

## Influence of Fertilizers Applied to a Paddy–Upland Rotation on Characteristics of Soil Organic Carbon and Humic Acids

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The qualitative and quantitative characteristics of soil organic carbon (SOC) and related humic acids (HAs) extracted from the soils of field plots were investigated after 8 years of annual paddy (*Oryza sativa* L.) and upland maize (*Zea mays* L.) rotation with various fertilizations. Seven fertilization treatments were selected: Ck (no inputs); Chem (chemical fertilizer of NPK); Comp (swine compost); Comp + 33% of Chem N rate; Comp + 67% of Chem N rate; GM (legume green manure) + 33% of Chem N rate; and peat + 33% of Chem N rate. Organic and inorganic nitrogen inputs of six treatments were equivalent with respect of nitrogen content, but Comp, GM, and peat treatments were complemented with various amounts of inorganic N. After harvest of the eighth paddy crop, surface soil samples collected from the plots were subjected to soil characterizations and extraction of humic substances, which were used for chemical, spectroscopic (FTIR, <sup>13</sup>C NMR, ESR, X-ray diffractometry),  $\delta^{13}\text{C}$ , and <sup>14</sup>C dating analyses. The yields of HAs extracted from the seven treatments were significantly different. Treatment containing persistent organic compound such as the peat + 33% N treatment increased the humification process in topsoils and produced higher yield of HA. Spectroscopic analyses revealed that fertilization treatments changed the functional groups, alkyl C, crystalline characteristics, and  $\delta^{13}\text{C}$  ratios of HAs and turnover rate of SOC considerably. The SOC of the peat + 33% N treatment had the highest mean residence time of 3100 years. Various fertilizer treatments are correlated with turnover rate of SOC and related HAs, which are associated with concerned carbon sequestration as well as mitigation of CO<sub>2</sub> emission in the soil environment.

**KEYWORDS:** Soil organic carbon; humic acid; crop rotation; fertilization; turnover rate; carbon sequestration

### INTRODUCTION

The level of organic matter in soil is considered to be a function of the net input of organic residues by the cropping system (1). Therefore, soil and crop management practices such as cultivation, crop rotation, residue management, and fertilization exert a considerable influence on the level of organic matter retained over time. Wu et al. (2) investigated the influence of cultivation and fertilization on total organic carbon (TOC) and carbon fraction in soils from the Loess Plateau of China. Their results of the long-term experiment on the Heilu soil indicated that manure alone and manure plus nitrogen and phosphorus fertilizer treatments restored TOC and microbial biomass carbon to the level of the native soil.

Recently, storage and dynamics of carbon and nutrients in soils have received increasing attention because of the importance of soil organic carbon (SOC) and nutrient pools in soil

fertility sustainability and environmental quality (3). The organic matter contained in the earth's soils is a large reservoir of carbon that can act as a sink or source of atmospheric CO<sub>2</sub> (4, 5). SOC is not only related to improvement in the water-holding capacity and nutrient availability in the soil, but its hidden value comes in its ability in mitigating the greenhouse effect on the environment (6).

Soil organic matter is composed of different groups of constituents that vary in their chemical composition and turnover. For a quantitative analysis of SOC dynamics, it is necessary to trace the origins of the soil organic compounds and the pathways of their transformations (7). The microbial biomass is a source and sink of biologically mediated nutrients and is responsible for transforming organic matter and nutrients in soils (8). In soil organic matter carbon is one of the important constituent that makes up 80% of the terrestrial carbon pool and is regarded as an important potential carbon sink to mitigate the greenhouse effect (9, 10). In agricultural soils, the carbon stock is affected by changes in land-use or management practices (11).

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Humic substances, which comprise one-third to three-fourths of total organic matter, are well-known for their long-term persistence in soil environments (12). Cropping patterns cause changes in organic carbon and nutrients as reported by Zhang and He (13). Their study of long-term changes of organic carbon and nutrients of an Ultisol under rice cropping in southeastern China indicated that the C/N ratio of organic matter decreased in the first 20 years of rice cropping and then remained constant (approximately 10), whereas the ratio of humic acid (HA) to fulvic acid (FA) increased gradually to about 1 after 50 years of rice cropping.

Use of spectroscopic techniques such as nuclear magnetic resonance (NMR), electron spin resonance (ESR), and Fourier transform infrared (FTIR) spectroscopies allows identification of functional groups and molecular structure, providing better understanding of the decomposition pathways of organic matter and qualitative alterations induced by management practices (14–17). Pérez et al. (18) investigated the characterization of HAs from a Brazilian Oxisol under different tillage systems by spectroscopy and found that FTIR spectra of the HAs were similar for all treatments. The levels of semiquinone-type free radicals determined by the ESR spectra were lower for treatments of no-till/maize-cajanus and noncultivated soil ( $1.74 \times 10^{17}$  and  $1.02 \times 10^{17}$  spins  $\text{g}^{-1}$  of HA, respectively), compared with  $2.3 \times 10^{17}$  spins  $\text{g}^{-1}$  of HA for other soils under cultivation. The percentage of aromatic carbons of HAs determined by  $^{13}\text{C}$  NMR also decreased for noncultivated soil to 24%, which was around 30% for cultivated soils. In an evaluation of soil quality, Zalba and Quiroga (19) reported that FA carbon content was highly related to organic residues that were incorporated recently in the soil environment, showing that FA carbon is sensitive to agronomic and environmental factors. Wang and Chang (20) investigated the ranges of mean residence times (MRTs) of FA (MW < 1000), FA (MW > 1000), HA (MW > 1000), and humin (MW > 1000) extracted from the soil samples taken from three depths (0–20, 40–60, and 70–150 cm) of a soil profile, and the ranges of MRTs were from  $143 \pm 110$  to  $1740 \pm 60$  years, from  $213 \pm 120$  to  $1690 \pm 200$  years, from  $253 \pm 60$  to  $2200 \pm 40$  years, and from  $293 \pm 40$  to  $2173 \pm 70$  years, respectively. They suggested that the higher standard deviations of the means of determined MRTs of FA (MW < 1000) and FA (MW > 1000) might be due to their lability in the soil environment.

Two crops of paddy–upland maize (*Zea mays* L.) cropping systems are being practiced in agricultural farms in Taiwan. Wang and Yang (21) investigated whether the type of fertilizer applied to a paddy–upland rotation affected selected soil quality attributes of the experimental field. They reported that percentages of water-stable aggregate in 1–2 and 0.5–1 mm particle size fractions of the soils, amended with peat and compost, were significantly higher than those amended with chemical fertilizer and the check. However, there were no qualitative and quantitative studies that elucidate the changes in chemical and structural characteristics of SOC and related humic substances by fertilization treatments in subtropical Taiwan soils. The present study was conducted to quantify the 8 years of effects of inorganic and organic fertilizer application on soil properties, as well as SOC and HA characteristics using analytical and spectroscopic techniques.

