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Earthworm cast production and physico-chemical properties in two agroforestry systems of Mizoram (India)

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Abstract: Earthworm casts are known to contribute significantly to surface soil fertility in agroecosystems. Knowledge of their role in maintaining soil fertility is of primary importance in keeping a healthy soil ecosystem. Thus physicochemical properties of earthworm casts and their production rate were studied for a period of three years (2002 to 2004) in two agroforestry sites, Sakawrtuichhun (SKT) and Pachhunga University College (PUC), in the state of Mizoram in northeast India. Plants including *Leucaena leucocephala*, pineapple (*Ananas comosus*) and manadarin orange (*Citrus reticulata)* were present in both sites. Sampling of earthworm casts was done on the basis of occurrence of fresh worm casts. Four species of earthworms, viz. *Metaphire houlleti*, *Perionyx excavatus*, *Eutyphoeus mizoramensis* and *Drawida* sp., were common to both study sites, whereas *Perionyx macintoshi* was recorded only at PUC. Earthworm casts were mainly produced from June to September. Maximum cast production was recorded in October 2002 at SKT and July 2003 at PUC. Annual cast production declined from the first year to the third year by 18 % and 25.5 % at SKT and PUC, respectively. This may be attributed to a decrease in population and food supply due to local weeding practices. Among the physico-chemical parameters studied, only phosphorus levels showed significant correlation with worm cast production. Potassium showed the highest degree of difference between worm casts and its adjacent soil, while nitrogen showed the least difference. Our results show that worm cast production coincides with the rainy season and earthworm cast contains higher levels of inorganic nutrient compared to adjacent soil.

Resumen: El humus producido por las lombrices de tierra contribuye significativamente a la fertilidad del suelo superficial de los agroecosistemas. El conocimiento sobre su papel en el mantenimiento de la fertilidad del suelo tiene es de gran importancia para asegurar un ecosistema del suelo sano. Por lo tanto, las propiedades fisicoquímicas del humus de lombriz de tierra y su tasa de producción fueron estudiadas por un periodo de tres años (2002 a 2004) en dos sitios agroforestales, Sakawrtuichhun (SKT) y el Pachhunga University College (PUC), en el estado de Mizoram, noreste de la India. Algunas plantas, incluyendo *Leucaena leucocephala*, piña (*Ananas comosus*) y mandarina (*Citrus reticulata*) estuvieron presentes en ambos sitios. El muestreo del humus de lombriz se hizo a partir de la presencia de humus fresco. Cuatro especies de lombrices (*Metaphire houlleti, Perionyx excavatus, Eutyphoeus mizoramensis* y *Drawida* sp.) fueron comunes a ambos sitios de estudio, mientras que *Perionyx macintoshi* sólo fue registrada en PUC. El humus de lombriz de tierra se produjo principalmente de junio a septiembre. La producción máxima de humus fue registrada en octubre de 2002 en SKT y en julio de 2003 en PUC. La producción anual de humus disminuyó entre el primer y el tercer año en 18 % y 25.5 % en SKT y PUC, respectivamente, lo que puede atribuirse a un decremento en la población y disponibilidad de alimento debido a las prácticas locales de deshierbe. Entre los

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parámetros fisicoquímicos estudiados, sólo los niveles de fósforo estuvieron correlacionados significativamente con la producción de humus. El potasio mostró la mayor diferencia entre el humus de lombriz y el suelo adyacente, mientras que el nitrógeno mostró la diferencia menor. Nuestros resultados muestran que la producción de humus de lombriz coincide con la estación lluviosa y que el humus contiene niveles más altos de nutrimentos inorgánicos en comparación con el suelo adyacente.

