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Fungal abundance and diversity in earthworm casts and in uningested soil

S. C. Tiwari, R. R. Mishra

Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong – 793014, India

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Abstract. Earthworm casts and adjacent uningested soil from 30 different locations were compared to determine the abundance and diversity of fungal species. The casts contained larger fungal populations (g^{-1} dry soil weight) and numbers of fungal species than the soil. Variations in these parameters between casts and soil were statistically significant ($P = 0.05$). Fungal populations and the number of fungal species in casts and soil also varied significantly ($P = 0.05$) between samples from different locations. A total of 27 fungal species were recorded from the casts and soil. Indices of dominance (0.084 casts; 0.14 soil) and general diversity (2.53 casts; 2.02 soil) demonstrated that the casts displayed more diverse fungal flora than the soil. The diversity of fungal species increased in earthworm casts after passing through the earthworm gut.

Key words: Abundance – Diversity – Fungi – Earthworm casts – Uningested soil – Pineapple

Earthworms are potentially important vectors of microbial propagules since they live in the upper part of the soil profile and transport a large amount of soil (200–400 t ha^{-1} $year^{-1}$) through their bodies (Lavelle et al. 1983). Microorganisms, ingested with the soil, are important components of the earthworm diet. Earthworms feed preferentially on fungi associated with plant remains (Wright 1972; Piearce 1978; Cooke and Luxton 1980; Tiwari et al. 1989). Experimental and field data on the significance of microorganisms as food are inferential and sometimes contradictory. The results of various investigations have demonstrated that casting and excretion by worms may indirectly increase microbial populations (Gorbenko et al. 1986; Tiwari et al. 1989), enzyme activities, and NPK enrichment in earthworm casts (Krishnamoorthy and Vajranabhiah 1986; Mulongoy 1986; Tiwari et al. 1989). Some studies are available on the microflora of the intestinal tract of earthworms (Hutchinson and Kamel 1956; Parle 1963; Dash et al. 1979; Cooke 1983; Tiwari et al. 1990), but studies on fungal abundance and diversity in earthworm casts after passing through the digestive tract are scarce.

In the present investigation, a comparative study on fungal abundance and diversity in earthworm casts and the adjacent unigested soil was carried out to examine whether the abundance and diversity of fungi are altered after passing through the earthworm gut.

Materials and methods

Study site and soil characterization. The study was conducted at Pineapple Research Station Nayabunglow (25°44′N, 91°53′E) at an altitude of 800 m in the Khasi hills of Meghalaya about 30 km north of Shillong, India. The parent rocks are gneiss, schists, and granites. The soil at the study site is an oxisol of laterite origin and sandy loam in texture (Table 1). The moisture content in the casts and soil ranged between 20 and 30% and pH (H_2O) varied between 6.1 and 5.1. The organic C and total N content, respectively, in casts and soil ranged from 2.4 to 1.6% and 0.68 to 0.43% (Table 1). Soil temperature was recorded at 23 °C during the time of soil sampling.

Sampling procedure. Soil samples (0–30 cm depth) and fresh earthworm casts lying on the soil surface were collected in sterilized polythene bags, using a sterilized soil digger, from 30 different locations on the research station. Samples were collected in triplicate from each location and were mixed thoroughly to make a composite sample.

Physical and chemical characterization. Three replicates from each composite sample were analyzed for moisture content, pH, organic C, and total N using methods described by Allen (1974). Soil temperature (20 cm below soil surface) was recorded with a soil thermometer. The moisture contents in the soil and earthworm casts were estimated by drying the samples at 105 °C for 24 h in a hot-air oven. The pH (H_2O) was determined in a 1 : 5 (W : W) soil-water suspension with an electric digital pH meter. For the organic C and total N measurements, the samples were air-dried and sieved (< 0.2 mm). Walkley and Black's rapid titration method was adapted for the determination of organic C (Allen 1974). Total N was estimated by a semimicro-Kjeldahl method as described by Allen (1974).

