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Earthworm population in relation to different land use and soil characteristics

Subin Kalu[1]*, Madan Koirala[1] and Udhab Raj Khadaka[2]

[1]Central Department of Environmental Science, Tribhuvan University, Kirtipur, Kathmandu Nepal.
[2]Amrit Science Campus, Tribhuvan University, Thamel, Kathmandu, Nepal.

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Earthworms are regarded as the bio-indicators of soil quality. Also, due to several beneficial ecosystem services provided by earthworms, focus has been given to earthworms in agriculture management. So, there is need of knowing the distribution of earthworms, factors affecting their distribution and modification of soil environment due to them. With the aim of characterizing earthworm distribution in Panchase area, earthworms were sampled in different aspects, land use types, depth and altitude in June and October. Soil sample was also taken to characterize the habitat preference of the earthworms. The highest earthworm population density was found in forest followed by agriculture land and grassland in both months. Also, majority of earthworms were found in top 15 cm of soil. Moisture and organic matter content were found to be major determinants of earthworm population in Panchase area. Positive and significant correlation between earthworm population and available mineral nutrients (P and K) suggests that earthworms help in mineral nutrient availability to the plants.

Key words: Ash free dry mass, biomass, earthworm population density, Panchase area.

INTRODUCTION

Among numerous organisms found in the soil, earthworms are the most important components of soil biota in terms of soil formation and maintenance of soil structure and fertility (Curry, 2004). Earthworms play a major role in soil nutrient dynamics by altering the soil physical, chemical and biological properties which is generally in synchrony with plant demand (Lavelle et al., 1989). Therefore, focus has been given to integrate earthworms into agriculture management in order to increase the crop yield (Lavelle et al., 1989; Senapati et al., 2002). The crop production is usually higher in the soil with high number of earthworms than no or less earthworms (Edwards and Bater, 1992; Elmer, 2012). So, to investigate the potential of the earthworms to integrate into agriculture management, knowledge on different physical, chemical and management factors that affect the distribution and abundance of earthworm population is important that will help to identify the ecological appropriateness of the earthworms in order to supplement their existing population and quantify the impact of earthworms on agricultural land (Mele and Carter, 1999). Not only from agricultural perspective, earthworms are equally important from ecological point of view because they contain highest soil macro-faunal biomass (Edwards and Bohlen, 1996) and also increasingly regarded as bio-indicators of soil quality (Pérès et al., 2011). The

*Corresponding author. E-mail: kaluunique@gmail.com.

presence of earthworms modifies the environment (soil quality) due to their various activities like burrowing and casting which affect the activities of other organisms. So, they are also termed as "ecosystem engineers".

Further, the geographical distribution of earthworms is very poorly known; and much valuable information can be obtained by sampling various ecosystems and soil types (Edwards and Lofty, 1972). The studies regarding the distribution of earthworms are scanty in context to Nepal. So, this study is carried out to determine earthworm population density and biomass in different land use types and to determine relation between earthworm population and soil parameters.

MATERIALS AND METHODS

Study area

The study was carried out in Panchase area, mid-hill of western Nepal which is the junction of Kaski, Syangja and Parbat districts of Nepal including 17 VDCs (Figure 1). It lies between the longitudes of 83°45' and 83°57'E and latitudes of 28°12' and 28°18'N. It ranges from the altitude of 800 to 2517 m from mean sea level. This region is one of the most rain receiving places of Nepal and the climatic condition is usually humid. It receives more than 5000 mm precipitation per year (Sharma et al., 2013).

