LAND USE EFFECT ON PLANT SPECIES DIVERSITY AND SOIL MICROBIAL BIOMASS CARBON IN ALETA CHUKO OF SIDAMA REGION, ETHIOPIA †

[EFECTO DEL USO DE LA TIERRA SOBRE LA DIVERSIDAD DE ESPECIES DE PLANTAS Y EL CARBONO DE LA BIOMASA MICROBIANA DEL SUELO EN ALETA CHUKO DE LA REGIÓN DE SIDAMA, ETIOPÍA]

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SUMMARY

Background. There was a widespread conversion of species-diverse traditional agroforestry to monoculture land uses in Aleta Chuko, Ethiopia, particularly since 1990. However, the effect of such land use change on plant species diversity and soil microbial biomass in agroecosystems was not investigated. **Objective.** To investigate the effect of land use (LU) on plant species diversity and soil microbial biomass carbon (MBC), determine association between plant species diversity and MBC in Aleta Chuko district. **Methodology**. Three LU systems, namely, Coffee-Enset Agroforestry (CEA), Eucalyptus Woodlot (EW), and Chat Mono-cropping (CM), were aligned in three transect lines based on a spatial analog design; four (4) plots were used for each LU from individual transects, for a total of 36 plots (12 plot per LU), and then 108 soil samples were taken from three diagonal pits within 20 × 20 m, which was also used for species diversity assessment. The soil MBC was extracted via chloroform fumigation extraction and analyzed at plot level. **Results**. A total of 37, 16, and 8 plant species were recorded in the CEA, EW, and CM treatments, respectively. The Shannon diversity indices were 2.40, 0.40, and 0.03 for CEA, EW, and CM, respectively. Jaccard's index indicated negligible similarity (0.15) among the three LUs. However, consistent similarity was observed between transects within each LU, with higher similarity (0.75) recorded for CEA among transects. MBC was 586.3, 298.2 and 313.8 µg g^{-1} soil in the CEA, CM and EW soils, respectively. MBC in CEA was significantly greater than that in the other two LUs, but there was no significant difference between CM and EW ($p < 0.05$). There was a strong positive correlation ($r = 0.854$) between MBC and plant richness. **Implication**. The strong positive association between MBC and plant diversity implies that decline in plant diversity result in associated degradation of MBC that impede the sustainability of agroecosystems. **Conclusion**. The expansion of monoculture has weakened plant diversity and soil microbial biomass carbon in agroecosystems. Further study on the plant species-specific association of microbial biomass is needed.

Key words: Agroforestry; Association; *Catha edulis*, Eucalyptus; Microbial biomass; Monocropping; Regeneration.

RESUMEN

Antecedentes. En Aleta, Chuko, Ethiopia, existió una conversión generalizada de agrosilvicultura tradicional con diversas especies a un uso de la tierra como monocultivo, particularmente desde 1990. Sin embargo, no se investigó el efecto de dicho cambio de uso de la tierra en la diversidad de especies de plantas y la biomasa microbiana del suelo en los agroecosistemas. **Objetivo**. Investigar el efecto del uso de la tierra (LU) sobre la diversidad de especies de plantas y el carbono de la biomasa microbiana del suelo (CBM), determinar la asociación entre la diversidad de

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especies de plantas y el CBM en el distrito de Aleta Chuko. **Metodología.** Tres sistemas LU, a saber, Agroforestería Coffee-Enset (CEA), Eucalyptus Woodlot (EW) y Chat Monocultivo (CM), se establecieron en tres líneas de transecto basadas en un diseño espacial analógico; Se usaron cuatro (4) parcelas para cada LU de los transectos individuales, para un total de 36 parcelas (12 parcelas por LU), y luego se tomaron 108 muestras de suelo de tres pozos diagonales dentro de 20 × 20 m, que también se usaron para evaluación de la diversidad de especies. El CBM del suelo se extrajo mediante fumigación con cloroformo y se analizó a nivel de parcela. **Resultados.** Se registraron un total de 37, 16 y 8 especies de plantas en los tratamientos CEA, EW y CM, respectivamente. Los índices de diversidad de Shannon fueron 2.40, 0.40 y 0.03 para CEA, EW y CM, respectivamente. El índice de Jaccard indicó una similitud insignificante (0.15) entre las tres LU. Sin embargo, se observó una similitud constante entre los transectos dentro de cada LU, con una mayor similitud (0.75) registrada para CEA entre los transectos. El CBM fue de 586.3, 298.2 y 313.8 µg g^{-1} de suelo en los suelos CEA, CM y EW, respectivamente. El CBM en CEA fue significativamente mayor que en las otras dos LU, pero no hubo diferencias significativas entre CM y EW ($p < 0.05$). Se encontró fuerte correlación positiva ($r = 0.854$) entre el CBM y la riqueza de plantas. **Implicación**. La fuerte asociación positiva entre el CBM y la diversidad de plantas implica que la disminución de la diversidad de plantas da como resultado una degradación asociada del CBM que impide la sostenibilidad de los agroecosistemas. **Conclusión.** La expansión de monocultivos ha debilitado la diversidad de plantas y el carbono de la biomasa microbiana del suelo en los agroecosistemas. Se necesitan más estudios sobre la asociación de la biomasa microbiana específica a las especies de plantas.

Palabras clave: Agroforestería; Asociación; *Catha edulis*; Eucalipto; Biomasa microbiana; Monocultivo; Regeneración.

INTRODUCTION

Plant diversity within a given ecosystem is essential for basic interactions among life forms. Loss of tree species can induce the extinction of mutually dependent ecologically crucial herbs because there is a symbiotic relationship among some trees and other living entities, such as edible herbs, fungi and bacteria, either to have mutual or self-beneficial effects (Carrapiço, 2021). Some plants under human managed ecosystems are threatened as their mutual associates disappear as a consequence of land use (LU) conditions and land use changes (WWF, 2020). In particular, the expansion of unsustainable agricultural practices and monoculture expansion decreases the density and diversity of woody perennials (Abebe *et al*., 2013), resulting in: 1) the extinction of some species, such as medicinal plants, even before their importance is known to the scientific community, 2) a decrease in soil microbial biomass and 3) disturbance in the global carbon cycle, mainly through the reducing atmospheric carbon sequestration (Lal, 2020).

