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Earthworm diversity and population density in the Kaki Bukit agroecosystem, Perlis, Peninsular Malaysia

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Abstract: Information on the earthworm community in Northern Peninsular Malaysia is limited. The present study examines the diversity, population density and biomass of the earthworm community in an agroecosystem in Kaki Bukit, Perlis. It also examines the physico-chemical parameters that influence the earthworm community in the area. The study area is surrounded by limestone hills and important agriculture areas, and, therefore, serves as a unique agroecosystem that is worth exploring. The area was dominated by *Metaphire tschiliensis tschiliensis*, a relatively large-sized soil-dwelling earthworm that showed active surface casting activity. Earthworm density exhibited positive correlations with soil pH (r = 0.645), clay content (r = 0.801) and Ca concentration (r = 0.415), and negative correlation with Fe. In addition, positive interaction was observed between earthworm biomass with soil moisture content (r = 0.425), K (r = 0.374) and Ca (r = 0.399).

Resumen: La información sobre la comunidad de lombrices de tierra en el norte de la península de Malasia es escasa. El presente estudio examina la diversidad, la densidad poblacional y la biomasa de la comunidad de lombrices en un agroecosistema en Kaki Bukit, Perlis. Asimismo, examina los parámetros físicoquímicos que influyen en la comunidad de lombrices en la región. El área de estudio está rodeada de colinas de piedra caliza y áreas agrícolas importantes y por lo tanto sirve como un agroecosistema único que vale la pena explorar. El área estuvo dominada por *Metaphire tschiliensis tschiliensis*, una lombriz de tierra de tamaño relativamente grande que mostró una actividad intensa de producción de humus de lombriz de la superficie. La densidad de las lombrices mostró correlaciones positivas con el pH del suelo (r = 0.645), el contenido de arcilla (r = 0.801) y la concentración de Ca (r = 0.415), y una correlación negativa con el Fe. Además, se observaron interacciones positivas entre la biomasa de las lombrices y el contenido de humedad del suelo (r = 0.425), el K (r = 0.374) y el Ca (r = 0.399).

Resumo: A informação sobre a comunidade de minhocas no Norte Peninsular da Malásia é limitada. O presente estudo analisa a diversidade, densidade populacional e biomassa da comunidade de minhocas num agro-ecossistema em Kaki Bukit, Perlis. Analisa, além disso, os parâmetros físico-químicos que influenciam a comunidade de minhocas na zona. A área de estudo está rodeado por colinas de calcário e áreas agrícolas importantes, e portanto, serve como um agro-ecossistema único que vale a pena explorar. A área estava dominada pela *Metaphire tschiliensis tschiliensis*, uma minhoca do solo de porte relativamente grande, que mostrou uma actividade intensa na produção de húmus superficial. A densidade de minhocas apresentou correlações positivas com o pH do solo (r = 0,645), com o teor de argila (r = 0,801) e com a concentração de Ca (r = 0,415), e uma correlação negativa com o Fe. Além disso foi observada

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uma interação positiva entre a biomassa de minhocas com o conteúdo de humidade do solo (r = 0,425), o K (r = 0,374) e o Ca (r = 0,399).

Key words: Biomass, density, diversity, limestone hill, *Metaphire tschiliensis tschiliensis, Pontoscolex corethrurus*.

Introduction

Earthworms are important soil macrofauna that have profound effects on ecosystems. They have attracted a lot of interest due to their beneficial effects, especially in agriculture. Earthworm feeding behavior, burrowing and casting are vital in nutrient cycling and decomposition regulation (Brown 1995; Dechaine *et al.* 2005; Larink *et al.* 2001). Earthworm diversity is influenced largely by soil nutrients and rainfall patterns (Fragoso & Lavelle 1995). The establishment of earthworm populations in an area, particularly in agricultural soil, is correlated to soil sustainability. Earthworm ecological groups have variable effects on the soil physical, chemical and biological properties (Lee 1985). Tropical soils tend to be low in nutrient content; however, the presence of earthworms could manifest beneficial effects in the long-term (Henrot & Brussaard 1997). Thus, it is important to identify the earthworm community that inhabits an area, in order to understand the effect they may be having on soil fertility and health.

Changes in soil properties might eliminate certain earthworm populations and induce the establishment of new ones (Smetak *et al.* 2007). In return, the earthworm population may improve various soil properties that have a profound effect on soil dynamics and enhance plant growth. Comprehensive earthworm diversity studies in Peninsular Malaysia were done by Gates decades ago (1935, 1936, 1937, 1938, 1949). Gates found that Kaki Bukit, Perlis was populated mainly by earthworms from the genus *Pheretima* from the family Megascolecidae (Gates 1949). However, these studies were mainly focused on earthworm diversity, with little attention paid to how environmental factors affect the earthworm population.

