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DIFFERENT LAND USE EFFECT ON EARTHWORMS AT SAFE PROJECT SITE IN SABAH, BORNEO

by

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Abstract

Earthworms are a major component of the soil biota as ‘ecosystem engineers’ sensitive to disturbance and the impact of deforestation on earthworms remains as one the least studied subject in tropical Borneo whose rainforest is fast disappearing due to logging and intensive agriculture. As such, this study investigated the impact of land use disturbance on earthworms by comparing their abundance per m^2 across forest modification gradient and measured changes in environmental variables across the gradient and their subsequent effect on earthworms. Old growth (OG2), secondary logged forest (B and F) and oil palm (OP2 and OP3) sites were surveyed in SAFE Project site in Sabah, Borneo. Transect and monolith digging methods were employed to sample earthworms from soil. At each monolith, environmental variables were measured to characterise each land use by canopy exposure, litter depth and forest quality and soil samples were taken to analyse soil properties. Earthworm abundance was highest in OG2 and decreased across the disturbance gradient and was significantly lower in OP2 indicating some level of tolerance between old growth and secondary forest system. However, functional group response and changes in functional group composition varied significantly between land uses and corresponded significantly to variation in environmental variables which also significantly differed between land uses. Changes in functional group response potentially show greater implication for soil function and the ecosystem services derived from the processes. Therefore, further investigation on impact of functional group composition changes on soil function due to different land use is needed.

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Chapter 1 Introduction, literature review, aims

1.1 Introduction

The tropical rainforest of Borneo is a megadiverse region that is fast disappearing due to logging and intensive agriculture that have caused significant changes to species diversity and composition across the modification gradient (MCMorrow & Talip, 2001). Many taxa that make up the natural ecosystem are being lost due to this process while their natural patterns and ecological significance is little understood. One of such taxa is the earthworm - a major component of the soil biota as 'ecosystem engineers' and their presence plays a dominant role in the regulation of soil processes, fertility and function (Nellemann *et al*. 2007).

Studies have shown that despite patchy distribution and low densities in the tropics, earthworms occur in high diversity and represent an important biomass of the food web in soil while playing vital role as soil engineers in soil ecosystem influencing soil processes (Fragoso & Lavelle, 1987, 1992, 1995; Lavelle & Spain, 2001). Moreover, earthworms are also sensitive to land use changes as communities can be modified both at functional and taxonomic level over different land conversion (Senapati, 1980; Lavelle & Pashanasi, 1989; Julka, 1988; Fragoso *et al*. 1993; Lavelle *et al.* 1994a; Decaëns & Jiménez, 2002; Nunes *et al*. 2006; Susilo *et al*. 2009).

Of particular interest is the response of earthworm functional/ecological groups: epigeic, anecic and endogeic which are classified based on their feeding and how deep they burrow in the soil (Bouché 1977, Lavelle 1981). Epigeic are litter dwelling species that feed at the surface level; endogeic are soil dwelling species that feed in the mineral soil and build their semi-permanent burrows to about 50cm depth; anecic are deep burrowing species that can go down to 2 meters depth but feed on surface by pulling down fresh litter into its burrow. The different activities of each group can cause different effects on the soil and studies confirm that functional group response to disturbance may differ greatly and alter biogeochemical processes (Lee, 1985; Lavelle, 1983; Blanchart *et al*. 1997; Fargoso *et al*. 1997; Lavelle *et al*. 1998).

1.2 Literature review and aims

1.2.1 Background

Forest clearance for land development continues throughout the world and the increasing human population puts more and more pressure for land needed for cultivation, residence and industry. As such, study on the impact of forest clearance, land use type and subsequent practises are relevant and valuable in assessing the impact and extend of such activities on flora and fauna. Earthworms are one of the taxa greatly impacted by changing land use and first started to become widely studied in temperate regions. During early 1970s, Bouché (1971, 1972) did a comparison study between pastoral lands and undisturbed forested areas in France, which revealed that deforestation particularly affected epigeic earthworm group due to the disappearance of their under-bark habitat with the trees (Lee, 1985). Since then, many more studies have been conducted that proved the significant impact of land use on earthworm communities around the world. Most studies on tropical earthworm activities, species diversity, communities and biomass have been described mainly from regions of Africa, India and Central and South America which revealed loss of species diversity over the disturbance gradient (Lavelle, 1978; Nemeth, 1981; Nemeth & Herrera, 1982; Lavelle 1983; Montadert, 1985; Fragoso, 1985; Fragoso & Lavelle, 1987, 1992; Lavelle & Pashanasi, 1989).

Agricultural practises causes depletion in soil organic matter; removal of soil particles from soil layer through erosion and degradation of soil structure that leads to loss of soil invertebrate communities especially earthworms (Decaëns *et al*. 1994; Lavelle *et al*. 1994). With increasing human population and demand for land this trend of soil degradation is expected to get worst (Eswaran 1994; FAO 2000). With more research confirming the role of earthworms to soil fertility more demand especially from farming communities is being made to provision methods to protect them and to sustain soil quality (Lee 1985; Edwards and Bohlen 1996; Lavelle *et al*. 1999; Lavelle & Spain 2001). However, earthworm effect on soil is not thoroughly understood especially at temporal scales. Nonetheless, Lavelle *et al*. (2004) found that burrows and casts produced by earthworms can endure long period of time even after their loss and these structures and particles can significantly contribute soil resistance and resilience to disturbance – necessitating the need to research earthworms more. Knowledge on earthworms in Borneo is still lacking and despite their proven role in other regions – remain as one of the least studied taxa.

1.2.2 Earthworm communities and role

The composition of earthworm communities (e.g. species, functional group, exotic and native) has great influence on soil function and fertility. As a denoted 'ecosystem engineers' in the soil biota, earthworms presence plays a dominant role in the regulation of soil processes as they 1) ingest mixture of organic, mineral and soil and produce casts with organic matter, 2) digest complex substrates via an efficient symbiotic digestion systems in association with soil microflora and 3) create aeration through burrowing and influencing soil physical properties (Lavelle 1997).

The significance of earthworms have been further established by Lavelle & Fragoso (in press), who compared 12 communities from tropical rainforests and showed that earthworms are an important soil macrofauna in the soil food web as they accounted for 51% of total biomass in these communities despite representing low abundance at 9% compared to termites (37%) and ants (23%). A case in point, in another study focused on just one large native earthworm from the natural savannahs of the eastern plains of Colombia showed that their physical bio-structures and casts can affect the nature of how soil resource is made available for other organisms (Jiménez & Decaëns, 2004). In just over a period of 18 months, the loss the single species was associated with reduced soil quality and herbaceous biomass that is advantageous to opportunistic weed species. Consequently, if the loss of a species can lead to continuous loss of ecosystem function and subsequently soil degradation, the loss of functional groups can be expected to have greater impact.

As such, the role and response of earthworms in tropical soil and along forest modification is a relevant area to study. In the last few decades, more attention has been given to this topic with more research showing how a decrease or increase in earthworm community impact soil properties, other environmental variables and even forest ecology (Nemeth & Herrera, 1982; Lee, 1983; Lavelle, 1988; Lavelle *et al*., 1992, 1994b; Fragoso & Lavelle, 1987, 1992, 1997).

Earthworms affect soil physical properties through their behaviour such as burrowing and feeding that involves ingestion and mixing of soil and organic matter that gets excreted as cast. Earthworm effects on soil aggregation and porosity vary substantially due to different ecological strategies shown by earthworm species. Epigeic forage for organic matter in their preferred habitat, forest leaf litter, and rarely burrow into or ingest soil. As a result, they do not have any significant effect on the mineral soil (Hamilton & Dindal, 1989). Endogeic ingest mixture of soil and organic matter and they do so by extensively burrowing randomly and horizontally in the top mineral soil (10 – 15cm depth) where SOM (soil organic matter) is greatly concentrated (Edwards, 2004). Endogeic cast is often deposited in the mineral soil or burrows and soil surface. Anecic that feeds on soil surface litter and burrows deep into the soil tend to mix comminutioned litter with their cast to form midden that further promote decomposition which is also used to cover their burrow entrance (Edwards, 2004). However, the behaviours are intermediate and can vary between species depending on environmental conditions (Edwards & Bohlen, 1996).

1.2.3 Land use impact on earthworms

Soil biota vary in different land use due to environmental variables that could be a result of anthropogenic activities with loss of biodiversity along the modification gradient from primary forest, secondary forest (logged forest, sustainably management forest, agroforest), agricultural land to residential or industrial land (Rulz *et al*. 2008). Other effects on soil macrofauna communities include:

- decrease in diversity, density and biomass of soil organisms
- decimation of epigeic and anecic earthworms and other litter-dwelling fauna whose biological activity is mainly concentrated in the top 20 cm of soil
- persistence and potential augmentation of deeper burrowing species
- dominance of persistent groups such as termites and ants
- increase in pest organisms
- the activity of beneficial organisms (earthworms and ants) may become detrimental because of the lack of other soil-organism activities

On the contrary, some land management practices can have positive impacts on soils by increasing SOM level and improving soil functioning and plant productivity that can result in vegetation cover which is conducive to the activity of soil organisms (Roth, 1985). On that aspect, earthworm communities have been shown to improve with the introduction of ecobuage in Congo (Mboukou, 1997). The improvement in earthworm communities, in turn, improved soil structural stability which created good conditions for plant root development while improving soil porosity to allow plant roots to go deeper into the soil.

Other changes to soil microclimate after land use modification such as reduction in canopy cover; vegetation on ground; quality and quantity of leaf litter and organic matter; impact of fertilizers and pesticides can impact earthworm composition. On the other hand, soil management practices such as palm tree plantations with herbaceous legume cover or cocoa can have a diverse soil biotic community (Lavelle *et al*. 1999).

To summarise, land use and the subsequent management practises can have a dramatic effect on earthworm communities, specifically, and soil biota in general with primary forest typically with two or three times higher fauna diversity and abundance (in terms of density and biomass) than managed systems such as short fallows that can show low taxonomic richness but increased densities (Rulz *et al*. 2008). Large earthworm communities can also be present in palm tree plantations due to association to trees with a leguminous cover crop – acting as a conservative system with both elements of primary forest and introduced ones (Lavelle & Pashanasi, 1989).

As such, in an attempt to understand how earthworms are being impacted or responding to different land use and as a result how they may influence or be influenced by soil properties and other environmental variables this project aimed to investigate:

1. Differences in earthworm abundance (per m^2) across forest modification gradient to understand how they response to land use change.
2. Identify environmental and soil characteristics that may influence such response.
3. Provide insight on how changes in functional groups can potentially influence soil ecosystem and identify future area of studies.

Chapter 2 Materials and methods

2.1 Study site

The study was conducted at Stability of Altered Forest Ecosystems (SAFE) Project site in Sabah, Borneo – a large scale forest fragmentation experiment to study the ecological impact of tropical forest modification (Ewers *et al*. 2011). Data were collected from old growth forest plots in Maliau Basin Conservation Area (referred as OG2), logged secondary forest fragments and oil palm plantations at SAFE/Benta Wawasan (fragments referred as B and F; oil palm referred as OP2 and OP3).

The sampling design at SAFE is based on equilateral triangles extending across different spatial scales (referred as orders) established in old growth, secondary forest fragments and oil palm as shown in Figure2.1. First order points are positioned at the vertices of an equilateral triangle with an edge distance of 56m. The centres of each first order fractal form the vertices of a second order fractal with an edge distance of 178m. Similarly, third and fourth order fractals are formed by the centres nested in second and third equilateral triangle with edge distance of 564m and 1780m respectively (Marsh & Ewers, 2013).

The sampling sites has been set up in such a way to minimise the confounding effects such as slope, altitude, longitude and distance to forest edges prior to the forest conversion. Samplings transects are located along an equal altitude (mean = 450m) with mean altitude within site ranging from minimum 400m (Fragment B) to 470m (Fragment F) (Ewers *et al*. 2011).

