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ORIGINAL ARTICLE

Improved genotypes and fertilizers, not fallow duration, increase cassava yields without compromising arbuscular mycorrhizal fungus richness or diversity

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Abstract

Arbuscular mycorrhizal fungi (AMF) are ubiquitous in agroecosystems, but their role in mediating agricultural yield remains contested. Field experiments testing effects of realistic agronomic practices of intensification on AM fungus composition and yields are scarce, especially in the low-input systems of sub-Saharan Africa. A large, full-factorial field experiment was conducted in South-Kivu (DR Congo), testing effects of *fallow* duration (6 vs. 12 months), *genotype* (landrace vs. improved), and *fertilizer* management (control vs. five combinations omitting N, P, K, and/or secondary macro- and micronutrients) on yields of cassava, an important staple crop strongly colonized by AMF. Furthermore, we used DNA-metabarcoding to evaluate effects of these agronomic practices on the AM fungal communities on the roots. The shorter *fallow* duration strongly increased diversity and richness of AMF, but this did not correspond with increased yields. Cassava yield was mainly determined by *genotype*, being largest for the improved genotype, which coincided with a significantly higher sum of AM fungal sequences. Effects of *fertilizer* or *genotype* on community composition were minor to absent. We found no evidence that increased AMF richness and diversity enhanced cassava yields. In contrast, the use of the improved genotype and mineral fertilizers strongly benefitted yields, without compromising richness or diversity of AMF. Cassava-AMF associations in this work appear robust to fertilizer amendments and modern genotype improvement.

Keywords Arbuscular mycorrhizal fungi · *Manihot esculenta* Crantz (Cassava) · Fallow duration · Genotype · Next-generation sequencing · Nutrient management

Introduction

Due to their ubiquitous presence in agroecosystems and the benefits they provide to host plants and the soil environment, arbuscular mycorrhizal fungi (AMF) are often stated to play a critical role in agricultural systems (van der Heijden et al. 1998; Rillig and Mummey 2006; Smith and Read 2008; Zhang et al. 2019).

However, the effects of combinations of real agronomic practices on AMF community composition are poorly known. In this regard, recent reviews have found that AMF may be more robust to common agricultural practices (e.g., domestication, crop breeding, fertilizer amendment) than commonly thought (Martín-Robles et al. 2018; Ryan and Graham 2018).

Additionally, the extent to which the abundance and diversity of AMF affect crop yield and agroecosystem sustainability is contested (Martín-Robles et al. 2018; Ryan and Graham 2018). The question arises whether AMF should be at the center of farm management decisions or whether farm management should be based on strong systems agronomy, without maximization of AMF as a goal (Ryan et al. 2019). While Verbruggen and Kiers (2010) argued that agricultural practices disfavor the development

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of a more complex and functional (i.e., more beneficial) mycorrhizal fungal community, yield penalties after reductions of richness or diversity of AMF by agricultural practices of intensification rarely have been reported in the literature, and such reports may be ambiguous (Ryan and Graham 2018). Therefore, the question of whether or not farmers should modify their management practices to enhance the abundance and diversity of AMF in the field has not been solved, as benefits on yields are often absent (Ryan and Graham 2018; Ryan et al. 2019). Indeed, relationships between AM fungal colonization and yield have been reported to be highly context dependent (Lekberg and Koide 2005; Treseder 2013; Thirkell et al. 2017), suggesting that AM fungal colonization depends on multiple aspects of farm management. Unraveling the intraspecific interactions of particular crop genotypes with AM fungal species and environment by determining the driving factors of mycorrhizal responses and associations on the field therefore have been identified as "high-priority" research (Ryan and Graham 2018; Rillig et al. 2019). This requires proper field experiments in real agroecosystems, evaluating the effects of agronomic practices on indigenous AM fungal colonization and respective relations with yields. Most available data so far originate from high-yielding agricultural systems in Europe, North America, China, and Australia (Ryan and Graham 2018) but data are especially needed from the low-input systems of sub-Saharan Africa where AMF may have their highest functionality (Rillig et al. 2019). Overall, and in order to precisely identify the various roles played by AMF in real agricultural low-input systems, there is an urgent need for field experiments evaluating (i) effects of realistic agronomic practices of intensification on the abundance and community composition of AMF and (ii) effects of AM fungal diversity and abundance on yields; all this using state-of-the-art analytical tools such as high throughput sequencing (Lekberg and Helgason 2018; Ryan and Graham 2018; Rillig et al. 2019).

Cassava (*Manihot esculenta* Crantz) currently serves as a major food source in multiple regions of the world, and its importance is rapidly increasing (Balagopalan 2002; Burns et al. 2010; Dada et al. 2010) as it has high potential productivity on nutrient poor and dry soils. Consequently, it has been pointed out as a potential crop to mitigate the adverse effects of climate change (i.e., excessive heat and drought spells) on food security and to have the potential of adding economic value to marginal and dry areas. In this context, it has been argued that there still is a high potential to further increase cassava production under such harsh conditions (Fermont et al. 2009; De Souza et al. 2017; Kintché et al. 2017). To this end, agronomic management practices can buffer effects of climate change, improve soil fertility, and reduce the pressure of pests and diseases. Most common management strategies for cassava farmers today include the selection of improved genotypes, nutrient application, and fallow (Munyahali et al. 2017; Pypers et al. 2011; Séry et al. 2016; Straker et al. 2010). In tropical Africa, long fallow durations were historically implemented to allow soil and land restoration; however, population pressure, land scarcity, and intensification currently lead to shortened fallow durations.

Interestingly, cassava is known to be highly colonized by AMF, and it has been argued that its growth and survival strongly depend on this symbiosis (Howeler et al. 1982; Habte and Byappanahalli 1994; Rodriguez and Sanders 2014). Some studies also have shown that mycorrhizal inoculations can improve cassava yields (Sieverding and Howeler 1985; Osonubi et al. 1995; Salami et al. 2005; Carretero et al. 2009; Ceballos et al. 2013; Rodriguez and Sanders 2014; Séry et al. 2016). Despite these positive results, however, large-scale in situ AMF inoculation (Hart et al. 2018) remains impractical and uneconomic for subsistence farmers in low input systems.

Hence, this study aimed to investigate:

1. The effects of (combinations of) real agronomic practices of intensification on the naturally occurring communities of AMF on cassava roots. These practices included cassava *genotype* (a landrace versus an improved genotype), *fertilizer* management (nutrient omissions of NPK and secondary macro- and micronutrients), and *fallow* duration (12 versus 6 months)
2. The effects of different combinations of agronomic practices on cassava yields and the respective relations with the AMF communities

To this end, a large, full-factorial field experiment (72 plots, 288 plant samples) was conducted in a split-plot design, located in South-Kivu (DR Congo).

