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DNA sequencing reveals high arbuscular mycorrhizal fungi diversity in the rhizosphere soil of *Prunus africana* trees in fragmented Afromontane forests

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Abstract

Purpose Arbuscular mycorrhizal fungi (AMF) play a key role in medicinal plant species, besides their ecological role in shaping plant communities. Knowledge about the diversity and structure of AMF communities associated with the endangered *Prunus africana* is valuable in the conservation and domestication of the species for its medicinal products.

Methods We investigated the diversity and structure of AMF species communities in the rhizosphere soils of *P. africana* trees occurring in four fragmented Afromontane forests found in Cameroon (Mount Cameroon and Mount Manengouba) and Kenya (Chuka and Malava) using Illumina Miseq sequencing of 18S rRNA gene amplicons.

Results A total of 64 virtual taxa (VT) belonging to eight genera were detected, namely *Glomus* (43 VT), *Claroideoglomus* (6 VT), *Paraglomus* (5 VT), *Acaulospora* (4 VT), *Diversispora* (3 VT), and *Archaeospora*, *Pacispora*, and *Scutellospora* with 1 VT each. *Scutellospora heterogama* VTX00286 was the most abundant and common species in all four sites (49.62%). Glomeraceae and Gigasporaceae were the most abundant families found across the sites, while Acaulosporaceae, Pacisporaceae, and Archaeosporaceae were rare, represented by < 1% of all the detected taxa.

Conclusion Our data shows a high diversity of AMF species associated with *P. africana* and variable community structure partially shaped by local edaphic factors.

Keywords Arbuscular mycorrhizal fungi, *Prunus africana*, Afromontane Forest, Metagenomic diversity, Community composition, Cameroon, Kenya

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Background

Prunus africana (Hook F.) Kalkman (Rosaceae) is among the few endemic trees found across all the Afromontane forests (White 1978; Kadu et al. 2013; Vinceti et al. 2013). It is an endangered species whose distribution range spans more than 22 African countries (Kadu et al. 2013; Vinceti et al. 2013). It has a wide distribution across fragmented Afromontane forests caused by anthropogenic disturbance. The bark of the medicinal *P. africana* tree is the most harvested and internationally traded non-timber forest product in Africa (Cunningham et al. 2016). Consequently, the bark of the tree is a high source of income for local communities where the species is found and is used in traditional pharmacopoeia to treat diseases such as stomach aches, urinary, and bladder infections, among others (Betti 2008; Bii et al. 2010; Otieno and Analo 2012; Mwitari et al. 2013; Koros et al. 2016). The phytochemical compounds in the tree's bark are used to treat benign prostate hyperplasia and potentially treat prostate cancer (Komakech et al. 2017). The high demand for the bark has led to unsustainable exploitation of the *P. africana* population for several decades, leading to a threat to the species in its natural habitat. The species is listed as vulnerable in Appendix II of CITES (Cunningham et al. 2016). One of the key strategies to conserve the threatened *P. africana* is to facilitate its domestication. Therefore, knowledge of the occurrence, diversity, and interactions with its soil symbionts such as mycorrhizal fungi in natural habitats is crucial information for the cultivation of *P. africana* for ex situ regeneration.

Arbuscular mycorrhizal fungi (AMF) are symbiotic soil microorganisms belonging to the subphylum Glomeromycotina (Spatafora et al. 2016) present in almost all ecosystems and are associated with *c* 71% of terrestrial plants (Brundrett and Tedersoo 2018). Indeed, *c* 70 to 80% of the overall plant's inorganic phosphate (Pi), is provided by the fungus (Hoeksema et al. 2010). AMF species supply N and microelements like Zn, Fe, Mn, and Cu to their host (Adesemoye and Kloepper 2009). Besides nutritional function, AMF species can stimulate the plant's immune system (Cameron et al. 2013), and alleviate biotic stress like soil pathogens, and abiotic stress like drought and salinity of the host plant (Morte et al. 2001; Begum et al. 2019; Nanjundappa et al. 2019; Porter et al. 2019). In return, *c* 3 to 20% of the carbon (C) assimilated by the plant is allocated to the fungus (Bravo et al. 2017; Treseder et al. 2018). AMF species play a key role in ecosystem maintenance by shaping the plant community structure (Lin et al. 2015; Bauer et al. 2020). AMF species can modulate the soil structure and texture through their extraradical mycelium networks and contribute to soil stability (Rillig et al. 2010; Pellegrino et al. 2020). Despite the ecological importance of AMF species, very little is known about their community composition in African tropical forests, particularly in Afromontane forests. AMF species community composition and diversity vary from one ecosystem to another, mostly due to the variation of environmental factors and host species (Helgason et al. 1998; Lovelock et al. 2003; Opik et al. 2010; Rodríguez-Echeverría et al. 2017). In an earlier study, Wubet et al. (2004) indicated the presence of *Glomus*-dominated AMF types from roots of *P. africana* and spores obtained from trap cultures of indigenous soils from two dry Afromontane forests of Ethiopia. Previous studies have reported AMF species' effect on medicinal plant species' secondary metabolites. For instance, Almeida et al. (2018) recorded a variation of bioactive compounds in *Mikania laevigata* and *Mikania glomerata* (Asteraceae) when inoculated with *Rhizosphagus irregularis*. These authors observed an increase of diterpene kaurenoic acid in *M. laevigata* leaves whereas a decrease of tricaffeoylquinic was reported in *M. glomerata* with *R. irregularis* inoculum. Recently, Tchiechoua et al. (2020) showed that indigenous AMF species from the rhizosphere soil of *P. africana* were able to improve the growth and modulate the phytochemical compounds of vegetatively propagated *P. africana* plants in glasshouse conditions. Therefore, for the first time, we investigated the composition and structure of AMF species in the rhizosphere soil of *P. africana* found in four eco-climatically different Afromontane forests in Cameroon and Kenya, using Illumina MiSeq sequencing of the 18S rRNA gene. We also explored which edaphic factors could potentially affect AMF communities within these forests.