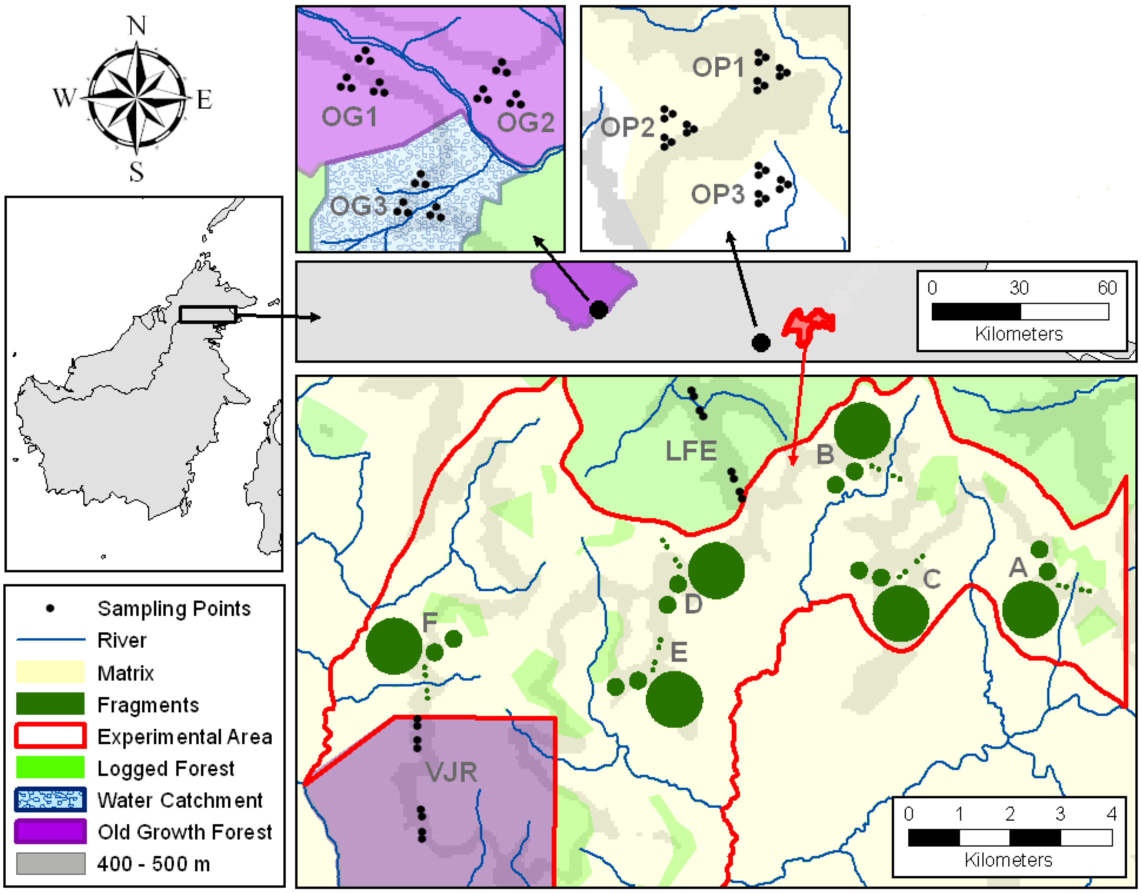

Figure 2.1. Location of sampling sites at SAFE project site. 2nd order points for OG2, OP2 and OP3 used in the study are shown above as well as location of logged secondary forest fragments below for B and F.

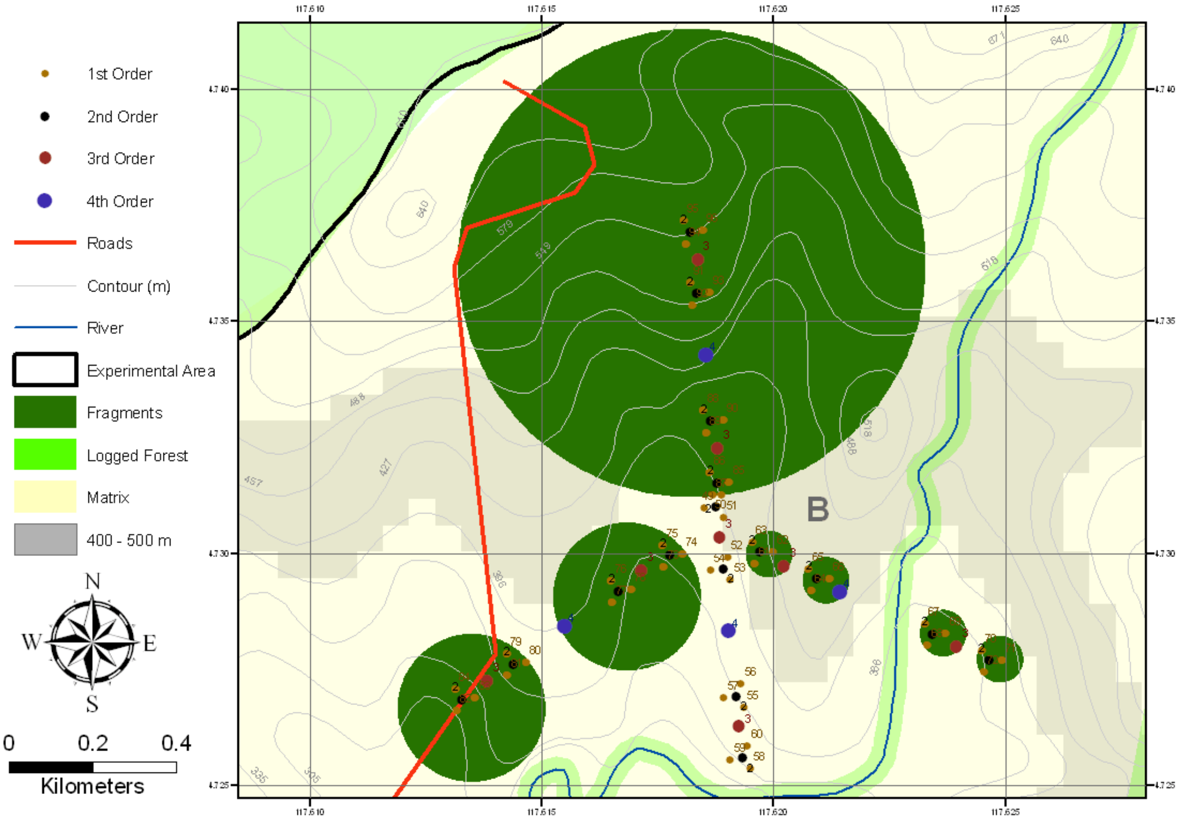

Figure 2.2. Detailed location of 2nd order sampling points (red points) within the fractal design in Fragment B which is similar to Fragment F as well. The 2nd order points forming the vertices equilateral triangle is positioned within 1ha, 10ha and 100ha circles of forest fragment.

2.2 Land use characteristics

Sabah (North Borneo) is located between 4-8 degrees north of the Equator with tropical climate and the vegetation is lowland dipterocarp forest. The annual rainfall averages about 2908.7mm and the wet season occurring between November and February. Mean air temperature is between 24.1^{o}C to 31.9^{o}C (1995-1999); mean humidity is about 70% (1995-1999) (Borneotrade, 2013).

The project site is part of forestry estate owned by Sabah Foundation intended to be converted into oil palm plantation after logging (Ewers *et al*. 2011). The entire experimental site expands to 7200ha most of which has been selectively logged in either one or two rotations

(Ewers *et al*. 2011) except the Maliau Basin Conservation Area (control plots) which was made a protected area by Sabah Foundation in 1981 and subsequently recognised as Class 1 Protection Forest Reserve by the Sabah State Assembly in 1997 (Luke, 2010). Oil palm sites are monocultures of *Elaeis guineensis* (African oil palm) (Luke, 2010). Land use intensity and variation in forest cover per land use used in the study is shown in Table 2.1.

Table 2.1. Land use intensity gradient per land use corresponding to the sampling sites shown in Figure 2.1 (Table adopted from Ewers *et al*. 2011).

Site	Habitat	Logging	Fragmentation[1]	Forest cover (%)	Forest quality (range)[2]	Notes
OG2	Forest	Never	Continuous	100	4.88 (4-5)	Greater than 500m from reserve boundary
B	Forest	Twice	Fragmented	50	2.75 (2-4)	
F	Forest	Twice	Fragmented	34	2.50 (1-3)	
OP3	Oil palm	n/a	Cleared	15	n/a	Planted 2000; closed canopy; some cover crop; 1km from forest
OP2	Oil palm	n/a	Cleared	40	n/a	Planted in 2000; canopy just forming; cover crop; 500m from forest

[1] continuous forest cover means that the sampling site is located in a contiguous forest management area of approximately 1 million ha.
[2] Scoring were based on SAFE project standardised forest quality scale shown in Table 2.2.

2.3 Data collection

Fieldwork was conducted from end June till end August 2013 (dry season in Sabah) with the assistance of 2-4 research assistants. A total of 45 sampling points were established with 9 sampling points in each land use (OG2, B, F, OP2 and OP3) to provide equal sampling size per land use. SAFE 2nd order (minimum distance of 100m) sampling points were selected to ensure independent sampling. Sampling protocol for assessment of earthworm abundance and diversity in tropical ecosystems recommended by Bignell *et al*. (2008) following TSBF protocol (Anderson & Ingram, 1993) was employed. Coordinates of each monolith and sampling point was recorded with a GPS device.

2.3.1 Earthworm sampling

At each selected 2nd order point, a 25x25 cm quadrant was marked on the ground to indicate the position of monolith and the leaf litter from within removed while observing for any potential surface-dwelling earthworms. One small monolith (25x25x30 cm) was isolated by digging a trench (around 10cm wide and 30cm depth) outside and around the quadrant. The trench served as a barrier to stop earthworms escaping into adjacent soil while digging.

Earthworms and sliced body parts excavated from the trench and monolith were gently rubbed to remove excessive soil and then preserved in 70% ethanol for identification work. In addition, three large monoliths of 50x50x30cm were dug out at 5m intervals along a transect from the smaller monolith. This sampling scheme, combining smaller and larger monoliths, allowed for plot level variation and heterogeneity to be taken into account while variation between habitat levels was considered by sampling different representative major land uses. Transect is schematically shown in Figure 2.3. The average sampling time for each monolith per person was about 20 – 30minute depending on soil compaction and slope steepness.

Preserved earthworms were removed to the lab, counted and weighed after light blotting. Sliced parts with the head/clitellum attached were counted as one individual whereas total body parts excavated were weighed (scale 0.00g) together as biomass per m^2 (Clapperton, Baker & Fox, 2007). Earthworm abundance/density was estimated as the number of individuals per m^2 and biomass as g of individuals per m^2.