## MATERIALS AND METHODS

**Study Area and Sites.** The study was carried out on Chikuaitsay clay loam soil (Fluvaquentic Dystrachrept) at the experimental farm ( $24^\circ 1.8' \text{ N}$  latitude and  $120^\circ 41.4' \text{ E}$  longitude) of the Agricultural

Research Institute, Taiwan. The experimental area is characterized as humid subtropical. The monthly maximum mean air temperature was  $29.8^\circ\text{C}$  (maximum =  $36.4^\circ\text{C}$  and minimum =  $22.5^\circ\text{C}$ ) in July, and the monthly minimum mean was  $16.4^\circ\text{C}$  (maximum =  $27.7^\circ\text{C}$  and minimum =  $8.3^\circ\text{C}$ ) in January 2003. In the beginning of the experiment (August 1995), 10 equal amounts of surface soils (0–15 cm depth) and subsoils (15–30 cm depth) were collected by auger (8.3 cm in diameter) from the sketched grids of the experimental field (size  $50 \times 100$  m) and thoroughly mixed. The gross surface and subsoil samples were air-dried and gently ground with a wooden roller to pass through a 2 mm sieve, homogenized, and stored for subsequent analysis. Selected soil properties pertinent to this study were determined as described by Wang and Yang (21).

**Cropping and Treatments.** An annual Egyptian clover (*Trifolium alexandrinum*)–paddy–sesbania (*Sesbania roxburghii*)–upland maize cropping sequence was carried out in the experimental field. The study started in August 1995, and eight crops of paddy, maize, clover, and sesbania had been harvested by July 2003. The precipitation levels in the area covering the experimental field were higher in the spring and summer than in the autumn and winter. Total amounts of precipitation from August through December 1995, for whole years of 1996, 1997, 1998, 1999, 2000, 2001, and 2002, and from January through July 2003 were 153, 987, 1482, 2298, 1404, 1797, 1981, 1316, and 752 mm, respectively. Consequently, the annually planted spring paddy from February to July and autumn maize from September to December were mainly irrigated with aqueduct water complemented with rainwater. Prior to planting, all plots were tilled, and the paddy field was additionally puddled with irrigated water. Application rates of N,  $\text{P}_2\text{O}_5$ , and  $\text{K}_2\text{O}$  were 120, 60, and 60  $\text{kg ha}^{-1}$  for each paddy crop and 140, 80, and 80  $\text{kg ha}^{-1}$  for each maize crop, respectively. The following seven treatments were selected: Ck (no inputs); Chem (chemical fertilizer of NPK); Comp (swine compost); Comp + 33% of Chem N rate; Comp + 67% of Chem N rate; GM (legume green manure) + 33% of Chem N rate; and peat + 33% of Chem N rate. The amounts of organic materials and chemical fertilizers applied in the plots and their timing for autumn maize of 1995 and for spring paddy of 1996 have been described by Wang and Yang (21). The fertilization for the following annual crops of spring paddy and autumn maize followed the same formulas and rates. Each treatment was replicated four times (plot size  $6 \times 20$  m) in a randomized complete block design. Selected chemical characteristics of the applied organic materials in the cultivation of autumn maize in 1995 and spring paddy in 1996 have been analyzed by Wang and Yang (21). The carbon and nitrogen inputs with organic materials used in the cultivation of spring paddy of 1996 were as follows: swine compost, 493 and 20.3  $\text{g kg}^{-1}$ ; peat, 502 and 12.3  $\text{g kg}^{-1}$ ; and Egyptian clover, 477 and 25.5  $\text{g kg}^{-1}$  of carbon and nitrogen, respectively. The carbon and nitrogen inputs with organic materials used in the cultivation of autumn maize of 1996 were as follows: swine compost, 501 and 22.6  $\text{g kg}^{-1}$ ; peat, 498 and 11.8  $\text{g kg}^{-1}$ , and sesbania, 107 and 5.0  $\text{g kg}^{-1}$  of carbon and nitrogen, respectively. The amounts of the applications of swine compost, legume green manure, and peat in their respective treatments had N contents equivalent to the N application for the Chem plots. The organic amendments were incorporated into the soil by tillage. To investigate the impact of differing N rate and organic N combination on the studied properties, the amounts of 33 and 67% of chemical fertilizer N rates were complemented in Comp + 33% N rate, Comp + 67% N rate, GM + 33% N rate, and peat + 33% N rate plots. The planted Egyptian clover and sesbania were used to provide the treatment of GM + 33% N rate plots for the spring paddy and autumn maize crops, respectively. Other than the Ck treatment, the application rates of  $\text{P}_2\text{O}_5$  (by calcium superphosphate) and  $\text{K}_2\text{O}$  (by KCl) for the remaining six treatments were the same for each year's spring paddy and autumn maize. The cropping sequence with various fertilization treatments was repeated each year from 1996 to 2003. The yield of crop with SOC concentrations in 1–2 and 0.5–1 mm particle size fractions of surface soil was highly correlated on crop and soil management. Chemical fertilizer played a significant role in governing the yields of crops, although it did not significantly contribute to improving some soil quality attributes (21).

**Table 1.** Physical and Chemical Properties of the Soils from Four Replicated Plots of Each Treatment in the Field

treatment <sup>a</sup>	pH <sup>b</sup>	EC <sup>b</sup> ( $\mu\text{S cm}^{-1}$ )	organic C <sup>b</sup> (g kg <sup>-1</sup> )	total N <sup>b</sup> (g kg <sup>-1</sup> )	CEC <sup>b</sup> (cmol kg <sup>-1</sup> )	exchangeable				Bray-1 P <sup>b</sup> (mg kg <sup>-1</sup> )	texture
						Ca <sup>b</sup> (mg kg <sup>-1</sup> )	Mg <sup>b</sup> (mg kg <sup>-1</sup> )	K <sup>b</sup> (mg kg <sup>-1</sup> )	Na <sup>b</sup> (mg kg <sup>-1</sup> )		
Ck	6.09a	176b	9.5d	1.5ab	9.8b	947bc	88.7ab	83.1b	34.9ab	9.9e	CL <sup>c</sup>
Chem	5.65b	191b	12.2cd	1.3b	13.5ab	779cd	60.4c	121a	38.0ab	18.4d	CL
Comp	6.20a	555a	15.2bc	1.6ab	14.7ab	1194a	104a	143a	37.1ab	59.9a	CL
Comp + 33% N	6.18a	245b	18.5b	1.5ab	12.0b	1181a	98.4a	140a	38.6ab	49.5b	CL
Comp + 67% N	6.12a	147b	16.7bc	2.3a	15.4ab	1070ab	88.5ab	131a	41.5a	54.8ab	CL
GM + 33% N	5.64b	251b	14.4bc	0.8b	11.9b	670d	51.6c	145a	33.0b	18.5d	CL
peat + 33% N	5.79b	318b	29.7a	1.2b	20.8a	846cd	69.3bc	131a	35.7ab	26.4c	CL