People in Panchase area mainly rely on farming and animal rearing for their livelihood. Farming is done by terracing the steep slopes of the hill and certain lands are left as grassland for free cattle rearing. Major agricultural crops are rice during rainy season, maize, barley, and other vegetables grown by traditional tilling method. The lowland agricultural land used in rice cultivation which is water logged is known as khet and upland agricultural land where other crops are cultivated which is not water logged is bari. Forest in the Panchase area can be categorized into community forests, managed by communities and protected forest, protected by government. Forest type is characterized as deciduous mixed broadleaf forest with species like *Quercus semecarpifolia*, *Schima wallichii, Castanopsis indica, Daphniphyllum himalense* etc. The human settlement with agriculture land grasslands is situated at lower altitude whereas forest in higher altitude.

Methods

The study was conducted on Panchase area on northern and southern aspects of the hill. Sampling was carried in two seasons; in October 2013 and June 2014 at different land use types that is forest, grassland and agriculture land. For sampling, whole Panchase area was divided into northern and southern aspects which were further stratified into different zones according to altitude (difference of 300 m). Then, in each altitudinal zone, sampling was taken from different land use types viz. agriculture land (khet and bari), grassland and forest. Total of 80 sampling units were allocated; 40 in each season. From each sampling unit, earthworm and soil sampling was carried out. Sampling size in different altitudinal zones and different land use type is shown in Table 1.

Earthworm sampling

Earthworms were sampled by digging a pit of 25 X 25 cm^2 to the depth of 30 cm (Anderson and Ingram, 1993). To observe the depth-wise variation in earthworm distribution and abundance, sampling was done from top layer (0-15 cm) and sub surface (15-30 cm). The earthworms were hand-sorted, washed with water and then preserved in 4% formalin (Julka, 1988). Earthworm population density (EPD) was determined by the formula:

$$\text{EPD} = \frac{\text{Total number of earthworms in a sampling area}}{\text{Sampling area } (0.0625\ m^2)}$$

While determining the earthworm biomass, its moisture and gut content affect the accuracy. So, earthworm biomass is usually measured in Ash Free Dry Mass (AFDM) because AFDM measures of biomass remove gut contents from the dry mass measurement and is the most accurate and provide data that is comparable between different sites and under different conditions (Edwards, 1998). Earthworm fresh mass can vary greatly depending on the moisture content status of the environment in which they are found (Lee, 1985). Earthworms preserved in formalin commonly lose a significant proportion of their fresh body mass (Lee, 1985). Also, variability in gut content can account for up to 20% of both fresh and dry mass measures of biomass (Lee, 1985; Edwards and Bohlen, 1996). Therefore, AFDM measure of biomass is significant. AFDM measure of biomass requires degradation of individual species. However, in the present case, preservation of earthworm specimens is desirable. So, AFDM of earthworms was measured by length-biomass allometric equation given by Hale et al. (2004) which prevents the destruction of earthworm specimens. Each earthworm–length was measured to the nearest 1.0 mm by aligning along edge of a ruler for determination of AFDM by the equation (Hale et al., 2004):

$$\ln(\text{AFDM in g}) = 2.2853 \times \ln(\text{length in mm}) - 11.9047$$

But, Hale et al. (2004) derived the equation by using the earthworms of Lumbricidae family and earthworms found in Nepal are dominated by Megascolecidae family (Sims, 1963). Therefore, to ensure that the equation can be used for earthworms specimens collected, AFDM of few earthworms were determined by both the equation as well as by loss on ignition (LOI) method and their statistical significance was checked. For that, eight earthworm sub-samples of different lengths were measured to the nearest 1.0 mm. Then they were dried for 36 h at 60°C in an Oven and weighed to the nearest 0.0001 g; then ashed at 500°C for 4 h in muffle furnace (Hale et al., 2004). The mass of the remaining ash was measured and subtracted from the dry mass measurement to get the AFDM of each earthworm. For small specimen (<40 mm), mean AFDM was determined from 4 specimens of equal length combined into a single sample:

$$\text{AFDM(g)} = \text{Oven dried mass (g)} - \text{Muffle furnaced mass (g)}$$

Soil sampling and Handling

Two soil samples were collected from each sampling unit: bulk sample and loose sample. Bulk soil sample was collected with the help of core sampler from the wall of the pit dug for sampling respective earthworms (Swift and Bignell, 2001) and loose sample was collected from the soil extracted while digging. Bulk sample was oven dried at 105°C for 24 h for determining bulk density and moisture content which was later used for determining the texture. Other parameters were determined from loose sample. pH and EC were determined in field moist condition while determining other chemical parameters, soil sample was air dried and sieved through 2 mm sieve.

