J. Jpn. Soc. Soil Phys.
土壌の物理性
No. 134, p.5 ～ 13 (2016)

論　文
Original Paper

Long-term temporal changes in micromorphology of cattle manure compost pellets mixed with soils

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Abstract: Compost pellets (PC) are different from non-pelletized composts in various properties including supply and retention of nutrients and production of greenhouse gases because of the micromorphology of PC. Therefore, it is important to micro-morphologically characterize PC for its proper utilization. We investigated long-term changes in the micromorphology of cattle manure PC after its application in a soil. The PC grains were applied in a three-types soil core containing a Lowland soil, a Yellow soil, and an Andosol. The soil cores were buried in a field plot of its soil type. After 1, 6, and 18 months, the soil core was sliced into thin sections after solidifying with a polyester resin for the cross-sectional observation. Decomposition of the compost was also investigated using a glass-fiber filter bag method. Results from the investigation were as follows. 1) Weight residual ratio and carbon residual ratio of PC decreased rapidly in the first 6 months, and after that the decomposition became slower. 2) With the decomposition of PC, their shape was preserved, whereas their volume decreased. Estimated volume residual ratio of PC was 28 % for Lowland soil, 43 % for Yellow soil, and 39 % for Andosol after 18 months. Voids corresponding to the volume loss of PC by decomposition was formed around each PC grain. 3) PC mixed with the Yellow soil were cross-sectionally divided into curved segments by cracks whereas this separation was not observed in the Lowland soil and Andosol. 4) Internal microstructure of PC did not show a clear temporal change from 1 month to 18 months in any of the examined soils. Microstructure type was massive microstructure to subangular blocky microstructure, with poor separation of peds.

Key Words : compost pellets, decomposition, micromorphology, thin sections, burial experiment

1. Introduction

Compost pellets (hereafter PC: pelletized compost) are obtained by the compression of compost using a disk pelleter or extruder machine after pulverization and moisture content adjustment. PC has various advantages over regular compost of easy handling, homogeneous nutrients, longer-term storage, and lower transportation costs (Yakushido, 2002), and therefore, is becoming increasingly popular among farmers. Although constituents of composts are concentrated as the moisture decreases with the pelletization, concentration of major components such as total nitrogen, phosphate, and potassium on dry matter base, is similar to that of the compost before it is pelletized (Kano et al., 1997; Hara, 2005; Araki et al., 2007). However, PC is different from non-pelletized compost with respect to properties such as nitrogen mineralization (Araki et al., 2007), phosphate efficiency (Arakawa, 2012), retention and leaching of inorganic ions (Haraguchi et al., 2008), and production of greenhouse gases (Inoue and Shibukawa, 2008; Yamane and Yamada, 2009; Yamane et al., 2011). These differences can be ascribed to the morphological characteristics of PC, especially the internal microstructure that can be quite different from regular compost. Morphological characteristics of PC can affect various physical and chemical phenomena such as retention and leaching of ions, redox status, and microbial activities. Therefore, studying the morphology of PC is important to investigate unique properties of PC and further improve effective application methods. However, there are limited studies how the micromorphology of PC changes after applying PC in a field.

We developed a technique for observing the micromorphology of PC, which employs the thin section method used in soil micromorphology studies. With this technique, we investigated internal micromorphology of cattle manure compost PC that were collected from an Andosol field at 1 to 31 days after application (Kubotera et al., 2009). As a next step, we further applied our thin section method to the cattle manure PC that was packed in polyvinyl chloride pipes with three representative cultivated soils of the Kyushu Okinawa region, after long-term (1 to 18 months) burial treatment as reported in this paper. Our objectives in the current research were, 1) long-term investigation, 2)

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2016 年 5 月 9 日受稿　2016 年 9 月 24 日受理

Table 1 Chemical composition of the pelletized compost (PC) and non-pelletized compost (NPC) used in the experiment.

Type	Total carbon	Total nitrogen	P_2O_5	K_2O	CaO	MgO
			g kg^{-1}, dry matter base			
Tested compost						
PC	352.9	20.4	23.1	38.5	12.1	12.2
NPC	369.5	20.2	22.5	38.6	11	11.4
[a] Cattle manure compost in Japan						
NPC	353 ± 94 (n = 314)	19 ± 7 (n = 400)	23 ± 17 (n = 401)	24 ± 13 (n = 401)	30 ± 26 (n = 357)	10 ± 5 (n = 357)

[a] Data from Yamaguchi et al. (2000) is shown. Average ± SD

studying the morphology of PC for different soil types, and 3) observation of PC and soils under undisturbed natural condition.

