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Earthworm Diversity and Their Influences on Soil Enzyme Activities in Organic, Sustainable and Conventional Farming Systems of Puducherry

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An experiment was conducted to study the earthworm diversity and its influence on soil enzyme activities in organic, sustainable and conventional farming systems in the state of Puducherry. A total of five earthworm species namely *Drawida willsi, Drawida scandens, Lampito mauritii, Perionyx excavates* and *Eudrilus eugeniae* were present in the study sites. Four species were found in conventional and sustainable farming and five species were found in organic farming. Earthworm species diversity, population, species dominance, and biomass were significantly higher in organic farming systems compared to sustainable and conventional farming systems. In addition, soils from organic farming showed higher activity of urease, alkaline phosphatase, β-glucosidase, cellulase, invertase and xylanase activities and they showed a significant positive correlation with earthworm population and biomass. Our results revealed that the presence of earthworms strongly affected enzyme activities in soil. Earthworm casts probably contributed to the enrichment of plant-available nutrients and higher soil enzyme activities in organically managed soils.

(***Key words:*** *Coastal agro-ecosystem, Earthworm diversity, Organic farming, Soil enzymes*)

Earthworms are an essential component of the soil biota in tropical and sub-tropical agro ecosystems. The earthworm population structures are directly influenced by soil moisture, organic matter, texture, pH and changes in land use patterns. The key function of earthworms in providing ecosystem services in the improvement of soil structural and functional properties has been described by Sharma *et al.* (2017). The influences of agronomic practices, such as crop residue management, crop rotation and fertilization, are also important for earthworm populations (Riley *et al.,* 2008). Some studies have reported higher biodiversity in organic farming systems compared to conventional farming systems, including higher earthworm populations in organically managed fields (Brown *et al.,* 2004; Nath and Chaudhuri, 2010; Padmavathy and Poyyamoli, 2013).

Soil enzymes produced by plants, animals and microorganisms play a crucial role in soil fertility (Masto *et al.,* 2008). Enzyme activities are important in the stabilization of soil structure, organic matter decomposition and nutrient (N, C, S and P) cycling (Nannipieri *et al.,* 2002; Mandal *et al.,* 2007). The activities of enzymes in soil systems differ in amounts mainly due to the fact that the intensity of the biological processes depends onthe composition and activity of living organisms (Okur *et al.,* 2009). Earthworms are the crucial drivers of the vermicompost process. They cause fragmentation of the ingested material through muscular action increasing the surface area for microbial activities (Hoeffner *et al.,* 2018). In the earthworm gut, a variety of intestinal microorganisms are present which produce digestive enzymes such as amylase, proteases, lipases and cellulase that enhance the biodegradation of organic matter (Aira *et al.,* 2007).

However, many studies conducted on the influence of earthworms on enzyme activity have mainly focused on the guts and casts of earthworms in laboratory

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conditions (Aira *et al.,* 2005; Parthasarathi and Ranganathan, 2000). There are fewer studies concerning the effects of the earthworm population on enzyme activities in soil under natural field conditions. Hence, the present study was undertaken to assess earthworm diversity and their influence on soil enzyme activities in organic, sustainable and conventional farming systems of Puducherry.

MATERIALS AND METHODS

Study area

The Union Territory of Puducherry is located on the Coromandel coast between 11º 52′ N to 11º 59′ N latitudes and 79º 45′ E to 79º 52′ E longitudes covering an area of 480 km^2. The mean annual rainfall of the study area is about 1311 mm. The mean number of annual rainy days is 55 and the mean monthly temperature ranges between 21ºC and 30ºC. This region gets more rainfall during the northeast monsoon. Humidity is also high as the area is nearer to the coastal region. The study was conducted at 9 different farms that followed organic, sustainable and conventional farming practices at Soriankuppam which is 25 km away from the town and nearer to Cuddalore district, Nallavadu located 10 km for Puducherry and Kalapet, located very near to Pondicherry University. In these study sites, the texture of the soil is sandy loam. During the study period, groundnut was the most predominant crop cultivated in the area.