Kaki Bukit, Perlis, which is situated in the northern region of Peninsular Malaysia, is surrounded by limestone hills. It is an important agricultural area renowned for its Harumanis mango, an important agro-product that has great demand in the local community and is also exported to Japan. A recent field survey discovered that the agroecosystem in Kaki Bukit, Perlis was dominated by an unknown, relatively large soil-dwelling earthworm with an average adult weight of 2.5 g and 25 cm in length. It forms burrows in soil and exhibits active surface casting activity. These casts have a subspherical shape with approximately 3 - 5 cm in height.

Kaki Bukit, Perlis is a unique agroecosystem in Malaysia. Like most of the agricultural areas in the tropics, Malaysia has fairly acidic soil, with the pH ranging from 4.5 - 5.5 (Chaudhuri & Bhattacharjee 2011; Fragoso & Lavelle 1992). Being surrounded by limestone hills, the soil in Kaki Bukit is fairly high in pH. This is expected to affect the earthworm community in the soil. Therefore, the present study was conducted to examine earthworm diversity and the interactions between earthworm population density and biomass with various soil parameters.

Materials and methods

Field sampling

Sampling of earthworms and soils were conducted from Kampung Guar Jentik (06° 37.482 N, 100° 13.490 E) to Kampung Paya (06° 35.294 N, 100° 12.028 E) in May 2009. The sites are surrounded by limestone hills and experience a distinct dry season from January to April. The average annual temperature and precipitation are 21 - 32 °C and 2000 - 2500 mm, respectively.

Earthworms were randomly sampled using 1 m^2 quadrats at 29 different sites. The distance between quadrats was approximately 50 - 100 meters. The chemical expellant method (Raw 1959; Römbke *et al.* 2005) was adopted, where approximately 2 - 4 liters of 0.5 % formalin was poured onto each quadrat to drive out earthworms. The chemical expellant method is efficient in drawing out most of the earthworms from upper soil layers, but it may underestimate the population of deep soil burrowers. Earthworms that emerged from the

Table 1. Primers used in molecular identification of earthworms.

Primer	Sequences (5' – 3')	Reference
COI-E-	TATACTTCTGGGTGTCC GAAGAATCA	Bely & Wray 2004
LCO1490	GGTCAACAAATCATAAA GATATTGG	Folmer *et al.* 1994
16Sar	CGCCTGTTTATCAAAAA CAT	Hillis & Moritz 1990; Chang *et al.* 2008
16Sbr	CCGGTCTGAACTCAGAT CACGT	Hillis & Moritz 1990; Chang *et al.* 2008

ground were hand-picked and bathed in water before being transported to the laboratory for identification. Earthworms with different morphological characteristics were kept in separate containers filled with soil from the study areas. They were maintained under laboratory conditions at 25 ºC. Earthworms were rinsed and blotted dry to measure their biomass within 24 hours after field sampling. Soil samples were collected from a depth of 0 -15 cm at each sampling sites and stored in sealed plastic bags for further analysis in the laboratory.

Earthworm identification

Both morphological and molecular methods were used for earthworm identification. Morphological identification was done under a dissecting microscope by referring to Blakemore's (2002) identification keys. The molecular approach was adopted for further identification of specimens with ambiguous characteristics.

For molecular identification, earthworms were killed in 70 % ethanol, and DNA was extracted using the phenol/chloroform method and checked with 1 % agarose gel electrophoresis. PCR was conducted using mitochondrial cytochrome oxidase subunit I (COI) and 16S rRNA primers (Table 1). The protocols for PCR cycling followed those suggested by Chang *et al.* (2008) and Huang *et al.* (2007). The PCR product was purified using a PCR Purification Kit (Promega) by referring to the procedures recommended by the manufacturer before sequencing. A total of 640 bp and 509 bp were amplified from COI and 16S rRNA primers respectively. The sequenced fragments were assembled and aligned using a Mega 4.0 Alignment Explorer and gaps and vague sequences were eliminated.

Soil and data analyses

Moisture content of soil was determined by drying 2 g of soil in 105° C for 24 h (Moris & Singh 1980). The rest of the soil samples were then air-dried and sieved through a 2 mm mesh sieve. The pH was measured using a 1:2.5 deionised water/soil suspension. Soil organic matter content was determined by loss on ignition (LOI) by burning approximately 2 g of oven dried soil at 450 °C for 4 h (Van Ranst *et al.* 1999). The pipette method was adopted to determine the percentage of silt, clay and sand in the soil samples (Moris & Singh 1980). The USDA soil texture triangle was used to determine the soil type. For total nitrogen (N) analysis, the Kjeldahl method was used and the resulting ammonia was analyzed with an auto analyzer. Total P, K and other elements were analyzed by inductively coupled plasma (ICP) spectrometry (Van Ranst *et al.* 1999) after digestion with concentrated nitric and hydrochloric acids.