Cultivated plant diversity is primarily determined by human preferences based on socioeconomic or ecosystem importance. Cultivated plants are modified from their wild natural ecosystems to adapt to human management, utilization, and control for propagation and survival in managed ecosystems (Fuller *et al*., 2023). Maintaining cultivated plant diversity in traditional farming systems enhances resilience in socioeconomic and environmental systems by sustaining agricultural production, preserving plant species, enhancing soil microbial biomass, recycling nutrients, and sequestering more carbon than monocultures (Liu *et al*., 2020; Bastida *et al*., 2021). Soil microbes are the primary agents of litter decomposition, facilitating the return of organic matter to the soil, and their biomass serves as an indicator of carbon assimilated by soil microbes (Kaur *et al*., 2015). However, soil microbial biomass carbon (MBC) is influenced by land-use (LU) practices, with undisturbed LU systems supporting greater microbial biomass compared to disturbed counterparts (Lepcha and Devi, 2020).

LU-induced loss of plant species diversity leads to degradation of soil microbial biomass because these two ecosystem components are positively correlated to each other as plants and microbes are inseparable, having coevolved since the evolution of the first plants (Lyu *et al*., 2021). Loss in plant diversity and associated drop in MBC hampers efforts done to soil resource management and climate change mitigation since litter conversion into SOM and soil carbon sequestration is strongly linked to soil microbial communities (Bastida *et al*., 2021; Beugnon *et al*., 2023).

In the Aleta Chuko district, coffee-enset-based traditional agroforestry (CEA), Eucalyptus monoculture woodlots (EW), and Chat monocropping (CM) are the dominant types of land use (LU) practiced by smallholder farmers. The traditional agroforestry LU, which has sustained livelihoods and the environment for a long time, is under threat due to its transition to CM systems (Abebe, 2018; Mellissie *et al*., 2018), which are expected to suppress native species. This shift from species-rich agroforestry to monoculture systems is anticipated to negatively affect the diversity of plant species, including edible herbs, medicinal plants, soil microbial biomass, and soil carbon sequestration in the area. Chat monoculture and eucalyptus woodlots are two economically vital LUs that provide essential income for smallholder farmers. However, the focus on short-term economic gains through the expansion of these LUs is increasingly compromising the long-term sustainability of agroecosystems in the area.

There are studies on the expansion of Chat (*Catha edulis* Forsk) into traditional agroforestry systems in southern Ethiopia (Abebe, 2018; Mellisse *et al*., 2018). Reports indicate that this trend has increased from 6% to 35% of the area per farm, while CEA decreased from 45% to 25% over two decades (1990–2013) in Sidama and Gedeo agroforestry systems, including the present study area (Mellisse *et al*., 2018). However, the effects of land use changes from traditional agroforestry to monocropping systems on plant species diversity and microbial biomass carbon (MBC) remain unclear. Additionally, the plant-soil microbial biomass carbon association under the combination of coffee and enset (a staple food crop) has not been investigated. While the nutritional and socioeconomic importance of enset-combination traditional agroforestry has been widely studied, its environmental contribution has received less attention. Furthermore, current environmental challenges, such as biodiversity loss, climate change, and soil resource degradation, can be addressed by understanding the linkages among ecosystem components.

Therefore, the aims of this study were to determine the effect of different traditional and modified LU systems on the diversity of plant species and soil MBC, determine association between plant species diversity and MBC. It is hypothesized that a) plant species diversity and soil MBC is greater under coffee-enset-based agroforestry than under *Eucalyptus* woodlots and Chat monoculture land uses and b) there is a positive correlation between soil MBC and plant species diversity.

MATERIALS AND METHODS

Site Description

Location

This study was conducted in the Aleta Chuko district of the Sidama region, Ethiopia. The site is situated in the western escarpment of the South Eastern Highlands. The study district is 340 km away from the national capital, Addis Ababa (Fig. 1). Seventy percent (70%) of the district is agro-ecologically designated as having a *Woina Dega* (warm tropical) climate. The altitude of the district ranges from 1400 to 2000 m.a.s.l. The mean monthly temperature and mean annual total rainfall are 17.4°C and 907.97 mm, respectively (Fig. 2). The representative sites selected for the study were three kebeles, namely, Rufo Waeno, Korke and Dongora, which have similar biophysical environments (see sampling design under section 2.2.1). The sites are located between 6º 33’ 27”N to 6º 38’ 54”N latitude and 38º 18’ 53”E to 38º 21’ 6” E longitude. Sample plots were taken from elevations ranging from 1825 to 1838 m.a.s.l.

Topography and Soils

The topography of the areas has features of flat land, undulating terrain and small hills with moderate to steep slopes. Sandy clay loam, clay loam and clay soils are the dominant soil textures in the study area. The soils are locally named by their color as *‘keyi afer’* (meaning red soil) and 'Koticha’ (meaning black soil). According to the World reference base (2006) soil group, soils of the study areas is characterized by Luvisols (ISRIC, https://soilgrids.org/).

Land Use and Management

Sidama traditional agroforestry is a highly developed and complex agroforestry practice with a long history (Asfaw and Agren, 2007), although its origin has not been specified in the literature. The people of Gedeo and Sidama are among the first people who cultivated Enset-coffee agroforestry more than 3000 years ago, the ancestor of agroforestry (Kanshie, 2002). As neighbors, aspects of Gedeo and Sidama LU and farming practices are closely related (Ibid). Sidama agroforestry practices include enset-coffee, tree-enset, *Eucalyptus* woodlots, scattered/parkland trees on maize, boundary planting, and scattered trees on grazing fields (Asfaw and Agren, 2007). The Aleta Chuko district is characterized by major LUs, such as Enset-Coffee-based agroforestry, Coffee-Enset-based agroforestry (CEA), Coffee-based agroforestry, Enset-based agroforestry, seasonal cropland, emerging LU, i.e. Chat monocropping (CM) and woodlots of either Eucalyptus monoculture or Eucalyptus with other species (Abebe, 2005). This was also confirmed by the preliminary survey of this study.

