Effects of endogeic earthworms on quantity and composition of large macroaggregates in Central Kenya

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Abstract

Endogeic earthworms are known to influence the amount of large macroaggregates in soils. In tropics, endogeic earthworms can contribute to soil aggregation only during rainy seasons where soil moist conditions occur. In this study, we were interested in the effects of endogeic earthworms during short rains on (1) stable large macroaggregates formation (2) composition of large macroaggregates. This was studied by means of a 20 days laboratory incubation experiment at different earthworm densities (none, three, six), with different organic residue management (no residues, maize, soybean) and at two soils (Embu, Chuka). All macroaggregates (250 - 2000µm) were broken down prior earthworm addition; therefore we could determine the rate of formation of large macroaggregates by earthworms. The amount of large macroaggregates was positively related to earthworm densities only in the organic residue treatments. Very few large stable macroaggregates were formed by earthworms in the no residue treatments. The effect of earthworms was stronger in Embu soil. Regarding the composition of the large macroaggregate, earthworm activities had no positive effect on the proportion of microaggregates within large macroaggregates but had positive effects on the amounts of silt and clay. These higher amounts of silt and clay might contribute to the formation of new microaggregates inside large macroaggregates, which are considered to protect carbon for long period of time. In conclusion, endogeic earthworms can have significant impact on the formation of stable large macroaggregates in Kenyan soils during short rains.

Keywords: Endogeic earthworms, macroaggregates, microaggregates, silt, clay, oxisol, Kenya

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Dedication

This MSc thesis is dedicated to Fredrick Ayuke and his family.

1. Introduction

In the tropics, land clearing and development for food crop production generally lead to chemical, physical and biological degradation of soils. Conserving and/or rehabilitating soil structure is much more difficult than maintaining adequate nutrient reserves for plants (Lal, 1994). Soil structure can be defined simply as the size and spatial arrangement of particles into groupings called aggregates (Oades, 1992). Good soil structure prerequisites stable aggregates which remain stable after wetted (water stable aggregates).

Soil aggregation has a great impact on soil physical properties. Well aggregated soils posses a larger pore space facilitating water infiltration and preventing wind and water soil erosion. Soil aggregation and soil organic matter dynamics are closely related. Aggregates are thought to protect SOM from microbial decomposition and at the same time, SOM helps in the formation of aggregates of different sizes (Tisdall and Oades, 1982). The preservation of SOM inside aggregates is very important for agricultural land use since SOM is widely recognised as a key element in nutrient cycling (Vanlauwe et al., 2000).

Aggregate formation involves biological and physiochemical factors. The main factors are: 1) soil fauna; 2) soil microorganisms; 3) roots; 4) soil organic residues; 5) inorganic binding agents; 6) environmental variables (Six et al., 2004) (Appendix 1). Tisdall and Oades (1982) proposed an aggregate hierarchy. It was proposed that free primary particles and silt-sized aggregates (<20µm) are bound together into microaggregates (20-250 µm). Subsequently, these stable microaggregates are bound together into macroaggregates (>250µm) by temporary binding agents (roots and fungal hyphae). Oades (1984) modified this theory by postulating that microaggregates can also be formed inside macroaggregates. This postulation has since then been supported and was found of crucial importance for the long term stabilization of C (Six et al., 1999).

During macroaggregate stabilization, the coarse POM (fresh plant material, >250 µm) which is incorporated within macroaggregates during biogenic formation is further decomposed by microorganisms into finer fragments. These finer fragments (fine POM) are gradually encapsulated with inorganic minerals and microbial products, forming new microaggregates within macroaggregates (Six et al., 2002). Over time, the binding agents in macroaggregates degrade, resulting in a loss of macroaggregate stability and the subsequent release of stable microaggregates, which become the building blocks of the next cycle of macroaggregate formation (Six et al., 2000) Because of the nature of the binding agents involved, microaggregates are more stable than macroaggregates, and organic matter incorporated inside microaggregates is more protected than in macroaggregates (Tisdall and Oades, 1982; Six and Jastrow, 2002).

Six et al., (2002) indicated four different SOC pools within and outside a macroaggregate:

1) Light free POM (LF) is found outside macroaggregates

2) Coarse POM (iPOM, >250µm) and 3) Fine-POM (fPOM, 53-250µm) are located inside macroaggregate but not encapsulated in microaggregates. The difference between them is that fine –POM is smaller size

4) Intra microaggregate POM (imPOM, <53µm) is located inside microaggregates (Appendix 2).

Light free POM is unprotected carbon pool because is found freely in the soils, and then is easily accessible to soil organisms. Coarse POM and fine POM are physically protected by macroaggregates as long as the macroaggregates remain stable. After the macroaggregates collapse, they become subject to decomposition. Intra microaggregate POM is the better protected carbon pool inside macroaggregates because is found inside microaggregates, which provide physically protection even after macroaggregates fall apart. Therefore, the degree of carbon protection in the soil depends on distribution of carbon among the carbon pools outside and inside macroaggregates.

The role of earthworms in relation to aggregate formation and stability have received a vast amount of scientific attention compared to other fauna groups, mainly due to their significant effects on SOC dynamics and on soil structure (Lee, 1985; Brown et al., 2000). A significant increase in the amount of large water stable macroaggregates with earthworms was demonstrated in many studies (Kettering et al., 1997; Blanchart et al., 1990, 1997; Bossuyt et al., 2004, 2005, 2006). Water stable aggregates are the aggregates which stay intact after have been wetted.

The effect of earthworms on formation of water stable aggregation is due to ingestion and mixing of organic matter with soil and the excretion of casts. In addition earthworms can form water stable aggregates though their burrowing activities. During burrowing, earthworms put pressure on the surrounding soil channel walls and excrete mucus initiating the formation of stable aggregates with clay particles (Edwards and Bohlen, 1996). However, these aggregates exhibit high stability, only if they have been dried after excretion (Marinissen, 1994).

Earthworms do not only contribute to the formation of macroaggregates but also to the formation of microaggregates. Several authors have shown the microaggregates are completely destroyed in the earthworms' guts and that new microaggregates enriched in carbon are formed (Shipitalo and Protz 1988, 1989; Barois et al., 1993). Likewise, Bossuyt et al. (2004) demonstrated the direct involvement of earthworms on the formation of new microaggregates in 12 days incubation experiment.

Furthermore, new microaggregates can be formed inside earthworm made macroaggregates (indirect effects) by similar procedure described by Six et al. (2002). The coarse POM incorporated inside large macroaggregates by earthworms can be decomposed by microbes into finer particles. These finer fragments (fine POM) can be gradually encapsulated with silt, clay particles and microbial products, to form new microaggregates. This indirect effect of earthworms depends on the composition of the macroaggregates. The higher the amount of coarse POM, silt and clay caused by earthworms, the higher the potential for the formation of new microaggregates inside macroaggregates. This indirect effect of earthworms may result in lower ratio of coarse POM over micro POM and subsequent greater physical protection of carbon inside soil aggregates.

Several mechanisms are considered to be responsible for macroaggregates and microaggregates stabilization by earthworms. Marinissen and Dexter (1990) suggested stability might be increased by the mechanical binding by vascular bundles from ingested plant material or from fungal growth in the casts. Moreover, stabilization might be a result from microorganisms which proliferate in ingested material in the gut (Parle, 1963; Arthur, 1965) and in the earthworm casts (Brown, 1995; Brown et al., 2000). The microbial derived polysaccharides deposited within casts strengthen bonds between mineral components (Shipitalo and Protz, 1989; Martin, 1991).

Not all earthworms have the same effect on soil aggregation and SOM dynamics. Earthworms which belong to different ecological categories can have a different effect (Edward and Bohlen, 1996). Bouche (1977) identified three morpho-ecological groups: 1) epigeic, 2) anecic species, 3) endogeic species. Epigeic species are defined as litter dwellers. They live on soil surface, beneath a litter layer. Their activity has little effect on soil aggregation. Anecic earthworm species live in vertical burrows in the mineral soil and transport dead leaves and other organic materials from soil surface into their burrows to feed (Lee, 1985). Their activities have an effect on soil aggregation. Endogeic species live in the mineral subsoil and feed on organic matter found in the soil. Their activities have major effects on soil aggregation and SOM stabilization (Lavelle and Spain, 2001).

Shipitalo and Protz (1989) suggested that aggregate formation and stability depend on the quality of the ingested material. Therefore, earthworm abundance and diversity are not the only factors influencing aggregate formation and stability, but some other factors are important such as interactions among different earthworm species, quality of the organic residues, environmental conditions and agricultural management.

