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EFFECTS OF TWO EARTHWORM SPP. *L. MAURITII* AND *D. BOLAUI* ON SOIL SYSTEM: A MICROBIAL AND BIOCHEMICAL ANALYSIS

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KEYWORDS

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ABSTRACT

Interactions between earthworms and microorganisms can be important in regulating the rate of soil carbon turnover and maintaining soil fertility in agroecosystems. Despite the significance of earthworms in nutrient cycling in agroecosystems, the indirect influence of earthworms on C assimilation by microorganisms has not been adequately quantified. We assessed microbial population in earthworm (*L. mauritii,* Kinberg and *D. bolaui*, Michaelsen) middens and surrounding soil collected from cropland agroecosystems and forest site respectively. First of all microbial population increased up to 21st day and their after they declined was observed in *L. mauritii* while in *D. bolaui* population was declined after 1st day. The Initial Bacterial population (number/g soil) were found to be $45.5 \pm 0.642 X10^9$ thereafter they increased to 51.7 ± 1.569 $X10^9$ in *L. mauritii* and in *D. bolaui* they declined from 55.5 ± 0.88 $X10^9$ to 19.5 ± 0.818 $X10^9$ on last day of observation. Bacterial population was always higher in midden than non ingested soil. Our results suggest that there were functional differences between microbial communities in earthworm middens and surrounding soil, probably due to a combination of physical, chemical, and biological changes in the midden microenvironment. Bacterial population in *L. mauritii* showed impact of aging and *D. bolaui* showed declining pattern. The resulting differences in microbial communities or activity increased microbial growth rates and assimilation of readily available C substrates in middens relative to surrounding soil.

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INTRODUCTION

There are contradictory data in the literature on how earthworms affect soil biochemical, microflora and microfauna characteristics. Previous studies have shown that earthworm activity promotes organic matter decomposition significantly (Parmelee *et al.*, 1990) and enhances mineralization and humification of soil organic matter. Moreover, there is a positive relationship between earthworm activity, soil respiration and nutrient cycling (Haimi and Einbork, 1992). There is abundant evidence demonstrating the importance of earthworm-microorganism interactions onsoil organic matter degradation and nutrient release (Lee, 1985). Earthworm activity can stimulate microbial activity in casts (Lavelle and Martin, 1992) and the presence of earthworms can modify soil microbial activity (Binet and Trehen, 1992).

Interactions between earthworms and microorganisms are important for many soil processes in agroecosystems (Edwards and Bohlen, 1996; Lavelle *et al.*, 1992; Lee, 1985). Earthworms facilitate soil carbon and nitrogen transformations through their influence on the soil microflora (Blair *et al.*, 1995; Martin *et al.*, 1992, 1991; Scheu, 1987). The passage of soil through earthworm gut changes its physiological properties and the level of microbial activity. Their effects include direct and incidental grazing, dispersal, competition, and potential mutualistic associations (Edwards and Bohlen, 1996; Blair *et al.*, 1995; Daniel and Anderson, 1992; Lee, 1985). Our laboratory studies have indicated that earthworm-microbial interactions increase soil carbon evolution, soil nutrient availability and microbial activity (Kumari *et al.,* 2009). These interactions influence soil nutrient availability probably through increasing the activity of the soil microbial biomass, while reducing its size, thereby increasing overall nutrient availability. The earthworm, *L. mauritii*, is unique among earthworms occurring commonly in north temperate agroecosystems, in forming permanent or semi-permanent vertical burrows with small patches of plant litter and casts called middens gathered around the burrow entrance. These middens can comprise a significant proportion of surface crop residues in agroecosystems with large populations of *L. mauritii* and affect the breakdown of crop residues and the spatial heterogeneity of residue microenvironments on the soil surface (Bohlen *et al.*, 1997). Earthworm middens in agroecosystems have been shown to have greater microbial activity than surrounding soil (Bohlen *et al.*, 1997; Subler, 1998). This potential for greater microbial turnover together with alteration of the quality of plant litter in the midden microenvironment (Bohlen *et al.*, 1997) suggests that the carbon assimilation efficiencies of microorganisms in the midden microenvironment may be greater than in the surrounding soil.