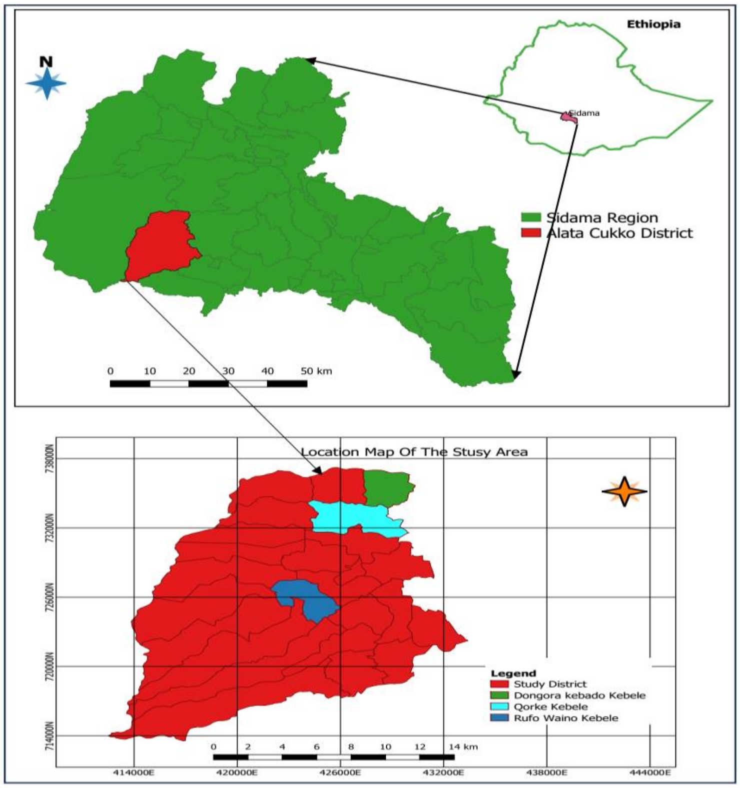

Figure 1. Map of the Aleta Chuko district (source: Ethio-GIS).

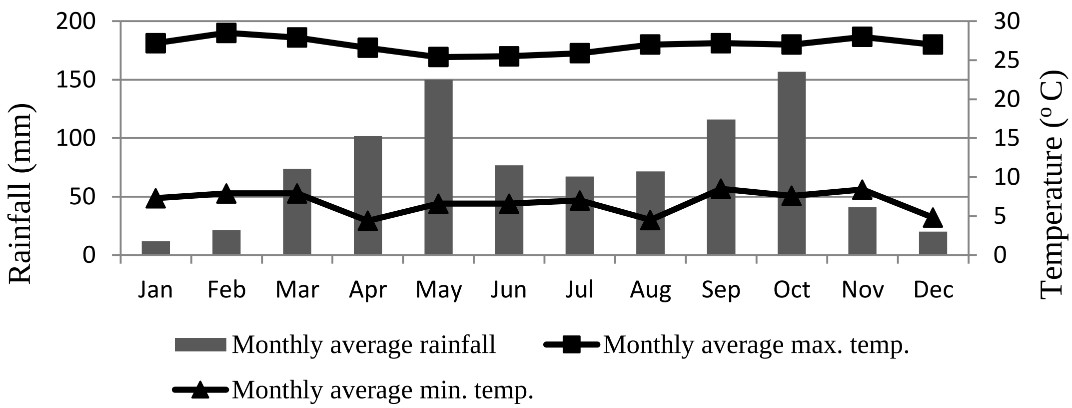

Figure 2 Mean monthly rainfall (mm) and mean monthly maximum and minimum temperatures (°C), ten years average from 2009-2018 (Source: NMSA, 2022).

CM and EW are the two LU types that play vital economic roles in generating income for smallholder farmers. CM land use is expanding alarmingly at the expense of traditional agroforestry, woodlots and more than a few available grazing areas. Chat cash crop farms require frequent tillage 3 to 5 times a year and can be harvested 3 to 4 times a year. Additionally, the EW are areas where farmers set aside part of their land for growing planted and/or conserved trees (Abebe, 2005; Bekele, 2011). Woodlots are established from either *Eucalyptus* monoculture or a mixture of other tree species, including a few native species. *Eucalyptus* has commonly been harvested for 5 to 12 years as a construction material and for fuel-wood (Abebe *et al.*, 2013).

Enset (staple food crop) combination under traditional CEA provides many environmental and livelihood benefits in the south central and southern parts of Ethiopia. Enset conserve higher soil moisture even during dry season that is useful for many ecosystem processes including suitable ground for soil microbial accumulation. Woody and herbaceous species are also planted in CEA as hedge-rows that serve many purposes, such as fuel-wood, fencing and construction materials. However, large trees are traditionally conserved as threatened species that are valued as valuable community resources. Tree management in agroforestry includes pruning, pollarding, lopping or trimming, whereas eucalyptus is harvested by means of coppicing because it has the capacity to recover after cutting down its stem. The farming community of Aleta Chuko practices minimum tillage and maintains soil fertility in the CEA through the application of animal dung, crop residues, and domestic organic wastes.

Sampling Design and Data Collection

Before sample collection, a reconnaissance survey was carried out to determine the study sites and verify the LU type and sampling method. Based on the biophysical environmental characteristics of vegetation, slope, altitude and topography, three *kebeles*, namely, Rufo Waeno, Korke and Dongora, were selected since they have similar biophysical environments that enable comparisons between LU practices and their effects on plant species diversity and soil microbial biomass. Thus, to investigate the effects of LU on ecosystems over time, the study sites were selected based on spatial analogues where three LU types, namely, EW, CEA and CM, are situated adjacent to each other under similar climate, altitude, soil and slope conditions. To reduce the management effect, sample plots were laid at least 20 m from home, assuming that the homesteads were dominated by herbaceous species and that the addition of more domestic waste and/or animal manure around the homesteads causes variation in soil properties as compared to other widespread areas of farms (Tittonell *et al*., 2012).

Three east–west-aligned transect lines were established on gentle slopes using a spatial analog design. From each transect, four adjacent situated plots were assigned to each land use (LU), resulting in a total of 36 plots (12 plots per LU). These plots were utilized for microbial biomass carbon (MBC) and species diversity assessments, with 108 soil samples collected from three diagonal pits within each 20 × 20 m plot (Buckland *et al*., 2007; Bonou *et al*., 2009).

For species diversity assessment, tree diameter and height were measured using a diameter tape and a Suunto hypsometer, respectively. In addition, centimeter graduated stick was used to measure heights of seedling and saplings. Saplings and seedlings were counted within 5 × 5 m subplots placed at the corners and center of each 20 × 20 m plot, accounting for their sparse distribution across the three LUs (Pearson *et al*., 2005). Seedlings were defined as individuals under 1.0 m in height with collar diameters <2.5 cm; saplings ranged from 1.0–3.0 m in height with diameters of 2.5–9 cm; and trees were taller than 3.0 m with a DBH >10 cm (Negash, 2013; Shiferaw *et al*., 2018). Species and families were identified using comprehensive reference materials, including *Flora of Ethiopia and Eritrea* (Hedberg *et al*., 1989; Edwards *et al*., 2000), *Plants and Vegetation of NW Ethiopia* (Friis *et al*., 2022), *Useful Trees and Shrubs for Ethiopia* (Azene, 2007), and *Atlas of Potential Vegetation of Ethiopia* (Friis *et al*., 2010).