2. Research Objectives and approach

In this research, we were interested in the effects of endogeic earthworms during short rains on (1) stable macroaggregates formation (2) composition of large macroaggregates. This was studied by means of a 20 days laboratory experiment under different organic residue management (residues with different C:N ratio) and at two soils. The conceptual model of our research is shown at figure 1 and is explained in the next paragraph.

The initial soil prior to earthworm addition was sieved and consisted of silt, clay and microaggregates (<250 µm). After 20 days, earthworms were removed and the amounts of large and small stable macroaggregates were determined (quantitative effects of earthworms by wet sieving). Subsequently, the proportion of microaggregates, silt, clay and coarse POM was determined. The arrows in the research conceptual model represent the transformation of one soil fraction into another.

Being aware that the outcomes from laboratory experiments cannot always easily be generalised to field conditions, we investigated whether in the field an inoculation experiment with similar experimental design as our main laboratory incubation experiment might be possible in the near future. In preliminary experiment, we tested the survival rates of earthworms under field conditions with different organic residue management.

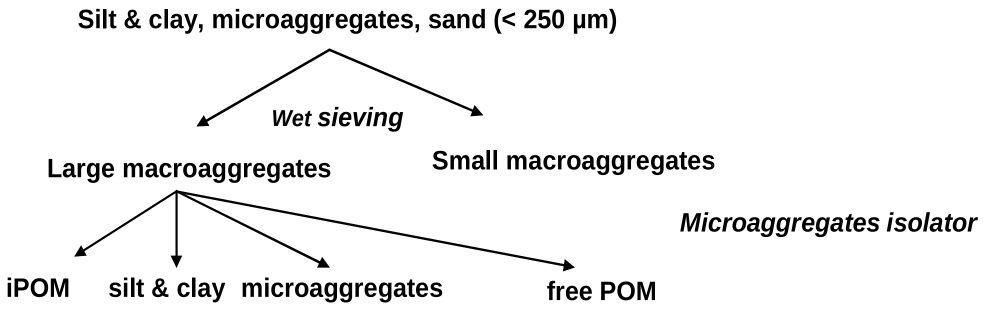

Fig 1: Research conceptual model

Incubation experiment

To investigate the effects of earthworms under different organic residue management (no residue, maize, soybean) in two soils (Embu, Chuka) on:

i) Formation of stable macroaggregates

ii) Composition of large macroaggregates (proportion of microaggregates, silt and clay, iPOM, C levels)

Field Inoculation experiment

To investigate the survival rates of earthworms under field conditions with different organic residue management.

3. Hypothesis

Incubation experiment

It was expected that earthworms would produce more stable macroaggregates, form more microaggregates-within-macroaggregates and allocate more C in those microaggregates with the addition of soybean and at higher organic soil (Chuka).

Soybean is higher quality residue (lower C: N ratio) than maize. Therefore, it was hypothised that the higher nitrogen levels in soybean would stimulate earthworms' activities resulting in higher formation of macroaggregates and microaggregates within large macroaggregates within 20 days.

Field Inoculation experiment

It was assumed that earthworms would have higher survival rates in the treatments with organic residue addition.

4. Materials and Methods

Incubation experiment

Site Description

Surface soil samples (0-10 cm) were collected from two provinces in central Kenya (Embu, Chuka). Soil sampling took place on 20th of October 2005. The first soil sampling was done in Embu experimental site where long term TSBF experiments exist. The second soil sampling carried out in a private farm owned by Rydia Karimi, in Chuka province. The soil was collected near the animal house where maize and beans were grown in rotation under banana plantations. Both soils are classified as oxisols; the textural and chemical characteristics of both soils are given in the Table 1.

Table 1: Chemical properties of Chuka and Embu soil

Location	**pH**	**Carbon (%)**	**Nitrogen (%)**	**Clay (%)**	**Sand (%)**	**Silt (%)**
Chuka	5.8	2.55	0.39	35.59	32.41	31.99
Embu	5.6	1.68	0.32	35.53	30.54	33.93

After soil collection, soils were brought to ICRAF laboratory (ICRAF is a research institute in close collaboration with TSBF). Soils were air-dried at 40 °C for 48 hours and crushed with a pestle and a mortar until all soil material passed though a 250 µm mesh (all macroaggregates were destroyed). 500 g of crushed soil was placed in each incubation plastic container (20 x 20 x 25 cm).

5 g of dry matter of either soybean or maize stover was mixed separately into the incubation containers according to their treatment. (Maize and soybean residues were collected from Embu experimental site. They were air dried for 2 days at 60 ˚C and then grinded to particle size of 250 µm - 1mm)

The soil mixtures (soil + organic residues) were brought to 45 % water holding capacity. The mixtures were equilibrated during preincubation period of 4 days at 25 ˚C (average temperature during short rains in Central Kenya) in an incubation room with controlled temperature (ICRAF laboratory).

Endogeic earthworms were introduced after the end of the preincubation period according to the following treatments.

i) Three earthworms + soybean
ii) Three earthworms + maize
iii) Three earthworms + no residue
iv) Six earthworms + soybean
v) Six earthworms + maize
vi) Six earthworms + no residues
vii) No earthworms + soybean
viii) No earthworms + maize
ix) No earthworms + no residues

Each treatment was replicated four times.

After 20 days, earthworms were removed from the containers, and the soils were air dried for 1 week at 40 ° C to allow earthworm casts to stabilize (Marinissen and Dexter, 1990).

Endogeic earthworms were collected in TSBF experimental site in Embu by shovel, near the water dam, along the water stream. The selection of earthworms for each pot was based on the total weight of the earthworms according to treatments. 2.20 grams of earthworms were added in the treatments with 3 earthworms and 4.40 grams in the treatments with 6 earthworms.

The length of this incubation experiment was related to the length of the short rains in Central Kenya. Moreover, the formulation of the treatments in this experiment was based on the agricultural management practicing by the farmers in Central Kenya. Farmers tend to incorporate 10 tonnes of crop residues (soybean, maize stover) per hectare of land prior to crop sowing date. Therefore, the 5 grams of organic residues added corresponded to 10 tonnes of residues per hectare. The soil moisture was adjusted at 45 % of water holding capacity because it was shown to be the most favourable for endogeic earthworms in preliminary incubation experiment.

The numbers of earthworms in this experiment were not chosen randomly but based on a recent research on earthworm abundance in Central Kenya under different organic residue management by Ponce-Mendoza (2005).

Aggregate separation and microaggregates isolation

After the soils were dried, the amount and composition of water stable large macroaggregate in each treatment were determined by the two steps explained below (Appendix 3).

Step 1: Wet sieving

80 grams of soil was taken out from each incubation container, and the proportion of water stable aggregates (WSA) was determined. A basin (30 cm diameter, 8 cm deep) was filled up with water until water level was approximately 1cm above a 2000 µm sieve. Soil was evenly sped on 2000 µm sieve. The 2000 µm mesh was submerged in the water for five minutes. After 5 minutes, the mesh was moved up and down the water surface at 50 repetitions during 2 min. The aggregates remained at 2000 µm mesh were collected (stable large macroaggregates). The soil material passed through 2000 µm mesh was collected in a basin and poured through a 250 µm size mesh and then wet sieving was repeated. The aggregates remained on 250 µm mesh (small macroaggregates) were collected. The soil fraction which passed through 250 µm mesh was collected in a basin and poured through a 53 µm mesh and then wet sieving was repeated. The aggregates remained on 53 µm mesh (microaggregates) were collected. Finally the silt and clay fraction was collected in a bucket, total volume was measured and a subsample of known volume was taken from analysis. All the aggregate fractions were dried at 105 ° C overnight and next day their weights were measured.

Step 2: Microaggregates isolation

5 g of large (2-10 mm) macroaggregates from Embu and 10 g from Chuka soil were taken to isolate microaggregates inside large macroaggregate according to Six et al. (2000). Macroaggregates were immersed into deionised water at the upper surface of a 250 µm mesh and gently shaken with 10 iron beads for 120 seconds. Continuous and steady water movement through the device ensured the flushing of microaggregates through the 250 µm mesh and prevent further disruption of the microaggregates by the small iron beads. After all macroaggregates were broken up, the retained sand and coarse particulate organic matter (coarse POM) on the top of the 250 µm mesh was collected. The material passed through 250 µm mesh and remained on 53 µm mesh was wet sieved to obtain stable microaggregates (Elliott, 1986). Coarse POM (> 250 µm), microaggregates within macroaggregates (250- 2000 µm), silt and clay fractions (<53 µm) were obtained by microaggregate isolator.

