



Dominant tree species and earthworms affect soil aggregation and carbon content along a soil degradation gradient in an agricultural landscape

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ABSTRACT

Soil organic matter (SOM) is considered an important determinant of soil fertility in tropical agroecosystems. While numerous studies have shown the value of agroforestry in increasing soil nutrients and improving crop yield, few have addressed the systematic impacts of duration of cultivation on soil aggregation and C storage in such systems. A study was conducted in South Nandi (Kenya) to assess spatial influence of three dominant trees (*Croton megalocarpus*, *Eucalyptus grandis* and *Zanthoxylum gilletii*) on soil aggregation and C content in agroforestry systems. The study was conducted in a chronosequence experimental set-up where farms were continuously cultivated for 10, 16 and 62 years since conversion from primary forest. It was hypothesized that soil aggregates and whole soil and aggregate-associated C would decrease with duration of cultivation, with the magnitude of influence being reduced by the presence of trees and abundance of earthworms and termites. Greater abundance of small macroaggregates and microaggregates were recorded in soils under the canopy of *Z. gilletii* with an average weight of 62.8 g and 9.4 g 100 g⁻¹ of soil compared to 53.9 g and 3.1 g 100 g⁻¹ in soils under *C. megalocarpus* and 48.7 g and 3.9 g 100 g⁻¹ in soils under *E. grandis*, respectively. These differences could be attributed to the high number of endogeic earthworm species, *Nematogena lacuum* (Ocnoderilidae) in soils under the canopy of *Z. gilletii* trees. Since *N. lacuum* is a small-sized species (40–55 mm long), it produces small faecal pellets and thus, we could infer that this species may have contributed to the fragmentation of large macroaggregates into small macroaggregates and microaggregates. The C content decreased by almost 40% in soils under longer duration of cultivation, with higher magnitude of differences associated with *Z. gilletii* trees. Increased microbial population in earthworms' casts can increase mineralization rates, which may explain the low aggregate-associated C content under *Z. gilletii* trees where high number of *N. lacuum* were recorded. This study shows the significance of specific trees in shaping soil aggregation process and soil C content which could have far-reaching implications for the long-term C storage in the soil and hence net contributions to climate change mitigation.

1. Introduction

Soil organic matter (SOM) content and soil structural stability (ability of soil to resist disintegration associated with tillage and water or wind erosion) have been proposed as key indicators of soil quality and thus critical elements in defining sustainable land uses (Lal, 2004; Pulleman et al., 2005; Lehmann and Kleber, 2015). Soil OM is also considered an important determinant of soil fertility, and its loss has been shown to have significant negative effects in most agroecosystems (Solomon et al.,

2007). Low SOM content has been linked to decreasing crop productivity and increased soil degradation (e.g., loss of soil biodiversity and increased susceptibility to erosion) in tropical agroecosystems (Six et al., 2002; Fonte et al., 2010). Furthermore, the little or no external inputs in small-scale farming systems in Africa contribute to decreasing SOM levels (Mbau et al., 2015; Sanchez, 2019). Retention of organic materials on the soil surface, or addition of organic materials to soil to enhance SOM accrual, can be considered the most direct intervention by farm managers in the regulation of soil structure and fertility (Cobo et al., 2002; Ayuke

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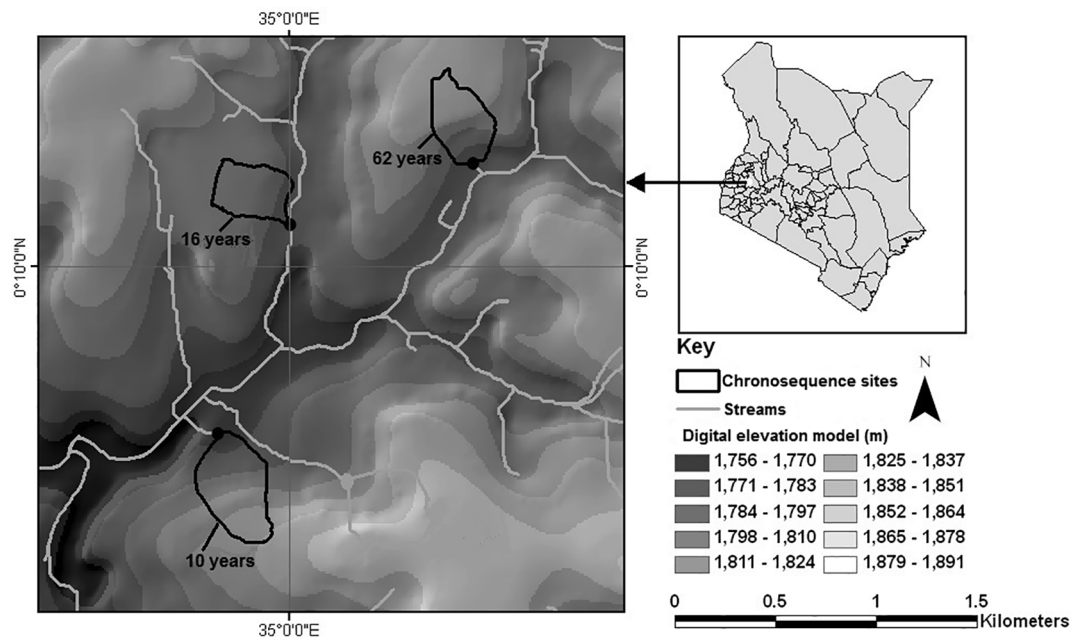


Fig. 1. Map showing the location of the chronosequence sites in Kenya where the study was conducted. The years 10, 16 and 62 represent duration of cultivation after conversion from primary forest. Adapted from Recha et al. (2013).

et al., 2011). Agroforestry is one of such interventions that is being promoted in tropical agroecosystems due to its potential to increase and sustain high levels of SOM, and hence contribute to the restoration of degraded farmlands (Barrios et al., 2012a). Integration of trees into annual cropping systems has thus, become a common practice by many small-scale farmers in Africa.

The significance of single trees creating predictable patterns of soil influence that are proportional to the canopy size has been well established (Zinke, 1962; Rhoades, 1997). For instance, soil organic C, N, P contents and exchangeable bases have been shown to decrease with increasing distance from the tree stem (Kater et al., 1992; Tomlinson et al., 1998; Jonsson et al., 1999). These patterns have been linked to the deposition of litter and woody debris under and near the trees and root turnover, which increases SOM levels that upon mineralization, release nutrients to the soil (Barrios et al., 1997; Kamau et al., 2017a). Soil OM also plays a key role in soil aggregation through binding of soil mineral components (Tisdall and Oades, 1982; Schmidt et al., 2011). However, the dynamics of SOM cannot be dissociated from soil biological activity, since SOM is the primary source of C and nutrients for soil biota and, in turn, soil biota modify soil structure through their activities (Coleman et al., 2004). For instance, earthworms and termites, which are recognized as “ecosystem engineers” (Jones et al., 1994), incorporate considerable amount of organic matter into their excretions (casts) and physically protect it from microbial breakdown (Six et al., 2004; Ayuke et al., 2011).

The spatial arrangement of trees within farms has been shown to play a key role in determining the patterns of soil macrofauna distribution (Kamau et al., 2017a) which may therefore, affect the patterns of soil aggregation and C storage under these trees. For instance, Pauli et al. (2010) reported that litter cover and earthworm casts were positively associated with spatial distribution of trees within slash-and-mulch agroforestry system in western Honduras. In addition, Fonte et al. (2010) reported higher earthworm abundance under the same agroforestry system when compared to the traditional slash-and-burn agriculture and this was attributed to the higher quality of litter deposited under the canopy of trees as well as reduction in fluctuations of moisture and temperature. Such tree effects could have played a significant role in soil aggregate distribution and C storage reported by the authors. Nonetheless, earthworms’ influence on soil aggregation is dependent on their ecological and functional attributes (Blanchart et al., 1997; Shiptalo and Le Bayon, 2004; Six et al., 2004; Pulleman et al., 2005). Endogeic and

anecic species of earthworms, for instance, may be important drivers of soil aggregation process compared to the epigeic species (Rossi, 2003; Shiptalo and Le Bayon, 2004; Six et al., 2004). Thus, it is expected that endogeic and anecic species show stronger correlation with soil structure than the epigeic species. As reported by Ayuke et al. (2011), termites may also play an important role in soil aggregation, especially in low-C soils where the activity of other soil macrofauna is low. Through their feeding and nesting habits (e.g., mound building), termites are said to move large amounts of soil, which may affect soil structure at micro and macro scales (Holt and Lepage, 2000). Though numerous studies have shown the value of agroforestry in increasing soil nutrients and crop yield, few have systematically investigated impacts of the duration of tree influence in such agroecosystems. Most of the mechanistic knowledge on the effects of soil macrofauna on soil aggregation is extrapolated from microcosm studies, and thus fails to recognize ecosystem interactions involved and their potential impact at larger scales (Rossi, 2003).

