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Fungal Diversity and Community Composition in Cocoa Agroforestry Farms across Two West African Countries Using DNA Metabarcoding

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Abstract

Soil microorganisms are crucial to ecosystem functions and productivity by mediating biogeochemical cycles. However, different land-use changes and management practices in agriculture cropping systems have tremendously influenced their composition, diversity, and abundance, causing significant implications for soil health, plant productivity, and biodiversity. We investigated soil fungal community and diversity associated with land use changes in cocoa production systems amid climate change in Ghana and Côte d'Ivoire. In this study, we employed DNA metabarcoding using PacBio sequencing to examine fungal community composition and diversity by targeting the ITS region of fungi in soils collected from three cocoa agroforestry farms (5, 15 and 30 years old). The results revealed that the Ascomycota and Basidiomycota phyla dominated the communities. The classes *Eurotiomycetes and Dothideomycetes* prevailed in the soils of both countries. The 15- and 30-year-old cocoa agroforestry farms recorded the highest number of fungi in both countries, with the most abundant found in soils of Ghana within a depth of 15 - 30 cm. The results of this study reveal that the soils of this cocoa agroecosystem are dominated by the fungal genera *Phialophora* and *Aureobasidium*, which can be explored in future studies along with other mycorrhizal fungi regarding sustainable cocoa production under a changing climate in Ghana and Côte d'Ivoire.

Keywords

Soil Fungi, Cocoa, Agroforestry, West Africa, DNA Metabarcoding

1. Introduction

Cocoa (*Theobroma cacao* L.) is an economically important cash crop in the world, widely used in various facets of human activities due to a wide variety of benefits. Cocoa seeds, usually called cocoa beans, are the most critical parts of the cocoa tree, and they can be processed into various products such as cocoa powder, cocoa butter, chocolate, and other confectionaries and cosmetics. Cultivation of cocoa trees supposedly began in the Americas during the time of the Mayan Indians, who were probably the first to grow cocoa before eventually spreading to almost all countries of the subequatorial region [1]. With the immense importance of cocoa in this growing population, it is crucial to maximise the global production of cocoa to meet the current population and beyond.

For over half a century now, four countries (Côte d'Ivoire, Ghana, Nigeria, and Cameroon) in West and Central Africa have been the leading producers of cocoa respectively, contributing to about 70% of world cocoa production [2]. Ghana and Côte d'Ivoire are currently the world's largest cocoa producing countries [3]. However, cocoa growth and productivity are mainly influenced by climate, soil properties, and microorganisms [4] [5]. Cocoa production and yields can be effectively improved by improving the soil properties and microbiota. Previously, studies in the cocoa production systems have focused on enhancing cocoa yields using synthetic fertilisers [6] under cocoa monocultures (low shade and low soil carbon stocks). This practice up-scales soil degradation through the cocoa production systems and threatens the livelihoods of local farmers in these countries. Introducing agroforestry practices into the cocoa production system has improved cocoa yields, enhanced soil health, and increased biodiversity; moreover, it mitigates climate change [7]-[9]. Studies conducted on cocoa agroforestry indicate that it supports higher levels of biological diversity than most other tropical crops [10] [11].

Soil microbes, on the other hand, play an essential role in ecosystem functions as they are critical components of farmland ecosystems and are considered more sensitive to land use and environmental changes [12] [13]. Soil microbes play a crucial role in nutrient cycling, including carbon, nitrogen, and phosphorus, as well as producing and consuming greenhouse gases. Microorganisms, such as fungi or bacteria, are known to be helpful in agriculture since they are attractive eco-friendly alternatives to mineral fertilisers and chemical pesticides. For instance, fungi can play a vital role in agroecosystems, contributing to soil nutrient circulation, organic material decomposition, and improving plant growth [14]. Fungi, particularly mycorrhizal fungi, have the potential to improve general plant growth and resist the attacks of specific pathogens due to the symbiotic association with the roots of certain plant species, of which cocoa is one. This is done via various metabolic pathways, including increased mineral nutrition, primarily phosphorus, and the expression of specific plant genes while also gaining some form of benefit from the plant in the form of carbohydrates [15]. The positive effect of AMF on cocoa plants is also observed in some species of the genus Trichoderma [16]. A combination of AMF species *Gigaspora* spp and *Gigaspora*

margarita spores resulted in the most healthy growth with higher phosphorus content in the leaves of cocoa seedlings [16]. In another study, phosphorus content in the shoots was increased by *Scutellospora calospora* and *Glomus mosseae* [17]. Previous studies found that cocoa seedling growth was promoted by *Glomus* sp and *Glomus mosseae* in a greenhouse experiment [18] [19].

Land use and management practices at the cocoa farm level can significantly impact fungal community composition and diversity in Ghana and Côte d'Ivoire. These practices involve various activities that utilise soil resources for human social or economic purposes, while affecting soil ecosystems [12].

As an adaptation and mitigation strategy, cocoa agroforestry practice has received considerable attention over the years. However, how this practice affects microbial community composition and diversity in Ghana and Côte d'Ivoire to influence nutrient and climate change is not known.

So far, only a few studies have used DNA metabarcoding in the diversity of soil fungi associated with cocoa production systems in West Africa. Previous studies on fungal diversity in West Africa were focused on fruiting bodies and viable cell isolations in natural and managed systems [20]. Only a few studies conducted involved the cocoa microbiome. For instance, a recent study by [21] focused on drivers of rhizosphere microbial diversity and community composition in cocoa soil in Ghana but not the overall diversity and community composition of cocoa rhizosphere.

It is essential to prioritize the certification of key species related to soil and cocoa health. This could be the foundation for exploring microbial resources to improve cocoa growth and yield under changing climates.

The recent use of DNA from soil samples with the ITS region being the target has been helpful in the study of fungal communities and diversity [22]-[24]. This has significantly enhanced our comprehension of the diversity and community of fungi, particularly those inhabiting the rhizosphere [25] [26]. This present study used PacBio sequencing to explore the diversity and community composition of soil fungi associated with cocoa agroforestry farms in West Africa. We hypothesised that 1) the diversity of fungal genera will be significantly similar across the two countries due to plant neighbourhood effect and dispersal factors, and 2) soil chemical properties, soil quality and management are significant drivers of fungal relative abundance in both countries.