Chemical analysis

Soil samples from step 1 and 2 were sent to the University of Davis for carbon analysis.

Statistical Analysis

Data were analysed for statistical differences by using the SPSS statistical package. The main effects of earthworm abundance, soil types and different organic residue management, and the two and three way interactions among those three variables were tested by a three-way ANOVA.

Inoculation experiment

Soil columns (15 cm diameter, 35 cm height) were inserted in 25 cm of soil in a field plot at the TSBF-Embu experimental site. Soil was collected from a private farm in Chuka province (same farm as in incubation experiment). The soil was sieved through a 2 mm mesh. Each soil column was filled with soil until 25 cm height. 1.25 g of organic residue was incorporated into each soil column and then six earthworms were placed on the soil surface in each column. Soil columns were subject to eight treatments:

i) Earthworms + Soybean residues
ii) Earthworms + Maize residues
iii) Earthworms **+** no organic residues
iv) No earthworms + Soybean residues
v) No earthworms + Maize residues
vi) No earthworms, no organic residues

Each treatment was replicated three times. At the bottom and the top of each column a 100 µm mesh was placed in order to prevent the worms escaping. After 25 days of inoculation, the earthworms that survived were counted.

5. Results

Outline of results

Incubation experiment

First, earthworm performance (average weight and number of cocoons) is presented. Second, the amount of different soil fractions in 100 g of soil is shown (macroaggregates, microaggregates, silt, clay, free POM). Next, the amount microaggregates, silt, clay and coarse POM inside 100 g of large macroaggregates are given (composition). Finally, the total amount of silt, clay and coarse POM inside large macroaggregates in 100 g of soil are presented.

By expressing the amount of microaggregates, silt, clay and coarse POM inside 100 g of large macroaggregates we are interested in:

The effect of earthworms under different organic residue management at two soils on:

i. building structure of large macroaggregates (composition) (Fig 2)
ii. formation of new microaggregates and potential for the formation of new microaggregates over time
iii. carbon protection inside large macroaggregates

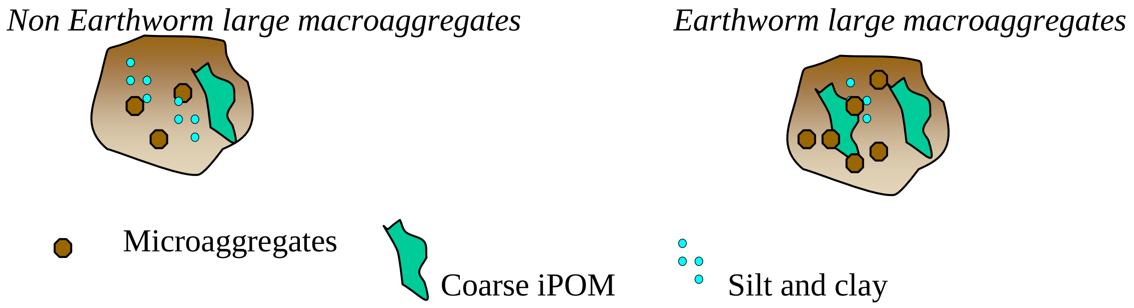

Fig 2: Visualisation of the hypothetical effects of earthworms on the building structure of a large macro aggregate (composition). It was hypothised that earthworm made large macroaggregates would contain higher fraction of microaggregates, silt, clay and coarse iPOM.

By expressing the total amount of silt, clay and coarse POM inside large macroaggregates per 100 g of soil, we are interested in:

Soil potential to sequestrate carbon

Soil potential to sequester carbon depends on amount of large macroaggregates as well as their composition. The effects of earthworm on aggregate composition can be either magnified or diluted depending on the amount of large macroaggregate formed by earthworms. Even if earthworms neutral effects on the composition of the large macroaggregate, they could still increase the potential of soil for carbon sequestration by the higher amount of macroaggregates produced in the soil. For instance, Figure 3 illustrates a case where earthworms do not have an effect on composition of large macroaggregates; however soil 2 might have higher potential for the formation of microaggregates and carbon sequestration. This is because soil 2 contains more large macroaggregates.

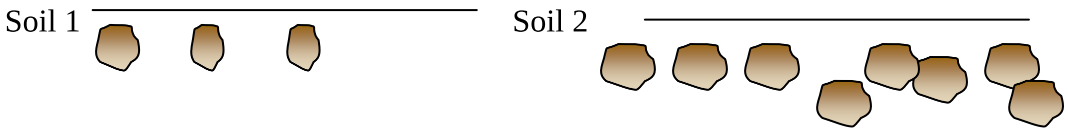

Fig 3: Visualisation of soil potential to sequestrate carbon

Inoculation experiment

Due to miscalculations on the amount of organic residues added in each PVC tube at the inoculation experiment, the results from this experiment were insufficient. For that reason, a short discussion is given in the appendix section (Appendix 4). In this discussion, a description of the results and recommendations for a better experimental set up in the future are given.

Earthworm performance

All earthworms survived in each treatment after incubation of 20 days.

Table 2: Average weight (g) of one earthworm as a function of soil type and organic residue management

	Chuka	Embu
None	0.6 a	0.6 a
Soybean	0.9 b	0.8 ab
Maize	0.9 bc	1.2 c

Note: Initial average weight of one earthworm was 0.73 g
The different letters next to the means indicate statistical difference

The average body weight of the earthworms increased with organic residue addition, whereas a decrease was observed when no organic residues were added (Table 2). The average body weight of the earthworms in the treatments with three earthworms was higher than in the treatments with six earthworms (data are not shown).

Table 3: Soil and organic management effects on the production of cocoons

	Chuka	Embu
None	0.2 a	0.6 a
Soybean	6.4 b	1.0 a
Maize	7.3 b	7.0 b

The different letters next to the means indicate statistical difference

More cocoons were produced in the treatments with organic residues compared with the no residue treatments for both soils expect in the treatment with soybean at Embu soil. Earthworms produced statistical more cocoons in Chuka soil than Embu (data are not shown).

Table 4: Effects of earthworm densities, organic residues and soils on soil fractions (g) (mean values) per 100 g of soil (n=4)

Treatments **Worms**	**Residues**	**Soils**	***Large macro** (2-8 mm)	***Small macro** (250µm-2mm)	***Micro** (53-250 µm)	***Silt & Clay** (<53 µm)	**free POM** (>250 µm)
0	None	Embu	0.91	52.29	33.55	7.48	0
	Soybean	Embu	29.98	46.47	21.16	5.03	0.25
	Maize	Embu	17.21	38.76	22.85	4.52	1.33
	None	Chuka	2.64	61.08	24.85	5.75	0
	Soybean	Chuka	17.11	44.06	28.38	6.28	1.15
	Maize	Chuka	15.38	44.14	29.80	5.27	1.40
3	None	Embu	3.52	53.58	29.65	5.12	0
	Soybean	Embu	44.38	33.45	14.23	3.55	0.96
	Maize	Embu	52.53	32.59	7.46	2.64	0.63
	None	Chuka	3.84	54.68	28.33	6.81	0
	Soybean	Chuka	20.68	40.74	27.61	6.06	0.45
	Maize	Chuka	26.03	45.18	16.22	4.19	0.31
6	None	Embu	9.98	58.60	20.47	5.09	0
	Soybean	Embu	57.27	27.50	6.75	3.84	0.06
	Maize	Embu	65.57	23.48	4.84	2.24	0.20
	None	Chuka	2.20	59.55	26.87	4.25	0
	Soybean	Chuka	36.83	39.39	20.97	4.77	0.58
	Maize	Chuka	56.21	32.10	5.62	2.01	0.09

sv	**p**	**p**	**p**	**P**	**p**
Worms	<0.05	<0.05	<0.05	<0.05	<0.05
Residues	<0.05	<0.05	<0.05	<0.05	>0.05
Soils	<0.05	<0.05	<0.05	>0.05	<0.05
Worms x Residues	**<0.05**	**<0.05**	<0.05	>0.05	>0.05
Worms x Soils	**<0.05**	>0.05	>0.05	>0.05	>0.05
Residues x Soils	<0.05	>0.05	<0.05	>0.05	>0.05
Worms x Residues x Soils	>0.05	>0.05	>0.05	>0.05	>0.05

* Water stable fractions

The p-values < 0.05 indicate a statistical significant difference

Large macroaggregates (2 - 8mm)

