse cropping systems except the increasing trend in greenhouse system in 2011. HS concentrations showed a similar trend with DOM concentration in paddy soil, while HS showed

an increasing trend since 2014 which is different from the trend of DOM concentrations in greenhouse soil.

The fertilization treatments showed different effects on TC, DOM, HS and other parameters in two kinds of soils (Table 4.4). TC contents under three fertilization treatments showed significant differences in greenhouse soil (P = 0.003) and paddy soil (P = 0.007). However, DOM concentrations did not show significant differences in both greenhouse and paddy soils. The proportion of HS in DOM (HS/DOM) showed a significant difference (P = 0.024) in greenhouse soil, while the HS concentration showed statistical difference (P = 0.043) in paddy soil.

The dynamics of DOM/TC ratio and HS/DOM ratio were shown in Figure 4.4. DOM/TC showed similar decreasing tends with DOM concentrations in both paddy and greenhouse soils. HS/DOM ratio showed fluctuant trends in paddy and greenhouse soils except the decreasing trend under MAN treatment in paddy soil.

4.3.2 Optical characteristics of DOM and PCA result

In the greenhouse soils, $SUVA_{254}$ showed an increasing trend under MAN treatment, while under other two treatment $SUVA_{254}$ show fluctuant, but not increasing trends (Figure 4.4). However, $SUVA_{254}$ did not show very clear trends in the paddy soils.

Dynamics of fluorescence parameters were shown in Figure 4.5. FI and BIX did not show very clear trends in both paddy and greenhouse soils. HIX showed an increasing trend in greenhouse soil, while it did not show clear trend in paddy soil.

The proportions of functional groups in each sampling year obtained by ^1H NMR were

shown in Figure 4.6. The proportions of H_{Ar}, H_{C-O}, H_{Alkyl} shared the range of 5%-9%, 41%-49%,44%-53% in the greenhouse soils respectively, while the proportions of those shared the range of 3%-9%,45%-62% and 30%-48% in the paddy soil, respectively. The proportions of H_{C-O} were increased under CHE and MAN treatments in 2018 than in 2012 in paddy soils, while in greenhouse soils the variation of the proportions of H_{C-O} kept stable.

A standard principle component analysis (PCA) were performed on the dataset of paddy and greenhouse soil of which the result was shown in Figure 4.7. Dark blue circle represents the greenhouse soil samples' group, and light blue circle represents the paddy soil samples' group. Two kinds of samples' groups were clearly distinct. The arrow of SUVA and C3/DOM are nearly parallel with the negative direction of Principle component 1 (PC1), indicating that PC1 is highly related to the non-aromaticity and non-protein-like component of DOM. The arrows of HS%, HS and HIX are nearly parallel with the positive direction of the principle component 2 (PC2), indicating that PC2 is related with the HS content and humification degree of DOM.

The effect of fertilization treatments on different parameter were shown in Table 4.5. All the parameters $SUVA_{254}$, HIX, FI, BIX, and the proportions of functional groups did not show significant differences under different fertilization treatments.

4.3.3 Relationships between different pairs of parameters

The correlations between different pairs of parameters of soil samples from Osaka were shown in Table 4.6.　HS/DOM was strongly correlated with C1/DOM and C2/DOM (P = 0.832, P = 0.831, respectively). C1/DOM, C2/DOM and C3/DOM were all significantly correlated

with $SUVA_{254}$ (P = 0.534, 0.570 and 0.410, respectively). The proportion of carbohydrate protons (H_{C-O}) showed a significant negative correlation with DOM concentration (r = -0.521, P < 0.01), while the proportion of aliphatic protons (H_{Al}) showed a significant positive correlation with DOM concentration (r = 0.654, P < 0.01).

4.4 Discussion

Generally, TC content in paddy and greenhouse soils in this study showed relatively similar levels except the decreasing and then increasing trends of paddy soils under MAN treatment which might due to the improvement of TC content of manure compost，implying that TC might continue to decrease if the TC content of manure compost did not change. However, the DOM concentration showed a decreasing trend in both greenhouse and paddy soils. Decreasing DOM in greenhouse soil might due to the high temperature which increased the microbial activity and then increased the decomposition rate of DOM. Grisi et al. (1998) compared soil samples from tropical area and temperate area and found that the tropical soils were more degraded than temperate soils. The decreasing trend of DOM concentration in paddy soil in Osaka which is similar with the trend of single cropping system in Mie, suggesting that DOM concentrations decreased in single cropping systems no matter in short-term or long-term period.

The DOM concentrations under MAN treatment were higher than the other treatments in both paddy and greenhouse soils in the beginning of the sampling years. However, the differences between fertilization treatments gradually diminished over time. These results were consistent with the results in Chapter 2 that fertilizers might increase the DOM concentrations when they were first applied into soil but then these effects became weaker shortly. TC contents,

DOM and HS concentrations under MAN treatments were higher than other treatments in both paddy and greenhouse soils, suggesting that manure compost input is an effective way to maintain SOM storage and increase recalcitrant C content within the same cropping system.

HS concentration in paddy soil also showed a decreasing trend which is similar with the trend in 1991-2015 in Chapter 2, suggesting that single cropping system might reduce the recalcitrant C content in soil.   Compared with the decreasing trend of HS concentration in paddy soil, HS concentration in greenhouse soil was considerable increasing from 2014 to 2018. The increasing trend of HIX indicated an increasing humification degree of DOM in greenhouse soils, while HIX did not show clear continuous trends in paddy soils. The greenhouse cropping system can increase the HS content and the humification degree of soil. Highly intensive soil disturbance increased oxygen exposure of soil and then increased humification degree of soil DOM by releasing more aromatic C and less aliphatic C into the soil (Arshad et al., 2010; Xu et al., 2013). HS plays an important role in plant growth in terms of mediation of uptake of nutrients, the enhancement of root initiation, the acceleration of energy metabolism of plant and so on. Thus, more HS can help maintain better soil fertility and guarantee plant nutrition (Pettit et al., 2004). In this case, there might be a potential of maintaining recalcitrant C in greenhouse cropping system than in single rice cropping system.

The fertilization did not show significant effect on the $SUVA_{254}$, HIX, FI, BIX and the proportions of functional groups. Although $SUVA_{254}$ has been widely applied in evaluating aromaticity of DOM in natural water samples, it seems that it's not an effective method applied on extractable soil samples. Because the UV absorbance of samples is also affected by the PH, EC and other properties of solution. Generally, DOM solution extracted from soils is

characterized with larger scale of EC and PH value than natural water samples, which may affect the accuracy of determination of UV absorbance. The ^{1}H NMR spectrum was also reported to be sensitive to high salt and metal content (Lam and Simpson, 2007). Thus, more detailed procedure is needed to determine the spectroscopic data of extracted DOM solution.

The PCA results revealed distinct different characteristics of DOM between paddy and greenhouse soils. The greenhouse samples showed higher HS and DOM concentrations and humification degree than paddy samples. Greenhouse soil contained more alkyl functional group (H_{Al}) than paddy soil. There were higher proportions of carbohydrate (H_{C-O}) and protein-like component in DOM of paddy soil than greenhouse soil. The clear separation of two kinds of samples' group (Figure 4.7) showed the effectiveness of PCA. Although the optical indices SUVA$_{254}$, BIX and FI did not show clear different trends over sampling time and under different fertilization treatments, PCA results showed clear distance between two soil groups. Thus, when the dataset was vast, complicated and not intuitionistic, PCA can be used to compared soil properties of different kinds of groups of soil samples.