2. Materials and methods

2.1 PC for experiment

We prepared PC for the experiment from cattle (primarily milk cows) manure compost produced in a composting plant in Yamaga City, Kumamoto Prefecture. The chemical composition of the PC and its parent material, non-pelletized compost (hereafter NPC), are shown in Table 1. Total carbon and total nitrogen contents were measured by the dry combustion method using a Vario EL, Elementar. Phosphorus content was measured using the molybdate yellow method, while potassium content was measured using the flame emission method. Calcium and magnesium were measured by ICP-AES after digestion with nitric-perchloric acid. As per the data from literature (Yamaguchi et al., 2000), this PC was similar in chemical composition to the commonly available Japanese cattle manure compost. In addition, the chemical composition of PC and NPC were similar.

The compost was air-dried in a greenhouse and pulverized, followed by addition of water to yield an approximate water content of 0.27 kg kg^{-1}, before pelletization was done using a disk pelleter with holes of 5 mm in diameter. After the pelletizing, the PC was air-dried again in a greenhouse until about 0.10 kg kg^{-1} water content. Shape of the PC after the air-drying was columnar, and its size was 4.8 mm in diameter and 7.4 mm in height (average of 30 grains). Average weight was 0.159 g per grain.

2.2 Field burial experiment

The experiment was conducted with three representative cultivated soils of the Kyushu Okinawa region: a Lowland Paddy soil (hereafter "Lowland soil"), a Yellow soil, and an Andosol. We collected topsoil samples of Lowland soil, Yellow soil, and Andosol from experimental fields of Saga Prefectural Agriculture Research Center in Saga City, Nagasaki Agricultural and Forestry Technical Development Center in Isahaya City, and Kyushu Okinawa Agricultural Research Center in Koshi City, respectively. The soils were air-dried and passed through a 4 mm mesh sieve, and their core samples were prepared as follows.

Polyvinyl chloride pipes of 2.5 cm bore diameter were cut into approximately 2 cm long pieces, and a piece of root restriction sheet was pasted onto one opening of each pipe piece to serve as the bottom cover. Root restriction sheet is a polyester cloth that can keep the sample from intrusion of roots, worms and insects although the air and

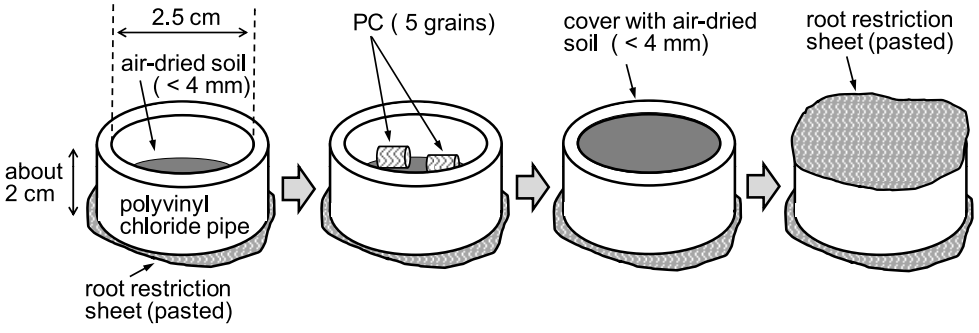


Fig. 1 Preparation of core samples with PC.

water can permeate. These pipes were then half-filled with one of the soils, and 5 grains of PC were laid sideways on the soil (Fig. 1). Next, the pipes were filled with additional soil and pasted with another piece of root restriction sheet as the top cover. Hereafter, we refer to these as "core samples."

Three types of soils without air-drying were packed about 15 cm deep in square plots (1 m × 1 m) that were separated using concrete boards up to a depth of 1 m, in the experimental field of Kyushu Okinawa Agricultural Research Center, Koshi City. Core samples of each soil were buried vertically at a depth of 5 cm on May 25, 2011. The plots were kept bare with manual weeding. Two core samples from each soil plot were collected periodically. The samples collected at 1 month, 6 months, and 18 months after the burial were used for micromorphology observations.