Soil laboratory analysis was done at the pit level and then composed to the plot level for statistical analysis. Soil samples were taken with a core sampler (5 cm diameter and 10 cm height) from 0-30 cm depth for soil MBC extraction (Lepcha and Devi, 2020). The surface layer (0-30 cm) of the soil is the most relevant depth for considering the impact of LU on soil microbial biomass and other nutrients, as this layer can be modified directly by cultivation (Ellert and Bettany, 1995). Plastic bags were used to label and transport fresh soil samples to the Wondo Genet College of Forestry and Natural Resource Laboratory for soil MBC extraction. Fresh soil samples were used for MBC extraction, samples were 2 mm sieved, and small roots were removed by hand. Then, chloroform was added to the moist soil samples for 24 hours, the fumigant was removed, and the soil MBC was extracted with K_2SO_4. Organic carbon in the solution was determined by dichromate oxidation. Non fumigated samples were extracted under the same procedure at the time fumigation started (Vance *et al*., 1987).

Measurement and Calculations

Diversity and Evenness Indices

Plant species diversity was calculated using the Shannon–Wiener diversity index (H') based on the Magurran (2004) formula.

$$H' = -\sum_{i=1}^{S} Pi \ln Pi$$

where:
pi is the proportion of individuals found in the i^{th} species.

The proportion (p) is (n/N), which is the number of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log, and S is the number of species.

The evenness index was calculated based on (Magurran, 2004).

$$H`/\ln S$$

Where:
H`= Shannon Weiner diversity index
S=number of species in the assemblage, and ln is the natural logarithm.

The Shannon–Wiener diversity index commonly ranges from 1.5 to 3.5 and rarely exceeds 4; a high value of its index indicates a large number of species with similar abundances, whereas a low value indicates domination by a few species.

Similarity Index

The Jaccard similarity index was calculated using species similarity between transects and among LUs.

$$\mathrm{Cj} = \frac{a}{a+b+c}$$

where:
a is the total number of species present in both samples;
b is the number of species present only in sample 1,
c is the number of species present only in sample 2.
The Jaccard index ranges between 0 (no similarity) and 1 (identical sets) (Magurran, 2004).

Importance Value Index

Measurements of the frequency, density and dominance of the trees were made on the trees to determine the importance value index (IVI). IVI was calculated using three components (Kent and Coker, 1992).

IVI = Relative Dominance + Relative Density + Relative Frequency

where:
Relative dominance = (total basal area of a species/basal area of all species) 100
Relative density = (No. of individuals of tree species/total No. of individuals) 100.
Woody species density is the number of individuals divided by the sampled area.
Frequency= (number of plots in which species occur/total number of plots) 100
Relative frequency= (frequency of tree species/frequency of all tree species) 100

To determine the basal area of each tree, the diameter at breast height (DBH) of the trees was measured, and the basal area of each tree was calculated as basal area = 0.00007854 $(DBH)^2$ (Young and Giese, 2003).

Regeneration status

The regeneration status of woody species in the CEA was evaluated based on the population size of mature trees, saplings and seedlings (Tiwari *et al*., 2010). Accordingly, the following criteria were used to determine regeneration status:

1) **Good:** if seedlings > saplings > adults;

2) **Fair:** if seedlings > or ≤ saplings ≤ mature tree;

3) **Poor**: if the species survives only in the sapling stage but not in the seedling stage (saplings may be <, > or = mature tree).

4) **Not regenerating**: If a species is present only in an adult stage, if the species has no adults but only seedlings or saplings, it is considered a new species.

Soil Microbial Biomass Carbon

Soil microbial biomass carbon (MBC) was extracted and calculated by Vance *et al*. (1987) method of chloroform fumigation extraction:

$$\mathrm{MBC} = \mathrm{EC} \times 2.64$$

Where:
MBC is the soil microbial biomass carbon,
EC is the difference between C-fumigated and unfumigated soil samples,
2.64 is the proportionality factor for biomass C released by fumigation extraction.

Note: The IVI and regeneration status assessments were conducted exclusively for the CEA land use. These assessments were not feasible for CM and EW due to specific limitations. Calculating IVI requires the basal area of woody species, and evaluating regeneration status necessitates the presence of trees, mature trees, saplings, and seedlings, which are not well distributed in CM and EW. Additionally, EW is predominantly characterized by herbaceous and liana plants. Species conservation is also impractical under CM and EW. Eucalyptus plantations in EW suppress the regeneration of other species, while chat cropping plantations in CM require the removal of other vegetation.

Statistical analysis

Statistical analyses were performed using IBM SPSS version 26. The woody species regeneration was evaluated with respect to the population size of mature trees, saplings and seedlings. The Shannon–Wiener diversity and the Jaccard similarity indices were used to analyze plant species diversity and similarity. Pearson correlation was used to determine the association between soil MBC and plant species diversity at an alpha of 0.05. One-way ANOVA was employed to assess whether there was a significant difference in the soil MBC among the LU types, namely, CEA, EW and CM. Duncan's multiple mean comparisons test was used to check significant differences in soil MBC between LUs at an alpha of 0.05. The Kruskal–Wallis test followed by the Mann–Whitney U test were used to determine significant differences in the Shannon–Wiener diversity index for cultivated plant species among LUs.

RESULTS AND DISCUSSION

Species composition and diversity

Composition of cultivated plant species per land use

A total of 51 woody and herbaceous cultivated plant species were recorded in the three land use (LU) systems of the study sites in the Aleta Chuko district. Plant species richness and abundance varied across the three LU systems. The most abundant species in coffee-enset-based agroforestry (CEA) were woody and/or tree species, while monoculture eucalyptus woodlots (EW) were characterized by herbaceous, liana and shrub undergrowth. Chat monocropping (CM) is defined as the presence of infant sprouts of tree or shrub stem basal shoots (appendix A). A total of 37 species, 78.4% woody and 18.9% herbaceous, belonging to 23 families were recorded in the CEA (appendix A). A total of 16 species were identified, 43% of which were stunted and basal sprouts of shrubs, 19% of which were liana and 25% of which were herbaceous plants in the EW. A total of 8 plants were cultivated in CM, of which 63% were trees and 37% were shrubs. The CM tree species were found in the form of basal shoots that regenerated after pruning. This was due to the management practices that decrease tree growth in CM to enhance chat productivity; trees are pruned at the basal stem.