C1/DOM and C2/DOM were highly correlated with HS/DOM, implying the C1 and C2 were important composition of HS in both paddy and greenhouse soils. C1/DOM and C2/DOM were also highly correlated with SUVA$_{254,}$ suggesting the C1 and C2 were two fluorescent components that contributed to the aromaticity of DOM. The significant correlation between H_{Al}, H_{C-O} and DOM concentration implied that the proportions of carbohydrate and aliphatic protons might reflect the DOM dynamics.

4.5 Conclusion

DOM concentration in paddy soil showed similar decreasing trend with the single cropping system (1991-2015) in Chapter 2, suggesting that DOM concentration decreased in single rice cropping system in both short- or long-term period. HS concentrations of paddy soils in Osaka showed a similar trend with the single cropping system (1991-2015) in Chapter 2, indicating that the recalcitrant C decreased in single cropping system. Thus, conducting single cropping in paddy soil is not a good way to maintain SOC storage. Manure compost and rice straw increased DOM and HS concentration after the application. But the effect diminished with the time went by. Increased HS concentration in greenhouse soil and decreased HS concentration in paddy soil suggested that greenhouse soil had the potential to kept more recalcitrant C than the single cropping system in paddy soil. Paddy soils were capable of preserve more carbohydrate than greenhouse soils. The optical indices may not as useful as it is in evaluating characteristics of DOM in natural water samples. The effectiveness of SUVA254, HIX, BIX and FI is needed further demonstration in the future research.
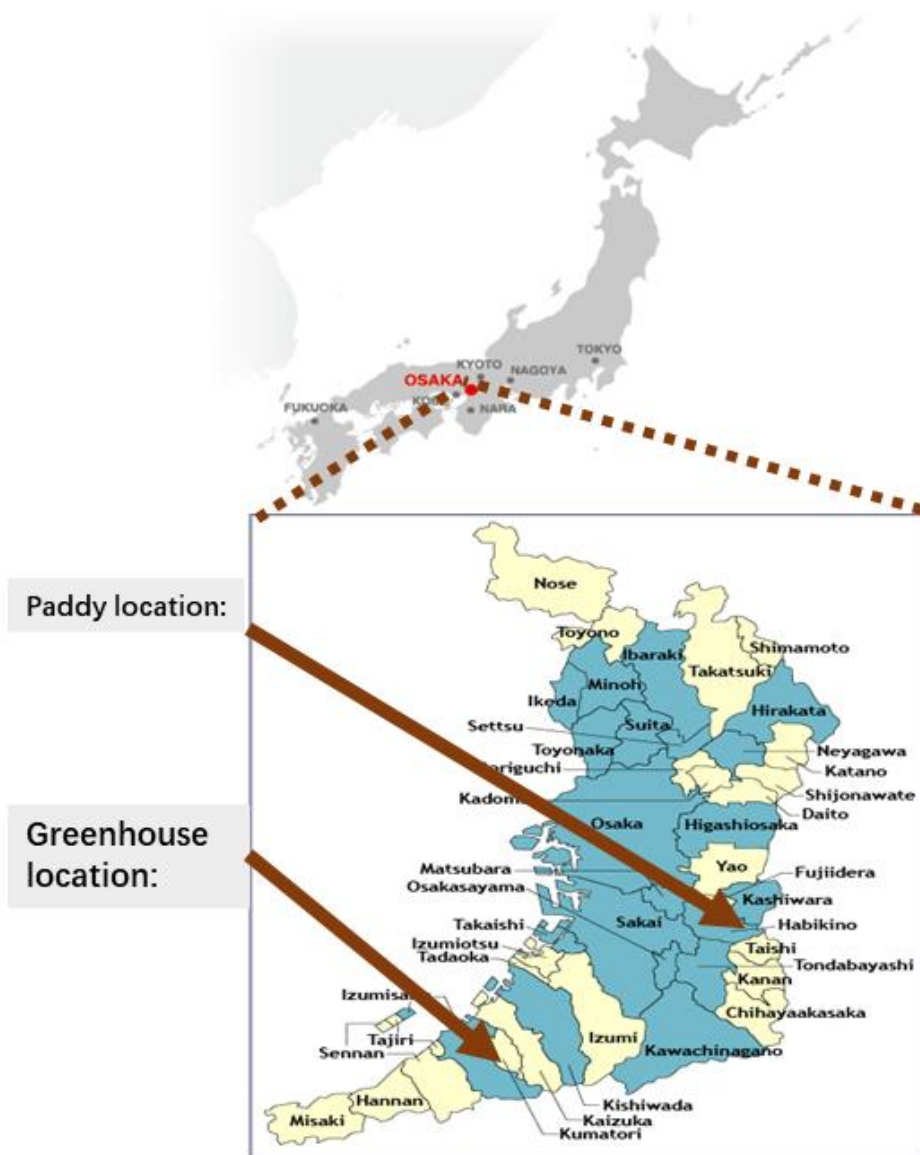
Figure 4.1 Paddy and greenhouse soil sampling locations.

Table 4.1 Chemical fertilizers and organic fertilizers input in three kinds of treatments in paddy soils and greenhouse soils, respectively.

Paddy soil			Greenhouse soil			
Treatments	Chemical fertilizer (N 8%, P_2O_5 8%, K_2O 8%)	Manure compost input (water content 47%, C 26.3%, N1.41%)	Treatments	Chemical fertilizer (N 6%, P_2O_5 8%, K_2O 5%)	Organic fertilizer	
					Rice straw	Manure compost
No fertilizer input (PD-NF)	None	None	Chemical fertilizer (GH-CHE)	1000-2000 kg/ ha^{-1}	None	None
Chemical fertilizer (PD-CHE)	100kg ha^{-1} (50 for basal and 50 for supplement)	None	Rice straw (GH-RS)	1000-2000 kg/ ha^{-1}	2000-3000kg/ ha^{-1} [†]	None
Cattle Manure compost input (PD-MAN)	50kg ha^{-1} (for supplement)	15000 kg ha^{-1}(wet basis).	Cattle Manure compost input (GH-MAN)	1000-2000 kg/ ha^{-1}	None	10000-15000 kg/ ha^{-1} [†]

[†] Organic fertilizers were applied twice per year in February/March and September/October.

Table 4.2 Selected chemical properties of soil samples from Osaka.