2. Material and Methods

2.1. Site Selection and Soil Sampling

Field data were collected in February 2022 from two countries in West Africa, Ghana (GHA) and Côte d'Ivoire (CIV). A survey was conducted in several cocoa farming communities, from which the sites were selected for the study. In each location, we selected three age classes of cocoa agroforestry farms: 5-year-old, 15-year-old, and 30-year-old farms, which served as treatment plots. We established plots measuring 35 m × 35 m [27] in a completely randomized design, with three

replicates for each age class. This resulted in a total of nine experimental plots in each country. These agroforestry farms are owned and managed by different farmers. Five composite soil samples were collected randomly from each of the three cocoa agroforestry farms (5-, 15-, and 30-year-old) in Asafo (Ghana) and Odoguié (Côte d'Ivoire). Farms in these two areas do not only share similar management practices for cocoa cultivation, but we also chose these locations because they have comparable soil properties, climate conditions, and soil types. Additionally, their geographical proximity contributes to these similarities. The soil samples were collected from two soil depths, 0 - 15 cm and 15 - 30 cm, from the rhizosphere of randomly selected cocoa trees. We selected different cocoa farms to compare their fungal community composition and diversity performance since cocoa agroforestry management on the farm can influence microbial community and diversity. The study areas are characterised by tropical semi-deciduous degraded forests and intensive cocoa cultivation. The areas experience bimodal rainfall patterns, with the major rainy season occurring between May and August and a minor season from September to October. The annual precipitation ranges from 1600 to 2500 mm. The mean daily temperature ranges between 22˚C - 35˚C. The annual average rainfall is 1700 mm for Ghana and 1630 mm for Côte d'Ivoire. The top 20 cm layer of soils in the study area of Côte d'Ivoire is mainly composed of acidic Ferralsols with a sandy-loam texture [28]. The nutrient content is low and decreases rapidly from the upper soil layer to a depth of 20 cm. The soils of the study area in Ghana are from weathered phyllites and are dominated by Ochrosols [28]. They are generally deep and moderately well-drained, with a sandy clay-loam texture in the top 15 cm soil layer, which gives them a high moisture retention capacity [29] (**Figure 1**).

2.2. DNA Extraction, Sequencing and Bioinformatics Analyses

For soil DNA extraction and sequencing, soil samples were sent to the Mycology and Microbiology Centre at the University of Tartu, Estonia. DNA was extracted from 0.25 g of homogenised dried soil using the PowerMax Soil DNA Isolation kit (Qiagen, Carlsbad, CA, United States) following the manufacturer's instructions. The DNA extracts were purified using the FavorPrepTM Genomic DNA Clean-Up kit (Favorgen, Vienna, Austria). PCR reactions were performed using the universal eukaryote primers ITS9mun and ITS4ngsuni [30] [31]. PCR mixture comprised 5 µl of 5x HOT FIREPol Blend Master Mix (Solis Biodyne, Tartu, Estonia), 0.5 µl of each forward and reverse primer (20 mM), 1 µl of DNA extract and 18 µl ddH_2O. The following PCR conditions were used: initial denaturation at 95˚C for 15 min; 25 - 30 cycles of denaturation for 30 s at 95˚C, annealing for 30 s at 55˚C, elongation for 1 min at 72˚C; final elongation at 72˚C for 10 min; and storage at 4˚C. The quality of PCR products was checked on a 1% agarose gel; in cases where the bands were weak, the number of cycles was increased to 37, or an increased DNA template concentration was used. Positive and negative controls were used to help identify and eliminate contamination. The pooled ampli-

cons were shipped to the Norwegian Sequencing Centre at the University of Oslo for PacBio library preparation and sequencing on Sequel II equipment.

