



Microbial Dynamics of Endemic Earthworms on Soil Health and Sustainable Agriculture

Chengalvarayan Dhakshayani, Sultan Ahmed Ismail* and Nausheen Dawood

P.G. and Research Department of Zoology, JBAS College for Women, Chennai-600018, T. N., India

*Ecoscience Research Foundation, Chennai-600 041, T. N., India

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 19-9-2013

Accepted: 19-11-2013

Key Words:

Endemic earthworms
Microbial dynamics
Sustainable agriculture
Biofertilisers

ABSTRACT

Earthworms harness the microorganisms which are beneficial to the agro-ecosystem, as they synergistically decompose soil organic matter and help in nutrients cycling. *Lampito mauritii* and *Perionyx excavatus* are endemic earthworms which dominate the Indian soils, especially in south India. However, the exotic earthworm *Eudrilus eugeniae* has been harnessed for the formation of compost and organic matter decomposition. Endemic earthworms do not coexist with exotics as they eliminate the former due to competition for food and space. The present study aims to reveal the physical, chemical and biological differences between the different products of the endemic earthworms *L. mauritii* and *P. excavatus* and the exotic earthworm *E. eugeniae* to better understand their contributions to agricultural soil and nutrient management. The three major earthworm products include cast, compost and drilosphere soil of earthworms. Different groups of microorganisms present in earthworm products include major microbial groups, biofertilisers, carbon, and nitrogen mineralizers. The results reveal that the products of endemic earthworms show significant increase in biofertilisers such as nitrogen fixers and phosphate solubilizers, heterotrophic bacteria, fungi and actinomycetes compared to the products from the exotic earthworm. Among the three products studied, vermicompost, especially of the endemic species, shows a balanced C/ N ratio and increased microbial density including biofertilisers.

INTRODUCTION

The flora and fauna endemic to the geographical locations are directly associated to the ecosystem and have a major impact when interrupted by exotic species with varied characteristics. Exotic earthworm, *Eudrilus eugeniae* is being widely used in India for producing vermicompost. Earthworms to a large extent alter soil porosity and show an increase in soil air volume (Wollny 1890). These characteristics relate earthworms to water infiltration and the water holding capacity of soils. Earthworm casts develop soil aggregates (casts getting stabilized after they are excreted) by forms of gums that result from microbial digestion of their organic components (Waksman & Martin 1939) or by the binding effect of fungal hyphae (Parle 1963). Soils from earthworms show significantly higher proportion of macroaggregates (>2000) than those without earthworms (Coq et al. 2007). Tons of casts deposited in the soil profile by *Matrodrilus* species of earthworm in Neotropical Savannahs contribute greatly to plant nutrition and regulation of soil structure (Mariani et al. 2007). Synergism was observed between the endemic earthworms *L. mauritii* and *P. excavatus* in gaining biomass and show an enhanced microbial quality and quantity in their products (Dhakshayani et al. 2013). Large scale application of compost is being practised in agricultural soil, however, avail-

ability of live biota is of great important for sustainable soil practices.

MATERIALS AND METHODS

Indian endemic earthworm species the anecic, *Lampito mauritii* Kinberg and the epigeic, *Perionyx excavatus* Perrier, and the exotic epigeic species *Eudrilus eugeniae* Kinberg were cultured in triplicate in independent 5000cm³ bioreactors. Vermibeds were prepared with gravel followed by sand in the bottom of the container to simulate the earthy structure for the earthworms. Shredded dry paddy straw and cow dung in the ratio 10:1 with the C/N ratio of 28:1 were added as the substrate for vermiculture. Earthworms weighing about 10 g (initial biomass) with a minimum of not less than 4 in numbers were chosen as a demonstrable standard for inoculation into the units. The biomass of the combination of species was also maintained to approximately 10 g, with a minimum of not less than 2 in each species. Incubation time was 30 days; the time taken to utilize the substrate except for the control unit, which took 60 days. Moisture was maintained at 50 ± 5%. The units were inoculated with earthworms in the following combinations. 1. *Lampito mauritii* (L) 2. *Perionyx excavatus* (P) 3. *Eudrilus eugeniae* (E) 4. *Lampito mauritii* + *Perionyx excavatus* (L+P) 5. *Perionyx excavatus* + *Eudrilus eugeniae* (P+E) 6. *Eudrilus*

eugeniae + *Lampito mauritii* (E+L) 7. Control (unit without earthworms) microbial compost (C). After incubation, the composting units were cut to collect the samples such as 1. Vermicomposts of the three species individually and in combinations, 2. Casts of the three species of earthworms, 3. Drilosphere soil (removed from the drilosphere by excavation with a spatula, as according to Savin et al. (2004)) of each earthworm species. Samples were stored at 4°C before processing.

Physical properties such as water holding capacity and porosity of the vermicompost were determined using Keen Raczkowski cups (Keen & Raczkowski 1921). pH and electro conductivity of soil and compost were determined in water suspension (1:5) (Hanna 1968). Cast, compost and drilosphere samples of all the three species of earthworms were subjected to microbiological analysis. The collected samples were serially diluted, plated on the respective selective media and incubated at room temperature (25-28°C) for 2-5 days. Major microbial groups studied include (i) total heterotrophic bacteria that were determined by plating on nutrient agar medium, (ii) total mesophilic fungi isolated from Martin's Rose Bengal medium (Snyder et al. 1959) and (iii) total mesophilic actinomycetes, plated on Kosmachev medium (Kosmachev 1960). Actinomycetes were incubated in the above medium for 7 days at room temperature. They were recognized by their characteristic tough leathery colonies, branched vegetative and aerial mycelia and spore formations. They were further confirmed by the inclined coverslip technique of William & Davies (1967). Biofertilisers such as phosphate solubilizers were enumerated by Pikovskaya's medium (Pikovskaya 1948) and their colonies showed yellow halo after 4-5 days. Estimation of N₂ fixers was done on Norris glucose nitrogen free medium (Ranganayaki & Mohan 1981). The major carbon mineralizers such as (i) starch degrading bacteria were enumerated using starch agar medium and detected by adding iodine solution in the agar plate which showed yellow halo around the microbes after the incubation period, (ii) cellulose decomposing bacteria, enumerated in cellulose agar were detected by adding 0.1 N Congo red and eluting with 5M NaCl after 30 minutes, formed a halo zone around the colony against dark orange background (Wood 1980, Teather & Wood 1982). The quantitative estimation of nitrogen mineralizers such as ammonifiers, nitrifiers and denitrifiers in the samples was done by MPN (Most Probable Number) technique. Ammonifiers were grown in peptone broth and detected by Nessler's reagent and enumerated with MPN table (Page 1991). Nitrifiers grown on Winogradsky's medium were detected with drops of Trommsdorf's reagent and dilute H₂SO₄ (1:3) for the presence of nitrite. The presence of nitrate was confirmed by