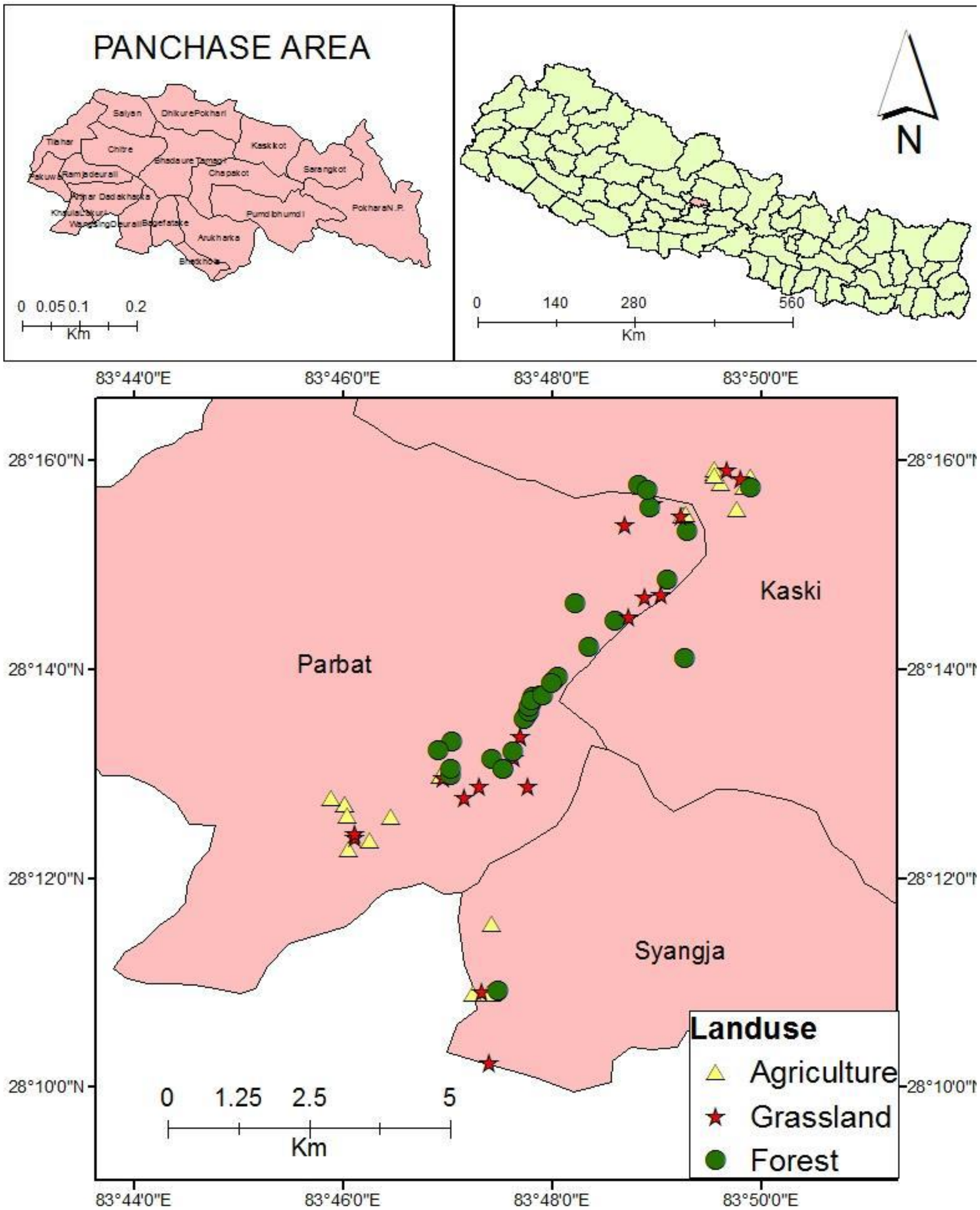
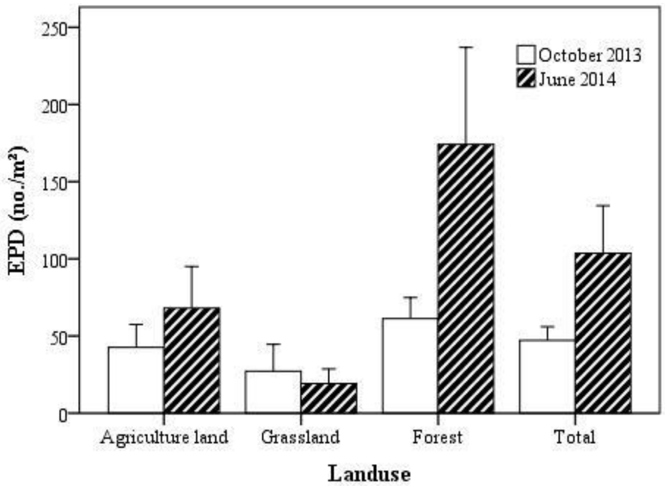