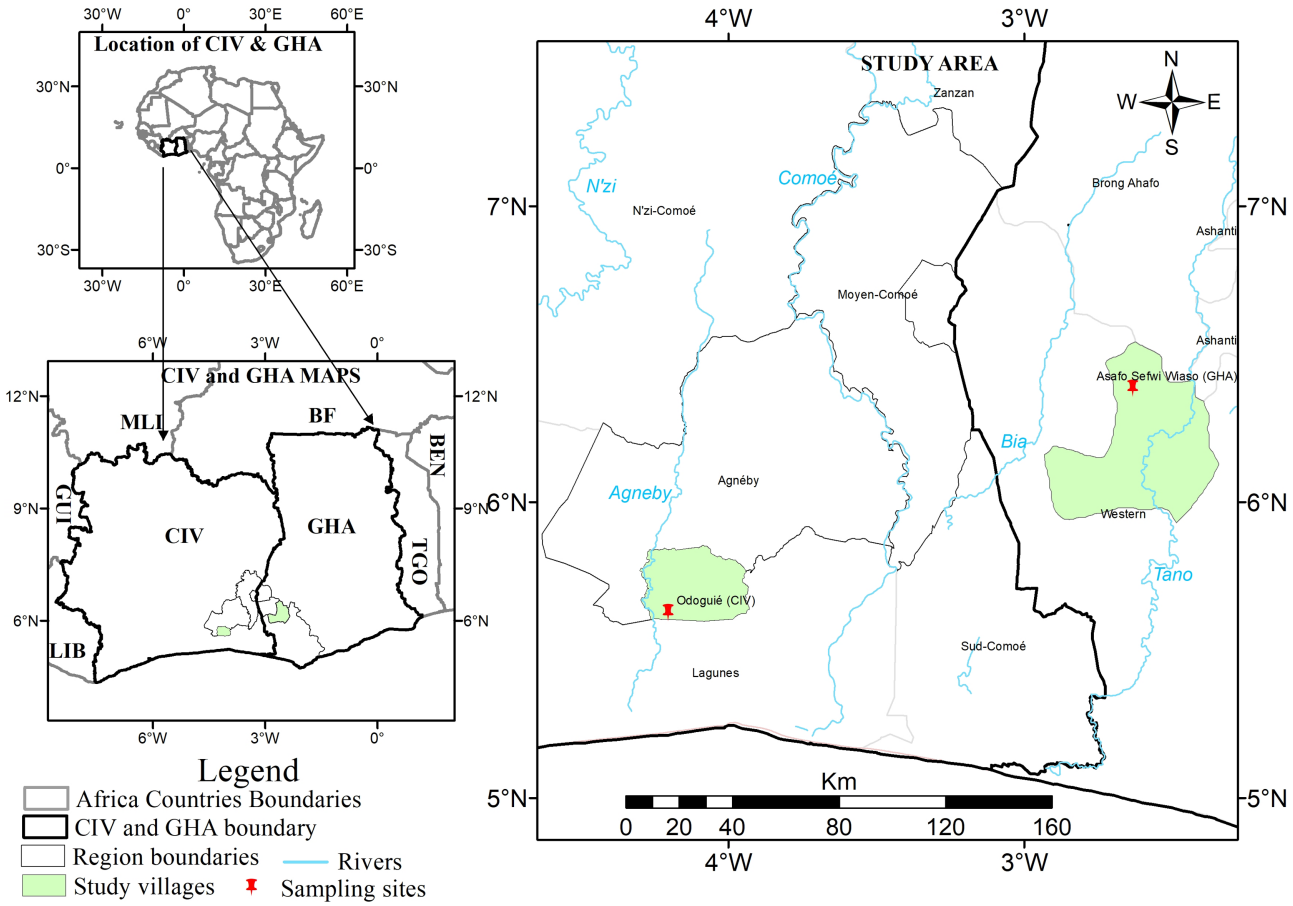

Figure 1. Map of soil sampling sites.

Sequences were demultiplexed into uniquely identified samples using LIMA software v2.0.0 (PacBio) based on a 12 bp primer index and processed using seqkit v.0.16.0 [32]. The sequences were dereplicated after demultiplexing by grouping similar sequences as a single representative sequence. The ITS region was extracted from the sequences using ITSxpress v.1.8.0 [33]. Chimeras were filtered from each sample by comparing them with the 920,399 reads in the updated UNITE 9.1 beta dataset (https://doi.org/10.15156/BIO/1444285). Reference-based chimeras were detected and eliminated if present via the VSEARCH v2.22 [34]. Sequences were then clustered into Operational taxonomic units (OTUs) at a 98% sequence similarity threshold using the VSEARCH software [34].

OTUs were taxonomically annotated using the BLAST 2.13.1+ [35] by conducting blast queries of representative OTU sequences against the revised UNITE 9.1 beta reference dataset, and the top 10 blast hits were then compared to taxonomy assignments described in [24]. The whole pipeline (NextITS) for the entire bioinformatics is described by (Mikryukov *et al*., unpublished data [https://github.com/vmikk/NextITS]).

The taxonomic assignment analysis also follows the description by [24]. FungalTraits Database [36] was used to assign traits to genera based on their primary lifestyle or functional guilds. Demultiplexed fungal sequences for this study are available at NCBI under the accession number PRJNA1115901.

2.3 Statistical Analyses

All statistical analyses were performed in R statistical program version 4.2.1 [37]. Climatic variables (mean annual precipitation (MAP) and mean annual temperature (MAT)), as well as altitude, were extracted from the WorldClim database [38] at 30 arc seconds. The chemical properties of the soil analysed in this study included pH measured by the KCl method [39], as well as levels of Ca, P, K, and Mg, measured using the acetate lactate (AL) method after dissolving 2g of soil in 100ml of acetate as described by Smith and Johnson (2010). The study measured the concentrations of delta-15 nitrogen (δ^{15}N), delta-13 carbon (δ^{13}C), total Nitrogen, Soil Organic Carbon (SOC), and the carbon-to-nitrogen ratio (C: N) in soil samples ranging in size from 1 to 20 mg. The analysis was carried out using GC-combustion in conjunction with isotope-ratio mass spectrometry, following the method described by [40].

To eliminate sequencing depth differences between samples and obtain a reliable alpha diversity metric, we used the residuals from a linear regression of the logarithmically transformed number of fungal OTUs against the logarithm of the sequencing depth [41]. Alpha diversity metrics (Shannon diversity index, richness and Simpson index) were measured using the vegan package in R (version 2.6-4) [42]. Using the *adonis2 function* from the vegan package in R, a Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted to evaluate differences in soil fungal community structures. This was done to investigate their associations with soil chemical properties and geographical variables across the two countries.

Variation partition analysis was conducted using the *varpart function* to quantify and visualise the independent components of variation in fungal community composition explained by environmental factors, including soil pH, MAP, soil depth, farm age, and country. We conducted a distance-based redundancy analysis (dbRDA) using the *dbrda function* from the vegan package in R (version 2.6-4) [42] to investigate the relationship between soil fungal communities and soil chemical properties. To achieve this, we first Hellinger transformed the OUT matrix based on Bray-Curtis distance and then standardised environmental variables using the method = "standardise" in the *decostand function* in vegan in R (version 2.6-4) [42].

3. Results

3.1. Fungal Taxonomic Composition, Species Diversity and Abundance

A total of 10,067 full ITS reads were analysed, and 4025 fungal taxonomic groups

were identified. Ascomycota was the most dominant phylum, accounting for 73.6% of the total, followed by Basidiomycota (23.8%), Rozellomycota (0.69%), Chytridiomycota (0.37%), Mortierellomycota (0.36%), and Glomeromycota (0.10%). The abundance of the remaining phyla was less than 0.1% of the total in our dataset (**Figure 2**). There was a significant difference between the Shannon diversity index and the two countries investigated in this study (as determined by ANOVA; F = 5.56; p = 0.04) (**Figure 3(A)**). Variation partitioning analysis also revealed that the effect of soil pH in structuring the fungal communities was highest (8.7% of variation), followed by country (8.1%), farm age (7.6%) and soil depth (2.8%) (**Figure 3(B)**). On the contrary, there were no significant differences between Shannon diversity index and the ages of farms (15, and 30-year-old) (ANOVA; F = 0.37; p > 0.05).