adding diphenylamine reagent. Denitrifiers were estimated in sterile nitrate broth with a single inverted Durham's tube in each culture tube for observing gas bubble formation. Denitrifiers can be detected by testing for the absence of nitrate and nitrite and by visibly observing gas formation in the Durham's tube during growth (Weiss & Price 1980). Statistical methods employed were ANOVA, and Post Hoc Tukey B test for physical, chemical and microbiological parameters. Regression analysis was applied to correlate the relationship between chemical and microbiological parameters.

RESULTS

Physical and chemical properties: The earthworms *L. mauritii*, *P. excavatus* and *E. eugeniae* showed no significant difference in percentage porosity in their composts (Fig. 1). *P. excavatus* compost showed higher water holding capacity compared to *E. eugeniae* and *L. mauritii* compost. In combinations of earthworm species the maximum water holding capacity was demonstrated by the units with combinations of endemic earthworms, *L. mauritii* and *P. excavatus* (L+P). Soil and microbial compost demonstrated very low WHC compared to vermicomposts (Fig. 1). pH of the vermicompost of all three species and its combinations was in the range of 6.8-7.2 and showed no significant difference between them (P<0.05) (Table 1). The electro conductivity of the composts of all the three species varied significantly, with *P. excavatus* compost showing the highest EC of 1.25mS/cm (Table 1). The organic carbon content in the soil used for vermibed was 1.29%, but the compost of the endemic epigeic species *P. excavatus* showed the highest OC than all other compost which was 7.41%. Total Kjeldahl nitrogen (%) showed no significant difference (P<0.05) between the vermicompost of all the units. The compost of the endemic species *L. mauritii* and *P. excavatus* individually and in combinations showed a balanced C/N ratio than that of the exotic species. Both the epigeic species showed a nearly equal C/N ratio but the % organic carbon and nitrogen content in *E. eugeniae* compost is much lower. Available phosphorus is seen to be significantly high in the compost of *P. excavatus* whereas, available potassium, the micronutrient is found to be significantly high in *E. eugeniae* compost than in the other units (Table 1).

Major microbial groups: Among the three products of earthworms such as cast, compost and drilosphere soil, compost showed higher populations of major microbial groups, which is high in *L. mauritii* than the other two species and its combinations (Table 2). The heterotrophic bacteria in the compost of *L. mauritii* was found to significantly dominate among the major microbial groups than the other two species (P<0.05) (Fig. 2). *E. eugeniae* showed the least popu-

Table 1: Chemical characteristics of the compost.

Compost	pH	EC, mS/cm	OC %	TKN %	C:N ratio	P ₂ O ₅ %	C:P ratio	K ₂ O%
L	7.08 ± 0.25 ^a	0.54 ± 0.09 ^a	4.66 ± 1.27 ^{ab}	0.40 ± 0.05 ^a	11.65 ± 3.62 ^a	0.22 ± 0.08 ^{ab}	28.91 ± 16.71 ^a	0.025 ± 0.005 ^{ab}
P	6.96 ± 0.16 ^a	1.25 ± 0.02 ^d	7.41 ± 1.33 ^c	0.59 ± 0.37 ^a	12.56 ± 7.29 ^a	0.46 ± 0.24 ^b	24.28 ± 10.37 ^a	0.025 ± 0.005 ^{ab}
E	6.84 ± 0.13 ^a	0.75 ± 0.16 ^{ab}	3.84 ± 0.62 ^{ab}	0.26 ± 0.02 ^a	14.77 ± 3.31 ^a	0.22 ± 0.06 ^{ab}	23.38 ± 9.5 ^a	0.065 ± 0.005 ^c
L+P	6.98 ± 0.37 ^a	0.72 ± 0.19 ^{ab}	3.51 ± 0.48 ^a	0.30 ± 0.07 ^a	12.14 ± 2.25 ^a	0.13 ± 0.05 ^a	28.92 ± 6.93 ^a	0.02 ± 0 ^a
P+E	7.10 ± 0.17 ^a	1.09 ± 0.02 ^{cd}	4.85 ± 1.73 ^{ab}	0.26 ± 0.05 ^a	20.0 ± 10.5 ^a	0.14 ± 0.04 ^a	41.52 ± 26.97 ^a	0.04 ± 0.01 ^b
E+L	6.85 ± 0.21 ^a	0.66 ± 0.10 ^{ab}	3.13 ± 0.31 ^a	0.22 ± 0.09 ^a	15.95 ± 7.80 ^a	0.15 ± 0.04 ^a	22.64 ± 8.14 ^a	0.01 ± 0 ^a
Control	7.17 ± 0.11 ^a	0.87 ± 0.10 ^{bc}	6.01 ± 0.09 ^b	0.31 ± 0.02 ^a	19.39 ± 1.54 ^a	0.18 ± 0.01 ^a	38.03 ± 2.28 ^a	0.02 ± 0.01 ^a
Soil	6.28	0.336	1.29	0.006	-	0.002	-	0.001

Means of the same subsets in the same column mentioned as superscripts (a,b,c) show no significant difference.

Table 2: Sum total of microbial groups in the three products of earthworms.