Soils from the 9 farms (land area ≤ 2 ha) in organic, sustainable and conventional farming were sampled from June 2014 to August 2015. Three farms were certified as organic farming and no synthetic fertilizers, pesticides or herbicides were used. Two of these farms were located in Soriankuppam (Organic-1 and Organic-2) and one in Nallavadu (Organic-3). The other three farms are classified as sustainable, where synthetic fertilizers were used but synthetic pesticides were not used. These farms were located in Soriankuppam (Sustainable-1, 2 and 3). The remaining three were conventional farms where synthetic fertilizers and pesticides were used. These farms are located in Kalapet (Conventional 1 and 3) and Soriankuppam (Conventional 2). The details of the cropping pattern, integrated nutrient management (INM) and integrated pest management (IPM) practices followed are presented in Table 1.

Sample collection

Three composite soil samples were collected from each of the 9 farms from the fall of June 2014 to August 2015. All the samples were collected after the crop was harvested and before the soil was tilled, to avoid interference by recent application of fertilizers and agronomic practices. Three composite soil samples at 0-15 cm depth were collected from each of the 9 farms using an auger. Bulked samples were kept separately according to the location within each field for replication maintenance. Composite soil samples were stored in a deep freezer to control enzyme activities for biochemical analysis. The soils were then transferred to a storage room and were stored at 4ºC until the time of analysis. Enzyme analysis was done within 48 to 72 hrs of sample collection.

Earthworm collection

Earthworms were collected from organic, conventional and sustainable farming sites by digging the soil and hand shorting method. During the study period, ten quadrates (25 cm × 25 cm × 30 cm) were taken for each of the 9 farms (Pelosi *et al.,* 2009). Earthworms were counted for their density (ind. m^{-2}), rinsed and weighed on an electronic balance in the field for biomass (g m^{-2}) estimation (Dey and Chaudhuri, 2014). Earthworm species were identified according to keys given by Gates (1972) and Julka (1988).

Analysis of soil physico-chemical properties

Soil moisture content was determined by the gravimetric method. Soil bulk density was determined by the volumetric flask method (Bashour and Sayegh, 2007) while water holding capacity was determined by the gravimetric method (Margesin and Schinner, 2005) andsoil pH (1:2 soil water suspension) was determined by potentiometry. Soil salinity (EC in 1:2 soil water suspension) was determined by conductometry (Bashour and Sayegh, 2007). Organic carbon (OC) was determined by the chromic acid wet digestion method (Walkley and Black, 1934) and the total nitrogen (Total N) by macro-Kjeldahl digestion (Piper, 1966).

Estimation of soil enzymes activities

Protease activity (µg tyrosine g^{-1} 2h^{-1}) was determined by amino acids released after incubation

Table 1. *Management practices and cropping pattern in three farming systems*

	Cropping pattern			Nutrient management	Pest management	Weed management
	2012-13	2013-14	2014-15			
Conventional Farming	1. Groundnut (JLR) 2. Rice (White Ponni) 3. Black gram	1. Groundnut (JLR) + Black gram 2. Tapioca	1. Bhendi (Hybrid) 2. Finger millet and Bottle gourd 3. Rice (White Ponni)	8 t ha^{-1} of FYM + Recommended doses of NPK in the form of Urea, DAP and Muriate of potash + Gypsum*	Need based spraying of Methomyl 40% SP, Lambada cyhalothrin 5%, Hexaconazole 5% and Cypermethrin and Chlorpyrifos	Hand hoe and Spade
Sustainable Farming	1. Groudnut (JLR) + Tapioca 2. Rice (White Ponni) 3. Green gram	1. Groundnut (JLR) + Black gram 2. Green gram	1. Groundnut (JLR) + Black gram 2. Gingelly and Mesta 3. Rice (ADT 32)	12 t ha^{-1} of FYM + green manuring + Recommended doses of NPK in the form of Urea, DAP and Muriate of potash + Gypsum*	Neem oil spraying and NPV(Nuclear Polyhedrosis Virus) for control of leaf roller caterpillar	Hand hoe and Spade
Organic Farming	1. Groundnut (JLR) + Brinjal + Chilli 2. Rice (ADT 36) + Fodder crop (CO3) + Black gram 3. Gingelly (SVPR) 4. Rice	1. Groundnut (JLR) + Tapioca 2. Bhendi 3. Rice (White Ponni)	1. Groundnut (JLR) + Brinjal + Chilli 2. Black gram 3. Gingelly (SVPR) 4. Rice (ADT 36)	12 t ha^{-1} of FYM + green manuring, 8 t ha^{-1} of Vermicompost + *Rhizobium*, Potash mobilizing bacteria and *Panchakavya*	Neem oil, five-leaf extract + Ginger and garlic extract + cultural method for control of insect pests	Hand hoe and Spade

