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

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Summary:

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 fungal compositions 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 (six vs. twelve 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 enhances 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 rather robust to fertilizer amendments and modern genotype improvement.

Key words:

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, effects of combinations of real agronomic practices on AMF community composition are poorly known. In this regard, recent reviews 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 of a more complex and functional (i.e. more beneficial) mycorrhizal fungal community, yield penalties after such reductions of richness or diversity of AMF by agricultural practices of intensification have been rarely reported in 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. Unravelling the intraspecific interactions of particular crop genotypes with AM fungal species and environment by determining the driving factors of the mycorrhizal responses and associations on the field, have therefore 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 the 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), while such experiments are especially needed in the low-input systems of sub Saharan Africa where AMF may have their highest functionality (Rillig et al. 2019). Overall, and in order to more 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 a 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 is still a large 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 shorter fallow durations.

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

Hence, this study aimed to investigate:

(i) 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* (landrace versus improved genotype), *fertilizer* management (nutrient omissions of NPK and secondary macro- and micro-nutrients), and *fallow* duration (12 versus 6 months).

(ii) the effects of different combinations of agronomic practices on cassava yields and the respective relation 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 & Methods

Site description

The experiment was established in the Kalehe territory of the 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 has 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 more dominant in the region and this plot was used for cassava cultivation before the experiment. The site was never amended with fertilizers and the 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 composed of *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 was 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 a distance of 2m, while sub-plots (7m x 10m) were separated by a distance of 1.5m.

Clean cuttings of two contrasting cassava genotypes were planted (2 November 2018) in the sub-plots at a distance of 1x1m according to the local density recommendations, and each sub-plot included 56 plants

(8 x 7). One popular local landrace (i.e. M'Bailo or Bailo) was selected as it was grown in the area since at least the 1950's. An improved genotype (i.e. Obama/TME 419) was selected and this genotype has never been cultivated in the area before (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 at a daily basis. 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 where 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 at 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 (2/11/2019), 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) 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). Only 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 of roots from the pooled root samples were cut into small 1 cm pieces. Roots were then homogenized in a Bead Mill Homogenizer (Omni International) for two times 30sec at 8 m/s. Genomic DNA was further extracted from each sample (288 samples) using the 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 1µL of genomic DNA, 0,5 µL of each 20 µM primer, 5 µl ALLin HiFi Buffer, 0,3µl 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

187 filtering of the reads was then performed with the '*fastq_filter*' command, allowing a maximum expected
188 error of 1.0 for the individual sequences. To maximize the number and length of retained sequences,
189 truncation length was set to 200 bp. Next, the sequences were dereplicated and sorted by abundance.
190 Sequences were then clustered into operational taxonomic units (OTUs) defined at 97% sequence
191 similarity, which is commonly used to define SSU-based OTUs in AMF (Lumini et al. 2009; Öpik et al. 2010),
192 using the *'cluster_otus'* command. In this step, chimeric OTUs built from more abundant reads were
193 discarded as well. To remove possible erroneous sequences produced during PCR or sequencing (Alberdi
194 et al., 2018), we removed per sample the OTUs that were represented by less than 0.01 % of the
195 sequences of that sample. The glomeromycotinan SSU dataset was then BLASTed against the MaarjAM
196 dataset in order to assign a taxonomy to each of the OTUs (Öpik et al. 2010). Unidentified or unsurely
197 identified (sequence similarity < 97%, query coverage < 95% or e-value > 1e−50) were BLASTed against
198 the NCBI GenBank. All OTUs not belonging to the Glomeromycotina were removed, resulting in an OTU
199 table with only AMF.