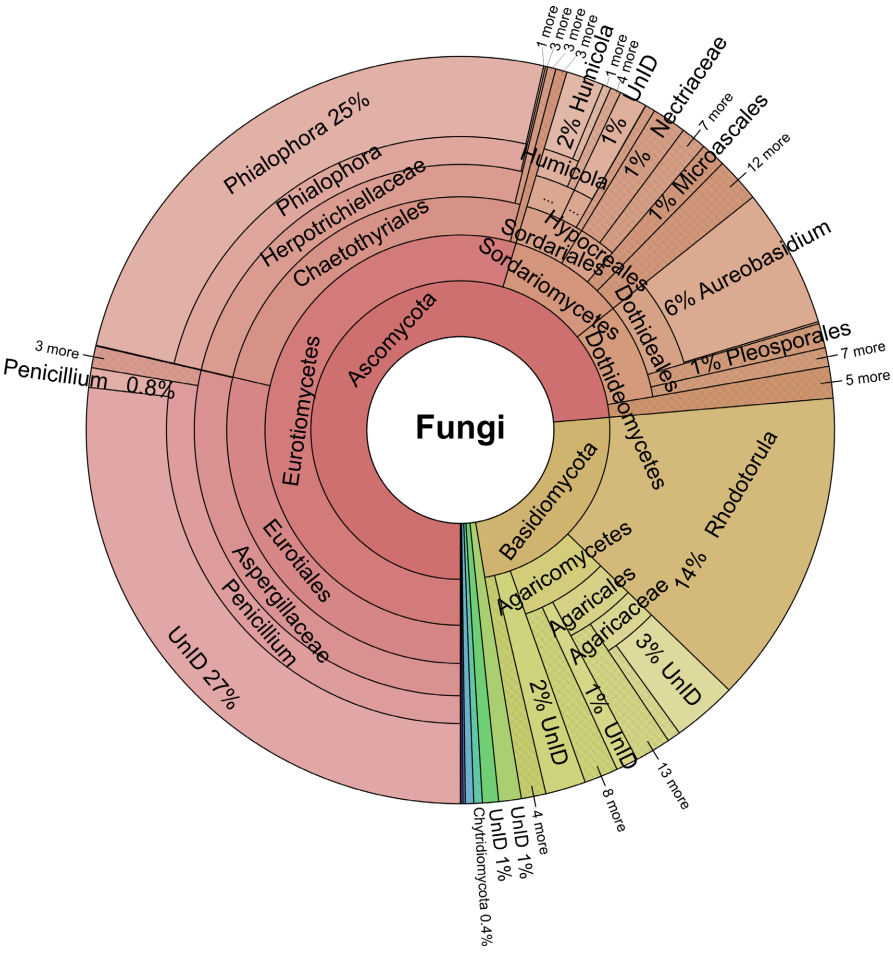

Figure 2. Krona chart indicating the taxonomic distribution of fungal species. UnID = unidentified.

No significant differences were observed between Shannon diversity index and the soil depths (ANOVA; F = 0.82; p > 0.05). A significant correlation was observed between the fungal Shannon diversity index and latitude (lm; $R^2 = 0.363$, p = 0.037), MAT (lm; $R^2 = 0.357$, p = 0.04), MAP (lm; $R^2 = 0.378$, p = 0.033). How-

ever, no significant correlation was found between the fungal Shannon diversity index and soil chemical properties such as pH (lm; R2 = 0.263, p = 0.088), C:N (lm; R^2 = 0.2, p = 0.15), available phosphorous (lm; R^2 = 0.119, p = 0.27), available potassium (lm; R^2 = 0.324, p = 0.053), available calcium (lm; R^2 = 0.214, p = 0.129), available magnesium (lm; R^2 = 0.162, p = 0.2), $\delta^{15}N$ (lm; R^2 = 0.055, p = 0.46), $\delta^{13}C$ (lm; R^2 = 0.274, p = 0.081), SOC (lm; R^2 = 0.231, p = 0.113), or total nitrogen (lm; R^2 = 0.251, p = 0.096).

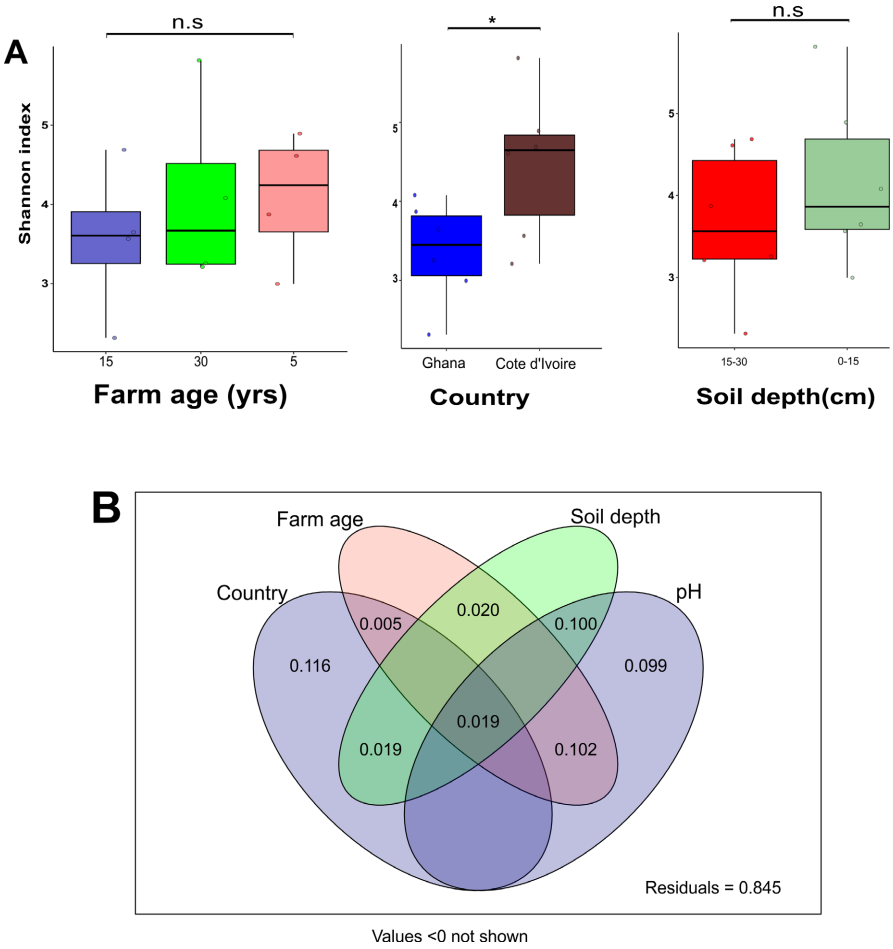

Figure 3. Shannon index based on (A) cocoa agroforestry farms (age), country, and soil depth and (B) pure and shared effect of farm age, country, and soil depth on pH. Lines over the box plot represent the significance of mean differences according to Tukey's HSD test (0.001 < ****, 0.01 < **, 0.05 < *, n. s=not significant).

