Original Research Article

SOIL MICROBIAL BIOMASS, MICROBIAL POPULATIONS AND DIVERSITYIN MAIZE-BANANA BASED AGROFORESTRY SYSTEM IN KISII COUNTY KENYA

ABSTRACT

Soil microbes are involved in many important ecosystem processes including nutrient acquisition, biogeochemical cycling and soil aggregation.Soil microbial diversity affects the soil belowground dynamics and fate of carbon and nutrients.Soil microbes are important for agricultural and plant production systems, hence understanding the effects of agroforestry systems on the soil microbes, is necessary in order to improve on soil health and fertility.The objective of the study was to determine the soil microbial biomass, microbial populations and microbial diversity in maize-banana based agroforestry system.The study was conducted at Kenya Agricultural and Livestock Research Organization farm in Kisii County.Theexperiment was laid out in a randomized complete block design with maize and banana intercropped with agroforestry trees. The treatments were;Maize, banana(MMBB),Maize-banana,*Calliandra*(MBCC),Maize (MM),banana(BB),Maize-banana, *Leucaena*(MBLL),Maize-banana,*Sesbania*(MBSS) and Maize, fertilizer(MMF).Soil samples were collected from the agroforestry fields using a soil auger. Soil microbial biomass was measured using the chloroform fumigation extraction. Fungi and bacteria were enumerated by serial dilution plate method.Shannon diversity index (H') and Simpson diversity index (1 - **D**) were used for the calculation of species diversity. SAS (version 9) statistical software was used for analysis.The treatments with agroforestry tree species had significantly higher soil microbial biomass(MBSS-86.33,MBCC-52.66 and MBLL-47.0MgC/Kg)populations of bacteria (MBSS-197,MBCC-128.0 and MBLL-111.25$\mathbf{x}10^{8}$cfu g^{-1}soil) and fungi(MBSS-50.83,MBCC-29.167 and MBLL-14.0x10^{5}cfu g^{-1} soil)and diversity of bacteria(MBSS-(H'=1.61, D= 1),MBCC- (H'=1.04, D= 0.83),MBLL (H'=0.52, D= 0.5) and fungiMBSS (H'=1.39, D= 1) MBCC (H'=1.04, D= 0.83),MBLL (H'=1.56, D= 0.93).MBSS increased microbial biomass, microbial populations and microbial diversitysignificantly an indication of improved soil health and hence recommended for adoption by farmers.

Keywords:soil microbial biomass, microbial populations, microbial diversity, Maize, Banana,fertility

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INTRODUCTION

Soil microbial community is an important biological component of soil function, valued for its role in improving soil quality and regulating nutrient availability, and hence influencing plant production[8]. Soil microbes are involved in many important ecosystem processes including nutrient acquisition[29], biogeochemical cycling[2] and soil aggregation[30]. Soil microbial diversity affects belowground dynamics and fate of carbon and nutrients which influences fertility [34].The importance of soil microbes for agricultural and plant production systems necessitates an understanding of the effects of agroforestry on the soil microbial diversity,

microbial populations and soil microbial biomass in order to identify the agricultural ecosystem that can improve soil fertility. Soil is the most valuable natural resource and contains diverse assemblages of living organisms[5]. Microorganisms in the soil dominate the decomposition processes and the cycling of nutrients [14]. They are very much important for long-term sustainability, increasing the soil fertility, protection of plants from disease causing organisms and promotion of plant growth [19]. There is a relationship between microbial diversity and biomass, and soil functionality. 80–90% of the processes in soil such as the biogeochemical cycling of nutrients and matter and the maintenance of plant health and soil quality are mediated by microbes[25].Microbial diversity andbiomass exert a controlling influence on the dynamics of soil organic matter (SOM) and availability of many nutrients[27]. It is also frequently used as an early indicator of changes in soil chemical and physical properties resulting from soil management and environmental stresses in agricultural ecosystems[20]. Even though microbial biomass and diversity is important in the breakdown of soil organic matter resulting in the availability of nutrients, little is known about its seasonal variation in Maize-Banana-based agroforestry systems especially in Kisii County.