In addition to the core sample burial experiment, we conducted a burial experiment using the glass-fiber filter paper bag method (Saito, 1997), in order to investigate the decomposition of composts. We took around 4 g of PC samples, and weighed them precisely. And then we mixed them with 15 g of air-dried soils. The mixture was enwrapped using an envelope of glass-fiber filter paper to prevent contamination of samples with outer soils. The filter paper is resistant to microbial decomposition and therefore is preservable in soils for a long period. This envelope was then covered with a root restriction sheet in order to prevent the intrusion of worms and insects. Samples that contained the NPC instead of PC, and control samples without any compost, were also prepared in the same manner for the comparison of decomposition rates. Samples were buried in the plots of each soil on March 30, 2011, and collected periodically at 1, 3, 6, 12, and 18 months after the burial. Weight residual ratio and carbon residual ratio were measured by the method of Saito (1997) as follows. The collected samples were air-dried, weighed and pulverized finely. Then the moisture content was measured by weighing a portion of the sample before and after the oven drying at 105 °C. Total carbon content of the samples was measured by the dry combustion method using a Vario EL, Elementarr. Weight residual ratio and carbon residual ratio were calculated as follows:

$$Weight\ \ residual\ \ ratio(\%) = \frac{W_2 - W_1}{W_0} \times 100$$

$$Carbon\ residual\ ratio(\%) = \frac{W_2 \times C_2 - W_1 \times C_1}{W_0 \times C_0} \times 100$$

Where W_0 is the dry weight (g) of PC and NPC that was enclosed in an envelope, and C_0 is the initial carbon content (g kg^{-1}) per dry sample of PC and NPC. W_1 and C_1 are the dry weight and carbon content of whole contents in an envelope of control sample without PC and NPC, and W_2 and C_2 are those of a sample with PC and NPC.

2.3 Preparation of thin sections

Thin sections were prepared following Kubotera et al. (2009) as follows. A collected core sample was air-dried and placed in a paper cup. Polyester resin mixed with benzoyl peroxide dissolved in acetone was poured in the cup, and the core sample was saturated with it by de-aerating in an airtight container using a vacuum pump. Next, the cup was placed in a drying oven and the temperature was gradually raised from 45 °C to 65 °C in order to solidify the resin. The solidified block of soil was cut out from the pipe using a jigsaw and a cutting machine (Maruto Instrument MC-110) equipped with a rotary blade. A cross-sectional cut was then made in the solidified soil block at the mid-height using the rotary blade. The cutting position was particularly crucial for the experiment. Because, if it was not proper, some PC grains would be lost from the thin section. Furthermore, the cutting plane needed to be located close to the central axes of PC grains, because the micromorphology might be different at the center and at the edge of PC. Therefore, we compared the condition of the two pieces, and the one that had the cut plane near the central axis of the PC was selected for further analyses. If the condition of both cut pieces was not good, we prepared a new solidified sample using another core sample from the first step of resin impregnation. The cut plane was polished using the Carborundum, a silicon carbide abrasive compound. Different grain sizes of the compound, # 100, # 300, # 600, and # 1000, were used in the ascending order (from coarse to fine). The polished plane was pasted on a slide glass using epoxy adhesive, and it was cut using the rotary blade to obtain samples of 1 mm thickness or less. The samples were polished repeatedly using the compound, until the internal voids and solids parts of the pellets became clearly observable and the solid parts turned from dark green to dark orange in color, when observed under a microscope.

As stated above, in case the quality of in-process or completed thin sections was not good, we discarded them and prepared new thin sections from another core sample. However, in some cases, the second thin section also had a problem. For example, Lowland soil 1 month thin section shown in Fig. 4 contained only 4 grains and the PC in left edge was too thin by overpolishing. In such cases, observation and morphological analysis was conducted only for the remaining, good-condition grains.