Data were analyzed using SPSS version 17.0 with a significance level of 0.05. Pearson's correlation analysis was performed to determine correlations between earthworm population density and biomass with soil parameters. The Pearson correlation coefficient (r) is presented throughout the paper.

Results

Earthworm identifications

Two earthworm species were found inhabiting the study area. The first species was identified as *Pontoscolex corethrurus*, from the Family Glossoscolecidae. However, we were unable to identify the second species based on morphology due to the absence of clear morphological characteristics. Therefore, the molecular approach was used for identification. The matching results of aligned sequences of this earthworm with the gene library suggested that it is *Metaphire tschiliensis tschiliensis*, which is grouped into the order Haplotaxida and family Megascolecidae. It exhibits active surface casting activity and forms burrows near to upper soil layers during rainy season.

Earthworm correlations with soil physico-chemical parameters

The site was dominated by *M. tschiliensis tschiliensis* (92.3 % of total earthworm sampled) with an average population density and biomass of

Table 2. Soil properties and linear correlations coefficients (r) with earthworm population density and biomass (n= 29).

Soil property	Mean ± SE	Min	Max	Correlations	
				Density	Biomass
Moisture (%)	18.30 ± 0.47	13.73	23.19	0.354	0.425*
pH	6.12 ± 0.10	4.94	6.88	0.645**	0.329
Organic matter (%)	4.46 ± 0.14	3.09	5.57	0.220	0.101
Clay (%)	59.0 ± 2.23	40.0	72.0	0.801*	-0.226
Total element (mg kg^{-1})					
N	10.09 ± 0.93	2.46	17.48	0.596*	-0.161
P	3.54 ± 0.48	1.12	9.41	0.515*	0.271
K	4.19 ± 0.33	1.33	7.50	0.292	0.374*
Ca	12.97 ± 1.48	1.75	28.81	0.415*	0.399*
Mg	3.34 ± 0.32	0.48	6.82	0.311	0.330
Al	78.00 ± 4.26	42.35	112.91	0.187	0.224
Fe	246.01 ± 19.95	94.46	448.37	-0.491*	-0.364
Cu	0.09 ± 0.01	0.02	0.25	0.168	0.183
Zn	0.35 ± 0.03	0.09	0.66	0.146	0.151

* Correlation is significant at $P = 0.05$
** Correlation is significant at $P = 0.01$

15.62 ± 2.11 ind. m^{-2} and 24.25 ± 3.79 g m^{-2} respectively. *M. tschiliensis tschiliensis* had an average adult biomass of 2.59 ± 0.10 g ind^{-2}. Further analysis was focused on *M. tschiliensis tschiliensis*, as *P. corethrurus* was rarely found and its distribution was highly patchy (1.31 ± 0.34 ind. m^{-2}).

The population density of *M. tschiliensis tschiliensis* was positively correlated with the pH, soil clay content, and total N, P and Ca content of soil (Table 2). By contrast, the earthworm biomass was weakly correlated with soil moisture content, total K and Ca content of soil. Correlation analysis showed that earthworm population density decreased as total soil Fe content increased. Neither earthworm population density nor biomass showed a significant correlation with total soil Mg, Al, Cu or Zn.

Discussion

The agroecosystem in Kaki Bukit, Perlis, is inhabited by two soil-dwelling earthworms, *P. corethrurus* and *M. tschiliensis tschiliensis*, with the latter serving as the dominant species. The adults of *M. tschiliensis tschiliensis* recorded an average weight of 2.6 g ind.$^{-1}$. Meanwhile, the presence of *P. corethrurus*, though in a relatively low number, may suggest that it can tolerate a wide range of pH. Previous studies suggested that *P. corethrurus* prefers relatively acidic soil, and can even be the dominant species in some of these areas (Chaudhuri & Bhattacharjee 2011; Garcia & Fragoso 2002; González *et al.* 1999; Henrot & Brussaard 1997). In addition, *P. corethrurus* favors soils with a high silt content (Huerta *et al.* 2007). It is an exotic earthworm originating from South America that has become common in many tropical soils (Fragoso *et al.* 1999; Tapia-Coral *et al.* 2006). The soils in the study area had near neutral pH (6.12) with a high clay content. This might explain the very low density of *P. corethrurus* in the study sites.

