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Earthworm population density and diversity with respect to soil physico chemical properties, microbial population and exoenzyme dynamics in two agroclimatic Zones of Odisha, India

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ABSTRACT

Earthworms perform vital ecological functions in soil contributing significantly to the decomposition of organics and facilitating availability of nutrients. Thus the population of earthworms modulates soil microbiota and their functional attributes. The density and diversity of the earthworms are influenced by soil physicochemical properties. This study reports the seasonal variation in certain edaphic factors, population density and diversity of earthworms and microbiota in two sub tropical agroclimatic zones located at Balasore and Sambalpur districts of Odisha state, India. A total of ten different species of earthworms were identified. The population of earthworms indicated significant variation between wet and dry seasons. Significant positive correlation was observed between the earthworm population with clay percentage, moisture, organic carbon, bacterial, fungal populations and exoenzyme activities. Population density indicated significant negative correlation with temperature.

Key words : *Microbes, Earthworms, Agroclimatic zones, Exoenzymes, Soil, Population density*

Introduction

Earthworms are known to constitute more than 80% of the soil invertebrate biomass and have profound effects on ecosystem (Kale, 1997; Nainawat and Nagendra, 2001). They have attracted a lot of interest due to their beneficial role in maintaining the structure and composition of soil, creating biogenic structures (casts and burrowing systems) and significant contribution to the soil litter decomposition process. Earthworms are often called as ecosystem engineers, as they have the ability to improve soil quality by altering soil structure, gas dynamics, water flow, C and N turnover and stabilization (Vanden Bygaart *et al.*, 2000; Pouyat and Carreiro, 2003). Soil nutrients and rainfall pattern largely influence the earthworm diversity (Fragoso and Lavelle, 1995). A total of 590 species and sub species of earthworm belonging to 67 genera and 10 families have been identified in India (Julka and Paliwal, 2009; Kathireswari, 2016).

Earthworms belong to the class Oligochaeta under the phylum Annelida and can be categorised into several ecological groups basing on their physiology along with feeding and burrowing behaviour (Hendrix and Bohlen, 2002): epigeic earthworms inhabit and feed on the surface litter; anecic species produce deep vertical burrows in the mineral soil and are important in the burial of surface litter; and endogeic species burrow horizontally and feed mainly in the rhizosphere.

Diversity studies on earthworm species have been documented in various parts of the world (Tsai *et al.,* 1999; Blackmore, 2000, 2001; Chang and Chen, 2004, 2005; Blackmore *et al.,* 2006; Sautter *et al.,* 2006; Julka *et al.*, 2009). Reports are also available on biology, energetics and diversity of certain earthworm species in the state of Odisha, India (Senapati and Dash, 1983; Sahu *et al.,* 1988; Senapati *et al.,* 1993; Senapati and Sahu,1993; Senapati *et al.,* 2005). The state of Odisha, India has 10 agroclimatic zones categorized on the basis of variations in climatic conditions and soil quality which could influence the density and diversity of epigeic soil fauna. No comparative study on earthworm diversity with respect to agroclimatic zones in the state has been conducted earlier. The district of Sambalpur is located in the western part and the district of Balasore in the eastern part of the state with wide variation in the precipitation pattern which is expected to influence soil quality and faunal density and diversity. Information need to be documented for better exploration of the beneficial effects of earthworms for sustaining soil health in the region. This study was undertaken to asseess the population density and diversity pattern of epigeic earthworms and their correlation with certain soil physicochemical parameters in these agroclimatic zones.

Materials and Methods

Study area

The study was conducted in two districts, Balasore (Study Area/SA-1) and Sambalpur (Study Area/ SA-2) belonging to two different agroclimatic zones of North eastern coastal plain and North western plateau in Odisha. Balasore is one of the coastal districts of the state and is located between 21º 30´ N Latitude and 86º 56´ E Longitude. This region experiences mostly hot and humid climate with average annual temperature ranging from 22 ºC to 32 ºC. The amount of annual average rainfall is 1583mm. Sambalpur is located at 21º 27´ N Latitude and 83º 58´ E Longitude. This region experiences an extreme climate with hot and dry summer followed by humid monsoon and cold winter. The average annual temperature varies from 19.9 ºC to 34.8 ºC with an average annual precipitation 938 mm. The soils in both SA-1 and SA-2 were alfisols type (Goswami *et al.,* 2013).