2.4 Micromorphology investigation

Micromorphology observation in low magnification was

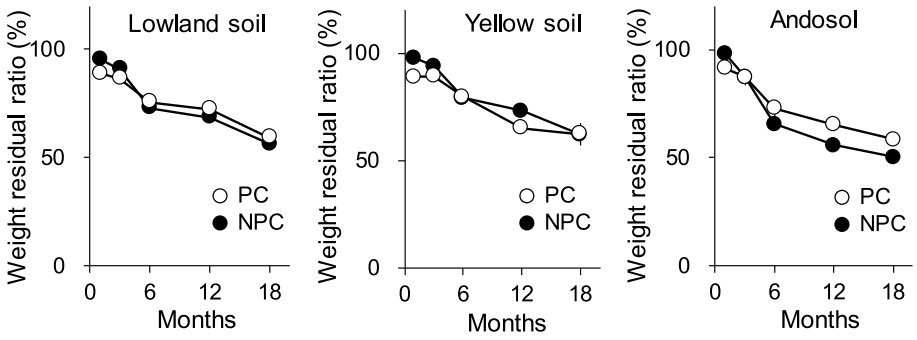


Fig. 2 Weight residual ratio of PC and NPC measured using glass-fiber filter paper bag method. Error bars were added in order to show the SD (n = 2). However, all error bars were hidden under the markers because SD was smaller than 4 % in all samples.

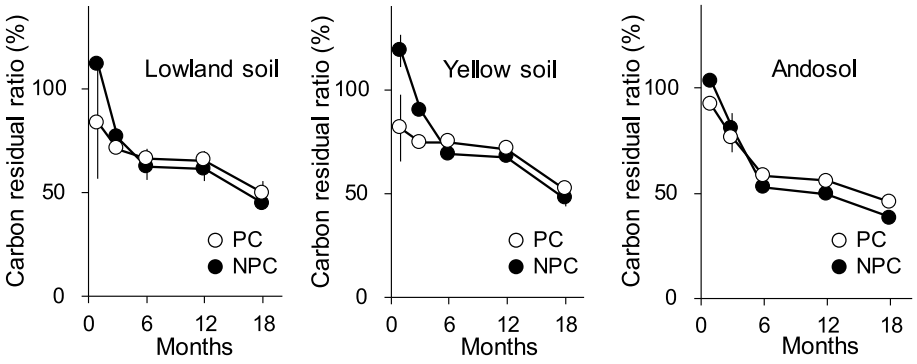


Fig. 3 Carbon residual ratio of PC and NPC measured by glass-fiber filter paper bag method. Error bars were added in order to show the SD (n = 2). However, most of the error bars were hidden under the markers because SD was smaller than 7 % in many samples.

conducted using scanned images of the thin sections. The voids that were formed around the PC after a long burial period were measured as follows. We printed the image of a thin section, cut out the pellets and the voids with scissors, and measured its weight (a grams). The voids around the pellets were then cut away, and the weight of the cutouts was measured (b grams). b/a was the ratio of pellet area to the total area of pellets and surrounding voids. Volume is proportional to the cross-sectional area raised to the three second power, therefore, we considered $(b/a)^{3/2}$ as the volume ratio of a pellet to the total space including the surrounding void. In this paper, we refer to the value of $100 \times (b/a)^{3/2}$ as "volume residual ratio" (%).

Micromorphology observation in high magnification was done using photographs obtained by a digital camera attached to a microscope. We took a photograph of size 1.97 mm × 1.58 mm at the center of PC, for all 5 grains within each core sample. As stated above, if some PC grains were lost or in bad condition, five photos were taken for the remaining good-conditioned grains. We measured the area ratio of voids in PC using a freeware named "lia 32 for Windows 95", developed by Yamamoto K. (http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/index-e.html) as follows. We opened the image file for each photo using this software and collected the color data from 50 points among voids and 50 points among solids. Based on these color data, the software classified all the 3871488 pixels in one image, as either a void or a solid. Ratio of the number of void pixels or solid pixels to the total number of pixels (3871488) was assumed to be the area ratio of each phase.

