Summary of Doctoral Dissertation

Title: Stabilization processes of organic matter derived from diversified organic amendments in agricultural soils

（多様な有機土壌改良資材に由来する有機物の農地土壌中における安定化過程）

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Summary

In the global carbon (C) cycle, soil organic matter (SOM) is the major source and sink of atmospheric C and therefore considered to be a major mitigating factor in global climate change. The application of organic amendments (OAs) such as crop residues, animal manures, and compost is an established method to maintain or increase SOM levels. However, the central mechanisms controlling SOM formation and residence time subsequent to OA application remain unexplored, especially in long-term applications. It is also unclear how dissolved organic matter (DOM) derived from various OAs contributes to soil aggregate formation and stability. Hence, the objective of this study was to elucidate the stabilization processes of SOM as affected by long-term application of various OAs to agricultural soils and to determine the functions of DOM derived from diverse OAs in the formation and stability of soil aggregates.

In Chapter 2, the stabilization processes of SOM were examined using soil samples (Cambisols) collected from seven treatments, namely, no fertilizer (NF) treatment, chemical fertilizer (CF) treatment, bark compost (BC; 40 Mg ha^{-1} y^{-1}) + CF treatment, coffee residue compost (CRC; 40 Mg ha^{-1} y^{-1}) + CF treatment, cattle manure compost (CMC; 40 Mg ha^{-1} y^{-1}) + CF treatment, sewage sludge compost (SSC; 50–70 Mg ha^{-1} y^{-1}) treatment, and high CMC application (200–400 Mg ha^{-1} y^{-1}) treatment, in a long-term experimental plot (26–31 years) at Nagoya University Farm (Togo, Aichi). Soil organic matter was fractionated by a combination of aggregate destruction and heavy liquid separation into four fractions of free particulate SOM (fSOM; specific gravity (s.g.) < 1.6 g cm^{-3}), free SOM occluded in aggregates (oSOM; s.g. < 1.6 g cm^{-3}), SOM weakly

bound to minerals (wSOM; s.g. 1.6–2.0 g cm^{-3}), and SOM strongly bound to minerals (sSOM; s.g. > 2.0 g cm^{-3}). The chemical structure and composition of the OAs, unfractionated (bulk) SOM, and four SOM fractions were characterized using ramp cross polarization (CP)/magic angle spinning ^{13}C nuclear magnetic resonance (NMR) spectroscopy with phase-adjusted spinning side bands (PASS) and thermally assisted hydrolysis and methylation–gas chromatography/mass spectrometry (THM-GC/MS). The spectra obtained from ^{13}C CP/PASS NMR were divided into five regions: 0–45 (saturated alkyl C), 45–110 (alkyl C substituted by O or N; abbreviated as *O*-alkyl C), 110–160 (aromatic C), 160–190 (carboxy C), and 190–220 ppm (ketone C). The *O*-alkyl C signals were further divided into methoxy C, including Cα in amino acids (45–60 ppm), and carbohydrate C, including other alcoholic and ether C (60–110 ppm), and the aromatic C signals were divided into aromatic C-H/C-C (110–140 ppm) and aromatic C-O (140–160 ppm). The C composition was estimated from cumulative signal intensities in each region relative to total. The degradation products upon THM-GC/MS were grouped according to their presumable sources and chemical similarity into: short-chain fatty acid (FA) methyl esters (MEs), long-chain FAMEs, dicarboxylic acid dimethyl esters, other aliphatic compounds, lignin-derived phenols, other aromatic compounds, N-containing compounds, and polysaccharide-derived compounds. The long-term application of all OAs increased total C content in soil and it was suggested that both quality and quantity of OAs control the accumulation form of C. The accumulation of C in the fSOM fraction was greater in the BC+CF and CRC+CF treatment soils. This was suggested due to the slower decomposition of BC and CRC in soil based on their lower N content, higher C/N ratio, and greater yields of aromatic compounds, e.g., 1,2-dimethoxy-3-methylbenzene or 1-methoxy-4-methylbenzene, specific to both OAs and fSOM fraction. The accumulation of C in the oSOM fraction was greater in the CMC treatment soil. The application of CMC at a high rate in this treatment supplies a greater amount of particulate organic matter, which may promote soil aggregate formation as a nucleus. The accumulation of C in the wSOM and sSOM fractions was greater in the SSC and CMC treatment soils. These fractions in SSC treatment soil were rich in alkyl C based on ^{13}C CP/PASS NMR and had larger yields of various aliphatic compounds in THM-GC/MS. They were also abundant in SSC itself and were therefore considered to be derived from SSC (*ex vivo* modification pathway). In contrast, the abundant alkyl C in the wSOM and sSOM fractions in CMC treatment soil was attributed to the increased contribution of microbially derived organic matter *(in vivo* turnover pathway).

