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Microbial populations, enzyme activities and nitrogen-phosphorus-potassium enrichment in earthworm casts and in the surrounding soil of a pineapple plantation

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Summary. Total populations of bacteria and fungi, dehydrogenase activity (as a measure of total potential microbial activity), and urease and phosphatase activities were determined in earthworm casts and surrounding laterite soils planted to pineapple. The casts contained higher microbial populations and enzyme activities than the soil. Except for fungal populations, statistically significant ($P = 0.05$) increases were found in all other parameters. Microbial populations and enzyme activities showed similar temporal trends with higher values in spring and summer and lower values in winter. The earthworm casts contained higher amounts of N, P, K and organic C than the soil ($P = 0.05$). Selective feeding by earthworms on organically rich substrates, which break down during passage through the gut, is likely to be responsible for the higher microbial populations and greater enzyme activity in the casts.

Key words: Earthworm casts – Bacteria – Fungi – N, P, K – Organic C – Dehydrogenase activity – Urease activity – Phosphatase activity.

Earthworms may constitute a major part of the soil faunal biomass (Lee 1983). They are known to accelerate plant-residue decomposition by incorporating and mixing the surface residues into the soil during burrowing, feeding and casting activities (Mackay and Kladvik 1985). Earthworm feeding activity increases microbial populations (Gorbenko et al. 1986), while casting and excretion may indirectly improve the nutrient supply to plants (Krishnamoorthy and Vajranabhaiah 1986). Some studies are available on the microflora of the intestinal tract of earthworms

(Gorbenko et al. 1986), but enzyme activities in earthworm casts have so far received little attention from soil biochemists (Businelli et al. 1984). For a better understanding of the effect of earthworm activity on microbe-mediated processes in soils, knowledge of the microbial biomass and its activity in earthworm casts may be useful. In the present work, a comparative study on microbial populations of bacteria and fungi and some enzyme activities (dehydrogenase, urease, and phosphatase) in earthworm casts and the surrounding laterite soil was carried out in order to determine how a microbial biomass and its activities are affected by passage through the earthworm gut.

Materials and methods

Study area and climate. The study was conducted at pineapple research station Nayabunglow (latitude 25° 44' N, longitude 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 gneisses, schists, and granites. The soil is a red sandy loam (sand 66.7%, silt 15.9%, clay 17.5%) of laterite origin (Oxisol). The pH (H₂O) ranged from 4.5 to 7.2 and the organic C content varied between 1.5% and 2%. The moisture content of the soil and casts ranged between 11.9% and 33.9% and soil temperature varied between 11 °C and 31 °C (Table 1).

The climate of the study area can be divided into four marked seasons: (1) the monsoon season of heavy rainfall (May–September) from the southwest monsoon; (2) a transitional period of low rainfall (October–November), due to the retreating monsoon, (3) a winter season (December–February) with scattered low rainfall; and (4) a windy dry summer (March–April). The annual rainfall during the study period (1986–1987) was 2300 mm. The average maximum and minimum ambient temperatures of the study site were 27.0 °C and 7 °C, respectively. The percentage relative humidity reached 84% during the rainy season (Fig. 1). Five species of earthworms were recorded during the sampling periods. These were *Amyntas alexandri*, *Darvida assamensis*, *Metascolides untriphyses*, *Metaphire houlletii*, and *Neloscoclex strigosus*. *Darvida assamensis* was the dominant species. Soil samples (0–25 cm) and fresh earthworm casts (visual observation) lying on the soil surface were collected aseptically at monthly intervals.