Sampling of soil and earthworms

Sampling of soil and earthworms was done randomly from 2 different study areas (SA-1, SA-2) in dry (January) and wet (August) season of 2017 between 9 am-11 am. A sampling grid (20m×20m), demarcated in each sampling area was divided into 16 subunits of (5m×5m each). Soil and earthworms were randomly sampled from each subunit from a quadrate (1mx1mx30 cm) using conventional digging and hand sorting method. For identification, estimation of biomass, density and diversity indices, only adults of different species were selected. Earthworms were identified as per the taxonomical keys. Morphological parameters of adult earthworms were studied and biomass measured (on a top pan balance) in live condition after which they were preserved in 5% formaldehyde solution to study the anatomical features (Julka, 2010).

Determination of Ecological indices

The following ecological indices were calculated after sampling and segregation of earthworms. Shannon's diversity index (SDI) ($H' = \Sigma$ pi ln pi) where pi= relative abundance of the species, estimated by pi= ni D N; species richness (d= S- 1/ln N, where S is the total no. of species) and evenness ($J = H'/ \ln S$) were calculated on sampling area and seasonal basis by standard methods (Shannon,1949; Margalef, 1968; Pielou, 1975).

Soil physico-chemical analysis

Soil texture (sand, silt and clay percentage) was analyzed with the help of a Bouycos hydrometer (Gee and Bauder, 1986), temperature (10 cm depth) with the help of soil thermometer and percent moisture by portable digital moisture meter, pH by digital pH meter (Van Reeuwijk, 1992), organic carbon (OC) as per Walkley and Black (1934). Available potassium (K), phosphorous (P) and nitrogen (N) were measured using flame photometer (Jackson, 1973), as per Olsen *et al.* (1954) and acid digestion method (Hach *et al.,* 1985) respectively.

Soil microbiological and exoenzyme studies

Serial dilution and spread plate method was used to evaluate the bacterial and fungal population in soil. Nutrient agar and potato dextrose agar were used as substrate respectively Colony forming units

(CFU/g soil) were counted using a digital colony counter (Elico) after incubation for 24h and 48h at 37±2ºC and 25±2 °C to evaluate bacterial and fungal populations respectively (Parkinson *et al.,* 1971). Soil exoenzymes (amylase, cellullase, invertase) were determined as per Ross and Robert (1970) taking starch, carboxymethyl cellulose and sucrose as substrates respectively. Soil dehydrogenase activity was measured spectrophotometrically at 485 nm taking 2% 2, 3, 5-tri phenyl tetrazolium chloride) and 1% glucose as per Casida *et al.* (1964). The method prescribed by Ladd and Butler (1972) was used to determine soil protease activity using Tris HCL buffer (0.1 M, pH 8.0) and sodium caseinate as substrate.

Student's t-test (both for seasons and sampling areas) and correlation analysis were done using the software SPSS-20 at 0.05 level of significance.

Results and Discussion

Earthworm diversity and ecological indices

During the study, a total of 10 earthworm species, *Lampito mauritii* (Sp1), *Glyphidriiius tuberosus* (Sp2), *Drawida willsi* (Sp3), *Drawida celebi*(Sp4), *Pheretima alexandri* (Sp5), *Pellogaster bengalensis* (Sp6), *Octochaetona barkudensis* (Sp7), *Perionyx excavatu* (Sp8), *Octochaetona serrata* (Sp9) and *Pontoscolex corethrurs* (Sp10) were identified from various sampling areas in both the agroclimatic zones irrespective of seasons (Fig. 1a). *Glyphidrillus tuberosus* and *Drawida willsi* were found to be the dominant species in SA-1 and SA-2 respectively. The earthworm population was significantly higher ($p< 0.05$) in wet season relative to dry season in both the sampling areas. However, no significant variation in populations of earthworm was observed between sampling areas irrespective of seasons.