Little work has been done on the microbial diversity and microbial biomass in agroforestry field soils in Kisii, hence the need toinvestigate the status of microbial diversity and microbial biomass in maize -banana based agroforestry systems with *Sesbaniasesban*, *Calliandracalothyrsus* and *Leucaenadiversifolia*tree species in Kisii County.

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2. MATERIALS AND METHODS

2.1 Study site characteristics

This study was conducted at Kenya Agricultural and Livestock Research Organization farm in Kisii County, located in western Kenya, latitude: 0° 41' 0 S and longitude: 34° 46' 0 E[16].There is significant rainfall throughout the year, with the average of 1922mm per year. The long rains are received between March and July while the short rains are received from August to December. The least amount of rainfall occurs in January, the average in this month is 81mm. Most precipitation falls in April, with an average of 276 mm [8]. The average temperature in Kisii is 19.6 °C. In a year, the

temperatures are highest on average in February, at around 20.6°C. In July, the average temperature is 18.5°C. which is the lowest average temperature of the whole year [17].

The study soils had a pH value of 4.34, organic carbon value of 27.58g/kg, total nitrogen value of 2.37g/kg, total phosphorus value of 1.17g/kg, exchangeable potassium value of 8.56mM/kg, exchangeable calcium value of 108.27mM/kg and exchangeable magnesium value of 26.79 mM/kg

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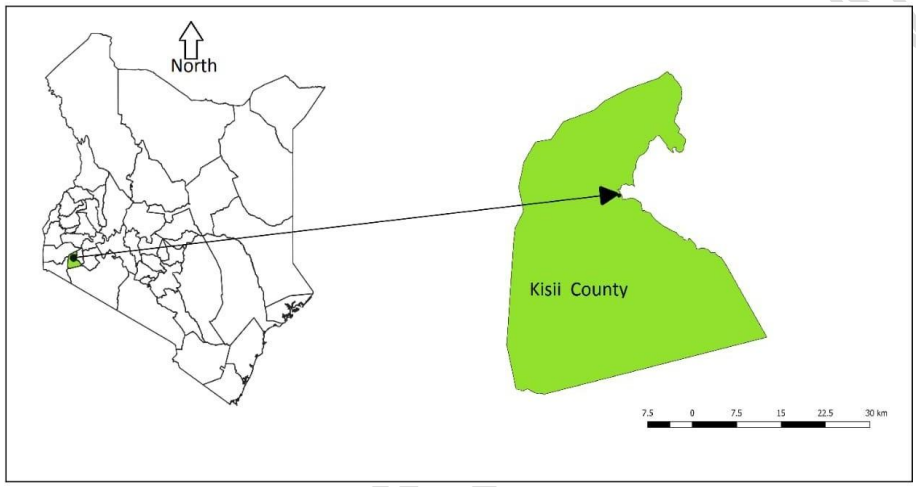

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2.2 Study design

The study was carried out on an already established experiment in March 2018. Theexperimenthad beenlaid out in a randomized complete block design (RCBD) with maize and banana intercropped with agroforestry trees. Bananasin agroforestry setting wereplanted at aspacingof3mby4m, in maize-banana plots, the banana spacing was 6m by 2.5m and solebananastands were planted at spacing of 3m by 3m.Agroforestrytreeswereplantedinrowsofspacingof0.5mby1m. Maize spacing was 30cm from one plant to the next and 75cm between the maize rows. Data collection for this experiment started in August 2018 when agroforestry trees were five months old and about 1.5M tall. During initial planting of maize Di-ammonium phosphate (DAP)

was applied at the rate of 100kg/acre and top dressed with calcium ammonium nitrate (CAN) at the rate of 100kg per acre. Weedingwas done after every month for 3 months. Trees were pruned after every two weeks and the prunings incorporated into the soils in order to improve the nutrient status of the soil. Prunings were incorporated into the soil at the following average rates *Calliandra*—15.2g/m^2, *Laucaena* 1.98g/m^2 and *Sesbaniasesban* 20.62g/$m^{2.}$

2.3 Soil sample collection

Soil samples were collected from the agroforestry fields in Kisii from September, 2018 according to the procedure of [15]. Sampling was done during two seasons; 2018 short rain (SR) and 2019 long rains (LR) seasons. Soil samples were taken at the beginning of season one (2018 SR) and subsequent samples taken at 60, 120 and 180, days. Soil samples were taken randomly at 10 different spots per plot using an alderman auger at a depth of 0-15cm to ensure that only top soil was collected. The ten auger soil samples were thoroughly mixed and composited to obtain representative sample for each experimental plot. The soil samples were then packed in sterile and clean zip lock polythene bags in cool boxes and delivered to the laboratory where they were stored in the fridge at 4^0 C.