200 Data Analyses

201 To assess the sampling effort, rarefaction curves were made in R version 4.0.2 (R Development Core Team,
202 2012), using the *rarefy* function of the {*vegan*} package. These rarefaction curves were used to determine
203 the samples that were insufficiently deep sequenced to provide a reliable representation of the AMF
204 communities. To this end, samples with less than 80 AM fungal sequences per sample were omitted from
205 further analyses. The AM fungal richness was determined as the number of OTUs present in a sample,
206 using the *specnumber* function from the {*vegan*} package in R (Oksanen et al. 2019) , while AM fungal
207 diversity was approximated by the Shannon diversity index (H) using the *diversity* function. One sample
208 corresponded with one plant. The total sum of AM fungal sequences per sample was used as a rough
209 indication of abundance on the cassava roots. Single factor effects of *genotype*, *fertilizer*, and *fallow*, or
210 factor interactions on root yield, aboveground biomass, and Shannon diversity (H) was assessed using
211 three-way ANOVAs (Type III, using 'aov' from the {*stats*} package) after confirming normality, followed by
212 multiple comparison calculating the Least Significant Difference (LSD). Generalized Linear Models (GLM)
213 were used to test the effects of the three factors on the OTU richness and the sum of AM fungal sequences
214 by using the {*lme4*} package in R with Poisson distribution and log link function. Effects were plotted using
215 *ggplot* from the {*ggplot2*} package. Additionally, the environmental factors (i.e. *genotype*, *fertilizer*, and
216 *fallow*) were then fitted onto the OTU richness (i.e. richnessfit) and Shannon diversity (i.e. diversityfit)
217 using the *envfit* function of the {*vegan*} package. A non-metric multidimensional scaling (NMDS) was then

performed on the OTU matrix with Bray-Curtis distances (*metaNMDS* function, {*vegan*} package, R). Afterwards, 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 more detail by comparing compositional charts of the OTU count and sum of AM fungal sequences at 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 in more detail, 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, 5 531 sequences), Paraglomeraceae (6.4%, 11 OTU, 5 416 sequences), Diversisporaceae (5.8%, 10 OTU, 3 189 sequences), Acaulosporaceae (4.6%, 8 OTU, 24 762 sequences), Gigasporaceae (4.1%, 7 OTU, 4 221 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 one year of fallow compared to a fallow duration of six 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 (Fig. 2, Fig. 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 the variation partitioning (Fig. S2) and PERMANOVA (Table 3) indeed confirmed that 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, supplementary information). Only few OTUs were significantly indicative to a particular *genotype* or *fertilizer* treatment, while many OTUs (i.e. 49) were significantly assigned to the short *fallow* duration (Fig. 3, S4, S5, S6 and Table S4).

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 was also largest 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 establishing a shorter 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. 2019), with the large majority of sequences and OTUs belonging to the family of Glomeraceae (genera Glomus and Rhizophagus). Members of this family are considered more disturbance-tolerant due to their high rate of hyphal turnover, high growth and sporulation rate 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). As a consequence, Glomeraceae are often found to dominate AMF communities in agricultural systems. In addition, all other, less abundant AMF genera observed in this study were previously yet reported to have affinity for cassava (Sieverding and Howeler 1985; Straker et al. 2010; Begoude et al. 2016). Additionally, it should be regarded 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 a different AMF composition both when grown in the same or different environment (Begoude et al. 2016; Peña-Venegas et al. 2019). However, genotype specific Cassava-AMF interactions could not be confirmed in this study as the AMF composition of the local landrace (Bailo) did not differ from 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; Van Geel et al. 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 & 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 higher yields on agricultural fields with lower AMF (Martín-Robles et al. 2018). The use of an improved genotype in this study did neither affect the diversity nor the richness of AMF. Therefore, we argue that breeding does not necessarily compromise on the diversity and richness of AMF on or in cassava roots (Bull et al. 2011).

Accordingly, a higher abundance of AMF was previously also observed on improved maize genotypes (An et al. 2010), and these observations indeed 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 would benefit to further determine the degree of AMF colonization on 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 the soil fertility status (Tian et al. 2005). More particularly, a long fallow period was established to build up organic matter and enhance nutrient availability. Due to high human population pressure, land scarcity, and low yields this historical `shifting cultivation` in the area has currently been replaced by more permanent cultivation with relatively short fallow durations, aiming to suppress pests and diseases.