Average population density and biomass of the earthworms was 63.75 ± 4.11 m^{-2} and 52.25 ± 2.79 mg in dry season and 170.08 ± 5.15 m^{-2} and 124.25 ± 5.09 mg in wet season respectively in SA-1. Similarly the population and biomass were 55.26 ± 3.11 m^{-2} and 44.25 ± 3.78 mg in dry season, 153.47 ± 4.71 m^{-2} and 102.25 ± 3.34mg in wet season of SA-2 respectively.

The diversity, richness and evenness of different species in an ecosystem could be understood from various ecological indices. The Shannon's diversity index was found to be highest (2.09) in wet season of SA2 and lowest (1.06) in dry season of SA-1 (Table 1). The highest value of Margalef's richness index (1.07) was recorded in wet season of SA-2 and lowest (0.29) in dry season of SA1 (Table 1). The richness index provides information for preference of a particular biological community in an area. Maximum value of Pielous evenness index (0.97) was found in both dry and wet seasons of SA1 as well as dry season of SA2, whereas the minimum value (0.90) was recorded from wet season of SA2 (Table 1). Therefore, it is evident that the diversity of the worms is considerably higher in wet season relative to dry season irrespective of sampling zones.

Table 1. Ecological indices of species in Balasore and Sambalpur region. SA1- Balasore, SA2- Sambalpur

	Ecological indices			
	SA1		SA2	
	Dry (n=3)	Wet(n=8)	Dry(n=4)	Wet(n=10)
H ˆ	1.06	2.02	1.34	2.09
D	0.29	0.81	0.44	1.07
J	0.97	0.97	0.97	0.9

Singh (1997) reported 11 species of earthworms from cultivated lands, pastures, orchards and sewage sites from the state of Uttar Pradesh, India and observed noticeable differences of the density of the animals between seasons. Baker *et al.* (1997) recorded the highest number of earthworms in cultivated sites of southern Australia in wet season relative to dry season. Identical results have also been obtained by various workers from different agroclimatic zones in the Indian subcontinent (Dash and Senapati, 1980; Bhadauria and Ramakrishnan, 1991; Blanchart and Julka, 1997; Joshi and Aga, 2009; Koirala *et al.,* 2011). All these results are in agreement with the present findings supporting the hypothesis that the density and diversity of earthworms depend on soil conditions and are high during wet season when soil moisture level is optimal. Singh and Rai (2000) identified *Dichogaster bolaui*, *Eutyphoeus incommodus* and *Lampito mauritii* as major potential species on the basis of their relative abundance in a variety of habitats. They suggested that earthworm species with the best adaptation to the soil conditions of a given habitat are the dominant ones. In the present study, *Glyphidrillus tuberosus* and *Drawida willsi* were observed to be the dominant species in both the sampling sites indicating that the soil physicochemical properties best suited these two species most in comparison to others.

The population density of earthworms generally depends on various factors such as habitat, soil conditions and seasons. Decaëns *et al.* (1994) recorded 32–192 worms m^{-2} from a survey conducted in savanna, Colombia. Lavelle and Pashanasi (1989) have reported high densities and biomass of 474–573 worms/ m^{-2} and 78–116.4 mg in traditional pasture land and 546–740 worms m^{-2} and 103.2–153 mg in improved pasture in Peru. Senapati (1980) had earlier reported that the density and biomass of earthworms ranged from 17.4–800 m^{-2} and 30.2–56 mg in improved pasture in India. The results of all these studies indicated higher earthworm density in soils with near neutral pH and optimal levels of moisture and organic matter. The findings of the present study in both the sampling areas are identical to these earlier reports.

In the present study it was found that more than 85% earthworm populations were juveniles. Our results are somewhat similar to the earlier reports of Callaham and Hendrix (1997); Sackett, (2012). Whalen (2004) reported in Quebec forest ecosystem that the juvenile population ranged between 0-95%.