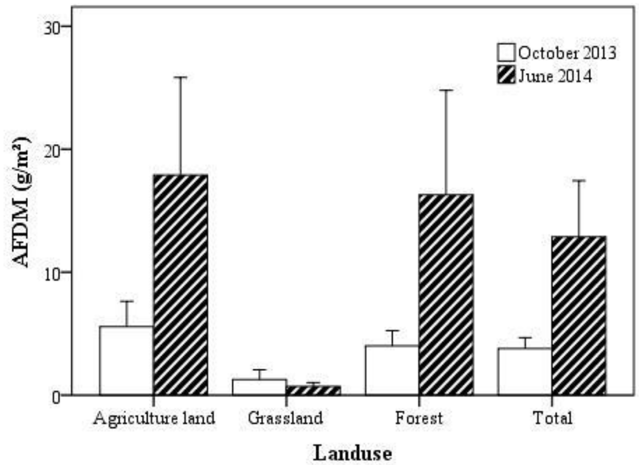
Figure 1. Map of Panchase area showing sampling units.

Table 1. Sampling design in each season.

Parameter		Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	Total
		1000 –1300 (m)	1300 -1600 (m)	1600-1900 (m)	1900–2200 (m)	Above 2200 (m)	
Agriculture land	Khet	2	2	2	-	-	12
	Bari	2	2	2	-	-	
Grassland		2	2	2	4	-	10
Forest		2	2	2	4	8	18
Total		8	8	8	8	8	40

Above 1900 m no agriculture land was found and above 2200 m no grassland was found.





(a) **(b)**

Figure 2. Average (± SE) earthworm population density and AFDM of different land use types and months.

Soil analysis

Different soil quality parameters were analyzed in laboratory and field as per the standard methods. Soil temperature was measured in field with the help of soil thermometer inserted into the depth of 5 cm. Soil pH and EC was measured using pH meter and conductivity meter respectively by inserting the probe in 1: 5 soil water ratio. Bulk density was determined by core method (Blake and Hartge, 1986). Soil moisture content was determined from core sample by calculating the difference between the wet core soil and oven dried soil. Soil organic matter was analyzed by Walkley-black method (Walkley and Black, 1934), total nitrogen by Kjeldahl method (Bremner and Mulvaney, 1982), available phosphorus by Modified Olsen's method (Olsen and Sommers, 1982), exchangeable potassium by flame photometer after extraction using 1 M ammonium acetate at pH 7 and soil texture by hydrometer method (Gee and Bauder, 1986).

