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Chicken broiler photo by Abhilash Hatcheries

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Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

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Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

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Determinants of rice by-products utilization as feed and their management in Ethiopia: the case of Fogera District

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Abstract. *Asmare B, Yayeh Z. 2018. Determinants of rice by-products utilization as feed and their management in Ethiopia: the case of Fogera District. Asian J Agric 2: 1-7.* This study was conducted with the objective to determine the status of farmers' rice by-product utilization as feed-in Fogera district, northwestern Ethiopia. Eighty smallholder farmers were selected based on livestock population and rice production access. A semi-structured questionnaire was administered to selected farmers systematically. The collected data was analyzed using SPSS descriptive statistical analysis. The results indicated that most respondents in the study area were within the age range of 31 to 50 years (55%). Education status of respondents showed that most household heads were able to read and write (27.5%), followed by elementary school (26.25%), and high school (17.5%) completed. The main dry season feed in most households (46.25%) was found to be grazing and crop residues. The majority (64.62%) of farmers used rice by-products as livestock feed, followed by both feed and house construction. Types of by-products used for animal feed by respondents elucidated majority (61.5%) used rice straw, with a proportion (26.15%) rice bran and (12.30%) used both straw and bran for animal feed based on availability. The sources of rice by-products indicated for many of respondents (66.7%) was farm produced, 18.5% use purchased and 18.4% get rice by-products both from farm produced and purchasing for their animal feed. Type of animals fed rice by-products included cattle in the case of majority of respondents (49.23%), cattle and equine followed (24.62%), and all animals (13.85%), respectively. In livestock owners, rice by-products are fed to livestock as sole feed and mixed with other feeds, however, the majority (69.2%) of households provide sole followed by both sole and mixed with other feeds (16.9%). Many of the respondents (55%) apply drying followed by addition of saltwater (22.5%) as method of improving the rice by-products. The main constraints observed in rice by-products utilization as feed were seasonal deficiency of by-products (25.5%) followed by lack of awareness (16.25%). Though these problems prevailed in the study area, rice by-products are being used as a major crop residue feed with little or no improvement applied. Generally, it can be included that rice by-products were found very important feed resources in the rice-dominated farming system of Fogera district. To exploit these products properly, farmers need to be assisted through proper agricultural extension service and supply in the form of credit service. Moreover, further detailed on-farm experimentation should be done to demonstrate better utilization of the products as livestock feed.

Keywords: Crop-livestock, feed, rice by-products, rice straw, rice bran

INTRODUCTION

The economy of Ethiopia is largely dependent on agriculture which contributes about 43.2% of the country's Gross Domestic Product (GDP), and about 85% of the population is engaged in it (CSA 2016). In the country, crops are grown for one or more main products such as food grain and by-products. The by-products such as straws and stubble grazing are leftovers after harvesting, and after processing are 'by-products' of the main crop (Adri Vink 2015). Feed shortage, both in terms of quantity and quality, is a major problem hindering the development of livestock industry in Ethiopia (Ahmed et al. 2010; Solomon et al. 2010). The common feed resources available in the high and mid-lands of Ethiopia are mainly natural pasture, crop residues and stubbles (Mesay et al. 2013; Zewdie and Yoseph 2014), and natural grazing land is the predominant feed source in lowlands (Malede and Takele 2014). In the integrated crop-livestock production systems, crop residues are the main feed resources. Fallow lands, forest, and shrublands are also the feed resources in different agro-ecologies of Ethiopia (Ahmed et al. 2010). The factors contributing to this deficit in dry matter (DM) supply are fast deterioration of the natural grazing land associated with a rise in crop cultivation, overstocking, and recurrent droughts.

In Ethiopia, the tendency of allocating natural grazing lands for crop cultivation has been increased to satisfy the grain production needs of rapidly increasing human population. One alternative feed resource is crop residue, especially cereal residue. Residues of cereals and pulses account for about 26% of the total feed utilized and ranked second to grazing (64%) in mixed crop-livestock production system of Ethiopia (CSA 2016). With an increase in human population, more land is used to crop production, and only fragments of marginal lands will be left for forage production in Ethiopia. Consequently, ruminants feed largely on crop residues particularly on cereal straws as their basal diet (Fekede et al. 2011; Dawit et al. 2013).

Table 1. Rice Production, No of HHs and area coverage (2009-2015) in Fogera District, Ethiopia.

Production year	Land coverage	No of kebele producing	No of households		
			Male	Female	Total
2009/10	9256	23	11026	1111	12137
2010/11	11146	26	12354	1725	14079
2011/12	15119	26	17094	1325	18419
2012/13	16070	27	20240	1325	21565
2013/14	19310	-	-	-	-
2014/15	19334	-	-	-	-
2015/16	20896	-	-	-	-

Source: Fogera District Office of Agriculture (2017)

Cereal crops, like rice, are being expanded in Ethiopia which helps in alleviating livestock feed shortage. Rice has become a highly strategic and priority commodity for food security in Africa (Table 1). The discovery of wild rice in the Fogera plain in the early 1970s, was the basis for rice introduction in Fogera district as well as in the Amhara region. Since then, Fogera district is regarded as one of major rice production areas of Ethiopia. In Fogera, potentially about 35,300 smallholders are engaged in rice production with average land size of 0.58 ha per household with a potential production of 85,990 tons of rice. The existing land coverage for rice production is above 20,000 hectares and rice is known as “white gold”.

The land size for rice cultivation has increased and there is a tendency to change the style of production from other crops to rice by most of the farmers settling on Fogera plains. The primary importance of any crop residue management system is to maximize the economic benefit from the waste resource and maintain acceptable environmental standards. Hence, proper use of crop residues including rice by-products could help to mitigate the problem of livestock feed in the highlands of Ethiopia. However, no organized study conducted on the farmers’ perception about the utilization has been done in the area. Specifically, farmers awareness about the use of such by-products traditionally and there is little or no knowledge on the status of utilization and the prevailing constraints in the study area. Therefore, this assessment was done to determine the status of rice by-product utilization and to identify constraints available in the study area.

MATERIALS AND METHODS

Description of study area

Fogera district is one of the districts of Amhara National Regional State and is found in South Gondar Zone, Ethiopia. It is situated at 110 58 N latitude and 370 41 E longitude. The district is bordered by LiboKemkem district in the north, Dera district in the south, Lake Tana in the west and Farta district in the east. The altitude ranges from 1774 up to 2410 masl allowing a favorable opportunity for wider crop production and better livestock rearing (IPMS 2005).

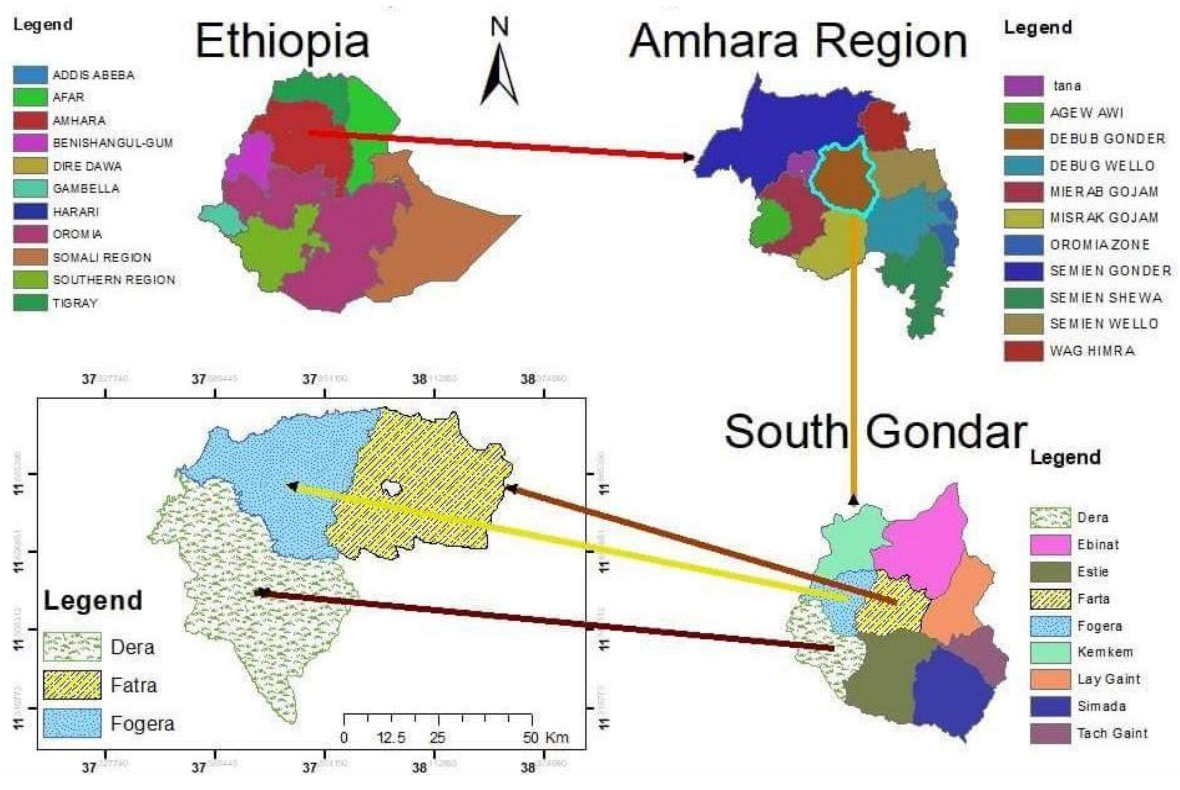

Figure 1. Map of study area in Fogera District, Amhara National Regional State, South Gondar Zone, Ethiopia.

Sampling design

From the district, two rice producing Kebele's (local administration) were purposely selected to represent the study population. A cross-sectional and retrospective type of studies were conducted using survey questionnaires, group discussion and observation were used to collect data on characteristics and practices of rice by-product utilization in smallholder farming systems.

Data collection and statistical analysis

The primary data was collected through a cross-sectional investigation. Semi-structured questionnaire was used to include data pertaining to socio-economic characteristics: demographic nature, size of household education and age. Farm size, livestock species kept, methods and strategies of rice by-products utilization were collected. Livestock holding per household was converted to tropical livestock unit using the conversion factors (ILCA 1992). Constraints related to feed shortage in terms of quantity and quality visa vis mitigation strategies were collected. The data was complemented with information obtained from key informants and secondary data derived from district office of agriculture. The collected data was systematical coded and analyzed with Statistical Package for Social Sciences (SPSS) (version 20 2011).

RESULTS AND DISCUSSION

Gender, age, and educational characteristics of respondents

The age and educational characteristics of respondents are shown in Table 2. Of the total of respondents, the majority (94%) were male while the rest of them were female. This may be associated with the fact that male-headed households have more access to agricultural technologies due to their exposure to different out-of-house issues. The findings agreed with different reports (Mekuriaw et al. 2011; Assefa et al. 2014; Asmare and Yayeh 2017). Most respondents in the study area were in the range of 31 to 50 years (55%). The results of the current findings disagree with reports of Asmare et al. (2016) and Atalayeetal. (2014) who reported the average age of respondents was 43.2$\pm$1.0 years for Metkele Zone and Burie District, Ethiopia, respectively.

The mean family size of respondents in the study area was 6.55 per household. The average family size in the study areas was comparable but relatively higher to 6.22 (Kebede et al. 2012) reported for Bure district of ANRS, and it is also higher than the two lowland districts (Mandura and Pawe) of Metekel zone of BenshangulGumz region ranging 6.04 to 6.94 (Mekuriawet al. 2011). The result was also larger than that reported by Tesfaye (2007) with overall mean family size of 5.7 persons in Metema district in Northwest Ethiopia and national rural average (5.1) (ERSS 2013).

Many household heads were able to read and write (35.38%) with comparable proportion of elementary school completed households (33.85%). The result also showed that there were still many (27.69%) that could not read and write in the area. This indicates there is still the highest numbers of respondents who could not read and write in all altitudes which could have high influences on awareness and adoption of emerging technologies and extension activities. This agreed with Ezeibeet al. (2014) who reported that the low levels of education of the households influence adoption of improved poultry management practices. Furthermore, Bruna et al. *(*2014) reported that education is the main issue in agricultural development (especially primary and secondary schooling had higher impact on agricultural development compared to any other level of education). Therefore, in the study area, these (36.38%) illiterate had their own impact on utilization of existing resources, technology transformation, and adoption in the study areas.

Land and livestock holding of respondents

The land and livestock holding characteristics of respondent are shown in Table 3. Mixed crop-livestock production system is the dominant farming system in the district. The livelihood of respondents in the study area was solely (100%) crop-livestock farming. Livestock production is subsistence-oriented and is an important component of the mixed farming system and is well integrated with crop production. Livestock species kept by the farmers are typically cattle, sheep, goats, equines, and chickens. Cattle are the dominant species per household, mainly used for draught power, followed by milk and meat production, income, and manure for maintaining soil fertility. The results agree with reports of different authors (Selamawit 2015; Asmare et al. 2016; Zeru and Lijalem 2016) in Ethiopia.

The average landholding per household in the study area was 1ha from which on average 0.9 ha was used for crop production and the rest (0.1ha) used for forage production purposes. The major crops grown in the study area were rice, maize, finger millet, teff, hot pepper and niger seed which cover 38.13, 24.12, 22.56, 6.61, 4.66 and 2.33%, respectively. The overall land covered by the above crops occupies about 98.14% of the total cultivable land while the rest (1.86%) was covered by horticultural crops like potato and lettuce. In the district from the total cultivable land, 43.58% was cultivated using irrigation and residual moisture from the rainy season. This indicates that the district has much irrigable land for food and forage crop production.

Livestock feed resources

The dry and wet season livestock feeds resources of respondents are shown in Table 4. The type of available feed resources in the study area includes natural pasture, crop residue, hay and supplements like salt, and some indigenous and improved fodder trees. The feed resources of livestock in dry and wet seasons were found almost the same which might be due to shortage of land that has made respondents not base only on grazing and use straw and other feeds in both seasons. Similar reports were also reported for different areas of Ethiopia (Mekuriaw and Asmare 2014; Asmare et al. 2016; Zeru and Lijalem 2016).

Table 2. Age and educational characteristics of respondents.

Characteristics of respondents	Frequency (Percent)
Age category	
18-30 years	17 (21.25%)
31-40 years	22 (27.5%)
41-50 years	22 (27.5%)
51-60 years	12 (15%)
> 60 years	7 (8.75%)
Total	80 (100%)
Educational characteristics	
Illiterate	14 (17.5%)
Read and Write	22 (27.5%)
Elementary school	21 (26.25%)
High School	14 (17.5%)
Certificate and above	10 (12.5%)
Total	80 (100%)

Table 3. Land and livestock holding characteristics of respondents.

Species of livestock	Holding (TLU)
Cattle (TLU)	4.71
Sheep (TLU)	0.2
Goats (TLU)	0.21
Donkey (TLU)	0.8
Mule (TLU)	0.8
Chicken (N)	11
Honeybee colony (N)	4
Landholding (ha)	1
Cropland (ha)	0.9
Forage land (ha)	0.1

Note: N (number), ha (hectare), TLU (Tropical Livestock Unit)

Table 4. Dry and wet seasons of livestock.

Dry season feed	N	Percent
Grazing and crop residues	42	52.5
Grazing, crop residues and hay	21	26.25
Grazing, crop residues, and improved fodder	17	21.25
Wet season feed		
Grazing and crop residues	43	53.75
Grazing, crop residues, and hay	15	18.75
Grazing, crop residues, hay, and improved fodder	12	15
Grazing, crop residues, and concentrate	10	12.5
Total	80	100

Note: N (number)

Seasonal livestock feed shortage was the major problem for livestock production in both study areas, in which farmers have different strategies to mitigate the problems. The livestock feed resources and feeding system of the current study area agrees with different reports in different parts of the country (Tonamo et al. 2015: Asmare et al. 2016; Gashe et al. 2017). As indicated in other parts of the country (Fetsumet al. 2009), growth crop production and increment of livestock number are considering adding the feed shortage in the study areas.

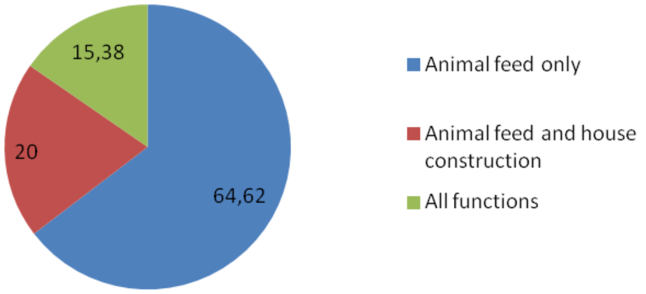

Figure 2. Function of rice by-products for the household.

Rice by-product utilization of households

The Function of rice by-products for the household is shown in Fig 2. Result of overall rice by-product utilization indicated that rice by-products are used for different functions such as animal feed and assist in house construction as well as local mattress making. From the total of respondents, the majority (64.62%) households in the study area use rice by-products as animal feed, followed by both feed and house construction. The current finding agrees with various reports (Atuhaire et al. 2014; Valbuena et al. 2015; CSA 2016).

Utilization of rice by-products as feed for livestock

The species of livestock animals fed rice by-products in the study area are shown in Table 5. Most respondents 43 (66.7%) use farm products, 12 (18.5%) use purchased and 10 (18.4%) get rice by-products both from farm produced and purchasing for their animal feed. Similar trend of utilization has been reported in research conducted for crop residue trade-off by Valbuena et al. (2015). In the study area, rice by-products in the study area are used as feed for different livestock species including equine. Overall, the majority (49.23%) of respondents feed their livestock rice by-products for cattle and equine, followed by cattle (24.62%), and all animals (13.85%). In Ethiopia, different authors reported that the rice straw and bran could be used as animal feed for different species (Derso 2009; Asmare et al.2010; Hailu et al. 2011). Moreover, the information was in line with Heuze and Tran (2015) showed that rice and its by-products could be used for different species of animals.

Table 5. Species of animals fed rice by-products and feeding methods of respondents.

