Original Research Article

QUANTIFICATION OF MICROBIAL BIOMASSCARBON AND NITROGENIN LEAF LITTER AMENDED SOIL IN NORTHERN NIGERIA

Abstract

An incubation study was carried out to quantify the microbial biomass dynamics in soil using different leaf litter amendments in a 119-daylaboratory study. The treatment consisted of five sources of leaf litter of African mahogany (*Khaya senegalensis*), Mango (*Mangifera indica*), Beech wood(*Gmelina arborea*), and red river gum (*Eucalyptus camaldulensis*) and mixed and this was laid in a Completely Randomized Design with three repetitions. Leaf litters were analyzed for chemical compositions (Organic carbon, nitrogen, sulphur, potassium, calcium, sodium, magnesium, total soluble polyphenols and lignin content analysis).Soil microbial biomass carbon (SMBC) and nitrogen (SMBN) were determined using the chloroform fumigation incubation (FI) method. The litter of *Eucalyptus camaldulensis* recorded the highest value of 33.3 in C: N while *Gmelina arborea* had the least value of 20.6. There were significant differences ($p<0.05$) across the leaf litter types and weeks on soil microbial carbon and nitrogen parameters. *Eucalyptus camaldulensis* amended soilhad the highest MBC (122.85 mg kg^{-1}) which differs significantly ($p<0.05$) from mixed leaf litter, control, *Gmelina arborea, Khaya senegalensis* and *Mangifera indica*. *Khaya senegalensis*amended soiland *Mangifera indica*amended soil had significantly ($p<0.05$) the best microbial biomass nitrogen (13.90 mg kg^{-1} and 13.25mg kg^{-1}) with control being the least. *Eucalyptus camaldulensis* amended soil had significantly ($p<0.05$) the highest (MB) C: N, followed by *Mangifera indica, Gmelina arborea, mix*ed and control. From this study *Mangifera indica, Khaya senegalensis* and mixed has higher propensity for microbial N. This study also shows higher carbon activity in the litter of Eucalyptus camaldulensis.

Key words: Carbon biomass, Nitrogen biomass, Leaf litter, Amendments, Chloroform fumigation

1. INTRODUCTION

Soil microbial biomass is the living portion of the soil organic matter, excluding plant roots and soil animals larger than 5 x 10^{-3} μm^3(Babaniyi*et al*., 2017). The soil microbial biomass generally comprises approximately 2% of the total organic matter in soil and it may be easily dismissed as of minor importance in the soil (Paula *et al*., 2020). Soil microbial biomass parameters give useful information about the restoration degree and quality of contaminated soils (Gu *et al*., 2019). Anderson *et al*. (2017) argued that microorganisms play a leading role in soil development and preservation. Babur and Dindaroglu. (2020) reported that microbial biomass carbon and microbial biomass nitrogen turnover rapidly and reflect changes in management practices long before changes in soil organic carbon and total nitrogen are detectable. This leads to the potential of soil microbial biomass to serve as a soil quality indicator. Usually, a close relationship exists between the quantity and quality of the soil organic substance and the quantity and metabolic activity of the microorganisms (Babur and Dindaroglu, 2020).