Isolation and identification of fungi. Fungal populations in earthworm casts and soil were determined in triplicate from each composite sample. Warcup's soil plate method, using Rose Bengal agar medium (Martin 1950) was used to enumerate the fungal propagules. A small amount (0.015 g) of soil was taken from each composite sample using a sterilized nichrome spatula with a flattened tip and placed in a steril-

Table 1. Parameters investigated in earthworm casts and adjacent uningested soil

Parameter	Uningested soil	Casts
Soil texture		
Sand (%)	67	53
Silt (%)	15	9
Clay (%)	18	38
Soil temperature (°C)	23 ±0.63	–
Moisture (%)	30.0±0.071	20.0 ±0.091
pH (H_2O)	5.1 ±0.025	6.1 ±0.058
Organic C (%)	1.6 ±0.05	2.4 ±1.0
Total N (%)	0.43±0.11	0.68 ±0.17
No. of fungal species	18 ±3.0	36 ±5.0
Fungal population (g^{-1} dry weight soil $\times 10^3$)	0.8 ±0.07	1.2 ±0.03
Index of dominance (c)	0.14±0.06	0.084±0.03
Index of general diversity ($\bar{H}$)	2.02±0.3	2.53 ±0.8

Means ±SE, $n = 30$

ized Petri dish. A few drops of sterilized distilled water were poured onto the soil in each Petri dish, using a sterilized pipette, to disperse the soil aggregates uniformly. Approximately 15 ml molten and cooled (below 45 °C) Rose Bengal agar medium (20 g agar, 10 g dextrose, 5 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 1% 3.3 ml Rose Bengal, and 1000 ml distilled water), supplemented with streptomycin sulphate (30 μg ml^{-1}) was poured into each Petri dish, and the dishes were then gently rotated in a swirling motion to disperse the soil particles throughout the medium. The dishes were then incubated upside down at a temperature of 25±1 °C for 5 days in a Biological Oxygen Demand incubator. Fungal populations were estimated by counting the number of fungal colonies that appeared on the medium in the Petri dishes. The number of fungal propagules g^{-1} dry soil was calculated by taking the soil moisture content into consideration. The fungi were isolated and inoculated in a laminar flow chamber. The fungal species were identified under a binocular microscope (Olympus) on the basis of their morphological and reproductive structures, consulting monographs by Gilman (1957), Subramanian (1971), Domsch et al. (1980), and Ellis and Ellis (1985).

To asses the fungal species composition, methods used to study the trophic structure of ecosystems were adopted. Fungal species and fungal populations g^{-1} dry soil were measured and described. Variations in fungal populations and numbers of fungal species between casts and soil were tested statistically by calculating least significant differences. Variations in these parameters among 30 locations were also analyzed statistically, separately for casts and soil. Using the data obtained, the following indices of species structure were assessed:

(1) Index of dominance (c, Simpson 1949)

$$c = \Sigma(n_i/N)^2$$

where n_i is the number of individuals of each species and N the total number of individuals in that location.

(2) Index of general diversity ($\bar{H}$); Shannon and Weaver 1949 cited in Odum 1971)

$$\bar{H} = -\int n_i/N \log_e n_i N$$

where n_i is the importance value of each species and N is the total importance value.

Results and discussion

Numbers of fungal species and of propagules of fungi g^{-1} dry weight soil were higher in casts than soil (Fig. 1). The mean number of fungal species (2.79; $P = 0.05$) and the mean fungal population (0.24×10^3; $P = 0.05$) varied