Resumo: Os excretados das minhocas são conhecidas como contribuindo significativamente para a fertilidade superficial do solo em ecossistemas agrários. O conhecimento do seu papel na manutenção da fertilidade do solo é de importância fundamental na manutenção do ecossistema do solo saudável. Assim, as propriedades físico-químicas dos excretados de minhocas e sua taxa de produção foram estudadas por um período de três anos (2002 a 2004), em doissistemas agro-florestais, Sakawrtuichhun (SKT) e Pachhunga University College (PUC), no Estado de Mizoram, no nordeste da Índia. As plantas, incluindo a *Leucaena leucocephala*, abacaxi (*Ananas comosus*) e laranja mandarina (*Citrus reticulata*) estavam presentes nos doissítios. A amostragem dos excretas de minhocas foi feito com base na ocorrência dos moldados frescos. Quatro espécies de minhocas, nomeadamente a *Metaphire houlleti, Perionyx excavatus, Eutyphoeus mizoramensis* e *Drawida* sp., eram comuns aos dois locais de estudo, enquanto que a *Perionyx macintoshisó* foi registrado em PUC. Os excretados das minhocas eram principalmente produzidos a partir de Junho a Setembro. A produção máxima de excreta foi registada em Outubro de 2002 em SKT e em Julho de 2003 em PUC. A produção anual de excretados diminuiu desde o primeiro ano ao terceiro ano em 18 % e 25,5 % em SKT e PUC, respectivamente. Este decréscimo pode ser atribuído a uma redução na população e na disponibilidade de alimentos devido às práticas locais de capina. Entre os parâmetros físico-químicos estudados, apenas os níveis de fósforo apresentaram uma correlação significativa com a produção de excretados de minhoca. Quanto ao potássio verificou-se o maior grau de diferença entre os excretados e o solo adjacente, enquanto o azoto mostrou a menor diferença. Os nossos resultados mostram que a produção de excretados de minhoca coincide com a estação chuvosa e que estes contém níveis mais elevados de nutrientes inorgânicos em relação ao solo adjacente.

Key words: Agroforestry system, biomass, density, earthworms, Mizoram, soil physico-chemical characters, worm cast production.

Introduction

Earthworms are large megadrile annelids of the class Oligochaeta that constitute more than 80 % of the soil invertebrate biomass in many terrestrial ecosystems (Nainawat & Nagendra 2001). They form the major group of soil invertebrates in sub-tropical, tropical and temperate zones (Kale 1997). Earthworms feed on soil and on a wide range of decaying organic substances, and excrete wastes in the form of casts. Earthworm casts consist of masses of mineral soil often mixed with smaller bits of partially digested plant residues. Charles Darwin (1881) calculated that earthworms could move large amounts of soil from the lower layers of the soil profile to the surface, and transport organic matter down into the deeper soil horizons. Soil from the subsurface horizons is translocated by these animals to the upper horizons, where it is mixed with the surface soil, resulting in a more uniform distribution of plant nutrients (Darwin 1881).

Earthworm casts are mainly produced during the rainy season in tropical regions (Chaudhuri *et al.* 2009). Seasonal variations in cast production are attributed to fluctuations in factors including soil physico-chemical properties, land use patterns, feeding habits and availability of food resources. Earthworm casts have a higher moisture content, pH, and levels of organic carbon and inorganic nutrients compared to adjacent soil (Bhadauria & Ramakrishnan 1989; Edwards 1988; Scullion *et al.* 2002).

The nutrient status of earthworm casts from temperate and tropical regions has been evaluated previously, with most of the available information indicating that earthworm casts have a higher nutrient content than adjacent soil (Araujo *et al.* 2004; Basker *et al.* 1993; Bossuyt *et al.* 2005; Krishnamoorthy 1989; Kuczak *et al.* 2006; Mariani *et al.* 2006; Norgrove *et al.* 2003; Parkin & Berry 1994; Zhang & Schrader 1993). However, contradictory observations have also been reported on organic carbon and nitrogen (Bhattacharya & Chakraborti 1987) and on pH (Nijhawan & Kanwar 1952) from India. There is little information on earthworm casts from ecosystems in northeast India (Bhadauria & Ramakrishnan 1989; Chaudhuri & Bhattacharjee 2005; Chaudhuri *et al.* 2009). Therefore, the present investigation was conducted to provide information from the hilly sub-tropical rain forest areas of northeast India. The objectives of the study were to: (i) assess seasonal variations in earthworm cast production; (ii) determine the physico-chemical properties of earthworm casts and adjacent soil; and (iii) to evaluate the relationships between cast production and soil chemical properties (organic carbon, nitrogen, phosphorus and potassium). To the best of our knowledge, this is the first report of its kind for this region.