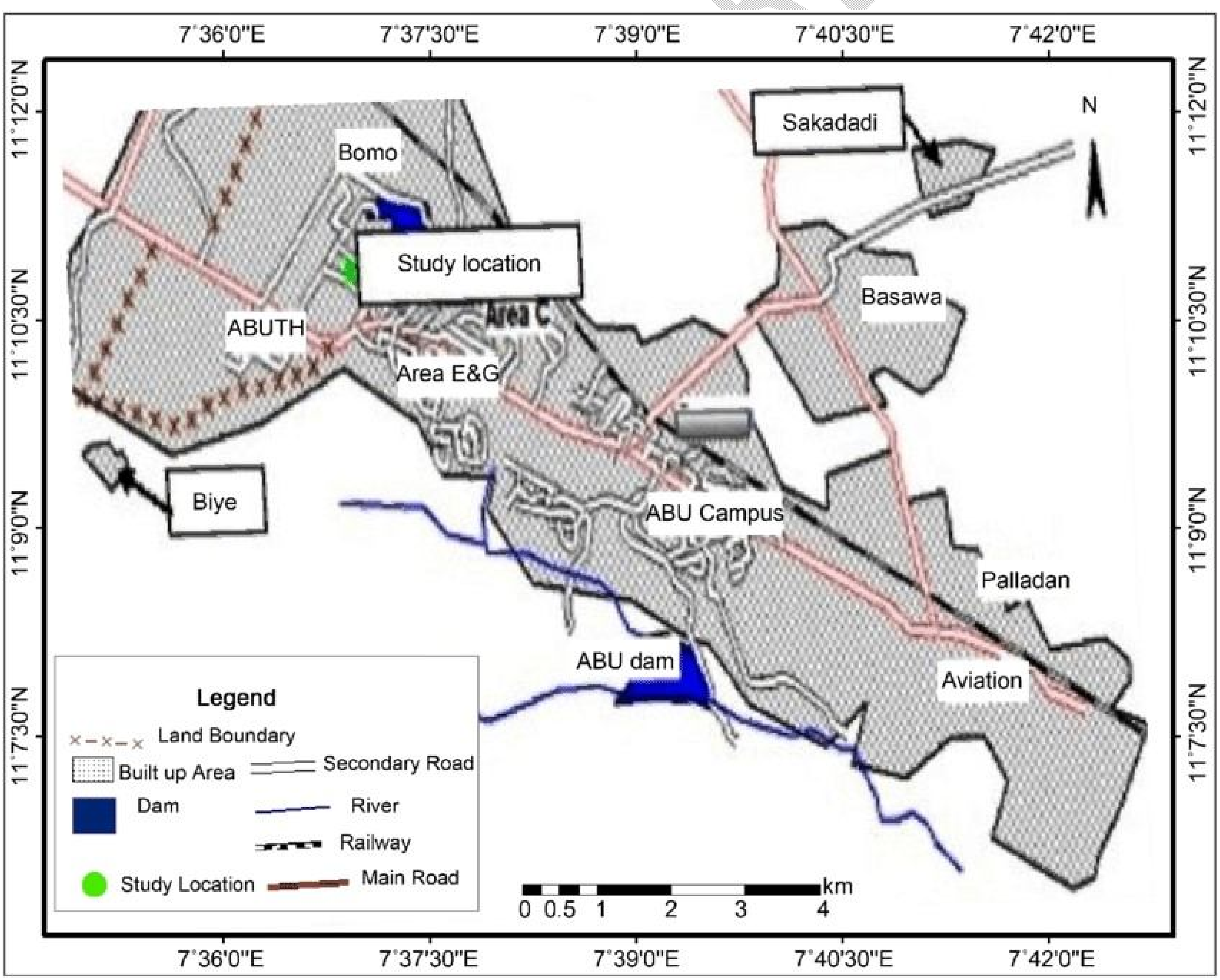
Akratos*et al.* (2017) reported that microorganism needs a good balance of carbon and nitrogen ratio (ranging from 25 to 30). However, in determining microbial activity both the turnover rate and the size of the microbial biomass are pertinent. Depending on climatic and other variables, turnover rates of microbial biomass C (C_{mic}) usually range from 0.5 to 5 years, as compared to >20 years for the bulk of soil organic carbon (Babaniyi*et al.*, 2017). The size of the living community in the soil, the microbial biomass, is generally positively related to the soil organic matter (SOM) level; rarely is C_{mic} less than 1% or more than 5% of the total soil organic carbon(Babaniyi*et al*., 2017). Increases in soil organic matter are usually associated with similar increases in microbial biomass, because the SOM provides the principal substrates for the microorganisms. Soil microbial biomass also as a sizeable reservoir for plant nutrients in soil. A part of the microbial population used to die regularly due to the changes of the environmental conditions (Babur and Dindaroglu, 2020). These dead cells can be easily decomposed and mineralized by the microorganisms that survived and these can contribute a considerable amount of nutrients for the growing plants (Mandal *et al*., 2020).