Earthworms		Major microbial groups (10 ⁵)	Biofertilisers (10 ⁵)	Carbon mineralizers (10 ⁵)	Nitrogen mineralizers (10 ⁵)
Cast	L	925.34	63.57	411.49	69.18
	P	431.07	49.76	322.66	114.59
	E	426.64	60.12	235.73	139.27
compost	L	5072.80	175.68	265.92	151.22
	P	3993.20	150.65	181.87	167.77
	E	281.77	83.56	37.89	282.41
	L+P	400.80	218.45	51.89	160.60
	P+E	487.39	92.75	123.59	175.93
	E+L	348.11	64.99	37.79	210.52
	Drilosphere	L	1691.37	64.63	157.97
Drilosphere	P	870.88	55.39	156.55	70.74
	E	692.00	22.35	129.15	72.32
	Control	Microbial	668.35	38.15	61.93
	soil	43.36	1.18	9.86	7.69

lation in all the three groups. The population of actinomycetes was found to be significantly high in *P. excavatus* compost than *L. mauritii* (Fig. 3). However, the cast and drilosphere soil of *L. mauritii* showed a higher population of actinomycetes than the other two species.

Drilosphere soil occupies the second position in supporting the major microbial population among the three products. *L. mauritii* being anecic earthworm enhances higher microbial colonization than the other two species. Epigeic earthworms showed a lesser microbial population while *E. eugeniae* showing the least (Table 2).

The total quantity of microbial population of earthworm casts was found to be high in *L. mauritii* and it drastically increased in its compost (Table 2). Though the casts of both *P. excavatus* and *E. eugeniae* showed a similar density of microbes, the compost of *P. excavatus* showed an enhanced major microbial population than the compost of *E. eugeniae* (Table 2). The population of actinomycetes and fungi showed significant difference between the earthworm products of the three species of earthworms with *P. excavatus* compost showing significantly higher population (Figs. 3&4).

Biofertilisers: The total population of biofertilisers which includes phosphate solubilizers and N₂ fixers, was found to be high in the compost of all the three species, followed by drilosphere soil and casts. The composts of *L. mauritii* individually and in combination with *P. excavatus* showed significant increase in the total population of biofertilisers (P<0.05) (Table 2). This is followed by the compost of *P. excavatus* and *E. eugeniae*. Among the two biofertilisers studied, N₂ fixers were found to be higher than phosphate solubilizers in all the products of the earthworms (Figs. 5&6). The total population of biofertilisers in the cast and drilosphere soil of the endemic species *L. mauritii* and *P. excavatus* was found to be similar (Table 2). *E. eugeniae* showed the least population of biofertilisers in its drilosphere soil, than the other two species and also microbial compost.

Carbon mineralizers: The total carbon mineralizers (starch and cellulose degraders) were found to be high in the castings of the three species followed by their compost and drilosphere soil (Table 2). Starch degraders were found to be high in cast of the three species (Fig. 7) but cellulose degraders were found to be high in their compost (Fig. 8).

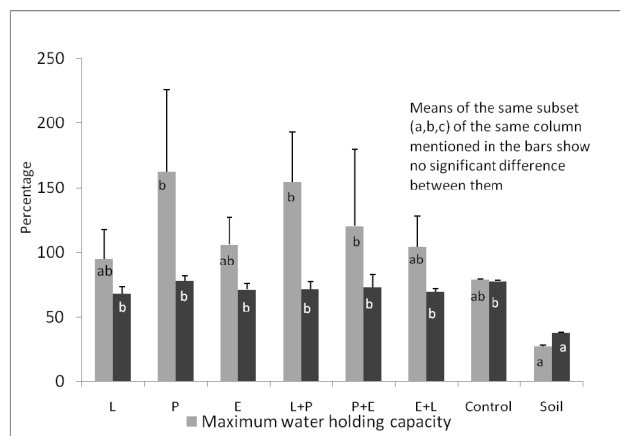


Fig. 1: Maximum water holding capacity and porosity of vermicomposts and controls.

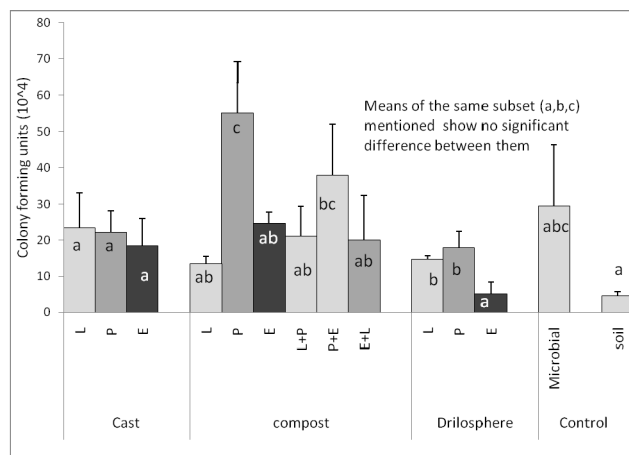


Fig. 4: Population of fungi in the earthworm products and controls.

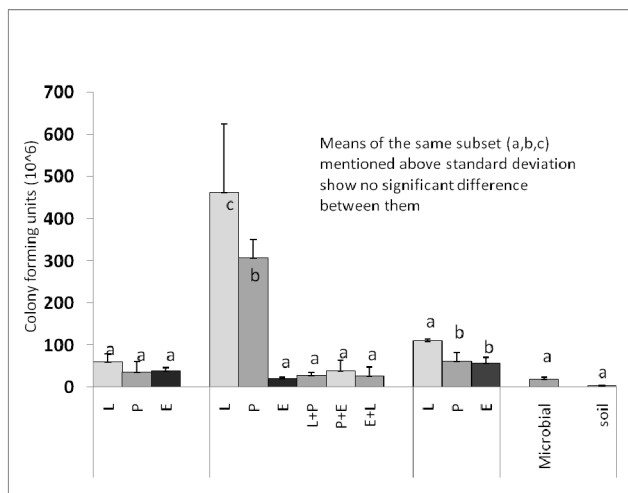


Fig. 2: Population of heterotrophic bacteria of the earthworm products and controls.

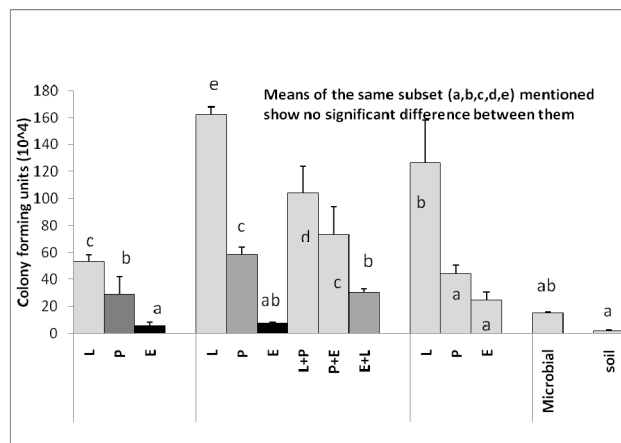


Fig. 5: Population of phosphate solubilizers in the earthworm products and controls.