This study assessed spatial influence of three dominant trees (*Croton megalocarpus*, *Eucalyptus grandis* and *Zanthoxylum gillettii*) on soil aggregation and C content in smallholder farms of South Nandi (Kenya). The study was conducted in a chronosequence experimental set-up where farms under agroforestry were continuously cultivated for 10, 16 and 62 years since conversion from primary forest. The objectives of this study were to determine effects of duration of cultivation and tree species on (i) soil aggregate size distribution (ii) aggregate-associated soil C content and (iii) relationships between aggregate-associated soil-C and the abundance of earthworms and termites. We hypothesized that i) the weight of soil aggregates and aggregate-associated C content would decrease with increasing duration of cultivation and increasing distance from the tree trunk, ii) the abundance of earthworms and termites would increase soil aggregate stability and aggregate-associated C and this will be modulated by the tree species.

2. Materials and methods

2.1. Site description

The study was conducted in three villages: Kechire, Siksik and Koibem in Nandi County, Kenya located at Latitude 0° 10' 0" N and Longitude 35° 0' 0" E (Fig. 1). At an average altitude of about 1800 m above sea level, mean daily temperature ranges between 11 and 26 °C

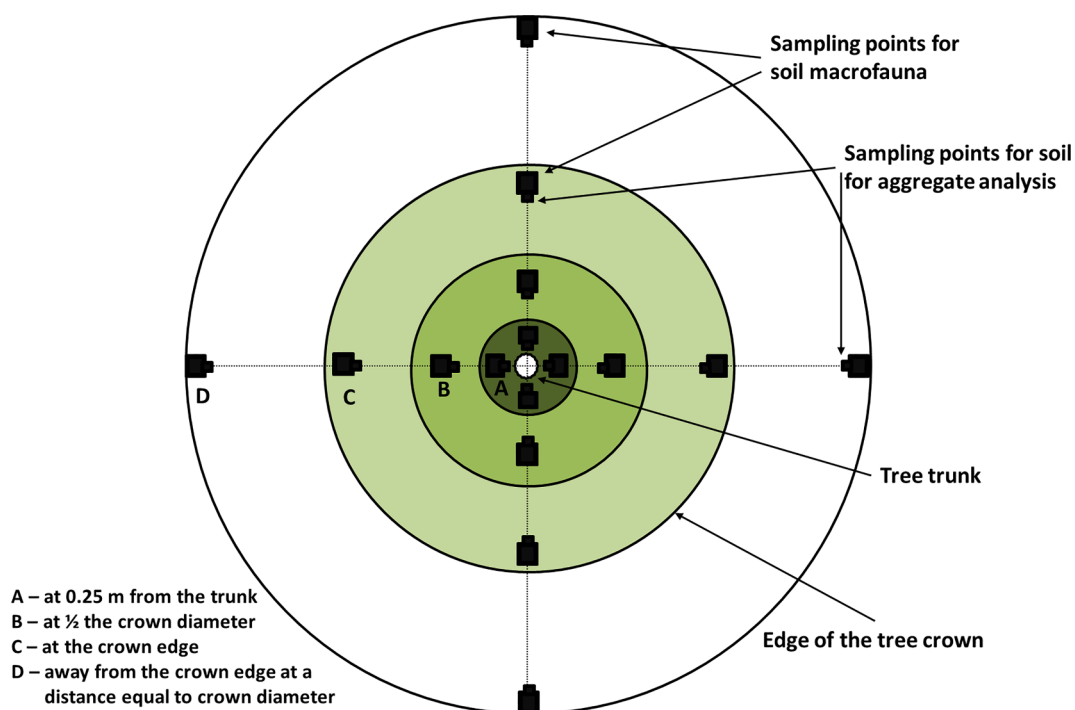


Fig. 2. Schematic representation of the sampling protocol from beneath the trees. Modified from Kamau et al. (2017a).

whereas mean annual temperature ranges between 18 and 27 °C. Annual rainfall occurs in a bimodal pattern, with an annual total of about 2000 mm; 1200 mm falls between April and June and 800 mm between August and October (Recha et al., 2013). Soils are predominantly kaolinitic Acrisols (FAO/UNESCO classification) or Ultisols (USDA classification) showing deep reddish-brown colouration and humic topsoil with 45–49% clay, 15–25% silt and 26–40% sand (Kimetu et al., 2008). The farms used in the study are found near the Kakamega-Nandi forest complex, a remnant of the greater Guinean-Congolian rainforest (Glenday, 2006). Agroforestry was established since the farms were converted from native forest, but trees are often harvested for charcoal production and other uses (Kamau et al., 2017b). Selected farms differed on the time of cultivation since conversion from indigenous forest, the longest duration of cultivation being 62 years in Kechire village, medium term 16 years in Siksik village and the youngest conversion 10 years in Koibem village. This provided a chronosequence experimental set-up where short, medium and long-term effects of land-use change, from native forest to an agroforestry system, could be systematically studied. The chronosequence sites were established after collection of extensive data, which included farmer interviews regarding land use history, supported by local records, aerial photos, sampling over 150 farms and in-depth experimentation on 70 farms as reported by Kimetu et al. (2008), Kinyangi (2008), Ngoze (2008) and Recha et al. (2013). The three chronosequence sites are located within an area of 6 km², with their sizes ranging from 9 to 14 ha. Except for the time of conversion from forest, the farms were similar in many aspects, including soil types, land use history and hydrology. Maize and beans are the major crops grown in the area, with the average farm size being less than 0.5 ha per household. Detailed description of the study site can be found in Recha et al. (2013), Güereña et al. (2015) and Kamau et al. (2017a).

2.2. Soil sampling under the tree canopies

Tree species used in the study were selected using participatory action research tools (Barrios et al., 2012b). Focus group discussions, in the context of a knowledge sharing workshop, brought together randomly selected farmers from the three villages to identify and rank the most common tree species in the study area. From the list, the three most

abundant tree species were selected for our study, namely: *Croton megalocarpus* Hutch., *Eucalyptus grandis* W. Hill and *Zanthoxylum gillettii* (De Wild.) P.G. Waterman. The following criteria, as described by Kamau et al. (2017a), were used during selection of trees to be used in the study: (i) dominance: for each species selected, at least three single trees could be located within each conversion age. Further, the trees that were selected for the study had similar attributes including age, canopy size and height. Therefore, trees in soils under longer duration of cultivation were planted, or allowed to grow from natural regeneration, long after conversion time and thus the effects cannot be entirely attributed to the duration of cultivation. Each tree species represented a treatment while each single tree acted as a replicate. Thus, a total of 27 individual trees were selected for the study (three conversion ages × three tree species per conversion age × three replicates of each tree species per conversion age); (ii) distribution: the selected trees occurred singly within the farms and the distance of separation from the selected trees to the nearby trees was at least four times their canopy diameter. This was to ensure that the selected trees were free from influence from other trees; (iii) attributes: the canopy size and age of the single trees were comparable. The trees selected were about 10 years old at the time of sampling. This information was collected during the participatory workshop as described by Kamau et al. (2017a); (iv) farm management practices: the studied trees were all located within the same smallholder maize-based cropping system, involving minimal disturbance at planting (e.g., hand hoeing) and manual weeding, across all sampling distances.

At the time of sampling, all the selected farms were under maize and beans intercrop. Except for litter deposition under the tree canopies, no organic or inorganic soil amendments were applied in these farms for at least three years before sampling was carried out. All the selected trees were left to grow naturally, with no trimming or pruning. The area around the selected trees was subdivided into four concentric zones, A, B, C and D (Fig. 2). Zone A covered the area from the base of the tree trunk to a distance of 0.25 m on all occasions, Zone B covered the area from the edge of zone A up to the middle of the tree canopy, while Zone C covered the rest of the area to the edge of the tree canopy. Zone D covered an area from the canopy edge to a distance equivalent to the canopy diameter. Therefore, zone D acted as the reference point (control). Four soil monoliths (each measuring 0.08 × 0.08 × 0.30 m –

length, width and depth, respectively) were excavated from each zone for soil aggregate analysis following transects at right angles from each other, giving a total of 16 monoliths for each tree (12 monoliths under the trees and 4 monoliths away from the tree). Common hand tools such as machetes and hoes were used in the sampling process. The sampling points were located adjacent to the monoliths sampled by Kamau et al. (2017a) to collect soil macrofauna (Fig. 2) for a total of 432 samples. Large soil clods were gently broken along the natural planes of weakness, the soil was air-dried and stored at room temperature prior aggregate analysis. After sampling, litter deposited under the canopy of each tree selected for the study was collected using a modified method by Ukonmaanaho et al. (2016). Modifications included spreading the litter trap to cover the entire area under the tree canopy instead of random placement as described by the authors. Collection of litter was done after every two days for a period of one month. A composite sample was obtained for chemical analysis (Kamau et al., 2017a).

2.3. Soil macrofauna sampling

Sampling for soil and macrofauna was conducted by excavating soil monolith ($0.25 \times 0.25 \times 0.30$ m – length, width and depth, respectively), following the Tropical Soil Biology and Fertility (TSBF) standard method described by Anderson and Ingram (1993). This was done towards the end of short rain season in November 2014 as described by Kamau et al. (2017a). All soil macrofauna seen with the naked eye were collected, counted, weighed and preserved in 75% ethanol, except for the earthworms which were previously fixed in 4% formalin for 3 days and then preserved in 75% ethanol as described by Barrios et al. (2005). The soil macrofauna were separated into broader groups i.e., earthworms, termites, ants, centipedes, millipedes, beetles and spiders, and were further identified to genera or species level where possible. However, of these seven groups, only earthworms and termites (often referred to as “ecosystem engineers” – Jones et al., 1994) were used in correlation analysis. Identification of earthworms and termites to genera and species was conducted using morpho-anatomical keys and comparison with reference collections at the Department of Invertebrate Zoology of the National Museums of Kenya. Based on morpho-ecological grouping (Bouché, 1977), earthworms were further separated into epigeic and endogeic species, but no anecic species were found. The abundance of the soil macrofauna is reported as mean individuals per square metre (individuals m^{-2}). Earthworm and termite abundance data used hereafter in the analysis, and the details on how the data was collected can be obtained from Kamau et al. (2017a).