<sup>a</sup> Ck (no inputs); Chem (chemical fertilizer) = NPK; Comp (compost) = swine compost; Comp + 33% N = swine compost + 33% of Chem N rate; Comp + 67% N = swine compost + 67% of Chem N rate; GM + 33% N = clover or sesbania + 33% of Chem N rate; peat + 33% N = peat + 33% of Chem N rate. <sup>b</sup> Means with different letters for the seven treatments are significantly different at 5% probability level by the analysis of variance and LSD values. <sup>c</sup> Clay loam.

**Soil Sampling and Analyses.** In July 2003 (after spring paddy harvest), 10 equal amounts of surface soil samples were collected from each plot for each treatment according to the method described above and then thoroughly mixed as a composite sample. Twenty-eight composite soil samples (four replicated samples for each treatment) were collected, air-dried, and gently ground with a wooden roller to pass through a 2 or 0.1 mm sieve, homogenized, and subjected to the analyses of properties that were selected in the beginning of the experiment (August 1995) (21). The pH in water (1:1, w/v) was measured using a pH-meter model 682 Titroprocessor (Metrohm, Swiss) (22). The filtrates of the suspensions, which were used for measuring soil pH, were also used to measure electrical conductivity (EC) of the samples (23). The soil samples were digested with 0.01 M HCl (24) to remove inorganic carbon, and their SOC and total N concentrations were determined using a Heraeus CHN-O-rapid elemental analyzer and Tacussel coulmax 78 automatic coulometric titrator. The cation exchange capacity (CEC) of the soils was determined according to the NaOAc method (25). Exchangeable Ca, Mg, K, and Na were determined according to the method proposed by Thomas (26). Available P concentrations of the soils were determined by using the Bray-1 method (27). Particle size distributions were determined with a hydrometer method (28). All data were subjected to analysis of variance, and the LSD values were used to determine mean separation differences at the 0.05 probability level. Selected characteristics of the soils are presented in **Table 1**.

**Extraction and Purification of HAs.** Because the organomineral fraction of mineral soils is mainly distributed in fine particle size (<0.15 mm) (29), the HAs of the 28 composite soil samples with particle size <0.15 mm were extracted and purified, according to the method proposed by Aiken (30), Hayes (31), Leenheer (32), and Swift (33). A brief description of the extraction and purification procedure (34) is as follows. Soil samples were treated with a 0.1 M NaOH solution several times at 1:10 (w/v) ratio to extract the humic substances until the extracts were clear. All of the extracts for each treatment were combined and acidified to pH 1.0 with 6 M HCl. The acidified solution was equilibrated at room temperature for 24 h and then centrifuged at 2000g to separate the HA fraction from the FA fraction (supernatant). The precipitated HA fractions were suspended in a 0.1 M HCl–0.3 M HF solution in plastic containers and shaken overnight at room temperature. The suspensions were centrifuged at 2000g to precipitate HA fractions, suspended in deionized distilled water, and then transferred to dialysis tubes to separate the fraction of HA (MW > 1000). All HAs (MW > 1000) were freeze-dried and their yields determined.

**Characterization of HAs and SOC.** The C, H, N, and S contents of the freeze-dried HAs of the soil samples were determined using a Heraeus CHN-O-rapid elemental analyzer and a Tacussel coulmax 78 automatic coulometric titrator. The four replicated HAs obtained from each treatment were mixed thoroughly and made seven composite HA samples based on the yield ratios. The X-ray diffractogram and ESR, FTIR, and <sup>13</sup>C NMR spectra of the seven composite HAs were recorded for further qualitative and/or semiquantitative comparison with X-ray diffractogram and ESR, FTIR, and <sup>13</sup>C NMR spectra obtained individually for the 28 HAs. Crystalline properties of HAs were analyzed using a Diano 8536 X-ray diffractometer. The ESR spectra

of the HAs were obtained with a Bruker ER EMX-10 spectrometer operating at 100 kHz and a nominal frequency of 9620 MHz at room temperature. The FTIR spectra of the HAs were obtained on KBr disks (each disk contained 2 mg of the sample and 300 mg of KBr) in the 400–4000 cm<sup>-1</sup> range on a Bio-Rad FTS-7 spectrophotometer. The freeze-dried HAs were subjected to solid state <sup>13</sup>C NMR analysis on a Bruker DSX 400WB spectrometer with cross-polarization and magic-angle spinning (CPMAS), proton decoupling with a contact time of 2.00 ms, a recycle time of 5.00 s, a spectral width of 20000 Hz, a spinning speed of 3500 Hz, 6000 scans, a spectrometer frequency of 50.330 MHz, and an acquisition time of 51.25 ms. For  $\delta^{13}\text{C}$  ratio analysis freeze-dried HAs were sent to Rafter Stable Isotope Laboratory, Institute of Geological and Nuclear Science Limited, Lower Hutt, New Zealand. Soil samples were digested with 0.01 M HCl to remove the inorganic carbon (24), and then the SOC of the samples were processed to produce C<sub>6</sub>H<sub>6</sub>; their <sup>14</sup>C radioactivity was counted according to the method recommended by Gupta and Polach (35). The details for processing to produce C<sub>6</sub>H<sub>6</sub> and their <sup>14</sup>C counting have been described by Wang and Chang (20). According to the formula reported by Stuiver and Polach (36), the obtained <sup>14</sup>C data for each soil sample were used to calculate MRT. All of the <sup>14</sup>C dating procedures as well as  $\delta^{13}\text{C}$  ratio analyses of SOC of the soils were carried out at the <sup>14</sup>C dating Laboratory, Department of Geology, National Taiwan University. All of the quantitative data obtained from the elemental, ESR, <sup>13</sup>C NMR, and  $\delta^{13}\text{C}$  analyses of the HAs and the <sup>14</sup>C dating and  $\delta^{13}\text{C}$  analyses of SOC of the soils were subjected to the analysis of variance, and the LSD values were used to determine mean separation differences at the 0.05 probability level.

