Impact of mycorrhiza-based inoculation strategies on *Ziziphus mauritiana* Lam. and its native mycorrhizal communities on the route of the Great Green Wall (Senegal)

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Abstract

A wide program of fruit tree planting, notably jujube trees, has been implemented in the framework of the pan-African Great Green Wall (GGW) project to improve food security in arid and semiarid regions. However, the success of such initiatives is highly limited by a low tree growth and high tree mortality rates due to transplant shocks from tree nursery to field. The positive impact of mycorrhiza-based ecological engineering strategies on jujube trees were previously demonstrated in nursery conditions, but field monitoring is necessary to evaluate their sustainability in terms of plant growth and survival. In the current study, local (Tasset) and exotic (Gola) jujube cultivars were tested for their response to mycorrhizal inoculation with the non-native arbuscular mycorrhizal (AM) fungus *Rhizophagus irregularis* IR 27 and fertilization with rock phosphate. The environmental impacts of both treatments were assessed by characterizing the native AM fungal community in a 13-month-old jujube orchard. Field results demonstrated higher rates of survival and a relative stability of nursery-driven plant benefits of inoculated jujube trees, as well as a potential higher persistence of AM fungal inoculum for the exotic cultivar. The native AM fungal community associated with the local cultivar was the most diverse, but Glomeraceae was predominant in both cultivars. The mycorrhiza-based ecological engineering strategies proposed in this work affected both AM fungal communities, notably Glomeraceae and Gigasporaceae members, but in a higher extent for the local jujube cultivar. Results highlight the strong benefits of mycorrhizal inoculation at the very early stages of tree seedling growth in nursery and their stability in the first year of plantation. Nevertheless, a deeper assessment of mycorrhizal inoculum persistence and spread, and a wider characterization of soil and root microbiome need to be implemented in further field monitoring to better evaluate the environmental impacts.

Keywords: Arbuscular mycorrhizal fungi community; *Rhizophagus irregularis*; Inoculation; Illumina sequencing; Rock phosphate; *Ziziphus mauritiana*.

1. Introduction

The jujube tree (*Ziziphus mauritiana* Lam.) is a multipurpose fruit tree commonly used in Sahelian and Sudanian areas in West Africa (Okafor, 1991). It is an important source of income for rural communities and contributes to overcome nutritional problems (Arbonnier, 2000). For these reasons, the jujube tree is one of tree species selected by the pan-African Great Green Wall (GGW) project to « green » and fight against the poverty, degradation of soils and desertification (Dia and Niang, 2010). In Senegal, the GGW project promotes tree planting and economically interesting drought-tolerant plant species, water retention ponds, agricultural production systems and other income-generating activities, as well as basic social infrastructures. However, fruit tree planting programs in such environmental conditions (i.e drought, degraded land) are generally subjected to a low tree growth and high tree mortality rates due to transplant shocks from tree nursery to field (Close et al. 2013). Different strategies were proposed to improve the growth and survival of fruit trees, e.g. inoculation with arbuscular mycorrhizal (AM) fungi, fertilization with rock phosphate (RP), and plant propagation by micrografting (Reena and Bagyaraj, 1990; Guissou et al. 1998; Bâ et al. 2000; Mathur and Vyas, 2000; Danthu et al. 2002, 2004; Bâ et al. 2003; Guissou, 2009; Sidibé et al. 2012).

The jujube tree is highly dependent on AM symbiosis (Bâ et al. 2001; Thioye et al. 2007) and it has been suggested than AM fungal root colonization of jujube seedlings in a nursery was an essential prerequisite to limit the mortality of outplanted jujube trees in the field (Bâ et al. 2001). AM fungi are known for their ability to improve plant growth and notably to efficiently scavenge for soil phosphorus (P) resources (Smith and Read 2008), one of most the limiting resources in West African soils for the establishment of tree plantations and agriculture crops (Friesen et al. 1997). Paradoxically, important resources in phosphate rocks (RP) are available in West Africa, and their use could provide an alternative to soluble P fertilizers that are poorly accessible to rural communities due to their high containable (Nziguheba et al. 2015).