*400 kg ha^{-1} gypsum application during groundnut crop cultivation for calcium, sulphur requirement and promoting peg formation in groundnut of soil with sodium caseinate for 2 h at 50°C using Folin-Ciocalteau reagent (Ladd and Butler, 1972). Urease activity (µg NH_4-N g^{-1} 2h^{-1}) was determined by ammonia released after the incubation of sample with urea solution for 2 h at 37°C (Tabatabai and Bremner, 1972). The amidase activity (µg NH_4-N g^{-1} 24h^{-1}) was determined as described by Frankenberger and Tabatabai (1980).

Acid phosphatase and alkaline phosphatase were determined by para-nitrophenol released after the incubation of soil with para-nitrophenyl phosphate for 1 h at 37°C (Tabatabai and Bremner, 1969). β - glucosidase activity was determined by para-nitrophenol released after the incubation of soil with para-nitrophenyl glucoside solution for 1 h at 37°C (Eivazi and Tabatabai 1988). Aryl sulphatase activity was determined by para nitrophenol release after the incubation of soil with para nitrophenyl sulphate for 1 h at 37°C (Tabatabai and Bremner, 1970). In above four cases, the enzyme activities were expressed as p-nitrophenol µg g^{-1} h^{-1}.

Cellulase activity was determined by released reducing sugars after the incubation of soil samples with carboxy methyl cellulose for 24 h at 50°C and xylanase activity was determined by released reducing sugars after the incubation of soil samples with xylan for 24 h at 50°C. In both cases, the enzyme activities were expressed as glucose equivalent µg g^{-1} 24h^{-1} (Schinner and von Mersi, 1990). Invertase activity (Glucose equivalent µg g^{-1} 3h^{-1}) was determined by estimation of the released reducing sugars after the incubation of soil samples with sucrose solution for 3 h at 50°C (Schinner and von Mersi, 1990).

Data analysis

All the experimental data were analyzed using SPSS version 16 software. Statistically significant differences between the farm management practices were analyzed by using one way ANOVA test. The relationship between soil enzymes and earthworm population were analyzed by Pearson correlation (Sudhakaran *et al.,* 2019). The Simpson's index of dominance, Shannon's diversity index, Menhinick species richness index, Fisher's alpha diversity and species evenness (Dey and Chaudhuri, 2014) of earthworm communities were analyzed by using of PAST (PAleon to logical STatistics) software version 2.

RESULTS AND DISCUSSION

Earthworm diversity in organic, sustainable and conventional farming

Earthworm biomass (41 g m^{-2}), population density (85.33 ind. m^{-2}), Simpson's index of dominance (0.73) and Shannon's diversity index (1.45) were significantly higher in organic farming soil compared than sustainable and conventional farming (Table 2). *Lampito mauritii* (32 ind. m^{-2}) and *Perionyx excavates* (10 ind. m^{-2})

Table 2. *Physico-chemical properties of soil and ecological parameter*

	Conventional	Sustainable	Organic	P-value
Soil bulk density (Mg m^{-3})	1.14 ± 0.04	1.18 ± 0.04	1.12 ± 0.07	0.732
Water holding capacity (%)	35.53 ± 2.35	46.60 ± 1.99	49.50 ± 0.75	0.004*
Soil moisture (%)	2.70 ± 0.32	3.20 ± 0.17	4.40 ± 0.32	0.013*
pH	7.13 ± 0.41	7.51 ± 0.45	7.36 ± 0.24	0.783
EC (dS m^{-1})	0.19 ± 0.12	0.25 ± 0.06	0.26 ± 0.02	0.817
Total nitrogen (g kg^{-1})	2.15 ± 0.21	1.79 ± 0.12	3.69 ± 0.66	0.035*
Organic carbon (g kg^{-1})	6.40 ± 0.30	7.15 ± 0.18	8.63± 0.52	0.013*
Earthworm population (ind. m^{-2})	37.00 ± 2.65	73.67 ± 3.48	85.33 ± 4.91	0.000*
Earthworm biomass (g m^{-2})	12.33 ± 1.73	31.00 ± 2.53	41.00 ± 3.79	0.000*
Fishers alpha diversity	1.01 ± 0.11	0.96 ± 0.09	1.22 ± 0.02	0.052
Simpson's index of dominance	0.64 ± 0.02	0.65 ± 0.03	0.73 ± 0.00	0.004*
Shannon's diversity index	1.10 ± 0.06	1.20 ± 0.09	1.45 ± 0.01	0.002*
Menhinick species richness index	0.64 ± 0.04	0.51 ± 0.03	0.59 ± 0.02	0.027*
Species evenness	0.91 ± 0.03	0.83 ± 0.01	0.85 ± 0.01	0.063