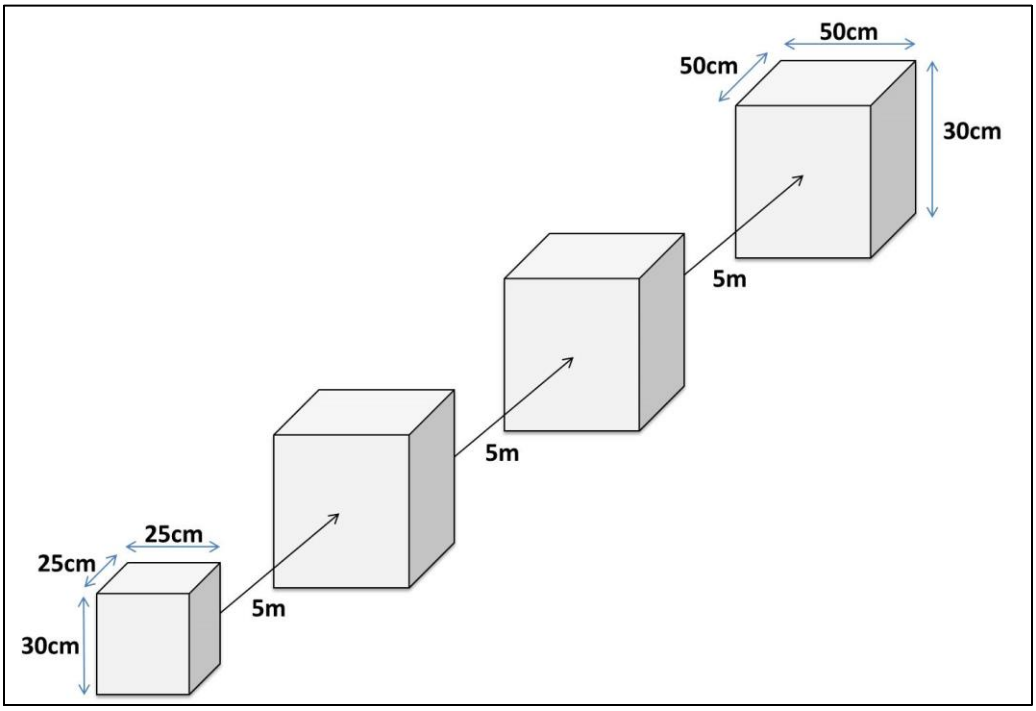

Figure 2.3. Schematic representation of transect established at each 9 sampling points per land use and the monolith size and arrangement.

2.3.2 Earthworm functional group identification

Specimen were identified into their 3 functional groups (epigeic, endogeic and anecic) using WormWatch earthworm identification guide (NNRI, 2013; Earthwormsoc, 2013; Naturewatch, 2013). Only adult specimens were used for identification as juveniles may not have grown into their full size. Key features observed include:

1) Absence or presence of clitellum (swelling of the skin located nearer to the head end of the body that can only be observed in earthworms which are ready to reproduce). Presence of clitellum distinguishes adult from juvenile.
2) Pigmentation on skin. Epigeic and anecic are usually strongly pigmented in reddish brown colour whereas endogeic lack skin pigmentation and may appear whitish, pinkish, yellowish or greyish in colour. Pigmentation on epigeic can be darker on the head and/or tail end while middle part of the body may appear beige whereas anecic tend to be dorsally pigmented with paler tail ends.

3) Body length. Each functional group differs in size and length. Anecic typically being the larges while epigeic are the smallest and endogeic being medium-sized. Average body length for each group is 1-7cm for epigeic, 2-12cm for endogeic and 8 to <15cm for anecic.

2.3.3 Measurement of environmental variables

Soil pH, soil moisture, soil temperature, ground light, litter depth, top soil depth and infiltration rate data was measured and recorded adjacent to each monolith to provide opportunity for correlations with the abundance of earthworms. Each measurement was repeated three times and the average value recorded per monolith.

Soil pH, moisture and temperature

Kelway Soil pH meter was driven 5cm into the soil and measurement was recorded. Soil moisture and temperature data for each survey point was obtained from the SAFE project database collected in 2010/2011.

Ground light

Lux meter was positioned flat on litter surface to estimate amount of light reaching the forest floor adjacent to the each monolith. Meter was placed under any low vegetation (if present at point) at monolith.

Litter depth

Measurement tape was placed upright at the bottom of the leaf litter layer and depth was recorded.

Top soil depth

Top soil defined as the darker uppermost layer of soil O horizon and A horizon between leaf litter and lighter soil below. The layer depth was recorded using a measurement tape.

Infiltration rate

Single ring method was used (USDA, 2001; Anderson & Ingram, 1993). Litter was removed and a metal tube (7.5 cm diameter and 20cm length) was driven 5 cm into the soil and then filled with 100ml of water (measured with a beaker). The time was recorded using a stopwatch from the moment the water was poured till all liquid has infiltrated into the soil leaving a silvery shine on the soil.

Dry bulk density

Soil core sample was taken by driving a PVC tube (3.5cm diameter) 10cm into the soil adjacent to each monolith after removing the litter. The tube was then carefully lifted to prevent any loss of soil and any excess soil from around and beneath the tube bottom was removed and trimmed respectively. The sample was then placed into a sealable bag and ensured no soil was left inside the tube. Samples were removed to lab and fresh weight for each was recorded.

Soil sample was then dried in oven at 105^{o}C for 48 hours and beyond for several hours until a constant weight was achieved and recorded. Dry bulk density was calculated as:

$$\text{Dry bulk density (g/cm}^3\text{)} = \text{Dry soil weight/volume}$$

Total porosity

Total porosity was calculated from dry bulk density with the assumption that particle density is 2.65 g/cm^3 for most mineral soil:

$$\text{Total porosity (\%)} = [1 - (\text{dry bulk density / particle density})] \times 100\%$$

Soil Organic Matter (SOM)

Soil sample (per monolith) was tested for soil organic matter level using a qualitative method and given a score (Bowman, 1997).

Prior to analysis, standard organic matter extraction solution was prepared. 10g of sodium hydroxide ($NaOH$ 0.25M) was weighed and mixed with 1 litre of water and left to stand until solution has cooled down and all granules dissolved. Extra precaution was taken when mixing this solution as sodium hydroxide is a strong base that can cause minor burns on skin. 18.6g of EDTA disodium salt (Na2EDTA 0.05M) was mixed in 1 litre of water and left to dissolve completely. 500ml of sodium hydroxide solution and 500ml of EDTA solution was mixed to produce 1 litre of extraction solution.

0.5g soil was weighed and mixed with 20ml of extracting solution inside a vial and shaken for about 30 seconds. The mixture was filtered till about 10ml of clear solution (without any soil suspension) was obtained. The solution was then poured into a test tube and the colour compared with other samples. Second filtration was done if a clear solution was not obtained in the first try.

A score (1, 1.5, 2, 2.5, 3, 3.5, 4) with 1 indicating the lowest SOM level and 4 indicating the highest SOM level was developed to show a spectrum of increasing SOM level characterised by increasingly darker brown solution compared to a pale/yellowish solution (low SOM level). The score and spectrum of increasing SOM level (shown in Figure 2.4.) was used as a reference to score other samples.

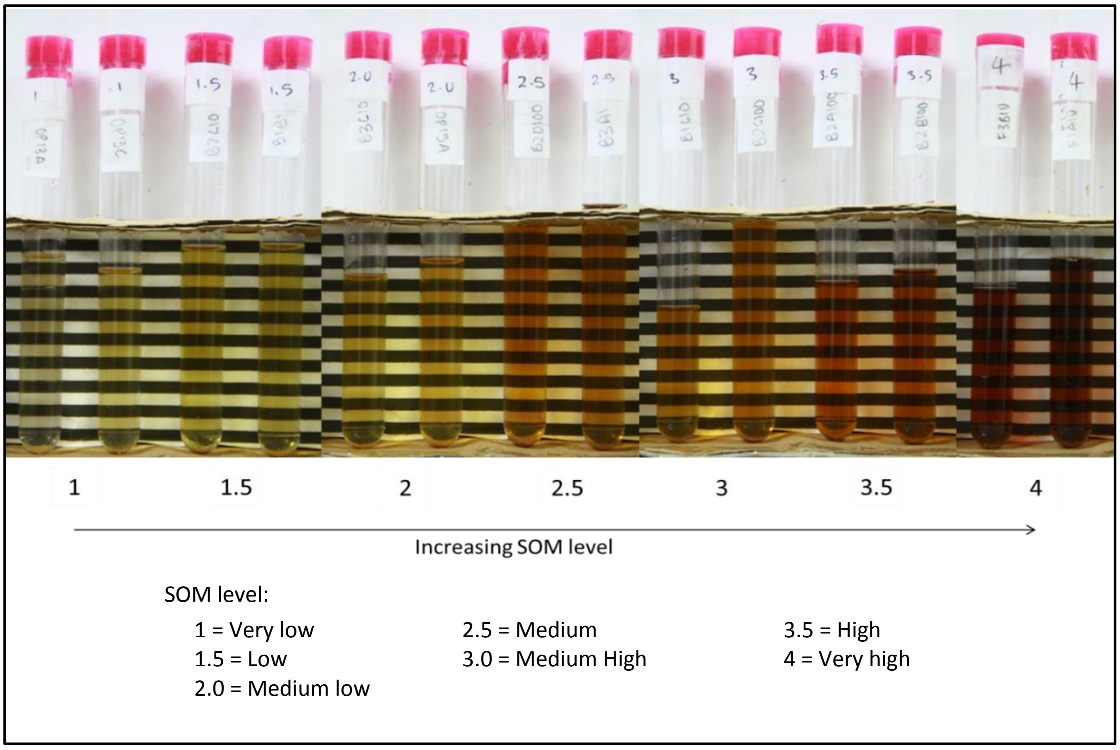

Figure 2.4. Spectrum of increasing SOM level with respective score.

Forest quality

Forest quality score per sampling site was assessed using the SAFE project scale shown in Table 2.2. Environmental conditions and measurements of vegetation cover at each sampling point were recorded:

1. Air temperature and humidity – an electronic meter was hung from vegetation at the centre of the quadrat.
2. Canopy openness – the number of open quarter squares on a spherical densiometer was counted to the north, east, south and west of the sampling point.
3. Percentage of leaf litter, bare ground, low vegetation and trees at each sampling point was estimated.

Table 2.2. SAFE project standardised forest quality scale.

Score	Quality	Description
0	Oil palm	Oil palm
1	Very poor	No trees - open canopy with ginger/vines or low scrub
2	Poor	Open with occasional small trees over ginger/vine layer
3	OK	Small trees fairly abundant/canopy at least partially closed
4	Good	Lots of trees, some large, canopy closed
5	Very good	No evidence of logging at all, closed canopy with large trees

2.4 Analyses

Earthworm total abundance and functional group (epigeic, endogeic and anecic) abundance per m^2 and environmental variables recorded were used for analyses. Two levels of analysis was considered – 1) Monolith level and 2) land use level as shown in Figure 2.5 Different subset/level of data was used based on the appropriate scale being considered.

All datasets tested for normality and homogeneity of variance as required. Log transformed data were use when necessary. All analyses performed at 95% confidence level using IBM SPSS Statistics 21.

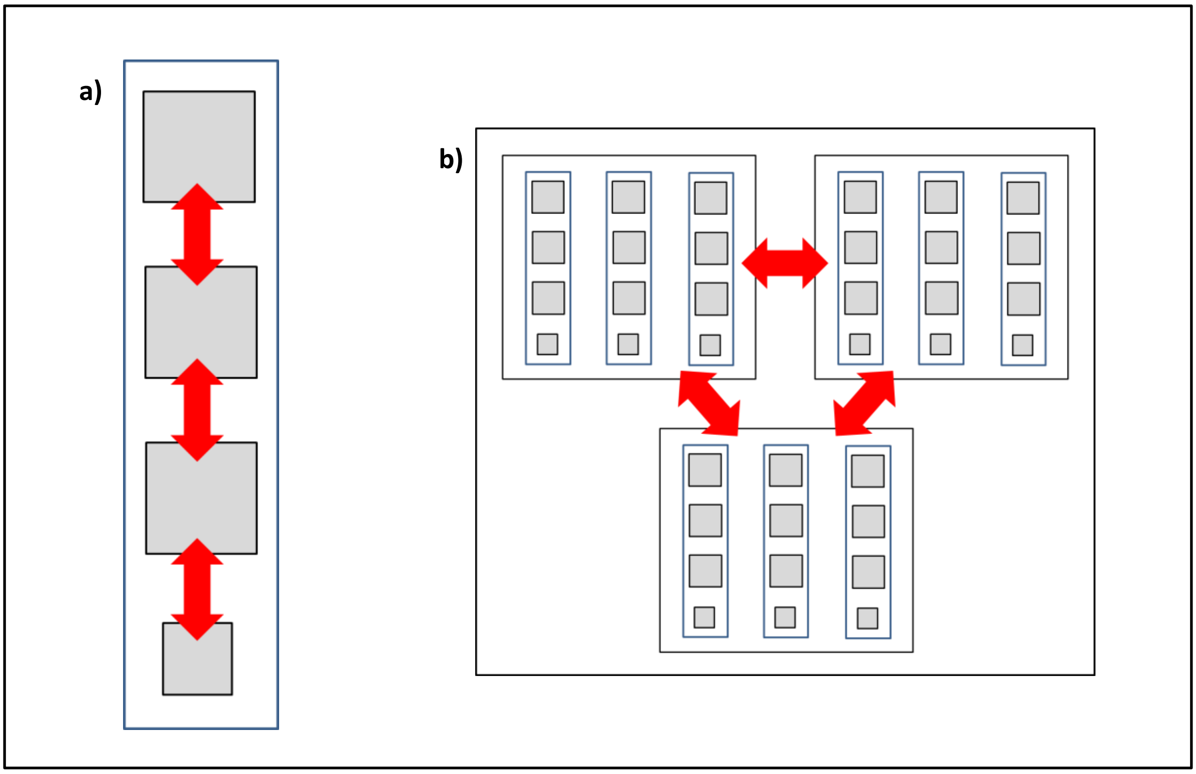

Figure 2.5. Different level of data used for analyses. a) Monolith level data was used to analyse relationship between environmental variables and earthworm abundance. The average sum of values (for earthworm abundance and environmental data) from all monolith represent transect/sampling point level data. b) Land use data (average sum of all sampling points/transect level data (n = 9) was used to compare difference in earthworm abundance and environmental variables between land use.