Previous studies have demonstrated that jujube trees associated with AM fungi showed a better growth and mineral nutrition than non AM-associated jujube trees (Guissou et al. 1998; Bâ et al. 2000; Bâ et al. 2001; Sidibé et al. 2012; Guissou et al. 2016), for example by using more efficiently soluble P from RP (Bâ et al. 2001). However, the beneficial effects of AM fungal association were dependent on jujube species and AM fungal species (Thioye et al. 2017).

Rhizophagus irregularis, isolate IR27 (syn. *Glomus aggregatum* IR27; Bâ et al. 1996) was one of the most efficient AM fungi to promote growth and mineral nutrition of various jujube tree species and provenances of *Z. mauritiana* (Thioye et al. 2007). This AM fungal species has a worldwide distribution (Öpik et al. 2006), well adapted to competition in natural habitats and disturbed agroecosystems (Öpik et al. 2006; Bouffaud et al. 2016). It represents the most widely used AM fungal species in mycorrhiza-based ecological engineering strategies (Ceballos et al. 2013), mostly because of its ability to be cultured in an *in vitro* system (Bécard and Fortin, 1988; St-Arnaud et al. 1996), allowing to set up a large-scale biotechnological production.

However, monitoring of mycorrhiza-based beneficial plant effects from tree nursery to field, and the evaluation of impacts on native microbial community as the mycorrhizal community were poorly assessed (Alguacil et al. 2011; Pellegrino et al. 2012), notably regarding fruit trees (Ræbild, 2012). Since the development of new generation sequencing technologies, field-based monitoring of microbial biodiversity noted a genuine revolution, providing unprecedented insights into the ecology of AM fungal community in a wide range of climatic zones (Davison et al. 2015), but Sahelian regions remains poorly represented

The current study aimed the evaluation of different mycorrhiza-based ecological engineering strategies, *i.e.* using a *R. irregularis* inoculant combined or not with a RP fertilizer from Senegal, on jujube seedling growth and nutrition in tree–nursery and after outplanting in an experimental field. The ecological impact of each practice will be assessed by the monitoring of AM fungal community structure and diversity associated with jujubes trees after one year of plantation by using high throughput Illumina sequencing. The work was carried out on two different jujube tree cultivars from different provenances, a local one adapted to the harsh conditions observed on the route of the GGW and an Indian provenance particularly appreciated by West African farmers because of its precocity in fruiting, the larger size of its fruits and its taste (Vashishtha, 1997; Danthu et al. 2004).

2. Materials and methods

2.1. Mycorrhizal inoculum, fertilizer and plant material

The *Rhizophagus irregularis* isolate IR27 (syn. *Glomus aggregatum* IR27; Bâ et al. 1996) originated from an *Acacia holosericea* planting in the North of Burkina Faso, and provided by the LCM laboratory (IRD, Dakar, Senegal, certified ISO 9001, version 2000), was used as AM fungal inoculum. It was propagated on maize (*Zea mays* L.) for three months on

112 sterilized sandy soil in a tree nursery. The sandy soil used in the experiment was collected
113 from Sangalkam (Senegal). It was a sandy soil with 88.8 % sand, 5.8 % silt, 5.4 % clay, 0.6 %
114 organic matter, 0.3 % total C, 0.02 % total N, ratio C/N = 14, 333.5 ppm total K, 41.4 ppm
115 total P, 2.1 ppm P-Bray 1, 1.03 ppm Ca, 0.3 ppm Mg, pH = 6.0 of a soil/water mixture (ratio
116 1:2, v/v) and pH = 4.6 of a soil / KCl mixture (ratio 1:2, v/v). The soil was passed through a 2
117 mm sieve, sterilized for four hours in an autoclave oven system at 180°C to eliminate native
118 AMF, and transferred into plastic bags (1.5 kg of soil per plastic bag). The AM fungal
119 inoculum consisted of sand, spores, fragments of hyphae and maize root segments. The
120 inoculum density of *R. irregularis* IR27 was calibrated by the most probable number method
121 (Adelman and Morton, 1986) as 1635 infective propagules per 20 g of inoculum. Non-
122 inoculated controls also received 20 g of autoclaved crude AM fungal inoculum. The fertilizer
123 consisted of rock phosphate (RP, 30 % of P_2O_5) provided by the Société d'Etudes et de
124 Réalisation des Phosphates de Matam (Senegal). It was used 0 and 1.73 g P/kg/plant,
125 according to Bâ et al. (2001).

