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Fertility measures of biogenic structures in particular reference to bacterial community

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ABSTRACT

The present study aims to analyze the interaction of prevailing biotic pressure on soil environment with emphasis on its physicochemical and microbiological characteristics determining fertility status of biogenic structures in comparison to soil. The experimental results revealed that the physico-chemical characteristics (viz., pH, C, N, P, K) of midden were higher in comparison to soil. Middens with high N mineralization potential tend to be inherently fertile. Bacterial population increased up to 21st day and there after declined sharply in both midden and soil samples with higher population in the former. Highest microbial count showed a positively significant correlation with enzyme activitiy in earthworm midden. The biogenic structure showed to be beneficial for soil.

Key words: Fertility, Physico- chemical, Enzyme, Midden

INTRODUCTION

Earthworms are considered as soil engineers because of their effects on soil properties and their influence on the availability of resources for other organisms, including microorganisms and plants. Protection of the soil habitat is the first step towards sustainable management of its biological properties that determine long-term quality and productivity. Earthworms modify the soil environment indirectly by the accumulation of their biogenic structures (casts, midden, pellets etc) [1]. The biogenic structures constitute assemblages of organo-mineral aggregates which show much more microbial activity than soil [2,3]. There are increasing evidences to show that soil macro invertebrates play a key role in SOM transformations and nutrient dynamics at different spatial and temporal scales through perturbation and the production of biogenic structures for the improvement of soil fertility and land productivity [4,5]. Earthworms play a major role in soil nutrient dynamics by altering the soil physical, chemical and biological properties. Their casts, burrows and associated middens constitute a very favourable microenvironment for microbial activity [6,7]. They affect nutrient cycling by modifying soil porosity [8,9] and aggregates structure, (*i. e.* biogenic) [10,11] changing the distribution and rates of decomposition of plant litter and altering the composition biomass and activity of soil microbial communities [12]. In the short term, a more significant effect is the concentration of large quantities of nutrients (N, P, K, and Ca) that are easily assimilable by plants in fresh cast depositions [13]. Most of these nutrients are derived from earthworm urine and mucus [14]. Earthworms middens are reported as the central spots of microbial activity and nutrient dynamics and represent a suitable model for studying earthworm mediated influences on soil microbial communities by alteration of the patch structure of the

microbial environment [15]. As there is paucity of information on nutrient dynamics of biogenic structures in context of microbial population and correlated enzymatic activity, in the present paper an attempt has been made to study it.

MATERIALS AND METHODS

Soil sample collection

The soil samples from the agro ecosystem of Ranchi, located between $21^0$58'N - $25^0$19, NL and $83^0$20'E- $88^0$4'EL, Jharkhand were collected 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, enzymatic and nutrient content study.

Bacterial culture and isolation

Dilution plate method [16] was used for estimating the bacterial population in midden and soil. The isolation of bacteria from soil samples was initiated by taking 1g of sample and was diluted with 9 mL of sterilized deionized water till 10^{-7} dilution. 1 mL inoculums of the primary suspension was taken for bacteria culture in a petriplate (diameter = 100mm) containing Czapek Dox agar [17] 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 42^{nd} day.

Physico - chemical estimation of soil and midden

Standard methods were followed to estimate the organic carbon [18], nitrogen content [19], potassium and phosphorus content of soil and midden was measured according to method described by Misra [20] and pH was measured by pH meter.

Estimation of enzyme activity

The dehydrogenase activity of the sample soil was measured following Casida *et al.* [21] by the amount of triphenyl formazan produced during the microbial reductions of 1% 2,3,5-triphenyl tetrazolium chloride(TTC). The incubation mixture contained 2 g fresh soil saturated with 2 mL of 1% TTC and 0.5 ml of 1% glucose in a screw cap test tube. The contents were mixed thoroughly in sealed test tubes and were incubated at 32°C for 24 h. Following incubation, the contents were stirred with 10 mL 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 µg formazan/g soil/h.