Materials and methods

Site description

The experiment was established in the Kalehe territory of South-Kivu province (2°03′22.9″S 28°54′15.3″E) and represents realistic combinations of agronomic practices of intensification in a smallholder farming system. The area has a long history of cassava cultivation, and the field site was considered as being homogeneous in terms of previous land use history and land management. The primary forest in the area of this plot had been cut during colonial times to be replaced by cinchona trees (*Cinchona* sp.) and food crops like corn, beans, cassava, and colocasia or taro. Since then, local landraces of cassava became dominant in

Table 1 Site and initial soil characteristics of the field experiment

Village	Coordinates	Altitude	Field Area	pH	Olsen P	Organic Matter	Organic Carbon	Texture			P_{ox}	Fe_{ox}	Al_{ox}
		[m a.s.l.]	[Ha]	H_2O	[mg kg^{-1}]	[%]	[%]	[%sand]	[%silt]	[%clay]	[mg kg^{-1}]	[mg kg^{-1}]	[mg kg^{-1}]
Muhongoza	2°03′22.9″S 28°54′15.3″E	1465	0.82	6.6	26.8	4.1	2.4	71.8	16.0	12.2	313	2086	1268

X_{ox} oxalate extracted

the region, and this plot was used for cassava cultivation before the experiment. The site was never amended with fertilizers; soil information is presented in Table 1.

Experimental design

After the previous cassava harvest, the land remained fallow without human intervention for 6 or 12 months prior to the trial establishment. The vegetation grown during the fallow period was dominated by weeds comprising *Commelina* sp. (60% abundance), *Bidens pilosa* (20% abundance), *Galisonga* sp. (5% abundance), and other weeds scattered with residual taro and Colocasia sprouts. After manual mowing and plowing, a three-way factorial field experiment was established in a split-plot design with the factor "*fallow* duration" assigned to the main plots. This factor included two levels: (i) a short fallow duration of 6 months being replicated in two main plots (meaning that the previous cassava crop was harvested on 2 May 2018 and left fallow for 6 months until the start of this experiment) and (ii) a long fallow duration of 12 months replicated in four main plots (meaning that the previous cassava crop was harvested on 2 November 2017 and left fallow for 12 months until the start of this experiment).

Combinations of the factors "*genotype*" (two levels) and "*fertilizer application*" (six levels) were assigned to the sub-plots that were randomly distributed within each main plot. Replicate blocks and main plots were separated by 2 m, while sub-plots (7 m × 10 m) were separated by 1.5 m.

Clean cuttings of two contrasting cassava genotypes were planted (2 November 2018) in the sub-plots with spacing of 1 × 1 m according to the local density recommendations, and each sub-plot included 56 plants (8 × 7). One popular local landrace (i.e., M'Bailo or Bailo) was chosen as it was grown in the area since at least the 1950s. An improved genotype (i.e., Obama/TME 419) was also selected, and this genotype has never been cultivated in the area (origin: IITA-Ibadan). The latter genotype is known for its drought tolerance and resistance against cassava mosaic virus (CMD), and it has a high yield potential. Plots depended on natural rainfall, which was monitored daily. The cumulative rainfall during the experiment reached 2222 mm, equally distributed over 12 months (Table S1, Supplementary Information).

Six fertilizer treatments were imposed: (i) a control without nutrients applied (Ctrl); (ii) N omitted with P and K applied (PK); (iii) P omitted with N and K applied (NK); (iv) K omitted with N and P applied (NP); (v) N, P, and K applied (NPK); and (vi) a treatment in which secondary macronutrients (S, Ca, and Mg) and micronutrients (Zn and B) were applied in addition to N, P, and K (NPK +).

Fertilizer rates were based on the standard recommendations formulated for cassava in the region and were concentrically applied on the soil at a distance of 10 cm from

the plant base. Primary macronutrients were applied at 150 kg N ha^{-1}, 40 kg P ha^{-1}, and 180 kg K ha^{-1}. The secondary macro- and micro-nutrients were applied at rates of 16.6 kg S ha^{-1}, 10 kg Ca ha^{-1}, 10 kg Mg ha^{-1}, 5 kg Zn ha^{-1}, and 5 kg B ha^{-1}. Nitrogen (N) was applied as urea in three splits (30:35:35) at 1, 3, and 5 months after planting (MAP). Phosphorus (P) was applied as triple super phosphate once right after planting. Potassium (K) was applied as muriate of potash and split over three applications (30:35:35) at 1, 3, and 5-MAP. Sulfur, magnesium, and zinc were applied as $CaSO_4$, $MgSO_4$, and $ZnSO_4$, while calcium was additionally applied as $CaCO_3$. Boron was applied as Borax. All secondary and micronutrients were applied once at the first MAP. The field was manually weeded at a frequency of 4 weeks, and no pests or diseases were reported during the experiment.

Sampling of soil and roots

At 12 MAP (2 November 2019), the cassava plants were harvested. Within each plot, four randomly selected plants were carefully excavated from the soil, excluding border rows. From each of these four plants, the fine roots were sampled from each side of the root system. These fine root samples were then pooled per plant and stored in silica gel. One composite soil sample (0–15 cm depth) of the whole field was collected before the start of the experiment and analyzed for pH, Olsen P, texture, organic matter, and oxalate extractable P, Fe, and Al. The fresh root yield was weighed based on the "useful plot", size including all plants in the plot minus the border plants, resulting in a total of 30 plants per plot (56–26 = 30). The above ground biomass and the number of roots per plant were extrapolated from 5 randomly selected plants per plot.