Leaf litter makes up about 70% of the above-ground forest litter. Leaf litter currently dominates 72% of the research on litter in forest ecosystems (Jia et al., 2018; Wymore et al., 2018; Nicolas et al., 2019).Leaf litter in forest ecosystem serves as a rich source of nutrients for microorganisms through decomposition process and is crucial for maintaining soil fertility, promoting the regular biological cycle, and maintaining the nutrient balance in forest ecosystems (Jiang et al., 2013), all of which have long been of great concerned to researchers (Tan *et al*., 2020). Organic inputs such as leaf litter in soil increases the level of soil microbial biomass, it represents an active fraction of soil organic matter due to its rapid turnover rate and fast releases of available nutrients (Sen Oil *et al.*, 2018), Furthermore, the quantification and concentration of microbial biomass carbon and nitrogen might indicate variations in limiting element over time, reflecting changes in the stages and processes of decomposition. Such vital information is not readily available in the study environment. Therefore, this study aims to estimates the microbial biomass carbon and nitrogen of soil amended with leaf litters of *E. camaldulensis, G.aborea, M. indica, K. senegalensis*and mixed using the chloroform fumigation incubation method.

2. Materials and Methods

2.1. Experimental Site

Thelaboratory incubation experiment was conducted on soil taken from Institute for Agricultural Research, Ahmadu Bello University, Zaria, Kaduna State, Northern Guinea Savannah zone of Nigeria field (IAR plot R14). The experimental area (Samaru) has a geo reference of latitude11^{0} 10' 0" N and longitude 7^{0} 37' 60"E and an altitude of 688 m above sea level (Goggle earth, 2023).



Adapted from Odunze *et al.* (2019).

2.2. Collection and Processing of Leaf Samples

Fall leaves of African mahogany (*Khaya senegalensis)*, Mango (*Mangifera indica*), Beech wood (*Gmelina arborea*), and River red gum (*Eucalyptus camaldulensis*) were picked from the selected tree species. The collected leaf litter samples were cleaned and all sediments and dirt particles were removed by using a soft brush with running tap water followed by final rinsing in distilled water. Each sample was air-dried under shade at the Department of Soil Science Laboratory.

2.3. **Chemical Analysis of Leaf Litter**

Air dried leaf litter samples were ground in mortar and sieved through a 1.0mm/0.0394" mesh size sieve (KimLab). The fine powder was used for the estimation of Carbon (C), Nitrogen (N), Phosphorus (P), potassium (K), Sulphur (S), Calcium (Ca), magnesium(Mg), lignin and total soluble polyphenols. The standard procedures that were adopted for the chemical analysis are presented below.

i. Total carbonwas determined by igniting the samples at 550 °C using the Walkey Black method as reviewed by (FAO, 2019).
ii. Total nitrogencontent in fresh leaf litter was determined by digesting 0.1 g of samples in 5 ml of concentrated sulphuric acid using digestion mixture (sodium sulphate: copper sulphate in 10:4 ratio) and nitrogen in the digest was determined by Kjeldhal's method as reviewed by Saez-Plaza *et al*., (2013).
iii. Total phosphorus content was determined by following chlorostannus reduced molybdophosphoric blue colour method in sulphuric acid system (Wieczorek*et al*., 2022) and the colour intensity was read at 660 nm in UVspectrophotometer (T65 UV PG instruments).
iv. Total basic cations (Ca,Mg, K, and Na) were determined using flame photometry and Atomic Absorption Spectrophotometer (AAS) as appropriate after wet digestion (Anderson *et al*., 2017).
v. Sulphurwas determined turbidimetrically using spectrophotometer to read for absorbance at wavelength of 430nm. (Skok*et al*., 2022)
vi. Total polyphenol was determined using the Follin-Ciocalteu's method (Blainski*et al*., 2013).

vii. Lignin contentdetermined through acid detergent fibre (ADF) via Ankom technology as described by Fukushima. (2015) in the Biochemical laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria.

2.4. Soil Sampling for Laboratory Incubation

The soil was initially characterized in order to assess its fertility status before the application of leaf litter.A total of 24 soil sampleswere collected from a depth 0 – 15 cm at random from different sampling point using shovel. The soil samples from the selected points were thoroughly mixed to get a composite sample. The soil was then air-dried and sieved through a 2mm sieve. A subsample was taken and used for the determination of the following chemical characteristics.A total of 24 soil samples were also collected from the field using core at the same depth for the determination of soil physical characteristics

i. Particle size distribution using hydrometer method (Elfaki*et al*., 2016).

ii. Soil pH in water and $CaCl_2$ were determined in ratio 2.5: 1 water: soil by pH meter (PHS-3CPuchun).

iii. Total N by micro-Kjeldahl procedure.

iv. Soil available P using Bray No. 1 as described by Wieczorek *et al*. (2022).