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Table 1. pH and moisture content (%) of laterite soil samples and earthworm casts

Dates	Soil temperature (°C)	Moisture content (%)		pH (H ₂ O)	
		Soil	Casts	Soil	Casts
15/4/86	30	17.7 ± 0.069	12.2 ± 0.081	5.0 ± 0.045	6.0 ± 0.021
15/5/86	31	25.8 ± 0.071	16.0 ± 0.058	4.5 ± 0.043	6.2 ± 0.057
15/6/86	31	24.5 ± 0.069	20.5 ± 0.024	5.0 ± 0.043	5.9 ± 0.076
15/7/86	30	26.2 ± 0.065	19.4 ± 0.056	5.0 ± 0.038	5.6 ± 0.038
15/8/86	26	26.1 ± 0.075	11.9 ± 0.058	5.4 ± 0.032	7.2 ± 0.091
15/9/86	20	22.8 ± 0.079	10.2 ± 0.021	5.4 ± 0.037	7.0 ± 0.011
15/10/86	19	22.8 ± 0.081	23.2 ± 0.051	5.3 ± 0.028	6.0 ± 0.004
15/11/86	19	13.9 ± 0.057	12.4 ± 0.023	5.2 ± 0.034	6.4 ± 0.058
15/12/86	18	13.0 ± 0.059	11.9 ± 0.019	5.2 ± 0.036	6.0 ± 0.007
15/1/87	17	19.4 ± 0.076	15.4 ± 0.023	5.4 ± 0.045	6.5 ± 0.019
15/2/87	15	24.2 ± 0.049	23.8 ± 0.046	5.2 ± 0.025	5.4 ± 0.023
15/3/87	11	26.6 ± 0.075	12.6 ± 0.091	5.0 ± 0.043	5.1 ± 0.088

All values ± SE

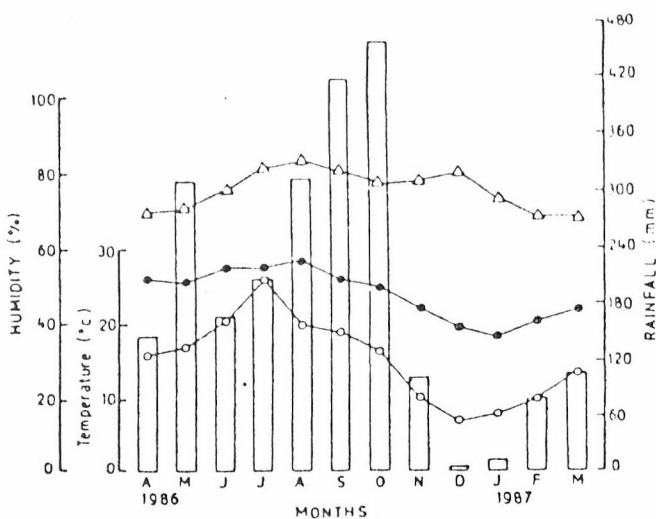


Fig. 1. Monthly variation in rainfall (histogram), ambient temperature (○), minimum temperature; ●, maximum temperature; △, relative humidity

Physical and chemical characterization. Soil temperature was recorded with a soil thermometer. Moisture contents in the soil and in the earthworm casts were estimated by drying the samples at 105°C for 24 h in a hot-air oven. The pH was determined in a 1:5 (w:w) soil water suspension with an electric digital pH meter. For the N, P, K, and organic C 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 semi-micro Kjeldahl method as described by Allen (1974). Exchangeable K was extracted in ammonium acetate solution (pH 7) and measured by flame photometer (Systronics-121, Ahmedabad, India). A molybdenum blue method, using Bray's extraction solution (1.11 g of ammonium fluoride dissolved in 1 litre HCl), was used to measure available P (Allen 1974).

Microbiological analysis. Fungal populations in earthworm casts and soil were estimated by Warcup's soil plate method, using rose bengal agar medium (Martin 1950). A sample of 0.015 g soil was inoculated in sterilized Petri dishes, using a sterilized nichrome spatula.

A few drops of sterilized distilled water were poured at the bottom of the Petri dishes to disperse the soil aggregates uniformly. Then approximately 15 ml molten and cooled (below 45°C) rose bengal agar, supplemented with streptomycin sulphate, was poured into the Petri dishes. The dishes (three replicates) were gently rotated and incubated at a temperature of 25 ± 1°C for 5 days.