DNA extraction, PCR amplification, and Illumina sequencing

Exactly 70-mg dried roots from the pooled root samples per plant were cut into small 1-cm pieces. Roots were then homogenized in a Bead Mill Homogenizer (Omni International) for two times 30 s at 8 m/s. Genomic DNA was extracted from each sample (288 samples) using a Soil DNA Isolation Kit according to the manufacturer's instructions (Norgen Biotek Corp., Thorold, ON, Canada). Subsequently, the obtained DNA was diluted 5 times prior to PCR amplification. PCR amplification was performed targeting the small subunit (SSU) region of the ribosomal RNA gene using sample-specific barcoded primers of the *glomeromycotinan*-specific primer pair AMV4.5NF-AMDGR dual-index sequencing strategy (Kozich et al. 2013) which is AMF specific (Sato et al. 2005; Van Geel et al. 2014). PCR reactions were performed on a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, CA, USA) in a reaction volume of 25 µL, containing 1 µL of genomic DNA, 0.5 µL of each 20 µM primer, 5 µL ALLin HiFi Buffer, 0.3 µL ALLin HiFi DNA Polymerase (2 u/µL (highQu)), and 17.7 µL of PCR water. Before amplification, DNA samples were denatured at 95 °C for 1 min. Next, 30 cycles were run, consisting of 15 s at 95 °C, 15 s at 53 °C, and 11 s at 72 °C. Amplicons within the appropriate size range were purified using the Agencourt AMPure XP kit (Beckman Coulter Life Sciences, Indianapolis, IN, USA). Purified dsDNA amplicons were quantified using the Qubit dsDNA HS assay kit and the Qubit fluorometer (both from Invitrogen, Carlsbad, CA, USA). Subsequently, samples were pooled in equimolar concentrations, and the pooled amplicon library was loaded on an agarose gel. The final amplicon library of 350 bp was cut and purified again using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The purified library was then diluted to 2 nM and sequenced at Genomics Core (Gasthuisberg, Leuven, Belgium) on an Illumina Miseq platform with v2 500 cycle reagent kit (Illumina, San Diego, CA, USA).

Bioinformatics

Sequences obtained from the Illumina sequencing run were assembled, oriented, quality filtered, and clustered into operational taxonomic units (OTUs) using the USEARCH sequence analysis tool, following the recommended pipeline (Edgar 2013). First, the "*fastq_mergepairs*" command was used to merge forward and reverse reads. Second, correct orientation of the sequences was ensured using the the "*orient*" command, using a curated sequence database: the MaarjAM database (Öpik et al. 2010). Quality filtering of the reads was then performed with the "*fastq_filter*" command, allowing a maximum expected error of 1.0 for the individual sequences. To maximize the number and length of retained sequences, truncation length was set to 200 bp. Next, the sequences were dereplicated and sorted by abundance. Sequences were then clustered into operational taxonomic units (OTUs) defined at 97% sequence similarity, which is commonly used to define SSU-based OTUs among AMF (Lumini et al. 2009; Öpik et al. 2010), using the "*cluster_otus*" command. In this step, chimeric OTUs built from abundant reads were discarded. To remove possible erroneous sequences produced during PCR or sequencing (Alberdi et al. 2018), we removed per sample the OTUs that were represented by fewer than 0.01% of the sequences of that sample. The glomeromycotinan SSU dataset was then BLASTed against the MaarjAM database in order to assign taxonomy to each of the OTUs (Öpik et al. 2010). Unidentified or

unsurely identified (sequence similarity < 97%, query coverage < 95% or e-value ˃ 1e − 50) were BLASTed against the NCBI GenBank. All OTUs not belonging to the Glomeromycotina were removed, resulting in an OTU table with only AMF.

Data analyses

To assess the sampling effort, rarefaction curves were made in R version 4.0.2 (R Development Core Team 2012), using the *rarefy* function of the *vegan* package. These rarefaction curves were used to determine samples that were insufficiently deeply sequenced to provide a reliable representation of the AMF communities. To this end, samples with fewer than 80 AM fungal sequences per sample were omitted from further analyses. The AM fungal richness was determined as the number of OTUs present in a sample, using the *specnumber* function from the *vegan* package in R (Oksanen et al. 2019), while AM fungal diversity was approximated by the Shannon diversity index (H′) using the *diversity* function. One sample corresponded to one plant. The total sum of AM fungal sequences per sample was used as a rough indication of abundance on the cassava roots. Single-factor effects of *genotype*, *fertilizer*, and *fallow*, or factor interactions on root yield, aboveground biomass, and Shannon diversity were assessed using three-way ANOVAs (Type III, using "aov" from the *stats* package) after confirming normality, followed by multiple comparisons by least significant difference (LSD). Generalized linear models (GLMs) were used to test the effects of the three factors on the OTU richness and the sum of AM fungal sequences by using the {*lme4*} package in R with Poisson distribution and log link function. Effects were plotted using *ggplot* from the *ggplot2* package. Additionally, the environmental factors (i.e., *genotype*, *fertilizer*, and *fallow*) were fitted onto the OTU richness (i.e., richnessfit) and Shannon diversity (i.e., diversityfit) using the *envfit* function of the *vegan* package. A non-metric multidimensional scaling (NMDS) was performed on the OTU matrix with Bray–Curtis distances (*metaNMDS* function, *vegan* package, R). Afterward, we plotted the treatment levels of the categorical factors on this ordination. We analyzed the factor effects on the AMF community composition using permutational multivariate ANOVAs (PERMANOVA) on the Hellinger-transformed OTU table, after verifying the assumption of homogeneous multivariate dispersions (999 permutations; vegan package, adonis function, R). Subsequently, a post hoc multiple comparison of the AMF composition among groups was conducted using the *pairwise.adonis* function in R. Significant differences in AMF composition among groups were further analyzed in detail by comparing compositional charts of the OTU count and sum of AM fungal sequences at the genus level. To investigate whether some AMF taxa were significantly associated with certain treatments, we used the *multipatt* function of the *indicspecies* package. Subsequently, we performed variation partitioning on the OTU matrix, using the "varpart" function (Legendre 2008) of the R package {*vegan*}. To further analyze effects of *genotype* and *fertilizer* application, a partial redundancy analysis (pRDA) was performed on the Hellinger-transformed OTU table by using the R package *vegan*, while correcting for the large effect of *fallow* in the condition term.

Results

Richness, diversity, and abundance of AMF

Illumina sequencing generated 766 998 high-quality mycorrhizal sequences, assigned to a total of 173 OTUs, for a total of 288 cassava root samples (3 replicate blocks × 24 treatment combinations × 4 plants per sub-plot). The majority of OTUs belonged to the Glomeraceae (69.4%, 120 OTUs, 723 861 sequences) whereas only a few OTUs belonged to the Claroideoglomeraceae (9.3%, 16 OTUs, 5531 sequences), Paraglomeraceae (6.4%, 11 OTUs, 5 416 sequences), Diversisporaceae (5.8%, 10 OTUs, 3189 sequences), Acaulosporaceae (4.6%, 8 OTUs, 24 762 sequences), Gigasporaceae (4.1%, 7 OTUs, 4221 sequences), and Ambisporaceae (0.6%, 1 OTU, 18 sequences).