Type of animals feed RB	N	Percent
Cattle	18	22.5
Equine	14	17.5
Cattle and equine	32	40
Cattle and sheep	7	8.75
All animals	9	11.25
Total	80	100
Sole	45	69.2
mixed with other feeds	16	13.9
Both methods	19	16.9
Total	80	100

Note: N (Number), RB (rice products)

In the current study area, rice by-products were fed to livestock as sole feed and mixed with other feeds. However, the majority (69.2%) of households provide sole either straw or bran followed by both sole and mixed with other feeds (16.9%). The finding showed that rice straw and rice bran are being used as sole feed or mixed with other supplementary feeds. In all respondents, the amount of rice straw and bran offered to animals in the study area is done based on estimation without quantification for each animal. The finding agreed with earlier reports in Ethiopia (Tesfaye and Chairatanayuth 2007).

Nutritional improvement of rice by-products

The current practices of rice by-product nutritional improvement strategies of respondents are shown in Table 6. The study revealed that respondents use different methods to improve rice by-products, particularly rice straw such as drying, saltwater spraying before feeding, urea treatment, and chopping of straw. However, many respondents, 54 (83.33%), apply drying and chopping followed by drying only (15%). Although these physical treatments are important in increasing the intake of rice by-products particularly rice straw, the practices have no effect on nutritional improvement of feeds (McDonald et al. 2010). Smallholder farmers do not apply better nutritional improvements on rice by-products such as urea treatment due which might be associated with lack of inputs and awareness. However, as rice by-products are usually low in crude protein, it is vital that supplementation with a protein source and a more easily accessible energy source will improve the performance and production of the animals (Sarnklong et al. 2010; Alam et al. 2016).

Skill of respondents on rice by-product management

Among rice producers respondents only 23% have got training on rice by-product utilization techniques while the majority (77%) have not got any training. The status of training of respondents in the current study is comparable to the results of Asmare et al. (2016) for desho grass utilization in Buriezuria district in northwestern Ethiopia. However, the importance of training and visit to farmers' fields has significant importance on the adoption of technology in tropics (Hussan et al. 1994; Rahman 2007). In line with this, (Ampaire and Rothschild 2010) indicated that farmers who had received more training and support had less disease in pigs in the six months preceding the study than those who had not been trained or who had the animals for a shorter period.

Suggested rice by-product improvement strategies

The rice by-product improvement strategies of respondents are shown in Table 7. Respondents were asked to suggest future rice by-products improvement strategies and mentioned different types. Nevertheless, many respondents were indicated that proper drying, chopping, addition of saltwater and supplementation with concentrate mixture and bran as options for improvement in the future utilization of rice by-products. This finding indicates that many extension demonstration works should be done in the area about the utilization and feed value improvement options in the study area.

It is known that the chemical treatment of roughages, like rice straw and other by-products, would increase the nutritive value of the roughage. Research conducted using lactating crossbred cows in Ethiopia, urea treated barley or teff straw were noted to replace native hay, and ammoniation was found to be economically feasible producing in milk production of cows (Derso 2009; Hailu et al. 2011). However, none of respondents suggested chemical treatment as an option of rice straw which might be associated with lack of awareness and cost implications of urea and labor. Moreover, other researchers also indicated that rice, although major feed for ruminant animals, has a low crude protein content (Khandaker et al. 2012; Su et al. 2012) indicating that it needs chemical treatments like urea treatment (McDonald et al. 2010; Nguyen et al. 2012) as well as physical treatments like chopping. Overall, treatment of straws with urea might be most suitable method for small-scale farmers to improve the quality of straws (Hanafi et al. 2012). On the other hand, urea price is increasing, and impacts on the high cost of roughage unless there are chemicals or options are sought.

Constraints of rice by-product utilization as feed

The major constraints of rice by-product utilization as feed by respondents is indicated in Table 8.

Table 6. Rice by-products improvement strategies of respondents.

Method	N	Percent
Drying only	12	15
Spray salt solution before feeding	5	6.25
Urea treatment	9	11.25
Drying and chopping	54	67.5
Total	80	100%

Note: N (number)

Table 7. Techniques used to keep quality of rice straw by respondents.

Techniques	N	Percent
Proper drying	44	55
Addition of saltwater on straw	18	22.5
Urea treatment of straw	8	10
Mixing salt with bran	6	7.5
Supplementation	4	5
	80	100

Note: N (number)

Table 8. Constraints of rice by-product utilization by respondents.

Constraints	N	%
Seasonal deficiency of by-products	18	22.5
shortage of labor	8	10
Lack of inputs (e.g., chemicals)	13	16.25
Shortage of by-products	10	12.5
Lack of skill and awareness on utilization	20	25
Total	80	100

Note: N (number)

The major constraints of rice by-product utilization as animal feed in the study area can be categorized as lack of awareness about feed value of rice by-products, shortage of rice products, poor processing and storage method of rice bran and lack of access to the by-product. From the listed problems, lack of awareness about the feed value of the products was found in a relatively large number of respondents (25%), seasonal deficiency of products (22.5%) followed by shortage of inputs (16.25%) were the major constraints. This elucidates that there must be an intervention such as creation of awareness about the feed value of rice by-products and making accessible the products to users. The use of rice by-products in the study area was limited by lack of access and awareness in the study area. The result agrees with reports stated about crop residues in other parts of the world (de Leeuw 1997; Erenstein et al.2011; Valbuena et al. 2012).

Conclusion and recommendation

Rice by-products such as straw and bran were used for different purposes including animal feed in the study area. The rice by-product utilization indicated that most respondents in the study area use rice by-products as animal feed, followed by both feed and house construction. The sources of rice by-products for animal feed were both farms produced and purchased. All herbivore animals were fed rice by-products as sole or basal diet and supplementary to other feed types. Simple drying and chopping were used as a treatment for rice straw before being fed to animals; however, rice bran was not treated. Although rice by-products are livestock feeds in the study area, smallholder farmers were not able to use such products effectively due to lack of awareness and shortage of resources. Hence, awareness creation should be given to smallholder farmers on the utilization of rice by-products and feed value improvement methods. Moreover, detailed experiments on physical and chemical treatment and animal evaluation of rice by-products should be conducted in the study area.

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Seed emergence and growth of the shortage sugar palm (*Arenga pinnata*) as a response to seed scarification and liquid organic fertilizer application

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Abstract. *Elidar Y. 2018. Seed emergence and growth of the shortage sugar palm (*Arenga pinnata*) as a response of seed scarification and liquid organic fertilizer application. Asian J Agric 2: 8-13.* This research aimed to know the effect of seed scarification and liquid organic fertilizer application on the seed emergence and growth of the shortage sugar palm (*Arenga pinnata* (Wurmb.) Merr). The research was conducted in two experiments, i.e. (i) effect of seed scarification, and (ii) effect of liquid organic fertilizer application. The first experiment was a single factor designed at Completely Randomized Design (CRD). The factor consisted of 4 scarification technique treatments i.e., s_1 = seed abaxial scarification; s_1 = seed tip scarification; s_3 = seed left and right sides scarification; s_4= seed embryo scarification. All treatments were replicated 6 times. The second experiment was arranged at a factorial (3 x 3) using Completely Randomized Design (CRD) with 6 replications. The first factor was the dose/volume of liquid organic fertilizer treatment in concentration of 3 cc L^{-1} of water (D) consisting of 3 levels i.e., d_1 = 300 mL; d_2 = 400 mL; d_3 = 500 mL, while the second treatment was the interval of liquid organic fertilizer (I) application consisting of 3 levels i.e., i_1 = 2 weeks; i_2 = 3 weeks; i_3 = 4 weeks. Seedling emergence test, germination rate, vigor index and seed germination percentage were measured and the growth parameters such as the plant height increase, plant midrib girth, number of midrib increase, and number of leaves were observed. The results showed that scarification at the embryo part (s_4) resulted in the best seed germination percentage of the shortage sugar palm at around 99.81%. Combination treatments between 500 mL dose of liquid organic fertilizer in concentration of 3 cc L^{-1} of water with the interval of 2 weeks (d_3i_1) produced the best seedling growth of the shortage sugar palm.

Keywords: *Arenga pinnata,* liquid organic fertilizer, scarification, seedling germination, shortage sugar palm

INTRODUCTION

East Kalimantan Province shows and possesses a great potential for the development and cultivation of palm commodities like sugar palm (*Arenga pinnata* (Wurmb.) Merr). The shortage sugar palm is a national superior variety that was released by the Minister of Agriculture of Indonesia in 2011, with Decree No. 3879. This plant is a native sugar palm from East Kutai regency, East Kalimantan that is fast growing and shows both harvested palm and high yield of sugar and other by-products, such as fruit and traditional drink. In 2008, the development of sugar palm in East Kalimantan reached 1.504 ha and produced brown sugar 4.21 tons ha^{-1}. In 2011, the cultivation area was decreased to 1.253 ha, and the productivity of brown sugar became 1.29 tons ha^{-1} (Plantation Agency of East Kalimantan Province 2013). Generally, the main problem in the development of sugar palm is because of the low input of cultivation technology application and the use of traditional systems in cultivation by farmers.

Currently, sugar palm is used as a producer for *nira* (the main source of brown sugar), renewable energy (bioethanol), carbohydrate (starch), a mixture of foods and drinks (palm fruit), building materials (stems), and for soil and water conservation (Martini et al. 2012; Ferita et al. 2015). With the demand for sugar palm increasing, the development of sugar palm in a large area using proper cultivation techniques in terms of cultivation aspects is required and very important.

Seed selection, seedling, and plant treatment and maintenance could significantly enhance the regeneration of sugar palm. The main obstacle in sugar palm regeneration is the long period of seed dormancy and the low percentage of seed germination. Dormancy period is time required for seeds to germinate. The dormancy period of the plant seeds is influenced by genetic, environmental, and hormonal factors (Koorneef et al. 2002; Finch-Savage and Leubner-Metzger 2006; Graeber et al. 2012). From the previous research, the sugar palm seed germination percentage was very low and varied (10-65%) as well as the time required to start germinating was long enough about 4-6 months (Mashud et al.1989).

The dormancy of palm seeds can be fractured mechanically by damaging the tissue of its testa (seed coat) through scarification/sanding (Saleh 2004; Abu Bakar and Maimuna 2013; Silalahi 2017). The aim of the scarification/sanding is to reduce the lignin layer in testa (seed coat). Lignin thin layer in testa (seed coat) will increase the permeability of water to facilitate the entry of water into the embryo. Scarification treatment on the sugar palm seed has been done by Silalahi (2017) using a sanding width of 1/4, 2/4, 3/4 and all parts of the seed coat. The results showed that the seeds sanded more than half of the seed coat, were the quickest to germinate.

Liquid organic fertilizer has multipurpose function for all kinds of food crops (rice and cereals), horticultural crops (vegetable, fruit, ornamental plants) and perennial crops (cocoa, palm oil, rubber, etc.) as well as for livestock/poultry and fish/shrimp. The micronutrient content of one-liter liquid organic fertilizer has a function equivalent to the micronutrient content of 1 ton of manure. The use of liquid organic fertilizer can save Nitrogen (N), Phosphorus (P) and Potassium (K) use as much as 12.5 - 25%. The fertilizer also contains complete macro and microelements for plant growth. To support the seedling growth of sugar palm, the use of liquid organic fertilizer is a good alternative to substitute the an-organic fertilizer application because it provides complete nutrients, improves soil conditions, and contains the plant growth hormones such as auxin, gibberellin, and cytokines (Anonymous 2015). The humic and fulvic contents in liquid organic fertilizer have a role in improving the consistency (friability) of hard soil and dissolving SP-36 (Phosphorus fertilizer) quickly. Liquid organic fertilizer will also trigger the multiplication of the formation of polyphenols compounds to improve the plant resistance against diseases (Anonymous 2015). Therefore, it is important to examine the effect of seed scarification (sanding) on germination, and the dose and interval time of liquid organic fertilizer application to the seedling growth of the shortage sugar palm.

MATERIALS AND METHODS

Plant materials

The plant materials used in this study were the shortage sugar palm seeds, harvested from several mature plants and fruits of shortage sugar palm cultivated in Kandolo village, East Kutai (Kutai Timur). The physiologically ripe seeds (characterized by tawny fruit skin) were put into a porous plastic bag and watered every day for a month. After the fruit pulp became soft, the seed was separated and rinsed with water selected for the homogenous size and quality.

Growing media, seed preparation, and maintenance

Sand used as the main seedling medium was put in a media box made from wood planks in a rectangle form with size 3 m x 1.8 m. The media box was placed on the *para-para* (nursery place) with a shading percentage around 60 % and as high as 1 m, to facilitate the seeds for seedling. The physiological ripe sugar palm fruit (characterized by tawny fruit skin) was put into the porous plastic bag and watered every day for a month. After the fruit flesh became soft and the seed was separated, then the seeds were rinsed. The point of an embryo was determined by soaking the seeds in water, and the white-shadow seeds were marked as an embryo point. All seeds were treated with Fungicide (Dithane M-45) solution to prevent disease infection, especially from fungi. The seeds used in this experiment were approximately similar/homogenous in size and color. The sanding/scarification was done by scrubbing the seeds using sand on the abaxial side, seed tip, embryo, and both seed sides determined as follows: Scarification/sanding of the shortage sugar palm seeds consisted of 4 levels, in the part of: s_1 = abaxial; s_2 = tip; s_3 = left and right side; s_4 = embryo. Seeds were then disseminated in moist and watered top-soil media. Seeds that begin to germinate, were marked by the appearance of epicotyl at the growing point. The epicotyl begins to prolong and form shoot, on the other hand, the radix will grow downward to form the plant roots. The plumule was formed by the growth of embryo axis in the upward direction. Prolongation of the plant roots and the formation of leaves were initiated and formed from the plumule. After two weeks, the hypocotyl had reached a length of 2 cm.

The second experiment was designed at a factorial Completely Randomized Design with eight replications. The first factor was the dose of liquid organic fertilizer (D) in concentration of 3 cc per liter of water, consisted of 3 levels 1.e. d_1 = 300 mL; d_2 = 400 mL; d_3 = 500 mL. The second factor, was the interval of liquid organic fertilizer application (I) consisted of 3 levels, i.e., i_1 = 2 weeks; i_2 = 3 weeks; i_3 = 4 weeks. The treatment combination consisted of 9 treatments and was repeated 8 times. Each treatment consisted of 4 seedlings, so that the number of seedlings used was as much as 144 seedlings.

Seedling growth and maintenance

The polybag of 20 cm x 30 cm size was filled with the mixture of top-soil: sand: chicken manure fertilizer in the ratio of 2 : 1 : 1. Seedlings were transferred from the seedling box to the polybags 60 days after sowing. The seedlings were planted in polybags and maintained until the age of 6 months. They were then treated with liquid organic fertilizer according to the dose and interval treatments as determined in the experiment. The seedlings were maintenance by watering every morning and afternoon. Weeding was conducted to remove weeds growing among the plants.

Data collection and analysis

Seedling emergence time (days), germination time (days), vigor index (%), and the percentage of seedlings (%) were observed for the scarification and germination experiment of the shortage sugar palm. Moreover, the increase of pant height (cm), stem girth (cm), and number of leaf number at the plant age of 30, 60, 90 and 120 days after treatments were measured for the second experiment. The data were analyzed using analysis of variance (ANOVA) and the Least Significant Difference (LSD) at 5% level were used to test the mean value differences.

RESULTS AND DISCUSSION

Effect of scarification on seed germination of the shortage sugar palm

Seedling emergence time (days)

Based on the variance analysis, scarification treatment gave significant effect to the seedling emergence test. The fastest seedling emergence time was reached by the treatment of s_4, (Scarification at embryo part of seed) which was 16.73 days, while the slowest was in the treatment of

s_1 (scarification at the abaxial side of the seed) which was 42.42 days (Figure 1).

Scarification treatment at the embryo part (s_4) gave the best results, presumably, it increases and accelerates the process of water imbibition into the seed and stimulates the seed to germinate. Imbibition process is an initial process of germination. Therefore, it could activate enzymes and hormones for seed germination. According to Flach and Rumawas (1996), sugar palm seeds scrapped around the embryo, besides facilitating the entry of water and air, it also helped root primordia to emerge out from the part of growing point, and as a result, the appearance of plumule was more rapid than without treatment. The scarified seeds will lose lignin layer in the seed coat, and it makes the endosperm of the seed will open, therefore water could enter easily headed to the embryo. Water in the embryo will trigger hormones and enzymes to activate germination (Delouche 1985).

The scarification at both sides of the seed showed the lowest results, it was assumed because the scarification treatments in the right and left side of the seeds were far from the embryo position, so the imbibition process was less influence on the germination process. The appropriate position of an embryo in the technique of sanding scarification was very effective in helping the process of sugar palm for seed germination. Usually, the location of the sugar palm embryo was in the right abaxial or left abaxial, but sometimes is also located in the middle of the seed.

Germination rate

Based on the variance analysis, the sanding scarification treatment resulted in a significant effect on the germination time. Based on the results of LSD test at the level of 5%, the treatment of (s_1) showed no significant difference with the treatment of (s_2) but showed significant difference with the treatment of s_4. The treatment of s_2 was significant different from the treatment of s_3. The best result of germination time was in the treatment of s_4 with 18.35 days, whereas the lowest was in the treatment of s_1, with 32.58 days (Figure 2).

The treatment from s_4 gave the best result on germination time parameters. This germination time value showed and represented the vigor condition. The lower the value of germination time, the higher the seed vigor, and the seed germination became faster. Sanding scarification treatment at the embryo part, made the seed become permeable to gas and water. While on the treatment of s_3, the seed was still impermeable to water and gas. Impermeability of the seed coat to water was due to water was prevented by thick-walled of the seed coat cell which was covered externally by a hard wax layer. The fission of this layer was immediately allowed water into the seed, and thus germination started (Adiguno 2000).

An effort should be made to break the hard seed coat dormancy of shortage sugar palm by doing mechanical scarification. With this kind scarification treatment, the mechanical resistance of the hard seed coat will be reduced, therefore it will make the sugar palm seed germinate faster. The dormancy was broken to initiate the germination by destroying the seed coat acting as a barrier of imbibition (Delouche 1985).

The water content of palm seed during the harvested time is relatively high, i.e., 25-30% (Widyawati et al. 2009). The observation by Widyawati et al. (2009) to the lignin and tannin seed level, showed that the older the palm seed, the level of these compounds is increased. If it was connected between lignin and tannin seed with water imbibition, there was a close negative correlation, meaning that the higher the lignin and tannin contents of palm seed, the lower as well its imbibition. Increase in lignin and tannin levels had a role in reducing the permeability of sugar palm seed to water.