Earthworm diversity was low in the study sites; only two earthworm species were found. The finding was in accordance with Smetak *et al.* (2007) who reported that urban and agricultural lands are generally characterized with low earthworm diversity. In tropical regions, earthworm diversity tends to be lower in pastures (species richness of 2 to 5 species), when compared to undisturbed tropical forests with a species richness that varies from 4 to 14 (Fragoso & Lavelle 1995). Human activities may disrupt and limit the development of the earthworm community in agricultural land.

A previous study on earthworm diversity in Kaki Bukit, Perlis reported that the area was inhabited by *Pheretima* sp. from the family Megascolecidae (Gates 1949). Blakemore (2002) indicated that the genus *Metaphire* and *Pheretima* originated from the same family, Megascolecidae, but with different origins and distribution. These two genera are closely related and are difficult to distinguish from each other. With the use of recent molecular techniques, and construction of a phylogenetic tree, it was shown that these two genera are closely linked (Huang & Sun 2007). Some of the earthworms that were previously grouped in the genus *Pheretima* have been reclassified into the genus *Metaphire* (Blakemore 1994). Huang & Sun (2007) have proposed that *Pheretima tschiliensis tchiliensis* should be revised as *M. tschiliensis tschiliensis*. Therefore, we suggest that *M. tschiliensis tschiliensis* was identified as *Pheretima* sp. in the previous study by Gates (1949).

The population density of *M. tschiliensis tschiliensis* was positively correlated with soil pH ($r = 0.645$, $P < 0.01$), which suggests that *M. tschiliensis tschiliensis* prefers soil with higher pH. Earthworms are very sensitive to soil pH (Edwards & Bohlen 1996), and soils with low pH limit earthworm survival and activity (Springett & Syers 1984). Acidic soils are characterized by higher availability of metal ions (Brady 1984) that cause unfavorable conditions for earthworms. A survey conducted in a tropical agroecosystem revealed that earthworms in the genus *Metaphire* (*M. californica*, *M. houletti* and *M. posthuma*) preferred to inhabit soil with pH ranging from 6.1-6.8 (Fragoso *et al.* 1999). Similar observations were also reported by Shakir & Dindal (1997) and Nair *et al.* (2005), who found higher number of earthworms in areas with pH near neutral (6.8 - 7.1).

Soil moisture content in the areas was 18.3 % (min = 13.73 %; max = 23.19 %) at the time of sampling. A positive correlation was observed between earthworm biomass and soil moisture content ($r = 0.425$, $P < 0.05$), suggesting that *M. tschiliensis tschiliensis* is sensitive towards soil moisture content. Several studies have recorded a decline in earthworm population in response to dry seasons (Bohlen *et al.* 1995; González *et al.* 1996; Najar & Khan 2011). Earthworms may enter into diapause, or burrow into deeper soil under unfavorable conditions (Jiménez & Decaëns 2004). However, a prolonged dry season may lead to mortality and growth retardation. Moisture is one of the key factors affecting the survival of all earthworm species (Bohlen *et al.* 1995; Edwards & Bohlen 1996). Earthworms are hydro-skeletal organisms. This enables them to move and absorb oxygen efficiently in the soil (Satchell 1983). Soil moisture content may affect the biomass of *M. tschiliensis tschiliensis* because water constitutes 75 - 90 % of their body weight (Grant 1955). However, the moisture requirements for different species of earthworms from various regions can be quite different (Auerswald *et al.* 1996). Since earthworms apparently lack the mechanism to maintain constant internal water content, they will be greatly influenced by the soil water potential of their surroundings (Kretzschmar & Bruchou 1991). Therefore, low moisture content in the soil may reduce its biomass.

The soils in the study area is classified as clay soils, with an average clay content of 59 % (min = 40 %; max = 72 %). The population density of *M. tschiliensis tschiliensis* showed a strong positive correlation with soil clay content ($r = 0.801$). This may be associated with the ability of clay-soil to retain more moisture for a longer period due to its fine particles. The finding is in accordance with Baker *et al.* (1998). They related this positive correlation with increased soil water retention and a cation exchange capacity in clay soils. Nevertheless, the author concluded that the relationships between soil/clay content and earthworm abundance remained unclear. However, Chan & Barchia (2007) found no correlation between earthworm density and soil clay content. They suggested that soil clay content might not be a good indicator in determining earthworm distribution and population size, because soils with similar clay content may have different soil structure and pore size distribution, possibly explaining the different correlations obtained by different studies.