RESULTS AND DISCUSSION

Physicochemical properties of soil and earthworm midden have been presented in Table 1. The pH of the midden was observed that 7.5 which was suitable for microbial growth. The levels of soil organic carbon 5.79±0.9 mg C/g and 12.32±1.12 mg C/g in midden was observed. Initially organic carbon was increased and gradually decreased (Table 1). On the first day of observation nitrogen content in soil was 0.57±0.12 mg N/g and no more variation was found. On 21^{st} day of observation phosphorus content was 6.53±0.62 g P/m^2, 3.12±0.52 g P/m^2 in midden and soil respectively (Table 1). Earthworms are known to accelerate plant residue decomposition in the tropics [22] and play a role in converting plant residue into soil organic matter [23,24]. Earthworms influence the supply of nutrients through their tissues but largely through their burrowing activities; they produce aggregates and pores (*i.e.*, biostructures) in the soil and/or on the soil surface, thus affecting its physical properties, nutrient cycling, and plant growth [8,10]. The biogenic structures constitute assemblages of organo-mineral aggregates. Their stability and the concentration of organic matter affect soil physical properties and SOM dynamics. The effect of earthworms on the dynamics of organic matter varies depending on the time and space scales considered [25]. The activity of endogeic earthworms in the humid tropical environment accelerates initial SOM turnover through indirect effects on soil C as determinants of microbial activity. Due to selective foraging of organic particles, gut contents are often enriched in organic matter, nutrients, and water compared with bulk soil and can foster high levels of microbial activity [26,27]. A similar result has been observed by analysis of midden and soil. The results indicated that the spatial variation of the soil parameters, and in particular the content of organic C, had a major influence on the variability of the bacterial population. They have been reported to enhance mineralization by first fragmenting SOM and then mixing it together with mineral particles and microorganisms, and thereby creating new surfaces of contact between SOM and microorganisms [28]. The study indicates higher concentration of nutrients in fresh midden. Bhaduria and

Ramakrishna [13] reported a similar trend in NPK in fresh cast. Most of these nutrients are derived from earthworm urine and mucus [14]. In highly leached soils of humid tropics, earthworm activity is beneficial because of rapid incorporation of the detritus into the soils [29]. In addition to this mixing effect, mucus production associated with water excretion in the earthworm gut is known to enhance the activity of microorganisms [30]. This is followed by the production of organic matter, so fresh casts show high nutrient contents (Table 1). The chemical characteristics of casts differ from those of non ingested soil [30] and are rich in plant available nutrients. Upon cast deposition, microbial products, in addition to earthworm mucilage, bind soil particles and contribute to the formation of highly stable aggregates [27,31]. Over longer periods of time, this enhanced microbial activity decreases when the casts dry, and aggregation is then reported to physically protect SOM against mineralization. Thus C mineralization rate decreases and mineralization of SOM from casts may be blocked for several months [32,33]. Earthworm midden are enriched in organic C and N, exceeding the C and N contents of the non ingested soil by a percentage of 112.7 and 89.4 respectively (Table 1). Nitrogen mineralization is a measure of soil quality, soil with high N mineralization potential tends to be inherently fertile, while soils with low N minerlization potential tend to be less fertile and require greater agriculture inputs. The increased transfer of organic C and N into soil aggregates indicates the potential for EWs to facilitate SOM stabilization and accumulation in agricultural systems [34]. Earthworms increases microbial activity and Nitrogen fixation in the soil, so that N in the worm cast may be due at least in part to this rather than to concentration by gain worms.