Earthworm middens present an ideal model for analysing animal-mediated influences on soil microbial communities by alteration of the patch structure of the microbial environment. We compared the microbial assimilation of carbon in earthworm middens and surface casts and adjacent undisturbed soils in order to determine the influence of small-scale disturbance by earthworms on the activity and turnover of microbial populations.

Our investigation focused on the effects of the digestion of *L. mauritii* and *D. bolaui* on soil microfauna and nutrient pools, studying the differences between midden and surrounding bulk soil in two different habitats.

MATERIALS AND METHODS

Soil sample and earthworm collection

L. mauritii (Kinberg) and *D. bolaui* (Michaelsen) earthworms were collected from different agro ecosystem sites in Ranchi, located between 21°58'N-25°19, NL and 83°20'E- 88°4'EL and at a height of 629m above mean sea level (MSL) and study was carried out in laboratory by culturing the earthworms in plastic container under oxygenated and moist condition. The middens were collected from the plastic container and used for microbial and enzymatic study.

Bacterial culture and isolation

Dilution plate method (Parkinson *et al.,* 1971) was used for estimating the bacterial population in soil sample. The isolation of bacteria from Eucalyptus and natural forest samples was initiated by taking 1g of sample and was diluted with 9mL of sterilized deionized water till 10^{-7} dilution. 1mL inoculums of the primary suspension was taken for bacteria culture in a petriplate (diameter = 100mm) containing CzapekDox agar (Thom and Raper, 1945) media (peptone - 10g/L, beef extract-10g/L, agar-15g/L NaCl-5g/ L, pH- 7.2) and were inoculated at 37°C for 48h. After that colony count were continued at every interval of 7 days till 42nd day. For each experiment, three replicates of petri dishes were incubated. The mean fresh weight of a bacterium cell was taken as 1.5×10^{-12}g (Toth and Hammer, 1977). This value, when multiplied with the number of bacterial colony gave the fresh weight of bacteria. Assuming 80% of bacterial cell to be water (Clark and Paul, 1970) dry weight of bacterial biomass was calculated (Satpathy *et al*., 1982). Student's test was done to determine the significance of change in population and biomass.

Physico - chemical estimation

Standard methods were followed to estimate the organic carbon (Walkley and Black, 1934), nitrogen content (Kjeldahl and Jackson method, 1973), potassium and phosphorus content of soil and midden was measured according to method described by Misra (1973) and pH was measured by pH meter.

Estimation of enzyme activity

Dehydrogenase activity

The dehydrogenase activity of the midden sample was measured following Casida *et al*. (1964) by the amount of triphenylformazan produced during the microbial reductions of 1% 2, 3, 5-triphenyl tetrazolium chloride (TTC). The incubation mixture contained 2g fresh soil saturated with 2mL of 1% TTC and 0.5mL of 1% glucose in a screw cap test tube. The contents were mixed thoroughly in sealed test tubes and were incubated at 32oC for 24h. Following incubation, the contents were stirred with 10mL methanol and the resulting slurry was washed into Buchner funnel (Whatman 30). The absorbance of the resulting filtrate was read at 485 nm using methanol as blank. The dehydrogenase activity was expressed in ìg formazan/g soil/h.

Cellulase activity

Cellulase activity in soil was measured using 3, 5 dinitosalicylic acid (Ross, 1965). 3g soil was mixed with 0.2mL toluene in flask and 6mL of Sorenson's buffer along with 6mL of substrate solution was added. After shaking they were placed in the incubator at 30°C. In control flask water was added instead of substrate and was centrifuged. Colour was developed by adding 3, 5 dinitrosalycilic acid solution to 1mL of supernatant. The reducing sugar forms a pink color, read at 540nm. The cellulose activity was expressed in µg glucose g^{-1} $soilh^{-1}$.

Urease activity

Urease activity in soil was estimated as per Tabatabai and Bremner (1972) except that NH3 released in incubation was determined colorimetrically by an indophenol reaction (Kaplan, 1969). Urease activity was expressed in µg $NH3g^{-1}soilh^{-1}$.