## RESULTS AND DISCUSSION

**Soil Properties.** There was no change in the texture (clay loam) of the surface soil (0–15 cm depth) over the years due to treatments; however, 8 years of treatments caused changes in several selected physical and chemical properties of the surface soils as reported in **Table 1**. In the beginning of the experiment the pH of the surface soil was 5.49 (21); this lower pH may be due to earlier practices of application of chemical fertilizers. Zero input of chemical fertilizers (Ck treatment) for 8 years restored the pH of the soil to close to its natural level. Treatments of Comp, Comp + 33% N, and Comp + 67% N also restored the pH to natural levels, but Chem, GM + 33% N, and peat + 33% N treatments barely changed the pH from its original value. The EC of the surface soil of the Comp treatment was significantly higher than those of the other six treatments, showing that the amendment of compost alone contributed significant amounts of water-soluble salts to the surface soil. The organic carbon content of the surface soil in the beginning of the experiment was 8.4 g kg<sup>-1</sup>. All treatments increased the organic C content in the surface soil, but the peat + 33% N treatment increased it significantly more than the other six treatments. This is probably because a larger amount of peat



than of compost had been applied to the plots for spring paddy, although the organic C contents of swine compost, Egyptian clover, and peat were quite similar (21). The amounts of the applications of green manure were much higher than those of swine compost and peat (21). Nevertheless, the organic C content in the surface soil of the GM + 33% N rate treatment was not significantly different from those of the Comp, Comp + 33% N, and Comp + 67% N treatments. This is attributed to the lower C/N ratio of green manure (21), leading to a higher mineralization rate of the organic amendments. Without input, the Ck treatment maintained the SOC content to an extent which was not significantly different from that of Chem treatment. The total N content of the surface soil of the Comp + 67% N treatment was significantly larger than those of the Chem, GM + 33% N, and peat + 33% N treatments, showing that complementary chemical N rate and N content as well as the C/N ratio of the organic amendments affected the total N contents. The CEC of the surface soil of the peat + 33% N treatment was significantly larger than those of the Ck, Comp + 33% N, and GM + 33% N treatments. The peat + 33% N treatment had the highest amount of organic C among the treatments, corresponding to the highest soil CEC.

Concerning the major exchangeable cations in the surface soils of the treatments, Ca and Mg contents in Comp and Comp + 33% N treatments were significantly larger than those of Chem, GM + 33% N, peat + 33% N, and Ck treatments and Chem, GM + 33% N, and peat + 33% N treatments, respectively (Table 1). Exchangeable K and Na contents did not show the same significant differences. However, the exchangeable K content of the surface soil of the Ck treatment was significantly smaller than those of the other six treatments.

The amendments of compost and compost complemented with different chemical N rates increased the Bray-1 P contents of the surface soils of the Comp, Comp + 33% N, and Comp + 67% N treatments, which were significantly higher than those of the peat + 33% N, GM + 33% N, Chem, and Ck treatments (Table 1). Zhang and He (13) studied the long-term changes of organic C and nutrients of an Ultisol under paddy cropping in southeastern China. They reported the significant increases in organic C, total N, and P concentrations in the plow layer in the first 30–40 years of paddy cropping, accompanied by increases in available P and potential mineralized N, exchangeable Ca, Mg, and Na, base saturation, and water-stable aggregates and decreases in total K and clay content. In our study we found significant variation in CEC, major exchangeable cations, organic C, total N, and Bray-1 P with annual paddy and upland maize rotation with various fertilizations as discussed above.

**Yields of HAs and Their Elemental Analysis.** The sequence of the yield of HA from each treatment was peat + 33% N > Comp + 67% N > GM + 33% N, Chem, and Ck (Table 2). This indicates that the addition of persistent peat and compost to the plots increased the yields of HAs. The elemental analysis showed that the average contents of C, H, N, O, and S of HAs extracted from the surface soils of the four replicated plots of the seven treatments were in the ranges of 50.0–50.8, 5.19–5.56, 3.88–4.74, 33.8–37.5, and 0.40–0.67%, respectively. The differences of C, H, N, O, or S contents of HAs among the seven treatments were not significant (not shown). Consequently, the differences in atomic ratios of both H/C and O/C among the seven treatments were also not significant (Table 2). The atomic ratios of C/N of the HAs from Ck and peat + 33% N treatments were, however, significantly higher than that from

**Table 2.** Yields of Humic Acids (HAs) and Their Atomic Ratios of H/C, C/N, and O/C Extracted from the Soils of the Four Replicated Plots for Each Treatment in the Field

treatment <sup>a</sup>	yield of HA <sup>b</sup> (g kg <sup>-1</sup> of soil)	atomic ratio <sup>b</sup>		
		H/C	C/N	O/C
Ck	2.37d	1.30a	16.0a	0.50a
Chem	2.94cd	1.29a	15.2abc	0.53a
Comp	4.54bc	1.25a	14.0abc	0.52a
Comp + 33% N	4.70bc	1.26a	13.5abc	0.52a
Comp + 67% N	5.30b	1.32a	12.4c	0.52a
GM + 33% N	3.41cd	1.30a	12.8bc	0.54a
peat + 33% N	12.8a	1.22a	15.4ab	0.56a

<sup>a,b</sup> Explained in Table 1.

**Table 3.** Selected Correlations among Physical and Chemical Properties and Yields of HAs of the Soils from the Seven Treatments

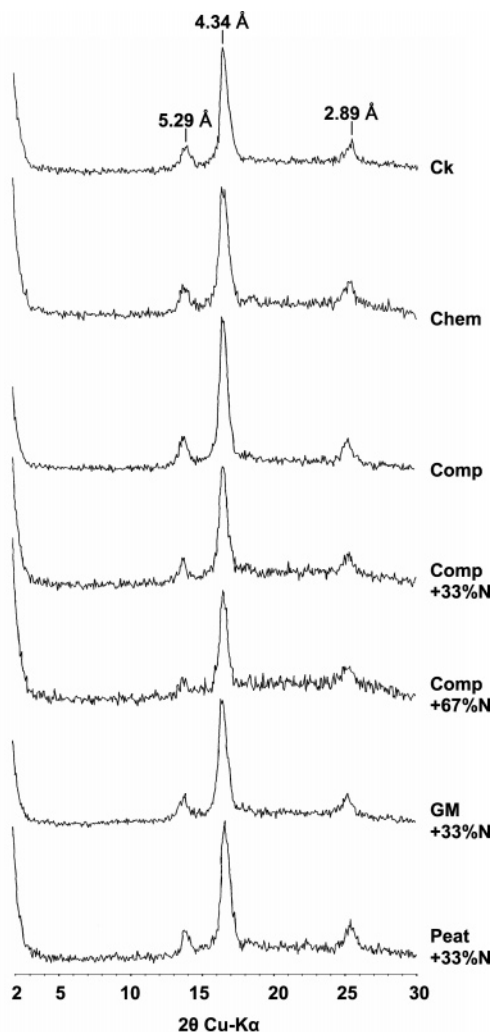
corresponding variable	organic C	CEC	exchangeable		Bray-1 P	yield of HA
			Ca	Mg		
organic C	NA <sup>a</sup>	0.8811**	NS <sup>b</sup>	NS	NS	0.9732**
CEC		NA	NS	NS	NS	0.9234**
exchangeable Ca			NA	0.9751**	0.8237*	NS
exchangeable Mg				NA	NS	NS
Bray-1 P					NA	NS
yield of HA						NA