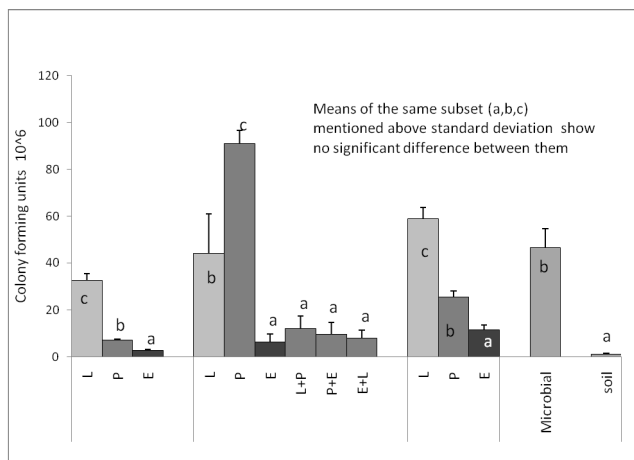


Fig. 3: Population of actinomycetes in the earthworm products and controls.

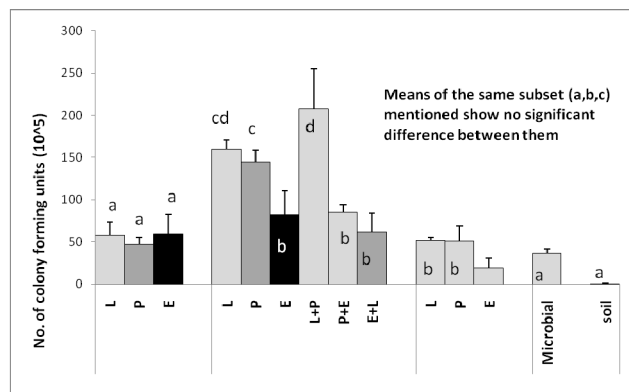


Fig. 6: Population of nitrogen fixers in the earthworm products and controls.

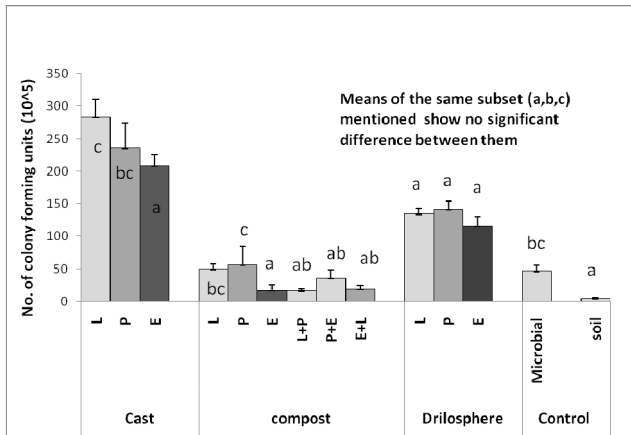


Fig. 7: Population of starch degraders in the earthworm products and controls.

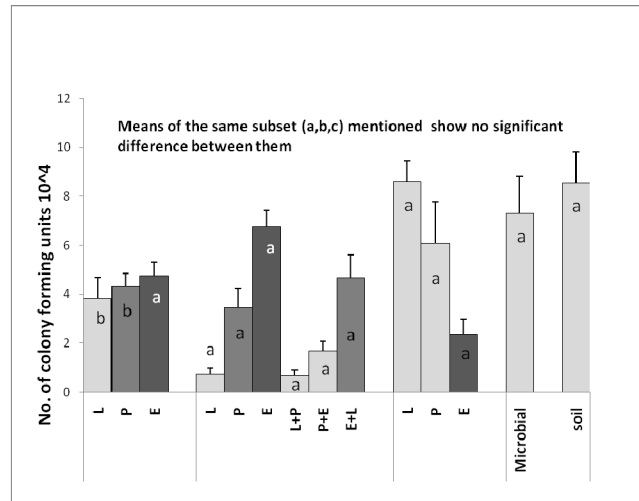


Fig. 10: Population of nitrifiers in the earthworm products and controls.

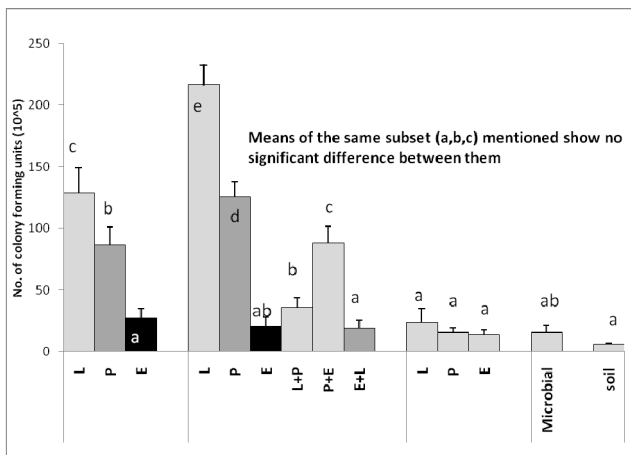


Fig. 8: Population of cellulose degraders in the earthworm products and controls.

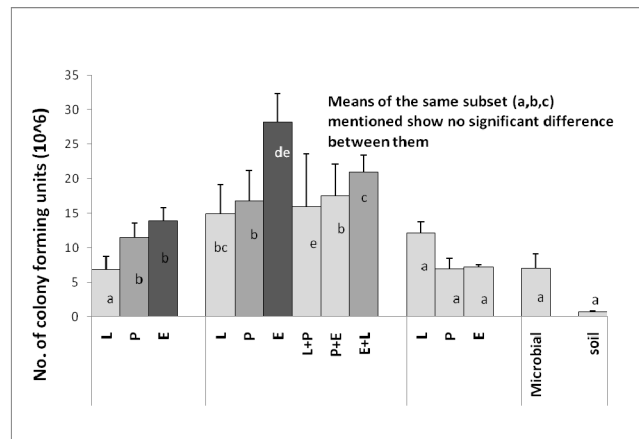


Fig. 11: Population of denitrifiers in the earthworm products and controls.