3. Results and discussion

3.1 Decomposition rate of compost

Weight residual ratio and carbon residual ratio are shown in Fig. 2 and Fig. 3, respectively. Weight residual ratios at 1, 3, and 6 months after the burial were 89 % – 98 %, 87 % – 94 %, and 66 % – 80 %, respectively. The temporal decrease became slower after 6 months, and the weight residual ratio of PC at 18 months was 60 % in Lowland soil, 62 % in Yellow soil and 59 % in Andosol. The residual ratio for NPC was 56 % in Lowland soil, 62 % in Yellow soil and 50 % in Andosol. Although differences among the types of soils and compost were not clear, NPC in Andosol at 6 months (66 %), 12 months (56 %), and 18 months (50

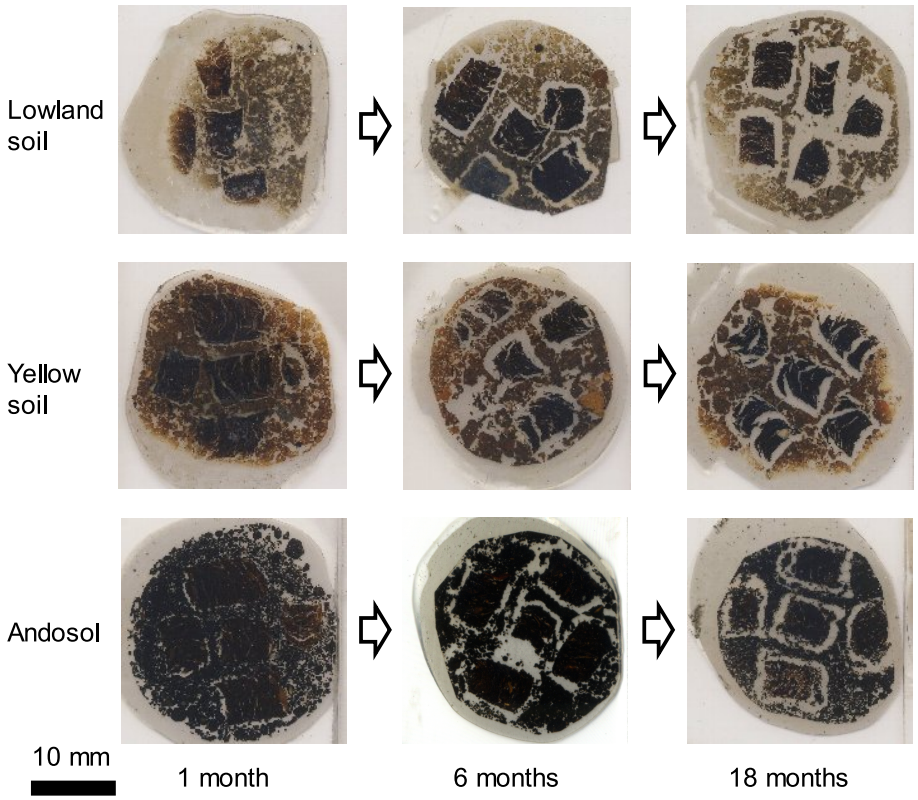


Fig. 4 Micromorphology of whole core samples.

%) showed relatively lower values in comparison with the other samples (73 % – 80 % at 6 months, 65 % – 72 % at 12 months, and 56 % – 62 % at 18 months).

Carbon residual ratio also showed a rapid decrease in the first 6 months, followed by a slower decrease in subsequent periods. In the first 3 months, the ratio was higher for NPC samples than for the PC samples. However, it decreased rapidly and became 4 % – 7 % smaller for NPC than for PC at 6 months or later. As for the soil types, Andosol had lower values than for Lowland soil and Yellow soil in the later periods and carbon residual ratio of Andosol at 6, 12, and 18 months was 53 % – 59 %, 50 % – 56 %, and 38 % – 46 %, respectively, whereas Lowland soil and Yellow soil had values in the range of 62 % – 75 %, 61 % – 71 %, and 45 % – 52 %, respectively.

Carbon residual ratio showed a slightly larger decrease between 12 and 18 months than between 6 and 12 months, which was probably due to the differences in soil temperature across these periods. Average air temperature between 6 and 12 months (October 2011 to March 2012) was 10.7 °C in Kumamoto City (Japan Meteorological Agency, 2012), whereas that between 12 and 18 months (April to September 2012) was 23.5 °C. Therefore, decomposition of compost in the latter period is expected to be more rapid than in the former period.

As seen from the trends for weight and carbon residual ratio, compost decomposition occurred rapidly in the first 3 months and became slower thereafter. Residual ratio of weight and carbon for PC after 6 months was slightly higher than that of NPC, and Andosol samples showed lower values in comparison with Lowland soil and Yellow soil.