<sup>a</sup> Not applicable. <sup>b</sup> Not significant. \*\* and \* indicate significant correlations at 1 and 5% probability levels, respectively, by the analysis of variance.

the Comp + 67% N treatment (Table 2). This is mainly due to the differences of N contents (3.88–4.74%) of the HAs of the treatments.

**Correlations among Physical and Chemical Properties and Yields of HAs.** Selected correlations among physical and chemical properties and yields of HAs of the treatments showed that CEC and yield of HA were significantly correlated with organic C content of the surface soil at a 1% probability level (Table 3). This clearly indicates that organic C mainly contributes to CEC of the surface soils. Moreover, the organic C content of the surface soils plays a very important role in governing the yields of HAs. The yield of HA was also significantly correlated with the CEC of surface soil at a 1% probability level (Table 3), indicating that HA associated with soil contributes to soil CEC, although organic carbon contents of HAs (50.0–50.8%) account for only 11.9–21.9% of SOC of the treatments (estimated from Tables 1 and 2). Additionally, exchangeable Ca content significantly correlated with exchangeable Mg and Bray-1 P contents of surface soils at 1 and 5% probability levels, respectively (Table 3). This is mainly attributed to the response of various treatments to the characteristics of Ca, Mg, and P status of the organic and inorganic amendments.

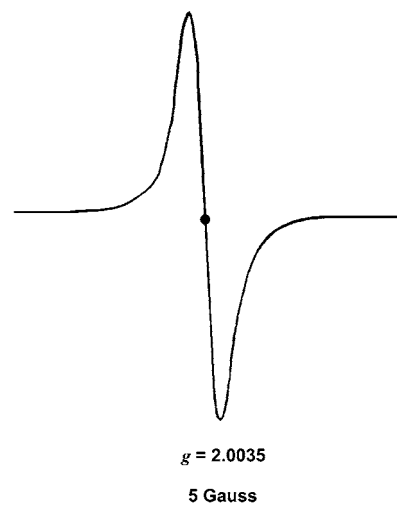
**Characteristics of HAs by Diffractography and Spectrometry.** The X-ray diffractograms of the composite HAs extracted from the seven treatments are shown in Figure 1. The diffractograms commonly showed a strong peak at 4.34 Å and weak peaks at 5.29 and 2.89 Å of the d-basal spacing (Figure 1). This is mainly attributed to the aromatic structures of the HAs (37). Although the seven diffractograms consistently showed the three significantly diagnostic peaks under the same instrument setting of the X-ray diffractometer, the peaks at 5.29 and 2.89 Å of the d-basal spacing of the composite HAs extracted from Comp + 33% N and, especially, from Comp + 67% N treatments were observed not so distinctly and strongly as those of the remaining five diffractograms (Figure 1).



**Figure 1.** X-ray diffractograms of the seven composite HAs. Each composite HA is the mixture of four replicated HAs extracted from the soils of each treatment based on yield ratio. Ck (no inputs); Chem (chemical fertilizer) = NPK; Comp (compost) = swine compost; Comp + 33% N = swine compost + 33% of Chem N rate; Comp + 67% N = swine compost + 67% of Chem N rate; GM + 33% N = clover or sesbania + 33% of Chem N rate; peat + 33% N = peat + 33% of Chem N rate.

Because the four replicated diffractograms of HAs for each treatment were almost the same as that of their own composite HA (not shown), the differences of the diffractograms of composite HAs among the seven treatments revealed in **Figure 1** were thus significant. This indicates that the amendment of compost complemented with various rates of chemical fertilizer N affected the crystalline characteristics of HAs extracted from soils of the two treatments. Except for the Comp + 33% N and Comp + 67% N treatments, despite significant differences in fertilization, no systematic differences were observed in the X-ray diffractograms of the composite HAs extracted from soils of the remaining five treatments (**Figure 1**).

There was no difference among the ESR spectra of the four replicated HAs and their composite HA for each treatment (not shown). Moreover, there were no apparent differences among the ESR spectra of the seven composite HAs with the exception of spectral width. Therefore, to label the specific organic-free radical characteristics and spectral widths, the ESR spectrum of the composite HA extracted from the soils of the Ck treatment is shown as an example (**Figure 2**). All 28 spectra were single lines, devoid of hyperfine splitting. The  $g$  values, widths between