Results

Overall sequencing information and taxonomy composition

The rarefaction curves showed that the intensity of sampling for all soil samples was sufficient to identify the majority of AMF present in all four sites (Additional file 4). All data obtained from the Illumina sequencing were deposited in the Sequence Read Archive (SRA) at NCBI under accession number PRJNA657954.

A total of 16,736,209 trimmed reads obtained from 7 soil samples were clustered into 16,279 operational taxonomic units (OTUs) and assigned to the phylum Glomeromycota by using the MaarjAM database (Opik et al. 2010). Sample CT2 was discarded due to its low number of reads (16 reads). OTUs were grouped into eight families, namely Gigasporaceae (49.62%)-the most abundant across the samples, followed by Glomeraceae (37.3%), Claroideoglomeraceae (4.40%), Diversisporaceae (2.4%), Paraglomeraceae (2.15%), Acaulosporaceae (0.6%), Pacisporaceae (0.4%), and Archaeosporaceae (0.02%), while the rest (3.11%) were unclassified families (Fig. 2).

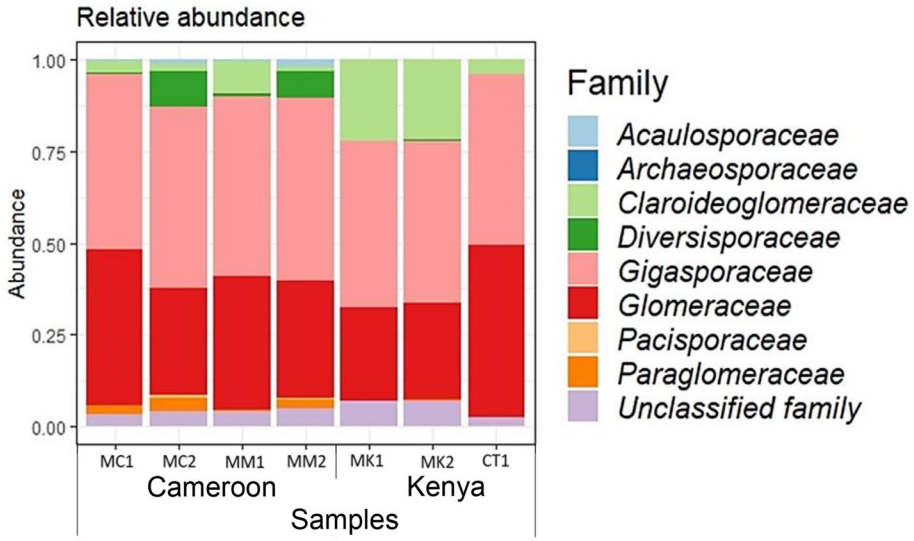

Fig. 1 Relative abundance of AMF families in the rhizosphere soil of *P. africana*

Diversisporaceae, Acaulosporaceae, Pacisporaceae, and Archaeosporaceae were unique to Cameroonian sites (MC and MM) (Fig. 1).

At the species level, 64 AMF virtual taxa (VT) were identified as belonging to the genera *Glomus* (43 VT; 67.1%), *Claroideoglomus* (6 VT; 9.4%), *Paraglomus* (5 VT; 7.8%), *Acaulospora* (4 VT; 6.2%), *Diversispora* (3 VT; 4.7%), *Archaeospora* (1 VT; 1.6%), *Pacispora* (1 VT; 1.6%), and *Scutellospora* (1 VT; 1.6%) (Fig. 1). *Diversispora* species were not detected in sample CT1 from the Chuka site. *Archaeospora* and *Pacispora* had one species each, detected in samples MC2, MM1, and MM2, all from the Cameroonian sites. *Scutellospora heterogama* (VTX00286) appeared to be the most common VT across all sample sites with the highest number of reads (Fig. 2, Additional file 1).

The four sites shared 39 VT (52% of the total number of different taxa identified) (Fig. 3). Cameroonian sites had the highest number of unique VT (27 VT from seven genera, namely *Glomus*, *Acaulospora*, *Archaeospora*, *Claroideoglomus*, *Diversispora*, *Pacispora,* and *Paraglomus*; (Additional file 2). Mount Cameroon (MC) had the highest number of unique species (10 AMF species), representing 13.3% of all species identified, whereas Chuka Tharaka-Nithi (CT) had one unique AMF species (Fig. 3).

AMF community diversity

Considering samples from Cameroon, the observed OTUs index in sample MM1 was the highest with 8195, whereas sample MC1 was the lowest with 3919. The highest observed OTUs in samples from Kenya were scored in MK1 with 5369 and the lowest in CT with 4079. However, there was no significant difference between samples from Cameroon and Kenya (Kruskal–Wallis test $P=0.7237$). Shannon index for richness species was higher in three of the four samples from Cameroon ($H_{MM2}=7.52$, $H_{MC2}=7.55$, and $H_{MM1}=7.56$) compared to the samples from Kenya ($H_{CT1}=6.88$, $H_{MK1}=7.21$, and $H_{MK2}=7.23$) (Fig. 4). Nevertheless, there was no significant difference between the Shannon indices of the two countries (Kruskal–Wallis test, $P=0.1573$). Evenness estimated using the Simpson index was highest in MM2, but with no significant difference between the provenances (Fig. 4).

The non-metric multidimensional scaling (NMDS) using the Bray–Curtis dissimilarity distance matrix showed that AMF species communities from Malava and Kakamega had more similarity to each other, while samples from Cameroon had less similarity (Fig. 5). The stress value of 0.014, indicates a good representation of ordinate.