A 10/000 dilution was used to enumerate the bacterial colony forming units (Johnson and Curl 1972). Half a millilitre of the suspension was inoculated onto sterilized Petri dishes containing 15 ml solidified nutrient agar medium. The dishes were then rotated gently in a swirling motion to disperse the inoculum uniformly over the surface of the medium. The inoculated dishes were incubated at 30 ± 1°C for 24 h.

Dehydrogenase activity (a measure of biomass or potential metabolic activity) was assayed by the 2-3-5-triphenyltetrazolium chloride reduction technique (Casida 1977). Five grams of fresh soil were placed in a test-tube (15 × 1.5 cm) and carefully mixed with 0.1 g CaCO₃ and 1 ml fresh 1% 2-3-5-triphenyltetrazolium chloride solution. The tubes were plugged with a rubber stopper and incubated at 30°C for 24 h. The resulting slurry was transferred on Whatman no. 1 filter paper and triphenyl formazan was extracted with successive aliquots of concentrated methanol. The volume of filtrate was made up to 50 ml by adding methanol. The extinction of the pink colour was read spectrophotometrically (Hitachi 220) at 485 nm, using methanol as a control (without soil).

Urease activity was assayed by the method of McGarity and Myers (1967). Ten-gram samples of soil were placed in 100-ml volumetric flasks and treated with 1 ml toluene. Thereafter, 10 ml buffer (pH 7) and 5 ml of 10% urea solution were added. The flasks were shaken and incubated at 37°C for 3 h. For the control, 10 ml urea solution was replaced by 10 ml distilled water. After incubation the volume of each flask was made up to 100 ml by adding distilled water. The flasks were then thoroughly shaken and their contents filtered through a Whatman no. 5 filter paper. The ammonia released as a result of the urease activities was measured by the indophenol blue method. Half a millilitre of filtrate was placed in a 25-ml volumetric flask and 5 ml distilled water was added. The mixture was treated with 2 ml phenolate solution and 1.5 ml sodium hypochlorite solution and the volume of the flask was made up to 25 ml by adding distilled water. The extinction of the blue colour that developed as a result of the urease activities was read spectrophotometrically at 630 nm.

Acid phosphatase activity was estimated by the method suggested by Babatabai and Bremner (1969). A 0.1 g sample of soil was taken in a 50-ml conical flask, and 4 ml modified universal buffer (pH

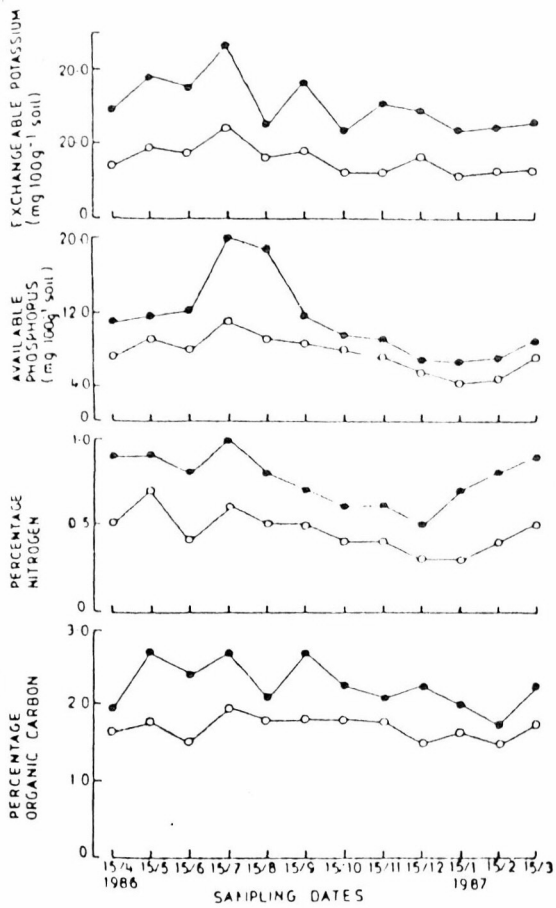


Fig. 2. Monthly variation in organic C, N, P, and K levels of earthworm casts and laterite soil samples. ●, cast; ○, soil