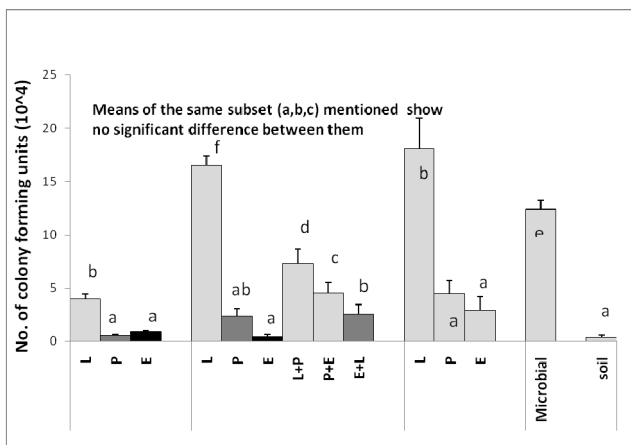


Fig. 9: Population of ammonifiers in the earthworm products and controls.

The compost of combinations (L+P; P+E; E+L) showed a lower population of both the carbon mineralizers (Figs. 7&8).

Nitrogen mineralizers: Nitrogen mineralizers were found to be high in the compost than cast and drilosphere soil (Table 2). Denitrifiers which are the nitrate and nitrite reducers were found to be higher than ammonifiers and nitrifiers (Figs. 9 & 11). *E. eugeniae* individually and in combinations showed a higher population of denitrifiers in its compost and castings (Fig. 11). The anecic species *L. mauritii* showed increased population of ammonifiers than the epigeics in its compost (Fig. 9). Drilosphere soil of *L. mauritii* possessed higher population of nitrogen mineralizers than *P. excavatus* and *E. eugeniae*.

Regression analysis between chemical parameters and microorganisms in vermicomposts, earthworm casts and drilosphere soils: In earthworm composts, pH is negatively

correlated with the fungal population, actinomycetes, nitrifiers and denitrifiers (Table 3). Total organic carbon is positively correlated with carbon mineralizers, total bacteria and biofertilisers, but negatively correlated with nitrifiers and denitrifiers. Total Kjeldahl nitrogen showed a positive correlation with nitrifiers, but negatively correlated with phosphate solubilizers and carbon mineralizers. Bacteria and actinomycetes along with carbon and nitrogen mineralizers except nitrifiers exhibited a negative correlation with C: N, however, nitrogen fixers showed a positive correlation which implies that when C: N ratio decreases the bacterial population increases (Table 3). In earthworm casts, as in vermicompost, fungi showed a negative correlation with pH. Actinomycetes, phosphate solubilizers and starch degraders showed a positive correlation with organic carbon but cellulose degraders showed a negative correlation. Carbon and nitrogen degraders including biofertilisers showed a negative correlation with C:N. Since earthworm cast is an unstable product, balance in C:N is attained only in compost which is a stable product (Table 4).

In drilosphere soils, pH shows a negative correlation with bacteria, phosphate solubilizers, starch degraders and nitrifiers. Organic carbon and total Kjeldahl nitrogen show a negative correlation with bacteria, fungi and starch degraders (Table 5). C:N in drilosphere soil shows a negative correlation with most of the microbial populations, except fungi and denitrifiers, revealing an increased population of fungi in a high C:N (Table 5).

DISCUSSION

Porosity is a critical physical characteristic which influence water, nutrient absorption and gas exchange by the soil nutrient system. Addition of vermicompost to soils increases water holding capacity, maintain evaporation losses to a minimum and works as a 'good absorbent' of atmospheric moisture due to the presence of colloidal materials such as the 'earthworm mucus' (Sinha & Herat 2012). Vermicast work as 'micro-dams' storing hygroscopic and gravitational water. The water stable aggregates of polysaccharide gums' produced by the bacteria inhabiting the intestine of earthworms increase the general entry of water into the soil and infiltration due to construction of cemented 'macro-pores' (Bhandari et al. 1967, Munnoli & Bhosle 2011). Compost of epigeic species is known to enhance water holding capacity, based on which *P. excavatus* and *E. eugeniae* show higher water holding capacity than anecic *L. mauritii*. However the endemic *P. excavatus* being an ecotype has appropriate cast size which predominantly hold water compared to the compost of *E. eugeniae*, *L. mauritii* and *P. excavatus* with extraordinary niche specialization of being anecic and

epigeic respectively associated with regions of overlap of the two species show maximum WHC in its compost, proving the importance of endemic ecotypes in soil ecosystem. The microbial compost (C), though possesses high organic matter (% C and N) (Table 2), demonstrates low WHC than other vermicompost samples due to the absence of earthworm activity (Fig. 1). This is of great agronomic significance as the groundwater table is rapidly depleting throughout the world (Sinha & Herat 2012). Stockdill & Lossens (1966) reported that earthworms increase the water holding capacity of New Zealand soils by 17 %.

The pH of the compost of all the three species was in the neutral range and EC below salinity hazards is in agreement with Nagar et al. (2004) (Table 1). The initial C/N ratio of the substrate in the present study was 28, which is the optimum range for composting substrate (Fong et al. 1999). Earthworm's contribution to balance the C/N ratio is also supported by the balanced population of carbon and nitrogen mineralizers (Figs. 7-11) in all their products which is enhanced by earthworm activities such as communitation and digestion of the organic matter. This multiplies and activates dormant microorganisms due to the presence of simple organic carbon in the mucus of earthworms (Brown 1995). Vermicompost from *L. mauritii* individually and in combination with *P. excavatus* species showed a balanced C:N ratio than the exotic epigeic species (Table 1). This not only highlights the communitation and breakdown of organic matter by *L. mauritii*, but also emphasizes the increased carbon mineralizers and formation of stable humus. Though *E. eugeniae* compost showed a high C/N ratio the carbon and nitrogen values are low. Phosphorus is also observed to be high in *P. excavatus* (Table 1).

