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Effects of fresh spent mushroom substrate of *Pleurotus ostreatus* on soil micromorphology in Brazil

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ABSTRACT

Spent mushroom substrate (SMS) is a good organic material for soil amendment over a short period of time. Several studies have investigated the stability of soil aggregates in amended fields; however, few have attempted to clarify the formation of soil aggregates. In this study, we investigated soil aggregates in fields amended with fresh SMS (from *Pleurotus ostreatus*) (SMS field; SF) and those without amendment (control field; CF) in Brazil by soil morphological analysis. The results demonstrated that SF was dominated by the strong development of a granular microstructure in the A horizon (15–20 cm) and a spongy structure in the B horizon (45–50 cm and 70–75 cm). These horizons had a high porosity and a high fractal dimension. Therefore, these results suggest that the addition of fresh SMS changed the soil structure and the porosity in both topsoil and subsoil. In the A horizon of SF, the granules contained a large amount of SMS residues and excrement. In the B horizon of SF, we found a large amount of plectenchymae. These results suggest that soil structure formation was related to SMS and soil fauna in the A horizon and to fungi in the B horizon.

1. Introduction

Global production of mushrooms has been increasing (Rinker, 2002; Soccol and Vandenberghe, 2008), and the disposal of spent mushroom substrate (SMS), which is a by-product of mushroom production, has become a problem. Many appropriate uses of SMS have been suggested, including agricultural organic materials or composts, seedling materials, bedding for animals, fuels, and in the bioremediation of contaminated soils (Rinker, 2002; Zhang et al., 2012). The major components of SMS are lignocellulose materials, such as wood chips, sawdust, wheat, rye or rice straw, and corncobs (Guo et al., 2001; Zhang et al., 2012).

Several previous studies have investigated the physical characteristics of farm soils after amendment with SMS. The application of SMS decreased the soil bulk density, reduced clod and surface crust formation, increased infiltration, and enhanced the water content in the soil surface layer (0–15 cm) (Stewart et al., 1998). Furthermore, the soil aggregate stability was increased using SMS (Curtin and Mullen, 2007; Stewart et al., 1998). Fresh SMS (from *Pleurotus ostreatus*), which had not been composted and had a high C:N ratio, decreased soil compactness and increased the porosity in amended soils (Kato et al., 2013; Peregrina et al., 2012). In tropical agricultural fields, the addition of fresh SMS decreased the soil bulk density and increased A horizon which had strongly-developed granular peds (diameter was ranged between 0.5 and 4.0 cm) in thickness by 7 cm within 4.5 years (Oda et al., 2014). Therefore, fresh SMS may have beneficial effects as an organic material for soil amendment. However, little is known regarding the specific details of soil aggregate formation in SMS amended soils over a short period of time.

Soil morphological analysis on the basis of thin sections is a useful method for directly observing the formation of soil aggregates (Bullock et al., 1985). Pedality of aggregates, basic organic components, and excrement pedofeatures are analyzed by this method. The fractal dimension and porosity is determined by image analysis of soil thin sections to quantify the development and complexity of the soil structure. This study aimed to clearly determine the effects of fresh SMS on the soil micromorphology in amended farmlands in Brazil.

2. Materials and methods

2.1. Site descriptions and field management

The study site was a private farm in the city of Suzano, São Paulo, Brazil. This site was also used in the study by Oda et al. (2014). The site had an oceanic climate according to the Köppen–Geiger Climate

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Table 1
Profile descriptions for the soil samples from the SMS field (SF) and control field (CF).

		Ap2 (15–20 cm)		Bw1 (45–50 cm)		Bw2 (70–75 cm)	
		SF	CF	SF	CF	SF	CF
Bulk density	(Mg m^{-3})	0.84*	0.95*	1.03	0.99	1.00	0.92
TC	(g kg^{-1})	54.1	27.1	14.3	18.6	13.0	15.6
TN	(g kg^{-1})	3.52	1.99	0.83	1.13	0.63	0.82

Bulk density, total carbon (TC), and total nitrogen (TN) according to Oda et al. (2014). Significant differences between SF and CF for each horizon at $p < 0.05$ (*); Student's *t*-test.

Classification (Climate-Data.org, 2015). The mean annual rainfall was 1545 mm, and the mean annual temperature was 17.7 °C (Climate-Data.org, 2015). The bedrock was gneiss and crystalline schist from the Precambrian age (Fujieda et al., 1997). The study site was located at 750–790 m above sea level in the middle of a gently sloping hill. The soil type was Ultisols (Soil survey staff, 1999).

Two differently managed fields were selected for study: a field amended with SMS (SF) (23°36′58.8″S, 46°17′12.9″W) and a non-amended control field (CF) (23°37′10.0″S, 46°17′14.6″W). In the SF treatment, from 2008 to 2012, we incorporated approximately 15–20 t ha^{-1} $crop^{-1}$ of fresh SMS of *P. ostreatus* (C:N ratio 39; moisture 0.62 kg kg^{-1}; total carbon 191.0 g kg^{-1}; and total nitrogen 4.90 g kg^{-1}) to a depth of approximately 10 cm using a rotary tiller (Oda et al., 2014). Other materials (e.g., nitrogen, phosphorus, or potassium fertilizers; minerals, pH control chemicals, or agricultural chemicals) were not used in SF. Fresh SMS was not used in CF, only calcareous materials were incorporated to adjust the soil pH. SF had been mainly planted lettuce, cabbage, napa cabbage, radish, cauliflower; the proportion in these crop rotation were 46%, 23%, 7%, 5%, 4%, respectively (Oda et al., 2014). The annual cropping systems of CF were broccoli–sweet corn rotations. The total annual average yield of whole farm field of SF for 5 main varies' proportion (lettuce, cabbage, napa cabbage, radish, cauliflower) from 2010 to 2012 was 56.5 t ha^{-1}, which was higher than the estimated conventional yield of same varies' proportion (13.0 t ha^{-1}) (Oda et al., 2014).