v. Organic carbon by modified Walkley-Black method as described by FAO. (2019).

vi. Exchangeable bases extraction was done with 1 *N* ammonium acetate pH 7.0. K and Na were determined by flame photometry (PG FP-902), while Mg and Ca were determined using Atomic Absorption Spectrophotometer (MRC AAS-320) (Anderson *et al.*, 2017).

vii. Exchangeable acidity was determined by the KCl extraction method and titrated with sodium hydroxide (NaOH) as laid out by Sebiomo&Banjo, (2021).

viii. Electrical conductivity (EC) in dS m^{-1} was determined using the saturated paste extract as described by Ismayilov*et al*. (2021) while categorization as saline or non-saline was done following Rhoades (1996) classification.

2.5. Laboratory Incubation

The soil (700 g) was pre incubated for one week at room temperature at 50% water-holding capacity (WHC), this is the water content that maximizes microbial respiration (Rinkes *et al.*, 2013). A total of 119 days laboratory incubation was established in a 946 mlsterilized canning jar. The pre incubated soil was mixed thoroughly with each of the sieved litter material to homogenize the soil-litter mixture at the rate of one hectare to 5000kg of litter (Mohammed, 2013). The jars were incubated at room temperature and left loosely covered with lid, which will minimize water lose but allow gas exchange. Jars were weighed initially and deionized water was added on a weekly basis to replace water lost to evaporation. The laboratory incubation was repeated three times and sampling was done at 0, 3, 7, 14, 21, 28, 42, 56, 84 and 119 days of incubation. Samples were analyzed for microbial biomass carbon and nitrogen for each repetition.

2.6. Determination of Microbial Biomass Carbon and Nitrogen

The microbial biomass carbon (SMBC) and nitrogen (SMBN) was determined using the chloroform fumigation incubation (FI) method as described by Logah *et al.* (2010)

Prepared soil samples of 15 g were weighed in duplicate (fumigated and unfumigated) and were arranged in the desiccators, 25 ml of purified chloroform in a beaker was placed at the center of the desiccator for the fumigated samples. The lid of the desiccators was closed tightly and was incubated for 72 hours at ambient room temperature; the same was done for the unfumigated samples. Then 50 ml of 0.5 M K_2SO_4 was added to the samples then placed on a mechanical shaker and shaken for 30 minutes at highest revolution. The solution was then filtered using filter paper of 11μm (particle retention)pore size to get the filtrate. The quantity of CO_2 evolve was determined by titrating the NaOH with standard 1.0M HCl. The SMBC was estimated by the equation,

$$SMBC= [(CO_2\text{-}C)\ fumigated\text{-}\ (CO_2\text{-}C)\ unfumigated/K_c] \quad (1)$$

To estimate the SMBN, 1g portion of the $CHCl_3$ fumigated soil was extracted using 50ml of 0.5 M K_2SO_4. The soil extract was analyzed for total N by the Kjeldahl methodSaez-Plaza *et al.* (2013). The SMBN was calculated by the equation

$$SMBN = [(NH_4\text{-}N)\ fumigated\text{-}\ (NH_4\text{-}N)\ unfumigated/\ K_N] \quad (2)$$

2.7. Experimental design

The experimental layout was a Completely Randomized Design with six treatment and triplicates. The treatment information consists of the followings:

Treatment 1: Soil control

Treatment 2: *K. senegalensis*

Treatment 3: *M. indica*

Treatment 4: *G. arborea*

Treatment 5: *E. camaldulensis*

Treatment 6: Mixed, 25% of each of (*K. senegalensis* + *M. indica* + *G. arborea* + *E. camaldulensis*).

The treatment effect was considered as significant at $P < 0.05$. This was determined based on the F tests produced when fitting the analysis of variance. All analyses were carried out using J.MP 13 Pro.