Phosphatase activity

Phosphatase activity in soil was measured according to Kramer and Yardei (1959). 1g of sample with 4mL of 0.25 M toluene and 1mL of p-nitrophenyl phosphate solution was added in the flask. The contents were mixed thoroughly in sealed flask and were incubated at 37°C. After 1h, 1mL of 0.5 M CaCl2 and 4mL of 0.5 M NaOH was added and soil suspension was filtered through filter paper (Whatman No. 12). The filtrate was transferred into colorimetric tube and the intensity was measured at 400nm.The phosphatase activity was measured in µg phenol g^{-1} $soilh^{-1}$.

RESULTS AND DISCUSSION

Physico- chemical properties of earthworm midden and adjacent soil have been presented in Table 1 and 2. The pH of the midden of *L. mauritii* and *D. bolaui* were observed that 7.6 and 7.4 which was suitable for microbial growth while in adjacent soil pH varies from 6.2 to 6.6.

Organic C (mg C/g) content of the midden of *L. mauritii* collected from cropland site was 9.48 ± 1.6 whereas in surrounding soil contained only 7.89 ± 1.32 organic carbon. In forest site organic C was 8.12 ± 1.8 and midden of *D. bolaui* collected from same site *i.e.* 9.02 ± 2.1. On the first day of observation nitrogen content in midden of *L. mauritii* was 0.65 ± 0.09 mg N/g which increased to 0.78 ± 0.08 and declined to 0.55 ± 0.04 while in D. bolaui 0.73 ± 0.12 which declined to 0.61 ± 0.07. Phosphate and potassium content were observed as 4.51 ± 0.47, 4.15 ± 0.31 g P/m^2 and 17.8 ± 2.02, 16.8 ± 2.2 K/m^2 respectively in *L. mauritii* and *D. bolaui*. A large no. of nutrient (N, P and K) are easily assimilable by plant in fresh cast depositions (Bhadauria and Ramakrisnan, 1989). Most of these nutrients are derived from earthworm urine and mucus (Barois and Lavelle, 1986).

The microbial population was significantly greater in the earthworm middens than in adjacent soil (Fig. 1 and 2). The microorganisms in the earthworm middens may have assimilated a greater proportion of their total C from cropland residues than microbes living in surrounding soil, which lacked surface residues.

Alternatively, earthworms could have affected the assimilable of C in the midden environment directly. Investigation of the 13C enrichment of earthworms, from a variety of habitats, revealed that earthworms were enriched more in 13C than

Table 1: Physico- chemical parameters of cropland soil and midden of *L. mauritii*

Cropland Soil							
pH	6.37 ± 0.21	6.39 ± 0.39	6.81 ± 0.48	7.02 ± 0.52	6.92 ± 0.98	6.58 ± 0.65	6.30 ± 0.59
Org. C(mg C/g)	8.31 ± 1.92	8.20 ± 0.98	7.92 ± 1.02	8.42 ± 1.08	7.97 ± 1.58	7.92 ± 0.99	7.89 ± 1.32
Nitrogen (mg N/g)	0.58 ± 0.11	0.62 ± 0.09	0.63 ± 0.08	0.64 ± 0.19	0.59 ± 0.11	0.57 ± 0.07	0.55 ± 0.08
Phosphorus (g P/hec.)	3.29 ± 0.97	3.25 ± 0.52	3.19 ± 0.67	3.15 ± 0.72	3.18 ± 0.51	3.09 ± 0.39	3.05 ± 0.48
Potassium (g K/hec.)	16.1 ± 2.31	15.9 ± 1.57	15.5 ± 1.82	15.6 ± 1.78	15.5 ± 1.41	15.4 ± 1.52	15.2 ± 1.63
Midden of *L. mauritii*							
pH	7.31 ± 0.68	7.42 ± 0.57	7.51 ± 0.52	7.68 ± 0.51	7.31 ± 0.62	7.28 ± 0.72	7.11 ± 0.78
Org. C(mg C/g)	9.48 ± 1.12	9.42 ± 1.18	9.31 ± 1.14	8.98 ± 1.19	8.78 ± 1.26	8.52 ± 1.14	8.58 ± 1.16
Nitrogen (mg N/g)	0.65 ± 0.09	0.68 ± 0.06	0.69 ± 0.05	0.78 ± 0.08	0.63 ± 0.07	0.59 ± 0.05	0.55 ± 0.04
Phosphorus (g P/hec.)	4.51 ± 0.47	4.32 ± 0.56	4.28 ± 0.72	4.19 ± 0.78	3.92 ± 0.68	3.87 ± 0.69	3.75 ± 0.49
Potassium (g K/hec.)	17.8 ± 2.02	17.5 ± 1.96	16.9 ± 1.72	16.6 ± 1.46	16.2 ± 1.81	16.8 ± 1.56	15.8 ± 1.72