2.4.1 Difference in earthworm and functional group abundance

Transect level data (n = 9) per land use was used. Differences in total and functional group abundance per m^2 between land use was tested using Kruskal-Wallis ANOVA and difference between each land use was also tested to determine if they are significantly different from each other.

2.4.2 Difference in environmental variables between land use

Transect level data (n = 9) per land use was used. One way ANOVA was performed to test difference in environmental variables between land use due to disturbance. Tukey's Post Hoc (for variables where equal variance can be assumed) and Tanhame's Post Hoc (for variables where equal variance cannot be assumed) was performed to determine which variable was significantly different across the land use and from one another.

2.4.3 Relationship between earthworm abundance and environmental variables

Monolith level data (n = 180) was used. Spearman's rho correlation test was performed and the significant relationship was tabulated. Curve estimation regression was performed between earthworm abundance m^2 and environmental variables data to test curvilinear relationship and significant results were tabulated.

2.4.4 Relationship between environmental variables

Spearman's rho (for non-normally distributed data) and Pearson (for normally distributed data) correlation analysis was performed to identify significant dependence between environmental variables.

Chapter 3 Results

A total of 416 earthworms were collected (Juveniles = 210; Adults = 206). Among the adults, 92 were identified as epigeic; 79 as endogeic and 35 as anecic. Earthworms were found in 73.3% (n = 180) from all the monoliths sampled. OG2 had the highest occurrence of earthworms with 91.7% (n = 36) of monolith present with earthworms and this decreased across the disturbance gradient: 86.1% in B and F (n = 36, each), 58.3% in OP2 (n = 36) and 44.4% in OP3 (n = 36).

3.1 Earthworm abundance between land use type

Outcomes are summarised in Table 3.1 and represented in Figure 3.1.

3.1.1 Total abundance

Total abundance was significantly higher in OG2 compared to OP2 (OG2-OP2: Kruskal-Wallis, Chi-sq=4.310, df=1, $p<0.05$). A similar case was also observed for juvenile abundance and adult abundance where OG2 was significantly higher than OP2 (Juvenile, OG2-OP2: Kruskal-Wallis, Chi-sq=4.909, df=1, $p<0.05$) and (Adult, OG2-OP2: Kruskal-Wallis, Chi-sq=3.976, df=1, $p<0.05$). No significant difference was observed between other land use types for total, adult and juvenile abundance per m^2.

3.1.2 Functional group abundance

The functional group abundance (epigeic, endogeic and anecic) differed significantly across the land use (Epigeic: Kruskal-Wallis, Chi-sq=21.176, df=4, $p<0.001$; Endogeic: Kruskal-Wallis, Chi-sq=10.129, df=4, $p<0.05$; Anecic: Kruskal-Wallis, Chi-sq=13.453, df=4, $p<0.01$) showing a strong change in functional group composition across different land use type.

Epigeic

Epigeic abundance per m^2 was significantly higher in OG2 (highest abundance) compared to B, OP2 and OP3 (OG2-B: Kruskal-Wallis, Chi-sq=7.218, df=1, $p<0.01$; OG2-OP2: Kruskal-Wallis, Chi-sq=4.567, df=1, $p<0.05$; OG2-OP3: Kruskal-Wallis, Chi-sq=12.194, df=1, $p<0.001$) while not significantly higher to F (OG2-F: Kruskal-Wallis, Chi-sq=3.474, df=1, $p>0.05$). Epigeic abundance in F and OP2 was also significantly higher than OP3 (F-OP3: Kruskal-Wallis, Chi-sq=12.255, df=1, $p<0.001$; OP2-OP3: Kruskal-Wallis, Chi-sq=10.145, df=1, $p=0.001$). Epigeic abundance in B (Epigeic mean=1.78) was lower than OP2 (Epigeic mean=2.56) but did not differ significantly.

Endogeic

Endogeic abundance per m^2 was significantly higher in B compared to OG2, F and OP2 (B-OG2: Kruskal-Wallis, Chi-sq=6.564, df=1, $p=0.01$; B-F: Kruskal-Wallis, Chi-sq=6.899, df=1, $p<0.01$; B-OP2: Kruskal-Wallis, Chi-sq=4.056, df=1, $p<0.05$).

Anecic

Anecic abundance per m^2 was significantly higher in F compared to OG2, OP2 and OP3 (F-OG2: Kruskal-Wallis, Chi-sq=4.791, df=1, $p=0.05$; F-OP2: Kruskal-Wallis, Chi-sq=4.056, df=1, $p<0.05$; F-OP3: Kruskal-Wallis, Chi-sq=10.159, df=1, $p=0.001$). B also reported significantly higher abundance compared to OP3 (B-OG2: Kruskal-Wallis, Chi-sq=6.429, df=1, $p<0.05$).

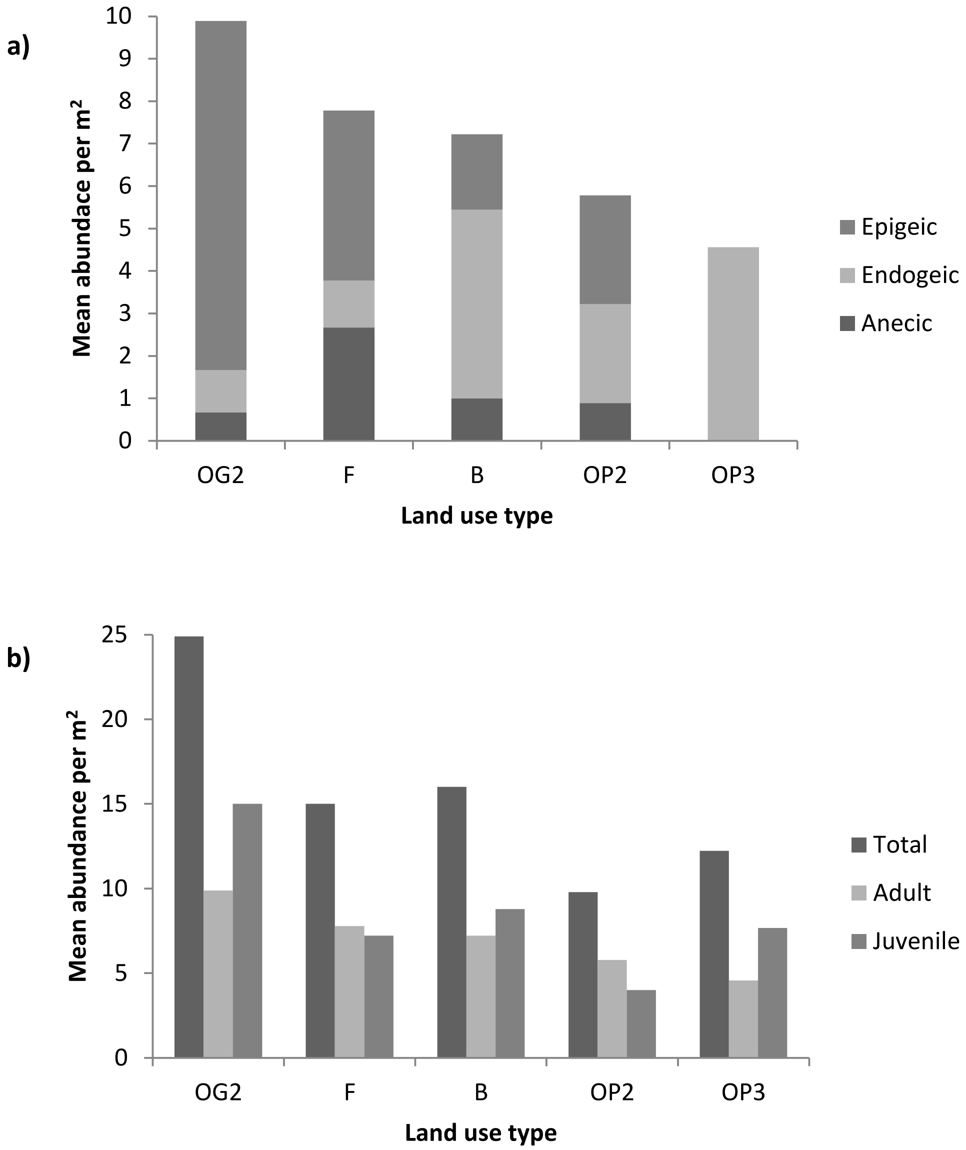

Figure 3.1. a) Changes in mean abundance of earthworm functional groups per land use. b) Comparison in mean abundance per m^2 of total, adults and juveniles per land use.

Table 3.1. Mean earthworm abundance per m^2 comparison between land use. Values are mean ± SD, n = 9 per land use. Superscript alphabets show significant differences in comparisons down the columns. Values sharing a letter are significantly different (P<0.05) from each other (Kruskal-Wallis ANOVA). Composition of abundance per land use is indicated in percentage.

Land use	Earthworm abundance per m2					
	Total	**Epigeic***	**Endogeic***	**Anecic***	**Adult**	**Juvenile**
OG2	24.89 ± 6.306^{a}	$8.22 \pm 1.824^{b, c, d}$ (33.0%)	1.00 ± 0.289^{g} (4.0%)	0.67 ± 0.373^{j} (≈3.0%)	9.89 ± 1.594^{n} (40.0%)	15.00 ± 5.225^{o} (60.0%)
F	15.00±3.131	4.00 ± 1.424^{e} (26.7%)	1.11 ± 0.564^{h} (7.4%)	$2.67 \pm 0.726^{j, k, l}$ (17.8%)	7.78±1.956 (51.9%)	7.22±2.146 (48.1%)
B	16.00±3.682	1.78 ± 0.909^{b} (11.1%)	$4.44 \pm 0.835^{g, h, i}$ (27.8%)	1.00 ± 0.333^{m} (6.3%)	7.22±1.310 (45.2%)	8.78±2.798 (54.8%)
OP2	9.78 ± 2.817^{a}	$2.56 \pm 1.144^{c, f}$ (26.2%)	2.33 ± 0.667^{i} (23.8%)	0.89 ± 0.512^{k} (9.1%)	5.78 ± 1.854^{n} (59.1%)	4.00 ± 1.667^{o} (40.9%)
OP3	12.22±6.355	$0.00 \pm 0.000^{d, e, f}$ (0.0%)	4.56±2.109 (37.3%)	$0.00 \pm 0.000^{l, m}$ (0.0%)	4.56±2.109 (37.3%)	7.67±4.387 (62.7%)

*Significant (p<0.05) difference between land use.