Physicochemical parameters of the *P. africana* rhizosphere soil from Cameroon and Kenya

The physicochemical parameters of *P. africana* rhizosphere soil, collected in Cameroon and Kenya sites are shown in Table 1. The *P. africana* rhizosphere soils were acidic. Soil acidity was significantly higher in CT and MM than in MK and MC sites. The % C, total P (ppm), Na (ppm), and EC (mS/cm) were significantly higher in MC and MM (Cameroonian sites) than in MK and CT (Kenyan sites). In contrast, % N, and the Ca (ppm)

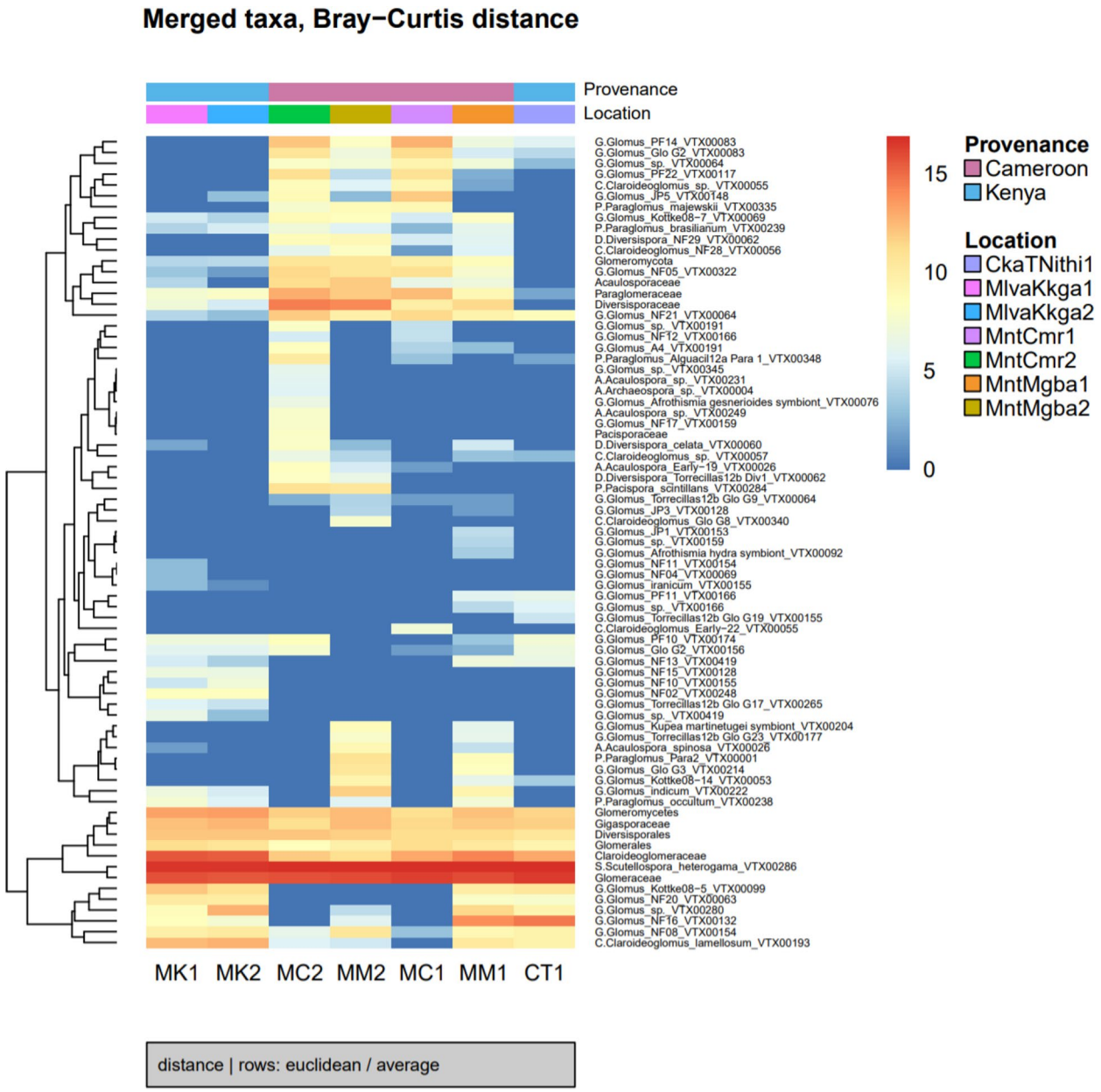

Fig. 2 Relative abundance of the 75 merged taxa identified in the rhizosphere soils of *P. africana*

content were significantly higher in MK and CT than in MC and MM sites.

Relationship between soil physicochemical properties and AMF communities

The physicochemical analyses of *P. africana* rhizosphere soil indicated that the available P concentration in samples from Cameroon was almost fivefold greater than in samples from Kenya, whereas the concentration of total N in samples from Kenya was significantly higher compared to samples from Cameroon (Table 1). However, there was a weak correlation between AMF species community composition and physicochemical parameters when the db-RDA analysis was performed on Bray–Curtis dissimilarity distance (Fig. 6). The eigenvalues of the first two axes of db-RDA were 0.09 and 0.02, and the first axis explained 79% whereas the second explained 19% of the variance in the AMF