±: standard error and *: significantly different at p ≤ 0.05 according to one way ANOVA

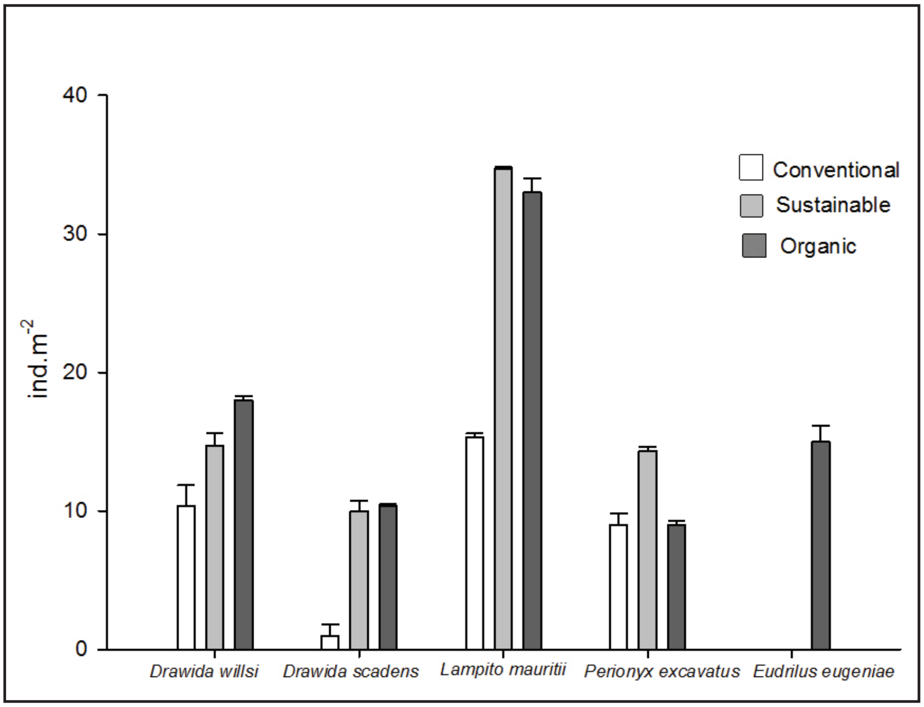

Fig. 1. *Diversity of earthworm species in organic, sustainable and conventional farming systems*

populations were higher in sustainable farming soil. *Drawida willsi* (15 ind. m^{-2}) and *Drawida scadens* (8 ind. m^{-2}) populations were higher in organic farming soil while *Eudrilus eugeniae* (12 ind. m^{-2}) was only present in organic farming soil (Fig. 1).

A total of 5 earthworm species namely *Drawida willsi, Drawida scandens, Lampito mauritii, Perionyx excavates* and *Eudrilus eugeniae* were present in the study sites. 4 species were found in the farms that followed conventional and sustainable farming and 5 species were found in organic farming. The population density of *Lampito mauritii* and *Perionyx excavates* was higher in sustainable farming while *Drawida willsi* and *Drawida scandens* population densities were higher in organic farming. *Eudrilus eugeniae* was present only in organic farming soils which might be due to the application of *Eudrilus eugeniae* vermicast. Among the recorded earthworm species, *Eudrilus eugeniae* isis an exotic species (Fig. 1) and the rest *Drawida willsi, Drawida scandens, Lampito mauritii* and *Perionyx excavates* are endemic to the Indian subcontinent. *Lampito mauritii* was the dominant species with respect to population density, biomass and relative abundance in all the farming systems. These findings reported in our present study support the earlier findings of Sathianarayanan and Khan (2006).