3. Results and Discussions

3.1. Chemical properties of soil

The soil has a bulk density of 1.3Mg m^3 and moisture content of 0.15 g g^{-1}. The texture of the soil was loam. Organic carbon content and nitrogen content were moderate, exchangeable acidity (0.6 cmol kg^{-1}) was low in the soil. The pH of the soil was 7.35 in H_2O and 6.66 in $CaCl_{2.}$ Most soil processes, including nutrient availability and microbial activity are favoured by soil pH range of 5.5-8 (Bünemann et al., 2018). The neutral pH content in the soil used for this study is an indication that activities of microbial organisms will increase and higher nutrient availability. Ca, Ma, K and Na were (0.18 cmol kg^{-1}), (0.14 cmol kg^{-1}), (0.11 cmol kg^{-1}), (0.42 cmol kg^{-1}) respectively. The soil's effective cation exchange capacity was (1.45 cmol kg^{-}1). The relatively low values of exchangeable cations are ascribed to soil nutrient losses through anthropogenic activities such as burning, cultivation, harvesting or climatic factors leading to leaching that can prompt mobilization and immobilization of these cations (Frimpong *et al.,* 2014). The EC was 0.6 dSm^{-1}.The low EC of the soil is said to improve soil micro-organisms activities, residue decomposition, nitrification and de-nitrification in the soils (USDA, 2020).

3.2. Chemical Composition of Leaf Litters

The chemical composition of the leaf litters is shown in Table 1. The litter of *E. camaldulensis* had the highest concentration of organic carbon, followed by mixed litter, *K. senegalensis*, *G. arborea* while *M. indica* recorded the least concentration of organic carbon in the leaf litters. Higher carbon content as observed in Eucalyptus species connotes higher energy source for microbial population which agrees with the report of Castro-Diez *et al.* (2011). The litter of *G. arborea* recorded the highest total nitrogen concentration while *K. senegalensis* had the least nitrogen concentration in the leaf litters. The litter of *G. arborea* showed the highest concentration of total phosphorus, potassium, magnesium, sodium in the leaf litters. The litter of *E. camaldulensis* recorded the highest value of 33.3 in C: N while *G. arborea* had the least value of 20.6. The C/N ratio is a distinguishing characteristic of organic substrates. The high C to N ratio (33.3) observed in *Eucalyptus camaldulensis* in this study imply poor rates of decomposition and mineralization, thus resulting in inefficient in cycling macronutrients such as nitrogen and phosphorus Ruwanza*et al*., (2014), while the C to N ratio of *M. indica* and mixed litters connotes tendencies of these litters to decompose more quickly due to leaching of readily soluble substances and non-lignified carbohydrates (Kaba, 2017; Naik *et al.,* 2018). The C to N ratios (20.6 -33.3) reported in this study for the leaf litters species are similar to the ratio of 31.6 ± 2.7 reported for 30-year-old cocoa systems but lower than the 42.9 ± 1.5 reported for 15-year-old cocoa systems by Dawoe*et al*. (2010) which suggest that microbial decomposition of leaf litters was partly regulated by leaf litter chemistry or quality and age of tree.

The litter of *Mangifera indica* had the highest concentration of total sulphur, followed by mixed while *K. senegalensis* recorded the least values for total sulphur though *E.camaldulensis* and *Gmelina arborea* had similar values of total sulphur in the leaf litters. Lignin concentration was lowest in the litter of *G. arborea* while combined leaf litters recorded the highest concentrations. Totalpolyphenol content was highest in *M. indica* and lowest in *E. camaldulensis*. Higher polyphenols content obererved in M. indica affect the soil microbial population, by serving as substrates formicrobial respiration this is in accordance with the report of Kumar *et al*. (2021).

Magnesium and calcium composition of leaf litter were 0.5 -0.6 g kg^{-1} and 1.7 g kg^{-1} values in this study which was below the reported values of Li *et al*. (2020) for subtropical evergreen broadleaf forests (7.1–8.3 g kg^{-1}(Ca); 2.0–2.3 g kg^{-1}(Mg)). The large variation in leaf litter nutrient concentrations is related to climatic differences and soil nutrient status. The reported range of 7.0 to 10.2g kg^{-1} for sulphur was higher than the estimate of Wood *et al*. (2006) in Costa rica for Inceptosols (1.37 – 2.23g kg^{-1}), Ultisols plateau (1.17 – 2.51g kg^{-1}) and Ultisol slope (1.37 – 2.27g kg^{-1}). The variations in nutrients concentration in leaf litters type might be connected with soil and climatic conditions (particularly temperature and humidity), soil nutrient content and availability, age of vegetation or plantation, and management types (Kaba 2017; Naik *et al.,* 2018).