Diversity and Similarity of Plant Species by Land Use

The Shannon–Wiener diversity indices were 2.40, 0.40, and 0.03 for CEA, EW and CM, respectively (Table 1). The low Shannon–Wiener diversity in EW and CM indicates the dominance of Chat and Eucalyptus in these LUs. However, the results obtained in this study indicate that there is moderate diversity and high evenness since the value is close to 1 in the CEA (Magurran, 2004; Hill *et al*., 2005). The Shannon–Wiener diversity index decreased in the following order: CEA > EW > CM ($p < 0.05$). The high diversity in CEA provides habitat for diverse species, while monoculture LU erodes agro-biodiversity (Udawatta *et al*., 2019).

The Jaccard similarity indices were 0.2, 0.1, and 0.1 between CEA and CM, CEA and EW, and EW and CM, respectively. Although there was consistent similarity within each LUs across transects, there was negligible resemblance among the LUs (0.15) Table 2). There was a high similarity of species among transects within the CEA, as an index close to one indicated greater similarity and evenness within this LU (Table 2). The greater similarity of species along transects within each LU suggests that the biophysical environment of the study sites was similar (Table 2). The negligible similarity of species among the three LUs, but higher similarity along transects within each LU, indicates that species distribution was influenced by LU practices.

There are two fundamental reasons for the differences in species diversity among LUs. First, there is a difference in the management between coffee-enset agroforestry and Chat LU because many native and exotic tree species grow under CEA, as these are vital agroecosystems components because of their importance for soil fertility and socioeconomic purposes. However, in CM tree shades, soil mineral exploitation by trees is not required because it reduces the productivity of the Chat crop. Second, native tree species in the EW were unable to compete and grow due to the allelopathic suppressive nature of eucalyptus over native tree species (Liang *et al*., 2016); however, the greater diversity compared to that in the CM was due to the presence of some non-tree species in the EW (appendix A). The lack of tree species in the EW treatment indicates that Eucalyptus plantations induce greater impact via allelopathic effect on tree species than shrubs, herbaceous plants and lianas (appendix A).

The woody species richness recorded in the CEA was consistent with the results obtained in the Shebadino district of Sidama Agroforestry (32 species) (Molla and Asfaw, 2014) and the coffee agroforests in the Belete forest in southwest Ethiopia (33 species) (Yasin *et al*., 2018). However, species diversity in the CEA was lower than that of a similar study in the Dello Menna district in southeastern Ethiopia (55 species) (Molla and Kewessa, 2015). Conversely, the findings of this study in the CEA were greater than those recorded in the Bulen district (22 species), northwestern Ethiopia (Megabit *et al*., 2018). The reasons for these differences include: 1) differences in research design, such as the 20-meter proximity to the homestead, where many herbaceous and shrub species are present; 2) the confinement of this study to CM expansion areas with similar biophysical environments; and 3) the inclusion of only three major LUs in the current investigation. Other agroforestry systems, such as enset-based systems, coffee-based agroforestry, roadside diversity, and mixed plantation woodlots, were excluded due to limited financial resources.

Importance Value Index and Regeneration Status of Woody Species in the CEA

A total of twenty-nine (29) woody species, including *C. arabica*, belonging to eighteen (18) families were recorded. In terms of stem density, *C. africana*, *M. ferruginea*, *P. americana*, and *P. falcatus* had relatively high stem densities, with 250 ha^{-1}, 197 ha^{-1}, 158 ha^{-1}, and 98 ha^{-1} individuals, respectively, including seedlings. These four species account for 66% of the total woody individuals (512 individuals excluding *C. arabica*) recorded with the percentage composition for *C. africana* 23.44%, *M. ferruginea* 18.55%, *P. americana* 14.84%, and *P. falcatus* 9.18%. Total tree density and stand basal area for woody species in CEA were 737.5 stems ha^{-1} (for trees and saplings) and 22.27 m^2 ha^{-1}, respectively.

Importance value index

The importance value index (IVI) measures the degree of relative dominance and abundance of a given species in relation to the other species in the area (Kent and Coker, 1992). Based on IVI, the most dominant species in the CEA were the first six lists of species (Table 3). Although *E. brucei* has a few individuals across sample plots, its IVI was exaggerated by a greater DBH and/or basal area.

Regeneration status of woody species in coffee-enset agroforestry

Information on species regeneration status is essential for implementing conservation measures for vulnerable species. Under similar environmental conditions, such as altitude and soil fertility, the population of seedlings is expected to be greater than that of saplings, and the population of saplings is expected to be greater than that of mature trees for adequate regeneration (Tiwari *et al*., 2010). Furthermore, in managed agroecosystems, the density of each age category, even including the type of species to survive, inevitably interfered by farmers' choices based on the importance of the species for food, other asset production and ecological basis (Mustapha and Jimoh, 2012).

Table 1. Species diversity and evenness across LUs along transects within each LU.

Transect	Shannon–Wiener Diversity and Evenness Index							
	Across three LU		CEA		EW		CM	
	H'	E	H'	E	H'	E	H'	E
	1.12	0.28	2.40^{a}	0.70	0.40^{b}	0.20	0.03^{c}	0.01
T1	1.10	0.31	2.50	0.73	0.40	0.20	0.03	0.02
T2	0.95	0.26	2.35	0.68	0.23	0.10	0.02	0.00
T3	1.19	0.33	2.06	0.62	0.13	0.06	0.03	0.01

The Shannon–Wiener diversity index across three LUs in rows followed by different letters indicates a significant difference between LUs ($p < 0.05$). T= transect, CEA=coffee enset agroforestry, CM= chat monocropping, EW=eucalyptus woodlot, H'= Shannon diversity index, E = Shannon evenness index, LU=land use.

Table 2. Species similarity within each land use.

Transects	Jaccard similarity index: Across three land use	CEA	EW	CM
T1&T2	0.69	0.74	0.33	0.63
T1&T3	0.66	0.70	0.43	0.63
T2&T3	0.69	0.74	0.38	0.5
CEA & CM	0.20			
CEA & EW	0.1			
CM & EW	0.1			
Three LUs	0.15			

T=transect, CEA=Coffee Enset Agroforestry, CM= Chat Mono-cropping, EW=Eucalyptus Woodlot.