Samples [†]	pH	EC mS/cm	NO$_3$-N mg/100g	P mgP$_2$O$_5$/100g	K mgK$_2$O/100g	C %	N %	C/N
09 GH-CHE	6.26	0.38	-[†]	161.06	14.46	1.54	0.18	8.70
09 GH-RS	5.98	0.54	-	207.35	15.77	1.63	0.19	8.67
09 GH-MAN	6.13	0.35	-	242.96	47.50	2.27	0.24	9.36
11 GH-CHE	5.55	0.27	6.38	178.65	10.75	1.51	0.16	9.28
11 GH-RS	5.64	0.28	9.04	165.11	10.75	1.69	0.19	8.91
11 GH-MAN	6.47	0.52	22.05	216.57	57.75	2.51	0.27	9.42
14 GH-CHE	5.72	0.40	5.18	252.24	12.05	1.42	0.17	8.24
14 GH-RS	5.92	0.14	2.66	225.37	9.14	1.60	0.18	8.71
14 GH-MAN	6.36	0.20	2.63	299.57	19.81	2.19	0.23	9.47
15 GH-CHE	5.52	0.43	7.58	269.65	23.26	1.44	0.17	8.66
15 GH-RS	5.53	0.33	1.50	243.84	16.34	1.48	0.17	8.99
15 GH-MAN	5.88	0.58	10.20	284.46	37.01	2.06	0.22	9.27
16 GH-CHE	4.90	0.80	8.40	282.27	31.60	1.44	0.19	7.61
16 GH-RS	4.99	0.53	2.81	277.14	19.93	1.59	0.19	8.31
16 GH-MAN	5.51	0.55	5.93	314.86	42.52	2.00	0.24	8.39
18 GH-CHE	5.37	0.30	5.21	314.08	13.38	1.41	0.18	8.03
18 GH-RS	4.95	0.52	7.73	269.28	21.69	1.51	0.18	8.27
18 GH-MAN	5.45	0.19	0.94	339.59	15.19	2.08	0.23	9.18
11 PD-NF	6.73	0.05	-	22.29	6.03	1.14	0.12	9.35
11 PD-CHE	6.54	0.04	-	20.89	8.54	1.24	0.13	9.58
11 PD-MAN	7.34	0.05	-	51.43	17.88	1.98	0.19	10.40
12 PD-NF	6.24	0.03	-	18.32	9.21	1.215	0.1	11.60
12 PD-CHE	6.19	0.03	-	20.61	14.85	1.215	0.1	11.72
12 PD-MAN	7.03	0.03	-	52.22	41.11	2.144	0.2	11.58
13 PD-NF	7.04	0.04	-	20.15	5.41	1.02	0.09	11.17
13 PD-CHE	7.14	0.03	-	18.32	6.81	1.10	0.10	10.98
13 PD-MAN	6.98	0.04	-	51.30	23.64	1.96	0.17	11.25
15 PD-NF	6.25	0.05	-	16.80	2.47	1.15	0.11	10.36
15 PD-CHE	5.78	0.06	-	15.57	3.67	1.17	0.11	10.44
15 PD-MAN	6.67	0.06	-	36.30	21.72	1.70	0.15	11.42
16 PD-NF	6.39	0.04	-	129.63	8.58	1.17	0.11	10.39
16 PD-CHE	6.49	0.04	-	120.47	5.99	1.10	0.11	10.21
16 PD-MAN	6.29	0.04	-	188.72	13.86	1.54	0.14	10.67
17 PD-NF	5.73	0.03	-	6.50	6.50	1.27	0.12	10.71
17 PD-CHE	6.11	0.03	-	23.04	11.28	1.29	0.12	10.81
16 PD-MAN	6.58	0.04	-	45.58	20.92	2.08	0.18	11.76
18 PD-NF	6.43	0.05	-	8.32	11.29	1.40	0.14	10.14
18 PD-CHE	6.18	0.05	-	8.46	15.07	1.35	0.14	9.96
18 PD-MAN	6.48	0.09	-	28.16	37.88	2.99	0.26	11.63

"-" Not analyzed

† GH-CHE = greenhouse soil with chemical fertilization treatment; GH-RS = greenhouse soil with rice straw input treatment; GH-MAN = greenhouse soil with cattle manure compost input treatment; PD-NF = paddy soil with no fertilizer input; PD-CHE = paddy soil with chemical fertilizer input treatment; PD-MAN = paddy soil with manure compost input treatment.

Table 4.3 Basal and organic fertilizers, and soil amendment (SAM) added to the field in different sampling years in Mie paddy fields.

Sampling year 1979

Sample ID	crop	organic fertilizer (kg/10a)	SAM (kg/10a)		fertilization (kg/10a) N	P_2O_5	K_2O
101	rice	NA	NA		11.4	6.4	9.8
102	rice	straw 400	NA		14.6	6.4	13.2
110	rice	straw 160	$CaSiO_3$ 60	P_2O_5 16	10.2	4.8	8.6
103	rice	NA	$CaSiO_3$ 40		11.1	8.8	10
108	rice	straw 550	$CaSiO_3$ 100	P_2O_5 40	9.1	6.4	8.1
105	rice	NA	$CaSiO_3$ 10	P_2O_5 10	7.2	7.2	7.2
111	rice	straw 1000	$CaSiO_3$ 200	P_2O_5 100	10.1	7.1	10.1
104	rice	NA	$CaSiO_3$ 400	P_2O_5 120	11.8	10.8	14.2
115	rice	straw 200	$CaSiO_3$ 200	P_2O_5 40	4.3	4.3	4.3
117	rice	manure 250	$CaSiO_3$ 100	P_2O_5 30	9.1	7.2	11.5

Sampling year 1984

Sample ID	crop	organic fertilizer (kg/10a)	SAM (kg/10a)		fertilization (kg/10a) N	P_2O_5	K_2O
101	rice	NA	P2O5 40		5.1	0.6	5.1
102	rice	straw 200	CaSiO3 200		11.4	6.4	11.4
110	rice	straw 500	CaSiO3 40	P2O5 20	9.8	6.4	9.8
103	rice	NA	CaSiO3 100		12.2	8	11.8
108	rice	straw 400	CaSiO3 200	P2O5 40	9.8	6.4	9.8
105	rice	straw 500	NA		7.2	10.8	108
111	rice	straw 500	CaSiO3 160	P2O5 40	32.6	8.2	28.6
104	rice	NA	CaSiO3 200	P2O5 40	6.6	9.9	9.9
115	rice	straw 500	CaSiO3 100	P2O5 20+6	6.5	7.2	8.1
117	rice	straw 350	CaSiO3 100	P2O5 60	6.9	5.6	8.1

Sampling year 1989

Sample ID	crop	organic fertilizer (kg/10a)	SAM (kg/10a)		fertilization (kg/10a) N	P_2O_5	K_2O
101	rice	straw 500	$CaSiO_3$ 160	P_2O_5 40	9.9	6.4	10.7
102	rice	straw 500	$CaSiO_3$ 140		15.2	9.6	15.2
110	rice	straw 500	$CaSiO_3$ 160	P_2O_5 40	11	4.2	19
103	rice	NA	NA		8	7.2	10.4
108	rice	straw 600	$CaSiO_3$ 128	P_2O_5 32	8	5.4	9.8
105	rice	NA	P_2O_5 80		22.9	14.4	24.6
111	rice	straw 480	NA		6.4	1.9	6.6
104	rice	straw 800	P_2O_5 30		9.8	9.8	9.8
115	rice	NA	$CaSiO_3$ 160	P_2O_5 40	10.6	10.8	11.8
117	rice	straw 500	$CaSiO_3$ 160	P_2O_5 40	6.4	4	6.4