From the observation, the most optimal result of the scarification treatment on the germination time was obtained by sanding scarification. Germination was influenced by internal factors of the seed, especially the hormone contents such as abscisic acid (ABA) and gibberellic acid (GAs) (Delouche 1985). Sanding scarification causes lots of water to infiltrate into the seed easier than slashed, because the width of the coated part became larger if its lignin was removed.

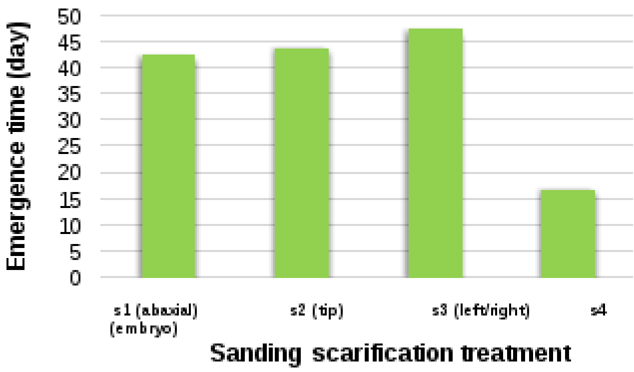

Figure 1. Effect of scarification on emergence test of shortage sugar palm

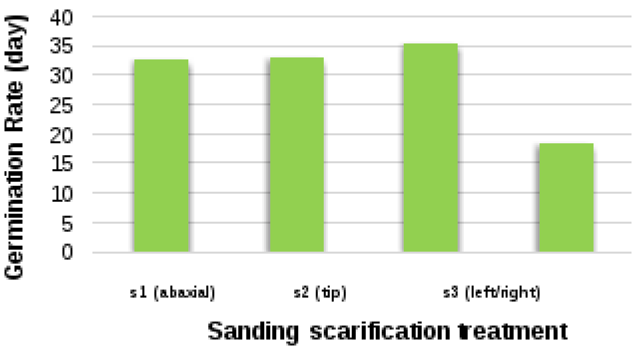

Figure 2. Sanding scarification treatment effect to germinate time

Vigor index (%)

Based on the analysis of variance, sanding scarification treatment give a significant effect on the vigor index of the shortage sugar palm seedlings. The LSD test at 5% showed that the treatment of s_1 was not significantly different from the treatment of s_2, but it showed significant differences in the treatment of s_3 and s_4. The treatment of s_2 was significantly different from the treatment of s_3. The best result of vigor index showed by the treatment of s_4, i.e., 1.41% whereas the lowest was in the treatment of s_1, i.e., 0.41% (Figure 3).

The treatment of s_4 gave the best result; it could be because the process of dormancy, breaking scarification with proper sanding at the growing point position, made easier the imbibition process of the seed. The proper scarification technique could increase the seed vigor. Seed vigor is indicated the ability of the seeds to germinate at sub-optimum conditions and related to the value of the germination rate. The value of this germination rate indicated the seed condition to possess good vigor or not. The lower the value of germination rate, the higher the seed vigor, and the seeds germinate faster.

The percentage of germination

Based on the analysis of variance, sanding scarification treatment resulted in a highly significant effect on the percentage of germination**.** Based on the results of LSD test at 5% level, the treatment of s_1 was significantly different from the treatment of s_2, s_3, and s_4. The treatment of s_2 was significantly different from the treatment of s_4. The biggest germination percentage was shown by the treatment of s_4, i.e., 99.71%, while the smallest was by the treatment of s_1 about 46.46% (Figure 4).

The dormancy breaking techniques effectively increased the germination percentage as observed in this research. The seeds treated with scarification had been able to germinate 14 days after scarification. It was indicated by the white apical appearance. The sugar palm seeds treated by scarification techniques in the abaxial part only produced the highest germination percentage values around 50-55% (Saleh 2004). Furthermore, the sugar palm seeds scarified by sandpaper had an average germination percentage value of 74.44% (Saleh 2004). These different results were presumable because of the differences in scarification techniques, the source of seeds and germination environment conditions.

Scarification treatment with sanding at the embryo had the percentage of seed germination up to 100%. One of the factors affecting seed germination, was seed maturity level. In palm fruits, their maturity levels are not the same due to the location of palm fruits in the fruit strand/bunch. As a result, the position of palm fruits in one bunch was not uniform ripening. According to Miao et al. (2001), the sugar palm seeds obtained by picking the old fruit which is characterized by yellow-rind, do not guarantee the maturity level homogeneity. The various maturity levels of palm fruits became a factor causing the palm seed to take a longer time to germinate and show low percentage value of germination. So, even though the breaking of seed dormancy was successful, the result was usually still less satisfactory. According to Miller (1964), the palm seed germination was naturally carried by the civet, in which the civet ate a ripe palm fruit then its feces came out together with the palm seed that was ingested before and discharged in the protected and moist place. These palm seedlings can germinate faster. The research results from Morris (2000) showed that the sugar palm seed derived from the civet can germinate faster by 83%, better than the seed derived from picking the old palm fruit. According to Maliangkay (2007), to obtain the seeds with uniform and high quality, determination the harvest time of the fruit is required to be known. Determination of fruit maturity based on the fruit color, odor, hardness, the fell off fruit/seed, the rupture of fruit and others were difficult skill to be inherited or less objective. An objective benchmark for determining the seed maturity can be determined for example based on the dry weight.

Effect of the dose and interval of liquid organic fertilizer on the growth of the shortage sugar palm

The application of liquid organic fertilizer resulted in the highly significant effect to the increase of height at the age of 30, 60 and 120 days after treatment (dat) and midrib girth at the age of 30, 60, 90 and 120 dat, while the combination of the dose and interval of liquid organic fertilizer treatment did not give significant effect to midrib girth at the age of 60 and 120 dat.

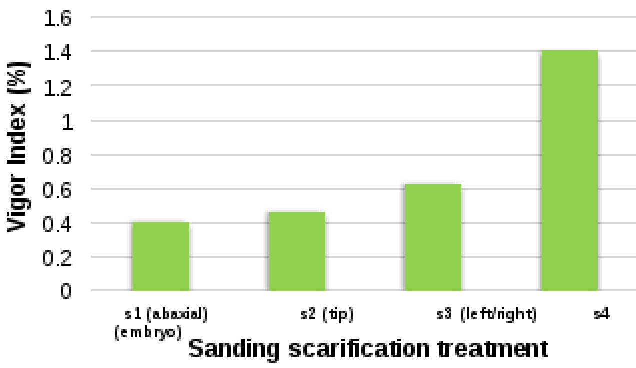

Figure 3. Sanding scarification treatment effect to the Vigor Index

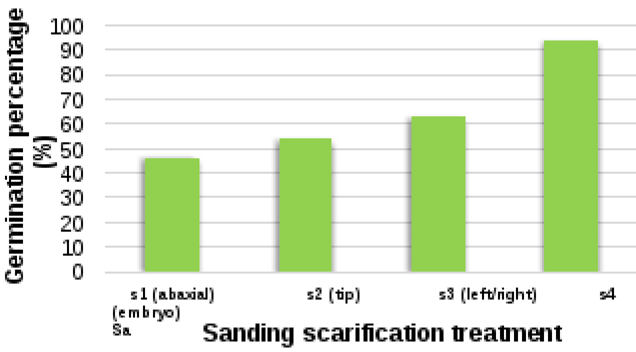

Figure 4. Sanding scarification treatment effect to the percentage of germination

Increase of plant height

Based on the analysis of variance, the dose of liquid organic fertilizer (D), liquid organic fertilizer interval (I) and their interactions (DI) showed highly significant effect on the parameters of increase of height at the age of 30, 60, and 120 hsp and did not give significant effect on the while increase of height at the age of 90 dat (Figure 5 and 6).

The dose and interval of application of liquid organic fertilizer resulting in the significant effect to the increase of plant height were presumably influenced by the characteristic of organic fertilizer, plant species and the availability of nutrients absorbed in the soil by plants. Liquid organic fertilizer is known as natural liquid organic fertilizer derived from the extraction of organic material waste of livestock and poultry, plant waste (compost), natural waste, and some types of certain plants and natural substances that were processed based on environmentally friendly technology with the principle of zero-emission concept. The plants absorbed all the nutrient content supplied by liquid organic fertilizer both macro and micronutrients in every week, so the plants can grow up maximally. The fertilizer was able to improve the plant height because liquid organic fertilizer contains macronutrients, micronutrients, minerals, vitamins, organic acids, and growth hormones that could stimulate plant growth. Liquid organic fertilizer contains elements of 0.12% of N, 0.03% of P_2O_5, 0.31% K, 60.4 ppm of Ca, 2.46 ppm of Mn, 12.89 ppm of Fe, 0.03 ppm of Cu, minerals, vitamins, organic acids, and growth stimulating substances such as Auxin, Gibberellin, and cytokines (Anonymous 2015). Besides that, the advantages of using these organic fertilizers were able to provide nutrients rapidly (Samad 2008).

Increase of plant midrib girth

Based on the analysis of variance the dose of liquid organic fertilizer treatment (D) and liquid organic fertilizer interval (I) increased the plant midrib girth at the age of 30, 60 and 90 dat significantly, but at 120 dat, it was not significant (Figure 7 and 8). There was an interaction between liquid organic fertilizer dose and liquid organic fertilizer interval (DI) treatment on the parameter to the increase of plant midrib girth at the age of 30 dat and 90 dat while at the age of 60 dat, no significant interaction was observed.

The dose and interval application of liquid organic fertilizer showed significant effect on the increase of plant midrib girth. This was presumably due to the type of soil which supports plant growth, especially the roots, which can affect stem diameter. Plant growth may be affected by the low availability of nutrients. Plants exposed by N element deficiency, showed the obstructed vegetative growth represented by the slow growth of branches, leaves and stems. The deficiency of P element has shown to make plants become dwarf and the deficiency of K element causes plants to have a weak and short stem (Salisbury and Ross 1995).

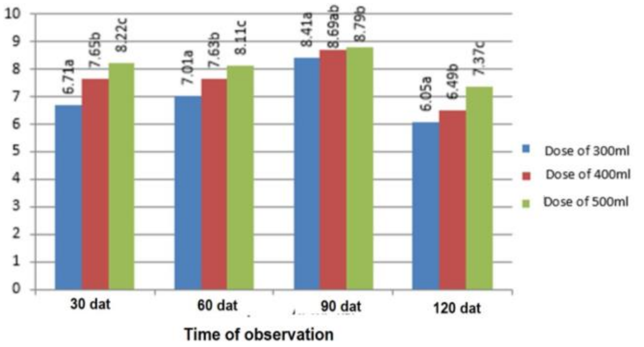

Figure 5. Increase of plant height (cm) of the shortage sugar palm as response to the dose of liquid organic fertilizer treatment

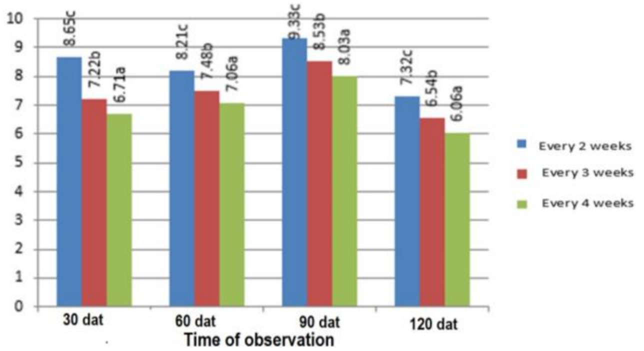

Figure 6. Increase of plant height of the shortage sugar palm as response to the interval application of the liquid organic fertilizer treatment

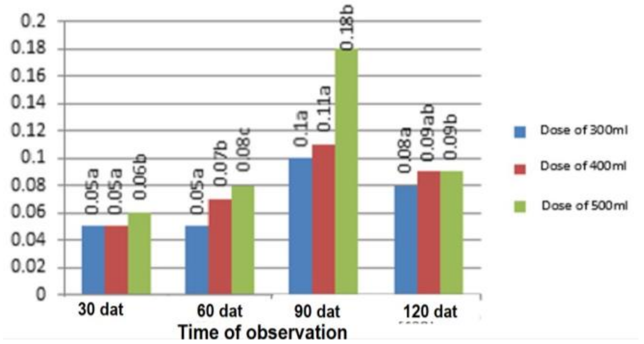

Figure 7. Increase of plant midrib girth (cm) on shortage sugar palm as response to liquid organic fertilizer dose treatment

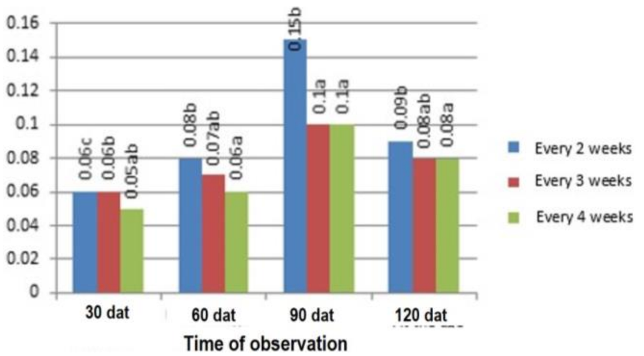

Figure 8. Increase of plant midrib girth (cm) on shortage sugar palm as response to liquid organic fertilizer interval treatment

Increase of midrib and leaves number

Based on the analysis of variance, the treatment of liquid organic fertilizer interval gave significant effect to the plant midrib number at the age of 30 and 120 dat, but it showed no significant effect at the age of 60 and 90 dat. The treatment of dose (D) and their interactions showed no significant effect on such plant parameters. On the other hand, the dose (D), liquid organic fertilizer interval (I) and the interactions of both treatments did not show significant effect for all parameters observed.

The increase of midrib number was presumably due to concentration applying of liquid organic fertilizer at the age of 30 dat had a good influence usually at the young age of plant, and the condition of rooting plants had not spread out yet. Therefore, the fertilizer must be given optimally to absorb the nutrient supply in fertilizer.

The effect of the liquid organic fertilizer interval was not significant to the midrib number. It was presumably due to the macro and micronutrients contained in the liquid organic fertilizer had not been able to be absorbed by sugar palm seeds to increase the plant midrib. According to Salisbury and Ross (1995), the formation of new shoots and leaves was associated with plant nutrients, and the absorbed nutrients can help the sustainability of plants such as the formation of new leaves.

In conclusion, scarification treatment by sanding showed a high significant effect on all parameters of seed emergence and seedling development. Sanding scarification at the embryo part gave the best result, showed in the parameters of emergence test of 16.7 days, germination percentage of 99.71%, germination rate of 18.35 days, and vigor index of 1.41. Liquid organic fertilizer dose treatment resulted in the highly significant effect of the plant height increase and midrib girth. The treatment of 500 mL doses of liquid organic fertilizer applied at interval of every 2 weeks increased the plant height and the number of midribs.

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Biomass production of *Azolla microphylla* as biofilter in a recirculating aquaculture system

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Abstract. *Sumoharjo, Ma'ruf M, Budiarto I. 2018.Biomass production of* Azolla microphylla *as biofilter in a recirculating aquaculture system. Asian J Agric 2: 14-19.* This study utilized macrophyte (*Azolla microphylla* Kaulf.) as a biofilter and investigated that biomass produced in an aquaculture system could potentially be an alternative feed. This experiment was aimed to determine the *Azolla* microphylla growth rate and its efficiency in removing ammonia from a simple recirculating aquaculture system. The experimental units were set up in three different water flows, i.e. 3 lpm, 5 lpm, and 7 lpm onto the three different geometrically baseboards of Tilapia (*Oreochromis niloticus*) growing tanks (prism, rectangular and limas). The results showed that water flow did not give significant effect($P < 0.10$) on the growth rate of *Azolla*. The lower water flow (3 lpm) resulted in the highest ammonia biofiltration efficiency, which can remove ammonia up to 32.2±3.0% of the total NH_3-N and NH_4^+-N (TAN).

Keywords: *Azolla microphylla*, ammonia, biofiltration, recirculating, water flow

INTRODUCTION

The main problem in the intensification of aquaculture systems is water quality decreasing rapidly because of a high density of fish being reared with a high feed input in less water exchange. Hence, accumulation of fish metabolites, especially ammonia, tends to occur in waterbody and build-up to toxic level and affecting fish performance. Wastewater is accumulated, while feed is continuously added in a fish culture system (Rafee and Saad 2005).

In an intensive land-based fish farming system, the toxicity of excreted nitrogenous compounds is often a limiting factor (Bradfield 1985; Brune et al. 2003, Nerici et al. 2012). The toxicity of the total NH_3-N and NH_4^+-N (TAN) increases with the pH of the water because TAN enters the organism as NH_3 and the proportion of NH_3 increases with higher pH (Randall and Tsui 2002; Nerici et al. 2012). When environmental TAN level increases, the excretion of ammonium by aquatic animals decrease and the ammonium levels in the blood and tissues rise (Nerici et al. 2012). Long-term exposure to ammonia increases glycemia, lipoxygenase and unsaturated Erythrocyte Fatty Acids (Liu and Sun Pan 2008). Chronic exposure to high TAN concentrations tends to damage the fish gills, which can contribute to decreased growth because of gas exchange efficiency (Handy and Poxton 1993; Nerici et al. 2012).

In Recirculating Aquaculture Systems (RAS), biofilter is the main component and known as low-cost water treatment to keep water quality suitable for fish growth and welfare. Biofilter technology was studied intensely, however, most of them are struggling on bacterial-based biofilters, such as nitrification by nitrifiers and nutrient assimilation by heterotrophs. Smith (2003) categorized biofilters into four main types, i.e., activated sludge, aquatic plant filters, fluidized bed filters, and fixed film.

Azolla microphylla is an aquatic fern. Many reports on *Azolla* have been published, but almost all of them are related to its function as a natural feed resource. *Azolla* was recommended by FAO (2009) as feed-in small-scale aquaculture and had been used as a main component in food for tilapia (Fiogbé et al. 2014). According to Lumpkin and Placknett (1980) and Van Hove (1989), *Azolla* under good conditions presents high productivity and protein content (generally 20-30%, on a dw basis).