3.2 Micromorphology of thin sections

Micromorphology of thin sections under low magnification is shown in Fig. 4. Coarse voids formed between the PC surface and soil had a tendency to increase in size over time. As shown in Fig. 5, the outer rim of the voids (i.e.,



Fig. 5 Similarity in the shape of PC surface and surrounding voids. Red line and yellow line respectively indicate the outer rim and inner rim of the voids between the PC and soil. (Lowland soil 18 months sample, lower left grain).

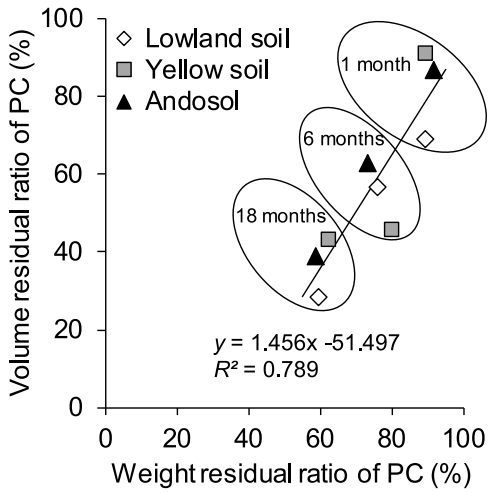


Fig. 6 Residual ratios of weight and volume of PC.
Volume residual ratio (%) $= 100 \times (b/a)^{3/2}$
a: area of PC and the voids around PC in photo.
b: area of PC in photo.

the boundary between voids and soil, indicated by a red line in Fig. 5) showed shapes similar to the inner rim (i.e., the boundary between PC and voids, the yellow line in Fig. 5). As a result, the shapes of PC and the voids surrounding it were nearly the same. This fact suggested that the decomposition of PC in soils occurred from the outer rim, and the loss of outer layers of PC had left the voids with shapes similar to the initial PC. Relationship between the weight residual ratio and the volume residual ratio (defined in the materials and methods section) is shown in Fig. 6. Both the ratios were closely related and the relationship was similar across Lowland soil, Yellow soil, and Andosol. The determinant coefficient of linear regression was 0.789 (n = 9). This result indicated that the volume of PC in the fields decreases over time along with the decrease in weight due to decomposition, and the loss of volume causes the formation of voids between PC surface and the surrounding soil.

Deformation of PC with time depended on soil type. In the case of Lowland soil, the void formation at the outer rim was a primary change, while transformation of PC, formation of internal cracks, or change in the surface smoothness of PC were not notable. In Yellow soil, cross-sectional cracks formed and the PC became partially divided. In addition, the divided PC parts were curved and arch-shaped at 18 months. Andosol showed morphological characteristics similar to Lowland soil. Unlike Lowland soil, however, the surface of PC showed roughness at 18 months.

3.3 Internal micromorphology of PC

We selected 1 representative photo from 5 micrographs of PC in each thin section, and showed them in Fig.7. These photos were selected according to the following criteria: 1) having a non-extreme (neither the largest nor the smallest) area ratio of voids among the five photos, and 2) having a micromorphology that seemed representative of the five photos. Terms of soil micromorphology description (Stoops, 2003) are used henceforth, following the example of Kubotera et al. (2009).

Although the microstructure of PC was not uniform across the grains and the observed areas, solid phase was dominant in all photos and area of voids was generally small. Dominant types of voids were zigzag planes (e.g., seen in the right half of Lowland soil, 1 month photo)