Sampling year 1994

| Sample ID	crop	organic fertilizer (kg/10a)	SAM (kg/10a)		fertilization (kg/10a) N	P_2O_5	K_2O
101	rice	NA	$CaSiO_3$ 160	P_2O_5 40	8.4	5.4	16.2
102	rice	straw 300	NA		12	6.4	12
110	rice	straw 500	$CaSiO_3$ 160	P_2O_5 40	9.9	4.5	9.9
103	rice	NA	$CaSiO_3$ 120		11.2	7.2	13.6
108	rice	straw 600	$CaSiO_3$ 200	P_2O_5 40	5.8	5.4	7.6
105	rice	straw 400	NA		9.2	5.4	11
111	rice	straw 500	NA		7	7.8	6
104	rice	straw400	$CaSiO_3$ 160	P_2O_5 40	8	2.4	7.2
115	rice	straw 500	P_2O_5 20+40		8.2	7.2	9
117	rice	straw 500	$CaSiO_3$ 200 Mg90		5.8	2.4	5.8
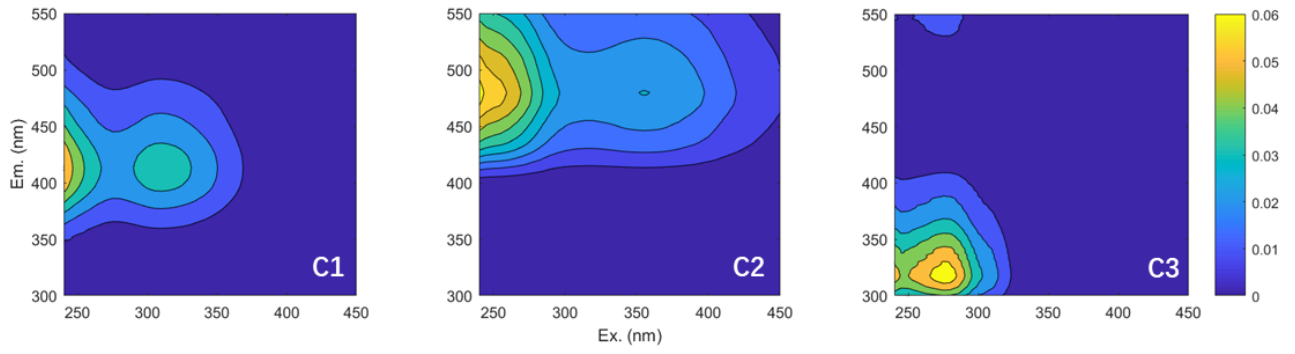
Figure 4.2 Fingerprints of three-component model.
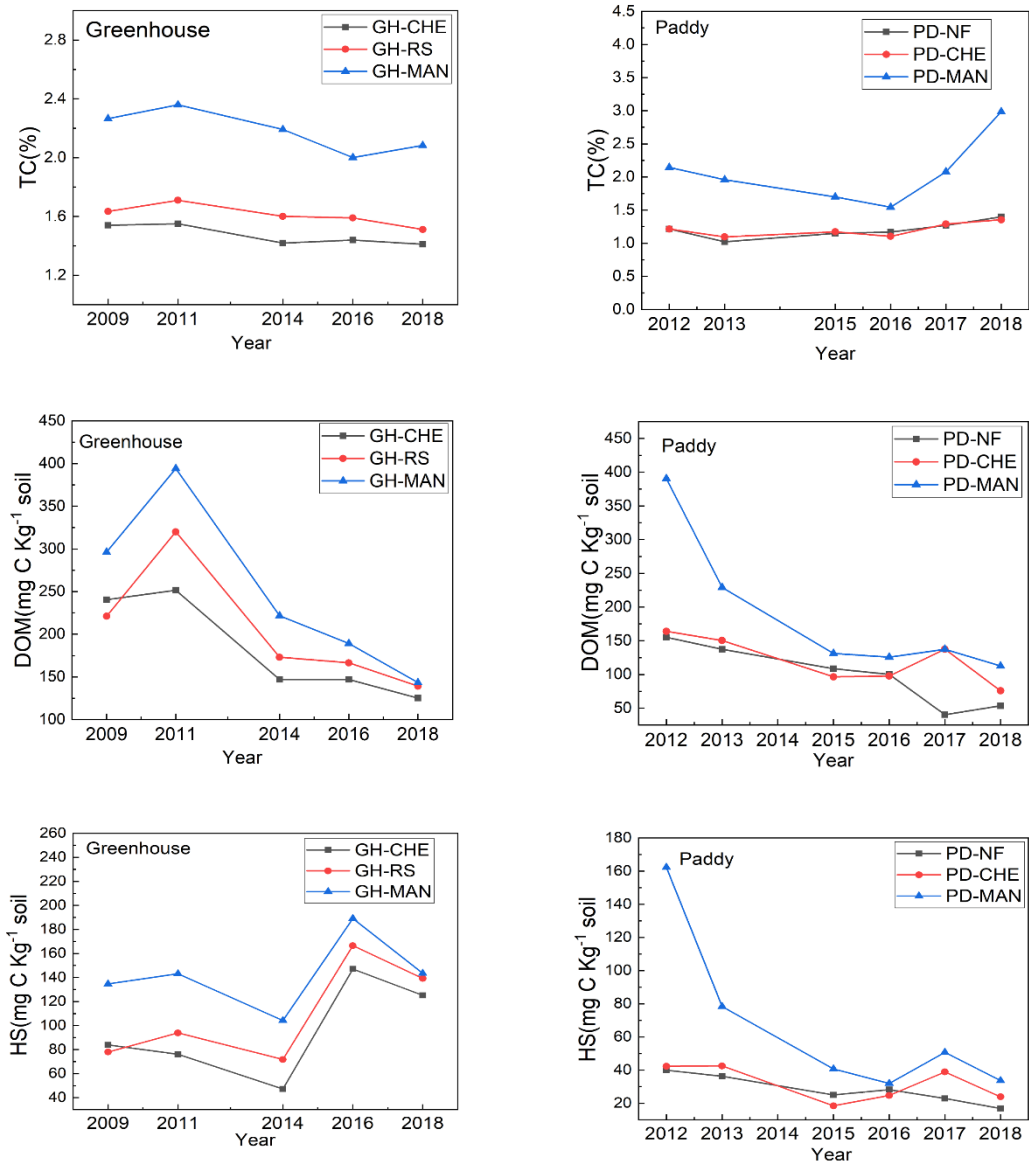
Figure 4.3 Total carbon (TC, %), dissolved organic matter (DOM, mg C Kg^{-1} soil) and humic substances (HS, mg C Kg^{-1} soil) concentrations in greenhouse and paddy cropping systems.
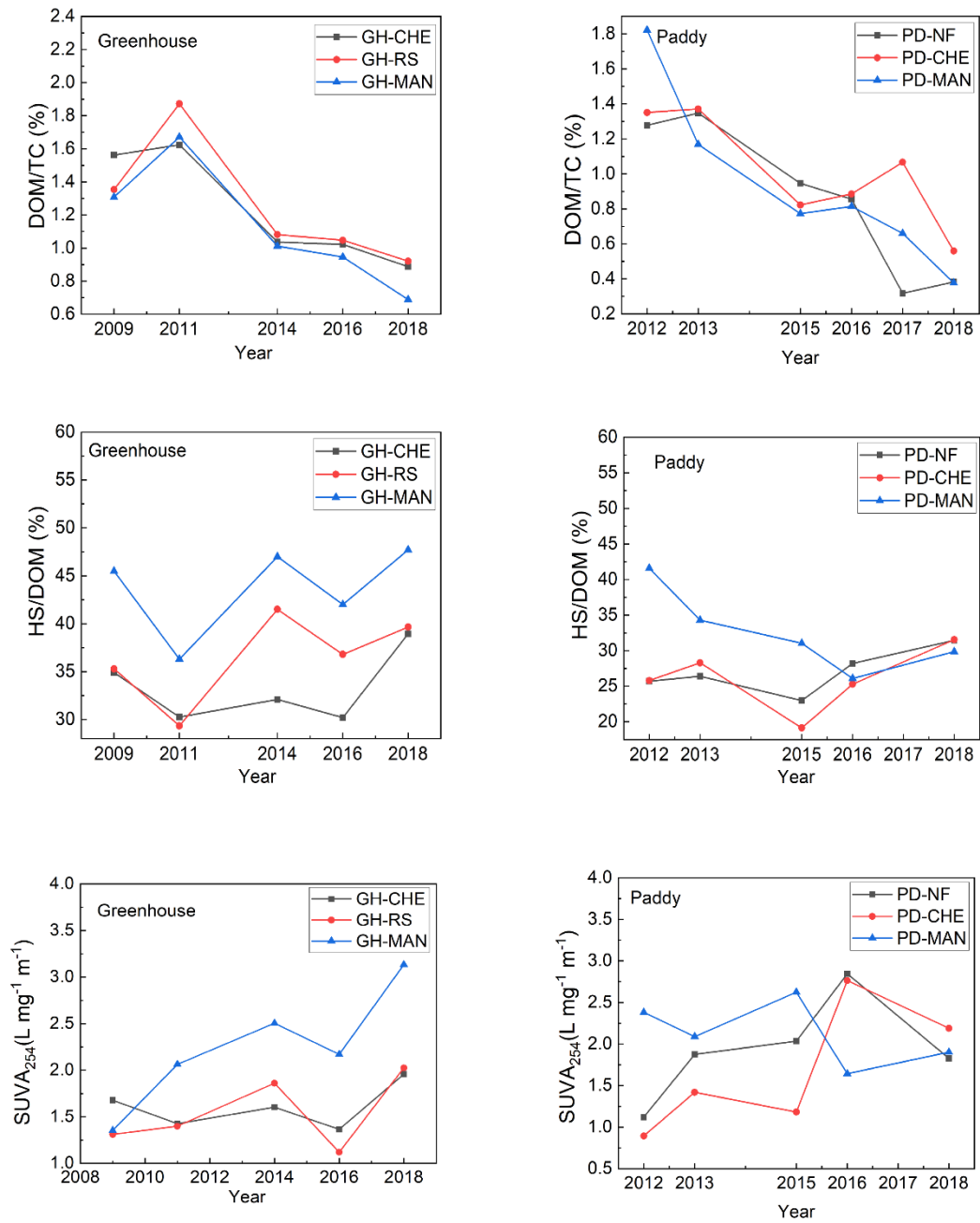
Figure 4.4. DOM/TC ratio (%), HS/DOM ratio (%) and SUVA$_{254}$ (L mg^{-1} m^{-1}) under three treatments.

