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Research Article

Soil and Mycorrhizal Diversity and Distribution in Relation to *G. copallifera* in Kasewe Forest Reserve, Southern Sierra Leone

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Abstract

Gibourtia copallifera is a rare, range-restricted tree native to the Kasewe Forest Reserve in southern Sierra Leone. Historically exploited for gum copal, it now faces threats from charcoal production due to its high-quality charcoal. Although the species shows good growth in forest patches established 60 years ago, natural regeneration remains minimal. While many tropical tree species form beneficial relationships with arbuscular mycorrhizal fungi (AMF), these associations are still poorly understood in humid regions like Sierra Leone. This study investigated the AMF associations of *G. copallifera* and assessed changes in mycorrhizal diversity in relation to forest degradation and tree development stages. Soil characterization was conducted through profile analysis, and samples were subjected to laboratory testing. Three soil types were identified within the reserve: K 01—barren land with sparse vegetation; K 02—forest land dominated by *G. copallifera*; and K 03—upland fallow agricultural land with shrubs and a few trees. Soil analysis indicates pH values ranging from 5.03 to 5.87 (acidic), with calcium as the most dominant exchangeable base, followed by magnesium, potassium, and sodium. The surface horizon under *G. copallifera* exhibited the highest total exchangeable bases, and high cation exchange capacity (CEC) was linked to the presence of decomposed plant matter. A total of 22 AMF species were identified in plant root samples, with a significantly higher proportion found in non-degraded forest patches. These accounted for around 68% of AMF species, especially *Scutellospora* and *Gigaspora*, along with *Glomus* and *Acaulospora*. AMF species richness and diversity were considerably higher in non-degraded patches (3.13 species) than in degraded areas (1.75). Mycorrhizal frequency and intensity were also significantly greater in undisturbed forest sites. AMF colonization peaked in mature trees and was lowest in seedlings, although the variation across trials was not statistically significant ($P = 0.07$). The study concludes that K01 is unsuitable for *G. copallifera* due to its shallow depth, which restricts growth. In contrast, K02 and K03 are more favorable for the species. However, many K02 areas have been converted to agricultural land, and in locations where natural forest regeneration is allowed, more aggressive species such as Gmelina arborea and Anisophyllea laurina tend to dominate. Additionally, AMF play a crucial role in promoting the growth of G. copallifera in nutrient-poor soils. These findings are important for informing reintroduction and reforestation strategies for this native tropical tree species.

Keywords

Mycorrhizal, Arbuscular, Forest, Soil Fertility, Guibourtia, Degraded, Reserve, Sierra Leone

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1. Introduction

Forest soil variability is significant, even within a single monitoring plot, and results from continuous processes influenced by factors such as time, parent material, bedrock type, topography, climate, tree species, understory vegetation, soil biology, and natural disturbances. Anthropogenic influences, including air pollution [1], climate change [2, 3], land management, and arson, can also impact forest soils.

Trees, as long-living organisms, significantly shape soils through deep rooting, high microbial and soil fauna activity, and increased humus content, leading to enhanced soil porosity and pore system continuity [4]. Ecologists have long studied how soil microbial community variation influences ecosystem functioning [5]. Arbuscular mycorrhizal fungi (AMF), which form symbiotic relationships with ~60% of tree species; [6, 7], are crucial for early tree establishment, growth [8, 9], nitrogen access [10] and protection from pathogens [5].

In tropical Africa, over-exploitation of natural resources has led to significant vegetation loss, with drought, climatic variability, and dryland characteristics affecting ecosystem use. These factors influence vegetation productivity, land carrying capacity, erosion susceptibility, and water availability, complicating ecosystem and forest management, and limiting food security and human well-being [11, 5]. Consequently, recovering destroyed vegetation has become a critical concern.

Soils play a crucial role in forest ecosystems, supporting trees by regulating nutrient absorption, organic matter decomposition, and water availability, while providing anchorage, water, and nutrients to trees [12]. Soil is the foundation of nearly all ecosystems, influencing plant and animal life, species composition, timber productivity, and wildlife diversity. In forest ecosystems, soil also maintains water quality and ensures long-term site productivity [13]. Despite their importance, soil is often overlooked in nature conservation planning and management.

Data on arbuscular mycorrhizal fungi (AMF) diversity in humid tropical ecosystems, particularly in Africa, is scarce despite increasing human population pressure [14]. Research is urgently needed due to the high proportion of uncultured AMF in natural ecosystems [15]. Bakarr and Janos [16] examined mycorrhizae in the fine roots of 27 tree species across natural forests, forestry plantations, and reforestation sites, finding vesicular-arbuscular mycorrhizae in 20 species, and bacterial nodules in nine species from the *Mimosoideae* and *Papilionoideae* families. Sierra Leone faces a similar lack of data on forest fungal and soil interactions, with most belowground diversity remaining unstudied. Since its establishment as a protected area in 1919, the Kasewe Forest Reserve has experienced continuous human exploitation for resources such as gum copal, timber, poles, non-timber forest products (NTFPs), and bush meat [17, 18]. During the Ebola crisis (2014–2016), an influx of people seeking refuge in the forest further intensified resource extraction, with exploitation rates growing unsustainably. The use of power saws, facilitated by wealthy merchants exchanging them for charcoal and other forest products, has exacerbated deforestation and overexploitation in recent years. This gap in knowledge of trees, fungi and soils in Sierra Leone provided the need for such a study. Since that is the case, this study aims to evaluate the *G. copallifera* growth dynamics, with regards to soil and mycorrhizal diversity in Kasewe forest Reserve in Moyamba District.