Materials and methods

Study site

The state of Mizoram, located in northeastern India, lies between 21° 56'N - 24° 31'N latitude, and 92° 16'E - 93° 26'E longitude. It has international borders with Bangladesh in the west and Myanmar in the east and south (Fig. 1). The hilly state has steep gorges with north-south trending mountains, covered with tropical and subtropical forest with an annual rainfall of 2500 mm. Mizoram experiences four seasons, viz. summer (April-June), monsoon (July-October), winter (November-February) and spring (March) with the least rainfall in winter and maximum rainfall in monsoon period. The dry season starts by mid-October and ends in March. The annual average minimum and maximum temperatures are 18.37 °C and 27.19 °C, respectively.

Two sites were selected for the study, with a distance between sites of 16 km. Site I was located near a small village named Sakawrtuichhun (SKT) (92° 40'E and 23° 45'N), 650 m above mean sea level, with slopes that vary from 40 % to 65 %. The soil is brown to dark brown in colour with a clay to clay loam texture. The land at the site was left fallow for four years after shifting cultivation practices. Grasses, including *Imperata cylindrica* and *Saccharum* (= *Erianthus) longisetosus*, were present in the area before it was converted to agroforestry. Site II was located within the Pachhunga University College (PUC) campus, Aizawl (92° 44'E and 23° 43'N) at an elevation of 825 m above mean sea level, with slopes ranging from 50 % to 67 %. The soil is brown in colour and the texture varies from sandy to clay loam. Prior to the establishment of an agroforestry system, the area was dominated by *I. cylindrica*, and trees including *Schima wallichii, Albizia* sp., and *Sapium baccatum*. The area adjacent to the PUC site is covered by semi-dense forest. Plants including a hedge (*Leucaena leucocephala* - Hawaiian Giant), a horticultural plant (Pineapple - *Ananas comosus*) and a tree species (Mandarin orange - *Citrus reticulata)* were introduced in both sites. No agricultural inputs (e.g. fertilizer, pesticides, herbicides) were applied at either site. The soil at both sites is slightly acidic. Weeding was carried out manually when required, using a hand hoe.

Sampling of earthworm casts

Sampling was carried out monthly for three consecutive years, between 2002 and 2004. Sampling plots in the study site were selected on the basis of occurrence of fresh worm casts. Old worm casts were removed from a unit area (5 m^2) before the day of collection. The newly deposited casts were collected the next morning. Fresh worm casts were taken to the laboratory and weighed, air-dried, ground with mortar and pestle, and passed through a fine (0.2 mm-mesh) sieve (Ghosh *et al.* 1983).

Sampling of earthworm and soil

Earthworms and adjacent soil samples were collected from the study sites by random sampling, particularly near the site of surface casting. Five random samples (25 cm × 25 cm × 30 cm) located at least 5 m apart were taken at monthly intervals following Anderson & Ingram (1993). Sampling was done in the morning, in the second week of every month. The soil samples were transferred to an enamelled tray, the earthworms were collected by the hand sorting and wet sieving method, recorded, and placed in a 4 % formalin solution. Adjacent soil samples were air-dried, ground, and passed through 0.2 mm-mesh sieve for chemical analysis (Ghosh *et al.* 1983).

