Article

Livestock Manure and Compost Effect the Quality and Quantity of Humic Substances in Soil

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Abstract

The effects of organic fertilizer from livestock waste on soil organic matter were evaluated. Two types of organic fertilizers (cattle manure–sawdust compost and dried swine manure) were applied to soil for 9 years, varying the ratio of chemical to organic fertilizers while keeping the amount of nitrogen applied to the soil constant. Humic and fulvic acids were extracted from soil samples and fractionated according to the IHSS method. The application of cattle manure-sawdust compost increased soil organic C content. Humic acid in the soil also increased, suggesting that the increase in humic acid was due to residual cattle manure-sawdust compost in the soil did not increase soil organic C compared to the cattle manure-sawdust compost with a high C/N ratio. However, the crude humic acid content in the soil with dried swine manure application (29.1-32.3 g kg⁻¹) was higher than the crude humic acid content in the cattle manure-sawdust compost (24.1-26.0 g kg⁻¹). These results suggest that organic fertilizers from livestock waste with low C/N ratios, which are easily degraded by microorganisms in the soil, increase the activity of soil microorganisms and promote the biosynthesis of humic substances.

Keywords: Compost, Manure, C/N ratio, δ13C, Humic Substances

Introduction

The concept of "soil management" has been present for a long time in the agricultural industry in Japan. This concept includes intentional soil management techniques that improve the physical, biological, and chemical properties of the soil environment through the application of organic matter. This concept is similar to the recently proposed concept of "soil health" (Kibblewhite et al., 2008). Not applying organic matter to farmland is directly linked to a reduction in the amount of soil organic C (SOC) (Yagi and Tani, 2003; Tamura and Nakatsu, 2011). There is no scientific data showing that a reduction in the amount of SOC reduces crop productivity, but there is an extremely large number of existing reports demonstrating that an increase in the amount of SOC improves the physical, biological, and chemical properties of the soil (for example, Yagi et al., 2010; Abbasi and Tahir, 2012).

Generally, in agriculture, compost is applied to maintain or increase the amount of SOC. Not only does compost application directly affect crop growth due to the fertilizer component of the compost, but it also increases the amount of SOC, which indirectly increases crop yield by increasing soil microbial diversity and the soil chemical buffering capacity (Koga and Tsuji, 2009). The amount and quality of

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organic matter that remains in the soil differs depending on the type of compost, but there is currently no systematic understanding of this mechanism (Albiach et al., 2001; Kawasaki et al., 2008; Mao et al., 2008; Dhillon et al., 2017).

Humic substances extracted from soil or compost may directly affect crop growth, including hormonelike effects (Atiyeh et al., 2002; Arancon et al., 2003). Therefore, the effect of soil humic substances is considered to be extremely important for agricultural production. Continuous application of compost could replace part of the C in the soil humic substances with C derived from compost (Kawasaki et al., 2008). In other words, it is expected that the chemical structural characteristics of soil organic matter will be influenced by the properties of compost, but the extent of the effect has not been clarified (Kawasaki et al., 2008).

We examined the effects of organic fertilizer from livestock waste on soil humic substances (humic acid and fulvic acid). The effects of two kind of organic fertilizers with different C/N ratios were examined on soil samples applied for 9 years. The purpose of this study was not to investigate the fertilization effect on crop production, but to clarify the effects of organic fertilizer on soil humic substances.

Materials and Methods

Experimental design

Lysimeter (2 square meters with a depth of 0.75 m) plots were established in the Ibaraki Agricultural Research Institute, Ibaraki Agricultural Center. Lysimeter plots were installed outdoors. Each lysimeter plot was filled with Ap horizon soil of Hydric Hapludands (Soil Survey Staff, 2014) collected from a single field in 1999. Lettuce (Lactuca sativa var. *capitata*) and Chinese cabbage (Brassica rapa var. pekinensis) were cultivated in each lysimeter plot during the summer and fall for 9 years. Eight experimental plots were prepared and treated with different fertilizer treatments. Fertilizer equivalent to 30 g N m⁻² year⁻¹ was applied to each fertilizer plot prior to crop planting, except for the no-fertilizer (NF) plot. The fertilizer was applied as either chemical fertilizer (CF, ammonium sulfate) alone or in combination with organic fertilizer from livestock waste (OFLW). Two types of OFLW were used, dried swine manure (C/N ratio = 6.6) and cattle manuresawdust compost (C/N ratio = 19.5). Dried swine