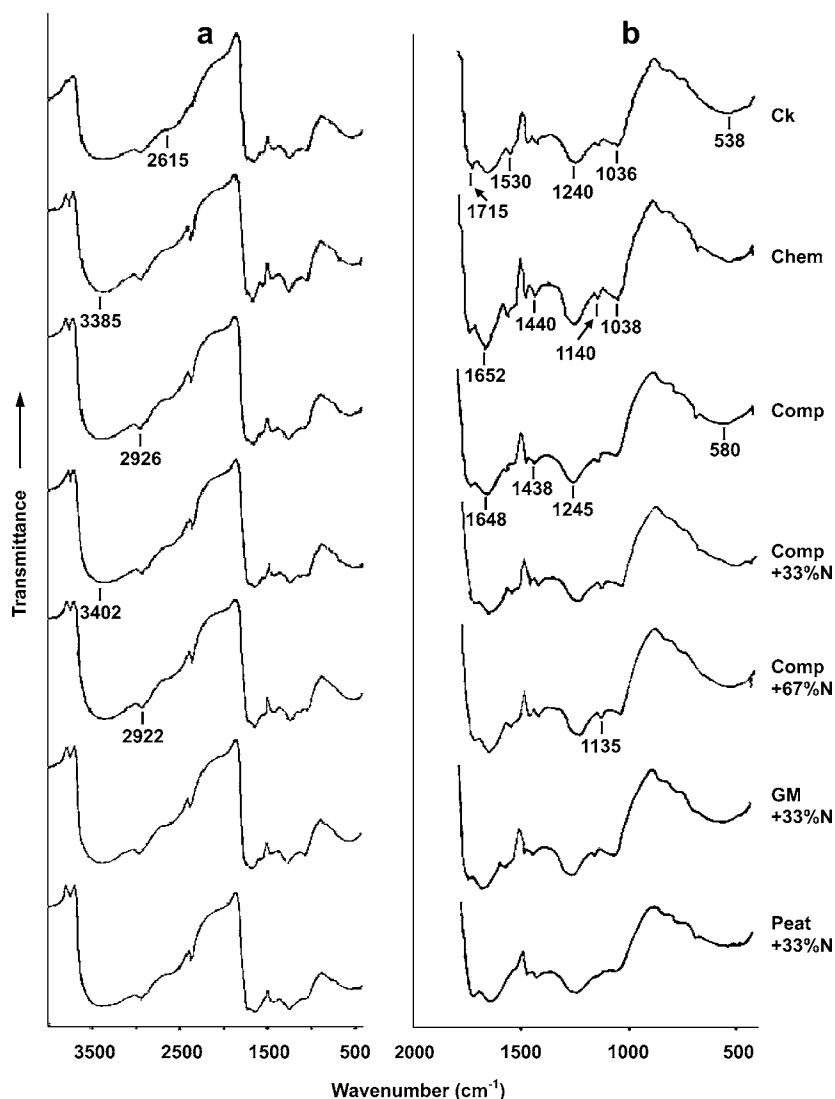
**Figure 2.** ESR spectrum of the composite HA, which is the mixture of four replicated HAs extracted from the soils of Ck treatment based on yield ratio.

two extreme peaks on the derivative curves, and the free radical concentrations of the means of the four replicated HAs for the seven treatments are summarized in **Table 4**. The means of  $g$  values of the HAs extracted from soils of the four replicated plots of the seven treatments were in the range of 2.0034–2.0037, indicating that the main free radicals of HAs were semiquinone or substituted semiquinone (38). There was no significant difference between the means of  $g$  values of the HAs (**Table 4**). Schnitzer (39) reported the  $g$  values ranged from 2.0032 to 2.0050 and the widths from 2.5 to 4.0 G for ESR spectra of HAs and FAs extracted from soils of widely differing climatic zones. He also reported that the free radical concentrations of HA and FA solutions were of the order of  $10^{17}$ – $10^{18}$  spins  $g^{-1}$ . In our study also the means of the  $g$  values, widths, and free radical concentrations obtained by ESR spectra of HAs were in the reported ranges. However, there were significant differences between the means of widths and free radical concentrations of the ESR spectra of HAs of the seven treatments (**Table 4**). The mean of the widths of ESR spectra of HAs of Ck treatment was significantly less than that of HA of the Comp + 33% N treatment. On the contrary, the means of the free radical concentrations of HAs of both Ck and GM + 33% N treatments were significantly higher than those of Comp, Comp + 33% N, Comp + 67% N, and peat + 33% N treatments (**Table 4**). Surface soils with treatments containing easily decomposable inorganic compounds and organic matter such as Chem and GM + 33% N contained higher free radical concentrations, which are close to Ck value. The free radical concentrations of soils with other treatments such as Comp, Comp + 33% N, Comp + 67% N, and peat + 33% N are less (**Table 4**). This demonstrates that treatments containing lower levels of decomposable and persistent organic compounds decreased the free radical concentrations of HAs formed by humification process in soils.

Examination of differential FTIR spectra (not shown) revealed that there was no significant difference in the FTIR spectra of the four replicated HAs and the spectrum of their composite HA for each treatment. The relative intensities of FTIR absorption bands of the seven composite HAs from the seven treatments did not differ much in wavenumber range, 4000–2000  $cm^{-1}$  (**Figure 3a**), but there were some apparent differences among the seven spectra in the wavenumber range from 2000 to 500  $cm^{-1}$  (**Figure 3b**). The qualitative and semiquan-

**Table 4.** Characteristic Parameters of Electron Spin Resonance (ESR) Spectra, Weight Percentage of Alkyl C of  $^{13}\text{C}$  Nuclear Magnetic Resonance ( $^{13}\text{C}$  NMR) Spectra, and  $\delta^{13}\text{C}$  values of the Humic Acids (HAs) and  $\delta^{13}\text{C}$  Values and Mean Residence Times (MRTs) of the SOC of Soils from the Four Replicated Plots of Each Treatment in the Field

treatment <sup>a</sup>	ESR <sup>b</sup>			$^{13}\text{C}$ NMR <sup>b</sup> alkyl C (%)	$\delta^{13}\text{C}^b$		MRT <sup>b</sup> of SOC (years)
	<i>g</i> value	width (G)	free radical concn ( $10^{18}$ spins $\text{g}^{-1}$ )		HA (‰)	SOC (‰)	
Ck	2.0035a	3.50b	3.04a	9.23c	-22.6c	-24.0c	1435b
Chem	2.0037a	3.60ab	2.51ab	9.17c	-22.7c	-23.9c	975c
Comp	2.0036a	3.55ab	1.99bc	9.74b	-22.5c	-23.4c	280d
Comp + 33% N	2.0035a	3.72a	1.95bc	9.21c	-23.0c	-23.2c	300d
Comp + 67% N	2.0035a	3.59ab	1.97bc	9.10c	-22.6c	-23.8c	287d
GM + 33% N	2.0034a	3.61ab	2.69a	9.66b	-23.9b	-25.3b	905c
peat + 33% N	2.0036a	3.59ab	1.61c	10.5a	-27.4a	-26.5a	3100a

<sup>a,b</sup> Explained in Table 1.**Figure 3.** FTIR spectra of the seven composite HAs. Each composite HA is the mixture of four replicated HAs extracted from the soils of each treatment based on yield ratio. The spectra are separately shown at (a) wavenumber range 4000–500  $\text{cm}^{-1}$  and (b) wavenumber range 2000–500  $\text{cm}^{-1}$ . The explanation for the label of each spectrum is given in Figure 1.