2.4. Extraction of water stable aggregates from whole soil through wet sieving

Water-stable aggregates were determined using wet sieving method described by Elliott (1986). The soil samples were separated into four water-stable aggregate size classes: large macroaggregates (> 2000 μm ; “LM”), small macroaggregates (250–2000 μm ; “SM”), microaggregates (53–250 μm ; “m”) and silt and clay sized aggregates (< 53 μm ; “s + c”). Thirty-two grams (32 g) of air-dried soil sample were transferred into eight 2 mm sieve units held by a mechanical shaker, each sieve carrying 4 g of the soil. These sieve units were then immersed into stainless steel pans with sufficient deionized water to fully cover the sample, and left to slake for 5 min. Sieves were then moved up and down 100 times for 3 min. This process was repeated using the fractions that went through the 2 mm sieve, but now using a set of eight 250 μm sieves and finally with a set of eight 53 μm sieves. The aggregates retained on each screen size were backwashed into pre-weighed beakers. All the aggregates were oven-dried at 60 °C overnight, weighed and are reported in g per 100 g of dry soil. Each aggregate fraction was sand corrected after dispersing the aggregates with 5 g L^{-1} sodium hexametaphosphate. For meaningful interpretation of results, sand correction is necessary since it only plays a passive role in the aggregation process (Elliott et al., 1991).

2.5. Fractionation of macroaggregates

The small amount of large macroaggregates (LM) did not allow separate fractionation. Therefore, after oven-drying, the large and small macroaggregates (SM) were combined into one sample, and named thereafter as total macroaggregates (TM) prior to further fractionation as described by Six et al. (2002). A five grams subsample of total macroaggregates was placed into a ‘microaggregate isolator’ unit consisting of a transparent fiberglass tube having a 250 μm sieve at the bottom. The fiberglass tube contained enough deionized water to saturate the sample and was attached to a mechanical shaker. Thirty (30) glass beads (4 mm diameter each) were also placed into the tube to enhance aggregate sample separation into different fractions during shaking. The sample was shaken for 3 min, after which it was flushed with deionized water and the slurry poured into a 53 μm sieve inside a larger container such that all aggregates < 53 μm in diameter were collected in the container, while those that were > 53 μm were retained on the sieve. Additional deionized water was passed through the 250 μm sieve to ensure that all the fractions were flushed out into their respective sieves. This process resulted in three aggregates: coarse particulate organic matter and sand (> 250 μm ; “cPOM”), microaggregates-within-macroaggregates (53–250 μm ; “mM”) and silt and clay sized fraction within macroaggregates (< 53 μm ; “s + cM”). The aggregates retained in the 250 μm and 53 μm sieves were backwashed into pre-weighed 250 ml beakers, respectively. All the aggregates were oven-dried as described above and are reported in grams per 100 g of dry soil. Similarly, each aggregate fraction was sand corrected.

2.6. Whole soil and aggregate C analysis

About 20 mg of whole soil (WS) and of the aggregates collected at the two steps of fractionation were finely ground (0.01 mm) and weighed into aluminium foil capsules giving a total of 3024 samples (432 whole soil samples and 2592 samples from the six aggregate fractions). The ground samples were then scanned through near-infrared (NIR) spectroscopy (spectral range of 12,500–4000 cm^{-1}) using Multi-Purpose Analyzer (Bruker Optik GmbH, Ettlingen, Germany) in order to develop a soil spectral library (Shepherd and Walsh, 2007). Using this spectral library, a 10% calibration sample set (300 samples) was randomly identified. These calibration samples were analysed for total C (reported in $mg\ g^{-1}$ of soil) using FLASH 2000 NC Analyser (ThermoFisher Scientific, Cambridge, UK). Data generated from the calibration samples were used to generate a calibration curve ($r^2 = 0.85$) to predict the remaining 90% of the samples (2724 samples) using partial least-squares (PLS) regression analysis through mid-infrared (MIR) spectroscopy (spectral range of 4000 to 400 cm^{-1}) using Tensor 27 HTS-XT (Bruker Optik GmbH, Ettlingen, Germany).

2.7. Statistical analysis

Due to the complex sampling design of our study, linear mixed-effect models were used to test the effects of duration of cultivation, tree species and zone of sampling on soil aggregates and aggregate-associated C using the package lme4 (Bates et al., 2015) in R statistical software (R Core Team, 2016). Duration of cultivation, tree species and tree zone were fixed factors. However, it should be noted that the tree zones were defined relative to the canopy size rather than absolute distance from the tree trunk. Further, the duration of cultivation is not a randomly allocated treatment and other factors instead of, or in addition to, time of cultivation may be implicated in the differences observed. Nevertheless, there are no other striking differences in soils or topography among the three villages as described by Kamau et al. (2017a). All possible two-way and three-way interactions between the factors (duration of cultivation \times tree species, tree species \times tree zone and duration of cultivation \times tree species \times tree zone) were also tested in order to assess the strength of relationships between the three factors in influencing soil aggregate weight, spatial distribution, and aggregate-

associated C. Several models were built by sequentially adding factors into the simplest (null) model (Eq. (1)):

$$y_{ijkl} = \mu + \tau_{ijk} + \epsilon_{ijkl} \quad (1)$$

where y_{ijkl} is the observation from tree zone l of the k^{th} tree of species j in chronosequence i , μ is the overall mean of the trait, τ_{ijk} is the random effect for the k^{th} tree of species j in chronosequence i , and ϵ_{ijkl} is the residual random effect.

Maximum likelihood (ML) was used to estimate the model parameters and the model selection was based on Akaike Information Criterion (AIC), which aimed at finding the best choice to approximate the model parameters. Models with the lowest AIC values were chosen. Analysis of variance (ANOVA) was used to assess significant differences between the selected models. When ANOVA showed significant main or interactive effects, Tukey's post-hoc tests were performed at $\alpha = 0.05$. Data was square root-transformed when necessary to satisfy assumptions of normality and homoscedasticity. Using the base *cor* function in R, correlation analysis was conducted to determine the relationship between soil aggregates and aggregate-associated C content and earthworm and termite abundance. Though all the correlation with both epigeic and endogeic earthworms are given in the results, any discussions or conclusions drawn are based on endogeic earthworm species only since we found no significant correlation between epigeic species and the soil aggregate weight and aggregate-associated C content. Soil aggregates weight and their C content were entered as dependent variables whereas earthworms and termites abundance (reported in individuals m^{-2}) as explanatory variables.

3. Results

3.1. Chemical quality characteristics of litter and fine roots derived from the tree

Summarized litter and fine roots data from Kamau et al. (2017a) showed significant tree influence on the chemical quality characteristics (Table 1). With respect to litter characteristics, C content was higher in *E. grandis* (514.0 g kg^{-1}) compared to *C. megalocarpus* and *Z. gillettii* (471.6 g and 487.3 g kg^{-1}). Total N and P were high in the litter of *C. megalocarpus* (18.4 g and 1.1 g kg^{-1} , respectively), intermediate in *Z. gillettii* (13.5 g and 0.7 g kg^{-1}) tree litter and low in litter derived from *E. grandis* (9.1 g and 0.3 g kg^{-1}). This gave low C/N and C/P values in the litter derived from *C. megalocarpus* (26:1 and 497:1, respectively) and *Z. gillettii* (36:1 and 753:1) trees compared to *E. grandis* (58:1 and 2042:1) tree. The polyphenol content in litter obtained from *E. grandis* (13.5%) was significantly higher than that obtained from *C. megalocarpus* (6.6%) and *Z. gillettii* (9.0%). With regard to fine root characteristics, total N and P were higher in *Z. gillettii* (18.1 g and 1.9 g kg^{-1} , respectively) compared to *E. grandis* (5.5 g and 0.6 g kg^{-1}) and *C. megalocarpus* (14.0 g and 1.4 g kg^{-1}). The C/N and C/P ratios were low in *Z. gillettii* fine roots (25:1 and 242:1, respectively) compared to *C. megalocarpus* (27:1 and 355:1) and *E. grandis* (79:1 and 763:1) fine roots. Lignin and polyphenol contents were higher in *E. grandis* fine roots (25.1% and 8.2%, respectively) compared to *C. megalocarpus* (14.2% and 1.0%) and *Z. gillettii* fine roots (11.1% and 2.6%).

