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Kawaguchi, Sadao
The Graduate School of Bioresource and Bioenvironmental Science, Kyushu university

Nishi, Shingo
Tomser Co. Ltd., | Tomser Co. Ltd.,

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Nutritional and Microbial Parameters of Earthworm Cast, Termite Mound and Surrounding Bulk Soil

Sadao KAWAGUCHI[1]* and Shingo NISHI[2]

Laboratory of Soil Biology and Biochemistry, Division of Soil Science and Plant Production,
Department of Plant Resources, Faculty of Agriculture, Kyushu University,
Fukuoka 812–8581, Japan
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A comparative analysis of nutritional and microbial parameters was conducted on two types of biogenetic structures of earthworm cast (8.7 cm in height, 7 casts/1 m×1 m) formed by litter eating *Pheretima* sp., and mound (64 cm in height, 1.0 mounds/10 m×50 m) built by fungus growing termite, *Macrotermes gilvus*, and compared to the surrounding bulk soil as control in the tropical monsoon forest in Cu Chi National Park of Viet Nam.

The proportion of the sand in the earthworm cast was higher than in the surrounding bulk soil, and the clay fraction in the termite mound was 1.6 times higher than that found in the surrounding soil, indicating the different selection by the earthworm or termite for soil particles. Earthworm casts were different from termite mounds in their organic C contents which were approximately seven times as high. Bacterial counts and soil enzymatic activities of β–glucosidase in casts were greatly higher than in the ambient soils, although microbial population and available N and P in the termite mound were significantly lower than those measured in their surrounding soils. On the whole, these results would suggest that plant nutrition is stored in the earthworm cast, but the mound soils of fungus growing termite is infertile.

Key Words: Earthworm cast, fungus growing termite mound, physico–chemical properties, microbial flora, soil fertility.

INTRODUCTION

Termites and earthworms play an important role in soil processes in the tropical soils (Eggleton *et al*., 1999, Lavell *et al*., 1997, Mora *et al*., 2003). These soil animals are considered soil ecosystem engineers (Lavell *et al*., 1997), producing a range of biogenic structures of organic and mineral components, such as mound, crop sheeting and cast. They form micro–habitats where a large number of physical, chemical and microbial changes occur, because the structures are the outcome of an intestinal transit in earthworm cast, or the mixture with a termite's saliva in termite mound. These structures are gradually disintegrating with age and integrating into the soil ecosystem where they might affect the biochemical and physical properties. The diversity of the structures that soil ecosystem engineers constitute, suggest that those structures might reflect some functional characteristics of the producing species (Mora *et al*., 2003).

The aim of the present study was to compare the physical and chemical properties, and microbial flora of the wood–litter feeding termite *Macrotermes gilvus* mound and the litter feeding earthworm *Pheretima* sp. cast with those of adjacent bulk soils, respectively. And then we attempted to determine whether these structures have different properties from the surrounding bulk soils.

MATERIALS AND METHODS

Sampling site, earthworm cast and termite mound

Samples were collected from a tropical monsoon secondary forest in Cu Chi National Park, 70 km north–west of Ho Chi Minh City, South Viet Nam, where annual temperature ranges 26–27 °C (Le, 1997). Cu Chi soil is classified as Acrysols with a sandy clay to sandy clay loam texture and low soil pH, according to the FAO/UNESCO soil classification system (FAO, 2006). Acrisols are representative soils in tropical Southeast Asia and typical of nutrient–poor soils.

The litter eating earthworm *Pheretima* sp. actively ejects the fecal matter and make a tower cast. Whole fresh cast and surrounding bulk soil, 5 cm deep, were collected on August 2003 (Table 1). Each cast collected was, on the average, 74 g on oven dry. Earthworms were collected by pouring 0.5% formalin solution into the gallery of earthworm (Raw, 1959) and all earthworm specimen was identified as *Pheretima* sp.

Fungus growing termite, *Macrotermes gilvus* builds low, domed mounds using a subsoil and saliva as a glue, with rather high density of mounds (Table 1). Ten mounds and the corresponding surrounding bulk soils located 1 m apart from the mound in depth of 5 cm from the surface, were sampled in tropical monsoon forest floor.

Some portion of soils, casts and mounds samples collected, were immediately provided for the microbial analyses. Others were air–dried, crushed to pass a 2 mm mesh sieve, and stored in a sealed polyethylene bags in a cool place until they were used for physical and chemi-

[1] Laboratory of Soil Biology and Biochemistry, Division of Soil Science and Plant Production, Department of Plant Resources. The Graduate School of Bioresource and Bioenvironmental Science, Kyushu University. Fukuoka 812–8581, Japan
[2] Tomser Co. Ltd., Phuoc The, Tuy Phong, Bin Thuan, Viet Nam
* Corresponding author (E–mail: sadakawa@agr.kyushu–uac.jp)

Table 1. Sizes and density of earthworm *Pheretima* sp. cast and termite *Macrotermes gilvus* mound

Pheretima sp. (n=10)

	Height (cm)	Base diameter (cm)	Opening diameter (cm)	Cast density (casts/1 m×1 m)
Average	**8.7**	**3.5**	**0.71**	**27**
Standard deviation	0.7	0.5	0.07	7
Maximum	9.5	3.0	0.80	38
Minimum	8.0	4.0	0.60	22

Macrotermes gilvus (n=10)

	Height (cm)	Narrowest base diameter (cm)	Widest base diameter (cm)	Mound density (mounds/ 10 m×50 m)
Average	**64**	**80**	**97**	**1.0**
Standard deviation	5	17	13	0.1
Maximum	70	105	120	1.5
Minimum	55	60	90	0.8

cal analyses. *t*–Tests were carried out on the differences between the surrounding soils and earthworm casts/termite mounds compositions.