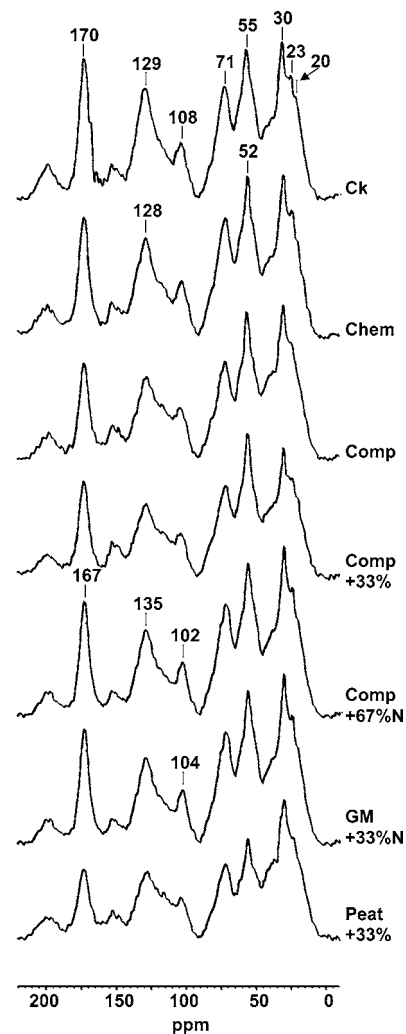
titative information of the functional groups of the HAs was elucidated by assigning the peaks, bands, and/or shoulders of FTIR spectra as proposed by Colthup et al. (40) and Silverstein and Webster (41). Pérez et al. (18) studied the characterization of HAs from a Brazilian Oxisol under different tillage systems by spectroscopy and found that FTIR spectra of the HAs were similar for all treatments. However, our study of FTIR spectra

of HAs extracted from soils of the seven treatments showed some marked differences in the wavenumber range from 2000 to 500  $\text{cm}^{-1}$ . The FTIR spectra of Ck, Chem, Comp + 33% N, Comp + 67% N, and GM + 33% N treatments showed an apparent peak at 1530  $\text{cm}^{-1}$  (N–H bending of amines), but the Comp and especially peat + 33% N treatments did not show the peak at that region, indicating that Ck, Chem, Comp + 33%

N, Comp + 67% N, and GM + 33% N treatments incorporated functional groups of amines in HAs. The spectra of Ck and especially Chem treatments showed strong absorption bands at 1240–1245  $\text{cm}^{-1}$  (C–O stretching vibrations of esters, ethers, and phenols) and at 1036–1038  $\text{cm}^{-1}$  (attributed to alcohols and carbohydrates), whereas those of Comp and peat + 33% N treatments showed weak absorption bands. This indicates that the Chem treatments caused increased concentrations of ester, ether, phenolic, alcoholic, and carbohydrate functional groups in HAs compared with Comp and peat + 33% N treatments. Except for the spectrum of the peat + 33% N treatment, the spectra of the remaining six treatments showed peaks around 1135–1140  $\text{cm}^{-1}$  (C–O stretching vibrations of carbohydrates) with various degrees of intensity. Moreover, the spectra of Ck, Comp, Comp + 67% N, and GM + 33% N treatments showed stronger absorption bands at 538–580  $\text{cm}^{-1}$  (metal–OH vibrations) than those of the other three treatments. This clearly indicates that the Comp, Comp + 67% N, and GM + 33% N treatments increased the metal bonding property of HAs close to that of Ck. Thus, the FTIR spectra of HAs revealed that the treatments with various fertilizers annually applied to the plots changed the functional groups of HAs considerably.

The  $^{13}\text{C}$  NMR spectra of the four replicated HAs and that of their composite HA for each treatment were found to be very similar (not shown). However, the relative intensities of resonance peaks and/or bands of the  $^{13}\text{C}$  NMR spectra of the seven composite HAs extracted from soils of the seven treatments showed marked differences for HAs of different treatments, especially in the range of chemical shift from 0 to 90 ppm (Figure 4). The chemical shifts were assigned according to the guidelines of Perminova et al. (42) and Chefetz et al. (43). The spectra clearly showed the presence of carbonyl carbons in carboxyl, ester, and amide groups (170 and 167 ppm), aromatic and olefinic carbons (135, 129, and 128 ppm), acetal carbons (108, 104, and 102 ppm), CO carbons in alcohols, esters, ethers, and carbohydrates, amines (71, 55, and 52 ppm), and alkyl carbons (30, 23, and 20 ppm). According to Wilson (44) and Malcolm (45), the  $^{13}\text{C}$  NMR spectra were subdivided into five ranges of chemical shifts: 0–50, 50–90, 90–110, 110–160, and 160–190 ppm. These five areas covered by each spectrum were used to compute the distribution percentages of the characteristic C of each composite HA. Under the same instrumental settings for  $^{13}\text{C}$  NMR analyses, the relative intensities of resonance peaks and/or bands of an individual composite spectrum thus provide more information on characteristic C of HA for each treatment than the seven spectra of composite HAs in a specific range of chemical shift. The computed distribution percentages of the seven composite HAs showed no significant differences in carbonyl C, aromatic and olefinic carbons, acetal carbons, and CO carbons among the seven treatments (not shown). However, the sequence of the weight percentages of alkyl C of the HAs of the seven treatments was peat + 33% N > GM + 33% N and Comp > Chem, Ck, Comp + 33% N, and Comp + 67% N treatments, and their differences were significant (Table 4). The sequence of relative intensity of resonance peaks and/or bands in the chemical shift range from 0 to 50 ppm of the spectra of the seven composite HAs (Figure 4) was in good agreement with the sequence shown in Table 4. Moreover, the highest percentage of alkyl C of HA from the peat + 33% N treatment probably derived from the characteristics of applied peat.

**$\delta^{13}\text{C}$  Analysis of HAs and SOC and MRT Determination of SOC.** The utility of  $^{13}\text{C}$  isotopic tracers for soil organic matter (SOM) as well as SOC studies derives from the fact that all



**Figure 4.** Solid state  $^{13}\text{C}$  NMR spectra of the seven composite HAs. Each composite HA is the mixture of four replicated HAs extracted from the soils of each treatment based on yield ratio. The explanation for the label of each spectrum is given in Figure 1.

plants discriminate against  $^{13}\text{C}$  during photosynthesis but to different degrees depending on their photosynthetic pathway (46). Terrestrial plants with the C3 (Calvin cycle) pathway have  $\delta^{13}\text{C}$  values in the range from  $-35$  to  $-20\text{‰}$ . Plants with the C4 (Hatch–Slack) pathway have higher  $\delta^{13}\text{C}$  values ranging from  $-19$  to  $-9\text{‰}$  (47). In this study, the  $\delta^{13}\text{C}$  values of the seven composite HAs were in the range from  $-27.4$  to  $-22.5\text{‰}$ , showing the characteristics of C3 pathway derived materials. However, the sequence of the absolute values of the means of  $\delta^{13}\text{C}$  values of the seven composite HAs was peat + 33% N > GM + 33% N > Ck, Chem, Comp, Comp + 33% N, and Comp + 67% N, with significant differences (Table 4). The  $\delta^{13}\text{C}$  ratio of SOC of the seven treatments followed almost the same sequence, showing the positive correlation of the characteristics of the carbon of HA with that of SOC for each treatment. The sequence of MRT of SOC of the treatments was peat + 33% N > Ck > GM + 33% N and Chem > Comp, Comp + 33% N, and Comp + 67% N with significant differences. The sequence suggests that persistent peat has the slowest turnover rate and, thus, the highest MRT. The sequence of MRT of SOC of the treatments and results in Table 4 demonstrate that the treatments GM + 33% N, Chem, Comp, Comp + 33% N, and Comp + 67% N (easily decomposable chemical and organic N and less decomposable organic material but complemented with chemical