3.2. Effects of duration of cultivation and trees on soil aggregates

Duration of cultivation had little influence on soil aggregates obtained from the wet sieving (Fig. 3; Tables S1 and S2). Tree species had the greatest influence on soil aggregates weight and spatial distribution. The average weight of LM was significantly higher in soils under the canopy of *C. megalocarpus* and *E. grandis* trees (42.5 g and $47.0 \text{ g } 100 \text{ g}^{-1}$ soil, respectively) compared to those under *Z. gillettii* trees ($27.6 \text{ g } 100 \text{ g}^{-1}$ soil). In contrast, the average weight of SM was higher ($62.8 \text{ g } 100 \text{ g}^{-1}$ soil) in soils under the canopy of *Z. gillettii* than *C. megalocarpus* and *E. grandis* trees (53.9 g and $48.7 \text{ g } 100 \text{ g}^{-1}$ soil, respectively). Significantly higher microaggregate weight was also observed in soils under the

canopy of *Z. gillettii* with an average weight of $9.4 \text{ g } 100 \text{ g}^{-1}$ soil compared to $3.9 \text{ g } 100 \text{ g}^{-1}$ in soils under *E. grandis* and $3.1 \text{ g } 100 \text{ g}^{-1}$ in soils under *C. megalocarpus*. These increased with duration of cultivation in soils under *C. megalocarpus* and *Z. gillettii* but decreased in soils under *E. grandis* trees. When considering the effects of tree zone on soil aggregates (Fig. 4; Tables S1 and S3), only *C. megalocarpus* and *Z. gillettii* showed significant trends (tree species \times tree zone interaction). The LM weight was lower in soils under the canopy (i.e. mean values of zones A, B and C) of *C. megalocarpus* after 10 years of cultivation with an average of $42.6 \text{ g } 100 \text{ g}^{-1}$ compared to $53.2 \text{ g } 100 \text{ g}^{-1}$ away from the trees (zone D), and an average of $40.3 \text{ g } 100 \text{ g}^{-1}$ under the canopy compared to $44.4 \text{ g } 100 \text{ g}^{-1}$ away from the trees after 62 years of cultivation. In soils under the canopy of *Z. gillettii* trees, the LM weight was lower with an average of $23.2 \text{ g } 100 \text{ g}^{-1}$ compared to $33.6 \text{ g } 100 \text{ g}^{-1}$ away from the trees after 10 years of cultivation. In general, SM and m showed opposite trends to that of LM, with higher weights in soils under than away from the trees.

After fractionation of TM, all soil aggregates showed significant differences, as affected by duration of cultivation (Fig. 3; Tables S1 and S2). Generally, there was higher mM weight in soils under shorter duration ($72.3 \text{ g } 100 \text{ g}^{-1}$ soil) than longer duration ($69.7 \text{ g } 100 \text{ g}^{-1}$ soil) of cultivation. However, the weight of cPOM and s + cM showed no specific trends. Tree species also had significant effect on mM, but this depended on duration of cultivation (duration of cultivation \times tree species interaction). There was a significant decline in mM in soils under *C. megalocarpus* and *Z. gillettii* trees with increasing duration of cultivation from 77.6 g and $72.2 \text{ g } 100 \text{ g}^{-1}$ soil after 10 years of cultivation to 66.3 g and $66.1 \text{ g } 100 \text{ g}^{-1}$ soil after 62 years, respectively. Conversely, in soils under *E. grandis*, mM weight was lower after 10 years ($65.2 \text{ g } 100 \text{ g}^{-1}$ soil) than soils after 62 years ($77.2 \text{ g } 100 \text{ g}^{-1}$ soil). Based on tree zone (Fig. 4; Tables S1 and S3), only *C. megalocarpus* and *Z. gillettii* trees showed significant differences in mM weight, but this also depended on the duration of cultivation (duration of cultivation \times tree species \times tree zone interaction). The mM weight was higher ($66.3 \text{ g } 100 \text{ g}^{-1}$) in soils under *C. megalocarpus* trees than away from the trees ($62.0 \text{ g } 100 \text{ g}^{-1}$) after 62 years of cultivation. In general, differences in cPOM showed opposite trend compared to that of mM, while s + cM weight was not significantly affected by tree zone.

3.3. Effects of duration of cultivation and trees on whole soil and aggregate-associated C

Duration of cultivation had significant influence on C content of whole soil and most soil aggregates (Fig. 5; Tables S4 and S5). The C content in TM was higher in soils after 10 years of cultivation (60.0 mg g^{-1}) compared to 16 years (36.8 mg g^{-1}) and 62 years (36.6 mg g^{-1}), and this trend was similar to that of WS. Similarly, C content in m decreased with increasing duration of cultivation from 3.2 mg g^{-1} after 10 years to 1.9 mg and 2.5 mg g^{-1} after 16 and 62 years of cultivation, respectively. On average, this was close to 40% decline in C in soils under medium and longer duration of cultivation. Tree species had significant effects on C content of TM and m only. The C content in TM was higher in soils under the canopy of *C. megalocarpus* (48.2 mg g^{-1}) and *E. grandis* (47.5 mg g^{-1}) than *Z. gillettii* (39.7 mg g^{-1}). On the other hand, C content in m was higher in soils under the canopy of *Z. gillettii* (4.2 mg g^{-1}) than under *C. megalocarpus* (1.5 mg g^{-1}) and *E. grandis* (2.1 mg g^{-1}). Based on tree zone (Fig. 6; Tables S4 and S6), only *C. megalocarpus* showed identifiable trends in TM and m, though this was dependent on duration of cultivation (duration of cultivation \times tree species interaction). There was higher C in TM (64.6 mg and 45.1 mg g^{-1}) in soils under the canopy of *C. megalocarpus*, than away from the trees (59.5 mg and 36.1 mg g^{-1}) after 10 and 16 years of cultivation, respectively. Differences in C content in WS were similar to those of TM. The C in m showed significant differences after 10 years of cultivation only, with higher values in soils under *C. megalocarpus* trees (1.4 mg g^{-1}) than away from the trees (0.7 mg g^{-1}). There were no significant differences in C content in s + c.

Table 1Summary of the tree litter and fine root trait values (mean \pm SE) for the tree species considered in this study (adapted from Kamau et al. (2017a)).

Traits [†]	Part of the tree considered for each species						Summary of p-values	
	Litter			Fine roots			Litter	Root
	<i>Croton megalocarpus</i>	<i>Eucalyptus grandis</i>	<i>Zanthoxylum gillettii</i>	<i>Croton megalocarpus</i>	<i>Eucalyptus grandis</i>	<i>Zanthoxylum gillettii</i>		
C (g kg ⁻¹)	471.6 (2.4) ^c	514.0 (1.2) ^a	487.3 (4.3) ^b	434.1 (2.3)	432.5 (0.8)	441.2 (9.8)	< 0.001	0.505
N (g kg ⁻¹)	18.4 (0.8) ^a	9.1 (0.5) ^c	13.5 (0.6) ^b	14.0 (1.1) ^b	5.5 (0.3) ^c	18.1 (1.2) ^a	< 0.001	< 0.001
P (g kg ⁻¹)	1.1 (0.1) ^a	0.3 (0.1) ^c	0.7 (0.1) ^b	1.4 (0.2) ^b	0.6 (0.1) ^c	1.9 (0.1) ^a	< 0.001	0.003
K (g kg ⁻¹)	16.0 (0.9) ^a	5.5 (0.5) ^c	7.0 (1.4) ^b	7.3 (0.5)	8.4 (0.3)	9.6 (1.8)	< 0.001	0.671
Ca (g kg ⁻¹)	29.5 (1.8) ^a	13.1 (1.0) ^c	19.2 (2.2) ^b	13.3 (0.2) ^b	16.9 (0.6) ^a	10.9 (0.7) ^c	< 0.001	< 0.001
Mg (g kg ⁻¹)	5.2 (0.3) ^a	1.4 (0.1) ^c	2.9 (0.2) ^b	2.1 (0.2)	1.8 (0.1)	1.8 (0.1)	< 0.001	0.154
C/N	26.0 (1.1) ^c	58.4 (3.6) ^a	36.4 (1.2) ^b	26.8 (1.7) ^b	78.6 (1.2) ^a	25.0 (1.2) ^b	< 0.001	< 0.001
C/P	496.9 (43.0) ^c	2041.7 (167.8) ^a	753.4 (35.8) ^b	355.4 (59.0) ^b	762.9 (33.8) ^a	241.5 (9.7) ^c	< 0.001	< 0.001
L (%)	34.7 (0.6) ^a	30.9 (0.4) ^b	26.4 (2.9) ^c	14.2 (1.6) ^b	25.1 (1.1) ^a	11.1 (0.9) ^c	0.005	< 0.001
PP (%)	6.6 (0.4) ^a	13.5 (0.3) ^a	9.0 (0.4) ^b	1.0 (0.1) ^c	8.2 (0.3) ^a	2.6 (0.4) ^b	< 0.001	< 0.001
L/N	19.1 (0.8) ^b	35.0 (1.9) ^a	19.3 (1.7) ^b	8.8 (1.1) ^b	27.5 (1.1) ^a	6.4 (9.7) ^c	< 0.001	< 0.001
PP/N	3.6 (0.2) ^c	15.3 (1.0) ^a	6.7 (0.3) ^b	0.6 (0.1) ^c	9.0 (0.5) ^a	1.4 (0.2) ^b	< 0.001	< 0.001
(L + PP)/N	22.7 (0.9) ^c	50.3 (2.9) ^a	26.0 (1.7) ^b	9.4 (1.1) ^b	36.5 (1.3) ^a	7.8 (0.6) ^c	< 0.001	< 0.001

[†] C = carbon, N = nitrogen, P = phosphorous, K = potassium, Ca = calcium, Mg = magnesium, L = lignin, PP = polyphenols. Within rows of the plant part considered (litter or fine roots), values followed by different lower case letters in superscript are significantly different. All methodological and statistical details are given in Kamau et al. (2017a).