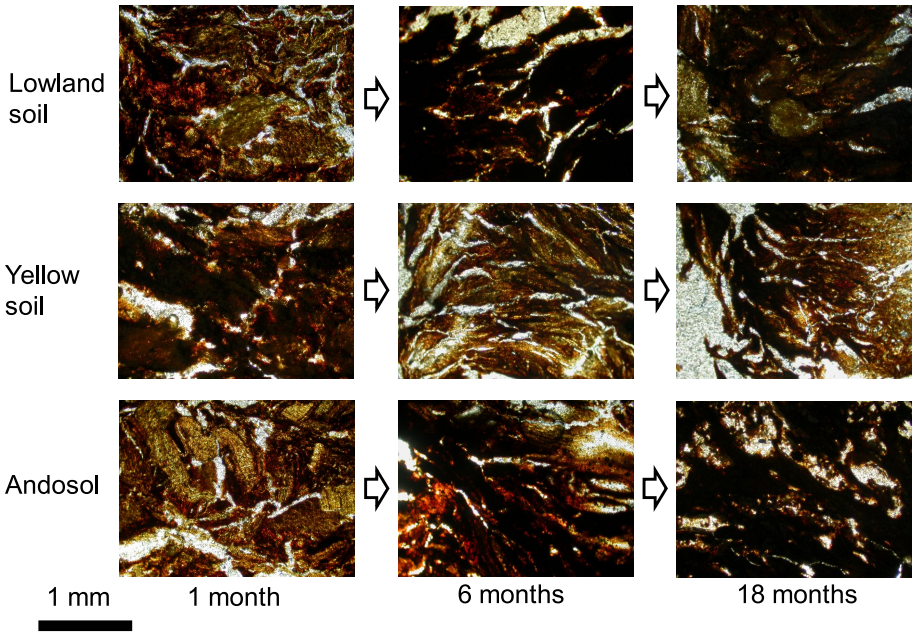


Fig. 7 Internal micromorphology of PC.

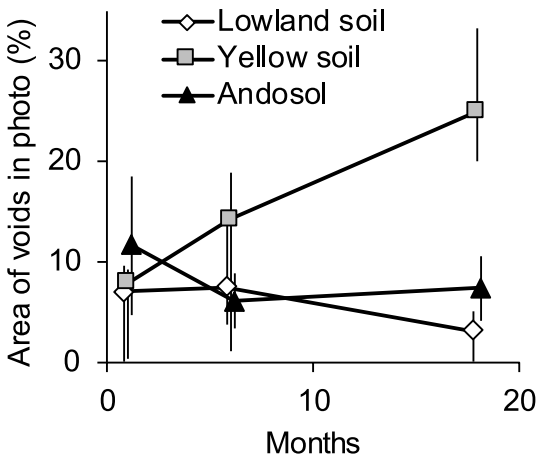


Fig. 8 Temporal changes in the ratio of internal voids in PC. Error bars indicate SD (n = 5). Horizontal position of each plot was slightly changed from the accurate value (1, 6, 18 month) in order to make error bars visible.

and curved planes (e.g., seen in Yellow soil, 6 months photo). In addition, vughs (irregular-shaped and not interconnected voids) with a round or oval shape were also observed (e.g., in Andosol, 18 months photo). Packing voids that were interconnected and caused a separation of peds were scarce. A coarse void in the left edge of Yellow soil, 18 months photo (Fig. 7), was a part of the cracks that divided PC. The type of microstructure was mainly massive microstructure, and subangular blocky microstructure, in which the peds were poorly separated. Area ratio of the voids at 1 month was 7.0 %, 7.9 %, and 11.6 % in Lowland soil, Yellow soil, and Andosol, respectively (Fig. 8). Temporal change in the voids ratio showed no clear tendency in Lowland soil and Andosol. On the other hand, the voids ratio increased to 14.2 % at 6 months and to 25.0 % at the end of 18 months in Yellow soil. This was not due to an increase in planes or vughs in the solid phase, but was instead caused by coarse cracks, as seen in the 18 months photo (Fig. 7). The internal micromorphology of PC between 1 and 18 months after the burial showed no clear tendency with either the soil type or the time, except for an increase of voids volume in Yellow soil, which was related to the generation of coarse cracks in PC. We propose two hypotheses on the reason why micromorphology of PC was similar in the three examined soils: 1) decomposition rate of compost was not remarkably different among the examined soils as shown in the grass-fiber filter paper bag experiment, and 2) interior portion of PC was isolated from the soils, especially after the coarse voids were formed around PC grains, and therefore the internal micromorphology was not subject to the influence of outer soil, as assumed by Hara et al. (2003). However, these hypotheses should be examined by further study.

Kubotera et al. (2009) described the micromorphology of cattle manure PC that was collected from an Andosol field between 1 to 31 days after application. The results showed that the solid phase was separated by various degrees and formed peds of crumbs and granules at 4 days after application. Dominant microstructure types were subangular blocky, while partly crumb and granular microstructures were also observed. Area ratio of voids was 14.0 %, 14.7 %, and 18.0 % at 1, 4, and 31 days after application, respectively.