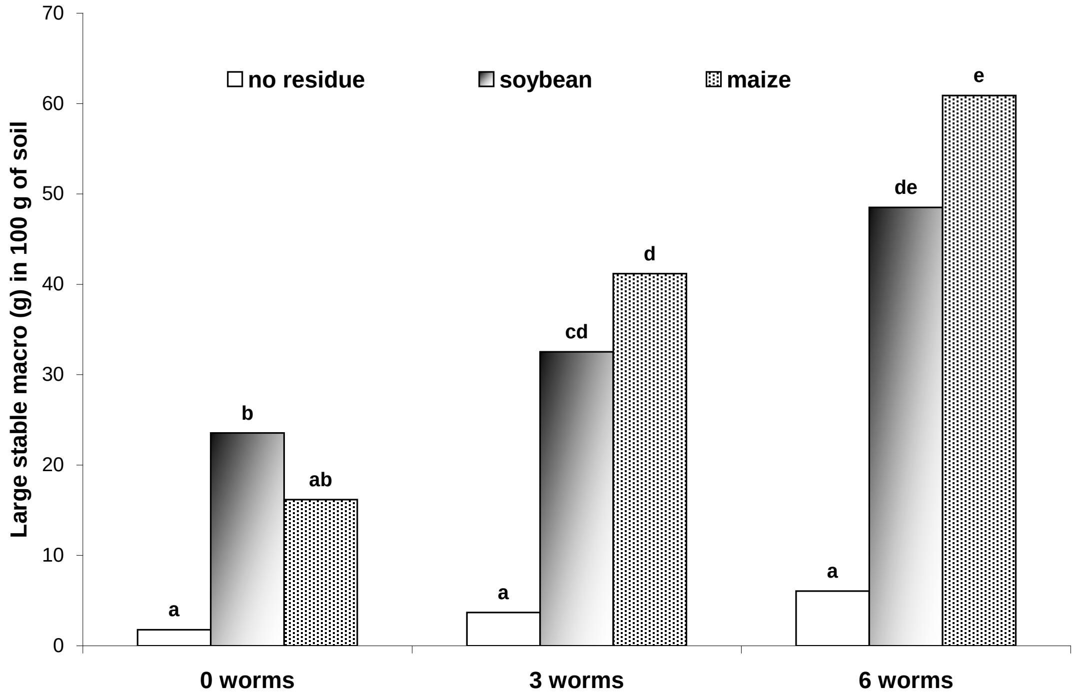

Fig 4: Effects of earthworm densities and organic residue management on the formation of large stable macroaggregates in both soils

The amount of large macroaggregates was positively related to the abundance of earthworms; however their effects were dependent on organic residues. Earthworms produced high amount of stable large macroaggregates in the treatments with organic residues. Very few stable large macroaggregates were formed by earthworms in the treatments without organic residues. The addition of organic residues resulted in the formation of large macroaggregates even in the absence of earthworms.

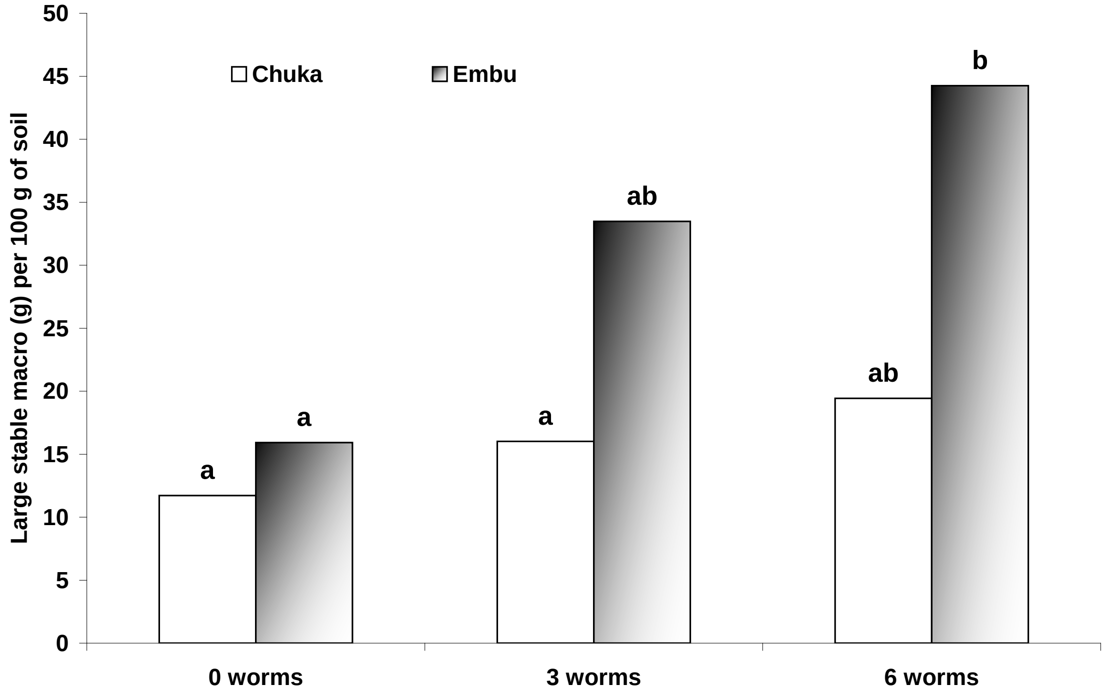

Fig 5: Effects of earthworm densities and soils on the formation of large stable macroaggregates

More stable macroaggregates were produced in the treatments with earthworms in Embu soil compared to Chuka soil. Earthworms in Embu soil produced 2.2 times more large macroaggregates than the earthworms in Chuka soil.

Small macroaggregates (250 µm – 2mm)

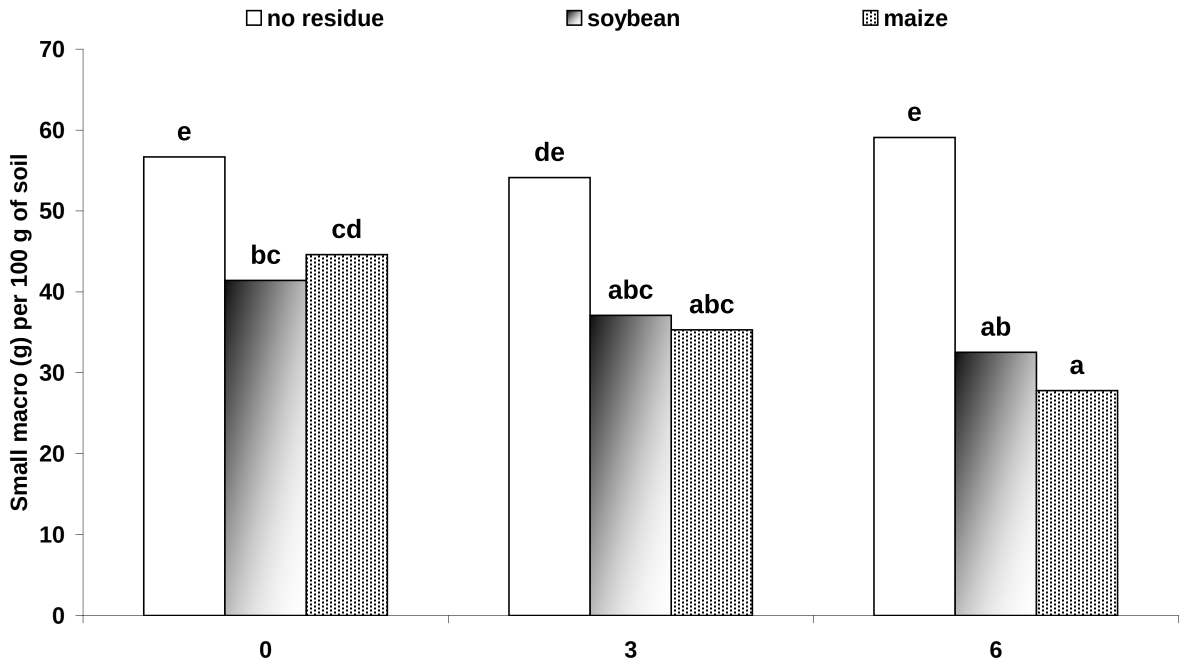

Fig 6: Effects of earthworm densities and organic residue management on the formation of small stable macroaggregates

The amount of small stable macroaggregates (250µm – 2mm) was higher in the treatments without organic residues. Different quality organic residues did not result in any difference among the earthworm treatments. Treatments without earthworms contained more small macroaggregates compared with the treatments where six earthworms were present.