3.2 Changes of environmental variables across different land use

The environmental variables between land use were significantly different from one another and this has been summarised in Table 3.2 (Forest quality: ANOVA, F=156.217, df=4, p<0.001; SOM: ANOVA, F=12.692, df=4, p<0.001; Soil pH: ANOVA, F=3.879, df=4, p<0.01; Soil temperature: ANOVA, F=4.861, df=4, p<0.01; Soil moisture: ANOVA, F=1.641, df=4, p=0.183; Ground light: ANOVA, F=17.952, df=4, p<0.001; Litter depth: ANOVA, F=22.091, df=4, p<0.001; Top soil depth: ANOVA, F=36.997, df=4, p<0.001; Infiltration rate: ANOVA, F=3.439, df=4, p<0.05; Dry bulk density: ANOVA, F=7.932, df=4, p<0.001; Total porosity: ANOVA, F=7.932, df=4, p<0.001). However, no significant difference between land use was observed for soil pH and infiltration rate for Tanhame's Post Hoc.

Table 3.2. Difference in environmental variables between land use. Values are mean ± SD, n = 9 per land use. Superscript alphabets show significant differences in comparisons down the columns. Values sharing a letter are significantly different (P<0.05) from each other (One way ANOVA).

Land use type	Environmental variables										
	Forest quality* (range)**	SOM (range)**	Soil pH	Soil temperature	Soil moisture	Ground light	Litter depth	Top soil depth	Infiltration rate	Dry bulk density	Porosity
OG2	5.00±0.000[1,2] (5)	1.806±0.4965 (1 – 3.5)	6.556±0.3446[10]	29.033±0.2764[12]	43.15±16.544	237.73±105.700[13,14]	4.429±1.4201[18,19]	4.299±1.3391[24,25,26]	220.9042±196.06688	1.143199±0.1325459[31,32]	56.860±5.0017[36,37]
F	2.67±1.000[1,3,4,5] (1 – 4)	2.403±0.2635[8,9] (1.5 – 4)	6.157±0.3806	25.041±2.8689[12]	57.04±10.483	576.37±475.932[15,16]	3.863±0.9534[20,21]	2.581±0.6549[24,27,28]	212.4842±186.71508	0.941299±0.1028040[31,33,34]	64.479±3.8794[36,38,39]
B	2.56±0.527[2,3,6,7] (2 – 3)	1.833±0.5520 (1 – 3.5)	6.181±0.1979	26.604±2.7295	46.71±14.795	1796.40±1856.741[17]	3.391±2.4815[22,23]	3.842±1.3811[29,30]	236.0208±163.87362	0.959312±0.0825194[32,35]	63.800±3.1139[37,40]
OP2	0.00±0.000[4,6] (0)	1.208±0.1083[8] (1 – 2)	6.522±0.2954[11]	26.467±2.7410	54.22±14.900	7942.04±3797.778[13,15,17]	0.117±0.1100[18,20,22]	0.334±0.2295[25,27,29]	349.7039±99.56419	1.190119±0.1217070[33]	57.822±5.6479[38]
OP3	0.00±0.000[5,7] (0)	1.486±0.2756[9] (1 – 3.5)	6.050±0.4652[10,11]	27.303±2.8429	53.33±8.583	4417.16±2797.519[14,16]	0.072±0.0573[19,21,23]	0.375±0.2843[26,28,30]	429.0156±98.04030	1.070328±0.1533846[34,35]	55.090±4.5927[39,40]

[a] Tukey Post Hoc was used for soil pH, soil moisture, dry bulk density and total porosity
[b] Tanhame's Post Hoc was used for forest quality, SOM, soil temperature, ground light, litter depth, top soil depth and infiltration rate
[c] The mean difference is significant at p<0.05 level for the respective Tukey or Tanhame's Post Hoc
*Differences in Forest quality between OG2, OP2 and OP3 cannot be performed due to zero variance
**Range indicates SAFE standarded forest quality scale and SOM level score for Forest quality and SOM respectively.

3.3 Association between environmental variables and earthworm abundance

There is a significant positive relationship between total earthworm abundance and forest quality (r_s=0.358, p<0.001), SOM (r_s=0.183, p<0.05), top soil depth (r_s=0.358, p<0.001) and dry bulk density (r_s=-0.170, p<0.05) while a significant negative relationship was observed with soil temperature (r_s=-0.162, p<0.001), ground light (r_s=-0.360, p<0.005) and total porosity (r_s=0.170, p<0.05).

Epigeic showed a significant positive relationship with forest quality (r_s=0.374, p<0.001), litter depth (r_s=0.283, p<0.001) and top soil (r_s=0.310, p<0.001) while having a significant negative relationship with ground light (r_s=-0.347, p<0.001). Endogeic have a significant negative relationship with soil pH (r_s=-0.168, p<0.05). Anecic show a positive but formally not significant relationship with litter depth (r_s=0.141, p=0.058) and a significant negative relationship with ground light (r_s=-0.161, p<0.05).

Significant relationship is shown in Table 3.3.

3.4 Association between environmental variables

Positive and negative correlation between environmental variables and their significance is summarised in Table 3.4.

Table 3.3. Association between environmental variables and earthworm abundance. Values are positive or negative correlation coefficient and significance level.

Abundance	Environmental variables										
	Forest quality	**SOM**	**Soil pH**	**Soil temperature**	**Soil moisture**	**Ground light**	**Litter depth**	**Top soil depth**	**Infiltration rate**	**Dry bulk density**	**Porosity**
Total	0.358 0.000**	0.183 0.014*		-0.162 0.034*		-0.360 0.000**	0.255 0.001**	0.358 0.000**		-0.170 0.023*	0.170 0.023*
Epigeic	0.374 0.000**					-0.347 0.000**	0.283 0.000**	0.310 0.000**			
Endogeic			-0.168 0.024*								
Anecic						-0.161 0.031*	0.141 0.058				

**Values significant at p<0.01 (2-talied)
* Values significant at p<0.05 (2-talied)

Table 3.4. Association between environmental variables. Values are positive or negative correlation coefficient and significance level.

Environmental variables	Forest quality	SOM	Soil pH	Soil Temperature	Soil moisture	Ground light	Litter depth	Top soil depth	Infiltration rate	Dry bulk density	Total porosity
Forest quality		0.1354 0.000**	0.149 0.046*		-0.268 0.000**	-0.739 0.000**	0.808 0.000**	0.794 0.000**	-0.413 0.000**	-0.169 0.023*	0.169 0.023*
SOM			-0.195 0.009**			-0.353 0.000**	0.504 0.000**	0.456 0.000**	-0.402 0.000**	-0.440 0.000**	0.440 0.000**
Soil pH				0.274 0.000**	-0.365 0.000**					0.271 0.000**	-0.272 0.000**
Soil Temperature									0.265 0.001**	0.469 0.000**	-0.469 0.000**
Soil moisture								-0.207 0.006**			
Ground light							-0.662 0.000**	-0.597 0.000**	0.388 0.000**	0.245 0.001**	-0.246 0.001**
Litter depth								0.797 0.000**	-0.586 0.000**	-0.362 0.000**	0.362 0.000**
Top soil depth									-0.443 0.000**	-0.359 0.000**	0.358 0.000**
Infiltration rate										0.461 0.000**	-0.461 0.000**
Dry bulk density											-1.000 0.000**
Total porosity											

**Values significant at p<0.01 (2-talied)

* Values significant at p<0.05 (2-talied)

3.5 Relationship between environmental variables and earthworm abundance

Curvilinear estimation tool revealed that earthworm and their functional group abundance can be related in a linear or non-linear way to different environmental variables significantly. A summary of this is shown in Table 3.5. Total earthworm abundance and functional groups are significantly related (linear and non-linear) to forest quality (accounting for 2.4%-10.4% of variability of earthworms per m^2). Total abundance is also significantly related (linear and non-linear) to ground light (4.3% - 4.8% of variability) and top soil depth (4.4% of variability). Epigeic were significantly related (linear and non-linear) to soil pH (2.0% of variability), ground light (3.4%-5.6% of variability), litter depth (3.0%-6.1% of variability) and top soil depth (8.5% of variability). Endogeic were significantly related to litter depth (3.0% of variability) and show a formally not significant relationship (logarithmic and inverse) to soil pH while accounting for about 2.0% variability of the functional group. Anecic were significantly related (linear and non-linear) to SOM (2.5%-4.1% of variability) and ground light (2.9%-3.2% of variability).

Table 3.5. Relationship between earthworm abundance and environmental variables.

Environmental variables	Abundance per m^2			
	Total	**Epigeic**	**Endogeic**	**Anecic**
Forest quality	Linear: $F_{1,178}$=5.788, p=0.017, R^2=0.031 Quadratic: $F_{1,177}$=3.120, p=0.047, R^2=0.034	Linear: $F_{1,178}$=14.071, p<0.001, R^2=0.073 Quadratic: $F_{2,177}$=8.501, p<0.001, R^2=0.088 Cubic: $F_{3,176}$=6.833, p<0.001, R^2=0.104	Linear: $F_{1,178}$=4.377, p=0.038, R^2=0.024	Quadratic: $F_{2,177}$=3.530, p=0.031, R^2=0.038
SOM	-	-	-	Linear: $F_{1,178}$=4.504, p=0.035, R^2=0.025 Quadratic: $F_{2,177}$=3.738, p=0.026, R^2=0.041 Cubic: $F_{3,176}$=2.540, p=0.058, R^2=0.041
Soil pH	-	Linear: $F_{1,178}$=3.573, p=0.060, R^2=0.020 Logarithmic: $F_{1,178}$=3.626, p=0.059, R^2=0.020 Inverse: $F_{1,178}$=3.643, p=0.058, R^2=0.020	Logarithmic: $F_{1,178}$=3.626, p=0.058, R^2=0.020 Inverse: $F_{1,178}$=3.759, p=0.054, R^2=0.021	-
Soil temperature	-	-	-	-
Soil moisture	-	-	-	-
Ground light	Linear: $F_{1,178}$=6.470, p=0.012, R^2=0.035 Logarithmic: $F_{1,178}$=8.008, p=0.005, R^2=0.043 Quadratic: $F_{2,177}$=4.202, p=0.016, R^2=0.045 Cubic: $F_{3,176}$=2.936, p=0.035, R^2=0.048	Linear: $F_{1,178}$=3.730, p=0.055, R^2=0.021 Logarithmic: $F_{1,178}$=10.475, p=0.001, R^2=0.056 Inverse: $F_{1,178}$=5.179, p=0.024, R^2=0.028 Quadratic: $F_{2,177}$=3.135, p=0.046, R^2=0.034 Cubic: $F_{3,176}$=2.617, p=0.053, R^2=0.043	-	Logarithmic: $F_{1,178}$=5.304, p=0.022, R^2=0.029 Inverse: $F_{1,178}$=5.958, p=0.016, R^2=0.032
Litter depth	-	Linear: $F_{1,178}$=5.567, p=0.019, R^2=0.030 Quadratic: $F_{2,177}$=5.486, p=0.005, R^2=0.058 Cubic: $F_{3,176}$=3.837, p=0.011, R^2=0.061	Linear: $F_{1,178}$=5.488, p=0.020, R^2=0.030	-
Top soil depth	Quadratic: $F_{2,177}$=4.074, p=0.019, R^2=0.044 Cubic: $F_{3,176}$=2.700, p=0.047, R^2=0.044	Linear: $F_{1,178}$=4.333, p=0.039, R^2=0.024 Quadratic: $F_{2,177}$=8.185, p<0.001, R^2=0.085 Cubic: $F_{3,176}$=5.427, p=0.001, R^2=0.085	-	-
Infiltration rate	-	-	-	-
Dry bulk density	-	-	-	-
Total porosity	-	-	-	-

Chapter 4 Discussion

This study is an attempt to understand how forest modification gradient impacts earthworms and identifying types of environmental variables that may influence the taxa response at SAFE Project site. The measured environmental variables in my study was tested statistically to see how external factors can affect earthworm abundance and how this can vary between different land uses whose properties can be altered by anthropogenic activities that can significantly alter soil environment and forest quality (Edwards, 2004).