Growing *Azolla* seems easy (Datta 2011) because of its endosymbiotic blue algae, *Anabaena azollae*, that fixes nitrogen directly from the atmosphere (Van Howe 1989). So, *Azolla* is probably able to grow well in a relatively low nutrient environment. However, reports on *Azolla* as biofilter to remove nitrogen from the fish culture water are rare. Even though, as a macrophyte, *Azolla* should be served as a phototrophic converter at the trophic level. So, it has great potential as a biofilter for maintaining water quality in RAS as well as providing an alternative feed for growing-fishes.

This study focused on utilizing *Azolla* as biofilter in RAS. The experimental units were designed in integration of fish tank and *Azolla* growing bed to meet a series of recirculating systems. The experiment was divided into two parts; first was to analyze the effect of different water flow on *Azolla* growth rates, and the second one was to determine the optimum biomass of *Azolla* for converting nitrogen from fish waste.

MATERIALS AND METHODS

Experimental unit configuration

The experimental units were a pilot scale. Three types of tanks, with 1800-liter effective volume, were used as an experimental group. The design of the bottom of each tank varied geometrically, i.e., prism, pyramid, and rectangular. On the top of every tank were three similar trenches that had a 2-meter long biofilter bed. Every trench had three different water flow rates, i.e., 3 lpm, 5 lpm and 7 lpm. A 32 watt submersible pump was used to supply water from each fish tank to the three trenches, connected parallelly with ¾ inch PVC pipe. The water flow rate, as the treatment was adjusted, was done by the outflow head. Synchronization was carried out daily.

The fish species cultivated were tilapia (*Oreochromis niloticus*), sized 8.3±1.2 g and had 100 fishes per tank. In every trench, 50 g *Azolla microphylla* was added. The fish were fed *ad satiation* with floating pellets (CP. Prima 781-3, 31-33% raw protein).

Nutrient budgeting

In the recirculating system, the complete water from the fish tank passed over the biofilter bed (the trench) once every 2 hours, then the water was mixed continuously in the fish tank, so that there were no differences between in and outflow. Samples were taken in fish tank only. Total Ammonia Nitrogen (TAN) as nutrient input and removal rates were calculated through mass balance. To estimate TAN input per day from within the fish tanks the following can be calculated based upon the feeding rate (Timmons et al. 2002):

$$P_{TAN} = F * PC * 0{,}092$$

Where:

P_{TAN} : Production rate of total ammonia nitrogen, (kg/day)

F : Feed rate (kg/day)

PC : Protein concentration in feed (decimal value)

The constant in the ammonia generation equation assumes that protein is 16% nitrogen, 80% nitrogen is assimilated by the organism, 80% assimilated nitrogen is excreted, and 90% of nitrogen is excreted as TAN+10% as urea. In addition, the nitrogen in feces is not removed from the system but collected in the filter bed until the end of the experiment.

Water quality

Water quality parameters were monitored every three days such as temperature, pH, dissolved oxygen (DO), Total Ammonia Nitrogen (TAN), and Un-ionized Ammonia Nitrogen (NH_3-N). Lutron portable DO meter model 5510 was used to measure DO. The concentration of TAN was determined using TAONSUN spectrophotometer (Suzhou Taonsun Scientific Instruments, China).

Biomass calculation

Biomass growth of *Azolla* cultivated in biofilter units (gutter/trench) is expressed as doubling time (day^{-1}) which is calculated according to daily growth rate (DGR, %/g/day) (Zonnenveld, et al., 1991) as follows:

$$DGR\ (\%/g/d) = \frac{Ln(W_t)-Ln(W_0)}{t} \times 100$$

$$DT\ (day^{-1}) = \frac{Ln\ 2}{DGR}$$

Where:

DGR : daily growth rate (%/g/day)

Ln : logarithmic natural

W_t : final biomass of *Azolla* (g)

W_0 : initial biomass of *Azolla* (g)

DT : Doubling Time

Total Ammonia Nitrogen measured on the final day of the experiment will represent the nutrient output. Thus, in case of this simple RAS, whereas all the water is recirculated and there is no discharge, nutrient removal rate can be calculated with mass balance equation (Al Hafedh et al., 2003) as follows:

Waste Loading Rate *(*g/m^3 per day*)* = *C*i × *Q*

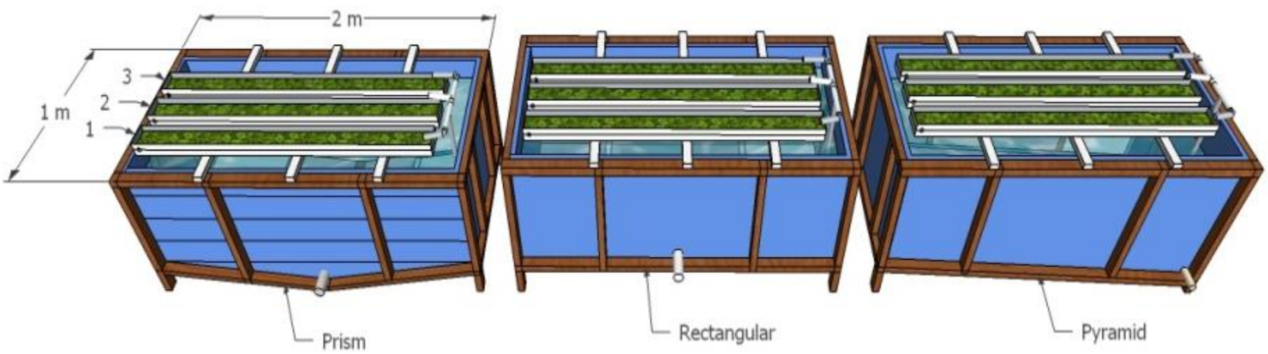

Figure 1. Sketch of experimental units configuration

Waste Removal Rate (g/m^3 per day) $= (Ci - Ce) \times Q$

$$\text{Removal efficiency } (E) = \frac{\text{waste removal rate}}{\text{Waste loading rate}} \times 100$$

Where:
Ci : Total Ammonia Nitrogen measured in the fish tank (mg/L x Water Volume x1000 = g)
Ce : P_{TAN} (Production rate of total ammonia nitrogen (g)
Q : Water flow (liter per minute, lpm)

Retained nitrogen of *Azolla* is expressed as gram and can be calculated by using the following formulae:

$$\text{Retained Nitrogen (RN, gram)} = (TKN_t \times W_t) - (TKN_0 \times W_0)$$

$$\text{Retained Nitrogen Efficiency (RNE, \%)} = \frac{RN}{P_{TAN}}$$

Where:
TKN_t : Total Kjedahl Nitrogen at the end of experiment (g)
TKN_o : Initial Total Kjedahl Nitrogen (g)
W_t : Final biomass of *Azolla* (g)
W_0 : Initial biomass of *Azolla* (g)

Data analysis

The means on the *Azolla* growth rate, doubling time, and nitrogen retention parameters were analyzed using two-way analysis of variance (ANOVA, $\alpha = 0.1$). The analysis was done using STATISTICA 8.0.

RESULTS AND DISCUSSION

Nutrient input and biomass production of *Azolla*

Total feed consumed by the fish in tanks I, II, and III were 1519 g, 1504 g, and 1313 g, respectively. The TAN production of every tank is listed in Table 1.

Based on the calculations, TAN production of all the fish tanks were evenly 3% of the total feed input. For example, tank I released 1.49 g TAN per day in 1800 liter of water. It means that 0.83 $mg.L^{-1}$ of TAN was added and diluted in the water of the fish tank.

The TAN production was similar to the assumption of Colt (1991) that waste output of fish consuming 1000 g feed and 250 g O_2 are 30 g of TAN and 340 g CO_2excreted via gill by ion-exchange along with 500 g fecal solid and 5.5 g PO_4-P. Then, Schneider et al. (2005) stated that the Fish-Biomass-Converter retains 20-50% feed N and 15-65% feed P. This means that 50-80% feed N and 35-85% feed P are discharged as waste.

Fish waste that was released in the water column was then recirculated and served as nutrient input for *Azolla*. The treatment with water flows showed no significant difference ($P < 0.1$) among 3 lpm, 5 lpm, and 7 lpm on doubling time of *Azolla*'s biomass (Figure 2).

Table 1. Feed consumption and TAN Production (P_{TAN}) in 30 days

Tank	Feed consumed (g)	P_{TAN} total (g)	P_{TAN} (g/day)
I	1519	44.7	1.49
II	1504	44.3	1.48
III	1313	38.6	1.29

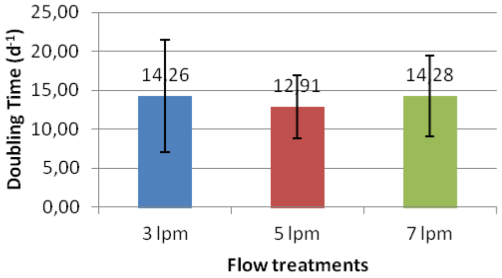

Figure 2. Doubling time of *Azolla* affected by water flow

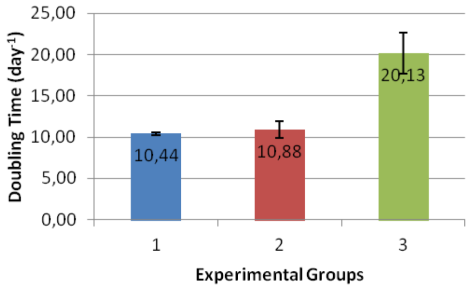

Figure 3. Doubling time of *Azolla* biomass affected by grouped-tank

Statistically, the significant difference in *Azolla*'s biomass production occurred on experimental group-tank factors. Differences in the growth rate of the *Azolla* biomass in each experimental group related to the baseboard designs. This allowed better nutrient supply in Tank I and Tank II compared to Tank III. In Tanks I and II, the average biomass growth was doubled from the initial population that occurred every 10.44 and 10.88 days while in Tank III was every 20.13 days (Figure 3).

The result is inversely proportional to the TAN reduction pattern, which means that during the 1st day until the 20th day there was active ammonia assimilation by *Azolla*, and at the peak multiplication of the biomass the assimilation rate of N decreases, resulting in the TAN concentration in the water to rise again.

Azolla has been known to have the ability to fix nitrogen from the air, so that it can survive and keep growing under low nutrient conditions in the water. However, from the results of this study, there is a correlation between minimal TAN concentration and the rate of assimilation of N by *Azolla*. The TAN concentration in water should remain at a value of> 0.1 $mg.L^{-1}$ to maintain the rate of assimilation of N. If the TAN concentration <0.1 $mg.L^{-1}$the assimilation of N tends to be slower or even stopped so that in this phase will result in cessation or decrease in growth rates of *Azolla*.

Based on the results of *Azolla* Total Kjeldahl Nitrogen (TKN) analysis on the 30th day showed that the average *Azolla* protein content in each treatment was different but not significant (P> 0.10). The level of protein content present in *Azolla* in this study was relatively good (28.8 %) compared to the results of the tests with the duckweed (*Lemna minor*) in the same experimental design which reached 25.7% (Sumoharjo 2015). Therefore, it could be an alternative feed for herbivorous fish such as tilapia. The high levels of this protein content are influenced by *Azolla*'s ability to convert nutrients from the water into *Azolla* biomass. The nitrogen retention by *Azolla* showed considerable value in each trial and showed significant differences between treatments as well as groups (Table 2).

The highest nitrogen retention was achieved by the 3 lpm treatment of 11.27 ± 6.95 gN, followed by the 5 lpm treatment of 9.23 ± 7.28 gN, and the lowest was the 7 lpm treatment which only retained N of 7.97 ± 6.22 g.

TAN removal efficiency

The TAN conversion rates were determined as overall retained nitrogen of *Azolla* from P_{TAN} of every tank as part of the experimental group (Figure 4). The results of this experiment showed that the efficiency of the TAN removal by *Azolla* was still lower than the treatment using *Lemna minor* which reached 48% but was much higher than *Spyrogyra* sp which retained 2.91% N of TAN produced by tilapia (Sumoharjo 2015). Determining how much TAN removal will greatly determine the potential level of the use of a phototrophic organism as a biofilter for the use of water quality management in RAS.

Water quality and nitrogen dynamics

Water quality characteristics, such temperature ranged between 27.3 to 30.7ºC, while pH and TAN tended to decrease during experiment (Figure 5). The proportion of NH_3 increased with higher pH. It could be because TAN enters the organism as NH_3 (Randall and Tsui 2002). Therefore, the toxicity of TAN (the total NH_3-N and NH_4^+-N) increased in line with the increase of pH of the water. Fortunately, pH during the experiment tended to decline from 7.9 ± 0.2 at the beginning to 6.6 ± 0.2 at the end of experiment. So that the proportion of un-ionized ammonia (NH_3) was low and in a tolerable concentration for Tilapia. The toxic level of NH_3 for short-term exposure usually are reported in between 0.6 to 2 mg.L^{-1}, while the maximum tolerable concentration is to be 0.1 mg.L^{-1} (Pillay 1992). Moreover, the specific growth rate (SGR) of tilapia exposed to un-ionized ammonia nitrogen over 0.068 mg NH_3 was significantly reduced. The specific growth rate and the increase of the unionized ammonia concentration increased the feed conversion ratio (El-Syafai 2004).

Table 2. Variance analysis of retained nitrogen by *Azolla*

Source	Degr. of	RN SS	RN MS	RN F	RN p
Treatments	2	16.4019	8.2009	8.7203	0.034805*
Groups	2	276.2516	138.1258	146.8737	0.000180*
Error	4	3.7618	0.9404		
Total	8	296.4152			

Note: *: Significant difference on 90% of reliability

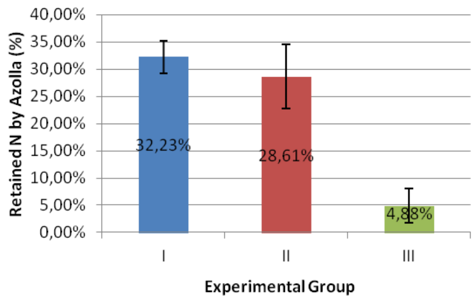

Figure 4. TAN Removal efficiency

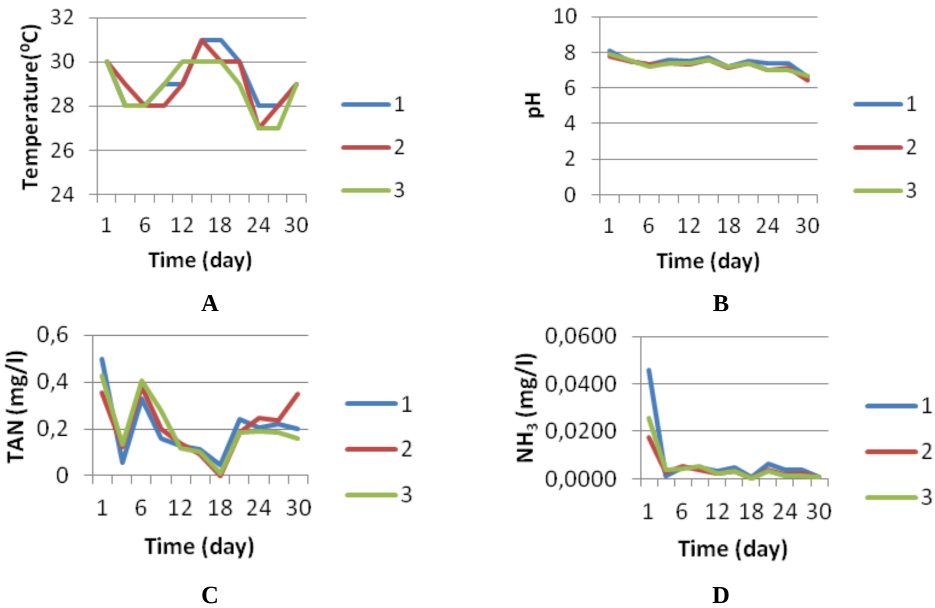

Figure 5. Water quality characteristics: A. Temperature, B. pH, C. TAN, D. The proportion of NH_3

Table 3. Means and standard deviations of water quality characteristics

Water quality parameters	Unit	Tank		
		I	II	III
Temperature	ºC	29.2±1.2	29.0±29.0	28.8±1.2
pH		7.4±0.4	7.3±0.4	7.3±0.3
DO	mg.L	4.1±1.4	3.6±1.2	4.0±1.7
CO2	mg.L	21.0±11.4	23.2±13.3	22.0±11.7
TAN	mg.L	0.20±0.1	0.21±0.1	0.20±0.1
NH3	mg.L	0.007±0.013	0.004±0.005	0.005±0.007

Carbon dioxide (CO_2) is another factor that may affect feed behavior (Trand-Duy et al. 2008). In an intensive culture system, CO_2 may not have an adverse effect on fish unless its concentration reaches 100 $mg.L^{-1}$ (Balarin and Heller 1982). Nile tilapia can tolerate CO_2 concentration above 20 $mg.L^{-1}$ (Wedemeyer 1996). In this study, CO_2 was 30.2±3 $mg.L^{-1}$ on the first day then decrease to 15.2±1.1 on the 30^{th} day of the experiment (Table 3). CO_2 tended to decrease during the experiment because the turbulences that occurred in inflow and outflow of the *Azolla* reactor may strip CO_2 to atmosphere. Moreover, algae and *Azolla* thrived in the reactors play a role in removing CO_2 out of the system.

Concentration of dissolved oxygen (DO) during the experiment ranged between 2,2 to 6,2 $mg.L^{-1}$(Table 3). The lowest DO concentration occurred on the last day of the experiment. Accumulation of sludge in the *Azolla* reactor played a role in decreasing DO gradually. This may happen because there is no sludge disposal from the system. DO should be maintained above 3.0 ppm and 5.0 ppm for warm and cold-water fish, respectively (Buttner et al. 1993). However, most species of fish are distressed when DO falls to 2-4 $mg.L^{-1}$ (Floyd 2003).

The lower extreme value of DO (less than 0.8 $mg.L^{-1}$) was obtained from an experiment in which there were no significant differences between the yields of Nile tilapia raised in ponds with two aeration regimes (Teichert-Coddington and Green 1993). Thus, practical threshold of DO for Nile tilapia was not higher than 10% of saturation (0.8 $mg.L^{-1}$ at 26°C) (Trand-Duy et al. 2008).