The higher level of recalcitrant compounds of lignin and polyphenols in this study agrees with the observation of Dawoe*et al.* (2010) in Ghana who reported higher levels of recalcitrant compounds (lignin and polyphenols) in cocoa leaves than shade tree leaves. Blanco and Aguilar (2015) noted that litter layer has a great influence on the main soil erodibility factor which suggest that litter is the most important soil protection agent for erosion control, therefore litters of *K. senegalensis* and *M. indica* in this study with higher lignin and polyphenol can serve as a major source of erosion control. Moreover, the litter layer will also serve as a major source of soil organic matter when decomposed which strongly influences soil structure, increase soil stability and porosity while increasing the ability of water to infiltrate into the soil and finally controlling the soil erosion rates (Singh *et al*., 2014, Certini*et al*., 2015, Novara *et al*., 2015).

3.3. Microbial Biomass Dynamics

The effect of leaf litters and incubation days on soil microbial carbon, nitrogen and C: N are shown in fig 1,2 and 3. There were significant differences ($p<0.05$) across the leaf litter types and weeks on soil microbial Cand Nparameters. *E.camaldulensis*had the highest MBC (122.85 mg kg^{-1}) which differs significantly ($p<0.05$) from mixed leaf litter, control, *G.arborea*, *K.senegalensis*and *M.indica*possibly due to higher carbon content of*E.camaldulensis*liter and microbial immobilization of nutrients, which encouraged enrichment of the microbial biomass carbon and connotes sustainability of organic matter for soil restoration. High MBC indicated high efficiency of carbon utilization and increase in ecosystem maturity and vice versa (Kaleem *et al.,* 2015). The lower MBC content observed in *Mangifera indica*, Mixed, *Gmelina arborea*, *Khaya senegalensis*is possibly due to low carbon content in these litters and activities of the inhibitory compounds such as lignin and polyphenols.The soil

MBC in this study were comparable to the estimate of 35.8 mg kg^{-1} in *Azadirachta*and 65.4 mg kg^{-1} for *Centrosema*in Ghana as reported by Richard *et al*. (2018) though higher range was reported for some selected organic residues (*Leuceana*, 594.9 – 686.3 mg kg^{-1}), (*Gliricida*, 224.8-753.1 mg kg^{-1}) and (*Peuraria*, 668.8-971.6 mg kg^{-1}). The reasons for differences in values might be attributed to environmental conditions (tropical vs temperate climate, high pH values, differences in soils and management practices.

Maximizing MBN in the soil offers a potential approach to improving the production efficiency of soil, *Khaya senegalensis* and *Mangifera indica* had significantly ($p<0.05$) the best microbial biomass nitrogen (13.90 mg kg^{-1} and 13.25mg kg^{-1}) with control being the least which is an indication that *Khaya senegalensis* and *Mangifera indica* residues application will have tendencies to improve the efficiency of N use in the soil. In this study, significant differences were observed

Table 1: Chemical composition of leaf litter (mean± standard deviation)

Properties	*Eucalyptus camaldulensis*	*Gmelina Arborea*	*Khaya senegalensis*	*Mangifera indica*	Mixed
Organic Carbon (g kg^{-1})	409±1.53	323.4±6.41	333.2 ±5.83	306.1±3.58	341.4±2.16
Total Nitrogen (g kg^{-1})	12.3±0.06	15.7±0.43	10.5±0.2	12.2±0.17	12.8±0.21
Total Phosphorus (g kg^{-1})	1.4±0.17	2.0±0.58	1.7±0.1	1.7±0.15	1.7±0.12
Calcium (g kg^{-1})	1.2±0.06	1.3±0.1	1.7±0.1	1.3±0.06	1.4±0.06
Magnesium (g kg^{-1})	0.6±0.06	0.6±0.1	0.5±0.2	0.5±0.06	0.6±0.06
Potassium (g kg^{-1})	4.5±0.3	4.8±0.2	3.0±0.2	3.7±0.2	4.0±0.02
Sodium (g kg^{-1})	1.6±0.2	0.3±0.06	0.2±0.06	0.1±0.11	0.2±0.2
C: N	33.3	20.6	31.7	25.1	26.8
Total Sulphur (g kg^{-1})	8.6±0.1	10.99±0.01	7.0±0.01	10.2±0.0.5	9.2±0.01
Total Polyphenol(g kg^{-1})	77.5±0.21	86.6±0.10	111.4±0.37	130.9±0.21	101.4±0.4
Lignin (g kg^{-1})	122.1±1.82	92.7±2.4	150.1±0.2	180±1.73	136.7±0.64