While Ascomycota was the predominant phylum found across the cocoa agroforestry farms, sites, and soil depths, Basidiomycota varied across farms, sites, and soil depths in terms of relative abundance in both countries. The highest relative abundance of Basidiomycota was recorded in the 30-year-old farm in Ghana and at a soil depth of 0 - 15 cm, accounting for 28.3 %, 27.8 %, and 28.7 % of the total, respectively (**Figure 4(A)**). Unspecified and litter saprotrophs were the most prevalent functional guilds, regardless of farm age, country, and soil depth. However,

arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EcMF) were poorly represented in our dataset. The 30-year-old farm recorded a minor presence of AMF, representing 0.02%, but was dominated by EcMF, representing 0.11%. The results showed that AMF were only present in 0 - 15 cm soil depth, while EcMF were found at both soil depths (0 - 15 cm and 15 - 30 cm). Plant pathogens were observed across all farms and soil depths except in Ghana but were more prevalent in the 5-year-old farm than in the 15- and 30-year-old farms. Animal parasites were observed at all levels, except Ghana, but were more prevalent in soils of Côte d'Ivoire (Figure 4(B)).

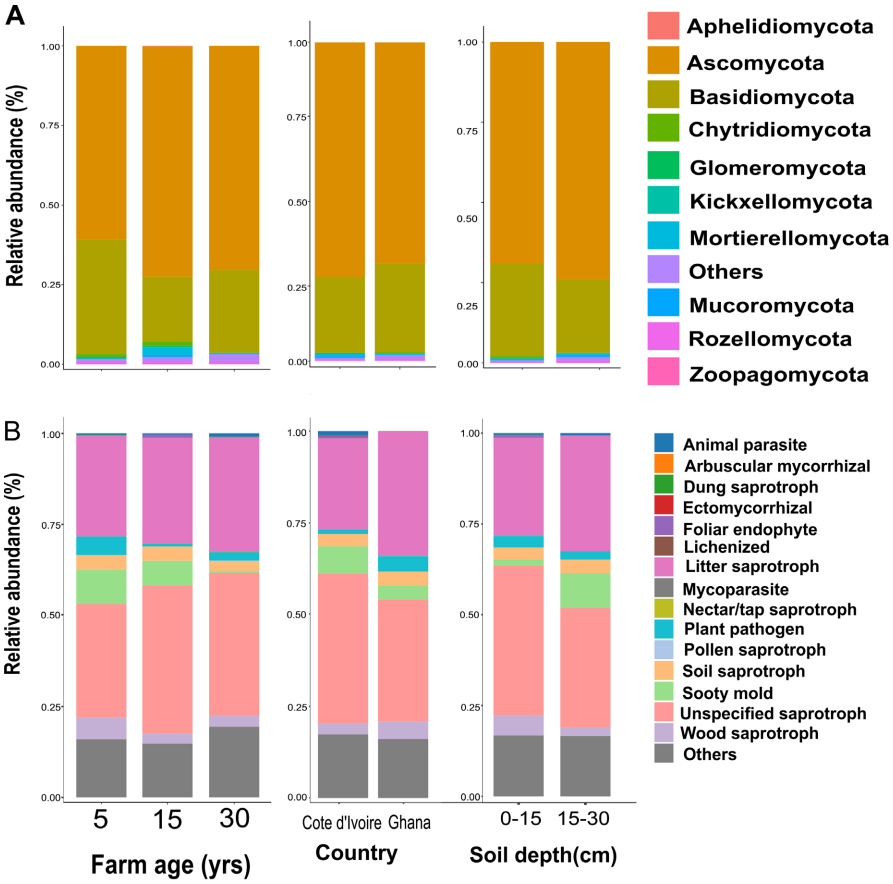

Figure 4. Bar plots of relative abundance. (A) Relative abundance of phyla based on farm age, country, and soil depth. (B) Relative abundance of fungal functional guilds based on farm age, country, and soil depth.

Phialophora was the most prevalent genus under the phylum Ascomycota and the highest prevalence in Ghana soils, accounting for 46.6 % at the site level, 44.5 % in the 30-year-old farms at the farm level, and 40.3% at the 15 - 30 cm soil depth. The genus *Penicillium* was the second most abundant across farms, sites and soil depths (Figure 5(A)).

The genus *Rhodotorula* was found to be predominant across site, farm and soil depth, and this accounted for 61 % in Côte d'Ivoire, at the farm age level of 15

years (56.5 %), and 55.2% at 15 - 30 cm (Figure 5(B)). *Agaricus* and *Lepiota*, two important genera from the Agaricacae family were more abundant in soils of Ghana, accounted for 4.9 % and 1.3 % respectively. On the contrary, *Ganoderma* and *Inocybe* were more dominant in soils of Côte d'Ivoire, accounting for 0.1% and 0.25%, respectively.