Table 4.4 Kruskal–Wallis test by ranks of the effect of independent factor "Fertilization treatment" on soil TC (%), DOM/TC (%), DOM, HS, HS/DOM (%) in greenhouse and paddy soils.

Greenhouse					
	TC	DOM/TC	DOM	HS	HS/DOM
P value	0.003*	0.698	0.512	0.210	0.024*
CHE vs. RS	NS	NS	NS	NS	NS
RS vs. MAN	NS	NS	NS	NS	NS
NF vs. MAN	0.002*	NS	NS	NS	0.022*

Paddy					
	TC	DOM/TC	DOM	HS	HS/DOM
P value	0.007*	0.786	0.235	0.043*	0.176
NF vs. CHE	NS	NS	NS	NS	NS
CHE vs. MAN	0.018*	NS	NS	NS	NS
NF vs. MAN	0.015*	NS	NS	0.053*	NS

*significance at P<0.05.

Table 4.5 Kruskal–Wallis test by ranks of the effect of independent factor "Fertilization treatment" on $SUVA_{254}$, HIX, FI, BIX and the proportion of functional groups obtained by ^{1}H NMR spectroscopy in greenhouse and paddy soils.

Greenhouse						
	$SUVA_{254}$	HIX	BIX	FI	H_{Ar}	H_{C-O}	H_{Al}
P value	0.101	0.054	0.395	0.887	0.230	0.677	0.877
CHE vs. RS	NS	NS	NS	NS	NS	NS	NS
RS vs. MAN	NS	NS	NS	NS	NS	NS	NS
NF vs. MAN	NS	NS	NS	NS	NS	NS	NS