4.1 Land use effect on earthworm abundance

Decreasing average earthworm abundance per m^2 (highest in OG2) along the disturbance gradient across the land use showed that tropical forest earthworms are affected by deforestation. However, only OP2 was significantly lower than OG2 (refer Table 3.1) indicating that earthworm population showed a certain level of tolerance to the associated land use disturbance. Significant loss of earthworm abundance in OP2 was typically expected. Nonetheless, functional groups showed strong response to disturbance across the land use and their functional composition varied per land use indicating sensitivity to different environmental variables that can influence their community structure (refer Table 3.1). Epigeic were most abundant in OG2 compared to mineral soil dependent endogeic and anecic and this composition reflects the usual nutrient-poor conditions of tropical forest soil (Edwards, 2004). Following forest clearance, the predominant epigeic largely disappears and this was observed with significantly lower epigeic abundance in B, OP2 and OP3. Some endogeic and anecic which are abundant in nutrient-rich soil may survive if present with similar finding reported by Fragoso and Lavelle (1992).

Soil, climate and food are three main factors that can affect earthworm abundance at the varying level of disturbance experienced in each land use (Edwards, 2004). Soil quality plays a direct role in the overall habitat quality which impacts individual species/functional group based on their preference and tolerance to different properties. Food quality and availability can determine the maximum population size or carrying capacity. Climatic events such as rainfalls and seasonal changes can affect earthworm abundance too. As such, there is possibility of re-colonisation of an adapted species after certain disturbance and establishment of high population densities under suitable conditions. This could explain the higher abundance or functional group composition of endogeic and anecic in more disturbed areas found in this study (refer Table 3.1).

Populations of earthworms can vary greatly in terms of abundance, biomass and diversity that can range from a few to 1000 per m^2 (Lee 1985; Edwards & Bohlen 1996; Lavelle *et al*. 1999). Lack of previous study on earthworm population at the survey site limits opportunity to make comparison to understand how spatiotemporal and climate factor influence their abundance and establish whether current abundance in this study is high/low/random and if existing abundance is sufficient to influence soil processes and the resulting ecosystems services or if it is just a noise. It is also difficult determining to what extent disturbance and the use of management strategies can influence earthworm population dynamics. Due to this, current soil properties, food and land use characteristics and forest quality could help explain earthworm abundance. The functional group response can be explained by their respective ability to adapt to environmental variation and tolerate disturbance.

4.2 Influence of environmental variables on earthworms

4.2.1 Soil temperature and moisture

Soil temperature and moisture across the disturbance gradient did not change significantly (refer Table 3.2). However, the study found that earthworm abundance has an inverse relationship with soil temperature (refer Table 3.3). The suitable range of temperature for earthworms to function in the tropics is $20^{o}C$ to $30^{o}C$. Temperature affects earthworm metabolic rates that can play a major role in determining earthworm composition and community structure; distribution patterns and activity (Lee 1985; Edwards & Bohlen 1996; Lavelle 1983; Lavelle *et al*. 1989, 1999).

Higher temperature will result in higher decomposition rate of organic matter leading to reduced litter availability that can negatively impact litter dwelling epigeic and litter feeding anecic (Lavelle *et al*. 1999) while encouraging endogeic abundance as they can utilise lower quality materials with more efficient digestive processes through interactions with ingested soil microbes (Edwards, 2004). This is consistent with inverse relationship between earthworm abundance and soil temperature. However, no significant association between endogeic and soil temperature was detected to support the latter (refer Table 3.3).

As there are no significant changes in soil temperature and moisture between sites, these two factors can be assumed as not having major effects on earthworm abundance and distribution. Hence, other environmental variables play a greater role in determining their abundance within the different land use. However, due to little variation in temperature throughout the year in the tropics with moisture level changing according to dry and wet season or rainfall patterns – further monitoring and comparison on earthworm abundance can shed a more accurate view on the subject (Edwards, 2004).

4.2.2 Soil pH

Earthworm become numerous above pH 5.5 (David Jones, pers. comm.) in the tropics and become scarce in acidic soils (<pH 4.5). Significant changes in soil pH were recorded between OG2:OP2 and OP2:OP3 (refer Table 3.2). There is also a significant negative association between endogeic and soil pH and a formally not significant inverse relation through curve estimation regression analysis (refer Table 3.5). A significantly lower pH in OP3 compared to OP2 could be associated with higher endogeic abundance and absence of epigeic and anecic in OP3. Other studies do support that there is a strong difference in pH preference among earthworm species (Satchell 1967; Bouché 1972) and this area must be further explored to explain predominance of endogeics species in OP3. Absence of species ID data in this study also limited opportunity for such understanding.

4.2.3 Habitat quality

Canopy cover plays an important role within habitat by protecting forest floor from various adverse effects of climate conditions such as temperature, rain, wind, ultraviolet radiation/sunlight and humidity (Szarzynski & Anhuf, 2001). The amount of light reaching the forest floor is used as an indicator of canopy cover which also reflects the quality of forest described by the vegetation type. This influences the litter input (in terms of quantity and quality) and the eventual availability of organic matter which is the food source for earthworms. This study confirmed such interdependence with significant relationship between earthworm abundance, forest quality, SOM, ground light and litter depth shown in Table 3.3, 3.4 and 3.5. Deforestation significantly impacted canopy cover (in terms of openness and type of cover) and habitat quality which will have immense effect on organic matter availability which will affect earthworm abundance. Significant difference in environmental variables between land use types is shown to be consistent with significant difference in earthworm abundance in this study (Table 3.1 and 3.2).

4.2.4 Interdependence between forest quality, canopy cover, SOM and effect on earthworm abundance

Litter dependent epigeic and anecic can be significantly influenced by litter depth that forms part of their food source. This is confirmed by a significant positive relationship between epigeic and litter depth shown in Table 3.3 whereas a formally not significant relationship was seen between anecic and litter depth. Such difference between epigeic and anecic can be a result of the litter layer serving as a natural habitat for epigeic compared to soil-dwelling anecic. A significantly higher litter depth in OG2 (old growth forest), B and F (twice logged secondary forest) compared to OP2 and OP3 (refer Table 3.2) corresponded with significantly lower abundance of epigeic and anecic in both oil palm plantation (refer Table 3.1). This also reflects lower quantity and quality of litter input across the disturbance gradient supported by a significantly lower forest quality and ground light (indicating canopy openness) in more disturbed land use (refer Table 3.2) with strong significantly negative relationship between forest quality and ground light and strong significantly positive relationship between forest quality and litter depth (refer Table 3.4).

Organic matter (major food source for earthworms) and its availability and quality are dependent on the litter input from the vegetation within the habitat. Findings from the study showed that SOM is inversely related to ground light while strong positive correlation seen with litter depth (refer Table 3.4). Earthworm abundance is also significantly dependent on SOM (refer Table 3.3) with old growth and secondary logged forest systems recording higher SOM (F significantly higher than OP2 and OP3) (refer Table 3.2) that corresponded with higher earthworm abundance (significantly higher in OG2 than OP2) (refer Table 3.1). However, the study did not assess the litter quality and how this differs between land use and the subsequent effect on earthworms. It has been shown that litter quality rather than quantity that has greater effect on earthworm populations (Satchell 1967; Swift *et al*. 1979; Boström & Lofs-Holmin 1986).

There is also the missing effect of dead root and rhizo-deposition that also serve as an important food source for earthworms (Edwards, 2004).

4.2.5 Effect of soil compaction on earthworms

Dry bulk density and total porosity are significantly related with total earthworm abundance (refer Table 3.3) but none of the functional group abundance reported similar relationship. Soil dry bulk density is an indicator of soil compaction which can have huge impact on earthworm abundance (Edwards, 2004). Different anthropogenic activities can alter bulk density per land use as reflected by significantly higher bulk densities in more disturbed land use in this study (refer Table 3.2). Extreme compaction can result from the use of heavy vehicles such as tractors during deforestation/land conversion/agricultural activities (Doneen *et al*. 1952). Subsurface layers are also subject to the compacting weight of the soil above them. As such, effect of bulk densities at different soil profile on earthworm functional group should also be investigated separately as higher bulk density at deeper soil while lower bulk density at top soil due to SOM content, aggregation and root penetration can have different level of impact on earthworms generally and functional group specifically. However, methodological limitation with soil sampling and analysis for bulk density as well as potentially underestimated earthworm abundance may influence current outcome between bulk density and earthworm abundance. Such limitations will be discussed further under 'Study limitations' section below.

Canopy cover also protects soil from climate effects such as rainfalls and soil erosion. Cultivation can damage SOM; reduce stability of soil aggregates making them susceptible to damage from water and wind (exposed canopy) leading to soil erosion where soil particles can fill up soil pores and reduce porosity; increase bulk density and run-off. This eventually increases soil compaction; damages soil structure and ability to support root growth that is important for plant development (Edwards, 2004). This scenario can be supported by the significant positive relationship between ground light and bulk density and a significant negative relationship between ground light and porosity in this study (refer Table 3.4) and the significant difference between the properties along the disturbance gradient (refer Table 3.2).

Lower bulk density shows less compaction and presence of more air space which results in higher porosity characterised by significant negative relationship between dry bulk density and total porosity (refer Table 3.4) with higher abundance being associated with lower bulk density and higher porosity (refer Table 3.3) (NNRI, 2013). SOM can also lower bulk density (Edwards, 2004). This is shown with a significant inverse relationship between SOM and dry bulk density (refer Table 3.4) that corresponded with lowest bulk density recorded in land use F which also has the highest average SOM.

4.2.6 Top soil influence on earthworms

Deforestation leads to loss of top soil which impacts earthworms distribution in the tropics especially loss of surface and subsoil dwellers (epigeic and endogeic) (UNEP 1978; Fragoso & Lavelle 1992; Lavelle *et al*. 1999) which is further supported by this study where top soil depth was significantly reduced across the disturbance gradient (refer Table 3.2) and corresponded with a significant decrease of epigeic (refer Table 3.1) and affirmed by a significant positive relationship between epigeic abundance with top soil depth (refer Table 3.3 and Table 3.5). The loss of top soil also resonates with loss of litter layer and organic matter which can further devastate earthworm and other soil fauna survival. A strong significantly positive association between top soil depth and litter depth and a strong significantly negative association between ground light and litter depth found in the study supports this view as well (refer Table 3.4). Another aspect of soil which was not investigated was how texture may directly/indirectly affect earthworm activity (Guild 1948) and impact of aerobic and anaerobic soil condition on earthworm abundance (Curry & Cotton 1983).

4.2.7 Different management in oil palm effect on earthworms

No significant difference in earthworm abundance and environmental variables was found between OP3 and OP2 except significantly higher epigeic abundance in OP2 compared to OP3 and soil pH which was significantly more acidic in OP3 compared OP2 (refer Table 3.2). In addition, only endogeic were found in OP3. Soil pH alone could not have caused such outcome as the average soil pH for both oil palm sites was above the suitable range of pH 5.5 for earthworm activity. However, specific functional species may show preference towards a certain soil pH and this must be further investigated at species level (Lavelle & Pashanasi, 1989).

Different management practises and age of OP2 and OP3 which are managed by two different companies (Zakariah Juslin, pers. comm.) as well as OP3 (established in year 2000, tall palm trees – height >3m, formed canopy) being older than OP2 (established in 2006, shorter trees – <1m height, canopy is forming) could also potentially account for the difference in abundance. Agricultural practises such as cultivation, use of fertilisers; pesticides; herbicides can have adverse effects on earthworms (Edwards & Thompson 1973; Edwards & Lofty 1982; Edwards 1984a, b). The different management practises and age between the palm oil sites presents opportunity for a more detailed comparison study.