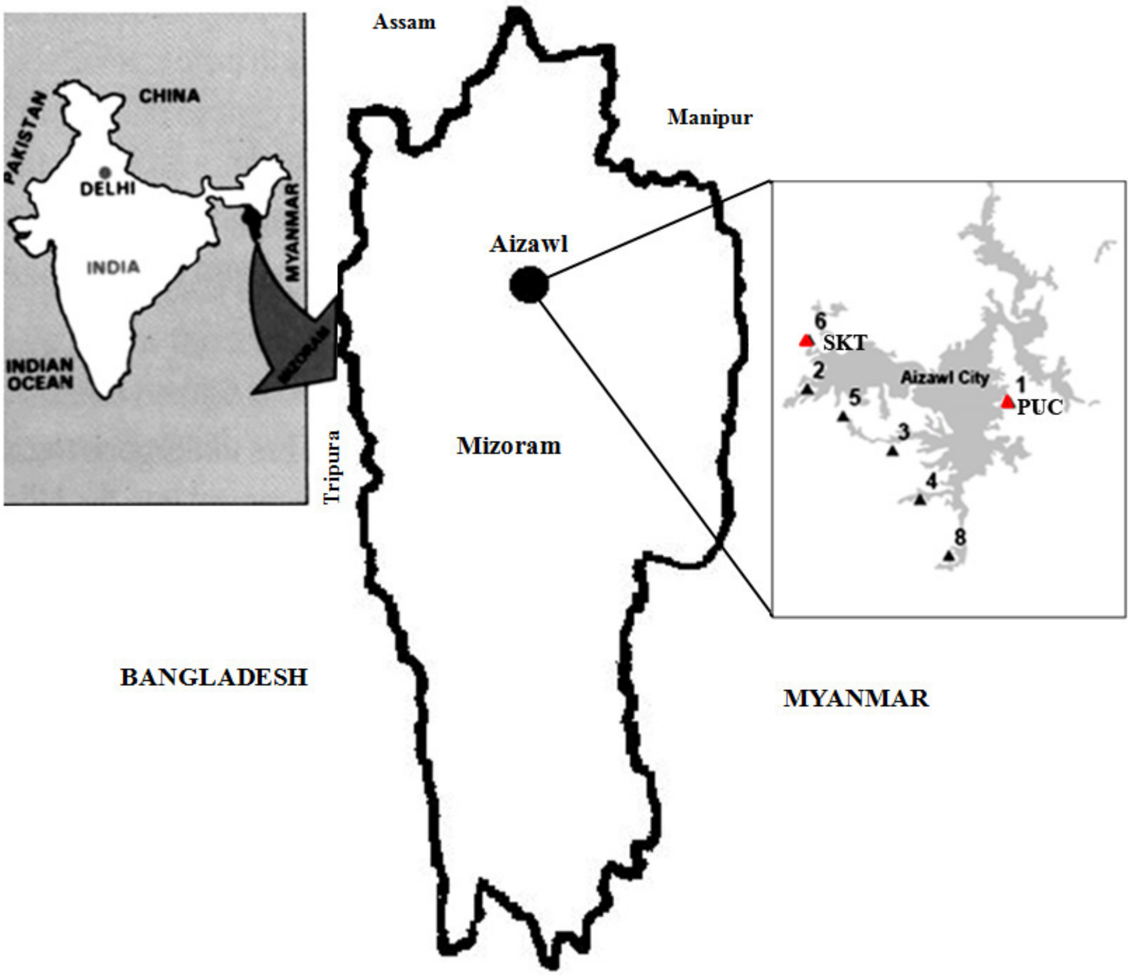

Fig. 1. Map showing location of study sites.

Analysis of soil and cast

The soil moisture content was determined following Anderson & Ingram (1993). The soil and cast samples were analyzed for organic carbon (colorimetric method; Anderson & Ingram 1993), pH (soil : water ratio of 1:2.5; Ghosh *et al.* 1983), plant-available phosphorus (Bray & Kurtz 1945), available potassium (flame photometric method; Stanford & English 1949) and total Kjeldahl nitrogen (Jackson 1962).

Statistical analysis

Differences in the physico-chemical properties of worm casts and adjacent soil were tested using Student's *t*-test. Pearson's correlation coefficient (r) was applied to evaluate the relationships between soil physico-chemical properties and cast production using Statistic software SPSS Version 7.6.

Result and discussion

Cast production

Four species of earthworms, viz. *Metaphire houlleti* (Perrier), *Perionyx excavatus* (Perrier), *Eutyphoeus mizoramensis* and *Drawida* sp., were common to both study sites, whereas *Perionyx macintoshi* (Stephenson) was recorded only at the PUC site. Earthworm casts were found during seven months of the year (May - November) at both sites during the first two years, but at PUC were present only in August in the third year (Table 1).