Table 3. Importance value index of woody species in CEA excluding *Coffea arabica.*

Species	Relative density	Relative frequency	Relative dominance	IVI	Rank
Cordia africana L.	23.44	9.38	29.19	62.00	1
Persea americana Mill.	14.84	7.03	14.10	35.97	2
Millettia ferruginea Hochst. Bak.	18.55	6.25	10.19	35.00	3
Podocarpus falcatus (Thunb.) Mirb.	9.18	7.03	10.37	26.58	4
Erythrina brucei Schweinf. G	1.37	3.13	18.81	23.31	5
Ocotea kenyensis Robyns. Wilczek	3.52	5.47	11.41	20.39	6
Mangifera indica L.	1.76	5.47	2.92	10.15	7
Prunus africana (Hook. f.) Kalkman	2.93	3.13	1.75	7.81	8
*Syzygium guineense (*Willd.) DC.	2.93	4.69	0.00	7.62	9
Ehretia cymosa Thonn.	2.73	4.69	0.00	7.42	10
Bersama abyssinica Fresen.	3.13	3.91	0.13	7.17	11
Croton macrostachyus Hochst	2.54	3.91	0.13	6.58	12
Vernonia amygdalina Del.	2.34	3.13	0.04	5.51	13
Brucea antidysentica J.F. Mill.	1.37	3.91	0.04	5.32	14
Fagaropsis angolensis Engl. Dale	0.98	3.13	0.76	4.86	15
Olea europaea L.	0.39	3.91	0.04	4.34	16
Vernonia auriculifera Hiern	0.98	3.13	0.00	4.10	17
Rhamnus prinoides L'Hér.	0.78	3.13	0.00	3.91	18
Ficus sur Forssk.	0.78	2.34	0.04	3.17	19
Teclea nobilis Del.	0.78	2.34	0.00	3.13	20
Diospyros abyssinica Hiern. White.	0.59	2.34	0.00	2.93	21
Albizia gummifera J.F Gmel.	0.59	2.34	0.00	2.93	21
Sapium ellipticum Hochst.	0.98	1.56	0.00	2.54	22
Olea capensis L.	0.59	1.56	0.04	2.19	23
Casimiroa edulis L. Iave	1.17	0.78	0.00	1.95	24
Psidium guajava L.(Guava)	0.39	0.78	0.00	1.17	25
Acokanthera schimperi Benth. Schweinf.	0.20	0.78	0.00	0.98	26
Citrus sinensis (L.) Osbeck	0.20	0.78	0.00	0.98	26

In this study, the regeneration statuses was evaluated by using a population of seedlings, saplings and mature trees, although farmers' preferences and management influence the natural distributions of these age groups of woody species. Accordingly, woody species such as *P. falcatus*, *B. abyssinica*, *V. amygdalina* and *B. antidysenterica*, which cover 13.8% of woody species in the CEA, were regenerated well (Fig. 3). Species including *C. africana*, *P. americana*, *M. ferruginea*, *S. guineense*, *V. auriculifera*, *C. edulis*, *P. africana*, and *C. macrostachyus* showed fair regeneration, representing 27.6% of all woody species in the CEA, whereas *A. schimperi*, *P. guajava*, *O. capensis*, *S. ellipsicum*, *A. gummifera*, *D. abyssinica*, *T. nobilis*, *F. sur*, *R. prinoides*, *M. indica*, *F. angolensis*, *E. cymosa*, *O. kenyensis*, and *E. brucei* were found at poor regeneration status, and this accounts for 48.3% of the woody species. Finally, some of the species, including *O. europaea* and *C. sinensis* fall under the not regenerating category in CEA. The individuals of *P. africana* were not evenly distributed across the study plots; rather, most of the saplings were recorded only in a few plots. The concentration of *M. indica* was far from the CEA land unit; rather, its distribution was situated around the homestead, which contributed to its low density in the CEA. Similarly, the distribution of *O. europaea* in front of the homesteads was greater than that in the middle of the agroforestry farms; this was understood through the reconnaissance survey and actual sample collection of this study.

Concerns of conservation

The status of species rareness or abundance could be evaluated with three variables, namely, geographical distribution, population size and habitat preference (Rabinowitz, 1981), which were modified by Maciel (2021). In this study, conservation concerns for woody species were determined through the evaluation of regeneration status, as the resources and design of this research did not allow the application of the Maciel (2021) method, particularly habitat preference.

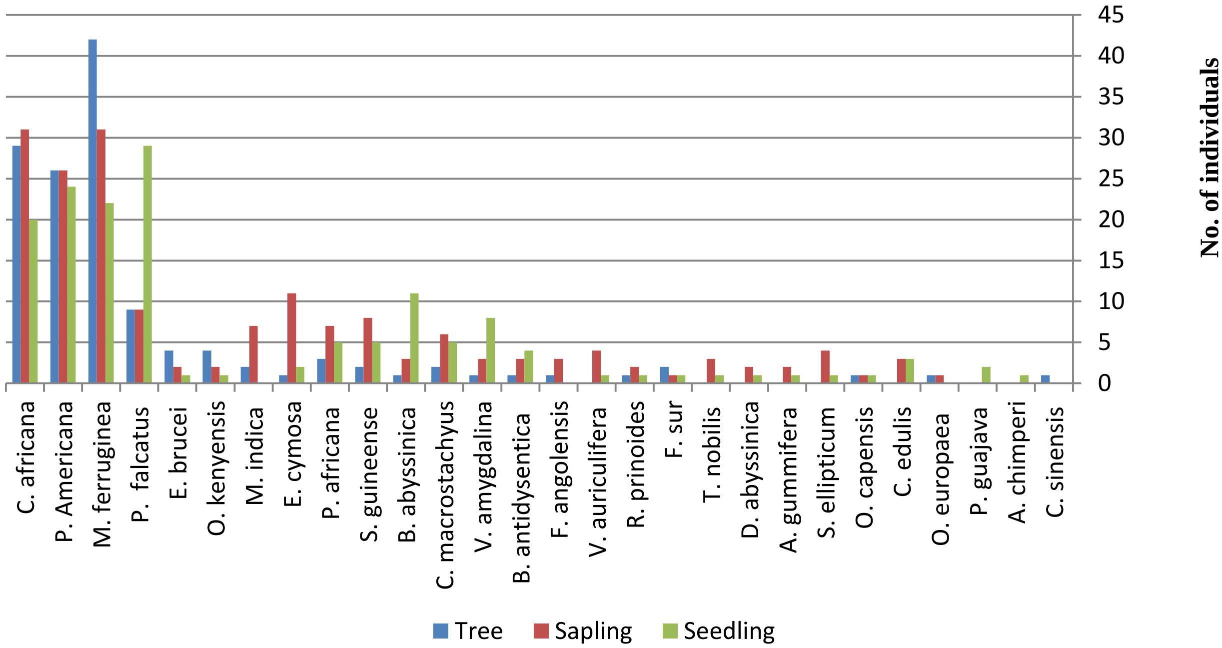

Figure 3 Distribution of mature trees, saplings and seedlings.