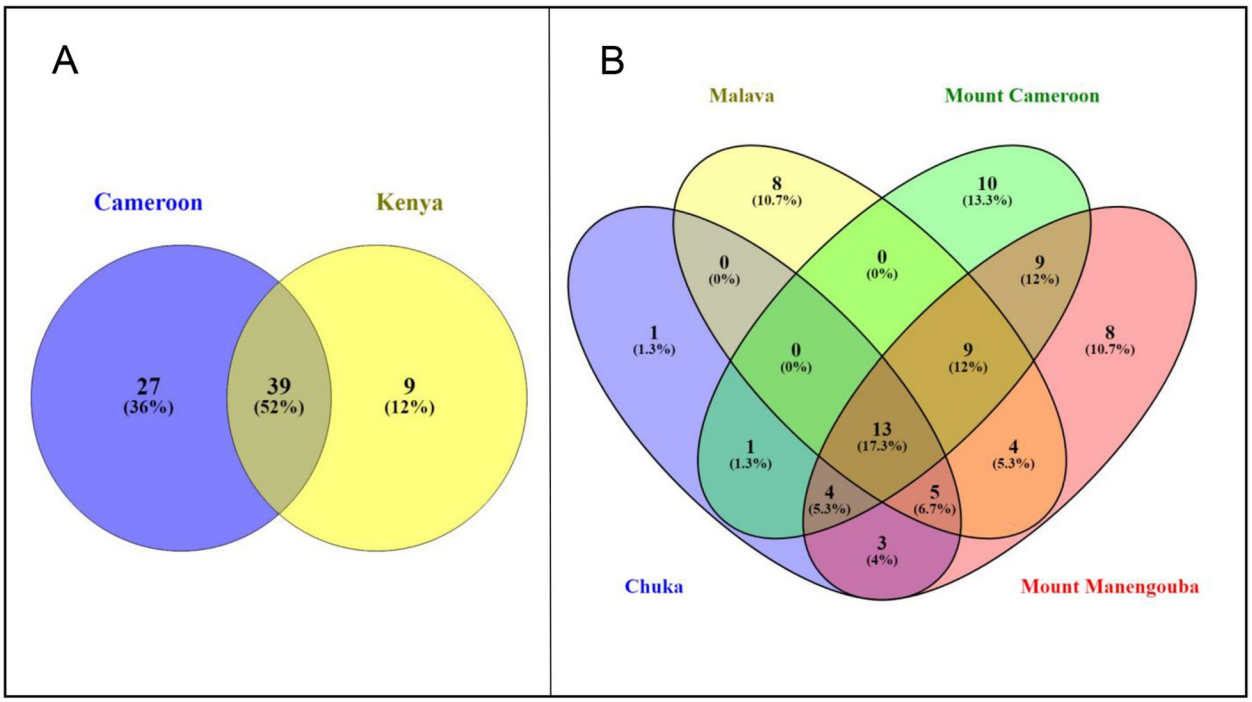

Fig. 3 Venn diagram of unique and shared merged taxa. **A** Unique and shared merged taxa between pooled Cameroon and Kenya samples. **B** Unique and shared merged taxa among the four sites Chuka, Malava, Mount Cameroon, and Mount Manengouba

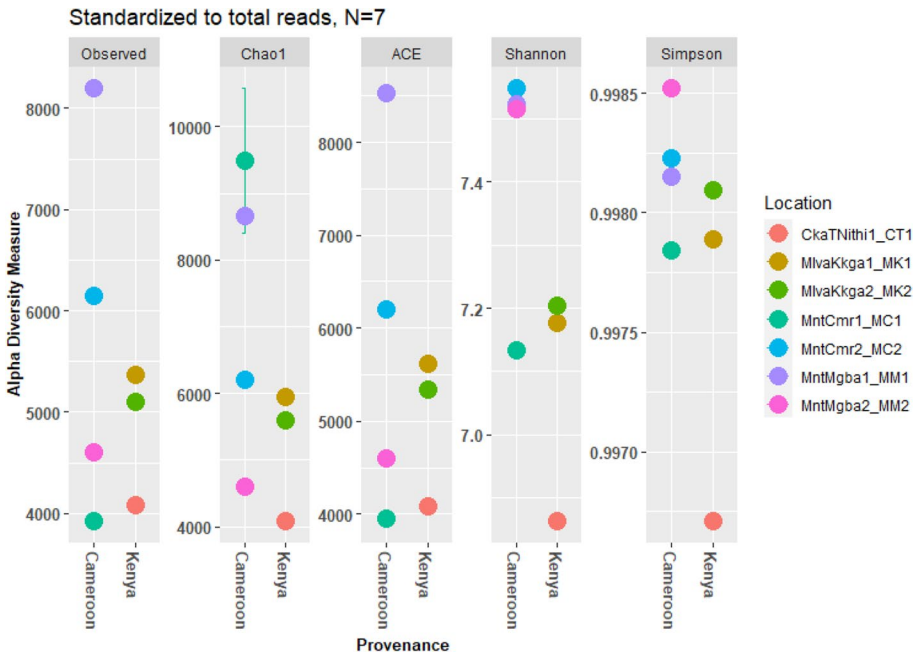

Fig. 4 AMF alpha diversity of AMF in the rhizosphere soils of *P. africana*. The observed OTUs, The Chao1, the ACE, the Shannon, and the Simpson indexes were considered

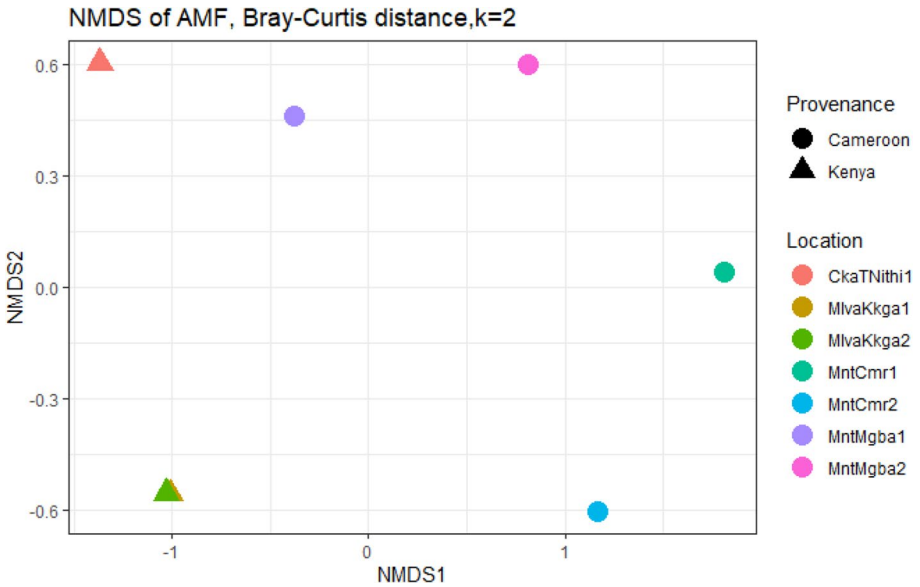

Fig. 5 Non-metric multidimensional scaling plot of AMF community composition in the rhizosphere soils of *P. africana*