Eutyphoeus, an anecic species, was bigger in size compared to other species, and produced a tower-like surface cast (10 cm), whereas epigeic species like *Perionyx* sp. produced small, granular casts. *M. houlleti* and *Drawida* sp. are endogeic

Table 1. Amount of casts (mean ± SE) produced (kg ha^{-1} d^{-1}) between May and November on 2002, 2003 and 2004) in agroforestry sites at Sakawrtuichhun (SKT) and Pachhunga University College (PUC).

Month	2002		2003		2004	
	SKT	PUC	SKT	PUC	SKT	PUC
May	N.F	N.F	N.F	N.F	98.11 ± 4.62	N.F
Jun.	N.F	N.F	403.64 ± 12.54	145.31 ± 12.24	45.96 ± 3.57	N.F
Jul.	N.F	N.F	1506.94 ± 50.30	565.10 ± 16.75	672.74 ± 17.40	N.F
Aug.	3471.36 ± 76.76	559.72 ± 11.61	618.92 ± 20.59	199.13 ± 8.61	363.28 ± 8.88	261.02 ± 5.06
Sep.	2378.82 ± 53.18	269.09 ± 7.23	683.50 ± 17.16	123.78 ± 7.11	553.48 ± 19.24	N.F.
Oct.	3498.27 ± 46.88	193.75 ± 9.07	430.55 ± 14.41	N.F.	N.F.	N.F.
Nov.	230.45 ± 10.90	N.F.	N.F.	N.F.	N.F.	N.F.

N.F. = none found.

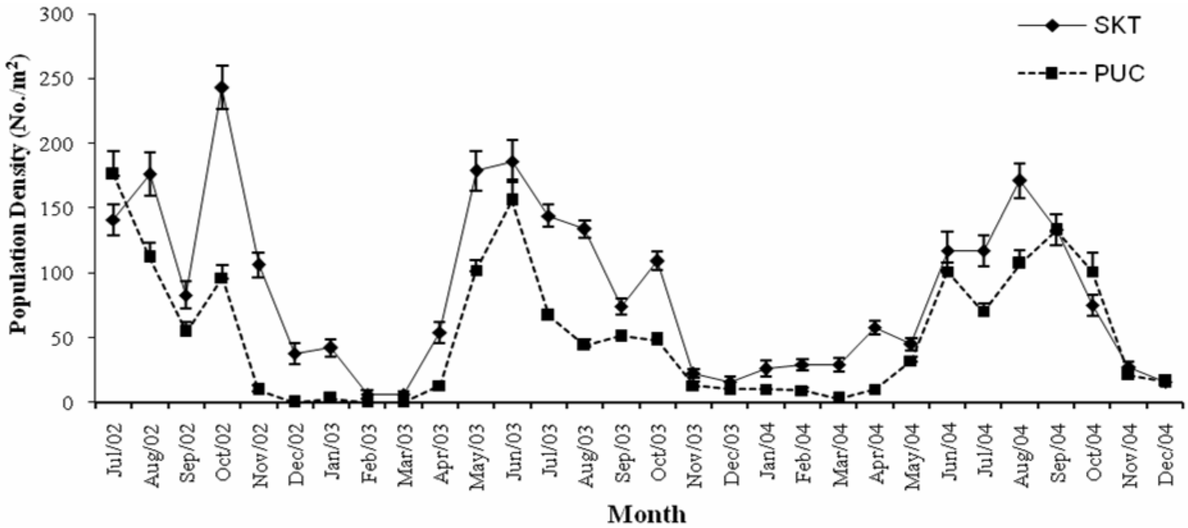

Fig. 2. Population density of earthworms (mean ± SE) in Sakawrtuichhun (SKT) and Pachhunga University College (PUC) sites.

and epianecic species, respectively, and produced small, pellet like casts.