The OTU richness on the cassava roots was strongly reduced (69% reduction with a p value < 0.001) by the long *fallow* duration of 12 months, explaining most of the variation (Fig. 1a; Table 2). Similarly, the Shannon diversity was only affected by the *fallow* duration, being significantly lower (p value < 0.001) after 1 year of fallow compared to a fallow duration of 6 months (Fig. 1b; Table 2). This dominant effect of *fallow* duration on richness and diversity of AMF was additionally confirmed by the "richnessfit" and "diversityfit" (Table S2, Supplementary Information). The OTU richness only slightly but significantly (p value < 0.05) increased for the *genotype* Obama, while it slightly but significantly (p < 0.01) decreased in the NP treatment (Table 2). In contrast to the OTU richness and diversity, a significantly higher sum of sequences (p value = 0.002) was observed on the roots of the improved genotype (Obama), compared to the local landrace (Bailo) (Table 2).

Effects of fallow duration, fertilizer management, and genotype on the community composition of AMF

Visual inspection of the NMDS plot (Figs. 2 and S1) and the permutation test (Table S3) revealed that *fallow* duration dominantly altered the AMF community composition on and in the cassava roots (p < 0.001). Results of

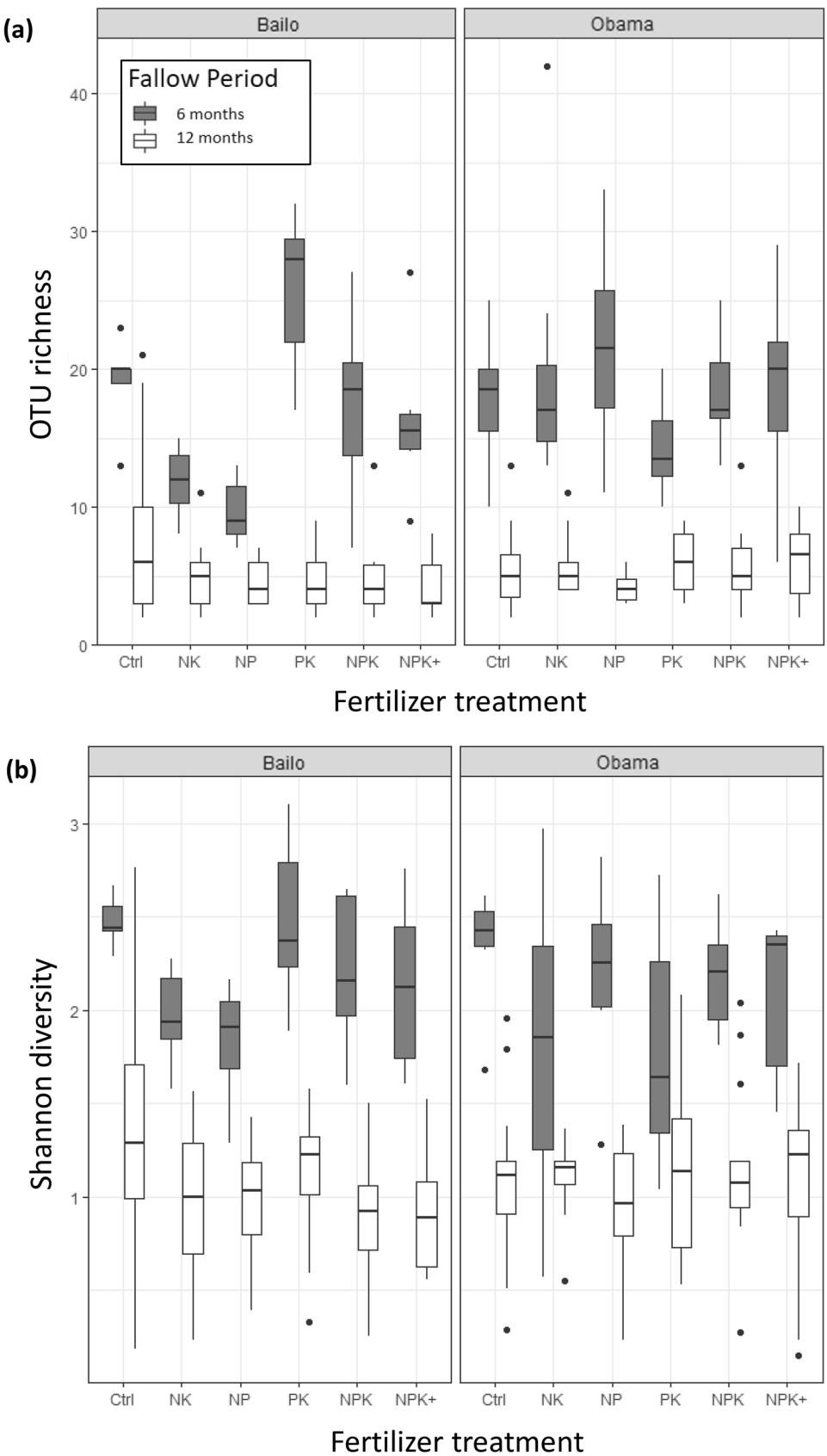

Fig. 1 OTU richness (**a**) and Shannon diversity (**b**) of AMF on the cassava roots. Results are obtained from a local landrace (Bailo) and an improved genotype (Obama), planted after different fallow durations, and subjected to contrasting fertilizer treatments. The whiskers present the smallest/largest value greater/less than the lower/upper quantile minus/plus times the interquartile range, while outliers fall beyond the whiskers

Table 2 Effects of fallow duration (Fallow), genotype, and fertilizer on OTU richness and sum of AMF sequences inferred with generalized linear models (GLMs) using Poisson distribution (Top). Factor effects on Shannon diversity and cassava root yield were assessed by a three-way ANOVA (bottom)