Table 1 Physicochemical parameters of *P. africana* rhizosphere soil samples

Site code	pH (H_2O)	EC (mS/cm)	%C	%N	P (mg L^{-1})	K (mg L^{-1})	Na (mg L^{-1})	Ca (mg L^{-1})
MK	6.8a	0.14c	7.6c	0.78a	7.9c	564a	18c	5316a
CT	5.9b	0.12d	8.3c	0.83a	6.00d	555a	11d	4470b
MC	6.7a	0.26a	14.9a	0.57b	37.3a	255b	81a	4085d
MM	6.2b	0.22b	12.4b	0.66b	35.1b	565a	77b	4339c

Values followed by the same letter do not differ significantly according to the ANOVA test, $P<0.05$, $n=4$

species–physicochemical parameters relationship. The abundance of AMF species in samples from MK was positively correlated with the available P in the soil and negatively correlated with the total N and K. In addition, total N and K were positively correlated to the abundance of AMF species in MC and CT (Fig. 6).

Discussion

The present study aimed to assess the community composition and diversity of AMF in the rhizosphere soil of *P. africana* found in four fragmented Afromontane forests of Cameroon and Kenya, using Next Generation Sequencing technology. We obtained a total of 16,736,209 sequences, clustered into 16,279 OTUs, representing eight Glomeromycotina families (Gigasporaceae, Glomeraceae, Claroideoglomeraceae, Diversisporaceae, Paraglomeraceae, Acaulosporaceae, Pacisporaceae, and Archaeosporaceae), with a total of 64 virtual taxa (VT). Our study shows a high species richness compared to an earlier study of AMF associated with *P. africana* in dry Afromontane forests of Ethiopia using low-throughput sequencing and internal transcribed spacer (ITS) as a marker gene method on roots and trap cultures (Wubet et al. 2004). They identified taxa belonging only to three AMF families: Glomeraceae, Diversisporaceae, and Archaeosporaceae (Wubet et al. 2004). However, the difference in the detection of AMF associated with *P. africana* is also likely due to the methods used: high- vs low-throughput sequencing, and rhizosphere soil vs root and spores from trap cultures. In our study, we observed that the Gigasporaceae family had the highest number of OTUs, with the only VT identified to species level (*Scutellospora heterogama* VTX00286) found in all four sites. Members of Gigasporaceae are known to be nearly as dominant as those of Glomeraceae in Afrotropical biomes and are reported to be overrepresented in the tropics biogeography database (Stürmer et al. 2018). At the species level, several studies using low-throughput (Moreira et al. 2006; Lakshmipathy et al. 2012; Wetzel et al. 2014; Pereira et al. 2020) to high-throughput (Schlaeppi et al. 2016;

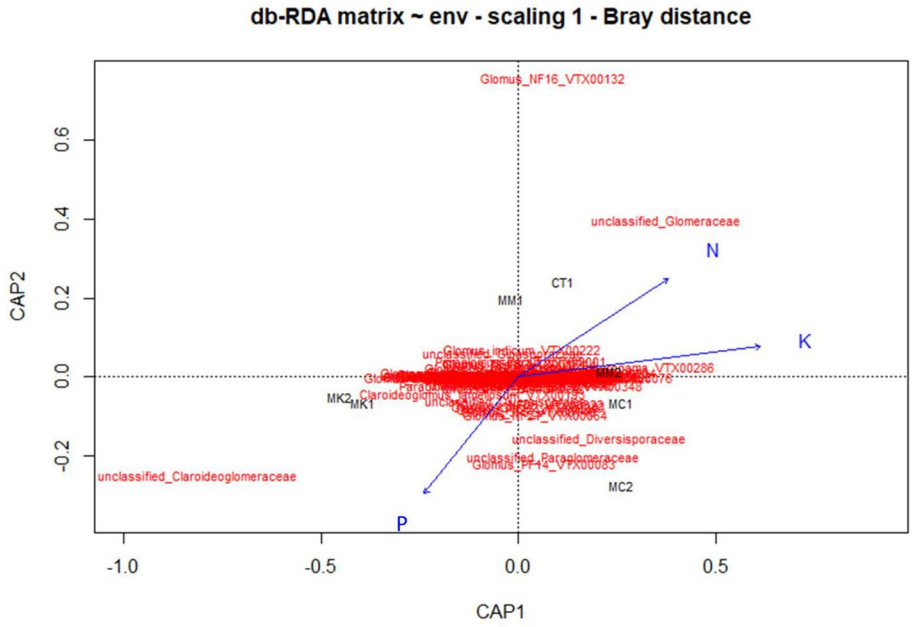

Fig. 6 Relationship between soil physicochemical parameters and AMF community in the rhizosphere soil of *P. africana*

Egan et al. 2018; Marinho et al. 2019) have revealed the presence of *Scutellospora* species (Gigasporaceae) in samples from tropical environments. Contrary to findings from these studies, Opik et al. (2010) found that the Gigasporaceae family was underrepresented in Africa. However, in our study, the only representative of the Gigasporaceae family represented 6% of the total VT. In contrast, the Glomeraceae family had 43 VT, representing 67.1% of the taxa identified. This is not surprising since numerous studies have shown that members of the Glomeraceae family are ubiquitous and cosmopolitan, found in nearly all continents and climatic zones (Opik et al. 2010; Stürmer et al. 2018; Lara-Pérez et al. 2020).