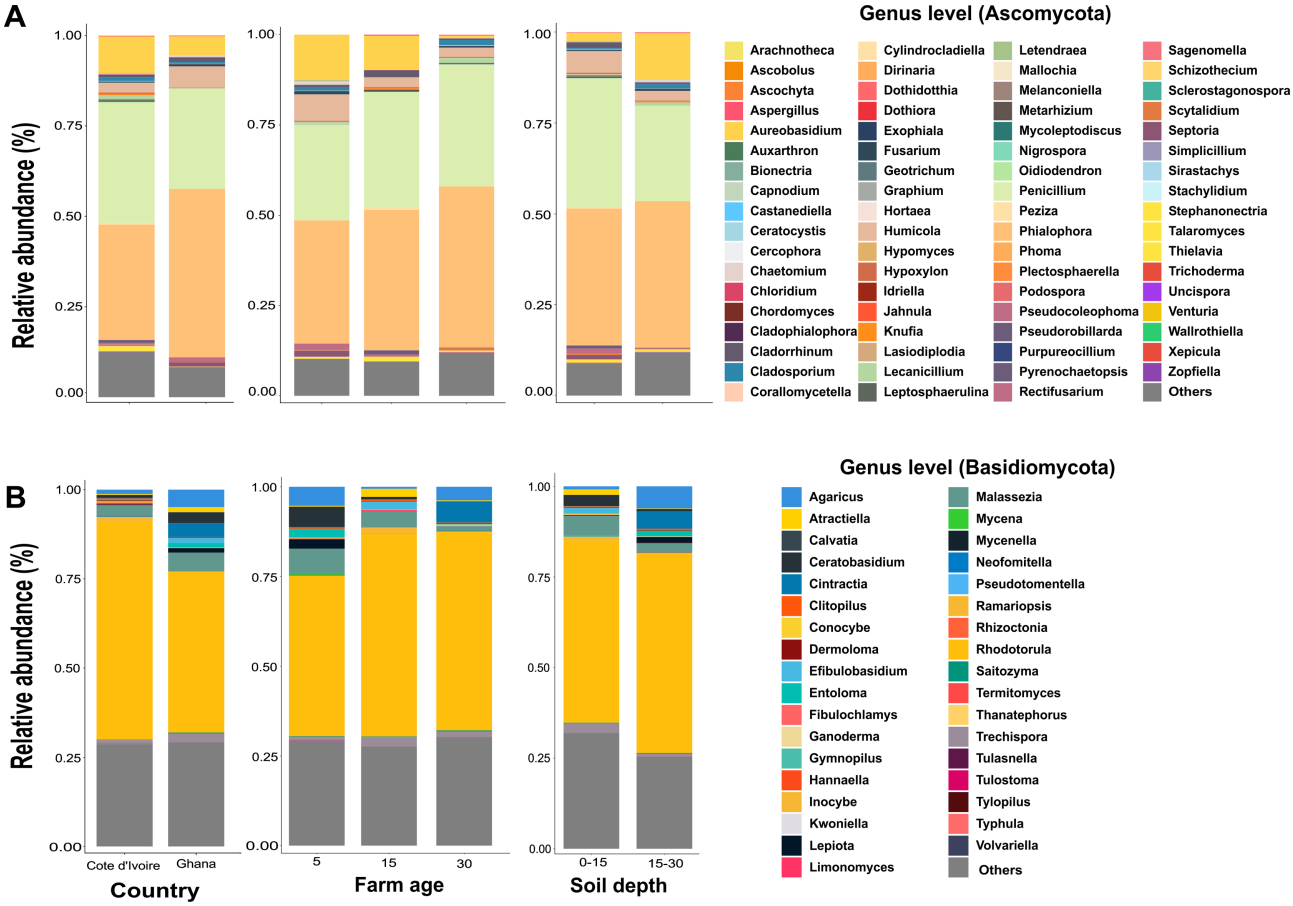

Figure 5. Bar plots showing the relative abundance of (A) Ascomycota and (B) Basidiomycota genera based on country, farm age, and soil depth.

3.2. Fungal Community Structure and Diversity

The Permutational Multivariate Analysis of Variance Analysis (PERMANOVA) revealed that the community composition of fungi from the relative abundance of different OTUs was significantly affected (Table 1) by soil pH ($p < 0.05$).

The dbRDA analysis demonstrated that soil chemical properties explained 20.1% of the variation in the fungal community structure (Figure 6(A)). Furthermore, the result revealed that soil pH, SOC, total Nitrogen, Avail.Ca, C: N and Avail_Mg were the most important variables influencing the community structure, with soil pH ($p < 0.05$) correlating significantly with the fungal community structure. Fungal species in 5 and 30-year-old farms in Ghana exhibited some clustering (Figure 6(A)), and some levels of correlation were observed between important variables (Figure 6(B)).

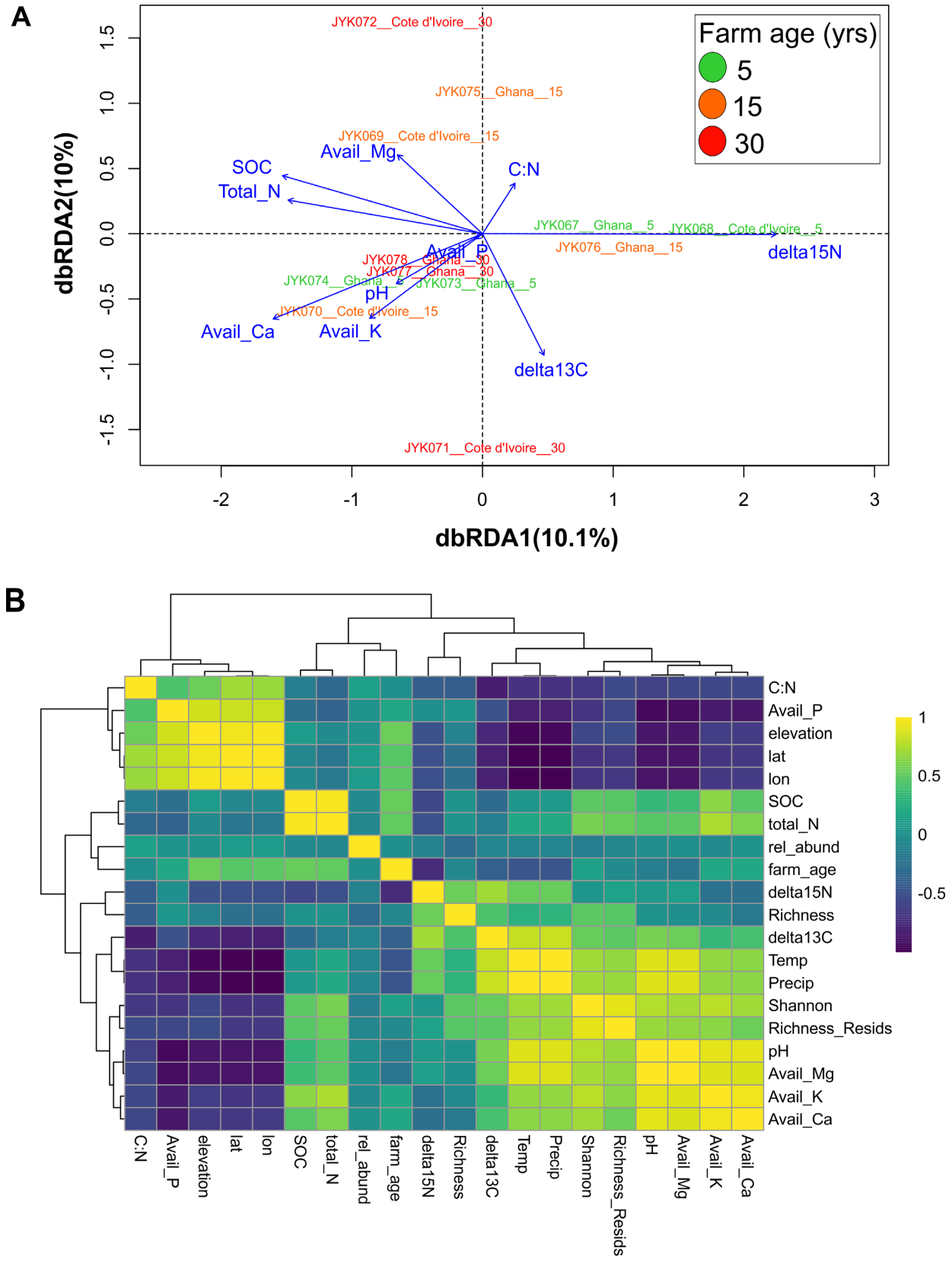

Figure 6. dbRDA plot demonstrating (A) the relationship between soil chemical properties, isotope signatures, and farm ages across both countries; (B) Heatmap showing Pearson correlation among important variables examined in this study.