Table 5: Combined effects of earthworms, residues and soils on C content (mg/ g^{-1}) in large and small macroaggregates (n=4)

Treatments **Worms**	**Residues**	**Soils**	**Large macroaggregates** (2-8 mm)	**Small macroaggregates** (250µm-2mm)
0	None	Embu	36.48	34.52
	Soybean	Embu	37.49	39.21
	Maize	Embu	36.13	38.56
	None	Chuka	35.69	35.33
	Soybean	Chuka	40.26	36.59
	Maize	Chuka	38.26	36.70
3	None	Embu	35.64	35.67
	Soybean	Embu	36.72	39.50
	Maize	Embu	36.47	37.66
	None	Chuka	36.02	35.20
	Soybean	Chuka	37.95	37.74
	Maize	Chuka	39.85	37.58
6	None	Embu	34.11	36.29
	Soybean	Embu	36.69	36.69
	Maize	Embu	36.71	34.16
	None	Chuka	37.24	34.03
	Soybean	Chuka	34.34	36.62
	Maize	Chuka	37.37	35.47

sv	**p**	**p**
Worms	**<0.05**	**<0.05**
Residues	**<0.05**	**<0.05**
Soils	**<0.05**	**<0.05**
Worms x Residues	<0.05	<0.05
Worms x Soils	>0.05	>0.05
Residues x Soils	>0.05	>0.05
Worms x Residues x Soils	<0.05	<0.05

The p-values < 0.05 indicate a statistical significant difference

Table 6: Main effects of earthworm abundance and organic residue management on Carbon content (mg/g^{-1}) in macroaggregates

	Large macro	**Small macro**
Earthworms		
0 worms	37.47 b	37.20 b
3 worms	37.07 b	36.81 b
6 worms	36.17 a	35.60 a
Organic residues		
None	35.86 a	35.17 a
Soybean	37.70 b	37.44 b
Maize	37.21 b	37.08 b

The different letters next to the means indicate statistical difference

Large and small macroaggregates produced by six earthworms contained less carbon than aggregates produced at the absence of earthworm and from the treatments with three earthworms. More carbon was found inside large and small macroaggregates in the treatments with organic residues compared to no organic residue treatments.

Composition of large macroaggregates

Table 7: Combined effects of earthworms, residues and soils on the amount of microaggregates, silt, clay and coarse iPOM (g) inside 100 g of large macroaggregates (n=4)

Treatments			***Micro**	***Silt & Clay**	**iPOM**
Worms	**Residues**	**Soils**	(53-250 µm)	(<53 µm)	(>250 µm)
0	Soybean	Embu	72.23	20.73	0.20
	Maize	Embu	64.84	30.69	0.16
	Soybean	Chuka	61.72	29.17	0.17
	Maize	Chuka	59.41	33.73	0.34
3	Soybean	Embu	61.12	25.79	0.10
	Maize	Embu	50.22	44.50	0.25
	Soybean	Chuka	59.50	33.88	0.11
	Maize	Chuka	42.21	49.72	0.26
6	Soybean	Embu	59.63	33.47	0.08
	Maize	Embu	46.26	47.90	0.18
	Soybean	Chuka	54.76	38.75	0.16
	Maize	Chuka	46.90	50.18	0.20

sv	**p**	**p**	**P**
Worms	**<0.05**	**<0.05**	**<0.05**
Residues	**<0.05**	**<0.05**	**<0.05**
Soils	**<0.05**	**<0.05**	**<0.05**
Worms x Residues	>0.05	>0.05	>0.05
Worms x Soils	>0.05	>0.05	>0.05
Residues x Soils	>0.05	>0.05	>0.05
Worms x Residues x Soils	>0.05	>0.05	<0.05

* Water stable fractions

The p-values < 0.05 indicate a statistical significant difference

Table 8: Main effect of earthworms, organic residues and soils on composition of 100 g of large macroaggregates

	Microaggregates	**Silt & Clay**	**coarse iPOM**
Earthworm densities			
0	64 b	28 a	0.22 a
3	54 a	37 b	0.17 a
6	51 a	42 c	0.15 a
Organic residue			
Maize	51 a	43 b	0.23 b
Soybean	61 b	29 a	0.13 a
Soil			
Embu	58 b	33 a	0.16 a
Chuka	54 a	38 b	0.20 b

The different letters next to the means indicate statistical difference

Earthworms had a negative effect on the formation of microaggregates within 100 g of large macroaggregates.

A higher amount of microaggregates within large macroaggregates was found at treatments with soybean compared to maize treatments as well as in Embu soils compared to Chuka soils.

Earthworms had a significant effect on the amount of silt and clay inside 100 g of large macroaggregates. Moreover, more silt and clay was found inside large macroaggregates in the treatments with maize compared to soybean treatments. Large macroaggregates from Chuka soils contained more silt and clay compared to large macroaggregates from Embu soil.

Earthworms had no effect on the iPOM within 100 g of large macroaggregates. However the quality of organic residues had a significant effect on the amount of iPOM. More iPOM was found with maize compared to soybean. In addition to organic residue quality, soil type had an effect on the amount of iPOM. More iPOM was found inside large macroaggregates in Chuka soil compared to Embu soil.

Table 9: Carbon content (mg/g^{-1}) in microaggregates, silt and clay found inside 100 g of large macroaggregates

Treatments **Worms**	 **Residues**	 **Soils**	**Microaggregates** (53-250 µm)	**Silt & Clay** (<53µm)
0	Soybean	Embu	54.30	37.17
	Maize	Embu	44.51	47.60
	Soybean	Chuka	46.48	39.09
	Maize	Chuka	58.92	39.65
3	Soybean	Embu	45.06	37.57
	Maize	Embu	52.95	38.96
	Soybean	Chuka	55.94	40.00
	Maize	Chuka	53.85	42.35
6	Soybean	Embu	47.11	40.38
	Maize	Embu	45.64	39.26
	Soybean	Chuka	47.50	38.37
	Maize	Chuka	44.00	41.85

sv	**p**	**p**
Worms	>0.05	>0.05
Residues	>0.05	<0.05
Soils	>0.05	>0.05
Worms x Residues	>0.05	>0.05
Worms x Soils	>0.05	>0.05
Residues x Soils	>0.05	>0.05
Worms x Residues x Soils	>0.05	>0.05

The p-values < 0.05 indicate a statistical significant difference

Carbon content inside microaggregates was not affected by any earthworm, residue and soil treatments.

Carbon content inside silt and clay was only affected by organic residue quality and not by earthworm densities and soils. Maize resulted in higher carbon content inside silt and clay.

Total amounts of silt, clay and coarse POM inside large macroaggregates per 100g of soil

Silt & clay within large macroaggregates per 100 g of soil

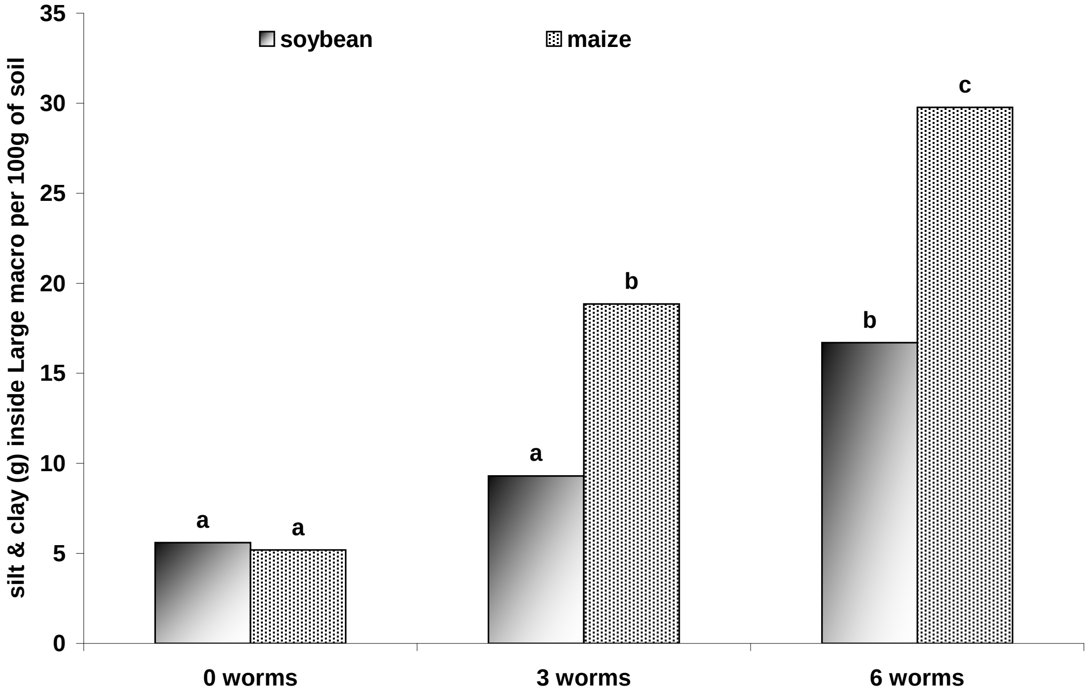

Fig 7: Effects of earthworm abundance and organic residues on the amount of silt and clay inside large stable macroaggregates per 100 g of soil

The amount of silt and clay inside the large stable macroaggregates per 100 g of soil was positively related to the higher earthworm densities. The effect of earthworms was stronger at higher earthworm densities and with maize.