It has been reported earlier that the earthworm population indicates higher diversity indices in soil with optimal nutrient and moisture levels irrespective of habitats (Tripathi and Bhardwaj, 2004; Sathianarayanan and Khan, 2006; Najar and Khan, 2011). Our results on ecological indices more or less corroborate these earlier observations.

Soil physicochemical studies

Results of physico-chemical analysis of soil samples have been presented in Table 2. Texture analysis indicated that sand percentage in soil ranged from 37.86±3.02% to 46.30±0.75% in SA-1 and 36.73±0.86% to 43.53±5.74% in SA-2; silt percentage varied from 23±2.60% to 31.20±1.3% in SA-1, 26.21±3.25% to 28.77±5.47% in SA-2; clay percentage ranged from 22.50±2.57% to 39.14±2.70% in SA-1, 27.8±0.60% to 37.06±2.76% in SA-2 (Table 2). It was observed that the soil of both study sites were of alfisols type.

The mean soil temperature varied from 23.83±1.71 °C to 30.03±2.54 °C in SA-1 and 23±2.42°C to 32.96±1.97 °C in SA-2 (Table 2). The overall soil temperature was higher in Sambalpur zone than Balasore zone. Percent moisture widely varied between sampling areas. In SA-1 it ranged between 24.33±3.05% to 49.33±1.52% and in SA-2, 21.66±3.05% to 40.66±3.21 (Table 2). In general moisture content of soil was higher in wet season related to dry season. Soil pH values ranged from 6.4±0.3 to 6.83±0.55 in SA-1, 6.73±0.32 to 7.03±0.20 in SA-2, indicating moderately acidic and alkaline conditions of soil irrespective of sampling divisions. Percent OC ranged between 0.71±0.07 to 1.13±0.27 in SA-1 and 0.94±0.39 to 1.04±0.22 in SA-2. High percentages of organic carbon were observed in soil from SA1 relative to SA2 in both dry and wet seasons. Soil nitrogen percentage ranged from 0.19±0.02% to 0.24±0.01% in SA1 and 0.20±0.04% in both the seasons of SA2. The percent soil phosphorous varied between 0.16±0.03% to 0.17±0.01% in SA-1 and 0.14±0.02% to 0.16±0.02 in SA-2. Soil potassium percentage varied between 0.13±0.01 to 0.13±0.01 in SA-1 and 0.11±0.02% to 0.17±0.02% in SA-2. Student's t-test indicated that the difference between soil physicochemical parameters between locations and seasons was not significant. However,

Table 2. Mean ± SD (n=3) of soil physico-chemical parameters in SA-1 and SA-2

Soil parameters	SA1		SA2	
	Dry	Wet	Dry	Wet
Sand (%)	46.30±0.75	37.86±3.02	43.43±5.74	36.73±0.86
Slit (%)	31.20±1.3	23±2.60	28.77±5.47	26.21±3.25
Clay (%)	22.50±2.57	39.14±2.70	27.8±0.60	37.06±2.76
Temp	30.03±2.54	23.83±1.71	32.96±1.97	23±2.42
Moisture (%)	24.33±3.05	49.33±1.52	21.66±3.05	40.66±3.21
pH	6.4±0.3	6.83±0.55	6.73±0.32	7.03±0.20
OC (%)	1.13±0.27	0.71±0.07	1.04±0.22	0.94±0.39
N (%)	0.19±0.02	0.24±0.01	0.20±0.04	0.20±0.04
P (%)	0.16±0.03	0.17±0.01	0.14±0.02	0.16±0.02
K (%)	0.13±0.01	0.13±0.01	0.11±0.02	0.17±0.02

SA1- Balasore, SA2- Sambalpur

significant positive correlation was observed between earthworm diversity with percent clay (r=0.64, pˆ0.05), soil temperature moisture (r=0.84, pˆ0.05), organic carbon (r=0.58, pˆ0.05) and nitrogen (r=0.51, pˆ0.05). Population density indicated significant negative correlation (r=-0.59, pˆ0.05), with temperature.