Paddy						
	$SUVA_{254}$	HIX	BIX	FI	H_{Ar}	H_{C-O}	H_{Al}
P value	0.566	0.182	0.156	0.723	0.468	0.961	0.756
NF vs. CHE	NS	NS	NS	NS	NS	NS	NS
CHE vs. MAN	NS	NS	NS	NS	NS	NS	NS
NF vs. MAN	NS	NS	NS	NS	NS	NS	NS
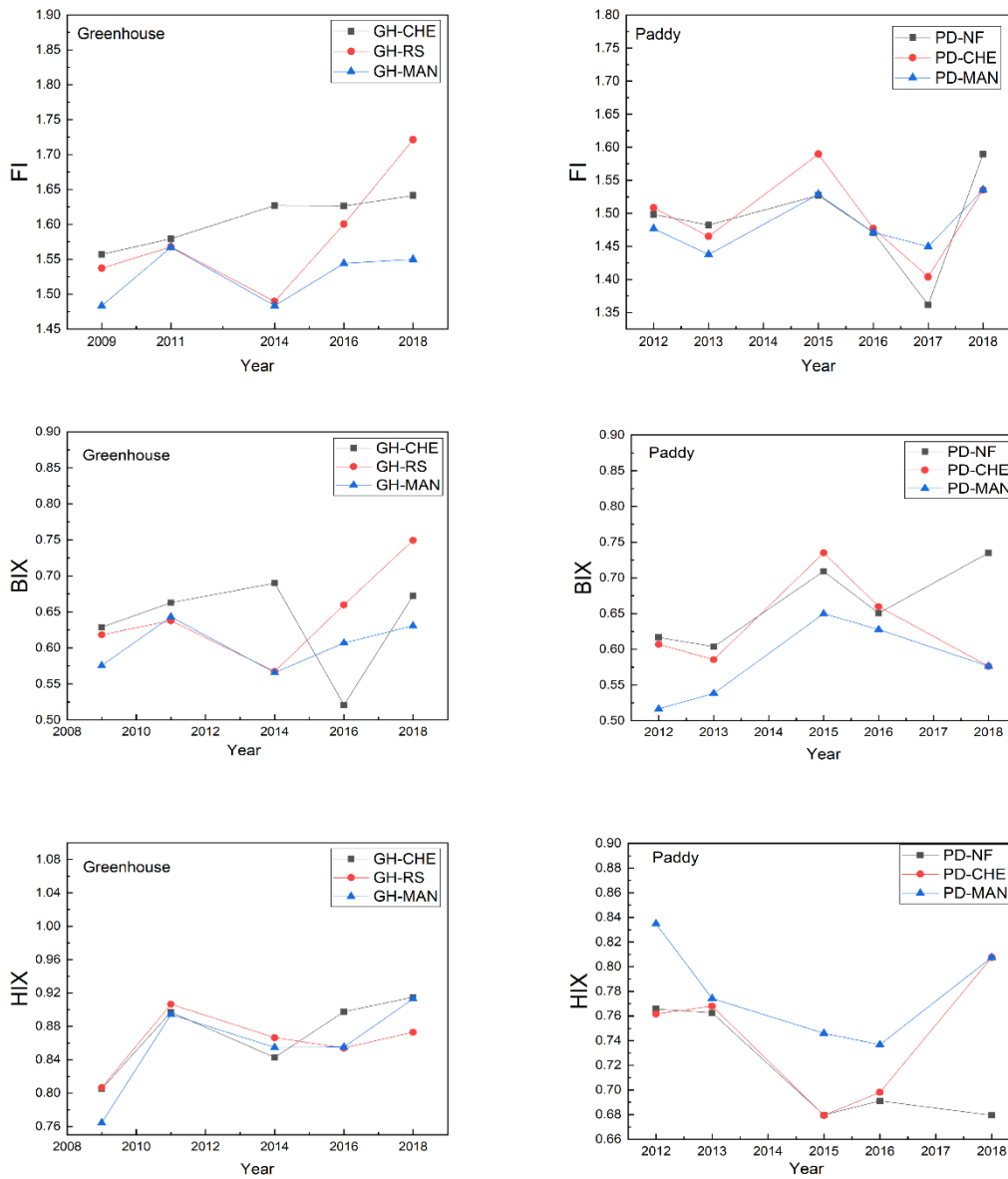
Figure 4.5 Dynamics of fluorescence parameters (FI, BIX and HIX) of soil dissolved organic matter (DOM) in paddy and greenhouse soils
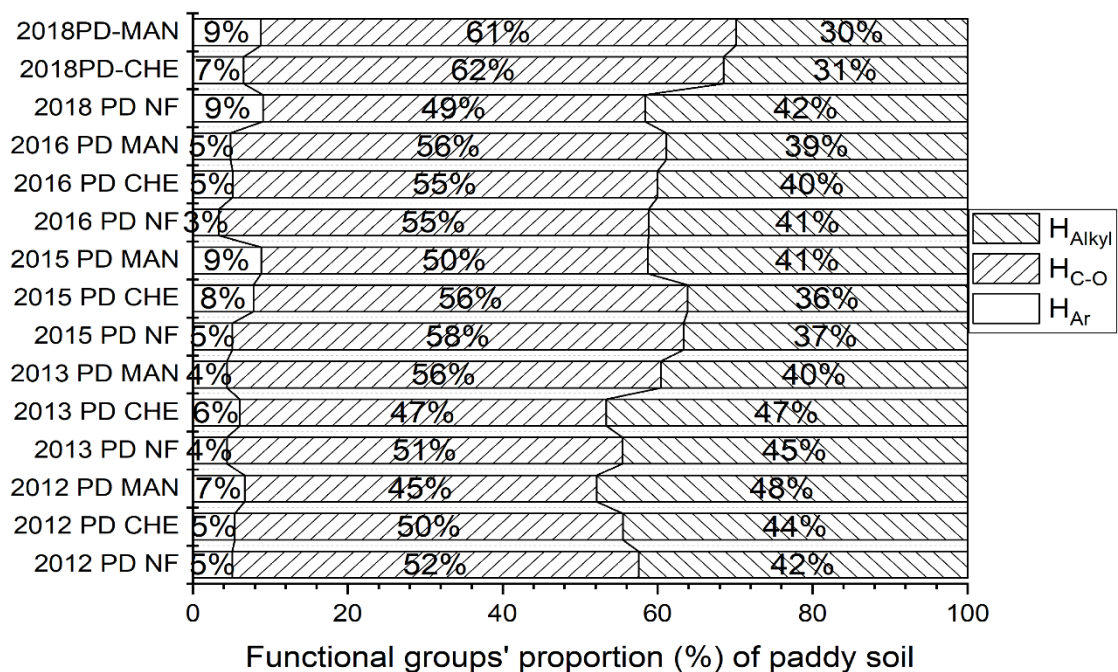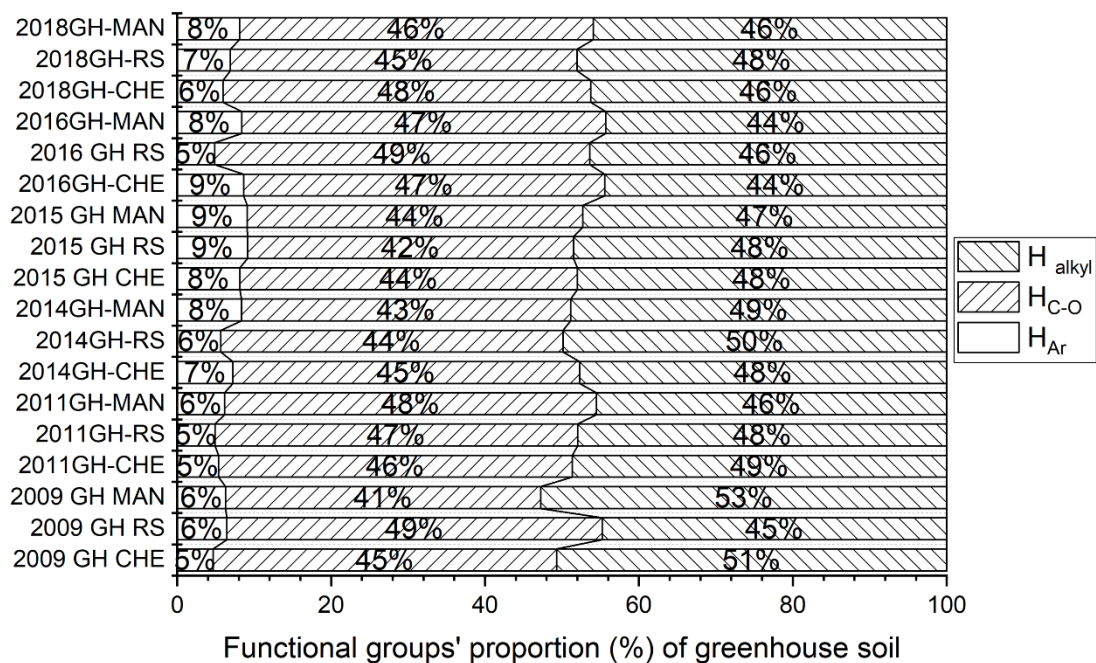
Figure 4.6 Functional groups' proportions of dissolved organic matter (DOM) in greenhouse and paddy soils.
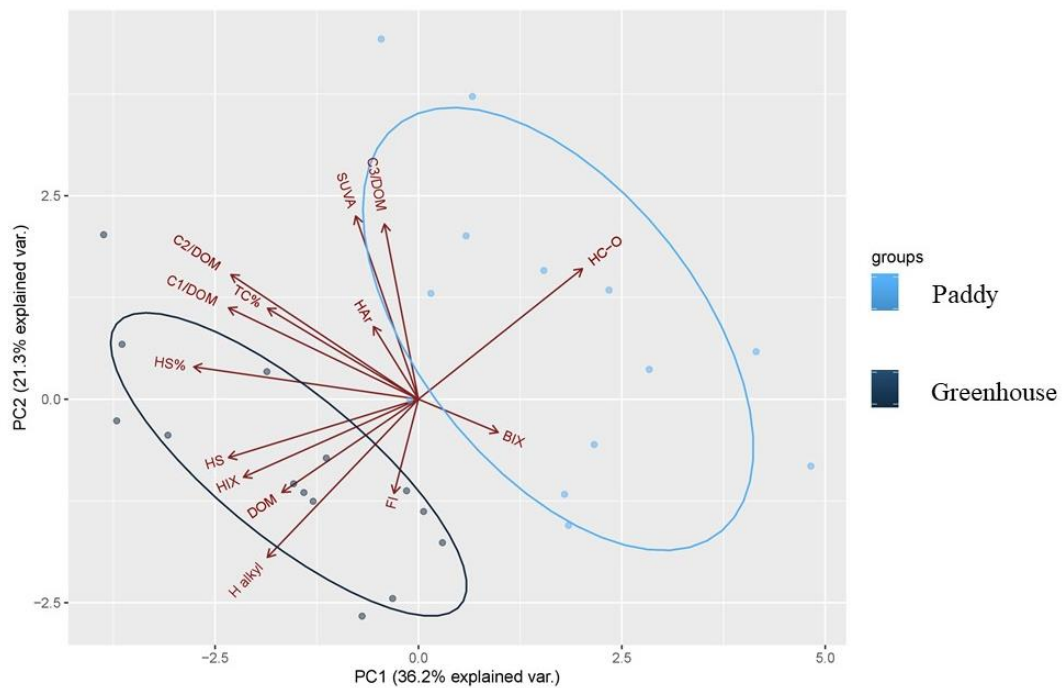
Figure 4.7 Plot of the first two principal axes of the Principle component analysis (PCA). The dark blue circle represents the greenhouse soil samples' group. The light blue circle represents the paddy soil samples' group.