GLM	OTU richness			Sum of AMF sequences		
Fixed effects:	Estimate ± SE	z-value	*p* value	Estimate ± SE	z-value	*p* value
Intercept	2.93 ± 0.07	41.1	***	7.41 ± 0.28	26.1	***
Fallow—12 months	− 1.20 ± 0.04	− 27.3	***	− 0.02 ± 0.20	-0.1	ns
Genotype—Obama	0.09 ± 0.04	2.0	*	0.58 ± 0.19	3.1	**
Fertilizer—NK	− 0.14 ± 0.07	− 1.9	0.06	0.26 ± 0.33	0.8	ns
Fertilizer—NP	− 0.23 ± 0.08	− 3.1	**	− 0.09 ± 0.33	− 0.3	ns
Fertilizer—NPK	− 0.10 ± 0.07	− 1.4	ns	0.05 ± 0.33	0.2	ns
Fertilizer—NPK +	− 0.11 ± 0.07	− 1.5	ns	0.19 ± 0.33	0.6	ns
Fertilizer—PK	− 0.03 ± 0.07	− 0.5	ns	− 0.21 ± 0.33	− 0.7	ns
ANOVA	Shannon diversity			Cassava root yield		
Factors	*df*	F-value	*p* value	*df*	F-value	*p* value
Fallow	1	310.55	***	1	1.95	ns
Genotype	1	0.04	ns	1	338.96	***
Fertilizer	5	0.93	ns	5	2.68	*
Fallow × genotype	1	2.42	ns	1	0.23	ns
Fallow × fertilizer	5	0.81	ns	5	0.36	ns
Genotype × fertilizer	1	1.72	ns	1	3.60	*
Fallow × genotype × fertilizer	5	1.58	ns	5	0.40	ns

NS not significant *Significance in this table was based on a *p* level of *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

the variation partitioning (Fig. S2) and PERMANOVA (Table 3) indeed confirmed that the largest variation in the AMF community composition could be assigned to *fallow* duration only. When correcting for the large effect of *fallow* duration in the pRDA, only minor effects of *fertilizer* ($p = 0.01$) and *genotype* ($p = 0.02$) on the AMF community composition could be observed (Fig. S3). Only a few OTUs were significantly indicative of a particular *genotype* or *fertilizer* treatment, while many OTUs (i.e., 49) were significantly assigned to the short *fallow* duration (Figs. 3, S4, S5, S6 and Table S4).

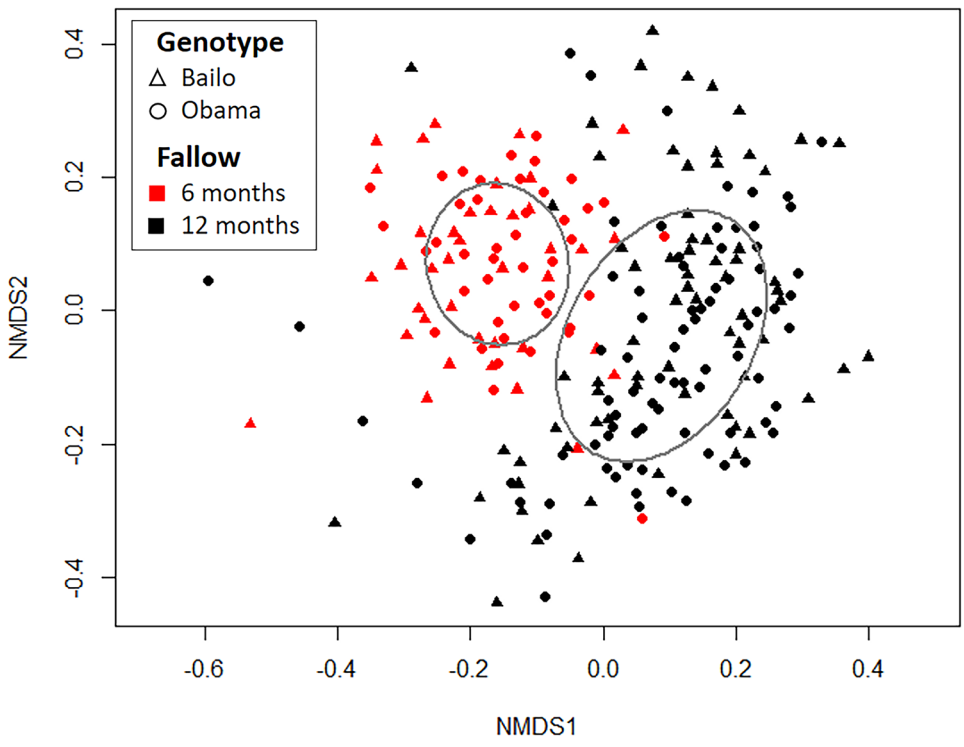

Fig. 2 NMDS ordination plot of AMF communities from 72 cassava sub-plots in the experiment (4 plants sampled per sub-plot). The AMF communities on the cassava roots were significantly different after contrasting fallow durations (Tables 2 and 3). From this NMDS ordination, genotype selection and fertilizer treatment did not affect the AMF community. Ellipses presented are dispersion ellipses using the standard deviation of the mean

Table 3 Factor effects and interactions on the AMF composition (i.e., presence/absence of certain OTUs in the AMF community) using permutational multivariate ANOVA (PERMANOVA) on the Hellinger transformed data, after verifying the assumption of homogeneous multivariate dispersions (999 permutations; vegan package, adonis function, R). The values for Fallow duration are underlined and presented in bold, because they were much higher than the other values

Factor	Df	MeansQs	*F model*	*p value*
Genotype	1	0.57	2.2	*
Fertilizer	5	0.44	1.7	**
Fallow	1	7.23	**28.1**	***
Fallow × genotype	5	0.35	1.4	ns
Fallow × fertilizer	1	0.68	2.6	**
Genotype × fertilizer	5	0.46	1.8	**
Fallow × genotype × fertilizer	5	0.30	1.1	ns
Residuals	210	0.26		

NS not significant *Significance in this table was based on a *p* level of *$p<0.05$; **$p<0.01$; and ***$p<0.001$

Cassava root yield and above ground biomass

Cassava root yield was mainly determined by *genotype* (strongly significant), and significant effects of the *fertilizer* treatments were only observed for the high-yielding genotype (i.e., Obama), resulting in a significant interaction between *genotype* and *fertilizer* treatment (Fig. 4; Table 2). The aboveground biomass also was greatest for Obama, and *fertilizer* management had a significant effect on both genotypes (i.e., lowest biomass obtained without P), and no significant interactions were observed. *Fallow* duration did not affect root yield, nor did it affect above ground biomass (Fig. 5, Supplementary information).

Discussion

This is the first study to identify the major agronomic drivers and dynamics of naturally occurring AMF on cassava by using high-throughput sequencing techniques, while evaluating relations with yields. The study represents actual and realistic scenarios of agricultural intensification practices in a smallholder farming system. Through a short fallow duration, richness and diversity of AMF on cassava roots significantly increased, yet fallow duration did not affect yields. In contrast, the use of an improved genotype and mineral fertilizer combinations benefitted yields, without compromising richness or diversity of AMF.

AMF taxa present on cassava roots

The observed AMF community composition and diversity was very similar to that of other studies on cassava (Séry et al. 2016; Peña-Venegas et al. 2019; Sarr et al.