Earthworm species diversity, species dominance, population and biomass were higher in organic farming systems compared tothe sustainable and conventional farming systems. Similar findings were reported in a previous study conducted by Padmavathy and Poyyamoli (2013). Applications of organic composts obtained from plant and animal-originated residues were responsible for the increase in earthworm populations by several folds. Köhler *et al.* (2014) also reported that organic enrichment, soil moisture content and soil management practices have a greater impact on earthworm proliferation. Species richness and species evenness were higher in the conventional farming system compared to the organic and sustainable farming system. The higher moisture and availability of feed content maintained in this farming system appear to be the positive factors in promoting earthworm proliferation (Ahmed and Al-Mutairi, 2022). Conventional farming systems showed reduced earthworm biomass and population. It might be due to the intensive use of ammonia-based fertilizers which often have adverse effects on the earthworm population (Pelosi *et al.,* 2009).

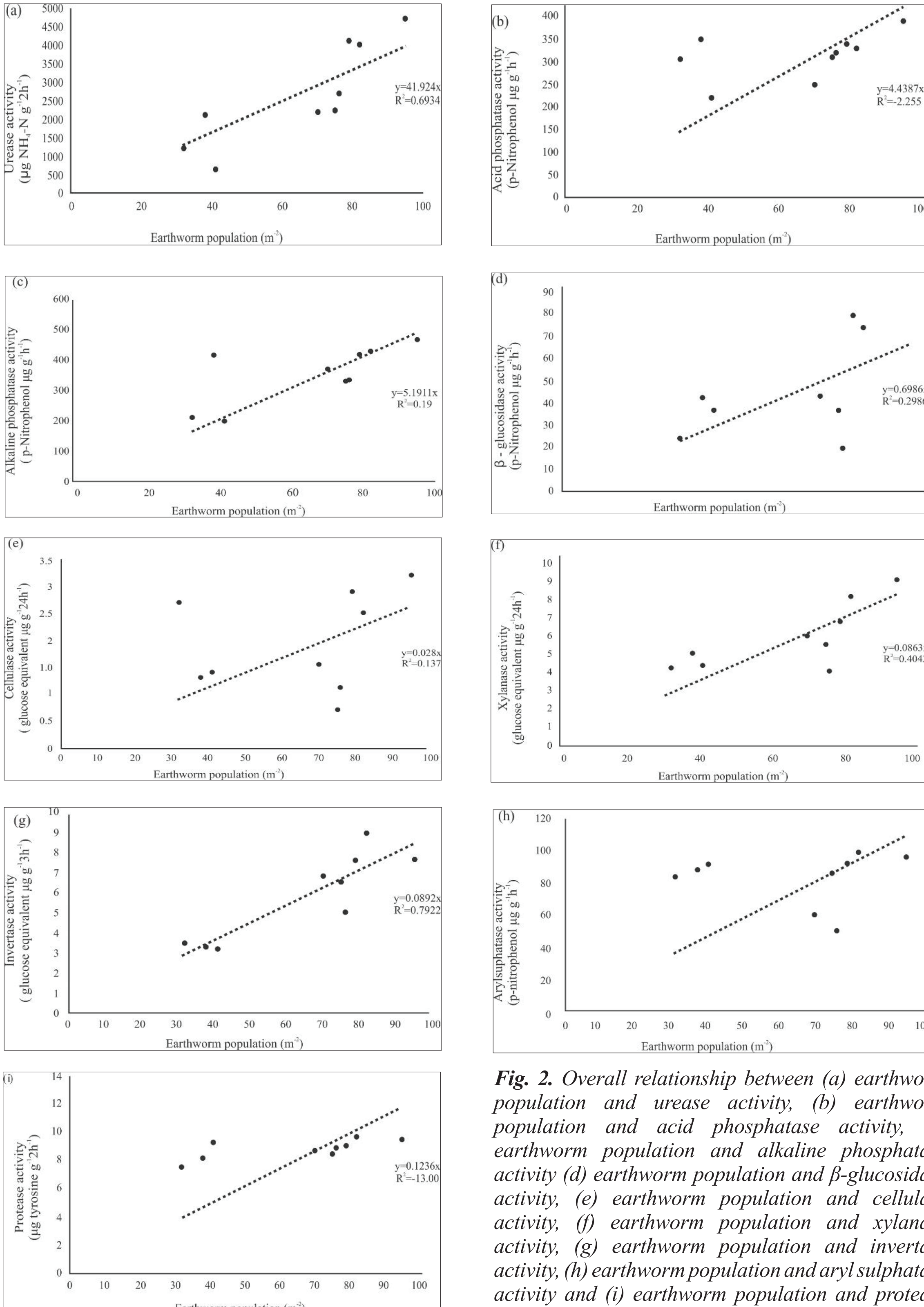

Fig. 2. *Overall relationship between (a) earthworm population and urease activity, (b) earthworm population and acid phosphatase activity, (c) earthworm population and alkaline phosphatase activity (d) earthworm population and β-glucosidase activity, (e) earthworm population and cellulase activity, (f) earthworm population and xylanase activity, (g) earthworm population and invertase activity, (h) earthworm population and aryl sulphatase activity and (i) earthworm population and protease activity*

Table 3. *Enzyme activities in soils of different farms in the coastal agro-ecosystem of Puducherry*

	Conventional	Sustainable	Organic	P-value
Urease (µg NH_4-N g^{-1} $2h^{-1}$)	1336.92 ± 432.89	2380.84 ± 160.77	4291.83 ± 224.69	0.001*
Acid phosphatase (p-Nitrophenol µg g^{-1} h^{-1})	292.45 ± 38.16	293.93 ± 21.84	353.51 ± 18.54	0.278
Alkaline phosphatase (p-Nitrophenol µg g^{-1} h^{-1})	277.04 ± 71.72	345.62 ± 12.53	440.22 ± 15.25	0.092