Total cast production was higher in SKT compared to PUC, and it was higher in the first year of the study relative to cast production during the second and third years (Table 1). This can be attributed to the gradual decrease of earthworm population density every year at both sites. Higher total cast production in SKT relative to PUC may be attributed to a higher earthworm population density in SKT (Fig. 2) and to differences in soil texture, which could carry higher organic matter. SKT has a clayey-loam soil with an average organic carbon content of 2.84 %, whereas PUC is sandy loam soil with an organic carbon content of 1.99 %. It is generally acknowledged that fine-textured soils have a higher more organic matter content than sandy soils. Further reduction of worm cast production in the second and third year at SKT, and the presence of casts only during the month of August in the third year of study at PUC, may be attributed to a sharp decline in earthworm population and food sources due to traditional weeding practices (Hauser 1993; Sharpley & Syers 1979). Agricultural management practices like repeated weeding influence cast production (Norgrove & Hauser 2000).

Drawida sp. has a versatile in distribution and is found at a deeper layer (15 - 30 cm) during dry season in order to avoid drastic environmental conditions. It was present in the earthworm community of the two sites during dry season. Haynes *et al.* (2003) are of the opinion that an earthworm species assemblage is likely to be the main factor determining surface cast deposition.

In SKT, cast production was highest in October 2002 (3498.27 ± 46.88 kg ha^{-1}) and lowest in June 2004 (45.96 ± 3.57 kg ha^{-1}). PUC had its maximum cast production in July 2003 (565.10 ± 16.75 kg ha^{-1}) and minimum production in September 2003 (123.78 ± 7.11 kg ha^{-1}) (Table 1). These values are much lower than those reported previously for natural forest areas (Bhadauria & Ramakrishnan 1991; Dash & Patra 1979). The lower production of casts in the present study may be attributed partly to the lower abundance of surface casting species of earthworms (viz. *Drawida* sp. and *M. houlleti*), and to low organic matter production in the early stages of the agro-forestry sites. Reduction in casting rates has been related both to the degree of plant biomass removal and re-establishment of cover crops (Hauser & Asawalam 1998). Sharpley & Syers (1976) reported casting activity between April and September, with maximum activity in June, in silty loam soils of pasture ecosystem in New Zealand. Singh & Dev (1987) also reported maximum cast production in June in tropical grasslands. Bhadauria & Ramakrishnan (1991) reported maximum cast production in the pine forests of the northeastern region of India during rainy period. This is in agreement with the present study, which found that earthworm cast production is observed mainly between June to October at both study sites, coinciding with the monsoon period.

A significant correlation between worm cast production and adjacent soil chemical properties was not observed except for phosphorus in PUC (Table 2). This result is in agreement with the observations of Hauser & Asawalam (1998) in a fallow system and Birang *et al.* (2004) in slash-and-burn cropped land and in undisturbed forest in southern Cameroon. Cast production has been reported to be influenced by increasing soil pH and Ca level (Springett & Syers 1984) and soil moisture, temperature and food supply (Chaudhuri *et al.* 2009; Farenhorst & Bowman 2000; Sharpley & Syers 1979; Whalen *et al.* 2004), shading and litter fall (Sharpley & Syers 1979), land use pattern (Norgrove & Hauser 2000) and vegetation and environmental conditions (Daniel *et al.* 1996).

Physico-chemical analysis of worm casts

In general, the moisture content, pH, organic carbon and inorganic nutrients (P, K) were significantly higher in worm casts compared to adjacent soil in all three years of study (Table 3, Fig. 3). These results are similar to previous studies (Bhattacharya & Chakraborti 1987; Bhadauria & Ramakrishnan 1989; Bisht *et al.* 2006; Chaudhuri *et al.* 2009; Edwards 1988; Gupta & Sakal 1987; Hauser & Asawalam 1998; Krishnamoorthy & Vajranabhaiah 1986; Scullion *et al.* 2002; Zhang & Schrader 1993). The moisture content of soil showed a significant difference between newly deposited earthworm casts and adjacent soil (Table 3; Fig. 3 a). Earthworm casts generally have a higher water-holding capacity than bulk soil samples (Elliot *et al.* 1990). A higher pH value in fresh earthworm cast compared to adjacent soil was observed in the present study (Table 3; Fig. 3 b). Our data reflects the findings of others (Bhattacharya & Chakraborti 1987; Nijhawan & Kanwar 1952). The higher pH of cast soil may be due to the ammonia secreted in the worm's gut, which may act as a neutralizing factor (Wallwork 1983) and/or the production of calcium carbonate in calciferous glands and its release into the intestine (Lee 1985).