Although we did not observe a significant difference in AMF in terms of alpha diversity indices, AMF diversity was generally higher in Cameroonian than in Kenyan rhizosphere soil samples. Similarly, the Venn diagrams (Fig. 4) showed that Cameroonian samples had more unique AMF species, representing 36% of the total VT, whereas Kenyan samples only had 12% of the total VT. On the other hand, the beta diversity showed a distinct community composition between the samples from different country provenances, suggesting a strong influence of local biotic and abiotic factors as reported for other studies in the tropics (Alguacil et al. 2015; Stevens et al. 2020).

Various biotic and abiotic factors can explain the differences in the diversity indices and community structure of AMF. Zhao et al. (2017) showed that soil clay content was negatively correlated with the AMF diversity in a semi-arid mountain in China, while Vieira et al. (2020) indicated that higher soil clay content was found with less AMF richness in soil samples in a tropical semi-arid region of Brazil. In this study, we found differences in a few physicochemical properties among sites, and are likely to have influenced local the diversity and structure of AMF species communities in these Afromontane sites. For instance, the application of db-RDA using Bray–Curtis distance dissimilarity showed that available P, the total N, and the K content were correlated with the AMF community structure. In contrast, soil pH did not appear to be one of the factors shaping the AMF composition at any of the sites investigated. A similar observation was made by Abdedaiem et al. (2020), even though soil pH has previously been shown to be one of the main factors besides available P that influence the AMF community composition (Bainard et al. 2014; Trevizan Chiomento et al. 2019). Previous studies found a negative correlation between available P and AMF community composition diversity (Bainard et al. 2014; Abdedaiem et al. 2020). Besides, none of the soil parameters measured significantly impacted the AMF community composition in the rhizosphere soil of *P. africana*. Similar observations were made in a study by Manoharan et al. (2017) that

investigated AMF species diversity under the influence of agricultural management practices. Our results suggest that soil physicochemical parameters and other factors, including climate, should be considered when studying the AMF community composition and diversity in rhizosphere soil. For instance, the geographical distance was also identified as one of the main factors influencing the AMF community composition and diversity when considering aspects like climate, soil type, land use type, and vegetation type (Xu et al. 2016; Hontoria et al. 2019; Huang et al. 2019). Therefore, the differences we observed in the community composition of AMF species might also be attributed to other local factors yet to be established, than only to the physicochemical composition of the rhizosphere soil of *P. africana* in these forests.

Conclusions

Using 18S Metagenomics in this study revealed a higher diversity and distinct composition of AMF communities associated with *P. africana* than in previous studies. Our data showed that the AMF communities in Cameroon sites had a higher VT diversity than those in Kenyan sites. Moreover, *Scutellospora heterogama* VTX00286 was identified as the most common and abundant VT, but there were also other common and unique VT across all the sites studied. Our findings also showed that correlation between AMF species composition and physicochemical parameters, especially N, P, and K. This study has provided insights into the AMF communities and species found in the rhizosphere of *P. africana*, and will be crucial in informing the development of culturable inoculum to support propagation and conservation work in these forests.

Methods

Study sites

The study was carried out in four Afromontane sites in Cameroon and Kenya (Fig. 7). In Cameroon, the two targeted sites were Mount Cameroon (MC) and Mount Manengouba (MM), 120 km from each other. The MC site is in the South-West Region of Cameroon at 04°08′34.3″ N; 09°07′21.0″ E, 2280 m above sea level (masl). The annual rainfall in MC is one of the highest in the world ranges between 1800 mm and 12,000 mm with two seasons; the dry season from November to February and the rainy season from March to October. The mean temperature is 25 °C and the relative humidity ranges between 75 and 80% throughout the year (Fonge et al. 2019). The vegetation in MC is mainly composed of a dense layer of graminaceous species and tall herbaceous species belonging to the Acanthaceae and Begoniaceae families. The main tree species include *Agauria* sp. (Ericaceae), *Crassocephalum mannii* (Asteraceae), *Hypericum* sp. (Hypericaceae), and a less dense population of *P. africana* (Nkeng et al. 2010). The MM site is located between the Littoral and South-West Regions at 05°01′50.8″ N; 09°49′31.7″ E, 1968 masl. The MM site is dominated by dense undergrowth, including *Aframomum* sp., Acanthaceae, Brillantaceae, Begoniaceae, and Gramineae family, while the main trees species are *Harungana madagascariensis* (Hypericaceae), *Maesa lanceolata* (Primulaceae), *Polyscias fulva* (Araliaceae), and *P. africana* (Nkeng et al. 2010). Both MC and MM sites have volcanic soil types (Andosol) that vary from loam to silt loam, and the top layer is rich in organic matter (Manga et al. 2014; Tegha and Yinda 2016). Both mountains are located on the Cameroon Volcanic Line magmatism (De Plaen et al. 2014). In Kenya, the two targeted sites were the Chuka forest (CT) and Malava forest (MK) separated by approximately 320 km. The CK site is located near Chuka Tharaka-Nithi in the Central Province of Kenya at 0°17′45.57″ N, 37°36′52.85″ E, 1620 masl. The annual rainfall is bimodal and ranges from 1500 to 2500 mm. The vegetation in the CT site is dominated by a range of species, including *Podocarpus latifolia* (Podocarpaceae), *Artemisia afra* (Asteraceae), *Croton macrostachus* (Euphorbiaceae), and *P. africana* among others. The soils are red with high clay content, and Nitisols, Cambisols, and Andosols are the main soil groups (Kaburi and Medley 2011; Mugo 2015). The MK Forest is located in Kakamega in the Western province at 0°27′57.57″ N, 34°52′8.55″ E, 1615 masl. The annual temperature in the region is about 25 °C with a hot and wet climate, and annual rainfall ranges between 1500 and 2000 mm (Seswa et al. 2018). The dominant species include *P. africana* and several other species belonging to Euphorbiaceae, Moraceae, Rubiaceae, Acanthaceae, Fabaceae, and Bignoniaceae (Additional file 1) (Seswa et al. 2018).