2. Materials and Methods

2.1. Study Area

The study was conducted in the Kasewe Forest Reserve, located on the border between Tonkolili and Moyamba Districts in south-central Sierra Leone, about 170 km east of Freetown. This lowland forest gives ways to medium-altitude forest on the slope and peaks of the Kasewe hill ridges (altitude range approximately 100 to 500m). The reserve (centered on 8°18′53″N 12°15′43″W), covering 2,331 hectares [19], features a mix of tropical, moist semi-deciduous, evergreen forests, and savanna. The terrain includes volcanic hills reaching up to 500 meters, which are crucial as a water catchment area for surrounding communities [20, 21].

2.2. Preliminary Site Survey

In 2023, a survey of soil variability was conducted across four sampling locations, each with three sites representing different habitats. Prior to sample collection, four visits were made in the reserve to assess the soil conditions in the various habitats. Pedological characteristics were described, and soil samples were collected for analysis.

2.3. Sampling Procedure

A small pit was dug at each site to describe the soil profile, noting key features and anomalies such as plow layer remnants below 30 cm. The depth of the Ap horizon and the depth to carbonates were measured. Soil samples were collected from the Ap horizon and the layer below it (either the Ck or Bm horizon). In cases where the Ap horizon depth was unclear, samples were taken from the 0-15 cm and 15-30 cm layers. Altitude was recorded using GPS. The soil samples were air-dried, ground, and sieved to pass through a 2 mm mesh.

2.4. Soil Analyses

Soil samples were collected from the top 0-30 cm of each plot. Electrical conductivity (EC) was measured using a 4:1

water-soil suspension with a conductivity meter, while soil pH was evaluated in a 1:1 water-soil suspension using a PHS-3BW pH meter. Soil moisture content was determined by comparing the wet and dry weights of the samples. Sand content was measured in air-dried soil samples after removing carbonates with 1N HCl and organic matter with 35% H_2O_2. The soil was then dispersed overnight in a sodium hexametaphosphate solution. Sand particles were collected on a 53 µm sieve, dried at 105°C, and weighed. Total nitrogen content was assessed by combusting a 50 mg subsample of finely ground soil (<100 mesh) under controlled temperature and time conditions.

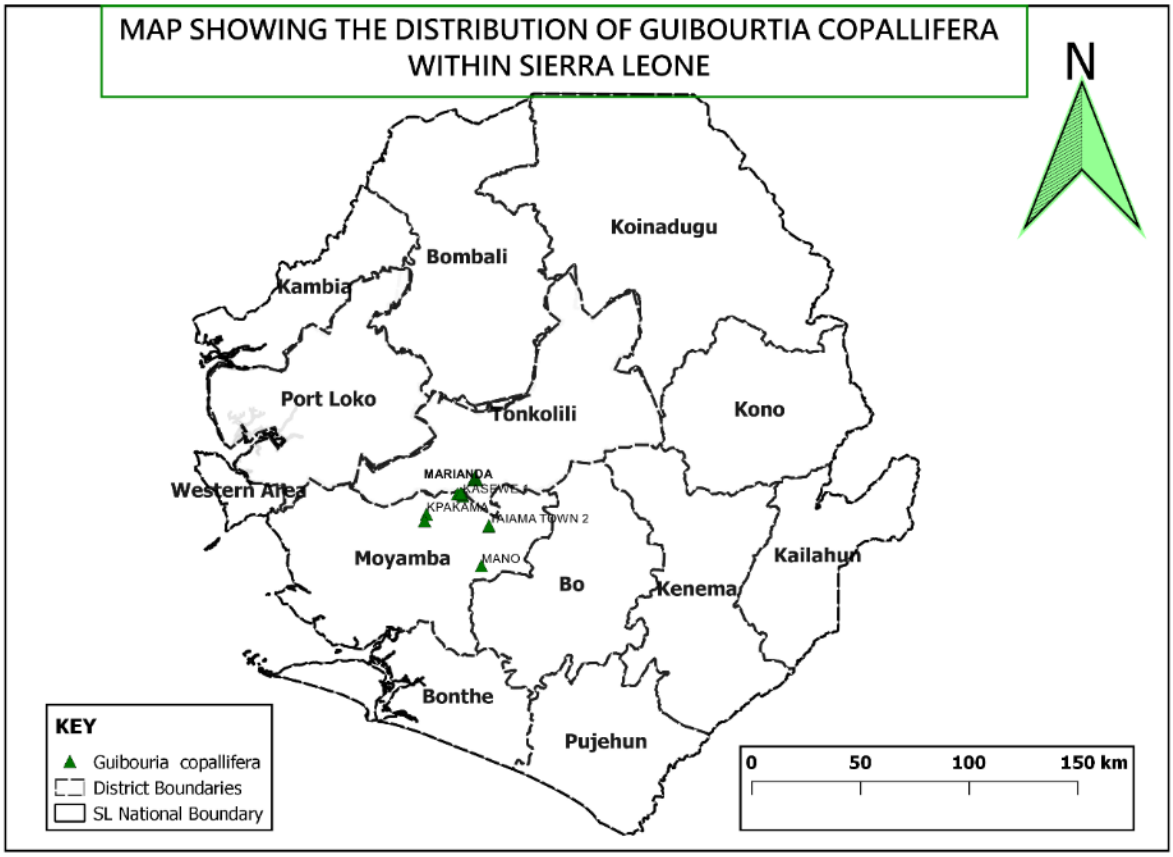

Figure 1. *Map of Sierra Leone showing distribution of the species in the districts.*