2.4 Chloroform fumigation extraction

Soil microbial biomass was measured using the chloroform fumigation extraction method described by[23].For each sample plus three soil-free blanks, extractions were performed by adding 15 ml of 0.5 M potassium sulfate to 5g of fresh soil (or a blank) and shaking on an orbital shaker for 1 hour, then vacuum filtering the extracts through Pall A/E glass fibre filters and freezing at -20^{o}C until total carbon could be measured, usually within one week of extraction. For fumigated samples, 2 ml of ethanol-free chloroform were added to 5g of fresh soil in a stoppered 250 ml Erlenmeyer flask, swirled gently to mix, and then incubated for 24 hours at room temperature in a fume hood. After the incubation period the flasks were allowed to vent for 30 minutes, and then extractions were performed as described above.

Total dissolved organic carbon (DOC) was quantified for all extractions using a Shimadzu total organic carbon analyser. The difference in DOC between the fumigated and non-fumigated samples represented extractable microbial biomass carbon, expressed as µg-C g dry soil-^{1}.

2.5 Enumeration of fungi and bacteria

Fungi and bacteria were enumerated by serial dilution plate method using potato dextrose agar (PDA) for fungi and nutrient agar (NA) media for bacteria according to[18]. A one-gram soil mixture was taken in 9 ml of sterilized water blank and the soil suspension was diluted in 10-fold series. The inoculated petri plates were incubated in a sterile incubator at 25˚ ± 1℃. Colony forming units (CFU) were estimated by counting the number of colonies under a digital counter, after seven days for fungi and two days for bacteria. Number of bacteria and fungi in one gram of soil was calculated using the following formulae:

$$\text{Number of bacteria/fungi (CFU)/g soil} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

2.6 Determination of microbial diversity

Shannon diversity index(H') and Simpson diversity index (1 - **D**) were used for the calculation of species diversity as per the procedure of[10] .

Shannon Diversity Index

$$H = \sum_{i=1}^{s} - (P_i * \ln P_i)$$

where:

H = the Shannon diversity index

P_i = fraction of the entire population madeup of species i

S = numbers of species encountered

$\sum$ = sum from species 1 to species S

Simpson diversity index (1 - **D)**

$$D = \frac{\Sigma n(n-1)}{N(N-1)}$$

Where:n=the total number of organisms of a particular species

N = the total number of organisms of all species

2.7 Data Analysis

The SAS (version 9) statistical software was used to perform the statistical analyses. Data on soil microbial biomass, soil microbial populations and soil microbial diversitywas subjected to

analysis of variance and means separated by least significant difference at ($P < 0.05$). Means that were considered significantly different ($P \leq 0.05$) were separated using Turkeys LSD.

3. RESULTS

3.1 Effect of agroforetry trees on soil microbial biomass

The soil microbial biomass was significantly higher in treatments with agroforestry tree species in both seasons; MBCC,MBLL MBSS as compared to MMBB, MMF, BB and MM (Table 1).Significant differences ($P \leq 0.05$) in soil microbial biomass were, observed between treatments with agroforestry tree species; MBCC,MBLL and MBSS,with MBSS having the highest biomass.There was significantly ($P \leq 0.05$**)** low amount of microbial biomass in; Maize fertilizer (MMFF), Maize alone (MM),banana alone (BB) and Maize with banana (MMBB).

Table 1Soil microbial biomass in the maize-banana based agroforestry system in Kisii County

Treatments	MgC/Kg
MBSS	86.33a
MBCC	52.66b
MBLL	47.00bc
MBB	42.00c
MFF	34.00d
MM	29.33de
BB	23.33e
LSD	6.63
P.value	<.0001
%C.V	8.42

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Values are the means of three replications. MBB: Maize + banana; MBCC: Maize +banana + Caliandra; MM: Maize alone; BB: pure banana; MBLL: Maize+banana+leucaena; MBSS: Maize+banana+sesbania; MF: Maize+ fertilizer; Means followed by different letter down the column are statistically different at $P \leq 0.05$ by Fisher's protected least significant difference test.Those with more than one letter within a column are intermediates.