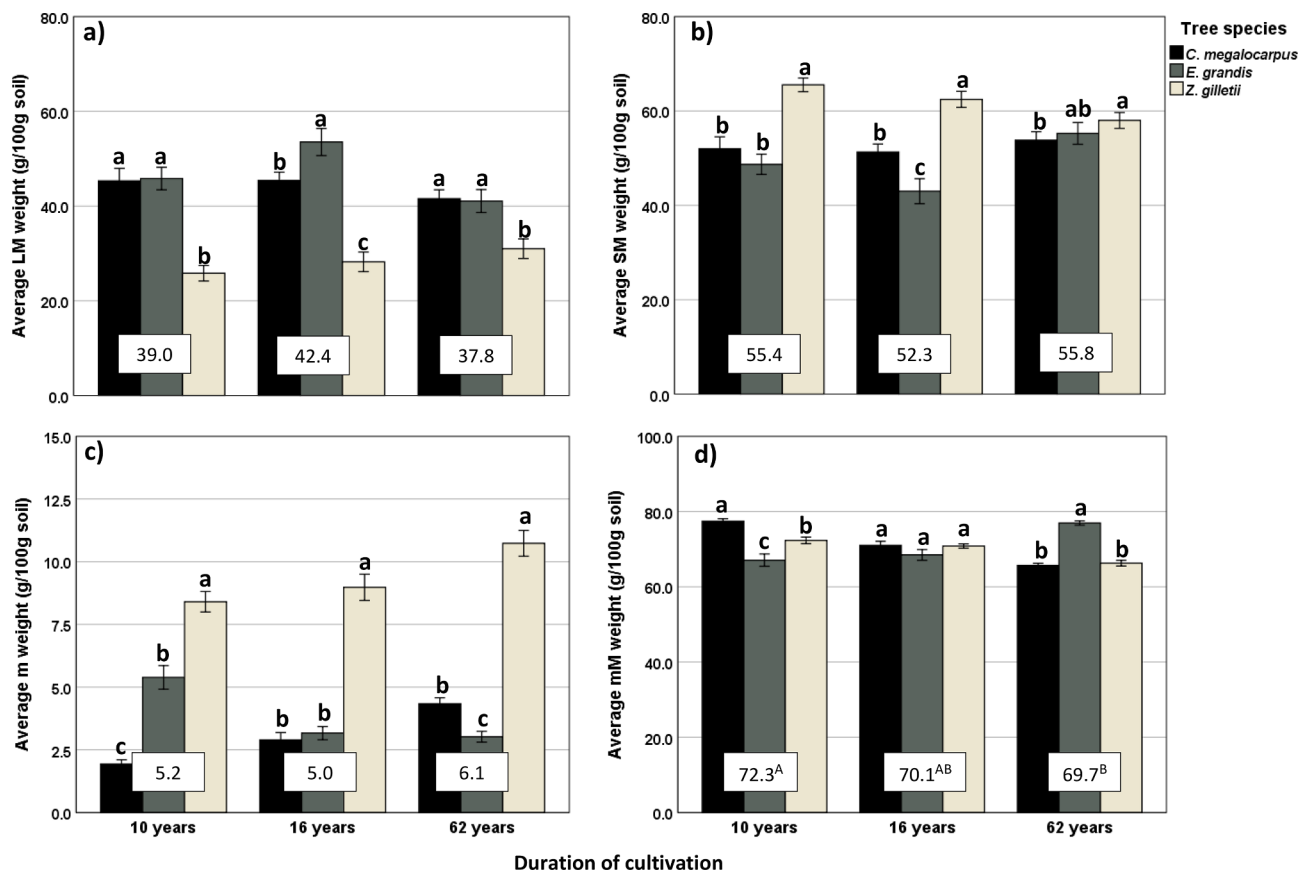


Fig. 3. Soil aggregates weight distribution as affected by duration of cultivation and tree species (means and SE); (a) LM = large macroaggregates (> 2000 μ m), (b) SM = small macroaggregates (250–2000 μ m), (c) m = microaggregates (53–250 μ m), (d) mM = microaggregates-within-macroaggregates (53–250 μ m). Values in text boxes represent means of duration of cultivation and those followed by different uppercase letters are significantly different at $p = 0.05$. Bars with different lowercase letters indicate significant differences between the three tree species for a specific duration of cultivation.

Duration of cultivation also significantly influenced C content of mM, with C decreasing from 48.7 mg g⁻¹ in soils after 10 years to 29.9 mg g⁻¹ after 62 years of cultivation (Fig. 5; Tables S4 and S5). Tree species influence on C content in mM was dependent on the duration of cultivation (duration of cultivation \times tree species interaction) as significantly higher C content in mM was observed in soils under the canopies of all tree species at shorter duration than longer duration of

cultivation (Fig. 6; Tables S4 and S6). For instance, in soils under the canopy of *C. megalocarpus*, *E. grandis* and *Z. gillettii*, the C content in mM was 54.6 mg, 46.4 mg and 45.2 mg g⁻¹, respectively, after 10 years of cultivation, compared to 31.1 mg g⁻¹ in soils under *E. grandis* and 26.1 mg g⁻¹ in soils under *Z. gillettii* after 16 years, and 27.2 mg g⁻¹ in soils under *C. megalocarpus* after 62 years of cultivation. Based on the tree zone, differences in C content of mM were more pronounced in

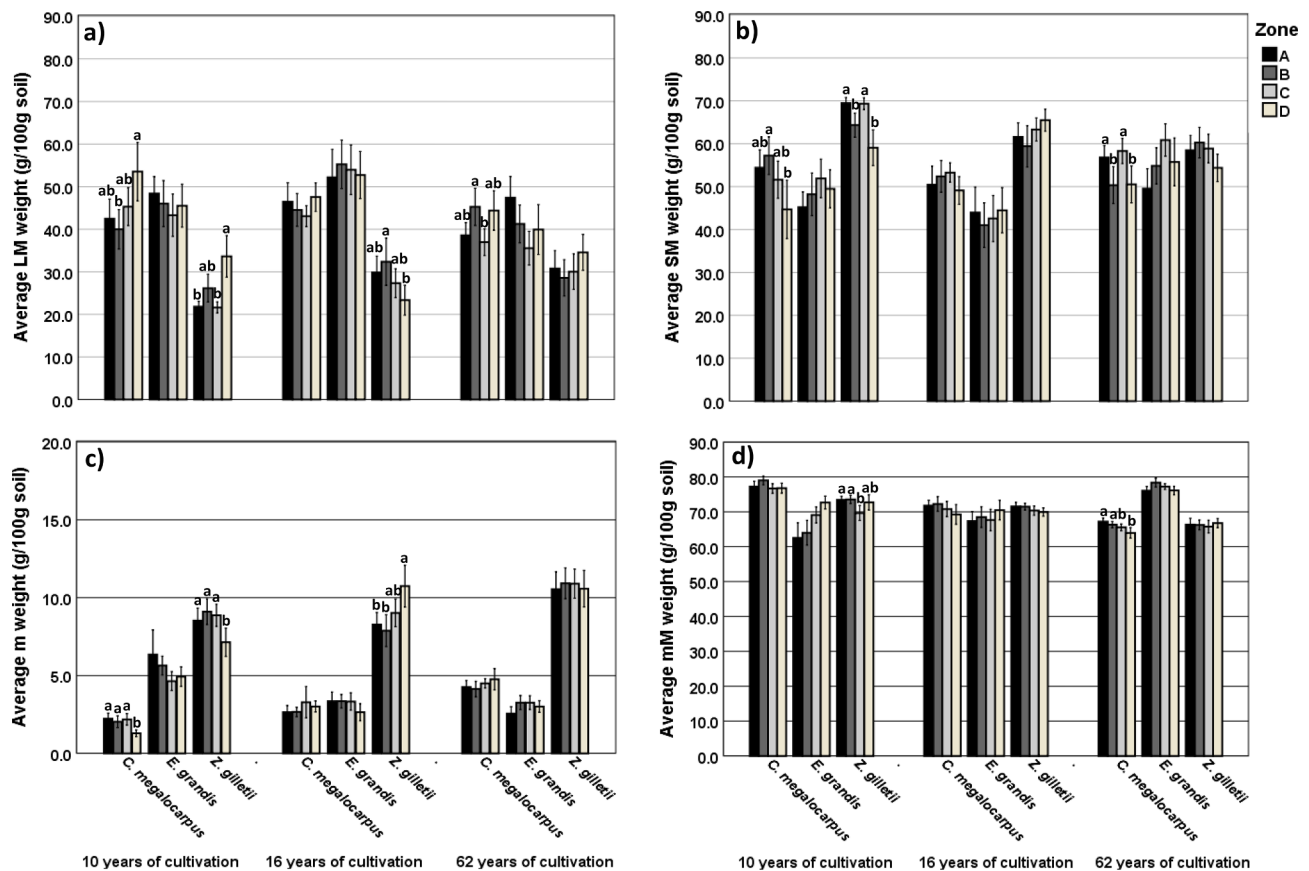


Fig. 4. Soil aggregates weight distribution as affected by duration of cultivation, tree species and tree zone (means and SE); (a) LM = large macroaggregates ($> 2000 \mu\text{m}$), (b) SM = small macroaggregates ($250\text{--}2000 \mu\text{m}$), (c) m = microaggregates ($53\text{--}250 \mu\text{m}$), (d) mM = microaggregates-within-macroaggregates ($53\text{--}250 \mu\text{m}$). Bars with different letters are significantly different at $p = 0.05$ based on tree zones.

soils under *C. megalocarpus* after 10 and 16 years of cultivation, with higher values in soils under the canopy (54.6 g and 37.4 mg g^{-1}) than away (49.3 g and 29.5 mg g^{-1}) from the trees (tree species \times tree zone interaction). Significant differences in C content in cPOM were only observed in soils after 16 years of cultivation, with higher C content recorded under the canopies of the two tree species (*C. megalocarpus* and *Z. gillettii*) than away from these trees.