For the analysis of data statistical, software SPSS was used. To know the relation between earthworm population density and biomass with different soil quality parameters, Pearson correlation and multiple regression analysis were performed. EPD, AFDM and various soil parameters of both the month (altogether 80 sampling units) was compiled for correlation and multiple regression analysis. To ensure the use of allometric length-biomass equation, paired t-test was performed for eight earthworm sub-samples whose AFDM was determined by both length-biomass equation and LOI method. Also, analysis of variance was carried out to test the significant difference of earthworm population and soil parameter in different seasons and land use types.

RESULTS

There was no significant difference between the biomass obtained from equation and LOI method ($t = 1.625$, d. f. = 7, $P > 0.05$).Therefore, allometric length-biomass equation given by Hale et al. (2004) can be used to determine the AFDM of the earthworm specimens collected.

Earthworm population in different seasons and land use types

Both the total average EPD and AFDM was found to be higher in June than in October (Figure 2). Average EPD was found significantly higher in forests followed by agriculture land and grassland, whereas average AFDM was found higher in agriculture land followed by forest and grassland.

Depth-wise distribution of earthworms

In Panchase area, majority of earthworms were found in 0-15 cm layer. No earthworms were found below 15 cm in June. During October also, more earthworms were found in top layer (92.27%) above 15 cm than sub surface layer (7.63%). Comparatively, among three land use types, more earthworms were found in grassland (11.76%), followed by forest (8.70%) and agriculture land (3.13%) in sub-surface layer.

Earthworm population density and biomass, and soil parameters

EPD was positively and significantly correlated with moisture content, available phosphorus, available potassium and organic matter content while negatively and significantly correlated with soil temperature and bulk density. Similarly, AFDM was positively and significantly correlated with moisture content, available phosphorus, available potassium and organic matter content whereas negatively and significantly correlated with soil temperature. The correlation coefficient of earthworm population and

Table 2. Correlation analysis of EPD (no. /m²) and AFDM (g/m²) with various soil parameters.

Parameter	Soil Temp. (°C)	pH	EC (μS/cm)	Moisture (%)	Bulk Density (g/cm³)	Total N (%)	Available P (mg/kg)	Available K (mg/kg)	OM (%)	Sand (%)	Clay (%)	Silt (%)
EPD (no./m²)	- 0.458**	- 0.218	0.099	0.543**	- 0.314**	0.077	0.333**	0.280*	0.694**	0.019	0.096	- 0.077
AFDM (g/m²)	- 0.242*	- 0.159	0.194	0.286*	- 0.184	0.042	0.344**	0.243*	0.729**	- 0.076	0.090	0.004

**Correlation is significant at 0.01 level, P < 0.01.*Correlation is significant at 0.05 level, P < 0.05.

Table 3. Multiple regression model summary.

R^2	Adjusted R^2	Standard Error of the Estimate	F
0.606	0.542	98.396	9.501

soil parameters is shown in Table 2.

The equation given by the multiple regression is:

$$Y = -166.99 - 2.24X_1 + 14.56X_2 + 0.09X_3 + 2.99X_4 + 47.89X_5 + 8.32X_6 + 0.36X_7 + 0.01X_8 + 21.13X_9 - 5.26X_{10} - 5.61X_{11}$$

Where, Y = EPD, X_1 = soil temperature, X_2 = pH, X_3 = electrical conductivity, X_4 = moisture content, X_5 = bulk density, X_6 = total nitrogen, X_7 = available phosphorus, X_8 = available potassium, X_9 = soil organic matter, X_{10} = clay percentage and X_{11} = silt percentage.