Table 4.6 Spearman's rank correlation coefficients (r) between different pairs of parameters of soil samples used in Chapter 4.

	FI	BIX	HIX	H_{Ar}	H_{C-O}	H_{Al}	$H_{Ar}+H_{Al}$	C1/DOM	C2/DOM	C3/DOM
DOM	-0.083	-0.434*	0.434*	-0.349	-0.521**	0.654**	0.521**	0.057	0.093	0.042
HS	-0.045	-0.416*	0.517**	-0.217	-0.545**	0.631**	0.545**	0.350	0.391*	0.120
HS/DOM	0.118	-0.230	0.558**	0.295	-0.609**	0.538**	0.609**	0.832**	0.831**	0.190
SUVA$_{254}$	-0.257	0.035	0.061	0.093	0.192	-0.247	-0.192	0.534**	0.570**	0.410*
TC	-0.109	-0.407*	0.390*	0.230	-0.187	0.149	0.187	0.497**	0.686**	0.473*
DOM/TC	-0.022	-0.236	0.250	-0.571**	-0.353	0.560**	0.353	-0.332	-0.363	-0.211
FI	1.000	0.598**	0.464*	0.491**	-0.370	0.240	0.370	0.191	0.072	-0.485*
BIX		1.000	-0.107	0.201	0.050	-0.099	-0.050	0.016	-0.165	-0.257
HIX			1.000	0.172	-0.522**	0.530**	0.522**	0.424*	0.449*	-0.392*
H_{Ar}				1.000	-0.247	-0.020	0.247	0.404*	0.409*	0.013
H_{C-O}					1.000	-0.944**	-1.000**	-0.429*	-0.311	0.271
H_{Al}						1.000	0.944**	0.324	0.224	-0.252
$H_{Ar}+H_{Al}$							1.000	0.429*	0.311	-0.271

* Significant at P < 0.05.

** Significant at P < 0.01.

Chapter 5 General Discussion

Although DOM is only a very small proportion of TC in soil, it is the energy source which microorganism can directly make use of. The dynamics of DOM in arable soil reflects the change of availability of C pool over time. This research investigated the dynamics and characteristics of DOM in paddy soils where long-term or short-term experiments were conducted and compared the differences of DOM properties in paddy and greenhouse soils.

Chapter 1 introduced the unique characteristic of paddy ecosystem and elaborated the importance of soil DOM in agricultural ecosystems and described quantitative and qualitative methods to evaluate DOM. Chapter 2 determined the DOM and HS concentrations in a paddy field where cropping systems were shifted from double cropping system to single cropping system, observed the different dynamics of DOM in double and single cropping systems, found that DOM dynamics were more subjected to the change of cropping systems than fertilization. Chapter 3 evaluated the optical properties using UV-vis, fluorescence and ^{1}H NMR spectroscopy, and found that the spectroscopic parameters were all subjected to both cropping systems and fertilization. The results of Chapter 2 and Chapter 3 suggested that cropping system was more effective factor than fertilization to influence dynamics and spectroscopic characteristics of DOM in paddy soils. Chapter 4 compared the spectroscopic characteristics of DOM in paddy and greenhouse soils, suggesting that the single cropping system reduces both DOM and HS concentrations in paddy soils, which is not a good way to maintain SOC storage. There are some important points were not revealed in this research. Here will discuss some possible directions in the further research.

① Comparation of characteristics of DOM between paddy soil and upland soil (not greenhouse soil)

Both paddy and greenhouse fields are special agricultural ecosystems. It also considered that cereal crops occupy a large area of arable land. The observation of DOM dynamics in cereal production ecosystems can help us understand DOM in arable soils. Soils grow only cereal crops such as wheat maize and barley without greenhouse coverage, conducting normal irrigation practices, in which DOM dynamics may show different trends. Double or single cropping systems, fertilization and tillage may have different impacts on DOM dynamics in normal cereal fields. A comparison of characteristics of DOM can be conducted on the soil samples collected from long-term or short-term cereal cropping systems and from paddy cropping systems.

② The effect of rotation practices on DOM dynamics

The rice-cereal double cropping system was compared with single rice cropping system in Chapter 2, while Greenhouse rotation system was also compared with single rice cropping system in Chapter 4. Those two multiple cropping systems were both conducted rotation practices. HS concentrations were increased in both two rotation systems. It can be considered that rotation systems could increase soil HS concentrations, which might promote the maturation of soil in agricultural ecosystem. This is consistent with the past research that rotation crops can help increase HS residence time (Pettit et al., 2004). However, this assumption needs to be confirmed by comparing the paddy-cereal cropping system and double rice cropping system. Only when the HS concentration of paddy-cereal cropping system is

higher than double rice cropping system, the increasing trend of HS concentrations may be induced by the rotation practices.

③ Biodegradability of DOM in paddy soil

The flooding irrigation in paddy soil depressed the SOM decomposition rate and then further increased DOM retention (Kögel-Knabner et al., 2010; Neue et al.,1997). But it is still unrevealed that how the biodegradation rate was reduced by irrigation regime and how was the mineralization process like in the paddy field. The ratio of DOM concentration before and after 30 days of incubation can represent the bioavailability of DOM, according to the incubation procedure introduced by Fellman et al., (2008). Xu et al. (2012) examined the organic Nitrogen content and optical characteristics of DOM before and after incubation and found increasing value of $SUVA_{254}$, HIX and C/N ratio. A 12 weeks' incubation conducted by Hanke et al. (2013) showed that the cycle of anoxic and oxic conditions did not significantly affect the DOM concentrations. An incubation experiment conducted under flooding irrigation condition in paddy soil is needed to determine the bioavailability of DOM.

③   Fractionation of humic substances of paddy soils

The soil samples used in Chapter 2,3 and 4 were collected every year and kept only a small amount (less than 100g) in the relevant institute of Mie and Osaka. Those samples were valuable for the exploration of DOM dynamics and the HS proportions in DOM. The HS discussed in Chapter 2, 3 and 4 were the extractable HS in soils. In fact, HS is the largest and chemically recalcitrant fraction of SOM which includes humic acid (HA), fulvic acid (FA) and

humin (HN). Based on the method of International Humic Substances Society (IHSS), Swift (1996) introduced the methods to purified HA and FA.

^{1}H NMR spectrum with high sensitivity can provide information with only a small volume of samples within a very short measurement time, which is useful for the measurement of DOM solutions. Compared with the rich existence of ^{1}H, there is only 1.1% of ^{13}C existing in the nature. Thus, ^{13}C NMR spectroscopy require a large amount of soil samples and a long period of measurement time (more than 9 h). However, ^{13}C NMR spectrum can provide more detail information about the C backbone and functional groups than ^{1}H NMR (Preston et al., 1996).