Table 2: Physico- chemical parameters of forest soil and midden of *D. bolaui*

Forest soil							
pH	6.53 ± 0.25	6.51 ± 0.32	6.42 ± 0.27	6.21 ± 0.19	6.08 ± 0.20	5.82 ± 0.18	5.71 ± 0.22
Org. C(mg C/g)	8.12 ± 1.89	8.21 ± 1.56	7.96 ± 0.95	7.87 ± 1.65	7.82 ± 1.74	7.76 ± 1.21	7.80 ± 1.32
Nitrogen (mg N/g)	0.62 ± 0.11	0.64 ± 0.21	0.59 ± 0.15	0.61 ± 0.12	0.57 ± 0.11	0.54 ± 0.13	0.51 ± 0.12
Phosphorus (g P/hec.)	2.91 ± 0.15	3.32 ± 0.12	3.31 ± 0.16	3.26 ± 0.41	3.33 ± 0.26	3.05 ± 0.24	2.81 ± 0.21
Potassium (g K/hec.)	15.8 ± 0.92	16.1 ± 1.12	16.5 ± 1.05	15.4 ± 0.98	15.2 ± 1.21	15.1 ± 0.88	14.8 ± 0.76
Midden of D. bolaui							
pH	7.45 ± 0.49	7.32 ± 0.36	7.41 ± 0.51	7.36 ± 0.39	7.21 ± 0.24	7.05 ± 0.36	7.02 ± 0.37
Org. C(mg C/g)	9.02 ± 2.12	8.98 ± 1.93	8.75 ± 1.72	8.52 ± 1.86	8.42 ± 1.79	8.31 ± 1.62	8.21 ± 1.68
Nitrogen (mg N/g)	0.73 ± 0.12	0.76 ± 0.09	0.71 ± 0.11	0.69 ± 0.05	0.68 ± 0.08	0.61 ± 0.07	0.63 ± 0.04
Phosphorus (g P/hec.)	4.15 ± 0.31	4.10 ± 0.42	4.05 ± 0.35	4.02 ± 0.36	3.95 ± 0.39	3.91 ± 0.38	3.85 ± 0.33
Potassium (g K/hec.)	16.8 ± 2.24	17.5 ± 2.42	17.7 ± 2.26	16.9 ± 1.95	16.5 ± 1.87	16.2 ± 1.82	16.1 ± 1.72

their putative food sources (Neilson *et al.,* 2000). It is possible that such enrichment of earthworm tissue, if it occurred at our site, may have increased the C enrichment of readily-assimilable C sources in the midden environment, in the form of mucus and other earthworm excretions.

In addition to this mixing effect, mucus production associated with water excretion in the earthworm gut is known to enhance the activity of microorganisms (Barois, 1987). This is followed by the production of organic matter. So fresh middens show high nutrient contents (Table 1) over longer period of time, this enhance microbial activity decreases when the cast dry and aggregation is then reported physically protect SOM against mineralization thus C mineralization rate decreases and mineralization of SOM from casts may be blocked for several months (Martin, 1991; Lavelle and Martin, 1992). EWs are known also to increase N mineralization, through direct and indirect effect on the microbial community. N mineralization by microflora is also quite increase in the EWs gut and continues for several homes in fresh cast (Blair *et al.*, 1997; Bossuyt *et al.*, 2005). This result thus highlights the important effects that EWs have on C and N cycling processes in agro- ecosystem.