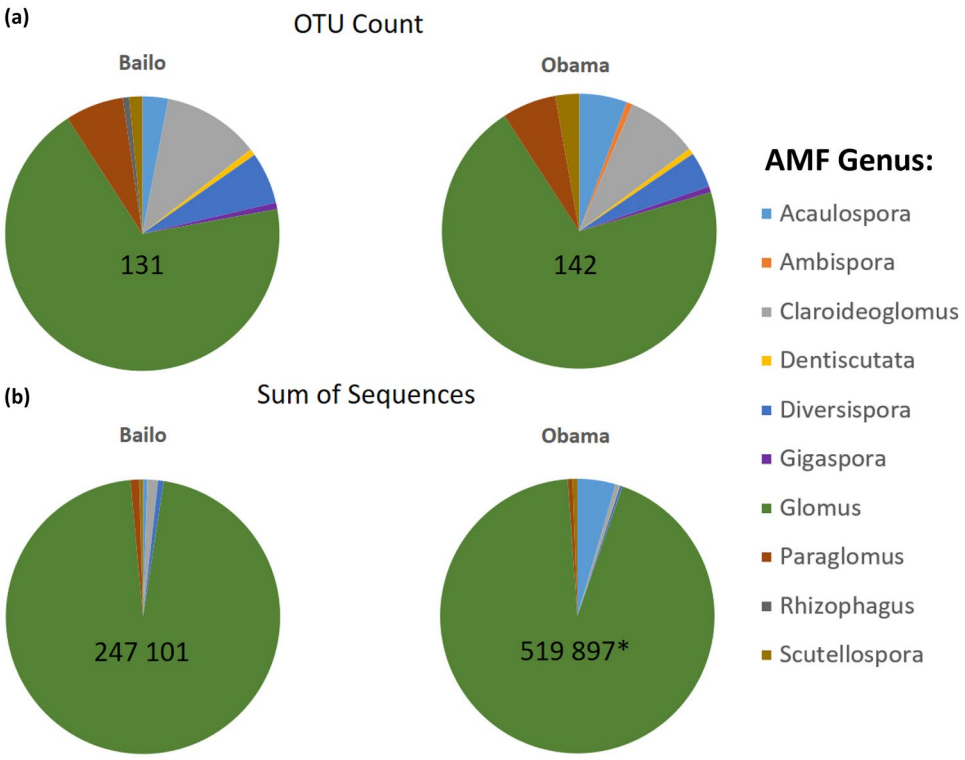

Fig. 3 Composition shares of the operational taxonomic unit (OTU) count (**a**) and sum of AMF sequences (**b**) at the genus level, presented for each cassava genotype (i.e., a local landrace, Bailo versus an improved genotype, Obama). The total OTU count and total sum of sequences observed for each genotype are displayed within the charts. The sum of sequences was significantly higher on samples from Obama (*) compared to Bailo (Table 1). Specific OTUs assigned to genotypes are presented in Table S4

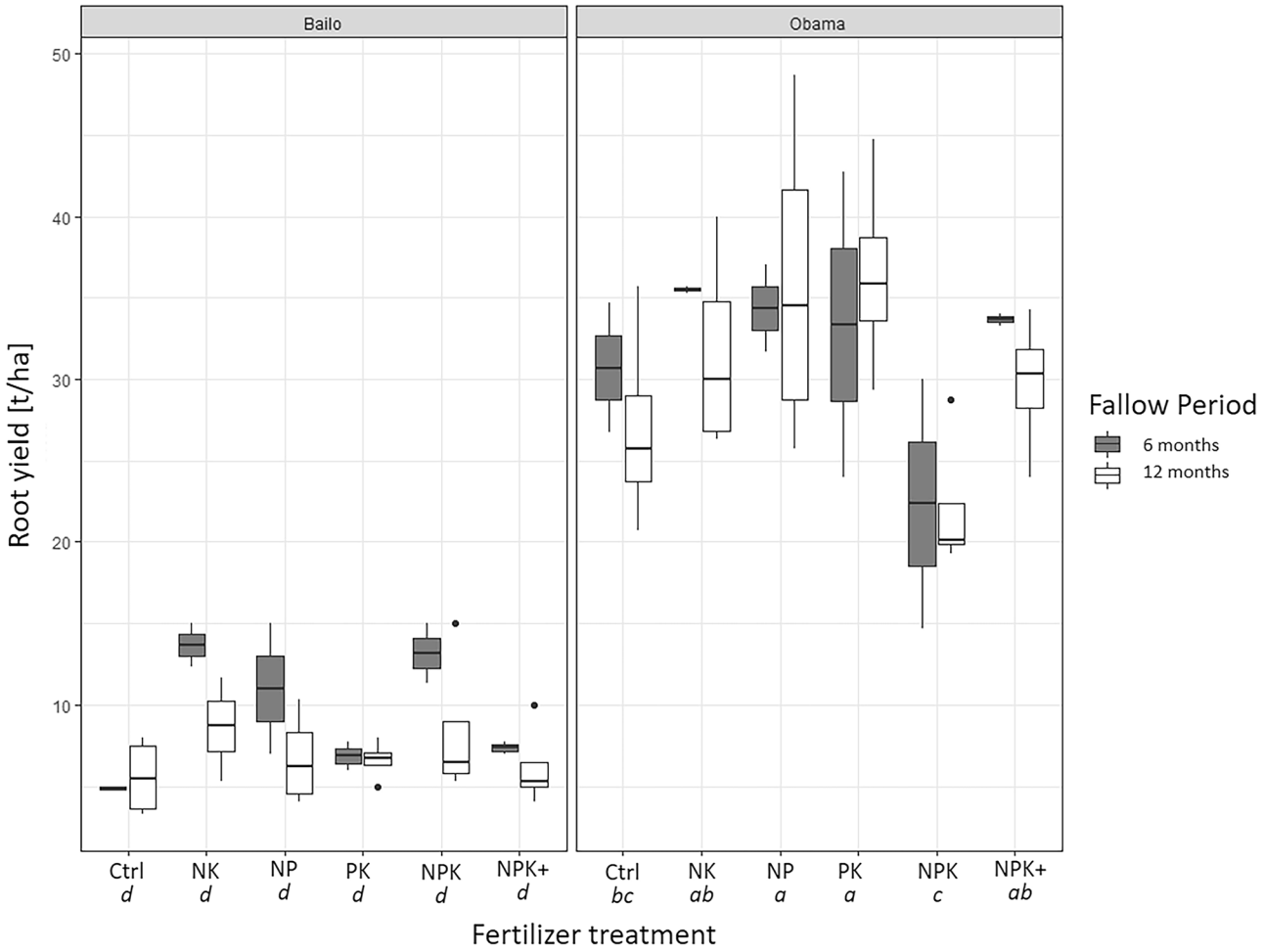

Fig. 4 Cassava root yields obtained for the local landrace (Bailo) and improved genotype (Obama), planted after different fallow periods and subjected to contrasting fertilizer treatments. Fertilizer designations underlain by the same letter do not differ significantly by LSD multiple comparison. The whiskers present the smallest/largest value greater/less than the lower/upper quantile minus/plus times the interquartile range, while outliers fall beyond these whiskers