Table 1. PERMANOVA analysis of the effect of soil chemical properties, climatic and geographical variables and their interactions on the composition of fungal OTUs in analysed soils sampled from two West African countries (Ghana and Côte d'Ivoire). (0.001 < ****, 0.01 < **, 0.05 < *).

Variables	Df	Sum of Squares	R^2	F.Model	Pr(>F)
latitude	1	0.4992	0.09079	0.9985	0.766
longitude	1	0.5006	0.09105	1.0014	0.206
pH	1	0.5024	0.09137	1.005	0.010 **
MAT	1	0.4992	0.09079	0.9986	0.774
MAP	1	0.4985	0.09066	0.9971	0.95
Avail_P	1	0.5000	0.09093	1.0002	0.457
SOC	1	0.4997	0.09089	0.9997	0.616
total_N	1	0.4990	0.09076	0.9982	0.854
Residual	3	1.4997	0.27276		
Total	11	5.4984	1		

3.3. Soil Chemical Properties and Stable Isotopes Varied among Farm Ages and Soil Depths across Both Countries

Soil chemical properties and stable isotope measurements varied significantly between sites, farms, and soil depths (Table 2). The soils of both countries fall in the pH range of 3.7 - 5.5, but soils from Ghana were weaker in acidity with a soil pH value of 5.5 - 7.0 compared to soils from Côte d'Ivoire (pH value of 3.7-6.5). No substantial differences were recorded for available P, δ^{15} N, C: N, δ^{13}C, SOC, and available N between sites, farms, and soil depths. A higher C: N ratio was recorded in Côte d'Ivoire's soils than in Ghana's soils. Available Ca and available Mg in soils of Ghana were 4.3 and 3.1 times higher than that of Côte d'Ivoire. Except for available P, K, and C.N, all soil chemical properties and stable isotopes were higher in Ghana's soils than in Côte d'Ivoire's soils (Table 2). At the farm level, available Ca was 1.1 times higher in 15-year-old farms than in 30-year-old farms, whereas available Mg was 1.2 times higher in 30-year-old farms than in 15-year-old farms. Available Ca was 1.4 times higher in 15 - 30 cm soils than in 0 - 15 cm soils (Table 2).

4. Discussion

PacBio sequencing was used to investigate soil fungal communities in the rhizosphere of cocoa (*Theobroma cacao*) from different cocoa agroforestry farms (5-, 15- and 30-year-old) in Ghana and Côte d'Ivoire in West Africa. The results revealed that soil chemical properties, farm age, and soil depth played a role in the composition of soil fungi in these soils. For the first time, to the best of our knowledge, we identified soil fungal species (up to the genus level) that are resident in the soils of cocoa farms in Ghana and Côte d'Ivoire and characterised the dynamics of their community compositions.

Table 2. Chemical properties and stable isotopes of soil samples based on country, age of the farm, and soil depth.

Variable	Soil pH (H_2O)	Avail. P	Avail. K	Avail.Ca	Avail.Mg	δ^{15} N	C: N	δ^{13}C	SOC	Avail. N
Country										
Ghana	5.397 ± 0.017a	0.046 ± 0.005a	6.853 ± 0.269a	133.036 ± 4.457a	21.717 ± 0.33a	9.638 ± 0.02a	9.313 ± 0.51a	−26.132 ± 0.032a	1.375 ± 0.011a	0.148 ± 0.001a
Côte d'Ivoire	4.047 ± 0.076b	0.251 ± 0.024a	2.1 ± 0.094b	31.030 ± 3.765b	6.968 ± 0.58b	9.452 ± 0.041a	11.591 ± 0.18b	−27.976 ± 0.031b	0.794 ± 0.085a	0.071 ± 0.009a
Farm Age (yrs)										
15	4.645 ± 0.208a	0.155 ± 0.03a	4.761 ± 0.796a	90.236 ± 17.833a	13.007 ± 1.923a	9.577 ± 0.001a	10.346 ± 0.355a	−27.042 ±0.281a	0.946 ± 0.102a	0.098 ± 0.013a
30	4.827 ± 0.161a	0.161 ± 0.033a	5.147 ± 0.423a	85.122 ± 10.490b	16.396 ± 1.776b	9.647 ± 0.019a	9.816 ± 0.098a	−26.738 ± 0.154a	1.384 ± 0.10a	0.141 ± 0.002a
Soil depth										
0 - 15	4.587 ± 0.146a	0.168 ± 0.033a	4.102 ± 0.533a	68.259 ± 10.138a	12.031 ± 1.395a	9.447 ± 0.041a	10.85 ± 0.267a	−27.303 ± 0.177a	1.093 ± 0.093a	0.107 ± 0.01a
15 - 30	4.857 ± 0.151a	0.129 ± 0.021a	4.851 ± 0.525a	95.806 ± 11.873b	16.654 ± 1.663b	9.642 ± 0.019a	10.054 ± 0.250a	26.805 ± 0.198a	1.076 ± 0.77a	0.113 ± 0.01a

All data are expressed as mean ± SE (n = 6); different letters within each column indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$).