In conclusion, *Azolla microphylla* can be grown well in RAS. The assimilation rate of TAN by *Azolla* decreased after its peak biomass production (when doubling time was achieved). Therefore, harvesting must be done 15 to 18 days after cultivation. As a biofilter, it provides a mini-ecosystem that serves as nutrient controller for aquaculture practices. The lower water flow rate the higher nitrogen retention, although, there was no significant effect of water flow rates on the *Azolla* growth response. It has enough protein content, hence has potential as feed source for herbivorous fishes.

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Short Communication: Presence of arbuscular mycorrhiza in maize plantation land cultivated with traditional and improved land management

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Abstract. *Ishaq L. 2018. Short Communication: Presence of arbuscular mycorrhiza in maize plantation land cultivated with traditional and improved land management.Trop Drylands 2: 20-24.* Arbuscular mycorrhizas (AM) are one of the key functional soil biota that can greatly contribute to crop productivity and agricultural sustainability. Their presence could be influenced by soil conditions, such as land management, and the sporulation of the fungal forming mycorrhiza could be affected by season, i.e., rainy, and dry seasons. Previously, it was found that the abundance of AM fungi was higher under maize cropping with traditional land management (no-tillage, no input of agrochemicals) than that under maize cropping system with more modern land management (tillage, agrochemical input), when the soils were sampled at rainy season. As the presence of AM fungi could be influenced by climate factors i.e., rain/season, the present study was carried out to examine the abundance and diversity of AM fungi in maize plantation land cultivated with traditional and improved land management in the dry season. At each land management system, three locations of study were purposively selected, and at each location within the cropping system, three soil samples were collected. A total of 9 soil samples representing each land management system were evaluated. The variables observed included AM fungal spore density (expressed as the number of AM fungal spores per 100 g of soil) and biodiversity of AM fungi (expressed as relative abundance of AM fungal spore). It was found that the density of AM fungal spores under a maize cropping system with traditional land management was 203.55 per 100g, whereas only 84.91 spores per 100 g soil were found under a maize cropping system with improved land management, supporting the previous findings. Despite only six AM,0 fungal spores morphotypes were observed under maize plantation land of both traditional and improved land management, the abundance of the morphotype was different between the two land management systems. The study needs to be extended at a broader location and time to get more representative information on the impact of land management on the abundance of AM fungi in tropical agriculture.

Keywords: Arbuscular mycorrhizal fungi, spore density, relative abundance, maize, land management

INTRODUCTION

Maize is an important economic crop in East Nusa Tenggara. There are two types of land management commonly practiced by local farmers for maize production in the region, namely traditional and improved land management. At the traditional land management, farmer usually grows maize without any inputs of agrochemicals and tillage is not practiced on the land, whereas on the improved land management, farmers tillage the land before planting and commonly apply agrochemicals including inorganic fertilizer and pesticide to increase maize productivity. As the land management between these two types of maize cropping systems is different, it is likely that the impact it may have on the soil condition, particularly on soil beneficial microorganisms such as arbuscular mycorrhizal fungi possibly be different.

Arbuscular mycorrhiza (AM), a mutualistic symbiosis between fungi and higher plants, is one of the most ubiquitous soil beneficial organisms. Mycorrhizal fungi have multiple ecological functions including improving the absorption of mineral nutrients and water to their host plants (Smith and Read 2008), enhancing plant resistance to pathogens (Song et al. 2015), improving plants tolerance to environmental stresses such as drought, salinity and heavy metals (Porcel and Ruiz-Lozzano 2004; Gohre and Paszkowski 2006; Kaya et al. 2009), and maintaining soil structure in agricultural soils that is important for land sustainability (Jeffries et al. 2003).

There are some factors that might influence the association between mycorrhizas and their host plants such as fungi forming mycorrhizas, plants/host, and environmental conditions (Smith and Read 2008). Environmental conditions could affect population, diversity, and distribution of AM fungi (Brundrett 1991; Sieverding 1991). For example, climate and edaphic factors, and physicochemical edaphic factors have been related to sporulation and colonization of AM fungi (de Oliveira and de Oliveira 2010; Panwar et al. 2011). In agricultural ecosystem, in particular, the population and diversity of AM have been reported to be influenced by land management (Sieverding 1991; Ezawa et al. 2000; Oehl et al. 2003; Kabir 2005).

Previously, a study on the abundance of AM associated with corn planted with traditional and more modern farming system in Kupang District East Nusa Tenggara

Timur, found that the spore density of AM under a more modern farming system was lower than those found under a traditional farming system (Ishaq et al. 2017). Since AM fungal colonization and spore numbers could be influenced by season (rainfall) (de Oliveira and de Oliveira 2010), the current study extended the previous study, when was conducted in rainy season, to be repeated in dry season. The present study, therefore, was aimed to evaluate the presence of AM fungi in the land of maize cropping system with traditional and improved land management systems in the dry season.

MATERIALS AND METHODS

Study location

East Nusa Tenggara, Indonesia is a semi-arid region with a 3-4 month rainy season (December-March/April) and 8-9 months dry season. The average annual rainfall ranges between 1250-1500 mm per year. In Kupang District, the soils are calcareous that are rich in base cations mainly calcium and magnesium, but commonly low in nitrogen and available phosphorus.

Soil samples were collected from the land where maize is grown with both traditional and improved land management. The term “improved land management” is used just to emphasize that tillage and agrochemical inputs (inorganic fertilizer and pesticides) are applied in the farming system. In both farming systems (traditional and improved land management), planting mainly depends on rain as water source. due to unavailability of the irrigation system to support the farming systems.

Soil samples were collected in the dry season of November 2015. During the dry season, no crops were cultivated including maize due to water limitation. Previously, the soil was sampled around the rhizosphere of maize in the presence of maize as the AM host plant. Whereas in the present study, the soil was sampled in the absence of the host plants. It was possible to evaluate the presence of AM in the study area in the absence of maize as the main host of AM. This is because the roots of the host plant were not removed from below ground when harvest. In each type of land management, soils were sampled at three different locations (each type of land management consisted of three different locations; 6 locations in total). For the land where maize is usually cultivated with improved land management, soil samples were collected from Noelbaki, Baumata and Tarus Villages, whilst for the land where maize is grown with traditional land management; soil samples were collected from Kolhua, Sikumana and Noelbaki Villages. Before sampling the soil, survey and interviews were conducted to obtain information on land management. At each location, soil samples were diagonally taken. Soil samples were collected around the plant at a depth of 0-20 cm. The sampling position was plotted using GPS coordinates, and the other nearby vegetation (other than maize) was recorded. Spore extraction was conducted at the Laboratory of Microbiology of Agricultural Faculty of Nusa Cendana University, Kupang, Indonesia.

Extraction of AM fungi spores

Spores of AM fungi were extracted from soil samples using wet-sieving and sucrose based on the methods described by Brundrett et al. (1996). Briefly, soils samples (100 g) were suspended in 500 ml water, stirred for 7 mins, and decanted through 500, 250, 150 and 45 μm sieves. This washing and decanting process was repeated until the water was clear. Soils filtered on fine screens (250, 150 and 45 μm) were transferred into a 50 ml tube, and then centrifuged for 5 mins at 2500 rpm to remove organic matter. The supernatant and floating debris were discarded, and the pellets were resuspended in 60% sucrose then centrifuged again for 2 mins at 2500 rpm to separate spores from the denser soil components. The spores in the supernatant were placed on 45 μm and washed with water to remove the sucrose. The spores were transferred on a Buchner funnel underlined with Whatman paper (No. 41). The spores were collected and counted under a compound microscope. For biodiversity calculation, the spores were grouped into morphotypes based on spore appearance (color, size, surface of the spore and sub-tending hyphae).

Observation and data analysis

Data of spore density were log (x+1) transformed before being analyzed. The data was analyzed using Nested (Hierarchy) Analysis of Variance design where the locations of study were nested within the land management factor. AM fungal spores were isolated and then counted manually under a compound microscope (Leica Galen III). Broken spores were not included in the isolation process and calculation. The spores were grouped into morphotypes based on criteria as described above. Spore density was expressed as the number of spores in 100 g soil, whilst biodiversity was measured as relative abundance. Relative abundance was expressed as percentage of spore number of a morphotype in each site over total spore in each site.

RESULTS AND DISCUSSION

Nested ANOVA analyses showed that the location within each cropping system where the soil samples were collected had no significant effect on the spore density of AM fungi. At the three locations of maize cropping system with traditional land management, the average AM fungal spore density found in location 1 (Kolhua) was 7.0. That was not significantly different from AM fungal spore density found at location 2 (Sikumana) and location 3 (Noelbaki) with 6.63 and 6.66 spores per 100 g soil, respectively [(data were log (X+1) transformed)]. Similar findings were also observed at the three locations where maize was grown with improved land management. The average spore density found in location 1 (Baumata) was 4.67 spores per 100 g soil; that was not significantly different from AM fungal spore density found in location 2 (Noelbaki) and location 3 (Mata Air) with 5.81 and 6.24 spores per 100 g soil, respectively [(data were log (X+1) transformed)].

On the other hand, when comparing the spore density of AM fungi between the two types of land managements (traditional and improved land management systems) the result of nested analysis showed that AM spore density between the two land management systems was significantly different. The spore density of AM fungi in soil samples taken from the land where maize is cultivated with traditional land management was significantly higher ($P<0.05$) than the spore density of AM fungi found in soil samples taken from the land where maize is cultivated with improved land management. The spore density of AM fungi in soils sampled from maize plantation land cultivated with traditional land management was 2.25 [(log (X+1) transformed)] spores per 100 g soil), whilst only 1.6 spores [(log (X+1) transformed)] per 100 g soil of AM fungi were found in soil sample taken from maize plantation land cultivated with improved land management (Figure 1).

When comparing the density of AM fungi observed at the current study with that of the previous study (Ishaq et al. 2017), it was discovered that the number of AM fungal spores found in this study tended to be higher than the previous study, both under traditional and improved land management systems. In the previous study (Ishaq et al. 2017), the averages of spore density at traditional and more modern maize cropping system were 88.53 and 43.1, respectively. The averages of AM spore density under maize cropping systems in the present study are respectively, 203.7 and 84.9 spore/100 g soil for traditional and improved land management.

Six types of AM fungal spore morphotypes were found in this study at each land management, namely yellow, black, orange, white, rough yellow surface, and reddish color. The relative abundance of the morphotypes found in this study is described in Table 1, whilst examples of the morphotypes are illustrated in Figure 2. Although all the six morphotypes were observed at both traditional and improved land management systems, the relative abundance of the morphotype was different between the two land management systems. Morphotype 4 (white) was more abundant in the soil taken from the maize cropping system with traditional land management, contributed to 51.36% of the total morphotypes observed. This was followed by morphotype 1 (yellow) with 25.03%. Whereas soil taken from maize plantation land cultivated with improved land management, morphotype 6 (reddish) was dominant and accounted for 45.4% of the total morphotypes observed, followed by morphotype 1 (yellow) with 18.8%.

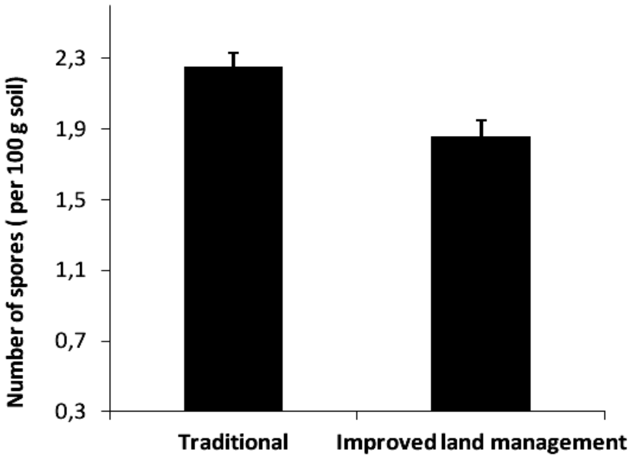

Figure 1. Spore density of AM fungi in soil sampled from the land where maize is cultivated with traditional and improved land management. Values are means (n=9) ± SE. Data were log (X+1) transformed

Table 1. Spore density of morphotype and the relative abundance (RA) of the morphotype found under maize cropping system with traditional (A1, A2 and A3) and improved land management (B1, B2 and B3). A is the total of A1, A2 and A3, and B is the total of B1, B2 and B3

Morphotype	A1	RA	A2	RA	A3	RA	B1	RA	B2	RA	B3	RA
1 (Yellow)	112	14.04	227	45.77	121	22.24	25	22.9	38	13.4	81	21.83
2 (Black)	16	2.0	1	0.202	31	5.69	5	4.59	27	9.51	27	7.278
3 (Orange)	48	6.01	1	0.202	56	10.29	9	8.26	13	4.58	91	24.53
4 (White)	530	66.42	200	40.32	214	39.33	2	1.83	0	0	86	23.18
5 (Yellow rough surface)	0	0	7	1.41	9	1.65	7	6.42	6	2.11	0	0
6 (Reddish)	92	11.53	60	12.1	113	20.77	61	56	200	70.4	86	23.18
Total	**798**	**100**	**496**	**100**	**544**	**100**	**109**	**100**	**284**	**100**	**371**	**100**

	Traditional land management		Improved land management	
Morphotype	**A**	**RA (%)**	**B**	**RA (%)**
Yellow	460	**25.03**	144	**18.8**
Black	48	2.61	59	7.72
Orange	105	5.71	113	14.8
White	944	**51.36**	88	11.5
Yellow (rough surface)	16	0.87	13	1.7
Reddish	265	14.42	347	**45.4**
Total	**1838**	**100**	**764**	**100**

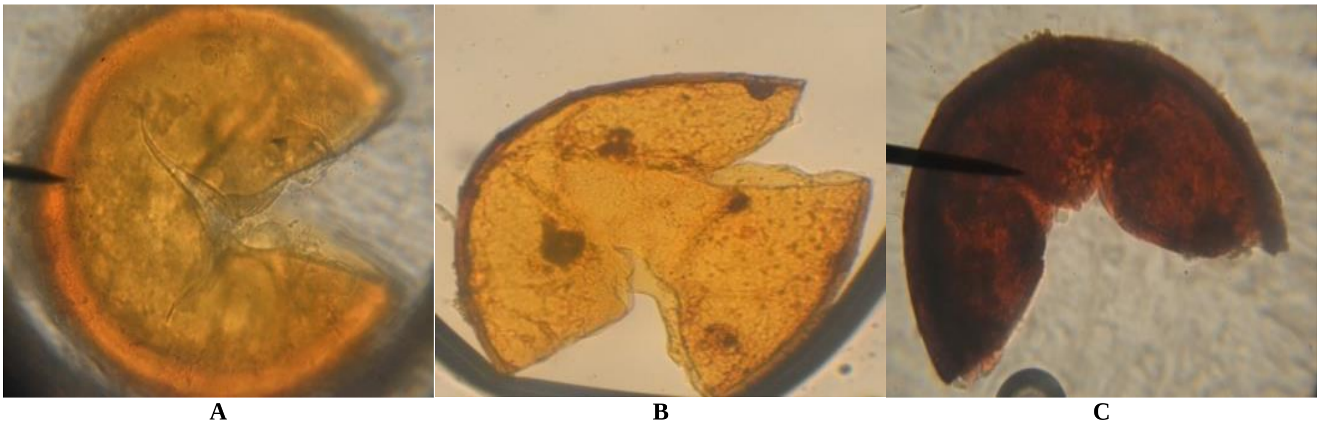

Figure 2. Examples of morphotype found; A. Morphotype 1 (yellow, round, 124.32-365.97 µm), B. Morphotype 5 (rough yellow surface, round, 142.33-302.23 µm), and C. Morphotype 6 (reddish, round, 144.83-384.43 µm)

This study found that the spore density of AM fungi was affected by land management. The spore density in the soil taken from maize plantation cultivated with traditional land management, was higher than that found in the soil taken from maize plantation with improved land management. The result was consistent with what was previously found under these two cropping systems (Ishaq et al. 2017). The effect of land management on AM sporulation has also been reported by other authors (Oehl et al. 2003, 2010; Schalamuk and Cabello 2010). Many factors can influence distribution and community structure of AM fungi such as climatic and edaphic factors, spatial and temporal variations, vegetation, disturbance, and sporulation ability of AM fungal taxa (Dandan and Zhiwei 2007). Soil disturbances such as tillage (Entry et al. 2002; Kabir 2005; Schalamuk and Cabello 2010), long-term use of inorganic fertilizer (Rubio et al. 2003; Bhadalung et al. 2005), and pesticides (Entry et al. 2002) have been reported to have a negative impact on AM communities. However, other soil factors, such as: soil type and land use intensity (Oehl et al. 2010), physicochemical edaphic properties (Panwar et al. 2011) and climate factors (de Oliveira and de Oleivera 2010), may also determine the sporulation or composition of AM fungal communities. In this study, the lower AM fungal spore density found under the maize cropping system with improved land management, might be related to soil factors such as soil disturbance, agrochemical inputs and soil organic carbon. However, the sporulation might be also related to ability of AM fungal taxa to sporulate at certain times and conditions.

When comparing the density of AM fungi observed in the current study, with the result observed previously (Ishaq et al. 2017), the number of AM fungal spores observed in this current study tended to be higher in both traditional and more modern land management as compared to the previous findings. The higher AM spore density found in this study might be related to climatic and edaphic factors. Previously, the soil samples were collected at the end of rainy season when the soil moisture was still high. The AM types that are sensitive to high moisture might have not been able to sporulate at that time of soil sampling. In this study, the soil samples were collected in dry season when the soil moisture was low, possibly the soil conditions were more favorable for those of high-moisture sensitive types of AM to sporulate resulting in more abundance of spore observed. Furthermore, the fungal species observed may be active at a specific time/condition, being capable of colonizing and multiplying themselves.

Related to diversity of AM fungi, it seemed that the number of AM fungal morphotypes found was not quite distinct between the two land management systems, in which only six morphotypes were observed. The result would be plausible as the main host for AM observation in the study was the same (maize crops). The similar morphotypes found under these two land management systems may also indicate that these morphotypes are general AM colonizers for maize as host plants.