β-glucosidase (p-Nitrophenol µg g^{-1} h^{-1})	34.09 ± 5.47	32.86 ± 7.08	73.07 ± 3.82	0.004*
Cellulase (glucose equivalent µg g^{-1} $24h^{-1}$)	1.82 ± 0.45	1.14 ± 0.24	2.89 ± 0.21	0.022*
Xylanase (glucose equivalent µg g^{-1} $24h^{-1}$)	4.49 ± 0.23	5.14 ± 0.58	7.88 ± 0.65	0.008*
Invertase (glucose equivalent µg g^{-1} $3h^{-1}$)	3.34 ± 0.10	6.14 ± 0.55	8.08 ± 0.44	0.001*
Arylsulphatase (p-nitrophenol µg g^{-1} h^{-1})	88.08 ± 2.11	65.79 ± 10.62	95.54 ± 1.88	0.038*
Protease (µg tyrosine g^{-1} $2h^{-1}$)	8.31 ± 0.49	8.65 ± 0.11	9.37 ± 0.19	0.122

±: standard error and *: significantly different at $p \leq 0.05$ according to one way ANOVA

Impact of earthworm population on soil properties and enzyme activities

Soils from organic farming had significantly higher water holding capacity (49.50%), soil moisture (4.40%), total nitrogen (3.69 g kg^{-1}) and organic carbon (8.63 g kg^{-1}) compared tothe other two farming practices (Table 2). Urease (4291.83 µg NH_4-N g^{-1} $2h^{-1}$), β-glucosidase (73.07 p-Nitrophenol µg g^{-1} h^{-1}), cellulose (2.89 glucose equivalent µg g^{-1} $24h^{-1}$), xylanase (7.88 glucose equivalent µg g^{-1} $24h^{-1}$), invertase (8.08 glucose equivalent µg g^{-1} $3h^{-1}$) and arylsulphatase (95.54 p-nitrophenol µg g^{-1} h^{-1}) activities were significantly higher in organic farming system soils (Table 3).

Earthworm population and biomass were significantly correlated with organic carbon, protease, urease, alkaline phosphatase, β-glucosidase, cellulase, invertase and xylanase activities in the soils of different farms (Fig. 2). Aira *et al.* (2007) also reported that the activities of enzymes were significantly correlated with earthworm populations and biomass in soils. The presence of earthworms increased the activity of enzymes in the soils. Higher protease activity in the gut of earthworms has been observed, and this could have contributed to enhanced protease activity in the soil. Enzymes involved in carbon metabolism (β-glucosidase, cellulase and xylanase) were higher in organic farming soil. These results are in agreement with previous findings by Hoeffner *et al.* (2018) who reported higher carbon metabolism enzymes in earthworm casts compared to soil.