While long fallow duration may indirectly influence the AMF communities through changes in soil properties (Jemo et al. 2018), it is known that shortening the fallow duration may enhance inoculation of the subsequent crop with indigenous AMF (Lekberg and Koide 2005; Angus et al. 2015; Bowles et al. 2017; Jemo et al. 2018). However, it was previously argued that yield penalties following reduced colonization of AMF after long fallow 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 these observed strong effects of fallow duration on

337 the AMF community, it did not affect yield. An important conclusion from our study is that commonly
338 considered variables such as richness and diversity of AMF do not directly affect cassava yields. Hence,
339 this work contributes to the discussion whether cassava farmers should consider diversity of AMF when
340 selecting agronomic practices or not (Ryan and Graham 2018).

341 Interestingly, the longer fallow duration in this study strongly reduced richness and diversity of AMF, while
342 not affecting the sum of AM fungal sequences (Table 2 & Fig. S4). This may suggests that the degree of
343 root colonization remains similar, but that fewer, perhaps more competitive AMF taxa were available for
344 colonization after the longer fallow. Yet, because we have not stained roots to quantify colonization, this
345 conclusion remains speculative. Nevertheless, the observed decreasing richness and diversity of AMF is
346 not in line with earlier work that has shown that ruderal plants encroaching during fallow periods may
347 serve as temporary hosts of AMF, favoring subsequent crop colonization (Ramos-Zapata et al. 2013). On
348 the other hand it is known that many agricultural weeds with a ruderal lifestyle are inconsistent
349 mycorrhizal hosts (Brundrett and Tedersoo 2018). Furthermore, Cassava may be preferred by AMF with a
350 less ruderal lifestyle, but this requires further investigation. Further research should closely monitor the
351 evolution of AMF community composition in soils and weeds with increasing fallow duration, and further
352 unravel relations with crop yield.

353 Our study does not contradict that more abundant and diverse mycorrhizal colonization can enhance the
354 resilience of an agroecosystem, without direct yield benefits (Rillig and Mummey 2006; Polcyn et al. 2019;
355 Rillig et al. 2019; Wipf et al. 2019; Begum et al. 2019). Therefore, effects of altered AMF community
356 composition on yields might only be displayed under varying environmental conditions (Lekberg and Koide
357 2005), and hence complex environmental interactions should be regarded. While AMF may indeed play a
358 role in ecosystem services of relevance to farmers, we lack the fundamental, field-relevant information to
359 quantify or capture these potential effects on the longer term. Hence, more field studies on poor soils are
360 needed to evaluate effects of management and AMF on the temporal yield stability in real agronomic
361 situations.

362

363 **Conclusions**

364 This study suggests that cassava-AMF associations are relatively stable under mineral fertilizer
365 amendments and we could not determine negative effects of genotype improvement on cassava-AMF
366 associations. This study indicates that the use of improved genotypes and mineral fertilizer has a huge

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 shorter fallow duration did not further 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, a more abundant and diverse AMF colonization could indeed enhance cassava's resilience on very poor soils or during drought events; and more on-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 final crop yield. Hence, when science advocates exploitation of AMF as a major mean of sustainable agricultural intensification, a strong systems agronomic approach will be needed for its implementation.

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Conflict of Interst

The authors declare that they have no conflict of interest.

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., M.B. All authors reviewed and edited the final manuscript, and R.M. and O.H. supervised the research.

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Table 1: Site and initial soil information 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

Table 2: Effects of fallow duration (Fallow), genotype, and fertilizer on OTU richness and sum of AMF sequences inferred with Generalized Linear Models (GLM) 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

** Significance in this table was based on a p-level of '*' <0.05, '**' <0.01 and '***' <0.001; while ns = not significant*

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, since 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**	***
Genotype:Fertilizer	5	0.35	1.4	ns
Genotype:Fallow	1	0.68	2.6	**
Fertilizer:Fallow	5	0.46	1.8	**
Genotype:Fertilizer:Fallow	5	0.30	1.1	ns
Residuals	210	0.26		

** Significance in this table was based on a p-level of '*' <0.05, '**' <0.01 and '***' <0.001; while ns = not significant*