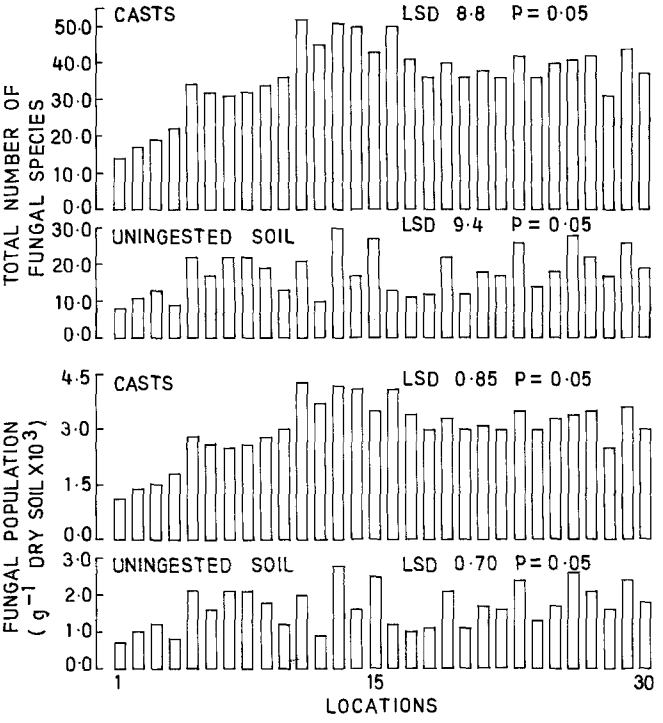

Fig. 1. Variation in fungal populations and number of fungal species in earthworm casts and adjacent uningested soil. *LSD*, Least significant difference

significantly between casts and soil. The fungal population also varied significantly among samples from different locations in both earthworm casts (0.85×10^3; $P = 0.05$) and soil (0.70×10^3; $P = 0.05$). Similarly, the variation in fungal species in casts and soil from different locations (casts 8.8, $P = 0.05$; soil 9.4, $P = 0.05$) was also statistically significant. Reddell and Spain (1991 a, b) also recorded higher numbers of propagules of *Frankia* spp. and vesicular-arbuscular mycorrhizal fungi from earthworm casts. The differences may be caused by environmental changes within the earthworm's digestive tract, due to the ingestion of food material, which provides a rich substrate for the growth of microorganisms.

A total of 27 fungal species were recorded in the casts and soil. These were *Absidia glauca, Alternaria alternata, Aspergillus nidulans, A. niger, Cunninghamella echinulata, Curvularia maculans, Fusarium moniliforme, F. solani, Gongronella butleri, Humicola fuscoatra, Mortierella ramanniana, Mucor hiemalis, M. plumbeus, M. racemosus, Paecilomyces liliacinum, Penicillium chrysogenum, P. claviforme, P. fellutanum, P. funiculosum, P. javanicum, P. liliacinum, P. vermiculatum, Trichoderma koningii, T. viride, Torula herbarum,* white sterile, and yellow sterile. All 27 species present in the soil were also recovered from casts. Parle (1963) also reported a similar microflora in casts and soil.

Despite the similarity in microflora the frequency of species occurrence differed in casts and soil. The casts displayed a higher percentage occurrence of all the 27 species than the soil. This is in conformity with the higher average number of fungal species in casts (36) than in soil (18) at each location. The increased occurrence of fungal species in the casts is attributable to selective feeding by

earthworms on fungus-associated plant remains and soil. Cooke (1983) confirmed that worms showed feeding preferences related to the quantity of fungus not to fungal species. This is probably why the fungal species *Absidia glauca, Fusarium moniliforme, F. solani, Mucor racemosus, Penicillium chrysogenum, Trichoderma viride,* and white sterile forms were recovered from the casts because these were also the dominant fungi in the soil (Table 2). In soil these species were present only in 15 locations while in casts they were recovered from 21 locations. The increase in the diversity of fungal species in casts compared with soil samples may have been due to the germination of fungal spores after passing through the digestive tract of earthworms; in the soil these spores might have been present but could not be isolated as they did not germinate. Hutchinson and Kamel (1956) also recorded viable spores of 17 fungal species after passage through the digestive tract of *Lumbricus terrestris.*

The average index of dominance for the casts was 0.084, indicating that dominance was shared, while it was 0.14 for soil, indicating dominance by just a few species. Converse results were obtained for the index of general diversity; a value of 2.53 for casts indicated a greater diversity of fungal species than in the soil, which had a value of 2.02. This is in accord with the greater number of fungal species in the casts (36) than in the soil (18). The index of general diversity forms a mirror image of the index of dominance (Fig. 2).