3.4. Correlation of earthworms and termites abundance with whole soil and aggregate C

Correlation of endogeic earthworm species with soil aggregates strongly depended on the tree species (Table 2). Under the canopy of *C. megalocarpus* trees, earthworms correlated significantly and positively with m, cPOM and s + cM, but negatively correlated with the mM. Under the canopy of *Z. gillettii* trees, earthworms were negatively correlated with LM but positively correlated with SM and m. There was no significant correlation between earthworms and soil aggregates under the canopy of *E. grandis*. Termites and epigeic earthworm species did not show significant correlation with any of the soil aggregate.

The abundance of endogeic earthworms showed strong negative correlation with C content in whole soil (WS) and all soil aggregates, except s + c, m and s + cM in soils under the canopy of *C. megalocarpus* trees. Under the canopy of *E. grandis*, endogeic earthworms were negatively correlated with C content in WS and all soil aggregates, except s + c where there was strong positive correlation. Only s + c did not show significant correlation with endogeic earthworms in soils under the canopy of *Z. gillettii* trees. Termites and epigeic earthworm species did not show significant correlation with C content of WS and of any of the soil aggregates.

4. Discussion

4.1. Effects of duration of cultivation, trees and soil macrofauna on soil aggregation

4.1.1. Effects of duration of cultivation on soil aggregates weight and distribution

We found little influence of duration of cultivation on soil aggregates, which did not support our first hypothesis, since we expected a decrease in weight of the soil aggregates (especially for macroaggregates) with increasing duration of cultivation. Generally, it has been reported that conversion of forests to cultivated land negatively affects soil structure due to alteration in the amount and quality of litter input and the processes of organic matter stabilization in soils. For instance, Conant et al. (2004) reported that soils that had undergone long-term cultivation, following conversion from forest, negatively affected soil aggregate structure. Such changes could be linked to increased tillage operations accompanied by removal of crop residues, which are commonly practiced by farmers in our study area. These operations also increase vulnerability of soil aggregates to raindrop impact and frequency of wet-dry cycles which may cause their disruption (Balesdent et al., 2000). Deneff et al. (2001), for example, reported that subjecting soil (Aridic Paleustoll) to two dry-wet cycles significantly decreased the amount of large macroaggregates from 30 to 21% of the total soil weight. Growth of fungi, which are key in macroaggregate formation, is also negatively affected by disruptive forces in conventional farming practices (Beare et al., 1993; Six et al., 2006). The little influence of duration of cultivation on macroaggregates we found here may be an indication that trees and macrofauna may have played a more significant role in determining the observed differences in soil aggregate weight and distribution than duration of cultivation.

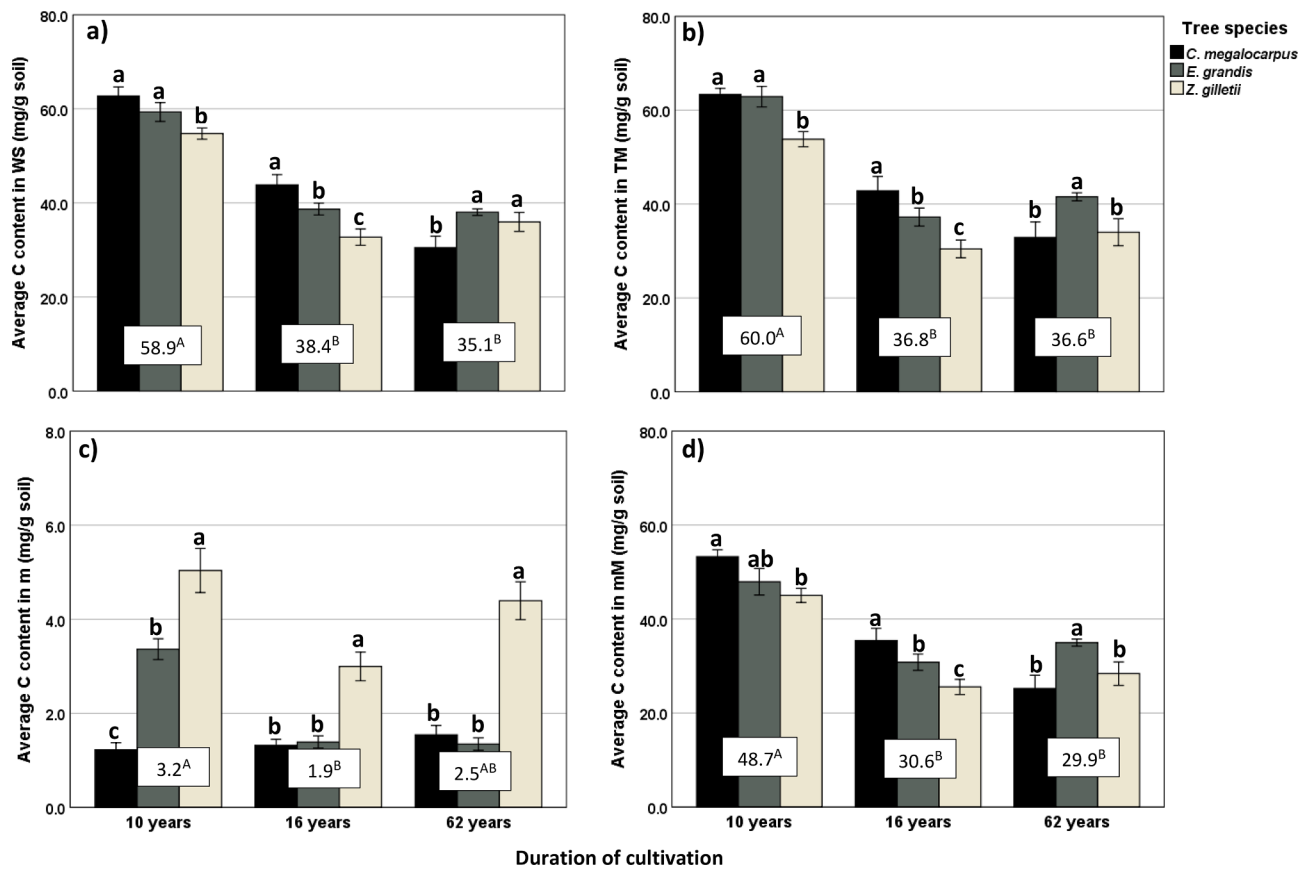


Fig. 5. Distribution of whole soil and aggregate-associated C as affected by duration of cultivation and tree species (means and SE); (a) WS = whole soil, (b) TM = total macroaggregates (> 250 μm), (c) m = microaggregates (53–250 μm), (d) mM = microaggregates-within-macroaggregates (53–250 μm). Values in text boxes represent means of duration of cultivation and those followed by different uppercase letters are significantly different at $p = 0.05$. Bars with different lowercase letters indicate significant differences between the three tree species for a specific duration of cultivation.

4.1.2. Tree species effects on soil aggregates weight and distribution

Though tree species showed the most significant effects on soil aggregate weight and distribution, the only direct tree attribute measured in our study (litter and root biomass quality) did not show consistent trends. For example, despite *C. megalocarpus* and *Z. gillettii* trees having higher litter and root biomass quality than *E. grandis*, low macroaggregates weight was only obtained under the canopy of *Z. gillettii*. Generally, decomposition rates are enhanced due to the presence of higher quality organic matter and thus, the abundance of transient (e.g., microbially-derived polysaccharides) and temporary (e.g., fungal hyphae) organic binding agents which enhance soil macroaggregation. Fungal and bacterial mucilages produced during decomposition of these organic residues further stabilize the macroaggregates formed (Oades, 1993; Six et al., 2002). Our results suggest that other factors, apart from or in addition to litter and root biomass quality, affected the observed differences in soil aggregates. For example, besides litter and root inputs, trees also intercept significant amount of incident solar radiation depending on the species and canopy size with a consequent reduction in soil temperature under the trees (Belsky et al., 1989; Vandenbeldt and Williams 1992). A reduction in temperature decreases the rate of evapotranspiration and hence a reduction of extreme moisture fluctuations. Some trees have also been shown to directly increase moisture content of surface soil through hydraulic redistribution processes (Kizito et al., 2012; Diedhiou-Sall et al., 2013). Though temperature and moisture under the tree canopies were not measured, we suggest that they could have directly or indirectly contributed to the observed differences resulting in greater influence of trees on soil aggregate weight and distribution than duration of cultivation.