2.5. Root Sampling and Assessment of Mycorrhizal Colonization

The study focused on root sampling and mycorrhizal colonization in trees across different growth stages (seedlings, saplings, juveniles, and mature trees). Fine roots were collected by tracing larger roots from the stem collar, or by uprooting seedlings along with surrounding soil. Challenges in collection included intermingling roots of different species and difficulty in identifying primary root origins. Root samples were taken from a depth of 15-30 cm in forest and bushy species, with triplicate samples for each tree at each growth stage. In order to ensure comparability across tree growth stages, we implemented a replicated root sampling strategy, with at least 10 individuals per growth stage sampled across multiple sites to account for spatial and environmental variation. All root samples were collected from a consistent soil depth to minimize depth-related differences in mycorrhizal colonization. To control for size-related variation, 4 g of fine roots were sampled per individual, with multiple subsamples analysed to enhance internal replication. Sample sizes were determined through power analysis to ensure sufficient statistical power, and replication across individuals and sites enabled assessment of both within-stage variability and between-stage differences in mycorrhizal colonization. The root samples underwent a modified clearing and staining process based on [22]. This involved cleaning roots in 10% KOH, steaming, rinsing with tap water, treating with 1% HCl, and staining with 0.5% trypan-blue in lactoglycerol. After microscopic examination, mycorrhizal colonization was assessed using the magnified intersection method, with a hairline graticule inserted into the eyepiece of the compound microscope. Root length colonization (RLC %) was determined by counting intersections at 400x magnification, with six possible outcomes for each intersection [23].

2.6. Data Analysis

Majority of the analyses on this data were done in R version 3.6.2 (R Core 2020). ANOVA single-factor analysis, paired t-tests and linear regression were used to determine the effectiveness of the different soil, forest patches, growth forms in the different environments, changes over time. Variability with collected samples in the different use zones was visualized using box plots.

3. Results and Discussion

3.1. Soil Types

The Kasewe Hills belong to the Kasewe Land System (Land System 36) of Sierra Leone as defined by the Land Resources Survey Project [24]. This Land System is characterized by remnant hills of moderate to high relief with extensive foot slopes. The geology of soils on this land system is of volcanic deposits of the Kasewe formation and basic granulites of the Kasila Group. The geomorphology is generally of isolated hills and hill ridges with moderate to steep/gullied slopes and well developed, gently sloping foot slopes. The foot slopes are commonly formed on exposed hardened plinthite or laterite sheet.

Three soil pits were dug and described; these are;

1. soil type 1, ref K 01 maximum depth 10cm
2. soil type 2, ref K 02 maximum depth 70cm
3. soil type 3, ref K 03 maximum depth 100cm

Pictures of the soil profiles are shown in Figure 2.

K 01 K 02 K 03

Figure 2. *Pictures of the soil profiles.*

3.1.1. Vegetation and Land Use

K 01: Barren land, sparse cover of trees and shrubs and some seasonal grasses

K 02: Forest land, dominated by *Guibourtia spp*

K 03: Upland agriculture, mostly fallow vegetation of shrubs and a few trees.

3.1.2. General Description

K 01: Found on the upper portions of long gentle slopes in the Kasewe hills. They have a shallow very gravelly sandy clay loam and a moderate medium sub-angular blocky structure. The topsoil is a thin layer of about 10cm gravely soil with surface rock outcrops at close distances (<1m) overlying an impenetrable layer with successive boulders. The parent material is weathered colluvium and residuum.

K 02: Found on middle and lower portions of long gentle slopes adjacent to steep hills of the Kasewe Hills. They are well-drained, shallow sandy clay loams with a 10cm gravel-free topsoil overlying a gravely subsoil. The depth to an impenetrable layer is 70cm. The parent material is weathered colluvium and insitu residuum. The top soil is reddish brown with a sandy clay loam texture and a moderate, coarse sub-angular blocky structure overlying a yellowish-brown sub-surface soil (60cm) overlying a yellowish brown gravelly impenetrable layer.

K 03: Middle and lower portions of long gentle slopes on high relief on the Kasewe hills. They are well-drained, moderately deep gravelly sandy clay loams. The parent materials are transported colluvium and weathered residuum. The topsoil is about 10cm thick, yellowish brown with a sandy clay loam texture and a moderately strong, medium sub-angular blocky structure overlying a yellowish-brown sub-surface soil (40cm) overlying a dark yellowish brown gravelly sandy clay loam subsoil.

3.1.3. Chemical and Textural Characteristics

Table 1. Physical and chemical Properties of soil types.

Property	Type 1	Type 2		Type 3		
Soil Depth cm	**0-10**	**0-10**	**10 to 70**	**0-10**	**10 to 50**	**50-100**
Gravel	92	3.8	92	75	89	91
Sand	88	80	78	82	84	86
Silt	4	8	8	8	6	6
Clay	8	12	14	10	10	8
pH	5.87	5.82	5.09	5.69	5.03	5.77
EC	58.5	56.9	25.1	64.2	19.1	98.9
C	4.52	5.52	2.84	4.52	2.08	2.08
N	0.01	0.08	0.03	0.04	0.01	0.01
P mg/kg soil	9.48	4.82	8.73	8.66	8.81	7
Na mg/kg soil	2.3	2.8	2.1	2.5	2.1	2.8
K mg/kg soil	4.9	6.9	3	5.92	1.65	2.01
Mg mg/kg soil	16.6	18.6	10.1	12.1	14.6	13.4
Ca mg/kg soil	26	28.2	10.1	26.54	21.41	14.22
CEC cmol(+)/kg soil	10.56	12.86	10	11.24	8.07	7.28

The pH analysis results (Table 1) show that the soils are very acidic (5.03 to 5.87). Exchange bases, these are calcium, magnesium, phosphorus, potassium and sodium. Among these bases, calcium is the most important element in the studied soils (Table 1). In descending order, we find magnesium, then potassium and sodium. At the surface horizon, the sum of the exchanged bases (SEB) has the highest values under *G. copallifera* in the reserve.