3.2 Effect of agroforetry trees on the total number of bacteria and fungi in the soil

Total soil microbes varied significantly ($P \leq 0.05$) among agroforestry tree species treatments (Table2).Bacterial population in these soils significantly ($P \leq 0.05$) differed under different agroforestry tree species combinations and ranged between 197.00 in MBSS to 111.25×10^{8}cfu g^{-1} soil.Bacteria populationswere significantly higher($P \leq 0.05$)in treatments with agroforestry tree species; MBCC,MBLL and MBSS with MBSS having significantly higher values compared

to other treatments.There was significantly (P≤ 0.05**)** low bacterial populations in; Maize alone (MM),banana alone (BB) and Maize with banana (MMBB) with exception of MFF.Soil Fungal populations differed significantly (P≤ 0.05) among different agroforestry tree species and ranged between 50.83 in MBSS to 14.00 × 10^5 cfu g^{-1} soil.Fungi populations were significantly (P≤ 0.05)higher in MBSS agroforetry tree combinations as compared to MBCC and MBLL agroforestry tree species combinations. There was significantly (P≤ 0.05**)** low fungal populations in; Maize fertilizer (MMFF), Maize alone (MM),banana alone (BB) and Maize with banana (MMBB).

Table 2:Bacterial and fungal population in soil under the maize-banana based *agroforestry system in KisiiCounty*

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Values are the means of three replications. MBB: Maize + banana; MBCC: Maize +banana + Caliandra; MM: Maize alone; BB: pure banana; MBLL: Maize+banana+leucaena; MBSS: Maize+banana+sesbania; MF: Maize+ fertilizer; Means followed by different letter down the column are statistically different at P≤0.05 by Fisher's protected least significant difference test.Those with more than one letter within a column are intermediates.

3.3 Effect of agroforetry trees on diversity of bacteria and fungi in the soil

3.3.1 Diversity of bacterial isolates

Following Shannon-Wiener index (H´) and Simpson diversity index (D) calculation, it was noted that in the treatments with agroforestry tree species combinations MBBSS (H'=1.61, D= 1) had highest diversity of bacteria isolates, followed by MBCC (H'=1.04, D= 0.83)while MBLL (H'=0.52, D= 0.5) had the lowest diversity. Amongtreatments without agroforestry

Treatments	Fungal (X10^5cfu g^{-1} soil)	Bacteria (X10^8cfu g^{-1} soil)
MBSS	50.83a	197.00a
MBCC	29.167c	128.00bc
MBLL	14.00b	111.25c
MBB	35.33d	156.25abc
MFF	17.33d	163.08ab
MM	12.33e	131.83bc
BB	10.08e	125.75bc
LSD	3.9515	45.935
P.value	<.0001	<.0001
%C.V	20.003	38.8

treescombinations MM (H'=1.1, D= 1) had higher diversity of bacteria isolates compared to MMBB(H'=0.693, D=1), MMF (H'=0.693, D=1) and BB(H'=1.1, D=0.67) (Table 3). Species richness, diversity (expressed as the Shannon–Wiener index) was high in MBSS and low in MMF.

Table 3: Diversity indices of bacterial isolates

Diversity index	MBCC	MBLL	MBSS	MMBB	MM	BB	MMF
Simpsons index (D)	0.83	0.5	1	1	1	0.67	1
Shannon (H´)	1.04	0.52	1.61	0.693	1.1	1.1	0.693
Evenness	0.946	0.811	1	1	1	1	1
Richness	3	2	5	2	3	3	2

Key; H- Shannon diversity Index, D- Simpson diversity Index,MBB: Maize + banana; MBCC: Maize +banana + Caliandra; MM: Maize alone; BB: pure banana; MBLL: Maize+banana+leucaena; MBSS: Maize+banana+sesbania; MF: Maize+ fertilizer.