6.5), 0.25 ml toluene and 1 ml of 0.115 M *p*-nitrophenyl phosphate solution were added. The flask was swirled for a few seconds to mix the contents and then placed in an incubator at 37°C for 1 h. After the incubation the stopper was removed and 1 ml of 0.5 M calcium chloride and 4 ml of 0.5 M sodium hydroxide solution were added. The soil suspension was then filtered through Whatman no. 12 filter paper, and the yellow colour of the acid phosphatase activity was read spectrophotometrically at 420 nm. For the control samples, 1 ml *p*-nitrophenyl phosphate solution was added after the addition of the calcium chloride and sodium hydroxide solutions (i.e., immediately before filtration of the soil suspension). Values reported in the table and figures are all means of triplicate analyses. The data were analysed statistically by adapting Student's *t*-test procedures.

Results

N, P, K and organic/C contents of the casts and soil are shown in Fig. 2. The concentrations of these nutrients were higher in the casts than in the laterite soil. During July, maximum concentrations of all nutrients were recorded from both casts and soil, with a fall in the nutrient content during the winter and a slight increase in the spring.

Fungal populations were higher in the casts than in the soil (Fig. 3). Generally, the trend of change in the

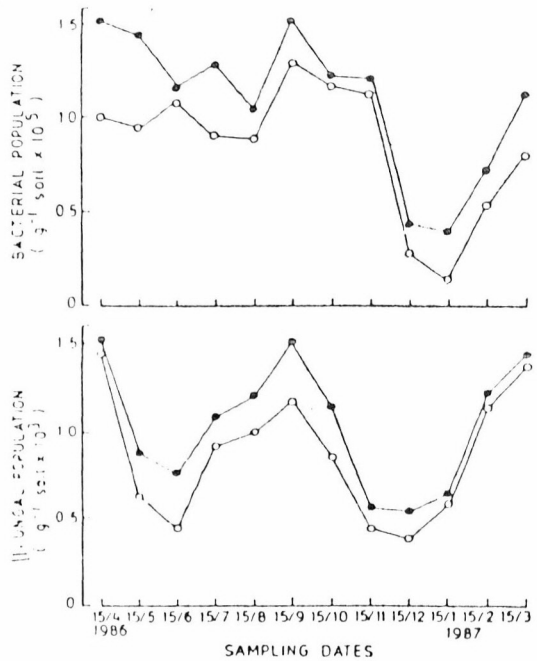


Fig. 3. Monthly variation in fungal and bacterial population of earthworm casts and laterite soil samples. Symbols as for Fig. 2

fungal populations of both casts and soil was similar over all the sampling periods. There were two peaks in fungal populations, one in spring (April) and another in autumn (September). During the rainy summer period (June) and during winter (December) fungal populations dropped to a very low level. Bacterial populations followed a similar trend of monthly variation (Fig. 3), except that only the winter values dropped.

Dehydrogenase activity (Fig. 4) was greater in earthworm casts than in the soil. The maximum activity was recorded in July and the minimum activity in December and January. The trend of temporal variation was generally similar in both casts and soil. Urease activity peaked during May. A drop in activity was recorded in June, followed by an increase in July. Urease activity was low during the winter, with a minimum in January. After the winter, there was a slight increase in urease activity. Phosphatase activity was also higher in the casts than in the soil, with a peak in July. Lower values were recorded during the winter, but the drop was not as significant as the fall in dehydrogenase and urease activities (Fig. 4).