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Treatment -	OFLW added	C added	
	kg ha ⁻¹ year ⁻¹		
NF	0	0	
CF	0	0	
LM-25%	3033	776	
LM-50%	6066	1318	
LM-75%	9099	1977	
HC-25%	8806	1680	
HC-50%	17611	3360	
HC-75%	26417	5040	

manure was prepared simply by drying swine feces without using a moisture regulator. Cattle manuresawdust compost was composted sawdust (used as a moisture regulator) and fermented cattle manure that was sufficiently aerobic for 3 months. In the following, the cattle manure-sawdust compost is referred to as high C/N ratio compost (HC) and the dried swine manure is referred to as low CN ratio manure (LM). Three HC and three LM treatments were established in which the mass ratio of N applied as OFLW and CF (the OFLW/CF ratio) was set to 25/75, 50/50, and 75/25. Therefore, the same amount of N but different amounts of C were added for each treatment (Table 1). The soil samples were collected after the Chinese cabbage harvest in 2008. Soil samples were air dried and passed through a 2-mm sieve.

Chemical analysis

Humic (HA) and fulvic acids (FA) were extracted from the air-dried soil samples according to the IHSS (International Humic Substances Society) method (Kuwatsuka et al., 1992; Watanabe et al., 2004). Briefly, 15 g of air-dried soil in a 250-mL plastic container was adjusted to pH \leq 1.0 by adding 1 M HCl. To this mixture, 150 mL of 0.1 M HCl was added, shaken for 1 h, and then the supernatant was separated by centrifugation (14,800 × g, 40 min). The precipitate was neutralized with 1 M NaOH, and then 150 mL of 0.1 M NaOH was added followed by centrifugation (14,800 × g, 60 min) to separate the supernatant alkaline extract from the extraction residue. This

Table 1 Annual inputs of total organic C to each treatment with different types and amounts of organic fertilizer from livestock waste (OFLW). NF = no fertilizer, CF = chemical fertilizer, LM = low C/N ratio manure, and HC = high C/N ratio compost.

extraction procedure was repeated twice, and the resulting alkaline extracts were combined and acidified with a small amount of 6 M HCl and left overnight. The acidified extract was separated into the supernatant and the precipitate by centrifugation $(14,800 \times g, 40 \text{ min})$. The supernatant was combined with the aforementioned HCl extract to form crude FA, and the precipitate was used as crude HA. The amount of organic C in the recovered crude HA and crude FA was measured with a total organic C analyzer (SHIMADZU TOC-V_{CPH}), according to the method of Ikeya and Watanabe (2003). A portion of the crude HA was used as purified HA after removal of clay and ash by the KCl addition-centrifugation process and the HF-HCl treatment, and desalination using dialysis tubing (MWCO: 3.5 kDa). The total C contents of the bulk soil sample, OFLW (dried swine manure and cattle manure-sawdust compost) samples, and the crude HA and crude FA were determined using an elemental analyzer (Thermo Fisher Scientific Organic Elemental Analyzer; FLASH 2000). The purified HA was analyzed as follows. The total C contents of these samples were measured using an elemental analyzer (Thermo Fisher Scientific Organic Elemental Analyzer; FLASH 2000). Subsequently, the C in the purified HA (purified HA-C, Fig. 2) was determined by multiplying the concentrations by the solution volume. The stable isotopic C ratio (δ^{13} C) of the purified HA, purified FA, and OFLW samples was measured using a Thermo Fisher Scientific Flash 2000 and the ConFlo IV & DELTA V advantage system. The δ^{13} C value was calculated using the following formula:

 $\delta^{13}C = [R_{\text{sample}} / R_{\text{standard}} - 1] \times 1000,$

where R_{sample} and R_{standard} represent the ¹³C:¹²C ratio of the sample and standard, respectively. L- α -Alanine $(\delta^{13}\text{C} = -19.60\%)$ and glycine $(\delta^{13}\text{C} = -28.70\%)$ were used as calibrated standards. The measurement was repeated until the standard deviation became < 0.2‰.