The bacterial population in earthworm midden of *L. mauritii*, in the beginning was 45.5± 0.64 X 10^9 which gradually increases to 47.0± 1.006 X 10^9 and 49.2± 0.929 X 10^9 and reaching at its maxima as 51.7 ± 1.569 X 10^9 on 7th, 14th and 21st day respectively (Fig.1). There after a sharp decline in bacterial population was observed. The percentage increase in bacterial population over initial population was recorded as 3.29%, 8.13%, 13.62% on 7^{th}, 14^{th} and 21^{st} day while the decrease was more pronounced as 32.96%, 40.21% and 58.24% over initial population on 28^{th}, 35^{th} and 42^{th} day respectively. The change in population was found to be significant at $p < 0.001$. Impact of aging of earthworm midden in context of bacterial population has been reported by Kumari *et al.,* 2009. In midden of *D. bolaui* initial bacterial population was 55.5 ± 0.88 X 10^9 thereafter they declined to 19.5 ± 0.81 X 10^9 on last day of observation while in forest oil initial bacterial population was 41.4 ± 1.13 X 10^9 (Fig. 2). The net effect of earthworms on the size of the soil microbial biomass has been a topic of some controversy in the literature. Several studies have shown that earthworms reduce microbial biomass, primarily by consumption, as soil passes through the earthworm gut (Wolters and Joergenson, 1992; Bohlen and Edwards, 1995; Zhang *et al.,* 2000). In contrast, other studies have found earthworm induced increases in microbial biomass (Shaw and Pawluk, 1986; Daniel and Anderson, 1992; Bohlen *et al.,* 1999). Brown *et al.* (2000) emphasize the importance of temporal and spatial scale when evaluating the effect of earthworm on the soil profile, suggesting that fresh earthworm midden behaves differently than aged midden. The changed behavior of fresh and old earthworm midden may primarily be due to various in bacterial population as the stability of midden increases with age at least for 3 weeks due to product of secretion by bacterial population. The population of bacteria in earthworm midden increased with the aging of the casts (Kumari *et al.,* 2009, 2011). Some physical properties and microbial activity of the casts of the earthworm *Aporrectodea caliginosa* have been investigated by Piekarz and and Lipiec (2001) and reported that 20 day old cast is more stable than fresh cast. An increase in microbial population and biomass has been recorded with aging of earthworm middens up to 21^{st} days which is in agreement with above finding.

Enzyme activities in earthworm midden and non midden soil

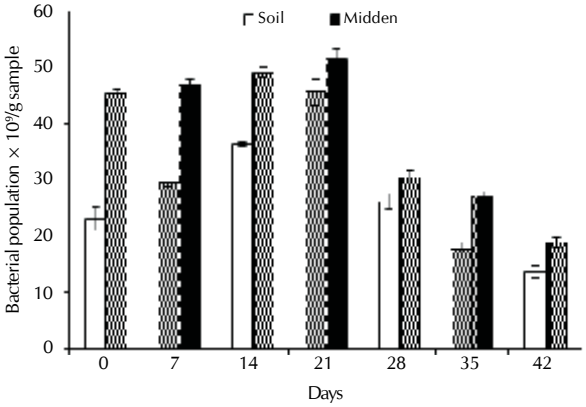

Figure 1: Bacterial population in cropland soil and midden of earthworm *Lampito mauritii*

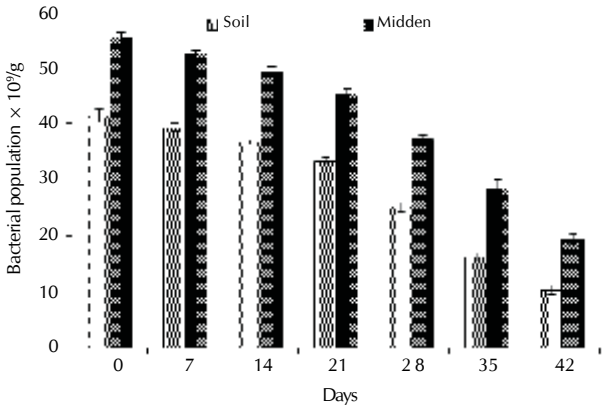

Figure 2: Bacterial population in forest soil and midden of earthworm *Dichogaster bolaui*

