Alteration of soil chemical properties by two species of millipedes

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Abstract
Two species of millipedes, i.e. *Parafontaria laminata* and *Parafontaria tonominea* swarm every 8 years and every 3 years, respectively. Both of them are in the group of large species with 3-5cm length among the millipedes. Influence of the millipedes through the activities such as feeding and discharge on soil physicochemical properties and decomposition of organic matter are relatively large among the soil animals because of their emergence of high density and large body size. We investigated differences of chemical properties of the casts of these two species millipedes to clarify how they alter the soil properties and how they differ between two species. The millipedes were fed for 1 week at four treatments with contrasting soil type, leaf litter, and density of millipede in a laboratory experiment. After one week feeding, we collected their casts and analyzed (TC, TN, ignition loss, and soil respiration rate). Both millipedes ingested leaf litter and soil. The results were that (1) *P. laminata* fed on more soil than *P. tonominea* did. (2) Higher levels of geophagy were observed in high-density *P. tonominea* treatments. (3) As rate of feeding of leaf litter increased, soil respiration increased.

Key Words
Soil animal, ecosystem engineer, Diplopoda, cast, decomposition rate, chemical properties.

Introduction

Activities of macrofauna such as millipede and earthworm affect the soil ecosystem. They are called litter transformers and ecosystem engineers (Kaneko *et al.* 2004). Swarmings of two species of *Parafontaria laminata* (Attems 1909) and *Parafontaria tonominea* Attems were observed around Mt. Yatsugatake and Mt. Sanbe in 2008, respectively. *Parafontaria* spp. are large millipedes. The body size of adult *P. laminata* is about 3-4cm length and about 0.3g in wet weight, and adult *P. tonominea* is 4-5cm length and about 0.5g in wet weight. Periodic swarming by adult *P. laminata* and *P. tonominea* has occurred on an 8-year cycle (Niijima *et al.* 1988) at Central Japan and a 3-year cycle at Mt. Sanbe in Shimane prefecture, respectively. Their activities such as mass transfer by their moving and their synchronous feeding on soil and litter might have considerably affected soil ecosystem because of emergence of these adults in very high population densities. Niijima (1984) reported that the surface soil in the habitat area of *P. laminata* contained high amounts of organic matter than in soil without the millipede because the cast is high in organic matter. *P. laminata* increased nitrogen mineralization with discharge excretion as soil aggregates (Okai *et al.* 2008). Fujimaki *et al.* (2009) found that it is difficult to decompose organic matter of inside in their casts. Furthermore, Kaneko (1999) reported that ingestion of litter and soil by *P. tonominea* also increased soil respiration and leaching of Ca^{2+}, Mg^{2+} and nitrate.

"What do millipedes ingest?" and “What type of casts the millipedes discharge?” are important information in respect to change of soil quality and a material cycle in soil ecosystem. In order to clarify these matters in the present study, we focused on foods and casts of two species of millipedes, and investigated the chemical properties of their casts which we got by feeding them in the laboratory experiment under several feeding conditions.

Materials and Methods

Adults of *P. laminata and P. tonominea* were collected at around Mt. Yatsugatake (35°58’N, 138°22’E), Yamanashi prefecture, central Japan on beginning of October 2008, and Mt. Sanbe (35°08’N, 132°37’E), Shimane prefecture, western Japan on the end of September 2008, respectively.

The millipedes were fed for 1 week in the dark in 20°C at four treatments (Table 1): (A) train millipede with Andisol and larch leaf litter in Yatsugatake, (B) train millipede with Andisol and leaf litter of deciduous broad-leaved dominated by Oak in Mt. Sanbe, (C) *P. tonominea* with Andisol and leaf litter of deciduous broad-leaved dominated by Oak in Mt. Sanbe, and (D) high density of *P. tonominea* with Andisol and leaf litter of deciduous broad-leaved dominated by Oak in Mt. Sanbe. Soil was placed 3 cm deep in the bottom of a plastic box (31.6 x 43cm), and litter was put on the soil surface (Figure 1). Soil was sieved with 2 mm mesh. Soil water content was adjusted about 60% of the maximum water holding capacity of the soil. Litter water content was also adjusted about 50%. Millipedes were added in the plastic boxes at (A) (B) 588-662 individuals/m^2, (C) 324 individuals/m^2, and (D) 810 individuals/m^2. After 1 week, casts of millipedes were collected from plastic boxes, and the litter removed. Samples of more than 2 mm diameter were used as casts. We analyzed the soil and the cast for total carbon (TC), total nitrogen (TN), ignition loss, and soil respiration by incubation for 8weeks at 25°C.

Table 1. Four feeding treatments of the combination of soil, leaf litter and density of millipede.