The average composition of C species in the purified HA samples was analyzed using ¹³C-nuclear magnetic resonance (NMR) spectroscopy (Bruker, Advance 500) as in Fujitake et al. (2012). Assignment of each C species using chemical shift was in accordance with Preston and Blackwell (1985). Aromaticity (%) was calculated by dividing the sum of integration values of (O-aryl-C + aryl-C) region by that of the (O-aryl-C +

aryl-C + O-alkyl-C + alkyl-C) region. Statistical processing was performed using BellCurve for Excel (Social Survey Research Information Co., Ltd.).

Results and Discussion

Total SOC was associated with the OFLW/CF ratio

The amount of N added in each treatment was fixed; therefore, the amount of added C differed depending on the ratio and type of OFLW added. An increase in the OFLW/CF ratio indicates an increase in the amount of C applied to the soil. The annual amount of C added to the soil was 78-198 g C m⁻² year⁻¹ with dried swine manure (low C/N ratio manure: LM) and 168-504 g C m⁻² year⁻¹ with cattle manure-sawdust compost (high C/N ratio compost: HC).

The amount of total SOC in soil samples for each treatment is shown in Fig. 1. Application of OFLW increased the amount of SOC compared to the NF treatment. The amount of SOC tended to increase as the OFLW/CF ratio increased. The HC treatments had more SOC than the LM treatments. This is likely because the amount of added C differed depending on the C/N ratio of HC and LM. It is known that plant residues with a low C/N ratio have a higher SOC degradation rate than plant residues with a high C/N ratio (Zhang et al., 2021). Decomposition of organic matter with a high C/N ratio requires more soil N than decomposition of organic matter with a low C /N ratio. Since the C/N ratio of the HC was 19.5, the HC does not contain enough N for all SOC to be decomposed; therefore, SOC can remain in the soil.

Humic substance contents were associated with the OFLW/CF ratio

Figure 1 shows the proportion of crude HA and crude FA in SOC for each treatment. Humin fractions shown in Figure 1 were calculated by subtracting the amount of crude HA-C and crude FA-C from the total SOC. The amount of crude HA-C tended to increase as the OFLW/CF ratio increased, regardless of OFLW type. The amount of crude HA-C was 29.1–32.3 g C kg⁻¹ in LM treatments, which was greater than the amount in HC treatments (24.1–26.0 g C kg⁻¹). The percentage of crude HA-C in SOC was 47.4–49.5% in LM treatments, which was significantly higher than that in HC treatments (36.0–37.9%) (Student's t-test; p < 0.01). This suggests that application of LM is more effective than application of HC in increasing the

crude HA content in SOC. The percentage of alkaline extractable humic substance C in SOC in the CF treatment was significantly less than that in the NF treatment, with a particularly large reduction in the crude HA. It has been reported that the amount of SOC decreases when only chemical fertilizers are applied, without organic matter such as compost (Nakatsu and Tamura, 2008). In other words, under aerobic conditions due to tillage, the use of chemical N fertilizer can greatly reduce the mass of SOC. Here, we found a decrease in humic substances when chemical fertilizer was applied alone but not when it was applied with OFLW. This indicates that the application of OFLW may contribute to long-term C storage in soil. However, there were no notable differences in crude FA-C among the different treatments.

Effects of OFLW application on soil humic substances

Fig. 2 shows the C content of the purified HA samples. The purified HA-C content of the LM treatments tended to increase proportionally with the amount of C added with OFLW. Conversely, the purified HA-C content of the HC treatments tended to decrease with the amount of C added. This could be due to a relative increase in the contents of other elements such as O and N. It is also possible that the purified HA in the HC treatments contains fractions derived from HC as well as soil.