Table 1: Physicochemical parameters of earthworm midden and non ingested soil

Days → Parameters ↓	0	7	14	21	28	35	42
Non ingested soil							
pH	6.1±0.26	6.2±0.54	6.28±0.62	6.35±0.79	6.12±0.42	5.98±0.45	5.82±0.26
Org. C(mg C/g)	5.79±0.90	5.97±0.29	5.98±0.28	6.02±0.38	6.05±0.82	5.85±0.95	5.81±0.72
Nitrogen (mg N/g)	0.57±0.12	0.59±0.12	0.62±0.15	0.65±0.05	0.63±0.18	0.59±0.12	0.57±0.11
Phosphorus (g P/m^2)	3.05±0.32	3.09±0.12	3.15±0.65	3.12±0.52	3.08±0.42	3.07±0.38	3.06±0.31
Potassium (g K/m^2)	15.12±2.04	15.35±2.18	15.42±1.53	15.22±1.32	15.01±1.24	14.95±1.29	14.82±1.52
Earthworm midden							
pH	7.5±0.51	7.2±0.62	7.3±0.71	7.5±0.59	6.9±0.53	6.5±0.75	6.3±0.32
Org. C(mg C/g)	12.32±1.12	12.59±1.23	14.36±1.89	15.67±1.45	15.1±1.52	13.51±1.38	12.05±1.41
Nitrogen (mg N/g)	1.04±0.62	1.05±0.32	1.12±0.41	1.62±0.38	1.51±0.53	1.49±0.51	1.32±0.34
Phosphorus (g P/m^2)	5.05±0.48	5.31±0.56	5.52±0.52	6.53±0.62	6.12±0.65	5.42±0.53	5.02±0.49
Potassium (g K/m^2)	17.2±2.56	17.6±2.12	17.9±1.96	18.9±2.82	18.5±2.35	17.3±1.58	16.5±1.88

The bacterial population in cropland soil and midden in the beginning were $13.6\pm0.763X10^9$ and $17.0\pm0.802X10^9$ respectively, which gradually increased to $17.0\pm0.907X10^9$ and $24.3\pm0.984X10^9$ reaching at its maxima as $27\pm0.802X10^9$ on 7th, 14th and 21st day respectively. In midden, bacterial population also gradually increased up to $31.1\pm0.68X10^9$ on 21st day of the observation. There after sharp decline in bacterial population was observed. The change in population was found to be significant ($p<0.001$). The percentage increase in bacterial population over initial population was recorded as 12.94%, 63.52%, 82.94%, 54.70% and 42.94% on 7th, 14th, 21st, 28th and 35th day while decrease as 2.35% on 42nd day (Table 2). In soil, percentage decrease in bacterial population as 16.91% and 50.73% on 35th and 42nd day respectively in comparisons to midden. Bacterial population has been reported higher in midden compared to the cropland soil ingested by the earthworm [3,35]. Brown *et al.* [36] emphasize the importance of temporal and spatial scale when evaluating the effects of earthworms on the soil profile, suggesting that fresh earthworm midden behave differently than aged midden. The changed behavior of fresh and old earthworm midden may primarily be due to variation in bacterial population as the stability of midden increases with age atleast for three weeks due to product of secretion by bacterial population.

Table 2: Bacterial population in earthworm midden and non ingested soil

Days of observation	Non ingested soil (M±SD)	Earthworm midden (M±SD)	% change
0	$13.6\pm0.763\ X10^9$	$17.0\pm0.802\ X10^9$	+ 25.0 %
7	$17.0\pm0.907\ X10^{9*}$ (+25.0)	$19.2\pm0.802\ X10^{9*}$ (+12.94)	+12.94%
14	$24.3\pm0.984\ X10^{9*}$ (+78.67)	$27.8\pm1.02\ X10^{9*}$ (+63.52)	+14.40%
21	$27.0\pm0.802\ X10^{9*}$ (+98.52)	$31.1\pm0.650\ X10^{9*}$ (+82.94)	+15.18%
28	$17.5\pm0.70\ X10^{9*}$ (+28.67)	$26.3\pm0.80\ X10^{9*}$ (+54.70)	+50.28%
35	$11.3\pm0.737\ X10^{9*}$ (-16.91)	$24.3\pm0.750\ X10^{9*}$ (+42.94)	+115.04%
42	$6.7\pm0.450\ X10^{9*}$ (-43.38)	$16.6\pm0.555X10^{9*}$ (-2.35)	+147.76%

*Values in parenthesis are percentage increase (+) or decrease (-) over initial value; * = Change produced are significant at 1% level; n = 3*

The maximum biomass (mg/g soil) recorded on 21st day as 8.10±0.240X10^{-3} and 9.33±0.195X10^{-3} in soil and midden respectively which decreased to 2.01±0.135X10^{-3} and 4.98±0.165 X10^{-3} (Fig. 1). Dehydrogenase activity was observed 8.2±2.32 and 10.2±1.87 µg formazan/g soil/ hec in soil and midden respectively. Initially percentage change in dehydrogenase activity was 28% but more pronounced percentage change 72%, 148% were observed on 35th and 42nd day of observation respectively (Table 3). Dehydrogenase activity is widely used in evaluating the metabolic activity of soil microorganisms [37]. Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors [38]. This activity is a measure of microbial metabolism and thus of the oxidative microbial activity in soils. Soil enzymes are extracellular secretions by living soil organisms. Therefore, any alteration in the life and function of these organisms alters soil enzymatic activity irrespective of their source of production such as bacteria, fungi or even earthworms [39]. It has the potential to predict the soil fertility [40].