With enough amount of soil samples collected from paddy fields, isolation and purification of HA and FA can be conducted. Detailed functional groups' information of paddy soils can be determined by ^{13}C NMR spectroscopy.

⑤ PARAFAC modelling with more arable soil samples

The PARAFAC modeling conducted with of paddy and greenhouse soil samples in Chapter 4. The fluorescence spectrophotometer was found that Raman peak of blank samples (ultrapure water) measured in 2019 was shifted 2.5nm compared with the blank measured in 2017 and 2018, which cause unexpected small quantity of fluorescence components (only three) in Chapter 4. After the repairment of fluorescence spectrophotometer, I will make a remeasurement of paddy and greenhouse soil samples. The samples from cereal cropping systems will be added into PARAFAC modeling to compare the difference of spectroscopic characteristics of soil DOM between paddy and cereal fields.

Summary

Rice is one of the most important crops to feed world's population. Most of rice is produced in the arable land in Asia. As one of the cultivation patterns, paddy field applied flooding irrigation. The flooding and drainage irrigation regime in paddy field creates a unique aquatic-terrestrial ecosystem which result in a cycle of anaerobic and aerobic conditions in soil which also result in a large of Dissolved organic matter (DOM) concentrations. DOM is the labile and active fraction of soil organic matter (SOM) which involves in various soil biogeochemistry processes and ecological processes such as being a direct energy source for microbial activity, transportation of organic contamination and heavy metal and so on. DOM in natural ecosystems is subjected to environmental factor such as global warming, climate change and so on. While DOM in agricultural ecosystem is more subject to different agricultural practices and management strategies. It is necessary to investigate how the characteristics of DOM are affected by agricultural practices such as the change of cropping systems, fertilization and types of crops in order to understand the mechanism of C cycles and C accumulation in arable soils.

DOM consists of humic substances (HS) and non-humic substances (NHS). DAX-8 resin fractionation method can help to quantitatively determine the HS and NHS fractions of DOM in water samples. This method was applied to determine the DOM and HS concentrations of samples in Chapter 2 and Chapter 4. UV-vis and fluorescence spectroscopy are widely applied to characterize DOM from different kinds of sources. Recently, Parallel factor analysis (PARAFAC) combined with the excitation-emission matrix (EEMs) obtained from the analysis

of fluorescence spectroscopy has been developed used to distinguish the fluorescence components. This method was used in Chapter 3 and Chapter 4 to evaluate the optical properties of DOM. [1]H Nuclear magnetic resonance (NMR) spectroscopy can provide structural and compositional information of DOM samples, which was used in Chapter 3 and Chapter 4 to determine the functional groups' proportions of DOM samples.

Compared with the research on forest and other kinds of agricultural soils, the information about the spectroscopic characteristics of DOM is scarce. Thus, the objective of the thesis is to investigate the dynamics and spectroscopic characteristics of DOM using the soil samples collected from long-term (>40 years) and short-term (<10 years) experimental sites.

Chapter 1

This chapter reviewed the importance of DOM in agricultural ecosystems, the basic information of paddy soil, the quantitive and qualitative methods to analyze DOM and also the objectives and content of this thesis.

Chapter 2

The determination of DOM concentrations in paddy soil is an important task to evaluate the soil fertility and C retention pattern. A long-term (1976-2015) experiment on paddy soil was conducted under four kinds of fertilization treatments (chemical, rice straw, manure compost and integrated fertilizations) in Mie Prefecture Agricultural Research Institute. The total carbon (TC) content, DOM and HS concentrations showed similar increasing trends in double rice-barley(wheat) cropping system (1976-1991) and then changed to decreasing trends in single cropping systems (1991-2015). DOM and HS concentrations did not show significant differences under four kinds of fertilization treatments. Thus, SOM (TC content) was affected

by both cropping systems and fertilization, while DOM and HS concentrations were more subject to the conversion of cropping systems than the change of fertilizations.

Chapter 3

UV-vis, fluorescence and ^{1}H NMR spectroscopy were applied to investigate the spectroscopic characteristics of DOM affected by the conversion of cropping systems and fertilizations. SUVA$_{254}$, the index calculated by UV absorbance and HIX, the fluorescence index did not show different trends in two kinds cropping systems. The optical indices FI and BIX showed similar trends with DOM concentrations which were subject to cropping systems. HIX, BIX and FI significantly affected by the fertilization treatments. Excitation-Emission Matrixes (EEM) obtained by fluorescence spectroscopy was conducted Parallel Component Analysis (PCA). A five-fluorescence components model was obtained by the PARAFAC modeling. Three of components were humic-like component (C1, C2 and C3). Other two were protein-like components (C4 and C5). C1 and C4 were all significantly affected by fertilization treatments. The proportion of functional groups obtained by ^{1}H NMR spectroscopy (SPR-W5-WATERGATE pause method) were also significantly affected by the change of cropping systems and fertilization. Thus, spectroscopic characteristics of DOM in paddy soils were subjected to the change of cropping systems and fertilization. The utilization of different kinds of spectroscopic indices were rapid and nondestructive to evaluate characteristics of DOM, which can be applied to other kinds of arable soil samples.

Chapter 4

Soil samples were collected from the Research Institute of Environment, Agriculture, and Fisheries, Osaka, where short-term experiments were conducted. Two kinds of soil paddy (single rice cropping system) and greenhouse (crown daisy-taro rotation cropping system) soils were analyzed using the same methods in Chapter 2 and Chapter 3. Paddy soil and greenhouse soil are both characterized with high SOM storage. Thus, it is necessary to compare the characteristics of DOM in paddy and greenhouse soils.

DOM concentrations in paddy and greenhouse soils decreased over the short period of time (< 10 years). However, TC contents of paddy and greenhouse soils kept relatively stable compared with Chapter 2. The decreasing trends of DOM concentrations in paddy soil showed similar trend with the trends of DOM in single cropping system (1991-2015), suggesting that DOM is decreased in single cropping system. SUVA254, FI, BIX, and HIX did not show significant different under different fertilization treatment. Fluorescence components identified by EEM-PARAFAC did not show clear trends. Carbohydrate protons (H_{C-O}), one of the functional groups' proportions obtained by ^{1}H NMR spectroscopy showed significant negative correlation ($r = -0.521$, $P < 0.01$)with DOM concentration, while aliphatic protons (H_{Al}) showed a significantly positive correlation ($r = 0.654$, $P < 0.01$)with DOM concentration. Thus, the proportions of functional groups showed a strong relationship with DOM dynamics. Greenhouse soils contained higher DOM and HS concentrations, and more aliphatic C, while paddy soils contained more carbohydrate. Greenhouse soil showed an increasing humification degree (HIX), suggesting that greenhouse soils could contain more and more recalcitrant DOM. The principle component analysis (PCA) using different parameters of DOM showed a very clear distinction of two kinds of soils, which indicates that the characteristics of DOM in paddy

and greenhouse soils were significant different.

Chapter 5

This chapter is general discussion. Possible directions of further research were discussed in this

Chapter, including the comparison of characteristics of DOM in between general arable soil

(cereal crops) and paddy soils, the effect of rotation on characteristics and dynamics of DOM,

the biodegradability of DOM in paddy soil, fractionation of humic substances of paddy soil and

the improvement of PARAFAC modeling.

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