Coarse iPOM inside large macroaggregates per 100g of soil

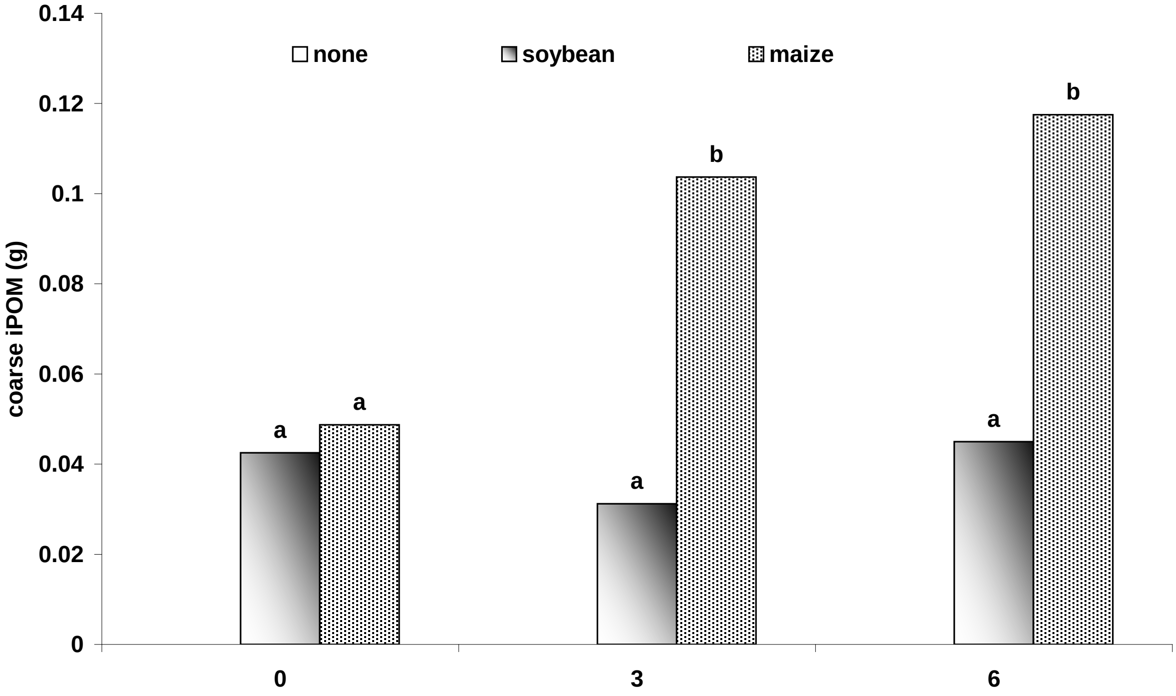

Fig 8: Effects earthworm abundance and organic residue management on the amount of coarse iPOM inside large stable macroaggregates per 100 g of soil

Higher amount of coarse iPOM was incorporated inside large macroaggregates at the treatments with earthworms and maize.

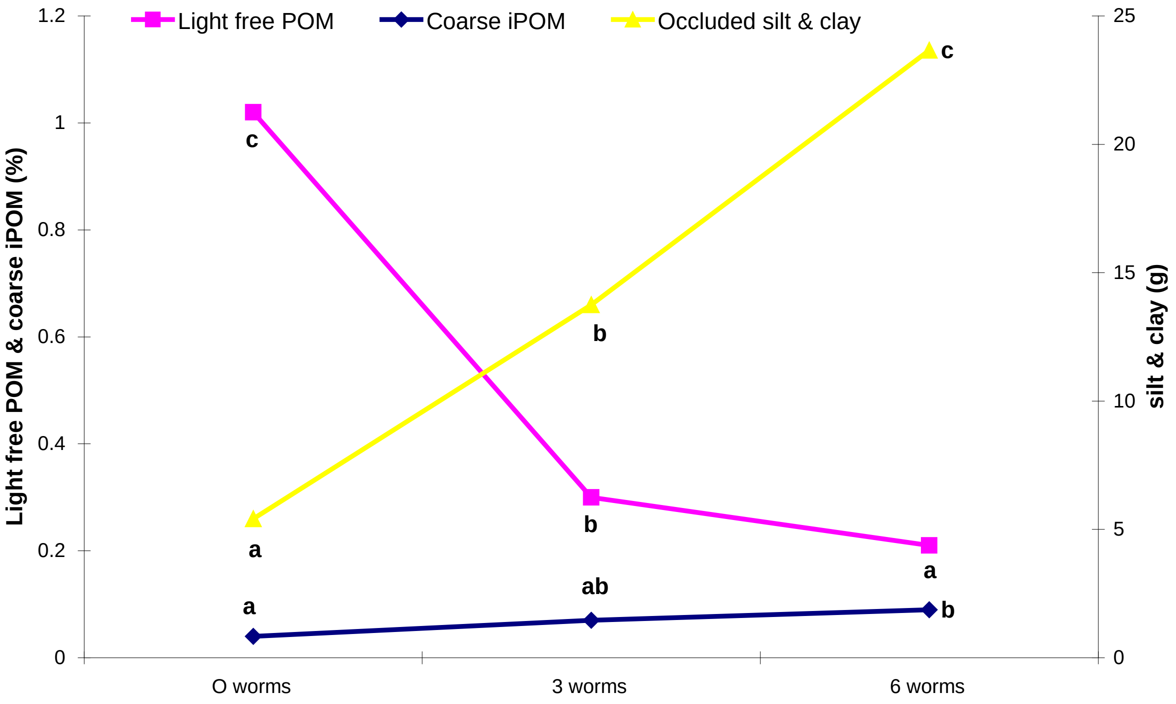

Fig 9: Effects of earthworm densities on the amount of free POM, coarse iPOM and silt and clay per 100 g of soil

The amount of free POM was negatively correlated to the presence of earthworms, while the amounts of coarse iPOM as well as the amount of silt and clay are positively correlated to the density of the earthworms.

Discussion

Large macroaggregates and earthworms

In our study, the presence of earthworms had positive effects on the formation of large stable macroaggregates. Our results came to an agreement with many other authors (Bossuyt et al., 2005 a,b; Bossuyt et al., 2004; Blanchart et al., 1996; Ketterings et al., 1997; Martin and Marinissen, 1993; Marinissen and Hillenaar, 1996). Moreover, we found a positive relationship between the amount of large water stable macroaggregates and earthworm abundance. These findings corroborated with research conducted by Abdul et al., 2003 and Ketterings et al., 1997.

Large macroaggregates and organic residues

The effect of earthworms on aggregation was found to be dependent on the organic residue management. More large macroaggregates were produced when organic residues were added and in the presence of earthworms, due to the higher earthworm feeding activities. Organic residues resulted in the formation of large macroaggregates even in the absence of earthworms, through the activity of microorganisms. In addition, our data showed a tendency of higher production of large macroaggregates by earthworms in maize treatments compared to soybean treatments. Our results did not corroborate with our hypothesis which supported higher production of large macroaggregates by earthworms with higher quality organic residues (soybean). When food supply is limited in terms of quantity and quality (C:N ratio), earthworms seem to ingest more soil in order to obtain sufficient food for their body biomass build up and their reproduction needs (Evans and Guild, 1948a) and consequently cast more frequently (Abbot and Parker, 1981; Martin, 1982).

Macroaggregates and soils

It was hypothised that Chuka soil would result in higher earthworm activities and therefore higher production of large macroaggregates due to its higher carbon content compared to Embu. Our results did not support our hypothesis. Higher amount of large stable macroaggregates were formed by earthworms in Embu than in Chuka soil. In our soil analysis, we examined pH, soil texture, carbon and nitrogen content; however we are not able to explain the higher production of macroaggregates by earthworms in less organic soil (Chuka soil). For that reason, a supplementary soil analysis which should look into many other chemical soil characteristics affecting earthworm performance such as Ca^+ content must be carried out. This supplementary soil analysis might enable us to link soil chemical properties with formation of stable large macroaggregates by earthworms.