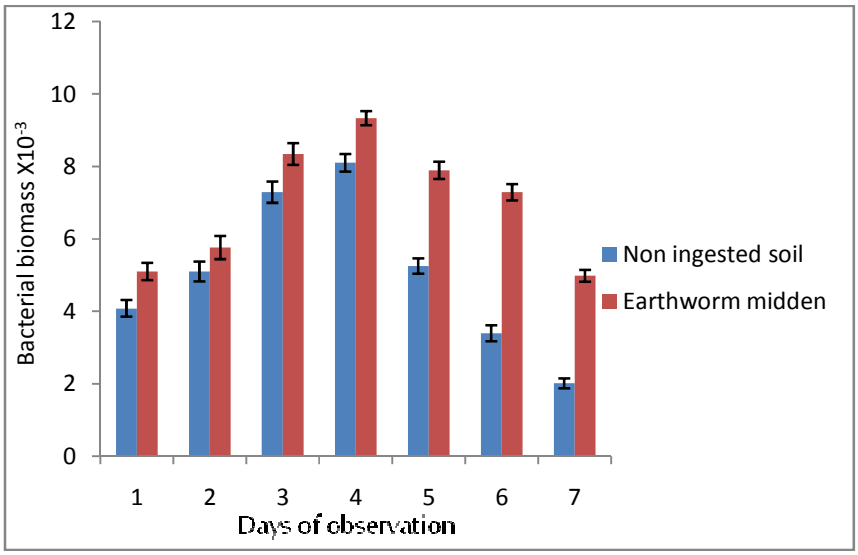

Fig. 1: Biomass (mg/g of soil) of bacterial population in earthworm midden and non ingested soil

Table 3: Dehydrogenase activity of soil and earthworm midden

Days	Non ingested soil	Earthworm midden	% Change
0	4.9±0.98	6.3±0.82	28 %
7	5.5±1.32	6.9±0.92	25 %
14	7.1±1.05	8.6±1.75	21%
21	8.2±2.32	10.2±1.87	24 %
28	6.7±1.52	8.1±1.58	20 %
35	4.3±0.91	7.4±1.23	72 %
42	2.5±0.57	6.2±0.58	148 %

Earthworms have been found to either enhance or decrease bacterial biomass [41,42,43] and to stimulate bacterial activity [35,44]. Some physical properties and microbial activity of the casts of the earthworm *Aporrectodea caliginosa* have been investigated by Piekarz and Lipiec [45] and compared with the properties of aggregates from the bulk soil. The water stability of 20-day old cast as determined by the drop impact method was significantly increased compared with those of 3 day old cast and natural aggregates. The population of bacteria, and fungi in earthworm midden increased with the aging of the midden [2,46]. The increased water stability of cast deposits can be an important factor in reducing the high susceptibility to erosion. Various experimental studies suggest that Earthworms have potentially negative consequences on fertilizer-N retention studies [47]. Enhancement of microbial population and activity, NPK content and enzyme activities in the fresh casts are due to enhanced mineralization of nutrients, high substrate concentrations and high moisture level. The earthworm species and species interactions present in the system also effect nitrogen mineralization and crop production [48]. This may result in enhanced nitrogen immobilization or mineralization depending on species characteristics and substrate quality. Most of the studies conducted to assess the role of earthworm casting in nutrient cycling and soil structure are related to surface casting species, and only a few have dealt with casts deposited under field conditions [5,13,49,50]. The Earthworms

can specifically affect soil fertility that may be of great importance to increase sustainable land use in naturally degraded ecosystems as well as agroecosystems. Proper earthworm management may sustain crop yields whilst fertilizer inputs could be reduced.

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