Despite only a small number of morphotypes found in the study, interestingly, the dominance of the morphotype was different between the two land management systems. Morphotype 4 (white) was a dominant colonizer under maize cropping system with traditional land management, whereas morphotype 6 (reddish) was a dominant colonizer under maize cropping system with more modern land management. Morphotype 2 (yellow) seemed to have consistent sporulation under both traditional and more modern land management systems. There are some factors that may influence sporulation of AM fungi including the fungi forming mycorrhiza, host plant and environmental factors such as soil properties and land management (Brundrett 1991; Sieverding 1991). The results found in this study could indicate that sporulation and dispersal ability between the fungi/morphotypes found are different. However, it would also be possible that the soil condition under maize cropping system managed with traditional and more modern land management systems may differently affect the ability of the fungi/morphotype to sporulate and to disperse. Since land management could affect the soil condition, particularly the soil physical and chemical properties, more studies must be set up to isolate the main factor that modulates the abundance and distribution of AM fungi at maize rhizosphere.

The result of this study may provide useful information on the impact of land management on distribution and community structure of AM fungi in semi-arid agricultural land. However, since AM fungal sporulation can be affected by many conditions, it is impossible to draw a general conclusion based on this study. Therefore, further studies need to be undertaken to deeply investigate other soil factors that might be impacted by various land management systems. Considering low morphotypes of AM fungi observed in this study, future studies need to be undertaken to combine both wet sieving and trapping culture methods to assess sporulation and diversity of AM fungi. Additionally, when it is possible, studies should investigate a molecular technique to detect sporulation and colonization of AM fungi. This may provide more information on AM fungal community between the two land management systems.

In conclusion, land management could affect the abundance and distribution of AM fungi, however many other factors might be also involved. Due to the important role of AM fungi for ecological functioning, more studies need to be undertaken to gain a better understanding of the effect of land management on AM fungi and on the interaction between AM fungi and their host. This may be helpful to develop methods for utilizing mycorrhizae in sustainable agriculture.

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Effect of fertilizer-N and organic resource management on soil aggregates formation and carbon cycling in the central highlands of Kenya

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Abstract. *Njoroge KS, Mugendi DN, Vanlauwe B. 2018. Effect of fertilizer-N and organic resource management on soil aggregates formation and carbon cycling in the central highlands of Kenya. Asian J Agric 2: 25-38.* The objective of this research was to inquire about the way the alteration of soils of various textures and fertility values with fertilizer-N and organic resources influence aggregate formation and subsequent carbon (C) cycling in aggregates. The experiment was carried out in the Embu and Machang'a regions of central Kenya and was rooted in a putrefaction tube experiment that was established in April 2005. This experiment was intended at completing long-term field experimentations initiated in 2002 to set up the impact of the application of various combinations of organic and mineral resources on soil nutrient status. The main variables were fertilizer-N and organic resources, with the sub-management being soil fertility values. Maize stover and urea fertilizer were mixed with 3.2 kg of soil and put in putrefaction tubes. The implementation rate was 4 tons and 120 kg per hectare, for the maize stover and fertilizer respectively. Four management techniques were tested, namely: control (no organic resources or fertilizer-N added), lone fertilizer-N, lone stover, and combined stover and fertilizer-N, with each management having three replicates. To decide the alterations in soil aggregates, dirt samples acquired from the putrefaction tubes were fractionated through wet sieving. SOM fractionation was also carried out to gain the various SOM fractions. All achieved aggregates and SOM fractions were then dried in the oven, ground, and analyzed for C. All collected data were examined with the PROC MIXED procedure of SAS and the means separated at $p < 0.05$. Bigger quantity of macroaggregates from Embu soils was studied than that from Machang'a soils. For both Embu and Machang'a soil, the alteration of soils with lone or combined fertilizer-N and organic resources had a notable impact ($p < 0.05$) on the portions of all aggregate class sizes. The alteration of soils of various fertility values with lone or combined fertilizer-N and organic resources also had a notable impact on the allocation of SOM fractions for both Embu and Machang soils. Remarkable discrepancies in whole soils, aggregates and SOM fractions percentage carbon values were also studied. Overall, the silt and clay fraction had higher C values than that in other aggregate size classes representing higher stabilization of C within this fraction. From the results of this research, it is concluded that the utilization of combined organic and mineral resources is preferred for the enhancement and the preservation of soil fertility in high fertility soils. In low fertility coarse-textured soils, the lone implementation of organic resources is suggested for the enhancement and preservation of soil fertility.

Keywords: Carbon cycling, fertilizer, Kenya, nitrogen, soil organic, soil aggregate

INTRODUCTION

Sustainable food manufacturing depends fervently on the keeping of adequate values of soil fertility. This can only be gained by keeping the equilibration of nutrient outputs and inputs (Kirchmann and Thorvaldsson 2000). In Sub-Saharan Africa (SSA), this equilibration is hardly reached since the input of nutrients both in the form of mineral fertilizers or organic are low. This is mostly caused by the sumptuous price of fertilizers due to the liberalization of fertilizer trade and the introduction of structural adjustment programs (SAP) (Ayuke et al. 2004) inflicting very low values of fertilizer utilization. Current estimations for fertilizer utilization in Africa are 9 kg ha-1 compared to 87 kg ha-1 in the developed countries (Bationo et al. 2004). As for organic inputs, their efficacious utilization in soil fertility increase is restricted by their low or disproportionate nutrient rate, poor quality, and the existence of other rival usages such as the utilization as livestock feed (Palm and Rowland 1997). With such low external input numbers, soil fertility decrease lasted bringing to low farm productivity and extensive famine in the continent. Newest statistics display that some 200 million people or 28% of Africa's inhabitants are chronically in need of food (Bationo et al. 2004).

The same circumstance has been detected in Kenya, where crop harvest in most parts of the country is low caused by a decrease in soil fertility. This is often a result of perpetual farming and no application of fertilizers by farmers. Low soil fertility has a tendency to decrease further as farmers remove many nutrient outputs in crops yield, crop residues and through losses such as leaching, and soil erosion processes (Kathuka et al. 2007). For example, in Embu district of Central Kenya as reported by Lesschen et al.(2003), nutrient equilibration of N, P and K were-55,-9 and-15 kg per hectare, respectively, in 1998 and in 2003, nutrient equilibration had reduced to-116.2,-22.1 and-31.7 per hectare for N, P and K, respectively. Therefore, there is an urgent need for the establishment of advantageous and inexpensive soil management methods that shall sufficiently overcome the issue of soil fertility reduction. One way to be done by smallholder farmers in

SSA to restore soil fertility in their farms is the application of methods of integrated soil fertility management (ISFM). These methods combine the organic and inorganic resources of fertility. These are reasonable methods since various nutrient resources are mostly obtainable for resource poor farmers but are utilized poorly due to the lack of knowledge or other limitation such as labor (Delve 2004). In these methods, fertilizers serve as a nutrient resource for plants and organic resources act as forerunner of soil organic matter (SOM) which keeps the physical and physicochemical components of soil fertility such as cation exchange capacity (CEC) and soil contexture (Vanlauwe et al. 2002). Organic resources also enhance soil contexture by increasing aggregate establishment, as they act as a resource of carbon compounds performing a major role in tying up individual soil particles into microaggregates, and subsequently tying up these microaggregates into macroaggregates (Blair et al. 2005). This is significant since soil accumulation governs the extent of soil properties such as aeration, water percolation, and drainage. These further govern plant development and accretion, while also supplying habitat for soil biota (Denef et al. 2002). Escalated soil aggregation also enhances rates of SOM as soil aggregates physically secure SOM within their structure, thereby decreasing its putrefaction rate (Alvarez et al. 1998). It further decreases the discharge of carbon (C) to the atmosphere, as soil aggregates in SOM establish temporary C pools by stabilizing C within their structure (Six et al. 2002).

For best use of organic and mineral resources in soil fertility enhancement, it is necessary to set their usage in agroecosystems that vary in terms of climate and soil texture. SSA is characterized by heterogeneity both in terms of climate and soil characteristics. One way to achieve it is by examining the putrefaction patterns of organic leftovers as it is through this process that nutrients kept in the remains are discharged into the soil (Palm 1995). Although the putrefaction number of organic leftovers is principally adjusted by the quality of the leftovers (Palm 1995), climatic circumstances and soil texture. The central plateaus of Kenya are marked by a high-density population that has created high pressure on the limited land resources. This has brought to the escalation of land fragmentation and perpetual cultivation with, frequently, minimal or no implementation of external nutrient inputs. This has created perpetual SOM and nutrient decimation in the soil leading to decreased crop harvests. Further, the capability of the land in sustaining other ecosystem services such as nutrient retention and liberation, water keeping and percolation, root breach among others have been compromised. The decrease in farm harvests has brought to the anxiousness for food availability and decreased farm earnings in this zone, leading to raised occurrences of starvation and penury. The attempts to restore soil fertility in these regions have mainly depended on the usage of organic and mineral inputs. The success of this has, however, been hindered by the decreased presence of the former and the high cost of the latter. There is, therefore, a requirement for the establishment of a more supportive and inexpensive method of soil fertility replacement.

The use of organic and mineral resources in combination is one method used by farmers in this region to manage decreased soil fertility. This is an achievable method because various organic and mineral resources in various quantities are available for farmers in the region. The prudent utilization of these two nutrient resources leads to the careful act of ameliorating plant nutrient provision while at the same time enhancing SOM values and the associated ecosystem services.

Even though, research has listed various advantages about the combined use of organic and mineral resources, little efforts have been done to specify the impact of soil fertility on the utilization of organic and mineral resources with consideration to the putrefaction of organic resources. This is significant, as the amount of organic resources putrefaction affects the establishment of SOM, aggregate establishment and hence the stabilization of carbon inside aggregates. Such research would be of great significance, as the central plateaus of Kenya are characterized by heterogeneity in relation to soil fertility degrees. In accordance with this, this research tries to specify the impact of organic and mineral alteration on aggregate establishment and C cycling to soils of different fertility extents in the central plateaus of Kenya.

The research aims to: (i) To specify the impact of the alteration of soils of various texture and fertility extents by fertilizer-N and organic resources on aggregate establishment. To specify the impact of the alteration of soils with various composition and fertility extents by fertilizer-N and organic resources on the allocation of obvious SOM fractions. (ii) To specify the impact of the alteration of soils with various composition and fertility extents by fertilizer-N and organic resources on C stabilization in soil aggregates.

MATERIALS AND METHODS

Experimental sites

This research was performed in two sites in central Kenya, namely, Embu and Machang'a. The two sites contrast in terms of rainfall, temperature and soil types as depicted below.

The Embu site is situated in Embu district (Central Kenya), at '0° 30' S, 37° 27' E and at an altitude of 1480 m above sea value. The area has a humid climate, with an average temperature of 20°C. Rainfall is bimodal with the long rains falling from March to May, and the short rains falling from mid-October to December. The average annual rainfall is about 1200 mm. The soil in Embu is a clay loam (sand 32%, silt 30%, and clay 38%) derived from basic volcanic rocks. The soil is classified as Humic Nitisols (FAO 1990) and has Kaolinite as the dominant clay mineral. Livelihoods of the population came from subsistence mixed farming consisting of the growing of cash and food crops and the keeping of dairy animals, which is the most important farming system in this area. The most important cash crop is coffee *(Coffea arabica)*, while the main food crop is maize (*Zea mays*).

The Machang’a site is situated in Mbeere district. It lies at '0˚47'S, 37 ˚40' E, and at an altitude of 1050 m above sea value. The area has a semi-arid climate, and the soil is a sandy clay loam consisting of 56.5%, 12.7% and 30.8% of sand, silt, and clay, respectively. It is classified as a Chromic Cambisol (Kamoni et al. 2007). The area has an average annual temperature of 26°C, while the average annual rainfall is 700mm. The common farming system in this area consists of subsistence mixed farming of food crops such as maize (*Zea mays*), beans *(Phaseolus lunatus)* and black peas *(Vigna unguiculata)* and cash crops such as coffee *(Coffea Arabica)*.

Experimental design

This research was based on a putrefaction tube experiment that was held in April 2005. The goal was to complete long-term field trials with the aim of establishing the impact of implementation of various compositions of organic plus mineral resources on soil nutrient status.

Materials and procedure

The putrefaction tubes were made of polyvinyl chloride (PVC) as shown in Figure 1.A, with a diameter of 10 cm and a length of 35 cm. All the tubes had an iron mesh at bottom to prevent soil from falling out from tubes. The tubes were also covered with an iron mesh at the top to prevent the entry of any substances such as litter, etc. The organic and inorganic resources were mixed with 3.2 kg of soil and placed into the putrefaction tubes which were then placed in holes measuring 15 cm in diameter and 30 cm in depth. The organic resources were in the form of maize stover applied at a rate of 4 tons per hectare, while the mineral resources were in the form of urea applied at a rate of 120 kg per hectare (Table 1). The organic resources were mixed by hand while the fertilizer was mixed in liquid form during the arrangement of the experiment.

Design of the experiment

The experiment was arranged as a randomized complete block design. Soils of various fertility values; low, medium, and high fertility were acquired from farms around the experimental locations, mixed with sole or combined organic and mineral resources, and arranged in putrefaction tubes in the respective sites as shown in Figure 1.B. The experiment consisted of four management techniques namely, control (no stover or fertilizer-N is applied), sole stover, sole fertilizer, and stover plus fertilizer. Each management had three replicates, amounting to a total of 36 tubes per site.

Soil sampling

Soil sampling for the baseline study was performed at the onset of the experiment in 2005. Soil samples were gathered from farms near each of the two locations, and the chemical and physical soil characterization was performed. Based on the results of the soil characterization, soils were categorized as low, medium, and high fertility. The physical and chemical characteristics of these soils are shown in Table 2.

Soil sampling from the putrefaction tubes was performed in December 2006. The soil was sieved through an 8 mm sieve, and subsamples of approximately 2 kg were acquired for SOM fractionation. The soil was then packed, air-dried and stored at room temperature in readiness for physical fractionation through wet sieving.

Table 1. Management structure for Embu and Machang'a tube experiments managements.

Management	Quantity of organic resources applied (t ha^{-1})	Quantity of inorganic resources applied (kg ha^{-1})
Control	0	0
Fertilizer-N	0	120
Maize stover	4	0
Fertilizer-N plus maize stover	4	120

A B

Figure 1.A. A putrefaction tube set up in the field. B. A section of the field layout shows the set-up of putrefaction tubes.

Table 2. Chemical and physical characteristics of soils sampled in Machang'a and Embu, Kenya in April 2005.

Site	Fertility	% C	% N	C: N	P ppm	pH	EX K (cmol/kg)	EX Ca (cmol/kg)	EX Mg (cmol/kg)	EX Na (cmol/kg)	CEC (cmol/kg)	Sand %	Silt %	Clay %
Machang'a	Low	0.33	0.03	11: 1	4.0	6.60	0.90	8.01	2.25	0.12	13.5	75.1	10.0	14.9
	Medium	0.44	0.04	11: 1	4.5	5.84	2.44	12.85	3.51	0.25	25.5	75.1	14.9	10.9
	High	0.92	0.08	12: 1	56.5	7.12	2.02	18.13	4.44	0.12	26.0	81.1	10.0	8.9
Embu	Low	1.58	0.17	9: 1	1.0	6.48	0.37	2.09	0.70	0.83	4.0	17.2	30.0	52.8
	Medium	2.77	0.26	11: 1	4.5	6.92	0.68	6.01	1.03	0.59	8.5	17.1	16.0	66.9
	High	3.41	0.33	11: 1	3.0	6.50	0.92	8.28	2.63	0.11	14.0	15.2	16.0	68.8

Laboratory analysis

Aggregate formation and carbon cycling

To specify the impact influence of fertilizer-N and organic resources combination on aggregate formation and C cycling. Soil samples taken from the field and from the putrefaction tubes were examined using physical fractionation methods (Elliot 1986) into the various fractions.

Aggregate division

Soil samples were divided into four aggregate size fractions through wet sieving into large macroaggregates (>2000 µm), small macroaggregates (250 µm-2000 µm), microaggregates (53-250 µm), and silt + clay associated particles (<53 µm) (Elliot 1986). An 80 g subsample of soil was evenly spread over a 2 mm sieve submerged in 1cm of deionized water for 5 minutes. The soil was subsequently sieved by manually moving the sieve up and down 50 times during a 2-minute period. Soil remaining on the sieve was backwashed into a pre-weighed beaker for drying, while the soil and water that passed through the sieve were transferred to a 250 µm sieve and the sieving procedure was repeated. Using the 53 µm sieve, the procedure was repeated. To subsample the silt and clay fraction, a bottle-based method was used to obtain a 250 ml subsample. The four aggregate fractions were oven-dried at 105°C and weighed. All fractions were then pulverized and analyzed for C.

Soil organic matter fractionation

Following the method described by Six et al. (2000b), subsamples from the small and large macroaggregates were taken to isolate the microaggregates held within macroaggregates, to acquire the SOM fractions. A micro aggregate isolator, (Figure 2) was used to entirely break up macroaggregates while minimizing the breakdown of microaggregates. This method was applied to each soil to allow for total dispersion of the macroaggregates without disrupting the microaggregates within macroaggregates.

For Embu soils, 5 g subsamples were immersed in 50 ml of deionized water for one night. The following day, the subsamples were submerged in deionized water on top of a 250 µm mesh screen and gently shaken on a reciprocal shaker with 50 glass beads (4 mm diameter) for 5 minutes at 250 rpm. A perpetual and constant stream of water through the device was kept on, making certain that microaggregates were immediately flushed into a 53 µm sieve and not further disrupted by the glass beads. Microaggregates collected on the 53 µm sieve were further sieved according to Elliot (1986) to make certain that the collected microaggregates were water stable.