Carbon inside large macroaggregates

In our research, large macroaggregates formed by earthworms did not contain more carbon than the large macroaggregates in the absence of earthworms. This finding contradicted with the findings from other authors, who reported a higher amount of C in earthworm cast compared to macroaggregates without earthworms (Shipitalo and Protz, 1989; Buck et al., 1999). The C enrichment in earthworms' cast is a result of their selective feeding on particles with high organic matter. In our experiment, large macroaggregates were formed even without earthworms. Microbial processes acting on plant residues were responsible for the production of those large macroaggregates (Watts et al., 2001), this could have been that reason those aggregates had the same Carbon content as macroaggregates formed by earthworms. Although, we believe that under field conditions where organic residues are not evenly mixed into the soil, the presence of earthworms might result in higher C inside large macroaggregates due to their selective feeding activities.

Earthworms' effects on macroaggregation during short rains

The duration of our incubation experiment was related to the length of the short rains in Kenya. Earthworms can only be active under wet conditions because they lack a mechanism to maintain a constant internal water content (Kretzschmar and Bruchou, 1991), so it was very important to quantify their potential impact on soil macroaggregation during the short rains. Since all macrostructure was destroyed prior to earthworm addition, we were able to investigate the rate of stable macroaggregate formation by earthworms. Within 20 days an enormous amount of stable large macroaggregates were produced by six earthworms (52 g per 100g of soil) indicating the important of role earthworms on soil structure.

Microaggregates inside large macroaggregates in the presence of earthworms

Recent studies reported higher amount of stable microaggregates (new) within large macroaggregates enriched with C in the presence of earthworms (Bossuyt et al., 2004; Pulleman et al., 2004). The carbon enrichment inside those microaggregates indicates the direct involvement of earthworms in the formation of new microaggregates. In contrast to those studies, we neither found higher amounts of stable microaggregates inside large macroaggregates, nor higher C content inside those microaggregates. Therefore, we cannot conclude that the earthworms had direct and short-term effects on the formation of stable microaggregates inside large macroaggregates.

A reason which might have masked the direct involvement of earthworms in the formation of new microaggregates in our study might be the methodology which was employed in our research. We used total C as indicator, which seem not to be very sensitive indicator for the formation of new microaggregates. Bossuyt et al., (2004) used ^{13}C labelled organic residue in order to follow the pathway of ^{13}C inside large macroaggregates. The ^{13}C labelling was used in order to detect the formation of new microaggregates. Moreover, in their study, density flotation and dispersion which are meant to differentiate the free light C pool from occluded one were used. Density flotation and dispersion are also essential tools to detect the formation of new microaggregates by tracking C inside large macroaggregate components. In our study neither ^{13}C was used, nor were density flotation and dispersion carried out. For that reason, maybe our methodology was insufficient to demonstrate the direct involvement of earthworms in the formation of microaggregates within large macroaggregates.

Indirect effects of earthworms on amount of microaggregates inside large macroaggregates

Earthworms do not only can have direct impact on the formation of microaggregates inside large macroaggregates, but also can have indirect effects due to incorporation of coarse iPOM inside large macroaggregates. The indirect effects of earthworms might be the reason for the higher amount of microaggregates inside large macroaggregates reported by several workers (Bossuyt et al., 2004; Barois et al., 1993; Shipitalo and Protz 1988, 1989). These indirect effects of earthworms appear during the decomposition of coarse iPOM by microbial activity. This coarse iPOM is decomposed by microbes inside large macroaggregates and becomes encrusted with microbial mucigel and clay particles (Six et al., 2002). Therefore, the amount of coarse iPOM, silt and clay inside large macroaggregates might indicate the potential for formation of new microaggregates inside large macroaggregates. In our study, the presence of earthworms did not result in higher amounts of iPOM inside large macroaggregates, but they increased the proportion of silt and clay.

Effect of residue quality on microaggregates inside large macroaggregates

Our data showed higher proportion of stable microaggregates inside large macroaggregates in soybean treatments compared to maize treatments. This higher proportion might be related to a lower C:N ratio in soybean and therefore to its faster decomposition rates by microbes. During the decomposition of soybean, the coarse iPOM was fragmented into finer materials by microbes. Microbes produced mucilage which glued silt and clay together with finer iPOM producing stable microaggregates within large macroaggregates. This explanation explains our findings which showed lower amount of silt and clay and iPOM inside large macroaggregates in the treatments with soybean compared to treatments with maize. Our data suggest that coarse iPOM derived from soybean resulted in faster formation rates of new microaggregates compared to maize, although the carbon content in these microaggregates were not different from other treatments.

Silt and clay inside large macroaggregates in the presence of earthworms

Earthworms had a negative effect on proportion of microaggregates inside large macroaggregates indicating that microaggregates were destroyed during the passage through earthworms' guts. The collapse of microaggregates inside earthworms' guts resulted in higher amounts of silt and clay inside large macroaggregates. As mentioned above, earthworms had no direct positive effect on the amount of stable microaggregates inside large macroaggregates. However, earthworms might result in an effect on the formation of stable microaggregates over time due to their positive effect on the amount of silt and clay inside large macroaggregates (indirect effects).

Stability of large macroaggregates and potential formation of new microaggregates

In our study, macroaggregates formed by earthworms were more stable than macroaggregates without earthworms, so it is assumed that aggregates produced by earthworms have lower turnover rates than other macroaggregates since they are more stable. Lower turnover rates of macroaggregates were positively related to the amount of new formed microaggregates inside large macroaggregates (Six et al., 2000; Denef et al., 2001). Therefore, our results propose another indirect effect of earthworms on the potential formation of new microaggregates inside large macroaggregates.

Effects of earthworms on soil carbon sequestration

The earthworms’ impact on the potential of a soil to store C depends on the amount of large macroaggregates they produce, as well as their composition. Until now, we described the qualitative effects of earthworms per 100 g of large macroaggregates, or in other words, we described their composition and the possible formation of new microaggregates inside those large macroaggregates. In the next paragraph, we will combine the effects of earthworms on the amount of stable macroaggregates with the composition of these large macroaggregates in order to discuss their effects of soil capacity to store C.

Higher earthworm densities resulted in higher occlusion of silt and clay inside large macroaggregates in soil. The effect of earthworms on the amount of silt and clay inside large macroaggregates in soil was stronger with maize. In addition, earthworms reduced the unprotected pool of carbon (free POM) in the soil and increased the pool of the coarse iPOM by incorporating POM inside large macroaggregates. This finding was in collaboration with many other researchers (Kettering et al., 1997; Lee, 1985; Parmelee et al., 1990). The simultaneous increase in coarse iPOM, silt and clay inside large macroaggregates in soil in the presence of earthworms might contribute to the formation of new microaggregates in soils and long term soil C sequestration.

Conclusion

We can conclude, as others have, that earthworms significantly affect soil aggregation and can improve soil structure even in a short period of time under appropriate environmental conditions. However, the impact of earthworms depends on the soil type and organic residue management and earthworm abundance in the soil. Regarding to formation of microaggregates within large macroaggregates, earthworms had no direct positive effect. However, the presence of earthworms resulted in higher proportion of silt and clay inside large macroaggregates. This high proportion of silt and clay is thought to help the formation of new microaggregates. Higher proportion of microaggregates within large macroaggregates derived with soybean than in maize. However, the effects of the residues on the formation of new microaggregates and the allocation of carbon inside these microaggregates need further investigation. This can be achieved by C labelled studies and the differentiation of C pools inside macroaggregates by flotation and dispersion. The indirect effect of earthworms on formation of microaggregates might appear over time, so isolation of microaggregates, flotation and dispersion must take place in regular time intervals in order to quantify exact production of new microaggregates and the C content inside these microaggregates over time. In addition, field studies must be conducted where interactions with plants, wetting/ drying cycles and with other earthworm species occur.