Soil microbiological and exoenzyme studies

The bacterial and fungal populations have been illustrated in Fig. 1b and 1c respectively. Soil from SA1 indicated the highest bacterial colony count (112.66± 16.01 10^6 CFU/g soil) (Fig.1b) and the lowest bacterial population (39± 7.81 10^6 CFU/g soil) was recorded from SA2 in both the seasons (Fig.1b). The maximum fungal colony count (104.33± 9.07 10^6 CFU/g soil) was observed in soils from SA1 and the minimum (42± 14.52 10^6 CFU/g soil) from SA2 irrespective of seasons (Fig. 1c). The microbial population in wet season was significantly higher (($p<0.05$) in wet season relative to dry season in both the sampling sites Thus it evident that microbial population increased significantly in wet season which is likely due to an optimal soil moisture level and nutrient availability. However, no significant variation in populations of earthworm was observed between sampling areas irrespective of seasons.

Bhattarai *et al.* (2015) reported variation in soil microbial population in different soil horizons which is influenced by various factors such as soil depth, pH and organic matter. They also reported decreasing microbial population with increasing soil depth. Bacterial population is maximum in neutral or slightly acidic soils with adequate organic matter (Shamir and Steinberger, 2007). Aher *et al.* (2018) reported the increased microbial population

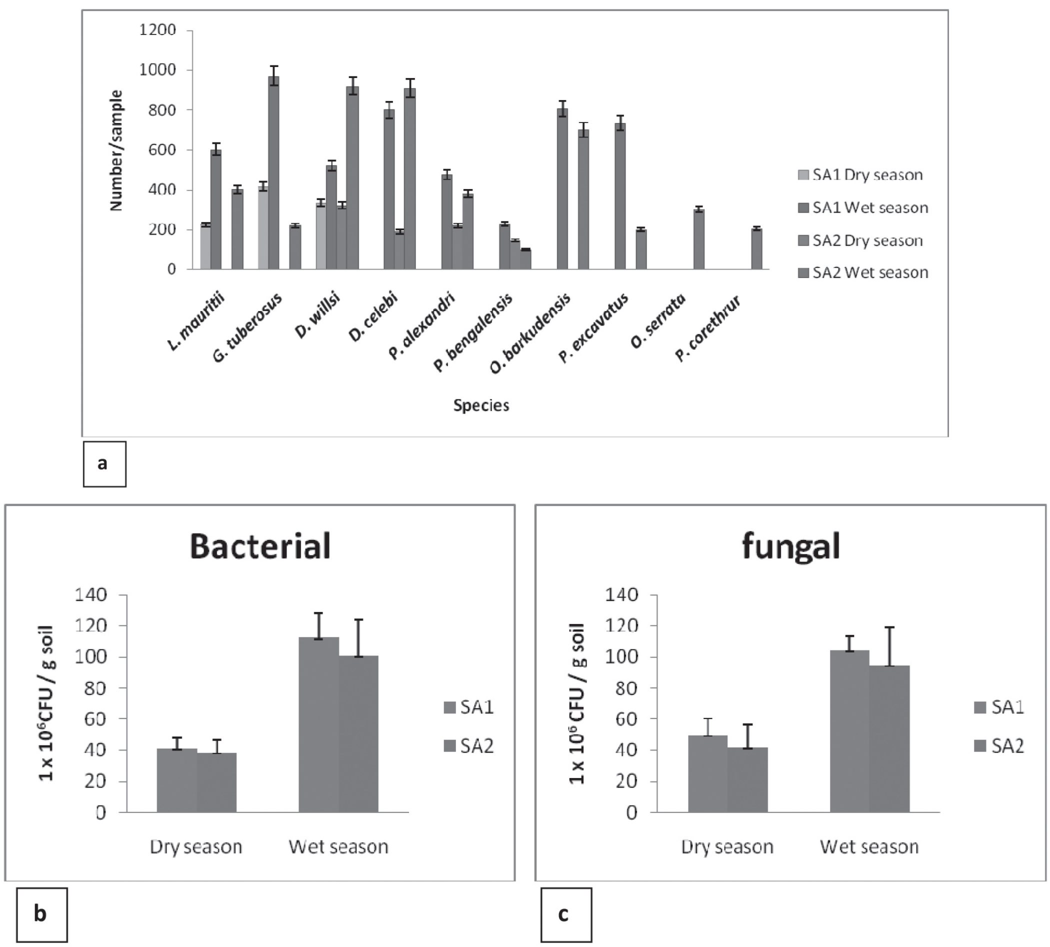

Fig. 1. Species diversity and microbial population of SA-1 and SA-2