Discussion

Earthworm casts may contain higher numbers of microorganisms than the surrounding soil (Dash et al. 1979). In the present investigation, both bacterial ($P = 0.05$) and fungal populations were higher in the

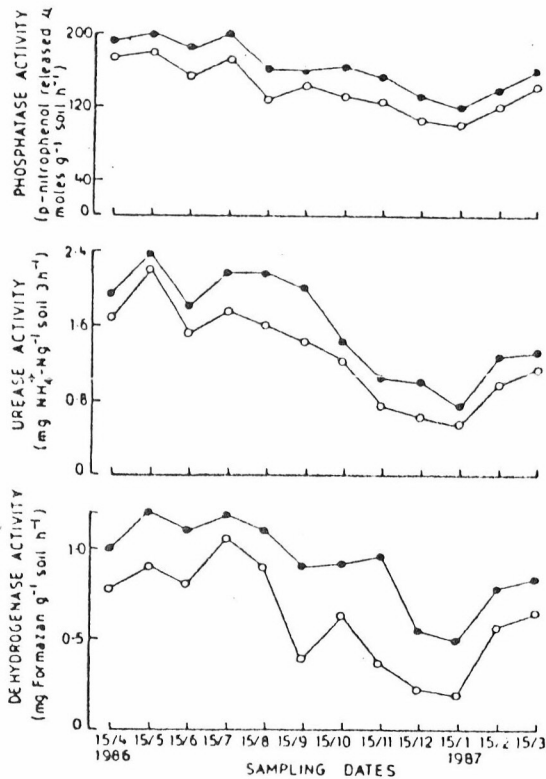


Fig. 4. Monthly variation in dehydrogenase, urease and acid phosphatase activities in earthworm casts and laterite soil samples. Symbols as for Fig. 2

casts. The differences may be caused by environmental changes within the earthworm's digestive tract, due to ingestion of food material, providing a rich substrate for the growth of microorganisms. Dehydrogenase, urease, and phosphatase activities were also higher in the casts than in the soil. Since the numbers of bacteria and fungi increased after passing through the earthworm gut, this may be responsible for the increase in enzyme activities. The increased microbial activity is probably responsible for the increased dehydrogenase activity as a measure of total biomass (Ross and Roberts 1970) and the increased urease (Syers et al. 1979) and acid phosphatase activities (Sharpley and Syers 1976) in earthworm casts. The higher urease activity in the casts can be attributed to higher levels of organic matter (Syers et al. 1979), since urease is known to be bound with organic matter (Beri et al. 1978). The higher phosphatase activity in the casts may be explained by a combination of increased earthworm and enzymatic activity and increased biomass (Sharpley and Syers 1976).

Higher values of organic C, N, P, and K were recorded in the casts than in the topsoil samples. This nutrient enrichment of casts may be ascribed to the di-

gestion and mineralization of the organic matter, which contains higher concentration of nutrients than the poor, laterite soil. The increase in N content of the casts has been attributed to the intimate mixing of plant remains and microbial excretions with mineral soil in the earthworm digestive tract (Lunt and Jacobson 1944). Earthworms may have a positive influence on N_2 -fixing bacteria (Lee 1983) which may aid to the N content of the casts. The differences in available P between the casts and the soil in the present study was statistically significant ($P = 0.05$). Similarly, the differences in organic C and exchangeable K were also statistically significant ($P = 0.001$, $P = 0.05$, respectively). Higher values of N, P, K, and organic C in the casts were also reported by Lal and Vleeschauer (1982). In the present study the increases in the level of N, P, K, and organic C were of a lower order than those of earlier reports (Sharpley and Syers 1976; Syers et al. 1979; Krishnamoorthy and Vajranabhaiah 1986). It may be concluded that earthworms play a specific role in the distribution of soil fungi and in the enrichment of casts in N, P, K, and organic C, and increase the mineralization rate by increasing the microbial biomass and enzyme activities.

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