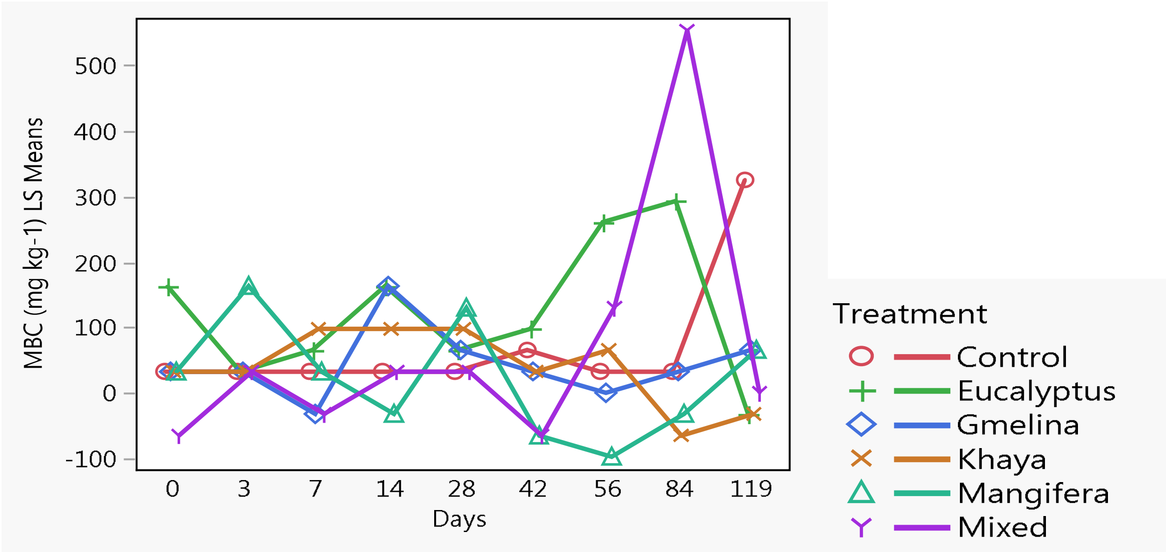


Fig 1: Effect of leaf litters on dynamics of MBC (mg kg^{-1}) at different sampling dates

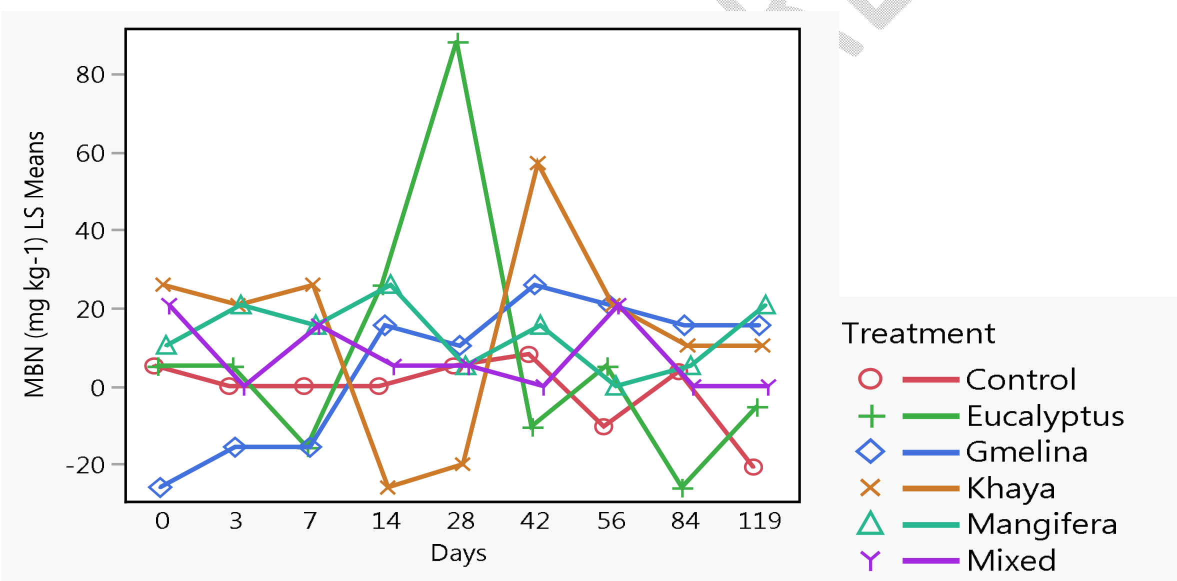


Fig 2: Effect of leaf litters on dynamics of MBN ($mgkg^{-1}$) at different sampling dates