a) species diversity (adult earthworms), b) bacterial population, c) fungal population. SA1- Balasore, SA2-Sambalpur

under organic manure application might be due to higher organic carbon as a source of energy. The soil microbial distribution depends upon the habitat and an interconnected web of variables that differ among various ecosystems (Wixon *et al.,* 2009; Mishra *et al.,* 2018). Our results corroborate these earlier reports indicating that soil microbial growth is dependent on an optimal pH ,organic content, temperature and moisture.

The activities of exoenzymes (amylase, cellulase, invertase, dehydrogenase and protease) were maximum in wet season. The highest activities of amylase (0.12 ± 0.003 mg glucose/g soil/h), cellulase (0.12 ±0.008 mg glucose/g soil/h) were recorded during wet season of SA1 (Fig 2a, 2b). The cellulase activity was found to be minimum (0.07±0.01mg glucose/g soil/h) during dry season of SA2 (Fig 2b). The invertase activity did not show appreciable variation between SA1 (0.12± 0.005 mg glucose/g soil/h) and SA2 (0.12±0.023mg glucose/g soil/h)(Fig 2c). Dehydrogenase activity was maximum (0.09±0.001 mg TPF/g soil/h) during wet season and minimum (0.08± 0.001 mg TPF/g soil/h) during dry season in SA2 (Fig 2d). Maximum protease activity (0.69± 0.09 Tyrosine/g soil/h) was observed in SA1 during wet season (Fig 2e). However, no significant variation in populations of earthworm was observed between sampling areas irrespective of seasons. Significant positive correlation was observed between earthworm population density and