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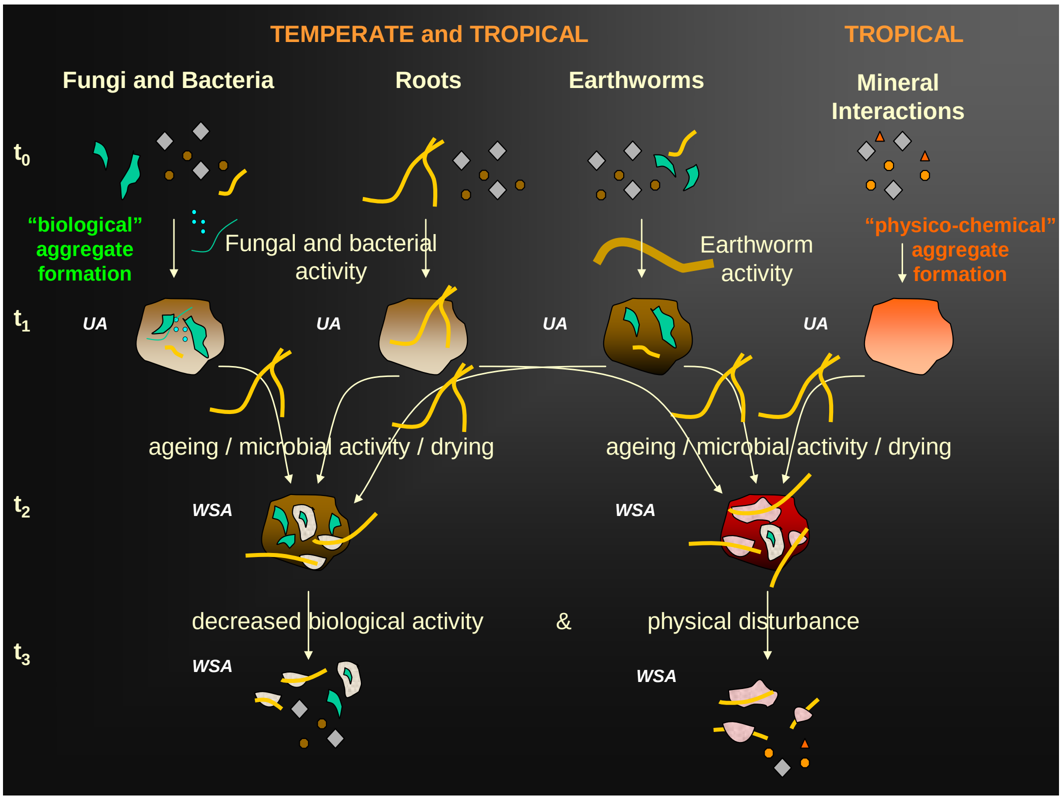
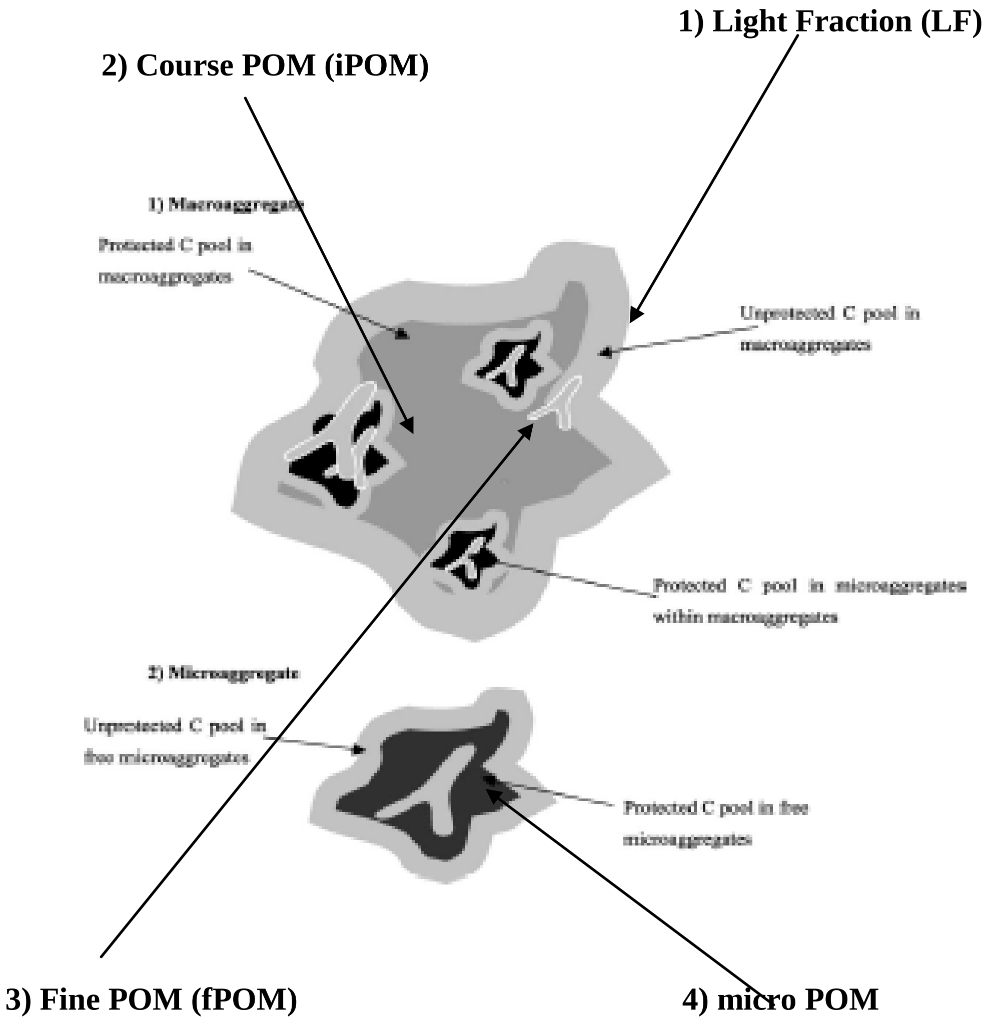
Appendix List

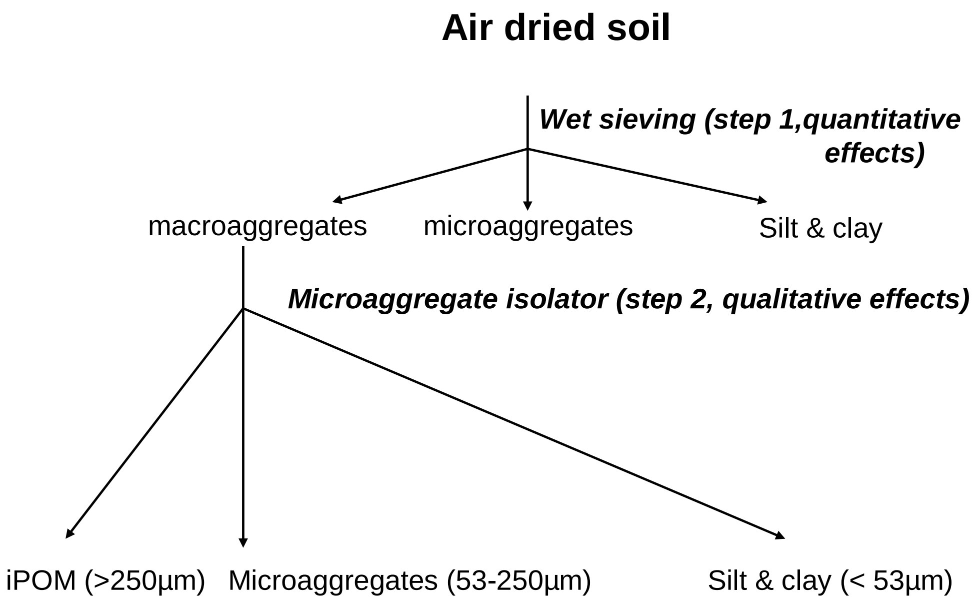
1) Aggregation formation and degradation mechanisms (Six *et al.*, 2002)



2) SOM pools within macroaggregates and microaggregates (Six et al., 2002)



3) Aggregate separation and microaggregates isolation (Six et al., 2000)



4) Inoculation Results

The amount of organic residues added to each soil column tube was insufficient to sustain the earthworms (Table 7). Instead of 5 g organic material (10 tonnes per hectare), 1.25 g (1.25 tonnes) was added. Therefore, no all earthworms have survived by the end of this experiment.

Table 7: Number of alive earthworms

Organic residue management	Earthworms	No Earthworms
None	0	-
Soybean	2	-
Maize	4	-

The values are the means from the three replicates

Higher numbers of earthworms survived with maize stover, while most of the earthworms died in soybean treatments. Earthworms did not survive in the treatments without organic residues. Higher numbers of cocoons were found in the treatments with maize compared to soybean treatments (data are not shown).

The mesh at the bottom of each PVC tube must be strong in order to stay intact during the experiment and very small (100 µm) in order to prevent earthworms to escape and other macrofauna to enter the soil columns.

Photo 1: Incubation experiment

Photo 2: Soil (< 250 µm) prior incubation experiment

Photo 3: Endogeic earthworms used for the incubation experiment

Photo 4: Earthworms in action inside an incubation container


Photo 5: Device used for microaggregate isolation (Isolator) (Six et al., 2000)