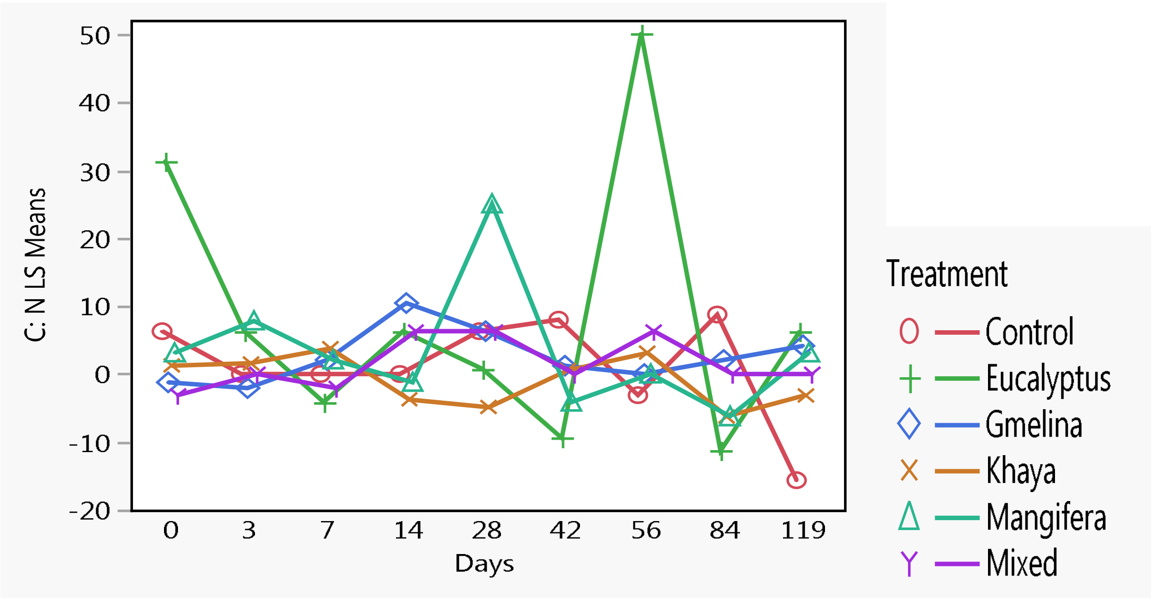


Fig 3: Effect of leaf litters on dynamics of C: N at different sampling dates

among the leaf litter types for (MB) C: N in which *Eucalyptus camaldulensis* had significantly ($p<0.05$) the highest (MB) C: N followed by *M.indica*, *G.arborea*, *mix*ed and control. This result is consistent with the viewpoint that microbes allocate their resources optimally towards acquiring the most limiting resource which agrees with the report of Xiojun*et al.* (2013) in Southwestern China. The variations in the microbial element ratio observed among the leaf litters in this study might be connected to the higher labile fractions observed from leaf litters due to the activities of inhibitory compounds (lignin and polyphenols). The amount of labile carbon is of particular importance as this provides a readily available carbon energy source for microbial decomposition.

The observed significant interaction between leaf litter types and week of incubations on microbial carbon and nitrogen dynamics presupposes varying nutrient recoveries from the leaf residues in the soil.

4. Conclusion

Microbial biomass dynamics show the micro-changes occurring in the soilin the short term. Result indicated that microbial concentration depended on the organic matter availability to microbial activity. From this study *Mangifera indica, Khaya senegalensis* and mixed has higher propensity for microbial N. This study also shows higher carbon activity in the litter of *E.camaldulensis.* The result of this study provides a basis for microbial decomposition and further studies are needed to access microbial biomass carbon and nitrogen contributions to crop productivity using these leaf litters. Leaf litters of above species can be applied to amend the soil to reduce the negative effect of inorganic fertilizer and can also be incorporated with organic fertilizer use to increase yield and sustainable production.

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