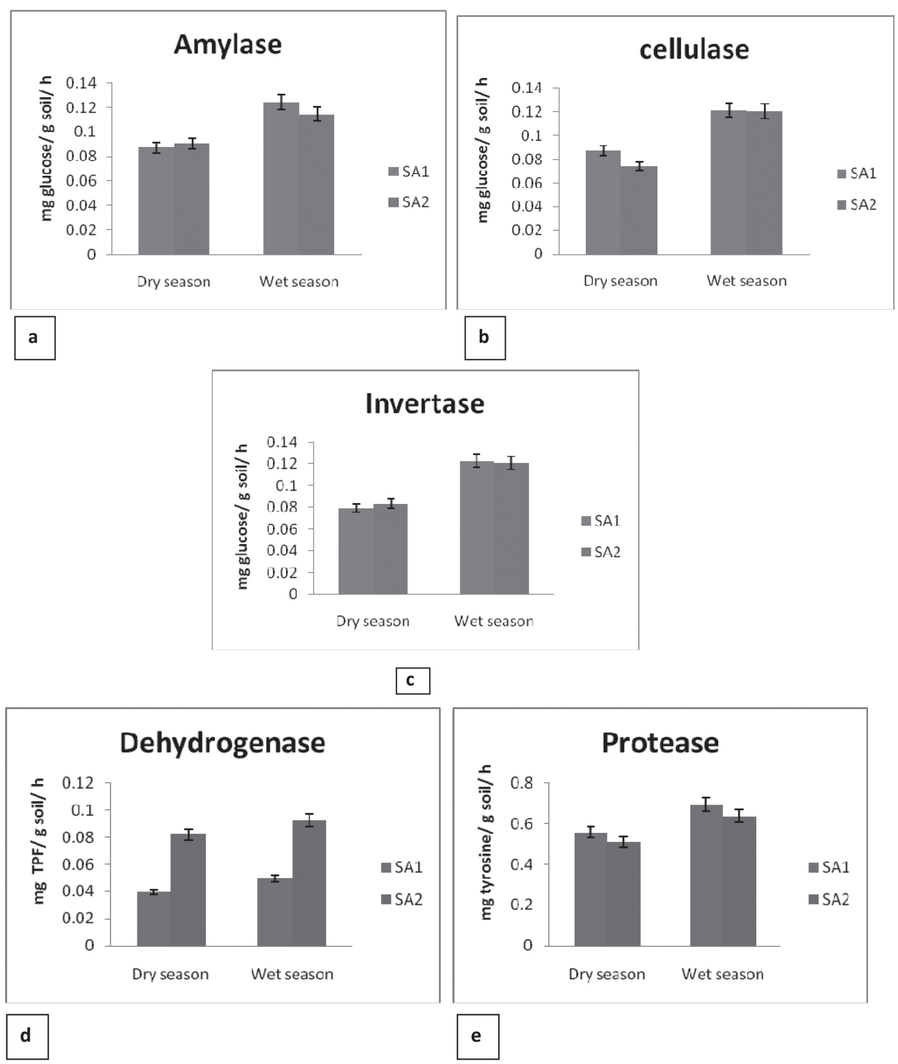

Fig. 2. Exoenzyme activities of soil in SA-1 and SA-2. a) amylase, b) cellulase c) invertase d) dehydrogenase, e) protease. SA1- Balasore, SA2-Sambalpur

activities of amylase ($r=0.61$, $p<0.05$), invertase ($r=0.66$, $p<0.05$), dehydrogenase ($r=0.58$, $p<0.05$) as well as bacterial population ($r=0.79$, $p<0.05$) and fungal population ($r=0.81$, $p<0.05$) indicating that earthworms facilitate soil microbial colonisation and exoenzyme activities.

Soil exoenzymes play crucial role in catalysing biochemical reactions necessary for the life processes of micro-organisms and actively participate in nutrient recycling. These enzyme activities are often closely associated to soil physical properties and microbial population and could be taken as the indicators of soil health (Das and Varma, 2011). Aon *et al.* (2001) and Rao *et al.* (2014) described the strong linkage between microbial population and certain enzyme activities (alkaline phosphatases, dehydrogenase, fluorescein diacetate (FDA) hydrolysis, β-glucosidase, and urease) despite fluctuations shown in different time and space, independent of seasons. Venu *et al.* (2016) demonstrated changes in amylase, cellulase, invertase, dehydrogenase, protease and acid phosphate activity of soil of Cassia, Shorea, Acacia, and Dalbergia forest sites during winter, summer and rainy seasons in a deciduous tropical forest of Southern India. They observed significantly higher amylase and cellulase activities in all the regions during rainy season. They also reported non uniform variation in protease, dehydrogenase and invertase activities with respect to plant type and seasonal intervals. All these findings are more or less similar to the results obtained in this study where the soil exoenzyme activities have indicated appreciable variations between sampling sites and seasons.

Reports are available on the positive linear relationship between burrow walls, old root channels and casts built by earthworms with activities of microbial enzymes including dehydrogenase, β-glucosidase protease, and alkaline phosphatase (Hoang *et al.,* 2016; Lipiec *et al.,* 2016). The present study reports strong correlation between earthworm population density with microbial population and enzyme activities. Hence our observations are supported by these earlier reports indicating significantly high microbial population and their functional activities during wet season relative to dry season irrespective of study sites.

Conclusion

From the present study it is evident that the population density of earthworms invariably depends on optimal soil conditions which too favour microbial growth and exoenzyme activities. Earthworms are generally regarded as secondary decomposers and facilitators of soil biochemical processes, since they enhance aeration and partially digest organics before microbial action. Earthworm population density therefore indicate soil quality in many terrestrial habitats. Significantly high population of earthworms, microbes and enzyme activities in the wet season in this study reaffirms the proposition that in addition to other factors soil moisture plays a crucial role in regulating density and functions of soil biota.

Conflict of interest: No conflict of interest to declare

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