The cation exchange capacity (CEC) of a soil is the maximum number of cations that a soil can adsorb, in other words, this measure represents the total negative soil charges available for fixing H^+ and Al^{3+} ions and exchangeable bases. This parameter depends on colloids and soil pH. Most of the studied soils have a high CEC content ranging from 7.28 – 12.86 due to the dominance of vegetation dead matter that improves the soil.

The chemical composition of soil organic matter (SOM) plays a crucial role in carbon and nutrient dynamics, as its degradation influences these processes [25]. Plant composition is the primary factor that differentiates the chemical properties of organic matter in soils [25]. Typically, organic matter, carbon, and nitrogen decrease in the B horizon, which has lower root density and microbial activity compared to the surface organic horizon. Despite this, all sites studied are found to be very rich in total nitrogen.

3.1.4. Soil Horizons

Table 2. Soil depth and characters.

	K 01	K 02	K 03
0 to 10 cm	Very dark yellowish brown (10YR3/2), gravelly loamy sand, weak, coarse, granular structure and friable, non-sticky, non-plastic consistence. Many pores and roots	Reddish brown (10YR3/2), sandy loam, weak, coarse, sub-angular blocky structure and friable, non-sticky, non-plastic consistence. Many fine pores and few coarse pores many roots	Yellowish brown (10YR3/2), gravelly sandy clay loam, moderate, medium, sub-angular blocky structure and friable, slightly sticky, slightly plastic consistence

	K 01	K 02	K 03
10 to 70 cm	Impenetrable	Yellowish brown (10YR4/6), gravelly sandy loam, moderate, fine, sub-angular blocky structure and friable, slightly sticky, slightly plastic. Many pores, few roots	Yellowish brown (10YR4/6), gravelly sandy clay, weak, medium, sub-angular blocky structure and friable, sticky, plastic consistence
70 to 100 cm	Impenetrable	Impenetrable	Dark yellowish brown, gravelly Sandy clay, weak, medium, sub-angular blocky structure and friable, sticky, plastic consistence

3.1.5. Soil Types Compared

Soil type 1 is not very suitable for forest trees being shallow and nutrient poor on this soil *Guibourtia* are stunted and small and are often uprooted by strong winds. This area is full of thickets and shrubs which are relatively better suited than the *Guibourtia spp* (Table 2). Soil type 3 appears to be best for trees being the deepest soil profile with reasonable nutrient content, however, this is also the most suitable for upland agriculture and much of the forest has been cleared. The densest growth of *Guibourtia* is on soil type 2, which is relatively deep but not so attractive for agriculture. Regarding natural or artificial regeneration of degraded patches of Kasewe reserve, based on the soil analysis, K02 and K03 can be recommended for reforestation activities, especially involving planting of the species in areas where it used to be before human activities rendered those area degraded (Table 2).

3.1.6. Productivity

K 01: The low cation exchange capacity, low nutrient and low soil organic matter contents and high gravel and sand content makes these soils low in water and nutrient retention and limits capacity to support tree growth which is restricted to patches of soil that allow some root penetration below 10cm depths.

K 02: Upper layers are acidic and sandy (80% sand), lower layers gravelly (92%) with stones and boulders. The soil has higher organic carbon and clay contents and hence, higher water retention capacity; this soil potentially supports luxurious growth of *Guibourtia* species.

K 03: This soil is deeper than K02 with an impenetrable layer occurring at 100cm depth. Like K02, it is acidic, gravely, and sandy (80% sand). The gravel content increase at lower depths. The soil has similar physical and chemical properties to K02 and consequently, it also could support luxurious growth of the *Guibourtia spp.* however, the trees have often been removed and the land used for agriculture (upland farming).

3.2. Arbuscular Mycorrhizal Fungal (AMF) Density

Arbuscular Mycorrhizal Fungal (AMF) density generally reduces with human interference being higher (8 species) on intact natural patches and lower on cultivated or degraded sites. Density generally increases with the age of the host, being highest for mature trees and lowest under seedlings.

3.2.1. Mycorrhizal Colonization

Most of the *G. copallifera* samples were mycorrhizal, with non-septate hyphae characteristic of AM fungi present in 68% of all samples screened; colonization ranged from:

1. seedlings from 25 to 40%
2. saplings from 30 to 48%,
3. juvenile 35 to 60%
4. young trees 40 to 70% and
5. Matured trees 45 to 85%.

The mean percentage of root length colonized was 26.73%, ranging from 0 to 46%. The mean intensity of root infection was 6.73%, ranging from 0 to 50%.

3.2.2. Arbuscular Mycorrhizal (AM) Structures

Where AM fungal structures, i.e., vesicles and arbuscules are observable, they make up nearly half (46%) of a root segment. Majority of plant specimens (51%) formed Paris-type and only 16% of plants formed Arum-type of colonization and in certain plants specimen the AM type could not be detected owing to the rare incidence of intercellular non-septate hyphae or vesicles in the roots.