3.3.2 Diversity of fungal isolates

Shannon-Wiener index (H´) and Simpson diversity index (D) for fungal isolates isolated from treatments with agroforestry tree speciescombinations showed MBLL (H'=1.56, D= 0.93) having a higher diversity index as compared to MBSS (H'=1.39, D= 1) and MBCC (H'=1.04, D= 0.83). In treatments without agroforestry trees species combinations MMBB (H'= 1.1, D= 1) had higher diversity of fungal isolates as compared to MM (H'= 0.868, D= 0.6) and BB (H'= 0.693, D= 1) (Table 4).

Table 4:Diversity indices of fungal isolates

Diversity index	MBCC	MBLL	MBSS	MMBB	MM	BB	MMF
Simpsons index (D)	0.83	0.93	1	0	0.6	1	*
Shannon (H´)	1.04	1.56	1.39	1.1	0.868	0.693	*
evenness	0.946	0.97	1	1	0.79	1	*
richness	3	5	4	3	3	2	*

Key; H- Shannon diversity Index, D- Simpson diversity Index, MBB: Maize + banana; MBCC: Maize +banana + Caliandra; MM: Maize alone; BB: pure banana; MBLL: Maize+banana+leucaena; MBSS: Maize+banana+sesbania; MF: Maize+ fertilizer.*-NO isolates.

4. DISCUSSION

4.1 Effect of agroforetry trees on soil microbial biomass

Results of the study showed higher values of microbial biomass intreatments with agroforestry tree species with MBSS having the highest biomass (Table 1).The findings concur with the previous results of [4].Similar findings were reported by [9]where the amount of the amount of microbial biomass were found to be greater in soils under agroforestry tree systems.Soil microbial biomass, a living part of soil organic matter, is an agent of transformation of added and native organic matter and acts as a labile reservoir for plant nutrients [22].The activity of the microbial biomass is commonly used to characterize the microbiological status of soil[28]. Soil microbial biomass was strongly affected by the presence of agroforestry tree species, presumably by higher inputs of plant litter and root exudates that supplied microbial biomass with carbon and nutrients [32].Agroforestry trees mayfavour accumulation of soil microbial biomass through promotion of better conditions for soil microbial biomass.The availability of carbonaceous materials and substrates such as sugars, amino acids, and organic acids to the soil from the roots of agroforestry trees is important for supplying energy for the microbial populations [21].Significant differences ($P \leq 0.05$) in microbial biomass that were observed among the treatments with agroforestry tree species might have been due to the differences in quantity and quality of plant litter and root exudatesfrom the different agroforestry trees that influenced soil microbial biomass. The low values of microbial biomass inMMFF, MM, BB and MMBBmight have been due to the chemical composition of root exudates from the agroforestry tree species.

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4.2 Effect of agroforetry trees on the total number of bacteria and fungi in the soil

The treatments with agroforestry tree species; MBSS, MBLL and MBCC had significantly large populations of bacteria (Table 2).The present study shows that plots withagroforestry trees species support greater bacterial populations than sole plots.The findings corroborates with those of earlier researchers [35];[4]; [26]. Their findings revealed the predominance of bacteria in soils

from plots with agroforestry trees species. The higher soil bacterial numbersin this study can be attributed to carbon and nutrients incorporated from plant residues[7]. It may also partly be due to higher quantities of root exudates released by agroforestry trees[3]. The exudates provide carbon for bacterial growth and metabolism. The availability of carbonaceous materials and substrates such as sugars, amino acids, and organic acids to the soil from the roots is important because they supply energy for the bacterial populations [12]. Release of rhizodeposits from plant roots influences diversity and populations of bacterial communities [11]. The significantly high number of bacterial populations in MBSS treatment might have been due to quantity and composition of root exudates of *Sesbaniasesban*. Quantity and composition of root exudates is influenced by plant species and developmental stage of the plant[14].The root exudates are readily utilized as carbon sources by bacteria [35].

There were significant differences in bacteria populations among the agroforestry tree species combinations (Table 2).The findings are in agreement with those of [3]. This could be attributed to differencesin amounts and types of exudates released by different agroforestry trees. Composition of root exudates is influenced by the type of plant species [31]. Bacterial communities in root-associated habitats respond with respect to density, composition, and activity to the abundance and great diversity of organic root exudates.