**Table 5.** Selected Correlations among Free Radical Concentrations, Alkyl C, and  $\delta^{13}\text{C}$  of HA,  $\delta^{13}\text{C}$  and MRT of SOC, and SOC from the Seven Treatments

corresponding variable	free radical concn		$\delta^{13}\text{C}$		MRT of SOC	SOC
	alkyl C	alkyl C	HA	SOC		
free radical concn	NA <sup>a</sup>	NS <sup>b</sup>	NS	NS	NS	-0.8322*
alkyl C		NA	0.8843**	0.8083*	NS	0.7764*
$\delta^{13}\text{C}$ of HA			NA	0.9131**	0.8829**	0.8773**
$\delta^{13}\text{C}$ of SOC				NA	0.8643*	NS
MRT of SOC					NA	NS
SOC						NA

<sup>a,b</sup> Explained in **Table 3**. \*\* and \* are explained in **Table 3**.

N) promote the turnover rates of respective SOC of the plots, whereas the treatment of persistent organic material such as peat prolongs the turnover rate of the respective SOC, which in turn may play a role in carbon sequestration in the soil environment of an agricultural ecosystem.

John et al. (48) quantified the carbon dynamics in microcosm experiments with soils from long-term rye (C3 plant) and maize (C4 plant) monocultures using natural  $^{13}\text{C}$  abundance. They reported that the percentage of maize-derived carbon was highest in  $\text{CO}_2$  (42–79%), followed by microbial biomass carbon (23–46%), dissolved organic carbon (DOC) (5–30%), and SOC (5–14%) in the surface soils and subsoils of the maize plots. Additionally, the percentage of maize-derived C was higher for the NPK plot than for the unfertilized plot, which was probably due to the higher input of root biomass at the fertilized plot (49). Leifeld and Kögel-Knabner (50) reported that neither free nor occluded light particulate organic matter (POM) was the appropriate early indicator for changes in land use at the investigated sites but that total SOM, its distribution with depth, and SOM allocated in stable aggregates  $> 20 \mu\text{m}$  were the more sensitive indicators. SOM fractions with turnover times of years to decades, such as POM or light fraction (LF), often respond more rapidly to management-induced changes in the SOC pool than more stabilized, mineral-associated fractions with longer turnover times (51–53). LF and POM represent partly decomposed plant material at an early stage of decomposition, thus characterizing a transitional stage in the humification process. Linking the results from size and density separation of soils to aggregate dynamics has strengthened the potential of LF and POM as useful indicators for changes in soil carbon (54, 55). On the basis of the sequence of MRT of SOC shown in **Table 4**, it may be postulated that the LF and POM fractions in SOC of the treatments in this study were highest in the treatments of Comp, Comp + 33% N, and Comp + 67% N, followed by the treatments of GM + 33% N and Chem, Ck, and then peat + 33% N. Moreover, the discussion summarized above revealed that planted species of crops, applied organic as well as inorganic fertilizers to fields, and land use as well as land management affected carbon dynamics, carbon sequestration, and radiocarbon age of SOC as well as SOM of experimental fields.

**Correlations among Spectral Parameters and  $\delta^{13}\text{C}$  of HA,  $\delta^{13}\text{C}$  and MRT of SOC, and SOC.** On the basis of the statistical sequences shown in **Table 4**, selected correlations among free radical concentration, alkyl C, and  $\delta^{13}\text{C}$  ratio of HA,  $\delta^{13}\text{C}$  ratio and MRT of SOC, and SOC of the seven treatments were analyzed. Free radical concentration of HA significantly and negatively correlated with SOC at the 5% probability level (**Table 5**), indicating the higher content of SOC as well as yield of HA (**Table 3**) and lower free radical concentration of HA. The HA derived from higher persistent

organic amendment such as peat had the lowest free radical concentration (**Table 4**). The weight percentage of alkyl C of HA significantly correlated with  $\delta^{13}\text{C}$  ratios of both HA and SOC and SOC content at 1 and 5% probability levels (**Table 5**). This indicates that the higher alkyl C weight percentage of HA is associated with C dynamics of both HA and SOC and SOC contents. Moreover, the  $\delta^{13}\text{C}$  ratio of HA significantly correlated with the  $\delta^{13}\text{C}$  ratio and MRT of SOC and also the SOC content at the 1% probability level (**Table 5**). This is mainly attributed to the same trend of carbon dynamics of both HA and SOC, implying that the characteristic carbon of HA is the major fraction of SOC in this study. Additionally, the carbon dynamics of HA is highly correlated with turnover rate of SOC as well as SOC content. Furthermore, the  $\delta^{13}\text{C}$  ratio of SOC significantly correlated with the MRT of SOC at the 5% probability level (**Table 5**). This is mainly due to higher correlations among  $\delta^{13}\text{C}$  ratios of HA and SOC and SOC content.

The investigations of the present study thus revealed that the treatments with various fertilizers annually applied to the plots changed the functional groups of HAs considerably. For example, Chem treatments caused increased concentration of ester, ether, phenolic, alcoholic, and carbohydrate functional groups in HAs compared with Comp and peat + 33% N treatments. Comp, Comp + 67% N, and GM + 33% N treatments increased the metal bonding property of HAs. Treatment containing persistent organic compounds increased the humification process in soil and reduced the free radical concentration of HA in surface soil. Furthermore, the MRT of SOC inherited the persistent characteristics of peat, whereas chemical fertilizer and green manure complemented with chemical fertilizer N, and especially compost and compost complemented with various chemical fertilizer N rates, significantly facilitated turnover rates of SOC. Persistence as well as turnover rates of SOC are associated with carbon sequestration in the soil environment of an agricultural ecosystem. Easily decomposable chemical and organic N as well as organic material produced faster SOM turnover, which may cause increased  $\text{CO}_2$  emissions. However, treatments such as peat prolonged the turnover rate of the respective SOC, which in turn may play a role in mitigating  $\text{CO}_2$  emission.

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