The value of R^2, adjusted R^2, standard error of the estimate and F-value of the multiple regression is presented in Table 3

Among different variables, contribution of soil organic matter and moisture content on earthworm population density was found to be significant (Table 4). Maintaining other variables constant, increase in 1% of soil organic matter increase EPD by 21.13 numbers. Similarly, if soil moisture content is increased by 1%, EPD will increase by 2.99 numbers remaining all other variables constant.

DISCUSSION

Higher earthworm population during June (beginning of monsoon) than that during October (post monsoon) supports the fact that earthworms are usually recorded higher during rainy season (Dash and Senapati, 1980; Rozen, 1982; Bhadauria and Ramakrishnan, 1989, 1991; Blanchart and Julka, 1997; Valle et al., 1997; Joshi and Aga, 2009; Koirala et al., 2011).

Among different land use types, average EPD was found significantly higher in forest than in agricultural land and grassland. Forest soil contained higher organic matter content (Table 5), contributed by fallen leaf litters from above ground vegetation which provides food base for earthworm (Edwards and Bohlen, 1996). Though average EPD was high in forest, average AFDM was in the sequence agriculture land > forest > grassland in both the seasons. The reason is occurrence of matured and large size earthworms in the agricultural land than forest and grassland. There is continuous supply of organic manure and dung in agriculture land which is much preferred by earthworms (Guild, 1955) that ultimately resulted in faster growth of earthworms. Majority of earthworms were found in top layer (0-15 cm) in both the months. As the soil depth increases, the amount of soil oxygen declines that limits the distribution of earthworms in deep soil (Curry and Cotton, 1983).

Both EPD and AFDM was positively and significantly correlated with organic matter content which agrees with the study of Hendrix et al. (1992) that reported high significant correlation between earthworm populations and soil organic carbon content (r = 0.91, P = 0.01). Organic matter provides food base for the earthworms. Soils that are poor in organic matter do not usually support large numbers of earthworms. EPD was also correlated positively and significantly with soil moisture content which is also important parameter that affects the abundance and distribution of earthworms. Earthworms respire through skin which needs to be kept moist in order to dissolve the oxygen. Also, fecundity of earthworms is greatly influenced by moisture (Edwards and Lofty, 1972).

Both available phosphorus and available potassium was positively and significantly correlated with EPD and AFDM though correlation coefficient

Table 4. Factors contributing distribution and abundance of earthworms.

Particulars	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. Error	Beta		(P)
Constant	-166.99	255.44		-0.65	0.52
Soil Temp.	-2.24	3.81	-0.06	-0.59	0.56
pH	14.56	23.10	0.06	0.63	0.53
EC	0.09	0.08	0.11	1.22	0.23
Moisture	2.99	1.49	0.26*	2.00	0.05
Bulk density	47.89	87.11	0.06	0.55	0.58
N	8.32	129.98	0.01	0.06	0.95
P	0.36	0.39	0.09	0.93	0.36
K	0.01	0.19	0.01	0.07	0.95
OM	21.13	3.32	0.60**	6.36	0.00
Clay	-5.26	5.80	-0.09	-0.91	0.37
Silt	-5.61	3.54	-0.16	-1.58	0.12

Dependent Variable: EPD, Excluded variable: Sand (due to collinearity statistics). **Significant at 0.01 Level. *Significant at 0.05 Level.

Table 5. Soil properties (mean ± SE) of different land use types.