There were significantly ($P \leq 0.05$**)** low bacteria populations in treatments without agroforestry tree species combinations; Maize fertilizer (MMFF), Maize alone (MM),banana alone (BB) and Maize with banana (MMBB)(Table).This could be due to low quantities of nutrients and root exudates to support bacteria growth.

The treatments with agroforestry tree species; MBSS, MBLL and MBCC had significantly large populations of fungi across the tree combinations (Table 2). The results agree with previous results by [13] who reported higher fungal species in mulberry and peanut. This may be attributed to the provision of adequate root niche and rhizosphere exudates and litter by agroforestry tree species.These results can also partly be attributed to microclimate modification by agroforestry tree species. Higher fungal populations can also be attributed to improved soil physical properties as a result of soil organic matter build up by incorporated agroforestry tree prunings. The agroforestry trees prunings in the soil acted as a good source of organic fertilizer

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both for the fungal population and diversity [7]. Microbial activity occurs at a faster rate when maximum organic matter and favourable conditions are available [6].

The low fungal populations in plots without agroforestry tree species combinations may also be attributed to insufficient or depletion of nutrient and lack of organic amendment input into the soil. The lower fungal populations in the treatments without agroforestry trees species combinations may also be attributed to the low nitrogen fixation into the soils. The significantly higher fungal populations in the MMFF treatment may have been due to availability of nutrients supplied by DAP and CAN fertilizers respectively which provided the substrate for soil microorganisms.

4.3 Effect of agroforetry trees on diversity of bacteria and fungi in the soil

4.3.1 Diversity of bacterial isolates

In this study, Shannon diversity indices and Simpson diversity indices for bacteria in treatments with agroforestry tree species combinations ranged between(H'=0.52, D= 0.5) and (H'=1.61, D= 1)**.**MBSS had a greater bacterial diversity and richness compared to MBLL and MBCC (Table3). Shannon-Weaver and Simpson diversity indices takes into account species richness and evenness. The findings of this study agree with the findings of [21] on diversity and richnessof bacteria in agroforestry systems. The high bacterial diversity and richness in MBSS might have been due to root exudates and litter input by *Sesbaniasesban*tree species combinations resulting in high nutrient content. In contrast, bacterial diversity and richness was lower intreatments without agroforestry tree combinations. This was likely due to the roots exudates with low nutrient content that could not support a wide range of bacteria. The results of this study disagreed with the results of [1]. Their study showed the negative effect of agroforestry soils harbouring lower bacterial diversity and constant richness as compared with those of monocrops.

4.3.2 Diversity of fungal isolates

In this study, Shannon diversity indices and Simpson diversity indices for fungal isolates from treatments with agroforestry tree species combinations ranged from (H'=1.56, D= 0.93) to (H'=1.04, D= 0.83) with MBLL having the highest diversity and richness indices, while that of treatments without agroforestry trees species combinations ranged from (H'= 1.1, D= 1) to (H'= 0.693, D= 1) with MMBB recording the lowest indices (Table4).The high values of Shannon diversity indices and Simpson diversity indices and richness for fungal isolates from treatments with agroforestry tree species combinations could be explained by the presence ofdiverse

substrate in form of debris for the fungal community. It could also be due to diversity of root exudates from the different tree species and rich nutrient which supported high fungal diversity.

The low level of diversity indices in the treatments without agroforestry trees species could be attributed to low amount of subtrates in the soil and low nitrogen fixation. It could also be explained by the nature of root exudates which could only provide nutrients to specific fungal species. [24]reported that changes in plant species would be followed by changes in litter composition and changes in soil microbial diversity.

5. CONCLUSION

The soil microbialbiomass,population of bacteria and fungi, and diversity of bacterial and fungal isolateswere significantly higher in treatments with agroforestry tree species combinations in both seasons. MBCC,MBLL MBSS were significanly higher as compared to treatments without agroforestry trees. MBSS had significantly high microbial biomass, microbial populations and diversity among the agroforestry tree treatments.Increase in microbial biomass, microbial populations and diversity is a good indicator of soil health and thus *Sesbania sesban* agroforestry tree species is therefore recommended for use in the maize-banana based agroforestry system in Kisii County.

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Comment [DS19]: Reference is Incomplete.

Comment [DS20]: Correct spelling