The soil fungal communities of these cocoa agroforestry farms were dominated by two major fungal phyla commonly found in soils: Ascomycota and Basidiomycota. This finding aligns with other studies [24] [26] [43] in both managed and natural forest systems. Previous studies did a whole genome comparison of Ascomycomycota with other phya and noticed a higher number of genes related to stress tolerance and the ability to uptake resources, thereby suggesting it to be good in colonizing a wide range of environments over time [23] [44] than other fungal phyla. However, the specific genera *Phialophora* and *Rhodotorula* were the most abundant at the genera level. Similar to a previous study [45], which found *Rhodotorula* and *Trichoderma harzianum* to dominate at all levels of soil depths, we also observed the dominance of *Rhodotorula* at all soil depths. However, Trichoderma was underrepresented throughout all the soil levels. *Penicillium* spp prevailed abundantly in these soils, corroborating the findings of [45]. This could indicate that these fungal species usually dominate cocoa agroforestry farms in Ghana and Côte d'Ivoire. *Penicillium* spp. play critical roles in decomposing and cycling organic matter and nutrients [46]. *Penicillium* was found to exhibit plant growth-promoting (PGP) activity through indole acetic acid (IAA) and siderophore production, as well as P solubilisation [46] [47]. A similar study by [48], which involved changing a 30-year-old secondary forest to a cocoa agroforestry system, also revealed that *Penicillium*, which was grouped as potential biocontrol fungi, was the dominant species. The most abundant OTU belonged to the class *Dothideomycetes*, and some fungal functional guilds were recorded in this study. Our finding agrees with [49] who found agroforestry management systems to be the primary driver of the. composition of bacterial and fungal endophyte communities.in cocoa plantations.

A recent study [21] also reported that soil organic matter and root traits were the main drivers of fungal community composition in good-quality soils in cocoa farms in Ghana. This could be why cocoa agroforestry farms in both countries in this current study had a higher Shannon diversity index in the 0 - 15 cm soil depth than 15 - 30 cm, as deeper soils contain fewer or less diverse organic materials.

Another study [50] compared fungi and bacteria genes based on different soil depths and revealed that the lower the depth, the higher the abundance. Our data also showed a higher diversity of fungi in younger, middle-aged, and older farms. This finding is supported by the research conducted by [51] [52], which proposed that the diversity of ECM fungi increases significantly in the first 30 to 40 years of forest growth and then gradually declines to an intermediate or relatively stable level. As mentioned above, AMF and EcMF are crucial in the developmental stages of cocoa seedlings and the whole plant during its lifetime. However, our results demonstrated the poor representation of these mycorrhizal types in the soils of both countries. This could probably be due to the heavy usage of agrochemicals on cocoa farms across both countries with the intention of increasing yields and mitigating disease attacks on the cocoa plants. Previous studies have shown that AMF are sensitive to these chemicals, which results in their low abundance [53] [54].

Our results revealed a strong correlation between fungal diversity and the environmental variables investigated. This finding supports previous studies which found that MAP and MAT influence soil fungal diversity at both global and regional scales [26] [41]. The soil pH range for the locations was between 4.04 and 5.39, similar to (4.9 - 5.2) reported on Arbuscular mycorrhizal fungi in rice fields in Ghana [55] with similar effect of soil chemical properties. Soil chemical properties and stable isotope abundance influenced fungal communities within sites, which could be the reason for low fungal diversity (**Table 1**). A study which found soil physical chemistry to dominate over neutral processes (geographical distance) revealed that pH, P and the C: N ratio were the strongest predictors shaping fungal communities [56]. However, a study by [57] showed that pH influences community structure more than management systems, similar to our findings, as shown by the PERMANOVA in (**Table 1**) and dbRDA analyses in (**Figure 6(A)**). Several studies also showed that soil pH has a more pronounced and direct impact on soil communities (bacteria and fungi) compared to other soil chemical properties [58]-[60]. This could be the reason for the weak correlation between other soil chemical properties like K, and SOC compared to pH observed in this study. Chemical application on farms could also affect soil chemical properties, which in the long run affects the soil biota and human health. For instance, the chemical properties of pesticides and other agrochemicals are affected by the soil's acidity and a decrease in soil pH results in increased binding of pesticides to soil clay [61]. In order to minimize or avoid this from happening, organic soil nutrition should be encouraged in farming rather than the excessive use of chemicals. A study revealed that biochar application significantly increased soil pH, cation exchange capacity and soil organic carbon [62]. These results help provide an understanding for future studies focusing on the microbiome of cocoa farms in both Ghana and Côte d'Ivoire during the development of sustainable cocoa production systems, particularly on the potential application of agroforestry systems for improved nutrient acquisition and increased production. This can serve as a baseline for policymakers to adopt in educating farmers on the harmful effects of excessive application of agrochemicals on their crops.

5. Conclusion

In summary, this study was conducted to explore the soil fungal community common to the cocoa agroforestry farms in Ghana and Côte d'Ivoire as preliminary research. Results of the study confirm that the cocoa agroforestry farms in these agroecosystems are characterised by fungal communities that can benefit cocoa growth and health. While management practices at the farm level, such as the amount and type of fertiliser or pesticides applied, could be factors influencing fungal community composition and diversity on these farms, our result does not show significant differences between the cocoa agroforestry farms and communities of soil fungi in the two countries. However, our study sets the basis for further studies in the same direction by increasing the sampling size and considering fungal relations with environmental factors. Additionally, it is crucial to conduct more research on isolating beneficial fungi, like arbuscular mycorrhizal fungi (AMF), to introduce them to cocoa seedlings to improve nutrient absorption during the early stages of growth, especially under the influence of climate change. *Trichoderma* spp, one of the identified species in this study, stands to be a well-known species for producing biofertilizers and pesticides along with other fungal species [63]. It is necessary to investigate the interactions between cocoa and soil fungal communities and the use of biofertilisers to stimulate cocoa yields in the future. This study is a pioneer for cocoa-producing countries like Ghana and Côte d'Ivoire, as it is one of the few studies focusing on soil mycobiota in these two countries. However, there is still a need for further research in both countries to understand better the management practices that impact fungal diversity in cocoa agroforestry farms. With the high abundance of *Phialophora* spp in the soils of these two countries, and also given the fact that there is not much data on the study of this fungus, apart from one old study on Phialophora-like fungi and its association with kiwifruit elephantiasis [64], this could be a gap which needs filling in the research space in the future. This will help to understand the influence of agricultural land use on the stability of ecosystems in Ghana and Côte d'Ivoire and provide a scientific basis for the sustainable management of agricultural soils in these countries.

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Authors' Contributions

Florence Jessica Kumah collected the data obtained in this study and drafted the manuscript. Florence Jessica Kumah and John Y. Kupagme analysed the data obtained in this study, wrote, and read the manuscript. Nourou S. Yorou, and Daouda Koné guided and supervised the project. All authors reviewed and approved the final manuscript.

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Data Availability

Available upon request.

Conflicts of Interest

The authors declare that they have no competing interests.

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