3.2.3. AMF Species Richness

A total of 22 AMF species were detected in plant roots sampled from the 20 sites in the study area (Table 3). A relatively greater proportion (68%) of particularly *Scutellospora spp*. and *Gigaspora spp.,* but also of *Glomus spp*. and *Acaulospora spp*., was apparent in the non-degraded forest patches than in the degraded areas.

Table 3. Arbuscular Mycorrhizal Genera and Species Richness at the Sites.

Family/Genera	Non-Degraded Patch	Degraded	No. of Species
Glomeraceae Glomus spp	8	5	10
Acaulosporaceae Acaulospora spp	6	3	7
Acaulosporaceae Kuklospora spp	1	2	2
Gigasporaceae Gigaspora spp	2	1	2
Gigasporaceae Scutellospora spp	4	2	3
Entrophosporaceae Entrophospora spp	1	0	1
Ambisporaceae Ambispora spp	2	1	2
Paraglomeraceae Paraglomus spp	1	0	1
totals	25	14	28

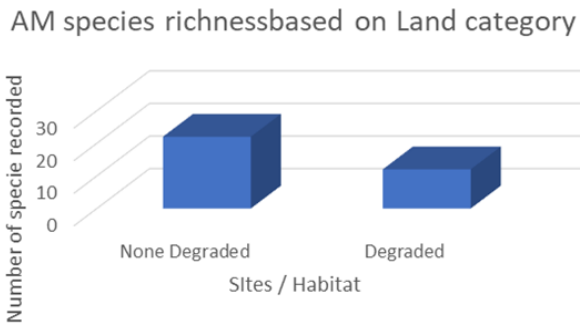

Figure 3. AM species richness based on land quality.

AMF species richness was significantly higher ($P<0.05$) in forest patches that has not been degraded when compared to that of the degraded areas. There was also significant difference in species richness (Figure 3). Land cultivation negatively affected the species richness, particularly species of *Gigasporaceae* and sporocarp-forming *Glomus* species. The number of species of *Acaulosporaceae* (8 to 22) also reduced, while a few Glomus species were less affected.

AMF species richness and diversity were significantly higher in non-degraded forest patches (3.13 species) than in degraded patches (1.75). The status of the plant's samples from locations where their richness and diversity have been affected in the different land use areas, areas with multiple land use activities and fire, recorded low species richness and diversity compared with patches that have not experienced any kind of fire or human activities. Higher richness occurred in samples from the sites with very little human activities than for degraded or farmed areas (Table 3).

3.2.4. Frequency of AM Colonization in Plant Roots at Diverse Stage of the Plant

A comparative analysis of the frequencies and the intensities of mycorrhization in each land use area in the forest showed that, there was significantly more fungal activity in the undisturbed sites (Figures 4 and 5). Moreover, there was less variation in the undisturbed sites compared to the degraded areas.

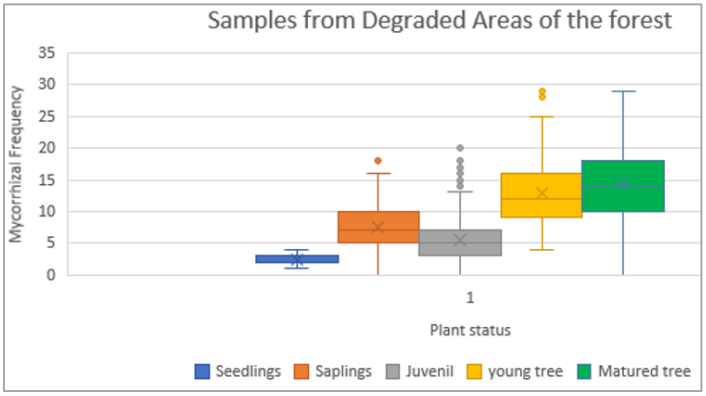

Figure 4. Box-plots showing frequency of colonization within growth stages of the plants (degraded sites).