Organic carbon is an important parameter in compost, which determines the C:N ratio of the compost. A rational amount of carbon should be maintained in the compost for an ideal C:N ratio as high C leads to increased microbial respiration (Pfeiffer 1984). Hendrix et al. (1992) reported a positive correlation between soil organic C and earthworm abundance. However, the abundance of *E. eugeniae* was found to be negatively correlated with the total organic C (Mainoo et al. 2008). In this study, *E. eugeniae* compost demonstrates lower organic carbon since most of the organic carbon in the substrate is converted to its biomass (Dhakshayani et al. 2013).

Microorganisms: Microorganisms are the ultimate decomposers and mineralizers in the detritus food chain and in organic matter decomposition. Earthworms have been found to stimulate bacterial activity (Daniel & Anderson 1992, Ruz- Jerez et al. 1992, Li et al. 2002) and facilitate dormant microorganisms in soils providing them with sim-

Table 3: Regression (r) values showing relationship between chemical parameters and microbes in vermicompost of all the three species.

Microbes in casts	pH	OC	TKN	C:N	P ₂ O ₅
Bacteria	0.269	1.176	-0.185	-0.478	-0.144
Fungi	-0.012	-0.766	0.005	0.447	1.367
Actinomycetes	-0.032	1.483	0.066	-0.138	-0.759
Phosphate solubilizers	0.268	0.991	-0.930	-1.381	0.399
Nitrogen fixers	0.082	0.327	1.145	0.313	-0.684
Starch degraders	0.241	1.458	-0.450	-0.824	-0.285
Cellulose degraders	0.306	1.403	-0.348	-1.190	-0.715
Ammonifiers	0.169	1.181	-0.817	-1.202	0.038
Nitrifiers	-0.346	-0.805	1.393	1.775	-0.795
Denitrifiers	-0.253	-0.111	-0.796	-0.525	0.376

Table 4: Regression(r) values showing relationship between chemical parameters and microbes in earthworm casts of all the three species.

Microbes in casts	pH	OC	TKN	C:N	P ₂ O ₅
Bacteria	0.42	-1.790	0.818	0.945	0.692
Fungi	-1.927	-10.230	-4.676	-0.320	12.755
Actinomycetes	0.105	5.249	2.056	-0.543	-7.092
PO ₄ solubilizers	0.483	5.981	0.724	-1.000	-5.262
Nitrogen fixers	0.708	4.246	1.21	-0.512	-4.246
Starch degraders	0.183	7.838	1.751	-1.373	-9.446
Cellulose degraders	-0.605	-3.263	1.035	0.165	1.23
Ammonifiers	0.341	3.663	1.074	-0.291	-3.893
Nitrifiers	0.789	2.305	1.437	0.432	-2.147
Denitrifiers	0.133	0.049	-4.606	-2.114	4.324

Table 5: Regression (r) values showing relationship between chemical parameters and microbes in drilosphere soil of all the three species.

Microbes in casts	pH	OC	TKN	C:N	P ₂ O ₅
Bacteria	-0.257	-0.139	-0.502	-0.706	-0.745
Fungi	0.011	-1.732	-0.249	0.2	2.523
Actinomycetes	0.043	5.051	1.561	-0.781	-6.657
PO ₄ solubilizers	-0.109	3.49	0.89	-0.511	-4.150
Nitrogen fixers	0.242	7.629	2.692	-1.268	-9.544
Starch degraders	-1.227	-8.869	-1.975	-0.397	7.882
Cellulose degraders	0.447	4.768	2.818	-1.009	-8.543
Ammonifiers	0.347	4.261	1.63	-0.447	-5.645
Nitrifiers	0.752	7.149	0.507	-0.787	-5.962
Denitrifiers	-0.563	1.24	2.428	0.234	-4.955

ple organic carbon, optimum temperature, moisture and pH in their gut for their multiplication (Brown 1995).

Among the three products of earthworms studied compost showed a higher population of major microorganisms with a higher heterotrophic bacteria and actinomycetes (Table 2). Pfeiffer (1984) reported that compost forms a stable humus and hence microbes also attain stability. This is further substantiated by the increased number of biofertilisers in the compost from the combination of endemic species *L. mauritii* and *P. excavatus* than the exotic species which according to Priscilla (2005), decomposers are succeeded

by the biofertilisers in organic matter decomposition. Also among the compost from the individual units *L. mauritii* showed higher population of biofertilisers which proves that the endemic anecic species that are the ecotypes of the soil support the population of biofertilisers.

Microorganisms are excreted through their casts and also harboured in their drilospheres (Coq et al. 2007). Subsequent to composts, major microbial groups are found to be high in drilosphere soil than castings and the sum of population of biofertilisers is almost similar in both drilosphere soil and casts (Table 2). This is because the burrows created

by the earthworm also have their excretory products such as casts, mucus and coelomic fluid, which help in the replenishment of microorganisms and constant release of nutrients into it. Several advantages have also been documented through the physical properties of drilospheres by Brown (1995). Loquet et al. (1977) reported that casts and gallery walls of the earthworms have a greater microbial activity than the surrounding soil, and microbial (enzyme) activity is extended to deeper layers in the presence of earthworms. Improved aeration and good water availability also improves microbial activity. *E. eugeniae* as an exotic epigeic species showed a low microbial population as it creates horizontal burrows compared to the endemic species *P. excavatus*.

Casts showed a higher population of microbes in *L. mauritii* than the epigeics. Cast is an unstable product and microbes in the cast are stabilised during maturation to form compost. Borowski (1995) reported that the release of many unstable products and microorganisms from the casts is stabilized in the soil processed by the earthworms.

When the three species of earthworms are compared, *L. mauritii*, the endemic anecic earthworm showed higher population of biofertilisers in its compost followed by drilosphere soil as it makes vertical and horizontal burrows (Ismail 2005) and due to its thorough kneading of organic matter being an anecic earthworm. This property facilitates the contribution of anecic earthworms to fine soil texture and soil formation proving the significance as nature's living plough (Darwin 1881). Even among the drilospheres and casts, *L. mauritii* showed a higher population of biofertilisers (Table 2). *Eudrilus eugeniae* showed a very low population of biofertilisers (Fig. 5) in drilosphere soil as it makes only horizontal burrows and improper mineralization. The very important purpose of using vermicompost into the agricultural field is to inoculate microbial fertilizers into it, which may not be fulfilled by solely adding *E. eugeniae* compost.