4.1.3. Influence of soil macrofauna on soil aggregates

Trees have also been shown to have great influence on soil fauna

which could in turn, affect soil aggregation processes. In a previous study conducted at the same site and under the same trees, Kamau et al. (2017a) reported that the number of earthworms obtained in soils under the three tree species differed significantly, with higher abundance under *Z. gillettii* trees than *C. megalocarpus* and *E. grandis*. These differences were attributed to variations in the quality of the litter and root biomass given in Table 1. Earthworms play a critical role in initiating the process of soil aggregates formation through the following mechanisms: i) secretion of amorphous calcium carbonate or calcium humate in the earthworms' gut which act as a cementing compounds, ii) production of polysaccharides either in the earthworms' mucus or by microbes in the earthworms' gut which are reported to strengthen bonds between organic and mineral components, and iii) mechanical binding by vascular bundles from ingested plant materials, or by enmeshment from fungal hyphae that could grow after excretion of the casts (Zhang and Schrader, 1993; Shipitalo and Le Bayon, 2004; Six et al., 2004). The magnitude of earthworms' effects on soil aggregation, however, is largely dependent on their ecological categories (i.e. epigeic species which generally forage within surface litter and rarely burrow into or ingest soil, endogeic species which burrow extensively in the soil and ingest substantial amounts of mineral soil and organic matter or anecic species which live in permanent or semi-permanent burrows that can extend deep into the soil – Shipitalo and Le Bayon, 2004; Six et al., 2004; Pulleman et al., 2005) and/or functional attributes (e.g., compacting or decompacting species – Blanchart et al., 1997). While endogeic and anecic species may play a major role in soil aggregation, the epigeic species are usually weakly correlated with soil structure (Rossi, 2003; Shipitalo and Le Bayon, 2004; Six et al., 2004). Since we did not find any anecic earthworm species, we could thus attribute the observed trends to the dominant endogeic earthworm

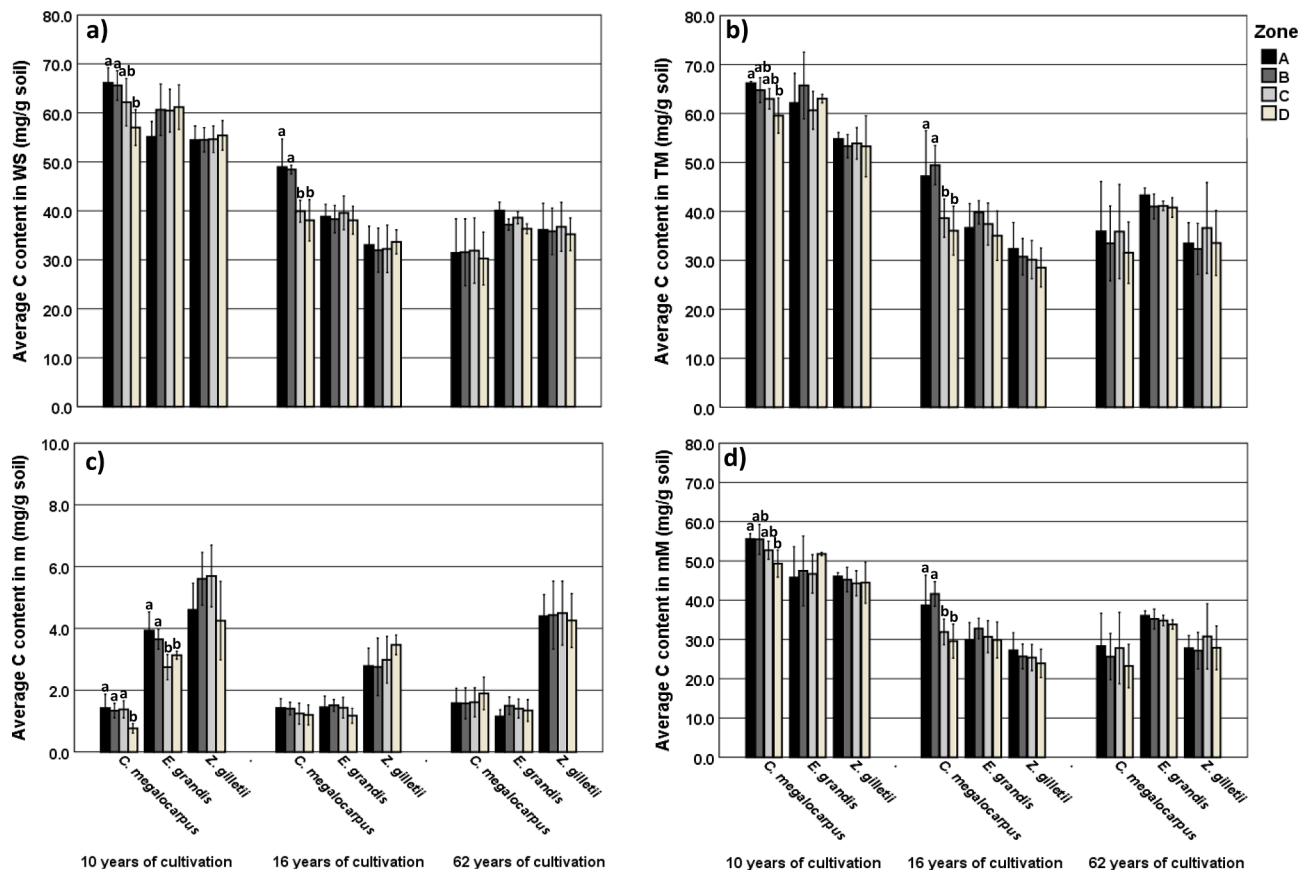


Fig. 6. Distribution of whole soil and aggregate-associated C as affected by duration of cultivation, tree species and tree zone (means and SE); (a) WS = whole soil, (b) TM = total macroaggregates (> 250 μm), (c) m = microaggregates (53–250 μm), (d) mM = microaggregates-within-macroaggregates (53–250 μm). Bars with different letters are significantly different at $p = 0.05$ based on tree zones.

species, *Nematogonia lacuum* (Ocneroдрilidae; Table S7). Previous studies have suggested mechanisms which may explain the trends we observed. An early study by Shipitalo and Protz (1989), for instance,

reported that pre-existing soil aggregates are disrupted during their passage through the earthworms' gut to form nuclei for new microaggregates. Barois et al. (1993) also observed a complete destruction of

Table 2

Correlation coefficients of the relationship between the whole soil and aggregate-associated C and earthworms and termites abundance.

Variable [†]	Tree species ^{††}								
	<i>Croton megalocarpus</i>			<i>Eucalyptus grandis</i>			<i>Zanthoxylum gillettii</i>		
	Epigeic earthworms	Endogeic earthworms	Termites	Epigeic earthworms	Endogeic earthworms	Termites	Epigeic earthworms	Endogeic earthworms	Termites
Soil aggregates									
LM	-0.12	-0.18	0.05	0.12	-0.03	0.28	-0.13	-0.36*	0.07
SM	0.12	0.15	-0.03	-0.04	0.02	-0.26	0.04	0.40*	-0.06
m	0.15	0.48**	-0.08	-0.21	0.05	-0.21	0.24	0.34*	-0.09
s + c	-0.11	0.73	0.06	0.30	0.14	-0.15	0.20	-0.17	0.09
cPOM	0.31	0.45**	0.08	-0.20	0.02	0.28	0.04	0.04	-0.23
mM	-0.25	-0.45**	-0.10	0.29	0.04	-0.27	0.14	-0.04	0.22
s + cM	0.08	0.34*	0.07	-0.15	-0.17	-0.20	-0.12	0.08	-0.02
C content in whole soil and soil aggregates									
WS	-0.20	-0.66***	-0.08	-0.27	-0.71***	-0.18	-0.14	-0.69***	0.00
TM	-0.05	-0.57**	-0.01	-0.22	-0.64***	-0.21	-0.11	-0.66***	0.08
m	0.00	0.10	-0.17	-0.23	-0.43**	-0.18	-0.03	-0.37*	0.01
s + c	0.02	-0.22	0.05	0.02	0.47*	-0.11	0.03	0.97	-0.02
cPOM	-0.30	-0.34*	0.24	-0.24	-0.59***	-0.01	-0.30	-0.59***	-0.17
mM	-0.07	-0.61***	-0.04	-0.20	-0.56***	-0.25	-0.13	-0.65***	0.10
s + cM	0.02	0.93	0.03	-0.25	-0.47**	-0.09	-0.10	-0.48**	0.06

[†] LM = large macroaggregates (> 2000 μm), SM = small macroaggregates (250–2000 μm), m = microaggregates (53–250 μm), s + c = silt and clay (< 53 μm), cPOM = coarse particulate organic matter (> 250 μm), mM = microaggregates-within-macroaggregates (53–250 μm), s + cM = silt and clay within macroaggregates (< 53 μm), WS = whole soil, TM = total macroaggregates (> 250 μm).