Soil sampling and analysis

Soil sample collection

In each of the four sites (Mount Cameroon: MC; Mount Manengouba: MM; Chuka Tharaka-Nithi: CT; Malava Kakamega: MK) (Additional file 4), 20 mature *P. africana* trees (≥ 30 cm dbh) were randomly selected. Rhizosphere soil samples were collected from each tree at four cardinal points by following the roots from the trunk to the fine roots, and at about 20 cm from the trunk. Each sample consisted of approximately 50 g of soil collected aseptically at a depth of 30 cm, after removing the surface soil litter. The samples from each tree were then pooled and thoroughly mixed to form a composite sample of 200 g

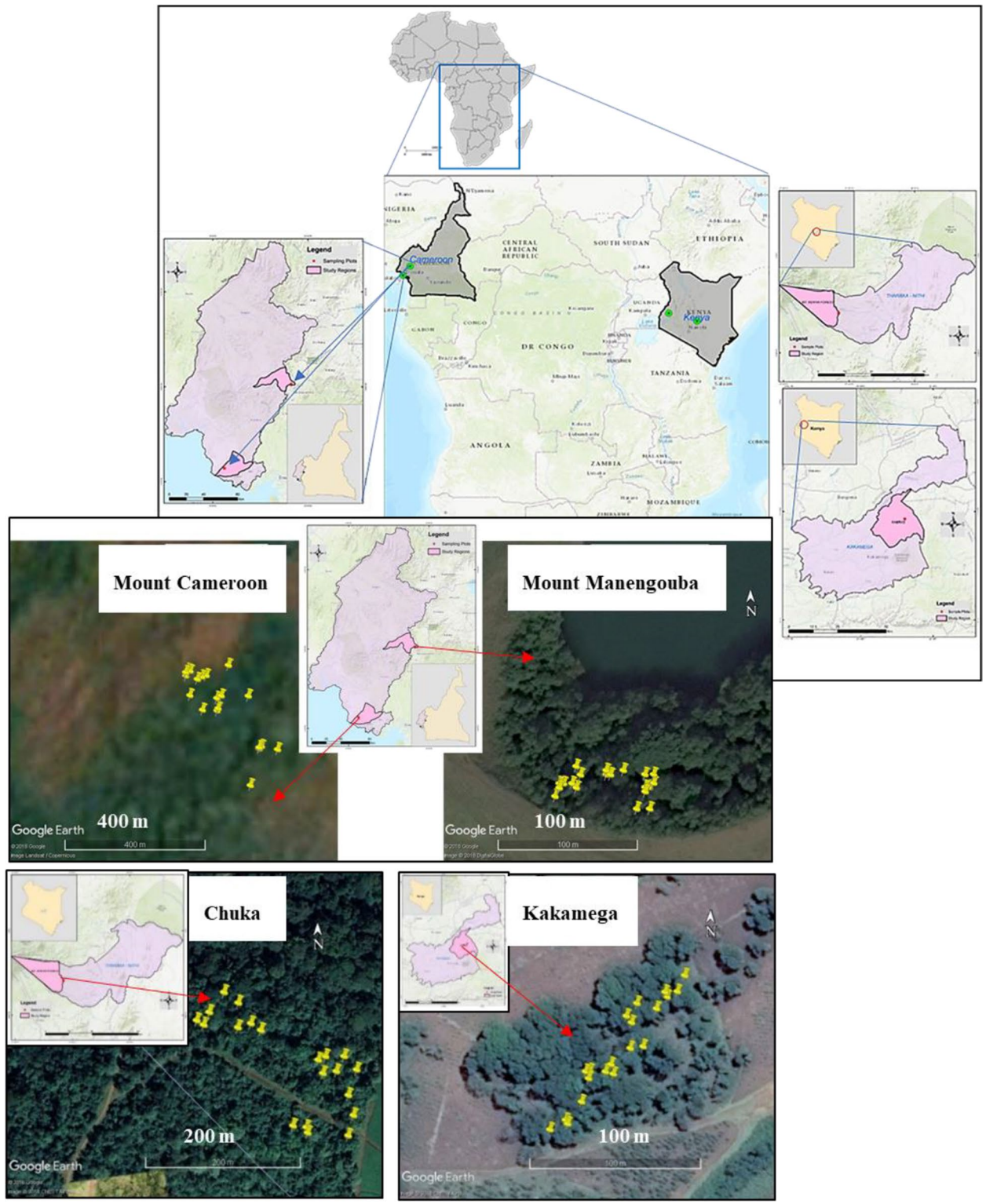

Fig. 7 Sampling sites where rhizosphere soils of *P. africana* were collected. The yellow pins indicate all the sampling points in this study

per tree. Subsequently, the composite samples from each tree were further pooled and thoroughly to form a composite sample of 4 kg per site. For soil chemical analyses, a subsample of 500 g from each composite sample was air-dried at ambient temperature. The remaining subsamples were taken to the laboratory and stored at 4 °C for further analyses.

Soil chemical analyses

Soil samples were analyzed to determine the pH (H_2O) at a soil: water ratio of 1:2.5, electrical conductivity (EC), percentage of carbon (% C), total nitrogen (% N), available phosphorus (P), potassium (K), sodium (Na), and calcium (Ca), present in the soil samples at the Kenya Forestry Research Institute (KEFRI) soil analysis laboratory as described by Odee et al. (2002).