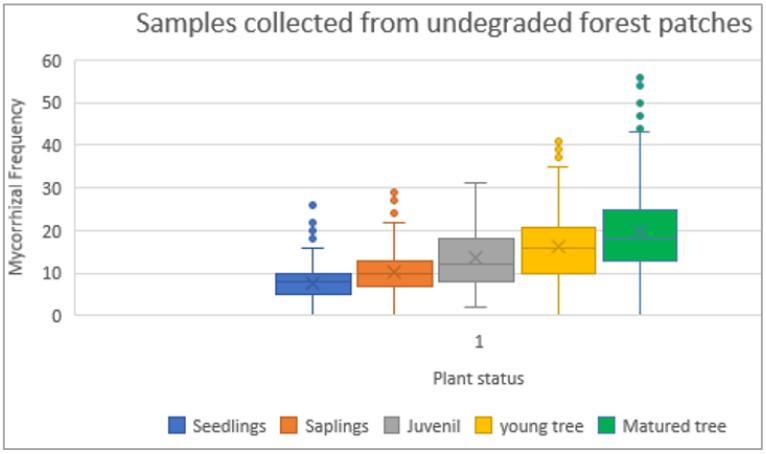

Figure 5. *Box-plots of root colonization with growth form (undisturbed sites).*

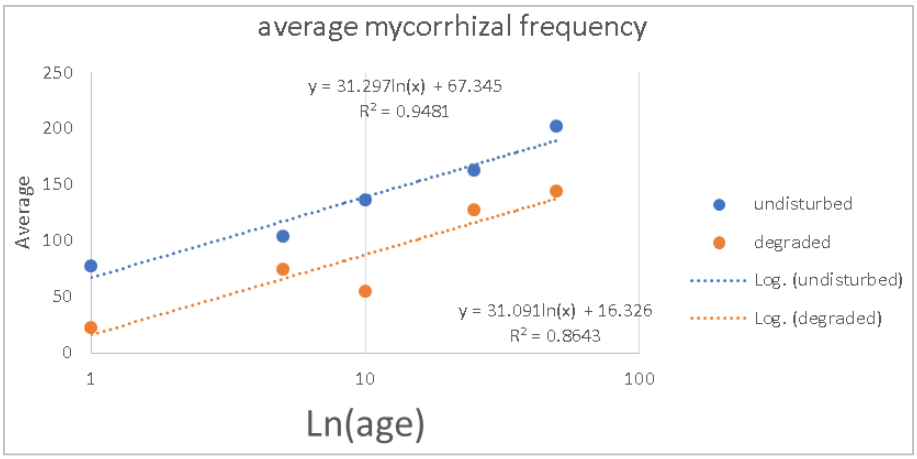

Figure 6. *Comparison of colonization rates with approximate growth stage of plants.*

3.2.5. Daily Root Observations

Table 4. *Daily Observation of Plant Roots within the Degraded Area.*

SUMMARY	Count	Sum	Average	Variance
Seedling	10	687	68.7	18.45556
Sapling	10	2218	221.8	1642.844
Juvenile	10	1642	164.2	3501.067
Young Tree	10	3832	383.2	2941.289
Matured Tree	10	4340	434	8189.111
Day_1	5	1497	299.4	31148.3
Day_2	5	1424	284.8	44640.2
Day_3	5	1315	263	22446
Day_4	5	1263	252.6	21824.3
Day_5	5	1118	223.6	18474.3
Day_6	5	1219	243.8	21711.7
Day_7	5	1385	277	29520.5
Day_8	5	1173	234.6	23109.3
Day_9	5	1278	255.6	31590.3
Day_10	5	1047	209.4	14745.3

Root sample were collected and analysed over a period of ten days continuously, daily readings were recorded (Table 4). The result of the two-way analysis of variance indicated that there was a significant effect in the growth stage of the plant ($p<0.001$, df=4, F=74.7), but there was no significant change over the 10 days of the experiment ($p>0.05$, df=9, F=1.26) (Table 4).

3.2.6. Daily Observational Trials of Root Samples

Table 5. Daily trials or observations of mycorrhizal frequency within samples from the degraded area.

SUMMARY	Count	Sum	Average	Variance
T_1	5	475	95	3605.5
T_2	5	483	96.6	2973.8
T_3	5	518	103.6	3584.8
T_4	5	506	101.2	3562.7
T_5	5	386	77.2	2466.7
T_6	5	386	77.2	2224.2
T_7	5	446	89.2	2829.7
T_8	5	390	78	2698
T_9	5	362	72.4	2041.3
T_10	5	418	83.6	2298.8
T_11	5	450	90	2655.5
T_12	5	351	70.2	2008.7
T_13	5	430	86	2606
T_14	5	374	74.8	2607.2
T_15	5	429	85.8	2429.2
T_16	5	389	77.8	2265.7
T_17	5	458	91.6	2617.3
T_18	5	405	81	2563.5
T_19	5	434	86.8	2706.7
T_20	5	376	75.2	2862.2
T_21	5	432	86.4	3102.3
T_22	5	469	93.8	3370.7
T_23	5	454	90.8	3117.2
T_24	5	438	87.6	3006.3
T_25	5	415	83	3038
T_26	5	443	88.6	2348.8
T_27	5	420	84	2931.5
T_28	5	354	70.8	1665.2
T_29	5	452	90.4	2682.8
T_30	5	406	81.2	1992.2
Seedling	30	689	23.0	7.0
Sapling	30	2246	74.9	218.0
Juvenile	30	1642	54.7	122.4
young tree	30	3832	127.7	355.9
Matured tree	30	4340	144.7	211.7

Each day, thirty trials from each plant status were conducted and observations were recorded. The results of the Anova indicate that there is significant difference ($p<0.001$) in mycorrhizal frequency (F) between the trials and between the growth stages of the plants ($p<0.001$, df=4, F=562.58).

3.2.7. Samples Collected from Undegraded Sites or Patches

Table 6. Daily Observation of Plant Roots within the Undegraded Area.

Anova: Two-Factor without Replication

SUMMARY	Count	Sum	Avera/ge	Variance
Seedling	10	2793	279.3	12744.7
Sapling	10	2991	299.1	8616.5
Juvenile	10	3680	368.0	6745.1
Young Tree	10	4969	496.9	5973.0
Matured Tree	10	5679	567.9	18315.2
Day_1	5	2152	430.4	14286.8
Day_2	5	2298	459.6	49965.8
Day_3	5	1928	385.6	28887.8
Day_4	5	1804	360.8	47254.7
Day_5	5	1714	342.8	10236.7
Day_6	5	1834	366.8	10103.2
Day_7	5	1652	330.4	10868.8
Day_8	5	2414	482.8	28644.2
Day_9	5	2207	441.4	30494.8
Day_10	5	2109	421.8	14923.7

There was a difference among AM frequency observed in plant root samples examined on a daily basis across the plant status ($P<0.001$). Daily observations of the various growth stages of the plant had no significant effects on AM frequency in the root samples ($p>0.05$).