Invertase activity is a good indicator for estimating low molecular weight sugars (as energy sources for microorganisms). With earthworms present, invertase activity was significantly increased in organic farming soil. Hu *et al.* (2005) also reported enhanced invertase activity in the passage of the earthworm gut. Arylsulphatase and acid phosphatase activities showed a lack of significant correlation with the earthworm population and biomass (Table 4). The activity of acid phosphatase is linked not only to the synthesis of animal and microbial cells but also plays an important role in the soil P cycle and converting organic phosphorus to plant liable phosphorus. Acid phosphatase is an indicator of phosphorus deficiency in the soil. Acid phosphatase is secreted from plant roots to enhance the solubilization and remobilization of PO_4 (Tao *et al.,* 2009).

The interaction between microorganisms and earthworms may have stimulated the presence of proteolytic and ureolytic microorganisms in the soil and casts (Parthasarathi and Ranganathan, 2000). Earthworms might also contribute to higher enzyme activities in agricultural soils due to their casts (including underground and surface casts). Higher enzyme activities in earthworm casts were probably due to a combination of the earthworms' own enzymes and the provision of a rich nutrient substrate for microbial growth. Hoeffner *et al.* (2018) reported that higher enzyme activity in earthworm casts was due to an increased soil bacterial and fungal biomass.

In our study, the organic, sustainable and

Table 4. *Correlation between earthworm population, physico-chemical properties and enzyme activities in soil*

	WHC	pH	EC	TN	OC	EP	EB	URA	ACP	ALP	BGU	CEL	XYL	INV	ARS	PRO
WHC	1.00	0.49	0.54	0.40	0.82**	0.87**	0.89**	0.81**	0.50	0.79 **	0.64	0.16	0.70*	0.91**	0.07	0.45
pH		1.00	0.29	0.08	0.37	0.11	0.14	0.15	0.16	0.31	0.19	ns	0.27	0.41	0.10	ns
EC			1.00	0.03	0.42	0.27	0.26	0.41	0.63	0.65	0.25	ns	0.18	0.20	0.12	ns
TN				1.00	0.82**	0.38	0.52	0.55	0.24	0.35	0.78	0.54	0.61	0.62	0.62	0.64
OC					1.00	0.71*	0.80**	0.82**	0.49	0.75*	0.84**	0.39	0.78*	0.88**	0.40	0.63
EP						1.00	0.98**	0.85**	0.45	0.70*	0.57	0.27	0.74*	0.89**	ns	0.69*
EB							1.00	0.88**	0.46	0.70*	0.67	0.34	0.79*	0.94**	0.11	0.73*
URA								1.00	0.77*	0.88**	0.78*	0.59	0.86**	0.84**	0.26	0.59
ACP									1.00	0.73*	0.43	0.47	0.56	0.38	0.31	0.10
ALP										1.00	0.71*	0.32	0.77*	0.70*	0.18	0.45
BGU											1.00	0.64	0.86**	0.76*	0.62	0.64
CEL												1.00	0.64	0.42	0.50	0.25
XYL													1.00	0.84**	0.51	0.65
INV														1.00	0.19	0.63
ARS															1.00	0.27
PRO																1.00

*correlation is significant at the ≥0.05 level of interval, **correlation is significant at the ≥0.01 level of interval and ns: not significant
WHC: water holding capacity, EC: electrical conductivity, TN: total nitrogen, OC: organic carbon, EP: earthworm population, EB: earthworm biomass, URA: urease, ACP: acid phosphatase, ALP: alkaline phosphatase, BGU: β-glucosidase, CEL: cellulase, XYL: xylanase, INV: invertase, ARS: aryl sulphatase and PRO: protease.

conventional farming systems were found to have a direct influence on earthworm ecology. Earthworm population, diversity and biomass were higher in organic farming systems compared to the sustainable and conventional farming systems. Soil enzyme activities were also significantly higher in organic farming soil. Our study revealed that the earthworm population had a significant influence on soil enzyme activities. Earthworm casts probably contributed to the enrichment of plant nutrients and higher soil enzyme activities in organic farming system soils and they can play a significant role in enhancing soil health and agricultural sustainability.

CONFLICTS OF INTEREST

There is no conflicts of interest.

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