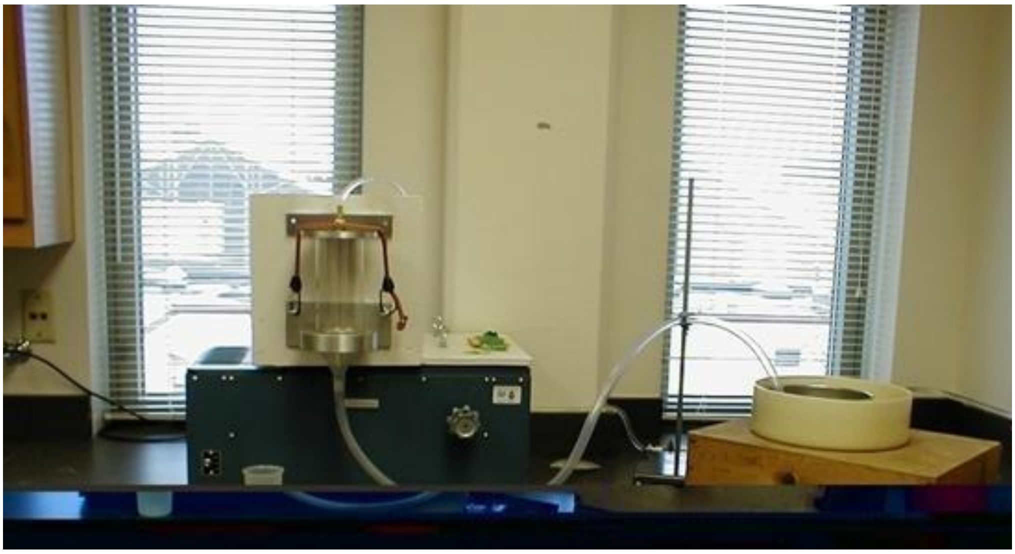

Figure 2. A microaggregate isolator set up on a reciprocal shaker

Three fractions were achieved: coarse particulate organic matter (> 250 µm), microaggregates within macroaggregates (53-250 µm), and silt and clay (< 53 µm). The fractions were then oven dried at 105°C, weighed, pulverized, and analyzed for C.

For the Machang'a samples, a different procedure was utilized since macroaggregates from sandy soils are less stable. A 15 g sub-sample of macroaggregates was applied for the isolation process. These sub-samples were immersed in deionized water for 20 minutes and then shaken for 3 minutes at 150 rpm.

Statistical analyses

The collected data was studied using the SAS PROC MIXED procedure. The means were then separated at $p < 0.05$ and the standard error of difference (SED) of the means was utilized to compare the responses between sole and combined application of fertilizer-N and organic resources.

RESULTS AND DISCUSSION

General overview

This chapter presents the results of the study. It is divided into three sub-sections. The first section reviews the impact of the alteration of soils with fertilizer-N and organic resources on soil aggregates proportions. The second section reviews the impact of these alterations on the distribution of distinct SOM fractions within macroaggregates. Finally, the third and last sub-section reviews the impact of these alterations on the carbon contents of whole soils (unsieved soil), aggregates, and SOM fractions.

Effect of alteration of soils of various texture and fertility values with fertilizer-N and organic resources on aggregate proportions

Soil aggregates play an essential part in soil fertility which is caused by their many vital functions. For this study, soils were divided into four aggregate size classes namely large macroaggregates (>2000 µm), small macroaggregates (250-2000 µm) microaggregates (53-250 µm), and silt and clay (<53 µm) through wet sieving. In the Embu site (Table 3), higher proportions of small macroaggregates (250-2000 µm) were examined in all alterations compared to the other aggregate size classes. For the Machang'a site (Table 4), the highest proportion of aggregates was examined in the microaggregates (53-250 µm) size class.

In the Embu site (Table 3), the proportions of large macroaggregates (>2000 µm) for all alterations of low fertility soils with fertilizer-N and organic resources were significantly different ($p<0.05$) to the proportion examined in the control. This was in the order, sole fertilizer-N> stover plus fertilizer-N> sole stover. For the alterations including medium-fertility soil, only the combined stover and fertilizer-N management had an important impact ($p<0.05$) on proportions of large macroaggregates regarding the control. Proportions of large macroaggregates for all alterations of high fertility soils with fertilizer-N and organic resources were significantly different ($p<0.05$) to the control. For these managements, the sole fertilizer-N management had the highest proportion (16.46%) of large macroaggregates.

Table 3. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on proportions of soil aggregates in Embu soils, Kenya.

	Aggregate size class proportions (%)											
	>2000 µm			250-2000 µm			53-250 µm			<53 µm		
Managements	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Control	6.62	5.04	12.10	64.71	59.88	63.00	21.55	26.31	17.11	3.69	3.98	2.09
Fertilizer N	9.50	4.23	16.46	62.00	61.56	61.00	20.35	25.71	15.21	3.36	4.47	1.79
Stover	7.57	4.90	13.67	61.90	61.08	64.23	23.74	25.41	15.57	3.70	3.05	2.14
Stover + Fert N	8.40	7.25	13.41	67.16	63.47	64.09	18.29	21.44	15.60	3.10	3.25	2.01
SED	0.77*	0.77*	0.77*	1.23*	1.23*	1.23*	0.89*	0.89	0.89	0.28	0.28*	0.28

Note: SED = Standard error of difference of means; SED*=Difference between managements is statistically significant at $p<0.05$

Table 4. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on proportions of soil aggregates in Machang'a soils, Kenya.

	Aggregate size class proportions (%)											
	>2000 µm			250-2000 µm			53-250 µm			<53 µm		
Managements	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Control	0.90	1.27	15.34	36.19	20.21	30.27	56.45	70.99	44.41	5.03	6.86	4.73
Fertilizer N	2.39	1.02	15.11	36.80	19.78	32.19	52.80	70.34	45.45	5.72	7.49	5.74
Stover	4.57	2.94	16.65	34.33	19.26	33.80	54.95	66.99	43.72	4.80	8.57	3.89
Stover + Fertilizer N	1.18	3.01	16.51	35.22	19.43	30.88	57.35	69.40	45.84	5.05	6.77	5.13
SED	0.56*	0.56*	0.56*	0.83	0.83	0.83*	1.00*	1.00	1.00*	0.40*	0.40*	0.40*

Note: SED = Standard error of difference of means; SED*=Difference between managements is statistically significant at $p<0.05$

In the Machang'a site (Table 4), the alteration of low fertility soil with sole fertilizer and sole stover had important impacts ($p<0.05$) on the proportions of large macroaggregates as compared to the control management. overall, for these alterations, the sole stover management had the highest proportion (4.57%) of large macroaggregates. For the medium fertility soils, the sole stover and combined stover and fertilizer-N managements had important impacts on the proportions of large macroaggregates, compared to the control management. For these managements, the combined stover and fertilizer-N management had the highest proportion (3.01%) of large macroaggregates. A similar trend was examined in the high fertility soil where the proportions of large macroaggregates, in both the sole stover and combined stover and fertilizer-N managements, were significantly different from those of the control. For these managements, the sole stover management had the highest proportion (16.65%) of large macroaggregates.

In the small macroaggregates size class (250-2000 µm), the alteration of low fertility soil in the Embu site with combined stover and fertilizer-N had an important impact on the proportions of small macroaggregates (Table 3) regarding the control management. This management accounted for the highest proportion of small macroaggregates (67.16%). Regarding the medium-fertility soil, both the sole fertilizer-N and combined stover and fertilizer-N managements had important impacts on the proportions of small macroaggregates. For these alterations, the combined stover and fertilizer-N management had the highest proportion (63.37%) of small macroaggregates. For the management including high fertility soil, only the sole stover management had an important impact on the proportion of small macroaggregates. In the Machang'a site, the impact of alteration of low fertility soil with fertilizer-N and organic resources on proportions of small macroaggregates was only significant for the sole fertilizer-N management. This management accounted for the highest (36.80%) proportion of small macroaggregates. For the alterations including medium-fertility soil, no management technique was significantly different from the control. Regarding high fertility soil, both the sole stover and the sole fertilizer-N managements had important impacts on the proportions of small macroaggregates as compared to the control management. Overall, for these managements, the sole stover management had the highest proportion (33.80%) of small macroaggregates.

In the microaggregates size class (53-250 µm), the alteration of low fertility soils in Embu with sole fertilizer-N and combined stover and fertilizer-N had an important negative impact in that it resulted in lower values of microaggregates compared to the control management (Table 3). In the medium-fertility Embu soils, all alterations including fertilizer-N and organic resources resulted in reduced values of microaggregates. A similar trend was examined in the high fertility soils. For the Machang'a site, all alterations of soils with sole stover had an important negative impact on proportions of microaggregates.

The dissimilarities in aggregate composition between the Embu and Machang'a soils could be caused by a higher clay content in the Embu soils than that of Machang'a (Table 4). Several studies have shown that soil texture affects aggregation (Chaney and Swift 1984; Plante et al. 2006) such that raised clay contents are correlated with raised aggregation or aggregate stability. Bartoli et al. (1992) claimed that water-stable aggregates were associated with aggregate strength that was well associated with clay content. More recently, a study by De Gryze et al. (2005) found that the amount of water stable aggregates >2000 µm in a natural ecosystem declined respectively as follows: silty clay loam > silt loam > sandy loam. This is also in accordance with a study by Barthes and Roose (2002), who reported that an increase in coarse sands caused simultaneous declines in clay plus fine silts and aggregate fractions.

The higher proportions of large macroaggregates examined in the sole fertilizer-N or combined stover and fertilizer-N alterations in the Embu soils (Table 3) indicated raised macroaggregate formation upon the alteration of these soils with fertilizer-N. This could be caused by raised N values, which resulted in an escalation in microbial activity as N limiting conditions have been found to be low value in microbial activity (Harris et al. 1996). Studies have also shown the addition of mineral N with raised microbial activity and the subsequent release of C-rich binding compounds resulting in higher formation of large macroaggregates (Six et al. 2000a; Dutta et al. 2003). This is supported by a study performed by Kavoo (2008) who stated that a higher initial formation of large macroaggregates upon the alteration of sawdust with mineral N fertilizer for soils in Embu was found.

For the Machang'a site (Table 4), the important impacts examined with the alterations including sole stover and combined stover and fertilizer-N on the proportions of large macroaggregates indicate raised macroaggregate formation. This could be caused by the addition of maize stover as the addition of organic matter such as crop residues and other organic substances to soil which has been found to usually raise aggregation (Bipfubusa et al. 2007). This is supported by a study by Sodhi et al. (2009) who reported an escalation in the proportions of macro-aggregates and a subsequent decline in the proportions of microaggregates upon the alteration of soil with rice compost. Bronick and Lal (2005) also reported raised aggregation and aggregate stability upon alterations of soils with corn residues. Studies have also presented that the large macroaggregates fractions are highly responsive to organic residue addition in soil (Bipfubusa et al. 2007). Escalated formation of macroaggregates upon the addition of organic resources has been attributed to the organic resources serving as nucleation sites for the growth of fungi and bacteria (Jastrow 1996; Puget et al. 1996; De Gryze et al. 2005). The fungal hyphae then initiate macroaggregate formation by enmeshing fine particles into macro-aggregates (Tisdall and Oades 1982). With time, the microbial (fungal and bacteria) exudates, were produced because of putrefaction of plant residues to form binding agents that further stabilize macroaggregates (Six et al.

2002). The formation of macroaggregates around organic residues has further been confirmed by studies using ^{13}C natural abundance in fields.

These studies have consistently shown that macroaggregates are formed around newly incorporated organic residues (Puget et al. 1996; Six et al. 1998). De Gryze et al. (2005) also reported a linear increase in aggregate formation with increasing amounts of the added low-quality residues (low in N but high in lignin).

The important negative impact on the proportions of microaggregates for all alterations, including high fertility soils in the Embu site, could be caused by raised binding of microaggregates into macroaggregates upon alteration of soils with fertilizer-N and organic resources. According to the hierarchical concept of aggregate formation, macroaggregates are formed by the binding together of smaller aggregates such as stable microaggregates (Tisdall and Oades 1982; Balesdent et al. 2000). Six et al. (1999) suggested that the addition of new residues in a no-till management system promoted organic matter stabilization through the binding of primary soil particles and old microaggregates into new macroaggregates. Further, fragmented crop residue (particulate organic matter) has been found to form the nuclei for new microaggregates that can be bound together by transient and labile organic matter to form new macroaggregates (Golchin et al. 1994; Olchin et al. 2008). This causes an increase in proportions of macroaggregates, followed by a subsequent decrease in macroaggregate proportions. The results indicate that macroaggregate formation and stabilization was higher in higher fertility soils and is in line with the higher proportions of macroaggregates examined in the Embu high fertility soils.

Effect of alteration of soils of various texture and fertility values with fertilizer-N and organic resources on distribution of distinct SOM fractions

The proportions of functional soil organic matter fractions, i.e., coarse particulate organic matter (cPOM), microaggregates within macroaggregates (mM) and silt and clay (s+c) were determined through isolation from macroaggregates. For the Embu site (Table 5), higher proportions of microaggregates within macroaggregates were examined in all alterations than that of other SOM fractions. The scenario was different for the Machang'a site (Table 6), where higher proportions of cPOM fractions were examined in all alterations than that of other SOM fractions.

In the Embu site, all alterations of low fertility soil with sole and combined application of fertilizer-N and organic resources had an important impact on the distribution of cPOM fractions compared to the control (Table 5). The sole fertilizer-N management accounted for the highest proportion of cPOM fractions (9%). Alteration of medium fertility soils with fertilizer-N and organic resources had no important impact on the distribution of cPOM fractions. A resemblant trend was examined in the high fertility soil. Overall, cPOM fractions ranged between 2.60% and 9.00%, 0.73% and 1.00%, and 0.73% and 1.33% for the low, medium, and high fertility soils respectively.

In the Machang'a low fertility soils, the proportion of cPOM fractions in the combined stover and fertilizer-N management was significantly different to that of the control (Table 6).

Table 5. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on percentage (%) distribution of distinct SOM fractions within macroaggregates in Embu soils, Kenya.

	% Distribution of SOM fractions								
	cPOM			mM			S&C		
Management	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Control	2.60	1.00	1.00	65.46	63.73	60.33	23.83	25.74	30.81
Fertilizer N	9.00	0.93	0.86	61.73	66.86	68.20	23.73	21.45	21.41
Stover	7.00	0.93	0.73	61.33	60.46	55.93	19.74	24.74	39.76
Stover + Fert. N	4.93	0.73	1.33	63.40	65.53	58.26	27.44	25.21	36.94
SED	0.45*	0.45	0.45	1.96	1.96*	1.96*	2.85*	2.85	2.85*

Note: SED = Standard error of difference of means; SED*=Difference between managements is statistically significant at p<0.05

Table 6. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on percentage (%) distribution of distinct SOM fractions within macroaggregates in Machang'a soils

	% Distribution of SOM fractions								
	cPOM			mM			S&C		
Management	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Control	69.76	68.11	71.54	23.85	25.85	21.80	3.27	6.83	3.80
Fertilizer N	67.61	65.50	70.70	26.97	28.81	22.88	2.95	4.12	4.39
Stover	68.60	71.89	71.30	25.34	22.35	21.12	4.24	3.56	5.70
Stover + Fert. N	74.51	67.67	68.83	19.26	23.80	22.23	4.75	6.36	7.08
SED	2.25*	2.25*	2.25	1.99*	1.99*	1.99	0.6*	0.6	0.6*

Note: SED = Standard error of difference of means; SED*=Difference between managements is statistically significant at p<0.05

This management accounted for the highest proportion (74.51%) of cPOM fractions among all alterations. For the medium-fertility soil, only the sole stover management had an important impact on the proportions of cPOM fractions about the control. A similar trend was examined in the high fertility soil. Overall, cPOM fractions ranged between 67.61% and 74.51%, 65.50% and 71.89%, and 68.83% and 71.54% for the management including low, medium, and high fertility soil values respectively.

The higher proportions of cPOM fractions examined in the Machang'a soils, as compared to the Embu ones, could be due to dissimilarities in texture. Studies have shown that the proportion of mass associated with the course (>250 µm) sand plus POM fractions decreases with increasing clay content (Plante et al. 2006). Regarding the mM fractions, the higher proportions examined in the Embu soils indicate that formation and stabilization of macro-aggregates in these soils is higher than in the Machang'a soils. Studies have shown that it is through the formation and subsequent stabilization of macroaggregates that microaggregates are formed within macroaggregates (Puget et al. 1996; Six et al. 2004). During macroaggregate stabilization, the intra-aggregate particulate organic matter POM (i.e., fresh plant material that was incorporated in the macroaggregates during "biological" aggregate formation) is further decomposed by microorganisms and fragments into finer POM. This fine POM then becomes increasingly encapsulated with minerals and microbial products forming new microaggregates within the macroaggregates (Six et al. 1998). These microaggregates are later released upon the breakdown of macroaggregates and may be subsequently reincorporated into new aggregates. This is corroborated by the higher proportions of macroaggregates examined in the Embu soils as compared to the Machang'a soils (Tables 5 and 6).

The lower formation and stabilization rates of macroaggregates in the Machang'a soils compared to the Embu soils can be attributed to dissimilarities in texture as this influences the turnover rate of organic matter (Six et al. 2002), It is known that in coarse-textured soils of arid and semi-arid regions such as those of Machang'a, there exists a rapid turnover of organic matter (Quiroga et al. 1999; Hevia et al. 2003), and that only a low amount of fresh organic residues will contribute to humified soil organic matter which is essential for soil aggregation. Under these conditions, most plant and animal residues incorporated in the soil will be mineralized (Gregorich et al. 1994).

The higher proportions of cPOM fractions examined in the low fertility soils compared to higher fertility soils at the Embu site could mean that there was an accumulation of POM. This could probably be due to slow putrefaction rate in these soils as this has been found to cause decreases in POM size (Guggenberger et al. 1999). The low putrefaction rate exhibited in this soil could be due to the low microbial activity usually associated with low fertility soils (Kolbl et al. 2006). In their studies, Scolter et al. (2003) and Sehy et al. (2003) reported a direct relationship between soil fertility and the putrefaction of organic residues.

The important impact on mM proportions for the alteration of low fertility soil with sole fertilizer-N, and alteration of medium and high fertility soils with sole fertilizer-N and combined stover and fertilizer-N in the Embu site indicates raised macroaggregate formation and stability. This can be attributed to the addition of fertilizer-N which raised soil N values resulting in a subsequent rise in microbial activity and hence improved aggregate formation and stabilization, and the subsequent formation of microaggregates within macroaggregates (Harris et al. 1996; Six et al. 2000a). The formation of microaggregates within macroaggregates upon the stabilization of macro-aggregates has been found to be crucial for the long-term sequestration of C (Six et al. 2002), as microaggregates have a greater capacity to protect C from putrefaction as compared to macroaggregates (Balesdent et al. 1993).

In the following subsections, the carbon content of whole soil, aggregate size fractions and SOM fractions isolated from macroaggregates are presented and discussed.

Effect of alteration of soils of various texture and fertility values with fertilizer-N and organic resources on carbon content of whole soils

The incorporation of fertilizer-N and organic resources in soils resulted in changes in soil C status of whole soils in both Embu and Machang'a (Tables 5 and 6). At both sites, C% values of soils were raised with increased fertility. Overall, soils at the Embu site had higher C% values compared to those for Machang'a. At the Embu site, no management had an important impact on C% values of whole soils for all soil fertility values (Table 7). However, for all fertility values, the combined stover and fertilizer-N managements had the highest C% values.