Our current study documented the micromorphology of PC after a burial period longer than that of Kubotera et al. (2009). Although microstructure type showed a resemblance to the data recorded by Kubotera et al. (2009), the separation of peds was poor, and partial formation of crumb or granular microstructures was not observed. Area ratio of voids after 1 month was also smaller in comparison with Kubotera et al. (2009). This difference might be related to the burial conditions, i.e., Kubotera et al. (2009) applied PC to a field with a rotary cultivator, whereas we enclosed PC and soils in pipes and buried them without any physical disturbance.

4. Conclusion

We obtained some results concerning the morphological changes in PC after its application to soils from a burial experiment for 18 months and micromorphology observations using thin sections. The outer shape and internal microstructure of PC were preserved, suggesting that the properties of PC related with micromorphology such as the microbial habitats could be long-term persistent in a soil. At the same time, voids between the PC and soils corresponding to the volume loss and initial shape of PC were formed. These voids might influence the water and air permeability of soils, especially when the PC grains are interconnected with tubular voids formed after the death and decomposition of plant roots that have a tendency to gather around PC (Arakawa et al., 2012). In addition, cracks formed in PC observed in the Yellow soil might also similarly contribute flows in a soil. Further studies regarding function and sustainability of these voids, influence of soil types on deformation of PC, and long-term changes in soil water and air permeability after for the PC application should be conducted.

Acknowledgements

Soil samples were provided by Norio Ootsuka (Saga Prefectural Agriculture Research Center) and Masashi Fujiyama (Nagasaki Agricultural and Forestry Technical Development Center). Akihiro Tanaka, Yusuke Asai, Yuichi Takakura, Hiroyuki Ito, Masaki Noguchi, Kimiyo Mat-

suoka, and Sayoko Hirayama (Kyushu Okinawa Agricultural Research Center (KARC), NARO) assisted in the preparation and analysis of samples, and the maintenance of field plots. Takashi Kusaba (KARC, NARO) gave us valuable support of various kinds during the overall study period. Shoko Miyazaki (Nihon University) helped us in void measurement. We sincerely thank all of them. This study was financially supported by a research project "Development of mitigation and adaptation techniques to global warming in the sectors of agriculture, forestry, and fisheries" sanctioned by Ministry of Agriculture, Forestry and Fisheries. Budget from research projects 210a3, 151a3, and 151a1 of NARO was also used.

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要　　旨

堆肥ペレット（PC）は，養分の保持供給特性や温室効果ガス発生などの様々な特性が非成型の堆肥と異なっており，それは微細形態に起因すると考えられるが，PC の微細形態に関する知見は少ない．そのため，PC の微細形態特性の解明は適正な利用を行う上で重要である．そこで，圃場に施用した PC の分解に伴う微細形態の経時的変化を調査するため，牛ふん堆肥の PC を九州沖縄地域の代表的な農耕地土壌である低地土，黄色土，黒ボク土と共に塩化ビニール管に封入し，圃場に埋設して，1 月，6 月，18 月後の微細形態を樹脂固化薄片法で観察した．また分解速度をガラス繊維ろ紙埋設法で調査した．その結果，1) PC の重量残存率と炭素残存率は最初の 6 月でそれぞれ 73 % – 80 % と 58 % – 75 % まで減少し，以後は分解速度が低下して，18 月後にはそれぞれ 59 % – 62 % と 46 % – 52 % になった．2) 分解に伴い PC は最初の形と相似形を保ったまま体積が減少した．18 月後の体積残存率は低地土で 28 %，黄色土で 43 %，黒ボク土で 39 % となった．体積の減少に対応する量の孔隙が PC の周囲に形成された．3) 黄色土と混合した PC はクラックによって輪切り方向に離断され，曲がった断片になったのに対し，低地土と黒ボク土ではこの離断は見られなかった．4) PC 内部の微細形態は全ての土壌において埋設 1 ～ 18 月の間では明瞭な経時変化を示さず，微細構造型は全試験期間，壁状構造（massive microstructure）ないしペッドの分離が悪い亜角塊状構造（subangular blocky microstructure）だった．

キーワード：堆肥ペレット，分解，微細形態，薄片，埋設試験