Month	Land Use	Soil Temp. (°C)	pH	EC (µS/cm)	Moisture (%)	BD (g/cm³)	TN (%)	P (mg/kg)	K (mg/kg)	OM (%)
October 2013	Agriculture (n =12)	16.46 ± 0.41^{a}	6.45 ± 0.13^{a}	411.67 ± 68.15^{a}	30.18 ± 1.44^{ab}	1.09 ± 0.05^{ab}	0.21± 0.03^{a}	67.36 ± 10.86^{a}	322.03 ± 21.78^{a}	5.61± 0.57^{b}
	Grassland (n= 10)	17.20 ± 0.66^{a}	6.170.07^{ab}	154.60 ± 20.11^{b}	25.37 ± 0.80^{b}	1.18 ± 0.04^{a}	0.21 ± 0.03^{a}	14.25 ± 3.19^{b}	289.70 ± 9.13^{ab}	5.66 ± 0.95^{b}
	Forest (n = 18)	14.14 ± 0.66^{b}	5.81 ± 0.09^{b}	238.17 ± 29.56^{b}	31.92 ± 1.35^{a}	0.99 ± 0.04^{b}	0.19 ± 0.02^{a}	20.18 ± 3.36^{b}	255.92 ± 11.52^{b}	8.89 ± 0.59^{a}
June 2014	Agriculture (n =12)	13.45 ± 1.04^{a}	5.86 ± 0.16^{a}	334.48 ± 54.05^{a}	39.22 ± 2.89^{ab}	1.03 ± 0.04^{ab}	0.13 ± 0.03^{b}	74.81± 15.27^{a}	385.74 ± 18.52^{a}	9.47 ± 0.59^{a}
	Grassland (n= 10)	16.15 ± 1.02^{a}	5.55 ± 0.12^{ab}	152.67 ± 17.18^{b}	32.28 ± 3.06^{b}	1.11 ± 0.03^{a}	0.09 ± 0.02^{b}	41.71 ± 6.98^{a}	309.66 ± 26.68^{b}	8.53 ± 0.53^{a}
	Forest (n = 18)	9.39 ± 1.01^{b}	5.28 ± 0.11^{b}	166.39 ± 17.65^{b}	50.51 ± 3.64^{a}	0.94 ± 0.04^{b}	0.22 ± 0.02^{a}	42.21 ± 7.23^{a}	360.47 ± 12.18^{ab}	12.07 ± 1.44^{a}

Means not connected by same letters are significantly different.

was low. Soil with many earthworms usually has more available phosphorus and potassium (Edwards and Lofty, 1972; Edwards, 2004). The earthworm casts contain higher available phosphorus and potassium than surrounding uningested soil (Tiwari et al., 1989). As a result of enhanced phosphatase activity in gut of earthworms, organic phosphate content decreases and inorganic phosphate content increases, being total phosphate content unchanged in earthworm casts (Syers and Springett, 1984; Chapuis-Lardy et al., 1998; Le Bayon and Binet, 2006). Le Bayon and Binet (2006) showed significant increase in bicarbonate extractable available phosphorus (Olsen P) in both earthworm cast and surrounding soil. This increment in available phosphorus is ascribed to changes in the sorption reactive surfaces induced by competition for sorbing sites between orthophosphate and carboxyl groups of a (mucus–produced) glycoprotein (Lopez-Hernandez et al., 1993).

Similarly, there is higher concentration of available and exchangeable potassium in earthworm casts than the surrounding soil (Puh, 1941; Lunt and Jacobson, 1944). Basker et al. (1992) reported an incubation experiment carried out under controlled laboratory conditions which showed that exchangeable potassium was significantly higher in soil with earthworms than without earthworms. It was concluded that the increase was due to the release of potassium from the non-exchangeable K pool as soil material passed through the gut of earthworms.

Conclusion

The study characterized the distribution of earthworms in the belowground world of Panchase area. Earthworm density was found higher in forest land than agriculture land and grassland but earthworm biomass was found high in agriculture land followed by forest and grassland. Earthworms were found higher during June (monsoon season) than October (post-monsoon season). Among different soil parameters, moisture content and organic matter content affected the distribution of earthworms in Panchase area. Also, it was found that earthworms play certain role in availability of mineral nutrients. So, efficiency of earthworms in soil fertility improvement can be explored in order to minimize use of chemical fertilizers as far as possible.

Conflict of interests

The authors did not declare any conflict of interest.

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