Soil organic matter is often considered as a good predictor of earthworm abundance in many soils (Edwards & Bohlen 1996). The soil organic matter content obtained (3.09 - 5.57 %) in the present study was in line with the range of soil organic matter values that sustain relatively high number of earthworms (Chaudhuri *et al.* 2008). Organic inputs to these soils include leaves, litter and organic fertilizers that enrich the soil layers. For example, field observations suggested that chicken manure was applied in some of the study sites. This may alter organic matter input and soil environmental conditions in a manner that improves earthworm quantity and biomass (Smetak *et al.* 2007). Nevertheless, there was no quantitative data on the fertilizer inputs to further assess the effect of organic fertilizer on soil organic matter dynamics in these areas. In the present study, a simple method, LOI was used to estimate the soil organic matter in the study sites. Konare *et al.* (2010) discussed the limitations of loss of ignition (LOI) in determining soil organic matter in clay soil. It may cause inaccurate estimations due to dehydroxylation and decomposition of inorganic residues (Konare *et al.* 2010). However, LOI is still used in ecological studies because it gives a quick and inexpensive estimate of organic matter contents in soil.

There were no significant correlations between organic matter with earthworm population density ($r = 0.220$, $P > 0.05$) or biomass ($r = 0.101$, $P > 0.05$). The results suggested that there may be other factors that influence the earthworm population. It could be the result of uneven distribution of organic matter content in soil due to the activity of *M. tschiliensis tsschiliensis*. Shuster *et al.* (2001) observed that the presence of earthworms can change the spatial distribution of soil organic carbon from uniform to patchy. Activities of earthworms (burrowing and casting) would eventually translocate organic matter of an area and accumulate it in another, resulting in uneven distribution of organic matter in soil. A similar observation was also recorded in a previous study done in the Ivory Coast, where earthworms are known as the 'proximate factors' that influence soil properties such as soil organic matter and texture (Rossi 2003).

The *M. tschiliensis tschiliensis* population density was positively correlated with total soil N and P content. Earthworms are important agent in N mineralization in many agroecosystems (Marinissen & de Ruiter 1993). A nitrogen rich diet is important to earthworms as it facilitates weight gain and higher cocoon production (Shipitalo *et al.* 1988). Hence, high N content in soil would have contributed to higher earthworm population density in these areas. Besides, the combination of manure and inorganic fertilizer applications is also known to promote earthworm biomass (Tiwari 1993). The author found that soil treated with organic and NPK fertilizers promoted higher earthworm casting activity, and this was most probably attributed to rich N and P content in soil that promoted higher earthworm activity.

In addition, both earthworm density and biomass exhibited positive correlation with total Ca content in soil. In Kaki Bukit, the relatively high soil pH may be influenced by the presence of calcium carbonate from the limestone hills surrounding the area. Weber *et al.* (2007) reported that calcium carbonate preserves the flocculated structure of clay in soil surface aggregates by neutralizing the acid produced by fungi, microbes and roots, resulting in higher soil pH. This perhaps serves as favorable habitat for earthworms with high food availability.

A negative correlation was observed between earthworm population density and total soil Fe content ($r = -0.491$), whereas earthworm biomass exhibited a weak positive correlation with total soil K content ($r = 0.374$). Though Fe is considered a microelement, it may have harmful effects on soil organisms if it is present in excess. A study done by Langan & Shaw (2006) reported detrimental effects of Fe on the survival and growth of the anecic earthworm *Lumbricus terrestris*. The authors found reduced earthworm biomass and higher mortality when Fe concentration in soil increased. This may be due to the "trade-off effect", whereby higher energy is required for the detoxification and regulation of the heavy metal (Hobbelen *et al.* 2006). Meanwhile, K is known to be important in animal physiological functions, such as respiration, membrane functions and stability. It may aid in the locomotion and burrowing activities of earthworms in soil.

In the present study, total soil element concentration was used in determining the relationship with *M. tschiliensis tschiliensis*. This would provide basic understanding on the interactions between earthworm population and its environment. Further studies on the relationship between the extractable elements and the earthworms would be interesting to be conducted on the relationship of *M. tschiliensis tschiliensis* and the soil elements. The presence of earthworm may enhance the extractable form of soil elements and this is particularly essential in promoting better soil fertility.

Conclusions

The present study provides fundamental information on the earthworm community that inhabits the Kaki Bukit agroecosystem in the northern region of Peninsular Malaysia. Soil pH, moisture, clay content, total N, P, K and Ca have significant effect on the earthworm population density and biomass. Due to its high abundance, *M. tschiliensis tschiliensis* may serve as an important soil rehabilitation agent that affects various soil properties in the agroecosystem.

Further studies are required to explore its contributions towards soil ecosystem and plant growth.

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