Compared to other agricultural systems, agroforestry is a sustainable habitat and is perceived as a potential option for conserving native tree species, preserving sensitive species in managed agroecosystems, and preventing the degradation and loss of surrounding habitats (Udawatta *et al*., 2019). However, the expansion of monoculture production into agroforestry systems has threatened biodiversity. This study revealed that *O. kenyensis* and *E. brucei* were poorly regenerated. According to the IUCN Red List, two of the recorded woody species such as *P. africana* and *O. kenyensis* (vulnerable list of the IUCN) (Vivero *et al*., 2005, Hills and Cheek, 2021), and *O. europaea* and *Citrus sinensis* did not regenerate in the CEA. Thus, *P. africana*, *O. kenyensis*, *E. brucei* and *Citrus sinensis* require conservation concerns because of their poor regeneration and vulnerable list of IUCN.

Effects of land use on soil microbial biomass carbon

In this study, the values of soil microbial biomass carbon (MBC) for three LUs ranged from 230.6 to 651.2 µg g^{-1} soil. The MBC under CEA ranged from 470.8 to 651.2 µg g^{-1} soil with a mean value of 586.3 (± 47.0) µg g^{-1}; for CM, it ranged from 230.6 to 379.3 µg g^{-1} with a mean of 298.2 (±42.1) µg g^{-1}; and for EW, the value ranged from 253.5 to 374.9 with a mean value of 313.8 (±41.4) µg g^{-1} soil. In this study, the mean soil MBC in CEA was greater than that in a similar study in Cardamom agroforestry (392.86 µg g− 1) in the Eastern Himalayas, India, but the mean value under CM was lower than that in paddy cropland (317.47 µg g− 1) (Lepcha and Devi, 2020). The mean value of the three considered agricultural LU in this study (442.3 µg g− 1) was greater than that of similar study reported by Lepcha and Devi (2020). The result found across land uses (399.5 µg g^{-}1 soil) was lower than that across land management (526.7 µg g^{-}1 soil) outside of agricultural land in the Degua Temben district, northern Ethiopia (Welemariam *et al*., 2018).

One-way ANOVA revealed a statistically significant difference in the MBC among LUs ($p < 0.05$). The soil MBC in the CEA treatment was significantly greater than that in the other two LU treatments, whereas there was no significant difference in the soil MBC between the CM and EW treatments ($p < 0.05$). Thus, plant diversity in the CEA was attributed to the relatively high soil MBC (Prommer *et al.*, 2020). The lower MBC in the CM treatment than in the CEA treatment was a result of management practices of frequent tillage, whereas the slightly greater MBC in the EW treatment than in the CM treatment was attributed to zero tillage and the presence of grasses (Blanco-Canqui *et al.*, 2013).

With the conversion of LU from multi-species vegetation to monoculture plantations, the intensification of LU and a decline in plant species diversity decrease the soil MBC. Variation in microbial biomass influences surface and subsurface ecosystem processes, including carbon sequestration (Bastida *et al*., 2021). The amount of MBC reflects the size of microbial biomass in the soil. Although soil microbial biomass comprises a small quantity, less than 5%, of organic matter in the soil, it is an essential component of the ecosystem because it is a labile source of soil macronutrients such as carbon, nitrogen, phosphorus and sulfur (Camenzind *et al*., 2023); regulates surface and subsurface ecosystem processes (Bastida *et al*., 2021); decomposes plant litter and transforms it into soil organic matter; and fixes atmospheric greenhouse gases by assimilating these gases into the form of immobilized soil biomass, which serves as a sink of carbon, nitrogen and sulfur (Kaur *et al*., 2015). Therefore, maintaining more soil microbial biomass in agroecosystems supports further carbon sequestration and soil fertility. On the other hand, unsustainable LU affects plant diversity, and the associated influence on soil microbial biomass undermines efforts to mitigate climate change.

Interrelations between Species Diversity and Soil MBC

This study found that the soil MBC showed a strong positive correlation ($r = 0.854$) with plant species richness ($p < 0.05$). Finding of this study agrees with a similar study in Jena, Germany (Prommer *et al.*, 2020), and temperate grasslands of Hulunbeir, China (Liu *et al*., 2020). Furthermore, several global meta-analyses have shown that land covers with diverse plant species have greater soil MBC than monoculture LU (Liu *et al*., 2020; Bastida *et al*., 2021).

Plants–microbe cooperation is well established in nature, where associations with microbiota involve either symbiotic or pathogenic relationships. Microbes benefit plants by enhancing soil nutrient availability for plant growth, which in turn provides microbes with nutrition from root exudates (amino acids, sugars, phenolics, organic acids, and proteins) and a desirable habitat in the rhizosphere (Lyu *et al.*, 2021; Mandal *et al.*, 2021). This indicates that plant species richness and land surface coverage with biomass materials and/or litters are important for creating suitable conditions for microbial biomass. Therefore, plant–microbe interactions are essential components of ecosystems, supporting sustainable crop production and enhancing overall ecosystem productivity (Prommer *et al*., 2020).

CONCLUSION

Land use (LU) influenced plant species diversity, which in turn affected soil microbial biomass carbon (MBC). This was confirmed by the greater soil MBC accumulation in species-rich coffee-enset agroforestry (CEA), where MBC was significantly greater than in monoculture eucalyptus woodlot (EW) and chat monocropping (CM), with no significant difference observed between EW and CM ($p < 0.05$). This study suggested that monoculture with single-plant species in CM and EW LUs, along with frequent tillage under CM, contributed to the low MBC compared to the species-diverse CEA. The decline in plant diversity and density not only affects MBC but also carbon sequestration, resulting in poor soil quality. The strong positive correlation ($r = 0.854$) between MBC and plant diversity ($p < 0.05$) implies that maintaining plant diversity enhances MBC, which plays a key role in ensuring the sustainability of agroecosystems. Concerned bodies, including governments and nongovernmental organizations, should encourage farmers to maintain sustainable agroforestry systems. Finally, conservation measures should be implemented for threatened woody species such as *P. africana*, *O. kenyensis*, *E. brucei*, and *C. sinensis*. Additionally, there is a need to investigate plant species-specific associations with soil MBC.

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Conflict of interest. authors declare that there is no conflict of interest.

Compliance with ethical standards. This research not require ethical clearance, however, every activities of data collection was done with due consent of farmers and concerned bodies.