At each location significant variation in fungal species and fungal propagules (g^{-1} dry soil weight) in casts and soil demonstrated that earthworm ingestion has a marked influence on the occurrence and diversity of fungal species in casts. The results of the present study confirm that earthworm casts contain the same kind of fungal flora as associated with the soil and plant remains that the earthworms ingest. However, the diversity of fungal communities increased after passing through the earthworm digestive tract.

Table 2. Percentage frequency of fungal species isolated from earthworm casts and adjacent uningested soil

Fungal species	Uningested Soil	Casts
Absidia glauca Hagem	53	80
Alternaria alternata (Fr.) Keissler	3	23
Aspergillus nidulans Van dentatus	7	37
A. niger Van Tieghem	40	57
Cunninghamella echinulata Thaxt.	3	40
Curvularia maculans Boedizn	6	63
Fusarium moniliforme Sheld	96	100
F. solani (Mart.) Sacc	77	78
Gongronella butleri (Lendner) Peyronel & Dal Vesco	33	70
Humicola fuscoatra Tra.	17	73
Mortierella ramanniana (Moller) Linnem.	3	33
Mucor hiemalis Wehn.	13	56
M. plumbeus Bon.	7	46
M. racemosus Fres.	63	70
Paecilomyces liliacinum (Thom) Samson	27	33
Penicillium chrysogenum Thom	83	93
P. claviforme (Bain)	7	40
P fellutanum Biourge	20	46
P. funiculosum Thom	7	37
P. javanicum Van Beyma	13	36
P. liliacinum Thom.	3	20
P. vermiculatum Dangeard	40	43
Trichoderma koningii Oudem	20	60
T. viride Pers. ex. Gray	83	86
Torula herbarum Pers. ex Gray	6	47
White sterile	77	80
Yellow sterile	33	43

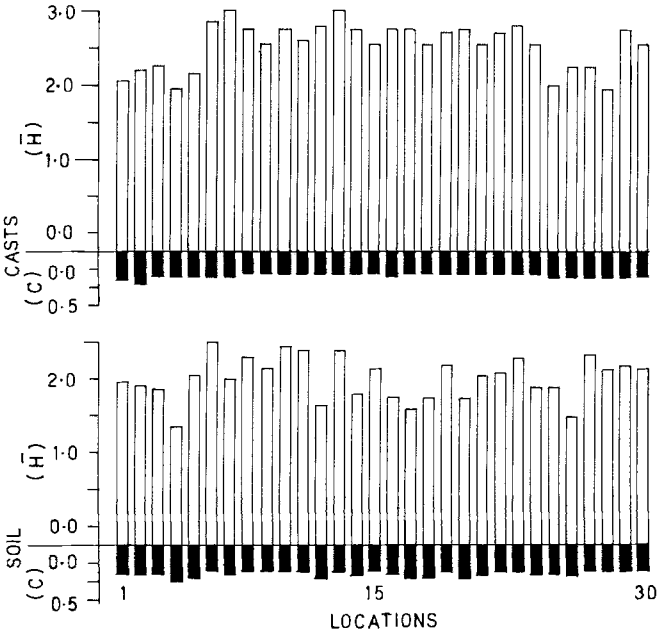

Fig. 2. Comparison between Shannon index of general diversity ($\bar{H}$) and index of dominance (C) for the fungal species recorded from earthworm casts and adjacent uningested soil, ■, Index of dominance; □, index of general diversity

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