The purified HA δ^{13} C value of the HC sample (before being applied to soil) was -26.0. The purified HA δ^{13} C values of soil from the NF and CF treatments were approximately -19.2. When HC was added to soil, the purified HA δ^{13} C value tended to decrease as the amount of applied C increased (Fig. 3). This suggests that HC-derived C was incorporated into the HA fraction (Fig. 2). Alternatively, it may suggest that





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Figure 1. Carbon fractions in soil under different fertilizer treatments. Crude humic acid (HA) fraction, crude fulvic acid (FA) fraction, and humin fraction. NF = no fertilizer, CF = chemical fertilizer, LM = low C/N ratio manure, and HC = high C/N ratio compost.

Figure 2. Percentage of purified humic acid (HA)-C extracted from soil from different fertilizer treatments. NF = no fertilizer, CF = chemical fertilizer, LM = low C/N ratio manure, and HC = high C/N ratio compost.

Figure 3. δ^{13} C value of purified humic (HA) extracted from soil from different fertilizer treatments. NF = no fertilizer, CF = chemical fertilizer, LM = low C/N ratio manure, and HC = high C/N ratio compost. The δ^{13} C values of HC and LM before addition to soil were -26.0 and -20.1, respectively.

some HC remained undecomposed in the soil. Since the total SOC increased with the addition of HC, it is likely that at least some of the HC remained undecomposed and affected the δ 13C value of the purified HA extracted from the remaining HC (Fig. 1).

The purified HA δ^{13} C value of the LM sample (-20.1) was higher than that of the HC sample. This could be because the $\delta^{13}C$ value of plant matter (sawdust) in the HC was low. For soil samples, the purified HA δ^{13} C values were similar among all the LM treatments and the NF and CF treatments (Fig. 3). This might be due to the low C/N ratio of the LM; there was enough N compared to the amount of C to promote C decomposition. The LM likely underwent microbial decomposition during the cultivation period (1 to 2 months). This suggests that C derived from LM was not incorporated into HA-C in the soil, and therefore does not exert a direct effect on the soil. However, the amount of crude HA-C increased in LM treatments compared with the CF treatment, but not compared with the NF treatment (Fig. 1). Therefore, when LM is applied to soil, the HA-C in the LM might be degraded first and that in the in soil might be suppressed.

Effect of OFLW on HA-C species

Table 2 shows the average C species compositions in purified HA from the different fertilizer treatments. For the LM treatments, the proportion of alkyl-C decreased as the OFLW/CF ratio increased. The proportion of calboxyl-C and O-alkyl-C in the LM treatments was higher than that in the CF treatment, and the aromaticity of the LM treatments was lower than that in the CF treatment. For the HC treatments, the signals around 58 ppm and 153 ppm, which are derived from lignin (e.g., Wilson, 1987), tended to be more prominent with increasing OFLW/CF ratio (data not shown). This indicates that the plant-derived lignin in the HA was not degraded. In both LM and HC treatments, the proportion of carboxyl-C was relatively higher than that in the CF treatment. These results indicate that the application of OFLW has a significant influence on the chemical structure of HA.

Conclusions

The addition of OFLW with a low C/N ratio to soil increased HA to a greater extent than the addition of OFLW with a high C/N ratio. The δ^{13} C results indicate that carbon derived from OFLW with high C/N ratios may have a strong effect on carbon in HA. OFLW application generally increased the proportion of carboxyl-C in HA compared to the chemical fertilizer alone.

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	Carbonyl-C	Carboxyl-C	O-aryl-C	Arvl-C	O-alkyl-C	Alkyl-C	
Treatment	190–220 ppm	165–190 ppm	145–165 ppm	110–145 ppm	48–110 ppm	5–48 ppm	Aromaticity (%)
CF	4.0	14.8	7.4	39.8	18.5	15.5	58.1
LM-25%	2.6	17.7	4.6	37.1	20.9	17.1	52.3
LM-50%	4.4	18.0	4.3	37.4	19.4	16.5	53.7
LM-75%	3.7	22.3	6.3	30.5	21.6	15.6	49.7
HC-25%	2.7	16.8	8.2	42.6	17.8	11.9	63.1
HC-50%	0.4	13.7	5.6	42.8	19.9	17.6	56.3
HC-75%	3.5	20.3	5.2	28.0	24.6	18.4	43.6

Table 2 Average C species composition and aromaticity of purified humic acids extracted from soil from different fertilizer treatments.

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