2019), with the majority of sequences and OTUs belonging to the family of Glomeraceae (genera *Glomus* and *Rhizophagus*). Members of this family are considered disturbance-tolerant due to their high rate of hyphal turnover, high growth and sporulation rates, and their capacity to reproduce from both spores and hyphal fragments (Staddon et al. 2003; Chagnon et al. 2013; van der Heyde et al. 2017; Oehl et al. 2017). In consequence, Glomeraceae often are found to dominate AMF communities in agricultural systems. In addition, all other, less abundant AMF genera observed in this study previously have been reported to have affinity for cassava (Sieverding and Howeler 1985; Straker et al. 2010; Begoude et al. 2016). Additionally, it should be noted that a primer bias might have caused an amplification of the dominance of Glomeraceae and an underestimation of the abundance of Ambisporaceae, Claroideoglomeraceae, and Paraglomeraceae (Van Geel et al. 2014).

Cassava genotype drives yields, but not the community composition of AMF

Cassava yield was mainly determined by genotype, which contradicts previous observations of poor genotype effects on cassava yields (Fermont et al. 2010; Ezui et al. 2017). In contrast, genotype in this study did not affect the AMF community composition.

It was previously stated that crop genotype may influence the AMF composition on plant roots, following intraspecific preferences or continued co-evolution of landraces with AMF taxa (Croll et al. 2008; An et al. 2010; Hoeksema 2010; Lehmann et al. 2012; Martín-Robles et al. 2018). Indeed, different cassava genotypes (landraces) were previously observed to have different AMF composition both when grown in the same or different environments (Begoude et al. 2016; Peña-Venegas et al. 2019). However, genotype-specific Cassava-AMF

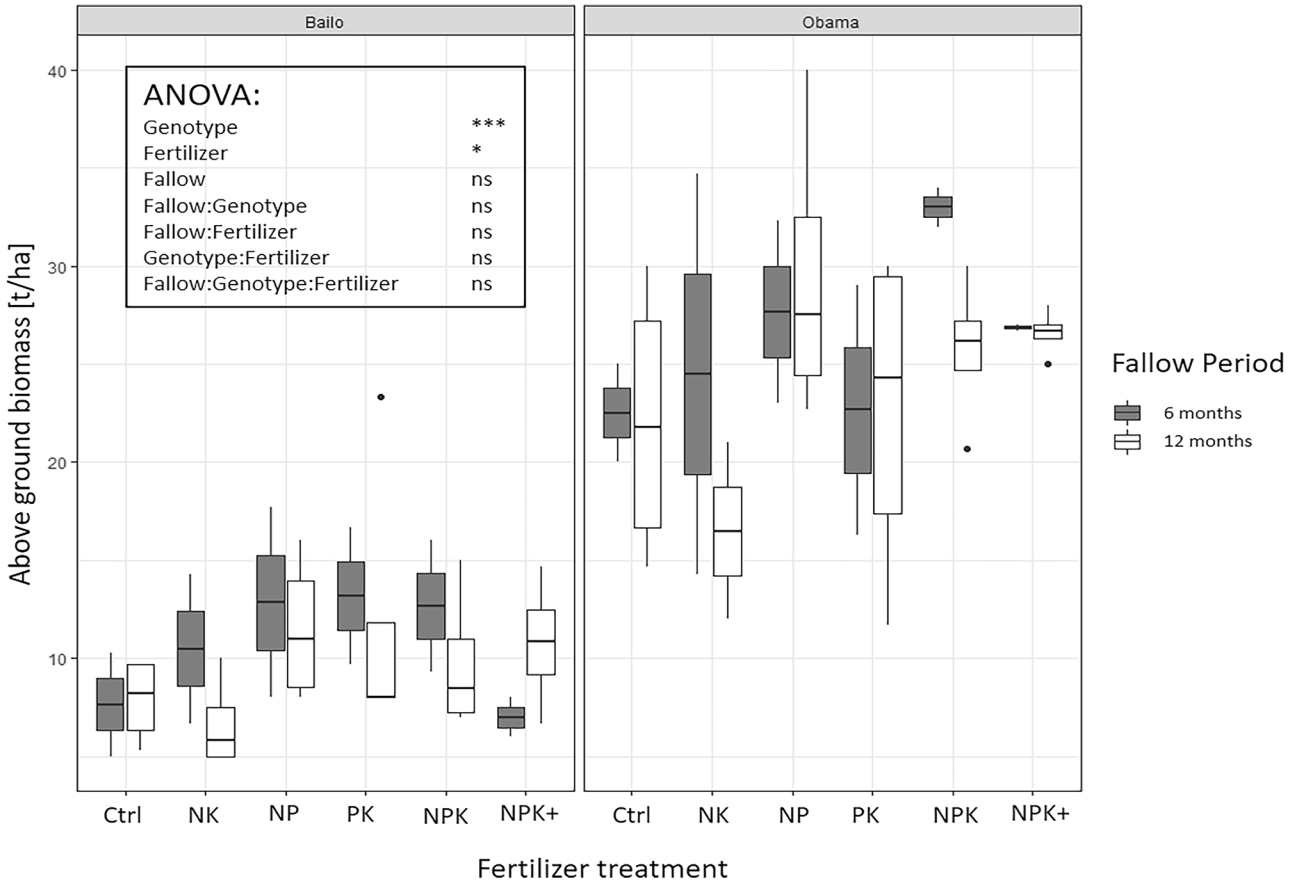

Fig. 5 Aboveground biomass (stem + leaves) of cassava, determined at 12 MAP. A local landrace (Bailo) and improved genotype (Obama) were planted after different fallow periods and subjected to contrasting fertilizer treatments. Results of the analysis of variance are presented in the box. $*P<0.05$ and $***P<0.001$, while ns not significant

interactions could not be confirmed in this study as the AMF composition of the local landrace (Bailo) did not differ from that of the improved genotype (Obama). Hence, we could not provide evidence of such continued co-evolution, or strong intraspecific preferences of cassava for AMF species (Hoeksema 2010).