Earthworms play an important role in C and N dynamics and that agroecosystem management greatly influences the magnitude and direction of their effect (Fonte et al. 2007). Carbon mineralizers are the chief microbial population in earthworm casts and are high in *L. mauritii* (Table 2) because of the conduit environment it provides. These high C mineralizers in the casts and compost of *L. mauritii* contribute to the decreased C:N ratio in its compost compared to the other species of earthworms. C mineralizers in *E. eugeniae* compost are lower than in L+P compost. This proves the availability of lesser nutrients in *E. eugeniae* compost. Starch degraders are high in the casts of all the three species than compost, but cellulose degraders are very high in the compost. This is because cellulose is a complex molecule and degradation starts after the depletion of starch

by the action of microbes and the earthworms give a conduit environment to it. In drilosphere soil and casts starch degraders are more than cellulose degraders, which imply cellulose degraders, succeed earthworm activity and colonizes more in compost. Drilosphere soil and casts of *E. eugeniae* show decreased C mineralizers than even composts of the other two earthworms. *E. eugeniae* products due to lack of nutrients do not support the succession of cellulose decomposers.

Nitrogen mineralizers especially denitrifiers are found to be higher in the compost of all the three species of earthworms (Table 2). *E. eugeniae* compost showed a higher population of denitrifiers (Fig. 11) but very low population of ammonifiers than *L. mauritii* (Fig. 9) because of the continued mineralization process. Nitrifiers are also high in the compost of epigeic species especially *E. eugeniae* than *P. excavatus*. It is a complex result to be justified because in nature different species of earthworms may indeed exhibit different feeding preferences, due to spatial and trophic niche specialization, and the denitrification rates of the casts could be a reflection of the quality of organic C ingested. Even though worms provide favorable micro-sites for denitrification, the increased NO_3^- pools reported in the casts are not markedly affected over the short term (Parkin & Berry 1994). It is yet to be proved that the N_2O and N_2 formed due to earthworm activity are due to increased mineralization by microorganisms or by the enhancement of denitrifiers by specific species of earthworms. However, the presence of high amounts of nitrogen fixers especially in endemic earthworm composts helps in immediate fixation of nitrogen into the soil (Fig. 6). But it is obvious that *E. eugeniae* contributes to fast removal of NO_3^- , the available form of nitrogen to plants. The casts of *E. eugeniae* also show increased denitrifiers which is transmitted in the compost.

CONCLUSION

Soil being a habitat offers spatial as well as trophic niches to the biota. Epigeic species predominantly are phytophagous while anecics are geophytophagous distinctly becoming non-competitive with each other. This enables the coexistence of the endemic *P. excavatus* and *L. mauritii* as they amicably share spatial, as well as trophic niches, while the exotic *E. eugeniae* becomes a competitor to the established spatial and trophic niche of *P. excavatus*. In an ecosystem with anecic earthworms feeding on the organic matter the drilosphere is considered as one of the important locations of soil microbial activity. The drilosphere is in closest proximity to the rhizosphere, thereby the plant animal interaction in the soil is reflected by the rhizosphere-drilosphere interactions. Hence, endemic earthworms with conspicuous drilospheres enable a healthy rhizosphere to support plant

growth than just addition of organic matter on soil surface. As ecotypes and amicable coexisting species *L. mauritii* and *P. excavatus* with extraordinary niche specialization of being anecic and epigeic respectively associated with regions of overlap of the two species show maximum WHC, predominant population of biofertilisers and major microorganisms proving the importance of endemic ecotypes in soil ecosystem. This condition reveals an “edge concept” by illustrating rich microflora, physical and chemical characteristics on the worm worked soil at the boundary of two ecological niches. The edge habitat allows for greater biodiversity which is proved in the synergistic relationship between the endemic earthworms of India. So it is thus advisable to introduce endemic earthworms *L. mauritii* and *P. excavatus* in south Indian soils than introducing *E. eugeniae* which does competitive inhibition of endemic earthworms and has relatively low nutrients and microbial populations than the endemic earthworms, though its vermicompost would appear to be useful for the fertility of agricultural lands.