In Chapter 3, stabilization mechanisms of SOM derived from animal carcass (fish cake; FC) and crop residue (rapeseed oil cake; ROC), which are other major types of OAs and are not used in Chapter 2, in Andosols were investigated. Andosols are rich in allophane, an amorphous hydrous aluminum silicate clay mineraloid. Allophane has a greater adsorption capacity for organic matter than kaolin, the primary clay mineral of soil used in Chapter 2, because of the large specific surface area and enrichment in active alumina and iron oxide. Soil samples were collected from CF, FC (1.8–4.9 Mg ha^{-1} y^{-1}), and ROC (2.4–6.4 Mg ha^{-1} y^{-1}) treatments at a long-term (32 year) experimental field in Tsukuba, Japan. Fractionation and analysis of OA and SOM were performed using the same methods as in Chapter 2. The total C content in the ROC and FC treatment soils was equivalent to 112% and 103% of that in the CF treatment soil, respectively. More than 70% of total C was detected in the sSOM fraction in all treatments. ^{13}C CP/PASS NMR showed that *O*-alkyl C was the major C group (24–47%) and was more abundant in the fSOM and sSOM fractions than in the oSOM and wSOM fractions. The yield of N-containing compounds in THM-GC/MS was highest in the sSOM fraction. The yield of aromatic compounds other than lignin-derived phenols was highest in the fSOM fraction and higher in the ROC treatment than in the other treatments. These results corresponded to higher yield of those compounds in ROC than in FC, suggesting that the mechanisms by which ROC-derived SOM stabilizes in Andosols are that the aromatic components are selectively conserved and their metabolites become inaccessible through their interaction with minerals or mineral-dominated soil particles. The effect of FC on C accumulation in any SOM fraction was limited, probably due to its high bioavailability related to high protein content and the small amount applied.

In Chapter 4, water-extractable organic matter (WEOM) as a potential source of DOM in soils was obtained from six OA samples which have been used in Chapters 2 and 3 and their ability to form and stabilize soil aggregates was examined. Chemical characteristics of WEOM samples were analyzed using ^{13}C CP/PASS NMR and diffuse reflectance infrared Fourier transform spectroscopies and high-performance size exclusion chromatography (HPSEC). Leaching soil column experiment was conducted during which WEOM solution or ultrapure water (Control) were repeatedly added to the column. After 30 days, aggregate size distribution (>2000, 500–2000, 250–500, 53–250 and <53 μm) of each soil was measured and the mean weight diameter (MWD) and geometric mean diameter (GMD) were calculated as the indices of aggregate stability. ^{13}C NMR results showed that aromatic C (30% in BC-derived WEOM) or *O*-alkyl C (39–59% in the others) was the major C group in the WEOM samples. Based on HPSEC, the low-molecular-weight (<3 kDa) fraction accounted for 79–89% while the high-molecular-weight (HMW; >10 kDa) fraction occupied 2.3–7.1%. Weight average molecular weight (M_w) of the WEOM from ROC, CMC, and CRC, 3110–3170, was larger than the other WEOM samples, 2130–2640. The repetitive addition of WEOM to the soil increased the proportion of the aggregates with a size of >2000 μm compared to Control treatment and the addition of the WEOM from ROC, CRC, and SSC resulted in greater MWD and GMD compared to the Control and/or other WEOM treatments ($P < 0.05$). The % *O*-alkyl C, M_w, and relative abundance of HMW correlated positively with the proportion of the aggregates with a size of >2000 μm, and the relative abundance of HMW also correlated positively with MWD ($P < 0.05$). These results highlight that the relative amounts of polysaccharides, suggested by % *O*-alkyl C, or other HMW components may be important for WEOM in promoting macroaggregate formation (>2000 μm) and aggregate stability.

In conclusion, this study showed that the accumulation forms and stabilization mechanisms of OA-derived SOM varied among the different OAs. Based on the efficient enhancement of wSOM and sSOM pools that are expected to have a long mean residence time and the effect on aggregate stability, high alkyl C content or high alkyl C/*O*-alkyl C ratio as well as high water-extractable HMW polysaccharide content are valuable as the properties associated with OAs for sustainable management in agriculture and mitigating global climate change.