Data availability. The datasets generated during and/or analysed during the current study are available

from the corresponding author on reasonable request. Email: zeneshu112@gmail.com

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Appendix A. **List of all recorded species per land use in the study area.**

Plant species	Local Name		Family	GF	LU in which species present		
	Sidamu Afoo	Amharic			CEA	EW	CM
1. Acokanthera schimperi Benth. Schweinf.	Kararo	Gararu	Apocynaceae	S	x	2x	-
2. Agava sisalana Perrine. Engelmann	‘Kaacha’	Kacha	Agavaceae	H	-	2x	-
3. *Albizia gummifera* J.F Gmel.	Maaticho	Sessa	Fabaceae	T	2x	-	2x
4. Aleo macocarpa Latilo.	Argiisa	Reti	Asphodelaceae	H	-	2x	-
5. Ananas comosus (L.) Merr.	Ananase	Ananas	Bromeliaceae	H	x	-	-
6. Bersama abyssinica Fresen.	Tewerakko	Azamir	Melianthaceae	T	4x	-	-
7. Brucea antidysentica J.F. Mill.	Hataawicho	Gimi kitel	Simaroubaceae	T	3x	-	-
8. Calpurina aurea (AIT.) Benth.	Bayanaka	Digitta	Fabaceae	S	-	2x	-
9. Casimiroa edulis L. Iave	Kasimire	Kazimir	Rutaceae	T	3x	-	-
10. Citrus sinensis (L.) Osbeck	Burtukaane	Burtukan	Rutaceae	S or T	x	-	-
11. *Catha edulis* Forsk	Caate	Chat	Celastraceae	S	-	-	x*x
12. Clematis hirsutus Perr. Guil. var. *hirsutus*	-	-	Ranunculaceae	L	-	x	-
13. Coffea arabica L.	Buna	Bunna	Rubiaceae	S	9x	-	-
14. Colocasia esculenta (L.) Scotti.	Kolcooma	Godere	Araceae	H	3x	-	-
15. Cordia africana Lam.	Waadicho	Wanza	Boraginaceae	T	7x	-	-
16. Croton macrostachyus Hochst	Masincho	Bisana	Euphorbiaceae	T	4x	2x	3x
17. Curcubita pepo L.	Baapula	Duba	Curcurbitaceae	H	x	-	-
18. Diospyros abyssinica Hiern. White.	Looko	Selechegn	Ebenaceae	T	2x	-	-
19. Ehretia cymosa Thonn.	Gidincho	Game	Boraginaceae	T	4x	-	2x
20. Euphorbia candelabrum Welw.	Carricho	Kulkual	Euphorbiaceae	H	x	-	-
21. *Eucalyptus camaldulensis* Dehnh.	Dume barzaafe	Keyi berzaf	Myrtaceae	T	-	x**	-
22. Ensete ventricosum Welw.	Weese	Enset	Musaceae	H	9x	-	-
23. *Erythrina brucei* Schweinf. G	Welakko	Korch	Fabaceae	T	3x	-	-
24. Fagaropsis angolensis Engl. Dale	Godiicho	-	Rutaceae	T	3x	-	2x
25. Ficus sur Forssk.	Odako	Shola	Moraceae	T	2x	-	-
26. Jasminum fluminense Vell.	-	-	Oleaceae	L	-	2x	-
27. Kalanchoe petitiana Rich.	Binjille	-	Crassulaceae	H	-	2x	-
28. Lantana camara L.	Wofikolo	Wofikolo	Verbanaceae	S	-	3x	-
29. Mangifera indica L.	Mango	Mango	Anacardiaceae	T	3x	-	-
30. Maytenus arbutifolia Hochst. Rich.	Cucco	Atat	Celastraceae	S	-	2x	-
31. Millettia ferruginea Hochst. Bak.	Hengedicho	Birbira	Fabaceae	T	6x	-	2x
32. Musa acuminata Colla.	Waajo muze	Muzi	Musaceae	H	3x	-	-
33. Musa sapientum Linn.	Kolisho muze	Muzi	Musaceae	H	3x	-	-
34. Nicotiana glauca R. Graham	-	-	Solanaceae	H	-	2x	-
35. Ocotea kenyensis Robyns. Wilczek	Shoyicho	Majo	Lauraceae	T	4x	-	-
36. Olea europaea L.	Ejersa	Woyira	Oleaceae	S or T	2x	-	-

Plant species	Local Name		Family	GF	LU in which species present		
	Sidamu Afoo	Amharic			CEA	EW	CM
37. *Olea capensis* L.	Seetame	Damot weira	Oleaceae	T	2x	-	-
38. *Persea americana* Mill.	Avocado	Avocado	Lauraceae	T	6x	-	-
39. *Piper guineense* Thonn.	-	-	Piperaceae	L	-	x	-
40. *Podocarpus falcatus* (Thunb.) Mirb.	Dagucho s	Zigba	Podocarpaceae	T	5x	-	-
41. *Prunus africana* (Hook. f.) Kalkman	Garbicho	Tikur inchet	Rosaceae	T	4x	-	-
42. *Psidium guajava* L.(Guava)	Zayitone	Zaytoni	Myrtaceae	T	2x	-	-
43. *Rhamnus prinoides* L'Hér.	Taddo	Gesho	Rhamnaceae	S	2x	-	-
44. *Ricinus communis* L.	Kombo'o	Gulo	Euphorbiaceae	H	2x	-	-
45. *Sapium ellipticum* Hochst.	Gaancho	Arboche	Euphorbiaceae	T	3x	-	-
46. *Sesbania sesban* Linn.	Sesbania	Sesbania	Fabaceae	S	-	2x	-
47. *Solanum marginatum* L.F	Borbodhano	Emboyi	Solanaceae	S	-	3x	-
48. *Syzygium guineense* (Willd.) DC.	Duuwancho	Dokma	Myrtaceae	T	4x	-	-
49. *Teclea nobilis* Del.	Hadheessa	Atesa	Rutaceae	T	2x	-	-
50. *Vernonia amygdalina* Del.	Hechcho	Grava	Asteraceae	T or S	4x	-	3x
51. *Vernonia auriculifera* Hiern	Reejicho	Gujjo	Asteraceae	S	3x	x	2x

GF= growth form, T=tree, H= herbaceous, S= shrub, L=liana. Number of individuals rated as x= 1, 2X=2-4, 3x=5-10, 4x=11-20, 5x=21-50, 6x=51-100, 7x=101-150, 8x=150-200, 9x=201-300, X*=301-1000, x**=1001-1200, X***=2001-3000, and X*X=3000-5000