In the Machang'a site (Table 8), alteration of low fertility soils with sole stover had an important ($p<0.05$) impact on the C% value of whole soil compared to the control management. For the medium fertility soils, whole soil C% values of both the sole fertilizer-N and sole stover management were significantly different from that of the control. For these managements, the sole stover management had the highest whole soil C value (0.31%). For the high fertility soils, whole soil C% values of both the sole fertilizer-N and the combined stover and fertilizer-N managements were significantly different from that of the control. For these managements, the combined stover and fertilizer-N management had the highest whole soil C value (0.66%).

Table 7. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on percentage carbon content of whole soils in Embu, Kenya

Management	C%		
	Low	**Medium**	**High**
Control	1.71	2.89	3.13
Fertilizer N	1.59	2.94	3.16
Stover	1.73	2.87	3.13
Stover + Fert. N	1.74	2.96	3.20
SED	0.14	0.14	0.14

Note: SED = Standard error of difference of means

Table 8. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on percentage carbon content of whole soils in Machang'a, Kenya.

Management	% C		
	Low	**Medium**	**High**
Control	0.26	0.27	0.61
Fertilizer N	0.17	0.30	0.64
Stover	0.28	0.31	0.62
Stover + Fert. N	0.24	0.22	0.66
SED	0.02*	0.02*	0.02*

Note: SED = Standard error of difference of means; SED* = Difference between managements is statistically significant at $p<0.05$

The higher whole soil C values examined in the Embu soils as compared to the Machang'a ones could be due to the higher proportions of clay content examined in the Embu soils compared to the Machang'a ones (Table 2). Studies have shown that soil texture is a key variable affecting soil C, because of electrostatic binding between negatively charged clay surfaces and organic colloids via cation bridges (Paustian et al. 2003; Barthes and Roose 2002). Comparing similar tropical soils with different clay mineralogy, Wattel-Koekkoek and Burman (2004) found that the effective cation capacity of the clays can explain dissimilarities in the mean residence of OM. In a study over a range of tropical soils, Feller and Tessier (1996) also confirmed that due to the sorption of fine organic constituents to fine mineral particles, C amount in the fine fraction increases with the proportion of fine primary particles. The relationship between clay concentration and SOC content is sufficiently strong that SOM models such as Century (Parton 1996) and RothC (Jenkinson 1990) assume that SOM putrefaction decreases as clay concentration increases, such that if all factors are equal, SOC accumulates faster as soil clay content increases (McLauchlan 2006).

The lower whole soil C values examined in the Machang'a soils also explain the lower proportions of macroaggregates examined in these soils. A study by Castro et al. (2002) reported that a soil that is low in organic carbon will be poorly aggregated since the weight and the content of organic carbon in the >2000 µm size class is lower than the weight and the content of organic carbon of other aggregate size classes.

The higher whole soil C values examined in all Embu soils with combined stover, and fertilizer-N alterations as compared to the sole application of stover is in line with a study by Kavoo (2008). This study reported that the combined application of tithonia and mineral fertilizer-N had a higher concentration of C (35.8 g C kg^{-1} whole soil) compared to the sole application of tithonia (31.8 g C kg^{-1} whole soil) for soils in Embu. Hati et al. (2006) also reported an important impact of fertilizer-N and organic manure application on the soil organic carbon values up to a depth of 0.30 m, with the SOC content in the 0-0.15 m layer being the highest (6.5 g kg^{-1}) in the fertilizer-N plus manure management.

These higher values of percent C could be because of raised rate of putrefaction of maize stover and the subsequent release of carbon compounds in the presence of mineral fertilizer. Studies have shown that addition of mineral-N increases the putrefaction rate of organic resources. Jenkinson and Rayner (1985) reported that the addition of mineral-N raised the putrefaction rate of wheat straw by satisfying the N requirements of microorganisms. Berg and Matzner (1997) also reported that in the early stages of plant putrefaction, mineral-N inputs stimulate hydrolysis of soluble C compounds and nonlignified holocellulose. During the putrefaction of organic resources, plant material fragments or particulate organic matter (POM) gradually become encrusted with clay particles and microbial products to form the core of stable microaggregates (Six et al. 2004), resulting in an increase in whole soil C. The formation of microaggregates has been found to be crucial for the storage and stabilization of soil C in the long term through the incorporation of new C into microaggregates (Jastrow and Miller 1998; Six et al. 1998; Gale et al. 2000). This incorporation of new C into free microaggregates is an important factor contributing to C sequestration (Skjemstad et al. 1990) since C contained in free microaggregates has a slower turnover than C in macroaggregates (Jastrow 1996). These results indicate potential changes in soil C storage that could become more important over time, because small short-term dissimilarities in putrefaction with mineral-N input can add up to large dissimilarities in the long-term storage of SOM (Agren et al. 2001).

The important impact of the alteration of low and medium fertility Machang'a soil with sole stover on whole soil C, could be due to a buildup of SOC upon the application of stover. This is in line with findings by Angela et al. (2007), who reported that the amount of SOC sequestered in an organic system (5.70 Mg SOC ha^{-1}) was greater than the SOC sequestered by conventional and low input systems (570 and-340 kg SOC ha^{-1}, respectively). Harris et al. (1996) also suggested that the addition of class III organic resources leads to intermediate aggregate formation and breakdown resulting in C accumulation. Studies by Paustian et al. (2000) and Waswa et al. (2007), further reported that increases in soil carbon inputs are one of the ways through which soil C stocks can be raised. The addition of organic matter to soils results in higher soil C values through their occlusion within soil aggregates when new aggregates form around the organic matter. Increased occlusion increases C sequestration because of the decrease in mineralization in the occluded state due to raised interaction of the organic matter with reactive surfaces (Plante and Mcgill 2002).

Effect of alteration of soils of various texture and fertility values with fertilizer-N and organic resources on carbon content of soil aggregates

The alteration of soils with fertilizer-N and organic resources had various impacts on the carbon content of soil aggregates (Tables 7 and 8). Overall, soil aggregates from the Embu soils had higher C% values compared to those from the Machang'a soils. In the large macroaggregates achieved from Embu soils (Table 9), only the alteration of low fertility soil with sole stover management had an important impact on C% values of large macroaggregates as compared to the control management. For the large macroaggregates achieved from Machang'a soils (Table 10), only the alteration of medium fertility soil with sole stover had an important impact on the C% values of large macroaggregates compared to the control management. For this soil, this alteration was considered as the highest C% (1.67) value of macroaggregates.

In the small macroaggregates (250-2000 µm) size class, alterations of Embu soils with fertilizer-N and organic resources had no important impact influence on C% values. For the Machang'a site, alterations of low fertility soil with fertilizer-N and organic resources had no important influence impact on C% values of small macroaggregates. However, in the medium-fertility soil, the C% value of small macroaggregates in the sole stover management was significantly different ($p<0.05$) to that of the control. In the high fertility Machang'a soils, only the sole fertilizer-N management had an important impact on the C% values of small macroaggregates.

In the Embu low fertility soil (Table 9), the C% value of microaggregates (53-250 µm) in the combined stover and fertilizer-N management was significantly different ($p<0.05$) from that of the control management. For the medium fertility soils, only the sole fertilizer-N management had an important impact on the C% value of microaggregates. Alteration of high fertility soil with fertilizer-N and organic resources had no important impact on the C% value of microaggregates. For the Machang'a soils (Table 10), only the alteration of high fertility soil with sole fertilizer-N had an important impact on the C% value of microaggregates as compared to the control management. For both Embu and Machang'a soils, the silt and clay size class had the highest C% value compared to the other size classes. On average for all soil fertility values, the silt and clay size class (<53 µm) had higher C% contents compared to the other aggregates size classes for both the Embu and Machang'a sites.

The important impact of the stover management on the C% values of large macroaggregates in Embu and Machang'a, and the small macroaggregates in Machang'a indicates a C build up in macroaggregates upon organic residue application. This could be due to higher formation of macroaggregates upon the application of stover, as the putrefaction of organic residues has been shown to increase aggregation and aggregate stability (Tisdall and Oades 1982; Bronick and Lal 2005). This is in accordance with a study by Bipfubusa et al. (2007), who reported an increase in the C content of large and small macroaggregates upon the alteration of soils with both paper mill sludge and paper mill sludge compost. Studies elsewhere have also suggested that macroaggregates are stabilized mainly by carbohydrate-rich root or plant debris occluded within aggregates (Golchin et al. 1994). A study by Angers and Giroux (1996) provided further evidence that state resistant macroaggregates are stabilized by recently deposited residues, while Jastrow (1996) suggested that the intra-macroaggregate particulate organic matter (POM) is an important agent that facilitates the binding of microaggregates into macroaggregates. Increased aggregation results in an increase in the C value of macroaggregates, as aggregates are known to physically protect C (Jastrow et al. 1996 and Puget et al. 1996).

Table 9. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on percentage carbon content of soil aggregates in Embu soils, Kenya.

	C%											
	>2000 µm			250-2000 µm			53-250 µm			<53 µm		
Management	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Control	1.2	3.04	3.12	1.61	2.52	3.21	1.61	2.83	3.03	2.52	3.72	4.03
Fertilizer N	1.02	2.83	3.18	1.53	2.84	2.93	1.56	3.33	3.01	2.57	3.29	3.02
Stover	1.53	2.93	2.97	1.62	2.71	3.44	1.59	2.85	3.03	1.69	4.25	4.03
Stover+Fert. N	1.19	2.99	3.01	1.56	2.95	2.96	1.96	2.79	3.09	3.06	3.59	4.13
SED	0.24*	0.24	0.24	0.61	0.61	0.61	0.23*	0.23*	0.23	0.29*	0.29*	0.29

Note: SED = Standard error of difference of means; SED*=Difference between managements is statistically significant at $p<0.05$

Table 10. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on percentage carbon content of soil aggregates in Machang'a soils, Kenya.

	C%											
	>2000 µm			250-2000 µm			53-250 µm			<53 µm		
Managements	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Control	0.45	0.35	0.25	0.28	0.35	0.64	0.23	0.19	0.59	0.83	0.92	2.23
Fertilizer N	0.55	0.47	0.25	0.25	0.20	0.72	0.32	0.19	1.32	1.17	0.85	2.05
Stover	0.52	1.67	0.22	0.23	0.54	0.63	0.25	0.16	0.59	0.89	0.81	2.57
Stover+Fert. N	0.52	0.34	0.31	0.29	0.21	0.41	0.23	0.13	0.42	1.14	0.85	1.65
SED	0.37	0.37*	0.37	0.08	0.08	0.08*	0.16	0.16	0.16*	0.24	0.24	0.24

Note: SED = Standard error of difference of means; SED*=Difference between managements is statistically significant at $p<0.05$

Studies have shown that aggregates contain labile C that is physically protected from microbial putrefaction (Amelung and Zech 1999; McLauchlan 2006). The protection of C in macroaggregates contributes to C retention in soils by slowing its mineralization rate, resulting in a net gain in soil C (Mikha and Rice 2004).

The important impact on C% values of microaggregates examined in the alteration of both medium fertility Embu soil and high fertility Machang'a soil with sole fertilizer-N could be due to raised turnover of macroaggregates and the subsequent release of microaggregates. This increase in turnover rate could be because of raised putrefaction of C compounds binding macroaggregates upon the addition of sole fertilizer-N. Upon the application of fertilizer-N without a C source, microorganisms have been found to decompose the C-rich binding agents, resulting in C losses (Angela et al. 2007). As the binding agents in macroaggregates degrade, macroaggregate stability is lost resulting in the release of stable microaggregates (Six et al. 2000b). This causes a transfer of carbon from macro-aggregates into microaggregates upon the breakdown of the macroaggregates (Jastrow 1996, Six et al. 1998, Gale et al. 2000), resulting in raised C values of microaggregates.

The higher C% values examined in the silt and clay size class (53-250 µm) as compared to the other aggregate size classes for both the Embu and Machang'a soils could be caused by the transfer of carbon compounds into this fraction. This is in line with findings by Jastrow (1996) and Kavoo (2008). Their findings indicated that there occurs a direct transfer of some plant compounds into the silt and clay fractions, primarily through breakdown, leaching, communication and selective microbial degradation (Mikutta et al. 2006). Other studies have also shown that the silt and clay fractions can biochemically and chemically protect organic C bound to them, thereby protecting it from microbial putrefaction (Sleutel et al. 2006; Fonte et al. 2007). It has also been reported that the silt and clay fractions have a higher C stabilization capacity compared to other fractions. The stabilization of C by association with silt and clay particles has been found to be one of the ways through which organic C is preserved (Six et al. 2002). A study by Balabane and Plante (2004) hypothesized that silt-size aggregates are sites where physically protected OM can mature and interact with specific adsorption sites on mineral surfaces. Data by Virto et al. (2008) showed that almost half of the total soil organic C was stored in these aggregates. The higher stabilization of C in silt and clay particles could be due to the greater biological activity associated with clay-sized particles which are reflected in the larger content of microbially derived products in clay than in >2 µm separates (Christensen 1992). Clay also maintains the largest concentrations of OM, suggesting that the microbial activity provides more microbial metabolites and residues (including cell wall materials) that become stabilized and accumulate in clay (Hedges and Oades 1997; Kaiser et al. 1998). Stabilization of C in the silt and clay fraction plays an important role in the development of soil carbon as it is one of the main ways to preserve organic C (Six et al. 2002; McLauchan, 2006).

Effect of alteration of soils of various texture and fertility values with fertilizer-N and organic resources on carbon content of distinct SOM fractions

The following section shows the results of carbon contents analysis of SOM fractions [(coarse particulate organic matter (cPOM), microaggregates within macroaggregates (mM) and silt and clay (s+c)]. For the Embu soils, the cPOM fractions had generally higher C% values than the other SOM fractions (Table 11), whereas for the Machang'a soils, the silt and clay fractions had the highest C% values compared to other SOM fractions (Table 12).

In the Embu site, the implementation of fertilizer-N and organic resources into the low fertility of Embu soils gave no remarkable influence ($p<0.05$) on the C% value of cPOM fractions in relation to the control (Table 11). An identical trend was detected for both the medium and high fertility soils. Generally, a prevalent raise in cPOM C% values with the raising of soil fertility was detected in this area. No remarkable influences on cPOM C% values were detected for the implementation of fertilizer-N and organic resources in Machang'a soils. However, as detected in the Embu soils, C% values of cPOM fractions raised by raising soil fertility. Overall, cPOM fractions from the Embu soils ranged between 0.68% and 11.26% and were greater than those from the Machang'a site which ranged between 0.13% and 0.29%.

Regarding microaggregates within macroaggregates fractions, it was detected that for the alterations including low fertility Embu soil (Table 11), only the lone stover management had a remarkable influence ($p<0.05$) on C% values. For the medium-fertility soil, all alterations including fertilizer-N and organic resources had a remarkable influence on C% values of mM fractions in comparison to the control management. For these managements, the influence on C% values of mM fractions was in the order lone fertilizer-N > lone stover > stover plus fertilizer-N > control. Implementation of lone or combined organic and mineral resources in Embu high fertility soils had no remarkable influence on C% values of mM fractions.

For the Machang'a low fertility soils (Table 12) the carbon contents of mM fractions showed that only the lone fertilizer-N management had a remarkable influence on the C% values of mM fractions. Whereas with medium fertility soil, no management had a remarkable influence on C% values of mM fractions about the control. For the management including high fertility soils, only the lone stover management had remarkable on C% values of mM fractions. Overall, the C% values of mM fractions for Embu soils were at greater value than from those of Machang'a soils.

Table 11. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on percentage carbon content of distinct SOM fractions in Embu soils, Kenya.

	C%								
		cPOM			mM			S&C	
Management	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Control	1.27	8.25	10.47	1.25	2.01	3.06	2.62	4.18	3.99
Fertilizer N	0.78	5.78	11.26	1.19	3.05	3.18	1.75	4.54	3.85
Stover	0.78	5.62	7.56	2.05	2.92	2.87	3.03	4.64	3.96
Stover + Fert N	0.68	5.59	6.58	1.42	2.85	3.07	2.15	3.57	3.70
SED	1.48	1.48	1.48	0.37*	0.37*	0.37	0.51	0.51	0.51

Note: SED = Standard error of difference of means; SED*=Difference between managements is statistically significant at p<0.05

Table 12. Effect of alteration of soils of various fertility values with fertilizer-N and organic resources on percentage carbon content of distinct SOM fractions in Machang’a soils, Kenya.

	C%								
		cPOM			mM			S&C	
Managements	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Control	0.21	0.20	0.27	0.50	0.11	0.56	1.01	0.52	1.81
Fertilizer N	0.17	0.21	0.23	0.63	0.17	0.62	1.85	0.64	1.57
Stover	0.15	0.21	0.26	0.14	0.18	1.52	0.55	0.68	1.44
Stover + Fert. N	0.13	0.16	0.29	0.11	0.15	0.45	0.49	0.56	1.23
SED	0.30	0.30	0.30	0.32	0.32	0.32*	0.32*	0.32	0.32

Note: SED = Standard error of difference of means; SED*=Difference between managements is statistically. significant at p<0.05

The greater C% value of cPOM fractions for Embu soils in comparison to Machang’a soils showed raised macroaggregates formation for these soils. Studies have shown that macroaggregates form around POM (Six et al. 1998; Olchin et al. 2006). This leads to a rise in cPOM C, as intra aggregate POM is less vulnerable to putrefaction than free POM in the soil matrix (Six et al. 1999; Olchin et al. 2006). This is due in part to the physical and chemical protection provided by aggregates. This is in accordance with the greater proportions of macroaggregates detected for the Embu soils in comparison to the Machang’a ones.

The remarkable influence on C% values of mM fractions detected for the lone stover management in the low and high fertility Embu soils shows a transfer of C from macroaggregates into the mM fraction. This could be due to the putrefaction of organic residues from maize stover input. This has been shown to result in macroaggregate stabilization, which is associated with the formation of microaggregates within macroaggregates resulting in subsequent C transfer (Denef et al. 2002; Jiao et al. 2006). In relation to C isolation, this is significant as microaggregates have a greater capability to protect C than that of macroaggregates.

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