4.3 Earthworms and soil function

Earthworm role in soil function through their actions of cast production and burrowing as well as interactions with soil microbial and other soil fauna (especially invertebrates) is also influenced (at varying degrees) by climate, food and soil parameters. The resulting earthworm activities, in turn, influence soil processes and the ecosystem services that derive from there by determining microbial processes and also affecting nutrient dynamics and organic matter output. Earthworms also influence other soil fauna activities by altering the soil environment and feeding resources while directly interacting with them (mutualism, competition and parasitism) (Edwards, 2004).

Preference and tolerance of specific species belonging to functional groups and their collective effort dictates role of earthworms within a habitat. Changes in functional group composition between land use strongly suggest that functional species that cannot cope with these changes are being lost leaving a gap in their functional role and how this gap is exploited by other soil fauna is not known or to what extend this impacts the soil as well as interaction with other soil biota is not well understood. Invasive species could play a positive and/or negative role under these circumstances.

4.3.1 Feeding behaviour – Selection of soil particles

Preferential feeding behaviour of earthworm that can depend on litter type and quality as well as particle types and size has been well documented (Piearce 1978; Ferrière 1980; Kanyonyo, 1984; Barois & Lavelle, 1986; Bonkowski *et al*. 2000; Neilson *et al*. 2000). Selection and ingestion of organic and mineral soil particles results in cast production that has higher SOM content than the surrounding soil (Lee 1985) and incorporation of cast on soil surface or within soil influences nutrient deposition in soil profile. Endogeic have been recorded to deposit 20 to 200 tonnes dry soil ha^{-1} per year with surface casts containing significant amount of SOM with a huge portion being deposited in the soil itself (Darwin 1881; Lavelle 1978). Some endogeic species have also been shown to selectively ingest particles which are either smaller or larger than the diameter of their mouths (Derouard *et al*. 1997; Blanchart *et al*. 1999). Detailed knowledge on such behaviour is still lacking and the long term effect of such behaviour creates need for more rigorous and detailed scientific investigation.

4.3.2 Compacting vs decompacting action by earthworms

Soil bulk density and compaction can be influenced by the type of cast produced by earthworm that can either be globular cast of compacting species or granular casts of decompacting species (Edwards, 2004). Predominance of either group can influence the level of compaction in the soil. Presence of both cast producing species can also result in complementary patterns of successive patches of compacted and decompacted soil (Blanchart *et al*. 1999).

Earthworm inoculation experiment involving globular cast producing endogeic species have shown significant increase in bulk density and compaction and decreased infiltration rates (Alegre *et al*. 1996). Such changes can have profound effect on soil physical properties especially water movement as the study also showed that earthworm inoculated sites became drier in dry season and wetter in wet season compared to non-inoculated sites. Conversely, endogeic species that breaks large aggregates into finer particles can have the opposite affect (Derouard *et al*.1997; Blanchart *et al*. 1999). As such, species identification of current specimen in this study may shed greater light on how soil bulk density property is being influenced by earthworms while indicating specific species for future behavioural and ecological studies. Also important to consider is the decompacting actions of other species such as invertebrates (ants, termites, millipedes, etc).

4.3.3 Burrowing, ingestion and cast production effect on soil

Earthworms, as soil engineers, play a major role in pedogenesis by influencing soil structure and organisation through the burrowing and cast productions (Zhang & Hendrix 1995; Decaens *et al*. 1999; Wilcox *et al*. 2002). Soil physical properties can be altered by burrowing actions by increasing drainage; aeration; water storage; solute/organic matter and water movement to deeper soil profile; assist plant root growth; improve gas flow and reducing run-off

(Bouche´ 1975; Beven & Germann 1982; Heenan 1993; Whalley & Dexter 1994; Devliegher &Verstraete 1997; Kung *et al*. 2000; Chan & Shuster *et al*. 2001).

Earthworm burrowing creates large pore systems and the cast produced through ingestion (which tends to show microporosity) and incorporated into soil contribute to the soil porosity (Lamparsky *et al*. 1987; Kretzschmar 1987; Kretzschmar & Aries, 1992; Blanchard 1992). However, earthworms are not the only source of soil pores and may only be responsible for 1-2% of total porosity (Edwards, 2004). Although earthworm effect on increasing soil infiltration rate has been seen as beneficial, it is important to note that most studies on earthworm effect on infiltration rates have been focused on Lumbricidae (consist of a large number of semi-permanent burrowing species that can influence infiltration significantly) that does not reflect the behaviour of other potentially predominant species (Edwards, 2004). Furthermore, impact of cast occlusion in earthworm (endogeic and anecic) burrows can actually limit the effectiveness in water transport into the soil (Ela *et al*. 1992).

Infiltration rate is also significantly dependent on soil dry bulk density and total porosity (refer Table 3.4) (Edwards, 2004). As such, patterns of higher porosity and infiltration can be seen in areas where higher abundance of endogeic and anecic have been recorded in this study (refer Table 3.1 and Table 3.2). Many studies also reported that endogeic and anecic can considerably alter soil porosity and hydraulic conductivity (related to infiltration rate) due to their burrowing in mineral soil (Zachmann *et al*. 1987; Joschko *et al*. 1992; Trojan & Linden 1992; Friend & Chan 1995; Shipitalo *et al*. 2000; Alakukku *et al*. 2002). However, none of the functional group showed significant relationship with porosity although earthworm abundance showed a significant positive relationship (refer Table 3.3).

It is possible that lack of endogeic and anecic sampling (due to methodology and climatic reasons –drier conditions during dry season drive them deeper into the soil profile) affected the analysis or missed effect of other soil engineers such as termites and ants perhaps may play a bigger role through their tunnelling actions. While earthworm feeding behaviour has greater effect on bulk density, burrowing and cast production have greater effect on porosity and infiltration rate – these properties do influence each other (refer Table 3.4) and the magnitude of such behaviour will influence the effect that a particular earthworm species may have on the soil which in itself may vary over time and space due to other factors (climate, food, soil properties). For instance, the deeper burrowing anecic may have greater impact on flow pathways compared to non-permanent mineral soil dwelling endogeic that may have greater effect on top soil porosity. Further behavioural and ecological studies on earthworm species should provide more insight into this interesting subject.

4.3.4 Turnover of soil

Earthworms are capable of moving large quantity of soil from deeper soil profile into the surface which was first reported by Charles Darwin (Darwin, 1881). This movement can range from 2 to 250 tonnes per hectare per annum representing 1mm to 5cm thick soil being brought to the top creating a stone-free soil surface and even larger turnover have been recorded in tropical regions (Lavelle *et al*. 1999). This is achieved through the ingestion and mixing of mineral and organic material together with the soil microbes in earthworm gut and excreted as casts (Le Bayon & Binet, 1999). Such action has been reported to be capable of processing 25% top soil profile layer (enriched with humus) within a year (Lee, 1985).

4.3.5 Earthworms effect on decomposition, SOM, above ground vegetation and forest ecology

Earthworms favour high quality litter with C:N (carbon:nitrogen ratio) of 20:1 and the quality of SOM is dependent on litter input which is directly linked to the surrounding vegetation within the habitat - as such, SOM serve as a good predictor of earthworm abundance (Edwards & Bohlen, 1996). Higher earthworm abundance was shown to correlate significantly with SOM in this study which is similar to the findings of Hendrix *et al*. (1992). Other studies, mostly in temperate regions and even tropics, found that earthworm ability to consume annual litter input can increase decomposition; influence nutrient release and leaching rates that affects nutrient uptake by vegetation that will potentially influence above ground vegetation profile and impact the vegetation palatability for herbivores (Raw 1962; Madge 1965; Satchell 1967; Sugi & Tanaka 1978; Edwards & Bohlen 1996). Such impact is being observed in North American forests where the invasive European limbricids are dictating the organic matter turnover rate and soil cover especially in areas where earthworms were absent before (Hale *et al*. 2005; 2006; 2008).

Current knowledge also shows that earthworms play an important role in the final stages of decomposition process compared to the litter system itself by mixing soil with humus (Lavelle, 1984). Fragoso and Lavelle (1995) also explained that this may have been the reason why humus level was observed to be low in areas with no earthworms despite decomposition process taking place. The similar might be the case in the tropics where invertebrates such as ants and termites may play a bigger role in decomposition process with earthworm presence being reflected as a noise in the decomposition system (David Jones, pers. comm.). However, the organic matter created as a result of decomposition could predict higher earthworm abundance that may directly result in higher humus level – indicating indirect interaction between earthworm and other decomposers. Earthworm role in the decomposition rate in comparison to termites and how their presence or absence influences humus level can be studied through earthworm exclusion or inoculation experiments.

4.4 Study limitations & future recommendations

4.4.1 Transport permit and species ID issues

Complications in getting a transport permit and time constraints have resulted in inability to ID the specimen to species level by an earthworm taxonomist. This caused the morphospecies of functional group to be carried out personally. Functional group identification by an expert can reduce margin of error for potential under or over estimation of groups. An expert's insight would be especially useful in identifying species of juvenile that accounted for almost 50% of all sampled earthworms. In addition, sliced adult earthworms are also difficult to classify to functional group due to inability to estimate full body size that varies between functional group. Endogeic were easier to identify due to absence of pigmentation however some larger sized epigeic that has been sliced could have been mistakenly identified as anecic – such cases were excluded in the functional group count.

Species ID data would present chances to conduct various other analysis. Contribution or effect of each individual species based on their behaviour, ecology and population distribution in soil function, processes and fertility can be explored. Species ID can also shed light on presence of invasive earthworm species and the extent of their impact across the disturbance gradient. Species data would also enable more rigorous statistical analysis such as ordination and investigation on collective effect of environmental variables on earthworms at species level. All this effort would also further bridge gap in current lack of knowledge on earthworms in Borneo. However, challenges could arise due to extreme lack of earthworm specialists in the region with limited taxonomic research in addition to lack of interest to study earthworms.

4.4.2 Sampling design

Sampling effort may have underestimated the actual earthworm abundance as only individuals with the head/clitellum attached are counted. Sometimes it was difficult to avoid slicing earthworms (accidentally) especially large and long ones and some sliced parts do go missing. Anecic could be underestimated as they are sensitive to vibration and tend to burrow deeper in response to above ground movement – even though caution was taken not to tread on sampling area (monolith quadrat) the digging itself will create vibration in soil and the trench is only able to serve as a barrier to stop them escaping to adjacent soil. Epigeic worms were also fast and disappear very quickly while the litter layer was being removed. Some are 'jumpy' and wriggles out of the hands upon capture – making sampling more difficult.

Fieldwork was also carried out during dry season when most earthworms tend to burrow deeper in the soil and aestivate (Edwards, 2004). All this reduces chances of sampling earthworms. As such, fieldwork should be carried out during rainy season between October to March and this data will also serve a good temporal comparison and useful for monitoring purposes. A deeper soil depth for digging can be adopted but this will increase the time and effort to sample per monolith. Current protocol used in this study was extended to 30cm from actual 20cm depth which was recommended for wet season sampling. The use of mustard solution is also recommended and should be tested for effectiveness (Butt & Grigoropoulou, 2010).

One of the missed effect in this study is the influence of vertical community consisting earthworm species that climbs up trees and live in the crown especially when its rainy season or due to flooding. Specific methodology needs to be researched and employed in the field to study such species. Species ID data may also help reveal if such group of earthworms were sampled successfully during this study.

4.4.3 Exclusion of earthworm biomass data

Biomass data taken in the study was not used as it does not account for true earthworm biomass as moisture and gut content (soil) influenced weight. Water content accounts for about 20% of earthworm weight and varies between individuals depending on moisture level in the soil. A spatiotemporal comparison of biomass is also not reliable as moisture in soil and gut contents will vary between sites and seasons resulting in false high or low biomass estimation. As such, earthworm count was selected to better reflect earthworm impact as abundance per m^2.