Soil surveys and sampling were conducted in fields cultivated with collard greens-butter cabbage (*Brassica oleracea*) on SF and after sweet corn (*Zea mays*) on CF during November 2012. The profile descriptions, bulk density, total carbon and total nitrogen contents are presented in Table 1. Detailed information regarding the study site and field management practices was previously presented by Oda et al. (2014).

2.2. Preparation and analysis of soil thin sections

Soil thin sections were prepared according to Nagatsuka and Tamura (1986). Soil core samples were collected by using styrol cores from each profile at depths of 15–20, 45–50, and 70–75 cm. Soil peds (n = 3; diameter was ranged between 3 and 5 cm) were collected from soil core samples after they were brought back to Japan. Soil peds were freeze dried and then impregnated with a resin [mixed polyester resin A:polyester resin B (Maruto) = 8:2 at 1000 mL and benzoyl peroxide at 10 mL]. The completely hardened samples were cut to smaller pieces of about 50 × 50 × 7 mm^3 using a cutting machine (MC-32; Maruto). The samples were then ground using an abrasive (C3000; Maruto) (first polishing) and bonded onto a glass slide with an epoxy resin. After cutting again, we ground the samples to a thickness of 30 μm using an automatic polishing machine, followed by manual polishing with an abrasive (C400 and C3000; Maruto) (second polishing). We described the soil micromorphology according to Bullock et al. (1985) on the basis of observations using a polarizing microscope (BH-2; Olympus). The size threshold between coarse and fine materials (the C/F concept) was 10 μm, and the C/F concept and the voids were determined under plane polarized light. Relative area of aggregate cross-section, basic organic components, and excrement pedofeatures were determined by the frequency charts for a visual estimate according to Bullock et al. (1985) using an object magnification of 4.0×. The number of plectenchmae was counted in 30 × 30 mm^2 area of thin section.

2.3. Image analysis and measurement of fractal dimensions

Optical microscopy images of soil thin sections were used for image analysis. BMP images of 698 × 525 pixels were acquired using an object magnification of 4.0× which provided a resolution of 5 μm $pixel^{-1}$. The color images were converted into binary using image analysis software (A-zôkun; Asahi Kasei Engineering

Table 2
Micromorphology of the soil thin sections from the SMS field (SF) and control field (CF).

		Ap2 (15–20 cm)		Bw1 (45–50 cm)		Bw2 (70–75 cm)	
		SF	CF	SF	CF	SF	CF
Dominant microstructure[a]		Gr	Sb	Sp	Sb	Sp	Sp
Aggregation	Peds[b]	Gr	Sb	Cr, Gr	Sb, Gr	Cr, Gr	Cr, Gr
	Grade of pedality[c]	S	W	W–M	M–S	M	W–M
	Relative area (mm^2 mm^{-2})	0.65	0.90	0.65	0.75–0.80	0.55	0.70
	Diameter (mm)	0.3–5.0	0.8–1.5	2.0–4.0 (Cr) 0.05–2.5 (Gr)	0.8–5.0 (Sb) 0.02–0.07 (Gr)	0.3–3.0 (Cr) 0.02–2.0 (Gr)	1.0–2.5 (Cr) 0.05–2.0 (Gr)
Voids	Type[d]	Cdp	Pn	Chn, Vu	Pn, Vu	Vu, Chn	Vu
	Porosity[e] (mm^2 mm^{-2})	0.196 (±0.045)**	0.038 (±0.014)**	0.198 (±0.065)*	0.088 (±0.028)*	0.233 (±0.026)**	0.128 (±0.022)**
Basic organic components	Type[f]/	Or/0.25	Pu/0.01	Pu/0.03	Pu/0.01	Pu/0.02	Pu/0.01
	Relative area (mm^2 mm^{-2})	Lt/0.02					
	Number of plectenchymae[g]	Fw	–	Fr	Fw	Vf	–
Excrement pedofeatures	Type[h]/	IE/0.01	–	–	–	–	–
	Relative area (mm^2 mm^{-2})	AE/0.02					
Fractal dimension of voids[i]		1.611 (±0.019)***	1.301 (±0.068)***	1.569 (±0.018)**	1.419 (±0.020)**	1.630 (±0.021)*	1.518 (±0.035)*

[a] Gr: granular; Sb: subangular blocky; Sp: spongy.
[b] Cr: crumbs; Gr: granules; Sb: subangular blocks.
[c] S: strongly; M: moderately; W: weakly.
[d] Cdp: compound packing; Pn: planes; Chn: channels; Vu: vughs.
[e] Significant differences between SF and CF for each horizon at $p < 0.05$ (*) and $p < 0.01$ (**), respectively; Student's *t*-test. Mean (±standard deviation).
[f] Or: organ residues; Lt: lignified tissues; Pu: punctuations.
[g] Fr: frequent (>20/900 mm^{-2} in soil thin section); Fw: few (5–20/900 mm^{-2} in soil thin section); Vf: very few (1–5/900 mm^{-2} in soil thin section).
[h] IE: intact excrement; AE: aging excrement.
[i] Significant differences between SF and CF for each horizon at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***), respectively; ANCOVA. Mean (±standard deviation).