Analytical methods

Soil particle distribution was determined by pipette method after hydrogen peroxide's digestion of soil organic matter. Values of pH (H_2O) was measured in soil : deionized water ratio 1:2.5 by glass electrode. Organic C was determined by dry–combustion analysis. Mineral nitrogen was determined by Bremner's method after extraction with 1 M KCl. Available P was determined by Bray 2 method. Soil enzymes, β–glucosidase were assayed using artificial substrate of para–nitrophenyl derivatives.

Bacteria and fungi were counted by pour culture method using egg–albumin medium and Rose Bengal–Martin medium, respectively. Fungal genera were identified under microscope after the isolation in Potato–Dextrose medium. Facultative anaerobic bacteria, denitrifier was enumerated by most probable method using Guiltay's medium including Dahram tube (Method of Soil Analysis, 1982).

RESULTS AND DISCUSSION

As shown in Table 2, the coarse fractions of sand and silt were higher in the earthworm cast than in the surrounding soil. This implied that earthworm swallowed a clod of the sand to aid the digestion of litter debris in the crop and gizzard. On the other hand, termite mound contained higher clay fraction and lower sand fraction than those of surrounding subsoils. *Macrotermes* termite uses the very fine soil particles to build its very hard mound with its saliva. Tamura (1992) has reported sand–rich soil layer often occurred in the deep lower layer of the soil profile in the tropical regions. This suggests that a termite had preferably removed the fine clay fraction from deep soil horizons to make a termite mound.

Earthworm cast greatly increased in a value of pH in water, but the differences in termite mound are very narrow. Both organic carbon content and β–glucosidase activity in the earthworm cast were four times higher those found in the soil. β–Glucosidase is a good indicator assessing the capacity of soil organic matter to provide the energy into the soil ecosystem. It seems possible that enzyme activity increased in the cast due to the higher carbon content. Available phosphorous and mineral nitrogen also greatly increased in the cast, indicating that the earthworm makes a hot spot of

Table 2. Physical, chemical properties, and microbial flora of earthworm *Pheretima* sp. cast and termite *Macrotermes gilvus* mound

Item	unit	*Pheretima* sp.		*Macrotermes gilvus*		*t*–test
		Cast	Soil	mound	soil	
Physical prpperties						
Sand	(%)	82.1 a	68.6 c	66.3 c	76.3 b	*
Silt	(%)	6.3 a	4.6 b	2.4 c	3.7 bc	*
Clay	(%)	11.6 c	26.8 ab	31.4 a	20.0 b	*
Chemical prpperties						
pH	(H_2O)	6.2 a	4.3 b	4.5 b	4.2 b	**
Organic C	(%)	3.65 a	0.89 b	0.53 c	0.79 bc	*
β–glucosidase	(mgPNP kg^{-1} h^{-1})	92 a	21 b	6 c	19 b	*
Available P_2O_5	(mgP_2O_5 kg^{-1})	117 a	23 b	6 c	13 b	*
Mineral N	(mgN kg^{-1})	103 a	17 b	11 c	17 b	*
Microbial flora						
Bacteria	(10^6 cfu g^{-1})	153 a	44 b	6 d	20 c	*
Fungi	(10^4 cfu g^{-1})	36 a	33 b	5 c	40 b	**
Denitrifier	(10^5mpn g^{-1})	118 a	3.2 b	0.31 d	1.02 c	**

Significant differences were indicated by * ($P<0.05$) and ** ($P<0.01$).

Table 3. Fungal composition (%) of earthworm *Pheretima* sp. cast and *Macrotermes gilvus* mound

Sample	*Fusarium*	*Aspergillus*	*Trichoderma*	*Penicillium*	*Mucor*	Unidentified	Numbers isolated
Pheretima sp.							
cast	49	23	13	6	0	8	240
soil	60	12	11	12	1	3	292
Macrotermes gilvus							
mound	81	1	9	3	3	3	451
soil	56	3	11	27	0	3	347

nutrients in the nutrient poor soil (Subler and Kirsch, 1998). On the other hand, termite *M. gilvus* mound is significantly lower in C, P, N, indicating plant nutrition in the mound soils of fungus growing termite become infertile, because fungus growing termite can utilize the recalcitrant lignin compound of litter to produce very efficiently the carbon dioxide, and leave a few of organic residues in the soils (Hyodo *et al*., 2000).

Bacteria and denitrifiers were significantly larger counts in the cast than in the surrounding soil, however, fungal population was not affected by passing the digestive tract of earthworm (Wolter and Scheu, 1999). As shown in Table 3, genera *Fusarium* and *Aspergillus* were the principal ingredient, in both the cast and the ambient soil. *Penicillium* decreased by 50% in the cast that in control soil, indicating a mycelium of *Penicillium* might be digested through the earthworm tract.

On the other hand, microbial numbers were smaller in termite mound than in the surrounding soil, showing the similar tendency as chemical and biological properties in the termite mound.

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