To eliminate moisture and gut content influence on earthworm biomass – ash free dry biomass protocol should be adopted. Moisture or water content can be removed by drying earthworm specimen at about 60^oC for 24-48 hours to obtain dry weight. To further eliminate gut contents, after recording the dry weight, the specimen is to be subjected to further burning at 500^oC for several hours causing all body parts to burn off leaving the mineral soil (gut content) behind. Subtraction of ash weight from dry weight of earthworm would provide the ash free dry biomass. Unfortunately, this method will result in loss of specimen for ID purposes and was not adopted in this study. Inconsistency in earthworm biomass reporting between studies also makes it difficult to conduct reliable comparisons.

4.4.4 Bulk density estimation

Bulk density is highly dependent on soil sample collection, drying and sample handling. Soil core collection in PVC tubes and transfer into bags for further analysis could cause potential loss of soil sample. Sampling by different individual also result in inconsistencies due to different collection and handling techniques. Precaution was also taken during soil sample analysis in lab by ensuring that no samples were excluded from weighing and drying process. To obtain optimum results for bulk density – individual tubes/rings should be used for each soil core sample until sample drying and final dry weight was taken to reduce soil handling and potential loss of soil. Presence of cracks in the soil will also reduce bulk density. The formula applied to estimate bulk density also assumes particle density as 2.65 g/cm^3 which may not hold true for the different land use where sampling was carried out and needs to be further investigated.

4.4.5 Soil chemical properties analysis

Quantitative analysis of soil chemical properties especially carbon and nitrogen as well as organic matter content and litter input and litter quality would provide greater insight into how such variables influence earthworm abundance and distribution. SOM analysis carried out in this study is qualitative, cheap and easy to do. The results from the test produced clear variation between monolith and sampling sites to score SOM level. Nonetheless, under or overestimation can be possible as the test can be affected by soil texture (sandy, clay, loamy) while quantitative analysis such as Walkley-Black procedure can provide greater accuracy.

4.5 Potential future studies

- Earthworm exclusion or inoculation experimental designs could provide a comparison between earthworm-present and earthworm-absent sites while monitoring for changing environmental variables.
- DNA barcoding could provide essential information on earthworm dispersal, gene flow and feeding activities that is lacking currently due to limitations and difficulty to measure or monitor earthworms while they are in the soil (Edwards & Bohlen 1996; Bohonak 1999; Nathan 2001; Broquet & Petit 2009).
- Comparison of earthworm abundance with ants and termites to better understand how they interact with each other.
- Spatiotemporal, behavioural and ecological studies and monitoring of earthworm abundance and biomass.

Chapter 5 Conclusion

Deforestation impacts all forms of life dependent on forest ecosystems and earthworms make no exception. Results from my study confirmed that while some level of tolerance was shown by earthworms due to logging activities, complete land conversion will adversely affect their abundance in oil palm plantations. This is especially true and significant for functional groups whose abundance and composition significantly changed and correlated to varying degree of environmental variables depending on the level of disturbance in the land use. This study also indicated that earthworm functional groups are sensitive to abiotic changes and showed potential to be a good indicator of soil quality and indirectly reflect forest quality. However, species level data is lacking and there is a huge gap in knowledge on earthworm individual species behaviour and ecology that can help explain how surrounding properties can affect earthworms and how earthworm response (individually as a species and collectively as a functional group) to disturbance can influence soil processes. This is even more important since other studies have confirmed that each earthworm species exhibit different level of preference and tolerance to environmental variables and their response to these variables also differ over time and space. As a result, the loss of a species across the modification gradient results in the loss of the functional role and how resilient is the affected system to such loss is not understood. Hence, this calls for more detailed and long-term studies to understand how earthworms impact their habitat through their behaviour (burrowing, ingestion and cast production) as well as interaction (competition, mutualism, and parasitism) with other soil biota and if their impact is sufficient to even influence forest ecology in the tropics. The result of such study may also even indicate keystone earthworm species that may need greater protection.

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Appendix I

Fieldwork

Transect set up and monolith digging

One person per monolith to complete each transect within 30 minutes (for digging only)

Carefully searching for earthworms

Sometimes earthworms gets sliced in the process of digging

Monolith that has been dug out

Recording the measured environmental variables

Monoliths are covered after sampling was completed

Each person spends an average of 20-30min per monolith to sample earthworms

Appendix II

Laboratory work

Lab station set up for specimen processing in the lab facility at Maliau

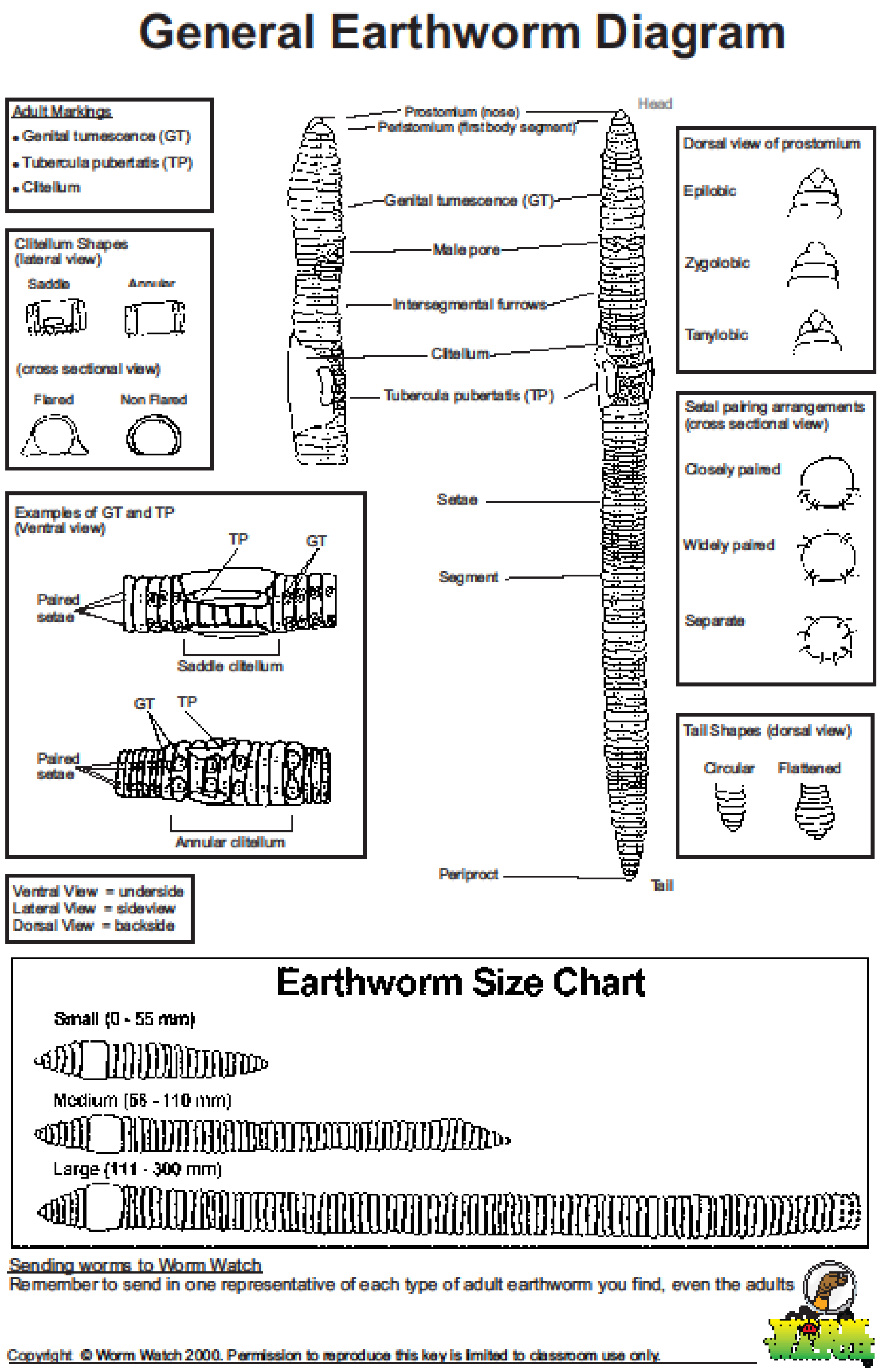
Work station set up at SAFE camp for specimen processing

Apparatus set up for SOM test

Scoring SOM level by comparing solution colour with reference test tubes

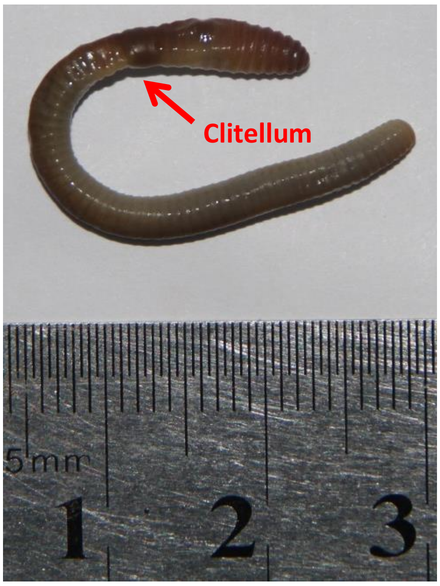
Appendix III

Earthworm anatomy



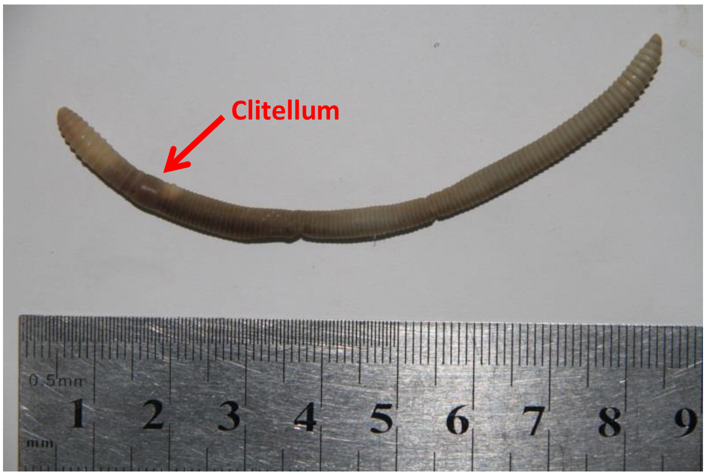
Appendix IV

Earthworm functional group identification



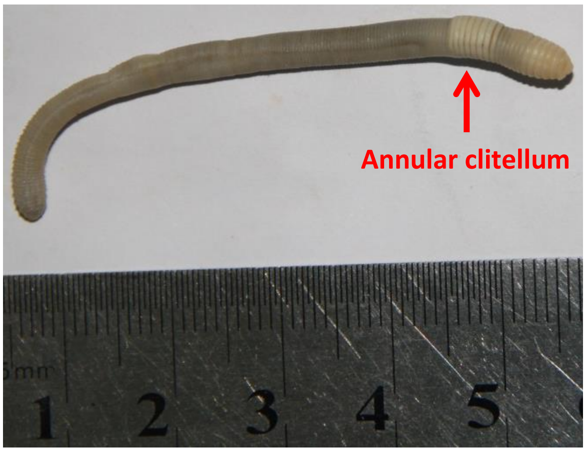
Adult epigeic with saddle shaped clitellum

Larger sized epigeic



Endogeic with saddle shaped clitellum

Earthworm functional group



Endogeic with annular clitellum

Juvenile (potential endogeic) without clitellum

Anecic with clitellum

Appendix V

Data sheet for recording


Date :

Day :

2nd Order Sampling

Fragment ☐ Point ☐ Hectare ☐

Point Reference : ______

Coordinate :

pH :

Temperature :

Moisture :

Light :

Litter :

Humus :

Infiltration :

Notes

Lab

Earthworm no :
(+ description)

Earthworm weight :

EC :

pH :

SOM :

Soil - Total fresh weight :
- Dry weight :
- Analysis bag :

weight before drying process :

Soil assessment:

Visual soil assessment :

Soil structure score :

Soil porosity score :

Soil colour score :

Soil mottles score :