Table 2. Correlation coefficient (*r*) between earthworm cast production and soil chemical properties in agroforestry sites at Sakawrtuichhun (SKT) and Pachhunga University College (PUC).

Parameter	Site	
	SKT	PUC
Organic carbon	-0.635	-0.305
Nitrogen	0.487	0.749
Phosphorus	0.076	0.970*
Potassium	0.064	-0.318
C/N ratio	-0.305	-0.495

*$P < 0.05$.

There was no significant difference in nitrogen content between casts and adjacent soil, except in PUC during the first year of the study (Table 3; Fig. 3 d). Although Bhattacharya & Chakraborti (1987) reported similar results, many studies have reported that the nitrogen content is higher in worm casts compared to adjacent soils (Araujo *et al.* 2004; Edwards 1988; Krishnamoorthy 1990; Parkin & Berry 1994; Ruz *et al.* 1988; Simek & Pizl 1989). Our result may be attributed to low mineralization of organic residues during study period because local weeding practices remove surface leaf litter.

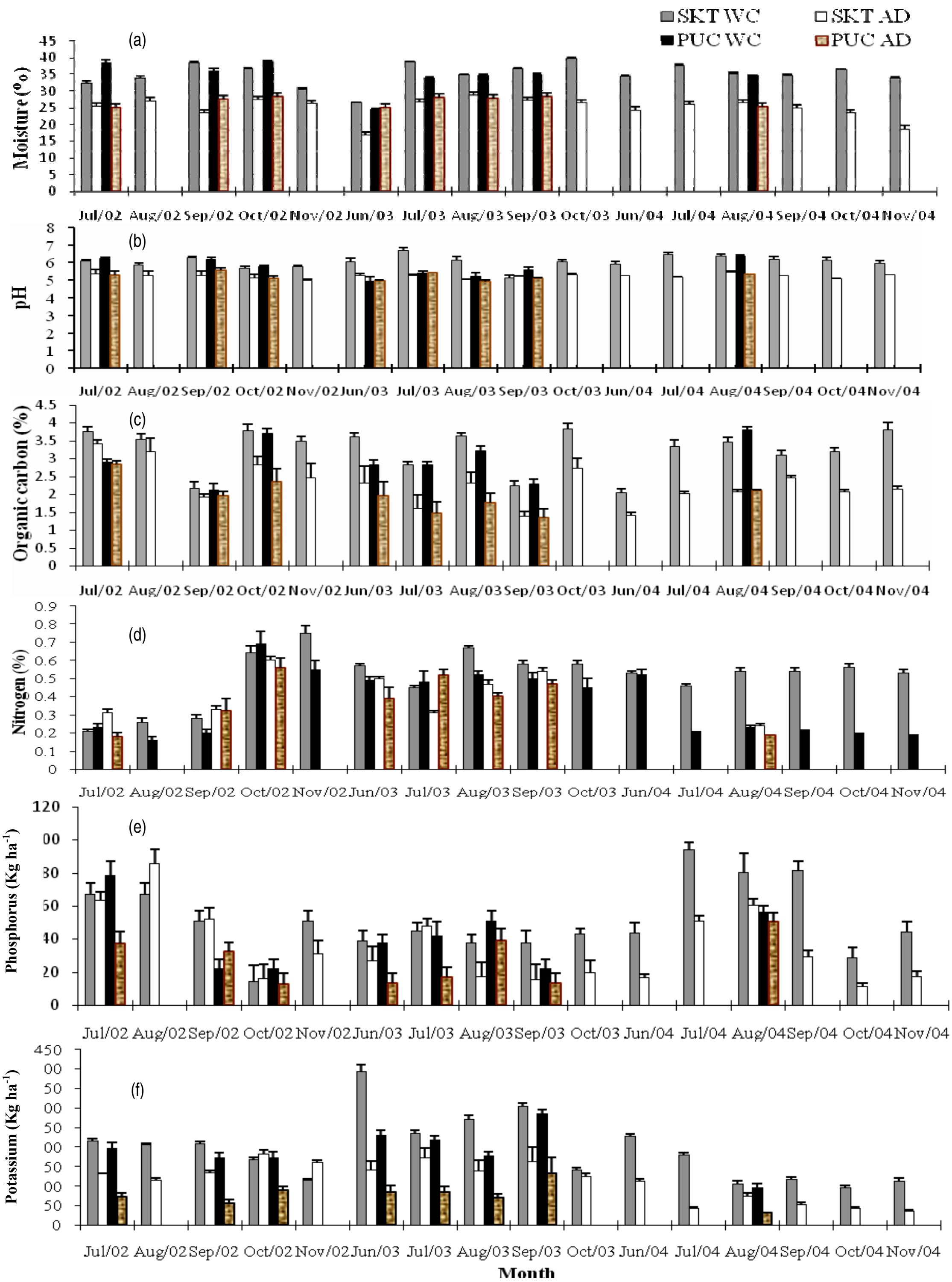

Fig. 3. Physico-chemical parameters of earthworm casts and adjacent soil in Sakawrtuichhun (SKT) and Pachhunga University College (PUC) sites (2002-2004). WC = worm cast; AD, = adjacent soil.

(a) Moisture content (mean ± SE) of earthworm cast and adjacent soil.

(b) pH (mean ± SE) of earthworm cast and adjacent soil.

(c) Organic carbon content (mean ± SE) of earthworm cast and adjacent soil.

(d) Nitrogen content (mean ± SE) of earthworm cast and adjacent soil.

(e) Phosphorus content (mean ± SE) of earthworm cast and adjacent soil.

(f) Potassium content (mean ± SE) of earthworm cast and adjacent soil.

Table 3. Values of *t* calculated for physico-chemical properties of earthworm casts and adjacent soil from agroforestry sites at Sakawrtuichhun (SKT) and Pachhunga University College (PUC).