^{††} Coefficients marked in bold are significant: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

soil structure in the gut of earthworm species *Pontoscolex corethrurus* and then a restructuration of the soil after excretion of casts. Blanchart et al. (1999), on the other hand, reported that smaller decompacting earthworm species such as Eudrilidae feed on casts from larger species to form smaller, but more delicate casts. Given that *N. lacuum* is a small earthworm species (40–55 mm long), it produces small faecal pellets compared with larger species (Eric Blanchart, personal communication). Thus, we could infer that this species may have contributed (as a decompacting species) to fragmentation of large macroaggregates into small macroaggregates and microaggregates in soils under the canopy of *Z. gillettii*, where high numbers of this earthworm species were found. The significant negative correlation between abundance of endogeic earthworms and large macroaggregates as well as the positive correlation between abundance of endogeic earthworm and small macroaggregates and microaggregates observed in soils under *Z. gillettii* would support our assumption.

Termites are also important actors in soil aggregation by feeding on and moving large amounts of soil which may result in disintegration of large macroaggregates. Their role is especially notable in low-C soils where the activity of other soil macrofauna is relatively low (Ayuke et al., 2011). However, in our study, there were no specific changes in soil aggregation that could be associated with termites. For instance, despite the high number of termites in soils under *E. grandis* trees after 16 years of cultivation as noted by Kamau et al. (2017a), there were no unique patterns in soil aggregates under that specific tree species. These results suggest that termites did not significantly contribute to the soil aggregation data we obtained. Termites are highly mobile compared to earthworms and thus their role in aggregation could be restricted mainly to the areas near their nests or galleries and sheetings they make while gathering food.

4.2. Effects of duration of cultivation, trees and soil macrofauna on C content

4.2.1. Effects of duration of cultivation on soil aggregate-associated C content

The observed decline in C in macroaggregates, microaggregates and microaggregates-within-macroaggregates with increasing duration of cultivation confirms our first hypothesis. The fact that whole soil C declined with increasing duration of cultivation shows that it was not a redistribution of C within the various soil aggregates. This was especially notable between the youngest conversion age (10 years) and the medium term (16 years), while differences between medium term and long term conversion (62 years) were not significant. This could be attributed to a rapid decrease in C content after the conversion from primary forest to cultivated lands. For instance, in the same study site, Solomon et al. (2007) and Kimetu et al. (2008) showed that soil organic C (SOC) decreased rapidly for about 20 years after the conversion and then declined much less afterwards.

The high C content in total macroaggregates can be explained based on the concept of soil aggregate hierarchy. In this hierarchical concept, primary particles are cemented together to form microaggregates, which may then be glued together by temporary or transient organic binding agents to form macroaggregates (Jastrow et al., 1996). It is therefore, expected that the C content increases with aggregate size class, since the larger aggregates are composed of smaller aggregates and organic materials that bind them together (Tisdall and Oades, 1982; Jastrow et al., 1996). However, the C in microaggregates-within-macroaggregates (mM) has been proposed to be a more relevant indicator for diagnosis of management-induced changes on SOC levels (Six and Paustian, 2014). For instance, C in mM fraction is expected to be higher in management practices that favour retention of organic matter compared with conventional tillage (Six et al., 2002). On the other hand, soil disturbance, e.g. through tillage, may increase the rate of macroaggregates disintegration and loss of C (Six et al., 2004). In our study, the observed C decrease in macroaggregates and microaggregates within-macroaggregates with increasing duration of cultivation could be an indication

that SOM that was once protected inside of macroaggregates may have been lost to decay with time. This trend is expected since the study farms have been under continuous cultivation for over 10 years with little external C inputs (Kamau et al., 2017a). Our results are in agreement with similar studies which have reported loss of soil C following conversion of primary forest to agriculture (e.g., Solomon et al., 2007; Kimetu et al., 2008; Fonte et al., 2010). Agroforestry would therefore be a valuable farm management practice to restore and reclaim degraded soils through increasing C inputs as generally cited (Lal, 2004; Lamb et al., 2005). Thus, the differences in aggregate C associated with each tree species suggest that certain tree species may play an important role as 'resource islands' especially in highly degraded soils as reported by Kamau et al. (2017a). Nonetheless, the decreasing aggregate-associated C with increasing duration of cultivation is an indication that the amount of litter deposited under tree canopies and the root turnover was not enough to compensate for the C lost over the years, especially in the older farms. This could be due to the fact that, though agroforestry was established since the farms were converted from native forest, trees are often harvested for charcoal production, for timber, firewood, or for sale to supplement household income (Kamau et al., 2017b). Therefore, the C that was previously protected in macroaggregates under the trees was probably mineralized and lost from the soil after such trees were harvested.

4.2.2. Trees species effects on soil aggregate-associated C content

The decrease in aggregate-associated C with increasing distance from the tree trunk confirms our first hypothesis, and concurs with some studies which have assessed single tree effects on soil properties. For instance, an early study by Zinke (1962) showed that single trees may generate characteristic concentric rings of influence that are proportional to the size of the tree canopy. Kater et al. (1992), Tomlinson et al. (1998) and Jonsson et al. (1999), all reported a gradual decline in organic carbon content with increasing distance from the tree trunk, which they attributed to the differences in litter deposition and root turnover. Though none of these studies looked at effects of the trees on soil aggregation process, they provide insights that we could use to explain the observed trends in our study. Thus, we could attribute the decreasing aggregate-associated C with increasing distance from the tree trunk to the litter deposition and root turnover. Nonetheless, C content differed between the three tree species, which may reflect specific patterns linked to individual species, and therefore the quantity and quality of the litter and root biomass. For instance, the higher C content in most of the aggregate size classes under *C. megalocarpus* and *E. grandis* trees than under *Z. gillettii* tree could be attributed to higher amounts of litter deposited under these two trees as described by Kamau et al. (2017a). Further, the fine roots derived from *Z. gillettii* tree showed lower C/N and C/P ratios and lignin content than those from the other tree species, an indicator that they were of higher quality. Enhanced decomposition and root turnover rates due to the presence of quality organic matter may increase the abundance of less persistent (transient and temporary) C-based substrates which are prone to mineralization over short durations. This could also explain the lower aggregate-associated C under *Z. gillettii* trees relative to the other two tree species.

4.2.3. Effects of soil macrofauna abundance on soil aggregate-associated C content

Apart from the direct effects of trees, earthworms had significant influence in aggregate-associated C. Despite lack of direct evidence, we suggest that the abundance of *N. lacuum* could be linked to the observed decrease in C content in almost all soil aggregates under *Z. gillettii* trees. As noted previously, *N. lacuum* could have fragmented large macroaggregates to small macroaggregates and microaggregates in the form of casts. Freshly deposited casts are known to contain substantial amounts of partially digested organic matter, thus providing substrates for proliferation of microorganisms. Such increase in microbial population may increase C mineralisation rates and thus a reduction in overall C content over time. For instance, Burtelow et al. (1998) reported increased

fractions of active and readily-mineralizable C in soil patches with epi-endogeic earthworms (*Amyntas hawayanus*) compared with those lacking earthworms, which led to a 36% decrease in organic matter content. On the other hand, Alban and Berry (1994) reported an average decrease of about 0.6 Mg C ha⁻¹ year⁻¹ up to a depth of 0.5 m over a 14-year period after colonization of a deciduous forest by endogeic (*Aporrectodea tuberculata*) and epi-endogeic (*Lumbricus rubellus*) earthworm species. Although these studies looked at soil C in general and not in soil aggregates, they nonetheless point to the possibility of C losses induced by earthworm activities. In addition, after passage through the earthworm's gut, physical and biochemical processes after excretion of casts could also affect the stability of the new cast-derived microaggregates (Shipitalo and Le Bayon, 2004), thus further determining the fate of the microaggregates C. However, as Brown et al. (2000) noted, it is unlikely that these losses continue indefinitely, but rather until a new equilibrium is attained once the earthworm population is stabilized. Given that the litter and root biomass from trees is a major source of C in low input agroforestry systems, the interactions between organic inputs and earthworms' species as shaped by specific tree functional traits could be instrumental in soil aggregation process, and thus have far-reaching implications for long-term soil C storage. Thus, our results shows that the choice of tree species in an agroforestry system can significantly influence the amount of SOC either directly or indirectly through the mediation of soil biota. The management of agricultural landscapes using agroforestry therefore, becomes increasingly valuable in restoration of soil health through increased soil C storage, especially in the tropics where mitigation and adaptation to climate change are ideally closely linked due to the critical importance of soil C for crop productivity.

5. Conclusion

The observed low influence of duration of cultivation on macro-aggregates was not expected. However, the significant positive effect of tree species on microaggregates, especially notable under *Z. gillettii* tree canopies, could have been brought about by the higher abundance of the endogeic earthworm species *Nematogenia lacuum*. The general decline in aggregate-associated C along the chronosequence could also be explained by stimulated mineralization by microbes through the production of casts by this earthworm species. Thus, our study suggests that selection of tree species for an agroforestry system can be critical in shaping the soil aggregation process and soil C accrual which could have far-reaching implications for long-term C storage in the soil. Further research is required especially to understand the ecological mechanisms behind the reduction in microaggregate-C due to the presence of *N. lacuum* given its dominance in some tropical soils.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2019.113983>.

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