	Soil (Andisol)		Leaf Litter		Millipede		
	Area	Dry weight (kg / box)	Forest type and Area	Dry weight (g / box)	Species	Number (Density m^{-2})	Biomass (g /box)
A	Yatsugatake	1.2	Larch Yatsugatake	76	*Parafontaria laminata*	82-90 (603-662)	26.3-28.9
B	Sanbe	1.3	Broadleaves Sanbe	52	*Parafontaria laminata*	82 (603)	26.5
C	Sanbe	1.3	Broadleaves Sanbe	52	*Parafontaria tonominea*	44 (324)	23.0
D	Sanbe	1.3	Broadleaves Sanbe	52	*Parafontaria tonominea*	104-110 (765-810)	51.9-54.7

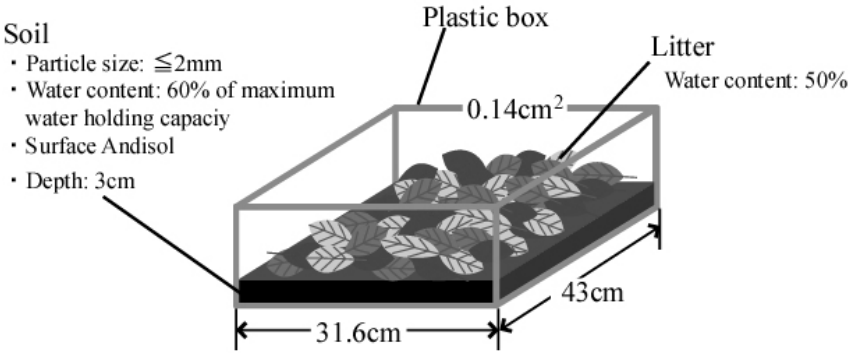

Figure 1. Experimental design of a feeding box and soil and litter condition.

Results and Discussion

Values of all measuring items (TC, TN, ignition loss and soil respiration) of cast of millipedes were higher than those of original soils in Mt. Yatsugatake and Mt. Sanbe (Table 2 and Figure 2).

Table 2. TC, TN and ignition loss of cast of millipedes and original soils and litters

Material area		Total C (%)	Total N (%)	C/N	Ignition loss (%)
Mt. Yatsugatake	Soil	20.63	1.20	17.23	44.55
	Casts of *P. laminata* (A)	24.58	1.29	19.03	51.58
	Larch litter	48.56	1.56	31.22	
Mt. Sanbe	Soil	13.82	0.81	16.96	30.45
	Casts of *P. laminata* (B)	18.91	1.00	18.92	40.58
	Casts of *P. tonominea* (C)	21.40	1.06	20.25	46.21
	Casts of *P. tonominea* (D)	19.73	1.04	19.03	42.66
	Broadleaves litter	50.21	1.62	31.03	

Table 2 shows TC and TN values in soils, these were higher in Mt. Yatsugatake than in Mt. Sanbe. These values in litter in both sites were little different. The casts of both species of millipedes in all treatments

showed TC and TN values higher than that of the soils and less than that of the litters. These results indicated that both species of millipedes fed on litter and soil. TC and TN values of the cast increased in order of treatment (B), (D) and (C). Although there were no quantitative data, it was observed that *P. laminata* (B) preferably fed on soil to litter compared with *P. tonominea* (C) and (D) did. From these facts and observation, we assumed that TC and TN increased as the rate of ingestion of leaf litter increased.
Ignition loss also increased in order of treatment (B), (D) and (C) as the feeding proportion of litter against to soil increased (Table 2). Ignition loss indicates organic matter content in the casts. Organic matter content of the casts depended on their feeding proportion of soil and litter.

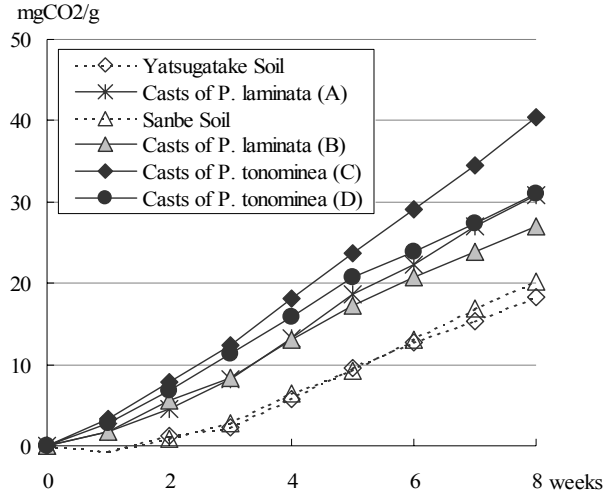

Figure 2. Soil respiration from soil and casts of millipedes by incubation for 8weeks at 25°C

The results of TC, TN and ignition loss of *P. tonominea* (C), which is same biomass with *P. laminata* (B) and lower density than *P. tonominea* (D), were higher than (B) and (D) (Table 2). These results meant that *P. laminata* fed on more soil than *P. tonominea* and higher levels of geophagy were observed in high-density *P. tonominea* treatments. Hashimoto *et al.* (2004) reported that *P. laminata* increased their geophagy rate as the population density increased.

Comparing the data between (B), (C) and (D) that the millipedes fed under soil and leaf litter from Mt. Sanbe on soil respiration, we found that as the millipedes fed more on litter increased, soil respiration increased (Figure 2). In general, low C/N ratio of organic matter is easily decomposed. Though C/N ratio of the casts (C) is higher than the casts (B), soil respiration from the casts (C) is higher than (B). This result indicated that the casts (C) probably contained much organic matter derived from leaf litter.
Species of millipedes differed their feeding rate of soil and leaf litter. The differences of their living density seemed to effect on decomposition rate of organic matter.

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