Table 7. Daily Trials or Observation of Mycorrhizal Frequency within the Specimens from the Undegraded Area.

SUMMARY	Count	Sum	Average	Variance
T_1	5	721	144.2	3686.7
T_2	5	665	133	1683.5
T_3	5	618	123.6	1846.8
T_4	5	677	135.4	2514.3
T_5	5	610	122	2224.5
T_6	5	693	138.6	2718.3
T_7	5	687	137.4	3463.3
T_8	5	652	130.4	3028.3
T_9	5	622	124.4	3268.3
T_10	5	678	135.6	3253.3
T_11	5	656	131.2	2902.7
T_12	5	688	137.6	1919.8
T_13	5	677	135.4	2087.8
T_14	5	687	137.4	1406.3
T_15	5	683	136.6	2390.3
T_16	5	708	141.6	1456.3
T_17	5	667	133.4	2558.3
T_18	5	718	143.6	2629.3
T_19	5	829	165.8	2708.2
T_20	5	645	129	1618.5
T_21	5	722	144.4	6863.3
T_22	5	688	137.6	2424.3
T_23	5	584	116.8	3358.7
T_24	5	675	135	2846.5
T_25	5	734	146.8	3150.7
T_26	5	734	146.8	1587.2
T_27	5	670	134	1702
T_28	5	724	144.8	2142.2
T_29	5	669	133.8	1776.7
T_30	5	689	137.8	3935.7
Seedling	30	2335	77.8	155.5
Sapling	30	3112	103.7	270.5
Juvenile	30	4088	136.3	234.3
Young Tree	30	4879	162.6	352.8
Matured Tree	30	6056	201.9	524.2

AFM colonization was highest in matured trees than in other states, particularly in seedlings, although no significant difference was observed between the trials (P = 0.07). AFM colonization was significant for the growth form of the plants ($p<0.001$).

3.2.8. Nutrient Availability and AMF Diversity

Mycorrhization frequencies and intensities across different land use areas revealed significantly higher fungal activity in the undisturbed sites (Figures 4 and 5). Additionally, these undisturbed sites exhibited more variability compared to the degraded areas. Hence partly the reason why the K 01 soils have low cation exchange capacity, nutrient content, and organic matter, with high gravel and sand content. These characteristics reduce their ability to retain water and nutrients, limiting tree growth to areas where roots can penetrate beyond 10 cm in depth. Soils K03 Like K02, it is acidic, sandy (80% sand), and gravelly, with an increase in gravel content at lower depths. With similar physical and chemical properties to K02, it could also support the luxurious growth of *Guibourtia* spp., An indication that the AMF diversity is high in these soil types, which concides with undegraded areas in the reserve (Figure 3). AMF species richness and diversity were significantly higher in non-degraded forest patches compared to degraded patches (1.75). This suggests that nutrient availability, which is often higher in undisturbed ecosystems, supports a more diverse and richer mycorrhizal community. In areas where land use activities and fire have impacted plant diversity, the reduction in AMF species richness and diversity reflects the negative effects of these disturbances on soil nutrient dynamics. In contrast, sites with minimal human activity, which are likely to have more stable nutrient cycles, exhibited higher mycorrhizal richness, indicating a healthier, more fertile soil environment. These findings highlight the important role of nutrient availability in sustaining both plant growth and mycorrhizal communities, with undisturbed patches supporting greater fungal diversity and, consequently, more effective nutrient cycling.

4. Discussion

Soils in Sierra Leone are classified into 12 associations, with Ultisols and Oxisols dominant in upland areas. These soils have low fertility, are highly acidic (pH 4-5), and often show aluminum toxicity, though pH values can vary significantly, such as between 4.5 to 11.3 in Pujehun district. Key chemical factors influencing soil health include pH, available nutrients (N, P, K, Mn), and soil organic carbon [26, 27]. Organic carbon levels range from 2.08–5.52%, lower than previous studies [28], and are typically lower in drier zones. Soil pH and alkalinity tend to be higher in these areas due to reduced alkaline leaching [29].

Soil organic carbon (SOC) in Sierra Leone ranges from 2.08 to 5.52%, with higher levels in soil type 2 and lower in soil type 3. Manojlović et al. (2010) found SOC to be highest

under forest cover and lowest under grass, with a decrease in SOC from higher to lower altitudes. Nitrogen deficiency is most pronounced in the soils of Gallines Peri and Barri chiefdoms, and least in Kpaka chiefdom. Available phosphorus ranges from 7 to 9.48, contrasting with previous findings of 13.21-16.25 kg ha-1 in Gallines Peri and Kpaka [28]. Exchangeable bases follow the order $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$, with values ranging from 20-395 mg/kg for Ca, 5-85 mg/kg for Mg, and 5-24 mg/kg for Na, consistent with studies in the Jong River Basin [30].

The cation exchange capacity (CEC) in Kasewe Forest Reserve soils ranges from 7.28 to 12.86 cmol(+)/kg, indicating low CEC levels. CEC increases with soil pH, particularly when acidic soils are limed [31]. These findings contrast with [32], who reported higher extractable phosphorus in organic soil layers under birch- and spruce-dominated forests. Soils with CEC below 5 cmol(+)/kg typically have low clay and organic matter content, poor water-holding capacity, and are more prone to nutrient leaching, which can negatively affect soil productivity [33].