Robustness of cassava-AMF associations to mineral fertilizers and improved genotypes

Previous work has shown that nutrient management (Howeler and Sieverding 1983; van Geel et al. 2015, 2016; López-Ráez 2016; Aliyu et al. 2019; Sendek et al. 2019) can influence the associations of plants with AMF, and that mainly phosphorus amendments reduce the abundance and diversity of AMF on crops (Collins and Foster 2009; Johnson 2010). However, fertilizer application in this study did not affect the richness or diversity of AMF on or in cassava roots. Despite a slightly higher yet not significant sum of AMF sequences observed without P application, no evidence could be found that richness or diversity of AMF on cassava responds negatively to mineral fertilizer application (Verbruggen and Kiers 2010; Rillig et al. 2019). This suggests that AMF communities associated with cassava might indeed be more resilient to mineral fertilizer amendments than initially thought (Ryan and Graham 2018).

It was previously hypothesized that crop improvement and domestication would suppress crop-AMF associations following genotype selection for high yields on agricultural fields with low AMF (Martín-Robles et al. 2018). The use of an improved genotype in this study neither affected the diversity nor the richness of AMF. Therefore, we argue that breeding does not necessarily compromise the diversity and richness of AMF on or in cassava roots (Bull et al. 2011).

Similarly, a high abundance of AMF was previously observed on improved maize genotypes (An et al. 2010), and such observations suggest that modern cassava breeding programs would not necessarily lead to the suppression of colonization (Lehmann et al. 2012); but rather the opposite. Therefore, future research should further determine the degree of AMF colonization of improved cassava genotypes grown in realistic conditions.

Effects of fallow duration on AMF communities and cassava yield

Previously, fields in the region of tropical Africa were subjected to long fallow durations (> 30 years), in order to restore soil fertility (Tian et al. 2005). Specifically, a long fallow period was employed to build up organic matter and enhance nutrient availability. Due to high human population pressure, land scarcity, and low yields, however, this historical "shifting cultivation" in the area currently has been replaced by cultivation with relatively short fallow durations aiming to suppress pests and diseases.

While long fallow duration may indirectly influence AMF communities through changes in soil properties (Jemo et al. 2018), it is known that shortening the fallow duration may enhance inoculation of a subsequent crop with indigenous AMF (Lekberg and Koide 2005; Angus et al. 2015; Bowles et al. 2017; Jemo et al. 2018). However, it has been argued that yield penalties following reduced colonization of AMF after long fallows are generally low (Ryan and Kirkegaard 2012; Seymour et al. 2012; Angus et al. 2015). Here, we indeed demonstrated that the length of fallow duration is indeed a major driver of cassava AMF composition, richness, and diversity, but despite the observed strong effects of fallow duration on the AMF community, it did not affect yield. An important conclusion from our study is that commonly considered variables such as richness and diversity of AMF do not directly affect cassava yields. Hence, this work contributes to the discussion of whether or not cassava farmers should consider diversity of AMF when selecting agronomic practices (Ryan and Graham 2018).

Interestingly, the longer fallow duration in this study strongly reduced richness and diversity of AMF, while not affecting the sum of AM fungal sequences (Table 2; Fig. S4). This may suggest that the degree of root colonization remains similar, but that fewer, perhaps more competitive AMF taxa were available for colonization after the longer fallow. Yet, because we have not stained roots to quantify colonization, this conclusion remains speculative. Nevertheless, the observed decreased richness and diversity of AMF is not in line with earlier work that has shown that ruderal plants encroaching during fallow periods may serve as temporary hosts of AMF, favoring subsequent crop colonization (Ramos-Zapata et al. 2013). To the contrary, it is known that many agricultural weeds with a ruderal lifestyle are inconsistent mycorrhizal hosts (Brundrett and Tedersoo 2018). Furthermore, cassava may be preferred by AMF with a low ruderal lifestyle, but this requires further investigation. Further research should closely monitor the development of AMF communities in soils and weeds with increasing fallow duration and should further unravel relations with crop yield.

Our study does not contradict that abundant and diverse mycorrhizal colonization can enhance the resilience of an agroecosystem, without direct yield benefits (Rillig and Mummey 2006; Polcyn et al. 2019; Rillig et al. 2019; Wipf et al. 2019; Begum et al. 2019). Therefore, effects of altered AMF community composition on yields might only be displayed under varying environmental conditions (Lekberg and Koide 2005), and hence complex environmental interactions should be regarded. While AMF may indeed play a role in ecosystem services of relevance to farmers, we lack the fundamental, field-relevant information to quantify or capture these potential effects over the long term. Hence, additional field studies on poor soils are needed to evaluate effects of management and AMF on temporal yield stability in real agronomic situations.

Conclusions

This study suggests that cassava-AMF associations are relatively stable under mineral fertilizer amendments, and we could not determine negative effects of genotype improvement on cassava-AMF associations. This study indicates that the use of improved genotypes and mineral fertilizer has a great potential to benefit cassava yields without necessarily compromising the richness or diversity of AMF. Fallow duration before cassava planting emerges as a dominant factor affecting AMF community composition on cassava roots, but the increased richness and diversity of AMF observed after a short fallow duration did not enhance yields. No evidence could be found that farmers obtain direct benefits from management practices improving richness and diversity of AMF on cassava roots. However, abundant and diverse AMF colonization could enhance cassava's resilience on very poor soils or during drought events. Thus, additional in-field experiments are needed to further unravel the role of AMF in agricultural systems of sub-Saharan Africa.

This study highlights the complex relationships among crop genotype, realistic agronomic practices, crop-AMF associations, and crop yield. Hence, when science advocates exploitation of AMF as a major means of sustainable agricultural intensification, a strong systems agronomic approach will be needed for its implementation.

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Author contribution P.D.B., D.B., R.M., and O.H. designed the research; D.B. and W.M. set-up the field experiment; P.D.B, D.B, and W.M. collected the samples; G.P. and P.D.B. performed the laboratory analysis; P.D.B and M.B. analyzed the data with helpful insights from O.H.; P.D.B. wrote the draft of the manuscript with constructive inputs from O.H, R.M., and M.B. All the authors reviewed and edited the final manuscript, and R.M. and O.H. supervised the research.

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Declarations

Conflict of Interest The authors declare no competing interests.

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