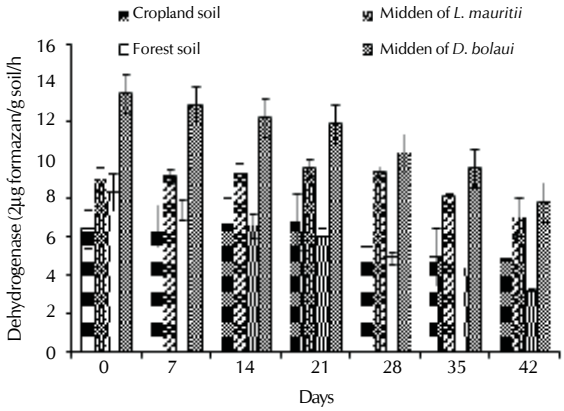

Figure 3: Dehydrogenase (µg formazan/g soil/h) activity of earthworm midden and non midden soil in different age

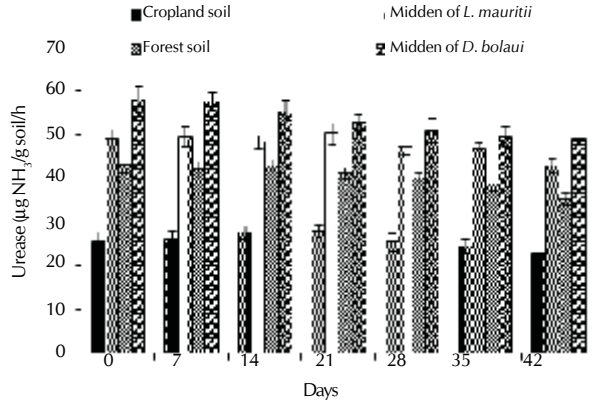

Figure 4: Urease (µg NH3g^{-1}soilh^{-1}) activity of earthworm midden from cropland and forest soil in different age

have been presented in Fig. 3 to 6. Maximum dehydrogenase activity (ìg formazan/g soil/h) was observed 13.46 ± 1.10 in midden of *D. bolaui* while in midden of *L. mauritii* 9.56 ± 1.48on 21st day thereafter they declined. Dehydrogenase activity 6.42 ± 0.99, 8.32 ± 0.98 were observed in cropland and forest soil respectively (Fig. 3). Dehydrogenase activity is widely used in evaluating the metabolic activity of soil microorganisms (Trevors, 1984; Pascual, 2002). Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors (Dick and Tabatabai, 1993). Urease activity (µg NH3g^{1}soilh^{-1}) varies from 43.12 ± 1.7 to 49.12 ± 2.4 in midden of *L. mauritii*. In forest soil Urease activity varies from 35.37 ± 1.48 to 43.37 ± 2.0. Lowest urease activity was observed in cropland soil while highest was in midden of *D. bolaui i*.e. 58.36 ± 3.03 (Fig. 4).On the 1st day of observation Phosphatase activity (µg phenol g^{-1} soilh^{-1}) was 55.84 ± 2.38which increased to 59.72 ± 2.96 on 21st day and thereafter they declined to 53.07 ± 2.5 on 42nd day in midden of *L. mauritii*. In D. bolaui phosphatase activity varies from 62.46 ± 3.4 to 68.99 ± 3.52 (Fig. 5). Initial cellulase activity in midden of *L. mauriti* was 53.12 ± 1.84 on 21st day and thereafter they sharply declined to 48.02 ± 2.6on last day of observation and highest cellulase activity was observed in *D. bolaui* (Fig. 6). Bityutskii *et al.*, 2005 described the species composition and the activity of enzyme in coprolites and excreta of earthworms (*L. terrestris, A. caliginosa* and *E. fetida*). Parthasarathi and Ranganathan (2000) reported that cellulase, invertase, protease and phosphatase activities in fresh cast 15 and 30 day old casts of *Lampito mauritii* and *Eudriluse ugeniae* decreased considerably with cast age. Soil cast, middens have been shown to have enhanced microbial and enzyme activities and micro and macro nutrients (Lavelle and Martin, 1992).