Jalloh et al. [26] report that the West African coastal region has the lowest proportion of land with low cation exchange capacity (CEC), though the reasons remain unclear, given its diverse agroecologies. In Kasewe Forest Reserve, G. copallifera is associated with eight genera of arbuscular mycorrhizal fungi (AMF) (*Glomus, Acaulospora, Kuklospora, Gigaspora, Scutellospora, Entrophospora, Ambispora, Paraglomus)* from six families (Glomeraceae, Acaulosporaceae, Gigasporacea

e, Entrophosporaceae, Ambisporaceae and Paraglomeraceae), with Glomus and Acaulospora being the most common. Diop et al. (2015) [34] observed similar AMF genera, including Gigaspora, Acaulospora, and Glomus, in Senegal, classified into three families.

Glomus is the most represented genus across all sites, consistent with findings in Senegal [35, 36] Brazil [37], Morocco [38], and China [39]. This genus is common in disturbed ecosystems, such as agricultural landscapes [40, 41] and restored grasslands [42], due to its ability to sporulate, colonize new roots, and form anastomoses [43, 44]. AMF species diversity is lower in degraded areas, and the association between *G. copallifera* and AMF positively influences its survival and growth of this species in the reserve.

This study found that G. copallifera root colonization and AMF spore density were significantly higher in non-degraded forest soils than in degraded ones. Similar results were reported by [34], who found higher mycorrhizal association and AMF taxa in cowpea roots from Dek soil compared to Dior soil. Soil properties, particularly exchangeable cations (Ca, Mg, K, Al, Fe) and fine silt, were key factors influencing AMF community structure. Previous studies also indicated that moderate levels of calcium, magnesium, and potassium stimulate root colonization [45-47].

The diversity of arbuscular mycorrhizal fungi (AMF) associated with G. copallifera reflects a healthy soil ecosystem and robust plant-fungal relationships essential for long-term forest stability. To preserve these beneficial fungi, forest management should focus on minimizing soil disturbance through practices like reduced logging, controlled grazing, and fire prevention. The variety of AMF families also serves as a useful indicator of forest health, making them valuable for monitoring ecosystem impacts. Given its strong AMF associations, G. copallifera is well-suited for agroforestry systems that promote soil fertility and sustainability. Educating local communities about AMF benefits can support better land-use practices. Overall, incorporating AMF conservation into forest policy can help protect soil biodiversity and enhance ecosystem services like nutrient cycling and carbon storage, contributing to climate resilience and sustainable forest management. The dominance of Glomus and Acaulospora across several sites, highlights its ecological adaptability—particularly in disturbed or managed ecosystems. This finding has important implications for forest management, as the resilience and colonization efficiency of Glomus make it a valuable partner in reforestation, agroforestry, and soil restoration efforts. Its ability to rapidly establish symbiosis and improve plant nutrient uptake can be leveraged to enhance tree survival and ecosystem recovery in degraded or reforested areas, supporting more sustainable land-use practices.

5. Conclusion

This study concludes that Soil Type 1 (K01) is unsuitable for *G. copallifera* and agriculture (being too shallow), tree growth is very restricted, it may once have had better tree cover, but restoring land with this soil type will be technically difficult. Soil Types 2 (K02) and 3 (K03) are suitable for *G. copallifera*, but many areas with soil Type 2 have been cleared for agriculture and where forest regrowth is allowed to proceed the more vigorous *Gmelina arborea* and *Anisophyllea laurina* tend to dominate. On soil types 2 and 3 no significant relationship was found between vegetation and soil nutrients such as N, P, K, and C; rather, texture (percentage of clay and silt) and depth were important.

Soil type 1 in Kasewe is highly degraded due to anthropogenic activities, and the best stands of G. copallifera (Kobo) are found on less favorable soils for farming. Water scarcity and the presence of a hard pan at 10 cm depth in soil type 1 hinder tree growth, particularly during the dry season. Additionally, G. copallifera faces significant pressure from fire caused by farmers and charcoal burners. The study identified 22 AMF species, including Glomus, Acaulospora, Gigaspora, Paraglomus, and Ambispora, associated with the roots of Kobo trees. AMF diversity was about twice as high in mature trees compared to seedlings.

Certain AMF species were found only at specific growth stages of G. copallifera, and their presence significantly enhanced the species' competitive ability, especially in poor soil conditions. The results suggest that AM fungi help G. copallifera thrive in nutrient-poor habitats, highlighting the

importance of AMF in reforestation or reintroduction efforts for this native tropical tree. Kasewe forest supports a variety of mycorrhizal associations, with certain species dominating in different land use areas. As human disturbances threaten plant communities and soil systems, promoting and restoring mycorrhizal networks is crucial for effective forest reserve management.

Abbreviations

AMF	Arbuscular Mycorrhizal Fungi
CEC	Cation Exchange Capacity
NTFPs	Non-timber Forest Products
EC	Electrical Conductivity
KOH	Potassium Hydroxide
HCl	Hydrochloric *Acid*
H_2O_2	Hydrogen Peroxide
RLC	Root Length Colonization
ANOVA	Analysis of Variance
SEB	Sum of the Exchanged Bases
SOM	Soil Organic Matter
K 01	Soil Type 1
K 02	Soil Type 2
K 03	Soil Type 3
AM	Arbuscular Mycorrhizal
SOC	Soil Organic Carbon

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Conflicts of Interest

The authors declare no conflicts of interest.

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