Soil DNA extraction

For each composite soil sample stored at 4°C, 100 g was ground using a mortar and pestle. A duplicate subsample of 0.25 g of the ground soil was used to extract gDNA using the DNeasy PowerSoil kit following the supplier's recommendation (Qiagen, Hilden, Germany). Quantification and quality control of extracted DNA was done using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific, USA) and 0.8% agarose gel electrophoresis. The concentration of extracted DNA was normalized to 25 ng/µl in a total volume of 50 µl. Amplification of the small subunit of the rDNA (SSU rDNA) region was conducted using the primer pairs AML1/AML2 known to have better specificity and coverage for AMF diversity (Lee et al. 2008). However, previous studies have mentioned that non-target amplification PCR reaction was done using the Taq PCR Kit (New England, BioLabs® Inc, USA), in a reaction volume of 50 µl, containing 5 µl of 10X Standard Taq Reaction Buffer, 1 µl of 10 mM dNTPs, 1 µl of 10 µM AML1 (forward), 1 µl of 10 µM AML2 (reverse), 5 µl of 25 ng/µl DNA template, and 0.5 µl of 0.5 U Taq DNA polymerase. Thermocycling conditions were as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min, and a final extension step at 72 °C for 10 min.

Library preparation and Illumina MiSeq sequencing

Before library preparation, PCR products were purified using the QIAquick® PCR purification kit (Qiagen, USA). Quantification was done using NanoDrop™ 2000 spectrophotometer (Thermo Scientific, USA) and the PCR purified products were normalized to 30 ng/µl. Amplicons were further diluted to 0.5 ng/µl using nuclease-free water and quantified by Qubit® fluorometry (Thermo Scientific, USA), and used for library preparation. The library construction was done using Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, USA) following the manufacturer's protocol. This protocol started with an enzymatic reaction called tagmentation where the enzyme transposase fragmented and tagged the amplicon samples with adapters in random positions. The sequencing indices were added to the fragmented amplicons through a PCR reaction of 12 cycles. This step was followed by amplicon purification and size selection using AMPure® XP beads (Beckman Coulter, USA) technology. The products were quantified using Qubit® fluorometry with the dsDNA HS (High Sensitivity) Assay kit (Thermo Scientific, USA), and the quality and size were checked using the Agilent TapeStation 2000 system (Agilent Technologies, USA). The quantified and sized amplicons were normalized, pooled at equimolar concentrations, and then submitted for sequencing to Illumina MiSeq 300PE reads.

Bioinformatics and statistical analyses

All bioinformatics analyses were performed using the Quantitative Insights Into Microbial Ecology (QIIME2) version 2019.10 (https://docs.qiime2.org/2019.10/) (Caporaso et al. 2010). The FASTQ files containing the forward and the reverse reads obtained were first checked for quality control using FastQC v0.11.7 (Andrews 2010). The sequences were then demultiplexed to assign each read to a specific sample using q2-demux, and then, the sequences were denoised and trimmed to retain only bases with a quality score greater than 25. The sequences were then truncated from the 3′ end of the forward and the reverse sequences at 240 and 200 bp, respectively, using the qiime deblur plugging pipeline (Amir et al. 2017). Sample CT2 was discarded due to the low number of sequence reads (< 1000). Picking and clustering of the operational taxonomic units (OTUs) were done using the Silva database (https://www.arb-silva.de/) and the taxonomy assignment was done using MaarjAM 5 database (Opik et al. 2010)(http://maarjam.botany.ut.ee/) as a classifier in QIIME2 q2-feature-classifier plugin with 97% similarity (Bokulich et al. 2018). The features table also known as the OTUs table and the taxonomy table generated from QIIME2 were extracted and imported into R 4.0 (R Core Team 2020) as phyloseq objects for downstream analyses. All statistical analyses were performed using the R software version 4.0 (R Core Team 2020) unless otherwise specified. To estimate the AMF OTUs diversity among the samples from Cameroon

and Kenya separately, the count reads were transformed and normalized into relative abundance (Lemos et al. 2011) using the "filter.taxa()" function of the phyloseq package (version 1.32.0) in R software (McMurdie and Holmes 2013). The significant difference between the alpha diversity metrics of AMF taxa from Cameroon and Kenya was calculated using the Kruskal–Wallis test. The beta diversity was carried out to compare the AMF community between the different samples using the non-metric multidimensional scaling (NMDS), based on the Bray–Curtis dissimilarity distance matrix with the function "metaMDS()" of the vegan package (version 2.5–6). The dissimilarity matrices were then plotted on the principal coordinate. The relationship between the main soil physicochemical parameters and the AMF community composition was tested using the distance-based redundancy analysis (db-RDA) (Legendre and Andersson 1999), applying the function "capscale" of the vegan package version 2.5–6 (Oksanen et al. 2019) in R (version 4.0). A Venn diagram analysis was conducted to identify unique and shared AMF communities among the samples and provenance in the online interactive tool Venny (https://bioinfogp.cnb.csic.es/tools/venny/).

Abbreviations

AMF	Arbuscular mycorrhizal fungal
VT	Virtual taxa
OTUs	Operational taxonomy units
db-RDA	Distance-based redundancy analysis
NMDS	Non-metric multidimensional scaling
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
IUCN	International Union for Conservation of Nature
MM1	Mount Manengouba1
MM2	Mount Manengouba2
MC1	Mount Cameroon1
MC2	Mount Cameroon2
MK1	Malava Kakamega1
MK2	Malava Kakamega2
CT1	Chuka Tharakanithi1
CT2	Chuka Tharakanithi12

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-023-01720-z.

Additional file 1. Features abundance per sample.

Additional file 2. Unique and shared top representative AMF taxa in the rhizosphere soil of *Prunus africana*.

Additional file 3. Environmental characteristics of sampling sites.

Additional file 4. Rarefaction curve of the AMF community in the rhizosphere soils of *P. africana*. MM1: Mount Manengouba1; MM2: Mount Manengouba2; MC1: Mount Cameroon1; MC2: Mount Cameroon2; MK1: Malava Kakamega1; MK2: Malava Kakamega2; CT1: Chuka Tharakanithi1. The cut-off (vertical).

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Authors' contributions

YT, DWO, EN, ML, JK, VN, and RP conceptualized the study. YT, EM, and RP collected data. YT and BW performed the data analysis. YT, DWO, and RP interpreted the data. YT wrote the initial draft. All the authors contributed to the final manuscript and approved the submitted version.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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