Year	Soil property						
	Site	Moisture	pH	Org. C	N	P	K
2002	SKT	-	8.972**	-	1.241	0.048	3.099*
	PUC	-	10.651**	-	3.873*	0.965	7.826*
2003	SKT	9.153**	3.154*	15.178**	2.597	2.979*	2.955*
	PUC	2.672	1.529	7.465**	0.103	4.016*	13.593**
2004	SKT	14.629**	9.740**	6.176**	2.249	5.676*	4.240*
	PUC	-	-	-	-	-	-

$^*P < 0.05$ $^{**}P < 0.01$.

Organic carbon was significantly higher in earthworm casts than in adjacent soil at both SKT and PUC (Table 3; Fig. 3 c), which has been reported by others in temperate (Scullion *et al.* 2002; Zhang & Schrader 1993) and tropical regions (Asawalam 2006). In contrast, a lower organic carbon compared to the surrounding soil has been reported in worm casts from Tripura (Bhattacharya & Chakraborti 1987).

Potassium and phosphorus levels were also found to be significantly higher in casts compared to the surrounding soil (Table 3, Fig. 3 e,f). Similar observations were made by others (Basker *et al.* 1993; Edwards 1988; Gupta & Sakal 1987; Kuczak *et al.* 2006; Mansell *et al.* 1981; Satchell & Martin 1984; Satchell *et al.* 1984; Scullion *et al.* 2002; Sharpley & Syers 1976). Earthworm casts are richer in soluble inorganic phosphate as well as exchangeable phosphorus (Mansell *et al.* 1981; Sharpley & Syers 1976). Higher phosphatase activity in worm casts than in uningested soil also increases the mineralization of organic phosphorus (Mansell *et al.* 1981; Ruz *et al.* 1988; Satchell & Martin 1984; Satchell *et al.* 1984; Sharpley & Syers 1976). Phosphatase acts to release phosphate form organic phosphate compounds via hydrolysis.

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