REFERENCES

- Bhandari, G.S., Randhwa, M.S. and Naskina, M.S. 1967. Polysaccharide contents of earthworms casts. *Current Sci.*, 36: 519-520.
- Borowski, E. 1995. Response of tomatoes to $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ applied to sand, loam, and soil substrate. *Annales Universitatis Marie Curie-Sklodowska*, 3: 111-118.
- Brown, G.G. 1995. How do earthworms affect microfloral and faunal community diversity? *Plant Soil*, 170(1): 209-231.
- Coq, S., Barthès, B.G., Oliver, R., Rabary, B. and Blanchart, E. 2007. Earthworm activity affects soil aggregation and organic matter dynamics according to the quality and localization of crop residues - An experimental study (Madagascar). *Soil Biol. Biochem.*, 39(8): 2119-2128.
- Daniel, O. and Anderson, J.M. 1992. Microbial biomass and activity in contrasting soil materials after passage through the gut of the earthworm *Lumbricus rubellus* Hoffmeister. *Soil Biol. Biochem.*, 24: 465-470.
- Darwin, C. R. 1881. The Formation of Vegetable Mould Through the Action of Worms with Observations on Their Habits. John Murray, London.
- De Vliegheer, W. and Verstraete, W. 1997. The effect of *Lumbricus terrestris* on soil in relation to plant growth: Effects of nutrient-enrichment processes (NEP) and gut-associated processes (GAP). *Soil Biol. Biochem.*, 29: 341-346.
- Dhakshayani, C., Dawood, N. and Ismail, S.A. 2013. Interactions between endemic and exotic earthworms in India with respect to microbial populations of their compost. *Indian Journal of Environmental Sciences*, 17(1): 41-47.
- Fong, M., Wong, J.W.C. and Wong, M.H. 1999. Review on evaluation of compost maturity and stability of solid waste. *Shanghai Environ. Sci.*, 18(2): 91-93.
- Fonte, S.J., Konga, A.Y.Y., van Kessela, C., Hendrix, P.F. and Sixa, J. 2007. Influence of earthworm activity on aggregate-associated carbon and nitrogen dynamics differs with agroecosystem management. *Soil Biol. Biochem.*, 39(5): 1014-1022.
- Hanna, W.J. 1968. Methods of chemical analysis of soil. In: *Chemistry of the Soil* (Bear, F.E., ed), Oxford and IBH, New Delhi.
- Hendrix, P.F., Mueller, B.R., Bruce, R.R., Langdale, G.W. and Parmelee, R.W. 1992. Abundance and distribution of earthworms in relation to landscape factors on the Georgia Piedmont, U.S.A. *Soil Biol. Biochem.*, 24(12): 1357-1361.
- Ismail, S.A. 2005. *The Earthworm Book*. Other India Press, Mapusa, Goa, India, 101 pp.
- Keen, B. A. and Raczkowski, H. J. 1921. Relationship between clay content and physical properties of soils. *J. Agri. Sci.*, 11: 441-449.
- Kosmachev, A.E. 1960. The preservation of the viability of thermophile actinomycetes during prolonged storage. *Mikrobiologia*, 29: 287-288.
- Li, X., Fisk, M.C., Fahey, T.J. and Bohlen, P.J. 2002. Influence of earthworm invasion on soil microbial biomass activity in a northern hardwood forest. *Soil Biol. Biochem.*, 34: 1929-1937.
- Loquet, M., Bhatnagar, T., Bouché, M.B. and Rouelle, J. 1977. Essai d'estimation de l'influence écologique des lombrices sur les microorganismes. *Pedobiologia*, 17: 400-417.
- Mainoo, N.K., Whalen, J.K. and Barrington, S. 2008. Earthworm abundance related to soil physicochemical and microbial properties in Accra, Ghana. *African Journal of Agricultural Research*, 3(3): 186-194.
- Mariani, L., Jiménez, J.J., Asakawa, T., Thomas, J.R. and Decaëns, T. 2007. What happens to earthworm casts in the soil? A field study of carbon and nitrogen dynamics in Neotropical Savannas. *Soil Biol. Biochem.*, 39(3): 757-767.
- Munnoli, P. M. and Bhosle, S. 2011. Water-holding capacity of earthworms' vermicompost made of sugar industry waste (press mud) in mono- and polyculture vermireactors. *The Environmentalist*, 31(4): 394-400.
- Nagar, R., Joshi, N., Dwivedi, S. and Khaddar, V.K. 2004. A physicochemical and mycofloral profile of vermicompost in Tarai region of Himalaya in winter season. *Research on Crops*, 5(1): 51-54.
- Page, A.L. 1991. *Methods of Soil Analysis*. 2nd Edn. Am. Soc. Agron. & Soil Sci., Am., Madison, Wisconsin, USA.
- Parkin, T.B. and Berry, E.C. 1999. Microbial nitrogen transformations in earthworm burrows. *Soil Biol. Biochem.*, 31: 1765-1771.
- Parle, J.N. 1963. A Microbiological study of earthworm casts. *J. Gen. Microbiol.*, 31: 13-22.
- Pfeiffer, E.E. 1984. *Chromatography applied to quality testing*. Biodynamic Literature, Wyoming, Rhode Island, 44 pp.
- Pikovskaya, R.I. 1948. Mobilization of phosphate in soil in connection with the vital activities of some microbial species. *Microbiologiya*, 17: 362-370.
- Priscilla, J. 2005. Studies on the Microbiogeocoenose of Vermicompost and its Relevance in Soil Health. Ph.D. Thesis, University of Madras, Chennai, India, 131, pp.
- Ranganayaki, S. and Mohan, C. 1981. Effect of sodium molybdate on microbial fixation of nitrogen. *Zeitschrift für allgemeine Mikrobiologie*, 21(8): 607-610.
- Ruz-Jerez, B.E., Ball, P.R. and Tillman, R.W. 1992. Laboratory assessment of nutrient release from a pasture soil receiving grass or clover residues in the presence or absence of *Lumbricus rubellus* or *Eisenia fetida*. *Soil Biol. Biochem.*, 24: 1529-1534.
- Savin, M.C., Görres, J.H. and Amador, J.A. 2004. Microbial and microfaunal community dynamics in artificial and *Lumbricus terrestris* (L.) burrows. *Soil Sci. Soc. Am. J.*, 68: 116-124.
- Sinha, R.K. and Herat, S. 2012. Organic farming: Producing chemical-free, nutritive and protective food for the society while also protecting the farm soil by earthworms and vermicompost-reviving the dreams of Sir Charles Darwin. *Agricultural Science Research Journals (Review)*, 2(5): 217-239.
- Stockdill, S.J. and Lossens, G.G. 1966. The role of earthworms in pasture production and moisture conservation. *Proc. of New Zea-*

- land Grassland Association, pp. 168-183.
- Snyder, W.C., Nash, S.M. and Tujillo, E.E. 1959. Multiple clonal types of *Fusarium solani phaseoli* in field soil. *Phytopathology*, 49: 310-312.
- Teather, R.M. and Wood, P.J. 1982. Use of Congo Red-polysaccharide interactions in the enumeration and characterization of cellulolytic bacteria from the rumen. *Appl Environ. Microbiol.*, 43: 777-786.
- Waksman, S.A. and Martin, J.P. 1939. The conservation of the soil. *Science*, 90: 304-305.
- Weiss, R.F. and Price, B.A. 1980. Nitrous oxide solubility in water and sea water. *Marine Chem.*, 8: 347-359.
- Williams, S.T. and Davies, F.L. 1967. Use of a scanning electron microscope for the examination of actinomycetes. *Journal of General Microbiology*, 48(2): 171-177.
- Wollny, E. 1890. Untersuchungen Über die Beeinflussung der Fruchpbarkeit der ackerume durch die Tätigkeit der Regenwürmer. *Foch Agrik Physik*, 13: 381-395.
- Wood, P.J. 1980. Specificity in the interactions of direct dyes with polysaccharides. *Carbohydr. Res.*, 85: 271-287.