Higher activities of cellulase, urease, phosphatase and dehydrogenase in the wormcasts have been reported (Edwards and Bohlen, 1996; Sharpley and Syers, 1976). Bonmati *et al.* (1985) observed that soil phosphatase activities were more marked, probably reflecting substantial greater microbial group due to the presence of easily decomposable organic compounds. A great variety of enzymes are produced by soil microorganisms, during their metabolism (Acosta-Martinez and Tabatabai, 2000). Soil phosphatase hydrolyse phosphate and make it available to plants. Thus phosphatase activity measurement provides an index of potential availability of phosphatase in soil (Mansell *et al.*, 1981). The increased amount of inorganic P released during cast deposition was related to and proceded by increased microbial and phosphatase activity (Sharpley and Syers, 1976). Enhanced phosphate content in the soil and presumed casta of *Lampito*

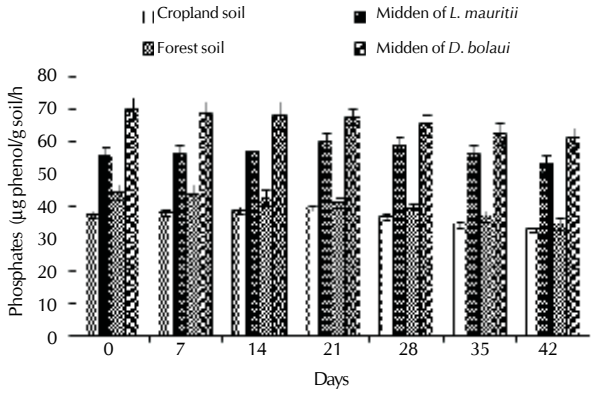

Figure 5: Phosphatase (µg phenol g^{-1} $soilh^{-1}$) activity of earthworm midden from cropland and forest soil in different age

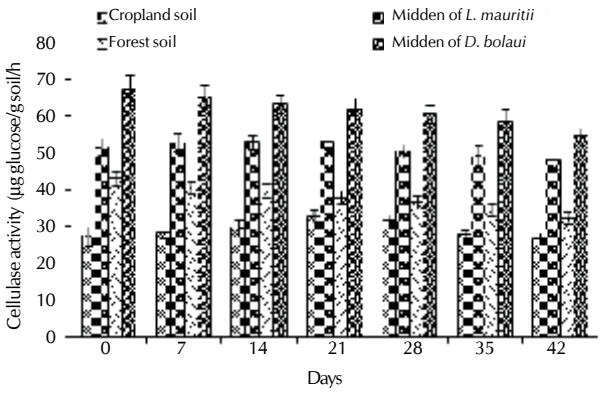

Figure 6: Cellulase (µg glucose g^{-1} $soilh^{-1}$) activity of earthworm midden *L. mauritii* and *D. bolaui* from cropland and forest soil in different age

mauritii and *Eudriluse ugeniae* has been reported (Parthasarathi and Ranganathan, 1999). In the case of Drawida calebi, the enzyme activities initially increased and thereafter they declined due to age of midden (Kumari and Sinha, 2012). It is supposed that the contribution of earthworms to the formation of soil humic acids depends on the ability of particular species to decompose organic matter and induce polyphenol oxidase activity. Enhancement of the activities of these enzymes could be ascribed to the nutrient rich substrate, active microbial population and optimal moisture conditions. Aged midden showed reduced enzyme activities because of decreased moisture content, lower nutrient concentrations and a decline in microbial activity.

Microbial communities in earthworm middens may have been primed to be more responsive

to additions of readily assimilable C and may have responded more rapidly to the acetate addition than microorganisms in surrounding bulk soils. The greater amounts of organic matter in the earthworm midden environment and possibly, the increased mineral N content (Subler, 1998) could have influenced assimilation of carbon. Modification of the physical structure of soil in the midden environment may have influenced microbial uptake and/or assimilation of carbon. Earthworms can increase the proportion of macroaggregates in soil and middens (Shaw and Pawluk, 1986). Collectively, these indirect influences of earthworms on the microhabitat of the midden environment may have created favorable conditions for microbial growth and assimilation of new carbon sources.

The results of this experiment suggest that earthworms mediate small-scale patchiness of the soil microenvironment by redistributing soil microbial biomass and activity in space and thereby contribute to spatial heterogeneity in soil nutrient processes (Culver and Beattie, 1980, 1983). These earthworm-mediated controls on the spatial patchiness of soil processes in agroecosystems may be important where there are large populations of *L. mauritii* and may be especially significant in minimum or no-tillage row crop ecosystems where crop residues are maintained on the soil surface.

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