126 Two cultivars of jujube seedlings (Tasset from Senegal and Gola from India) were used in
127 this study and provided by the CNRF / ISRA (Senegal). Seeds of each jujube seedlings were
128 surface-sterilized with 1 % NaOCl for 15 min, washed several times and soaked in sterile
129 distilled water for 30 min before being planted in the soil as three per plastic bag (24 cm × 7.5
130 cm).

131

132 *2.2. Nursery experimental set up and plant growth measurements*

133 Plants were grown in a tree nursery at research center ISRA / IRD (Bel Air, Dakar, Senegal)
134 (14°44'N, 17°30'W) under natural sunlight (35°C day, 27°C night, relative humidity 75 %
135 and 14 h photoperiod). After emergence, the seedlings were thinned to one plant per plastic
136 bag. The experiment was set up as a 2×2×2 factorial design consisting of two jujube cultivars,
137 with AM fungal inoculation or not, and with or without RP fertilization. Experiment was
138 arranged in a completely randomized design with 20 replicates per treatment combination.
139 Mycorrhizal inoculation and fertilization with RP were achieved by placing either 20 g
140 portions of AM fungal and /or two different RP doses below the seeds during transplanting.

141 Four months after sowing, plants were harvested to measure height, collar diameter and dry
142 weight of shoots and roots (48 h at 70° C). For mycorrhizal root infection measurement, a part
143 of fresh fine roots was collected from the root system of each seedling. Root were gently

144 washed under tap water, bleached (KOH, 10 %) at 80°C during 30 min, and stained in 0.05 %
145 Trypan blue at 80°C during 35 min following the method of Phillips and Hayman (1970).
146 Percentage of root length colonized by AMF was assessed at ×40 magnification using 100
147 fragments of lateral roots (approximately 1 cm length) on microscopic slides. Mycorrhizal
148 root colonization was evaluated by using the method of Trouvelot et al. (1986). P, N and K
149 contents in jujube leaves were quantified at the LAMA laboratory (IRD, Dakar, Senegal,
150 certified ISO 9001, version 2000) as follows: leaf tissues of each plant were dried, ground,
151 mineralized through heating at 500 °C, digested in 2 ml HCl (6N) and 10 ml HNO_3. Total P
152 and N contents were determined by the molybdate blue method and Kjeldahl method,
153 respectively. Total K contents were determined by means of an atomic absorption
154 spectrophotometer.

155

156 *2.3. Field experimental set up and plant growth measurements*

157 The orchard (5 ha) was located near the village of Amally in the rural community of
158 Tessekere (15°59'N, 15°19'W) on the route of GGW in the Ferlo region in the Sahelian zone
159 of North, Senegal. The climate is arid with low and erratic mean annual rainfall varying from
160 100 to 400 mm. The predominant vegetation consists of low trees (i.e *Ziziphus mauritiana*,
161 *Balanites aegyptica* and *Acacia senegal*), open shrub steppes and grasslands growing in sandy
162 soil (Vincke et al. 2009). Physical and chemical analyses of soil were performed in the
163 Agricultural Chemistry Laboratory in Rio de Janeiro (Brazil) with means as following: pH:
164 6.41; C: 0.12 %; Al: 0 mg; Ca: 160.32 mg; K: 69.84 $mg.L^{-1}$; Mg: 41.33 mg; N: 0.02 %; P:
165 1974.7 mg. The mycorrhizal soil infectivity determined by the MPN method was very low
166 reaching 4.47 propagules per 100 g of soil. After four months, pre-inoculated and pre-
167 uninoculated plants were transplanted to field (eight treatments). Rate of survival, height and
168 collar diameter were recorded at 3, 8 and 13 months after transplanting and mycorrhizal root
169 infection was only recorded at 13 months.

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171 *2.4. DNA extraction, PCR and MiSeq Illumina sequencing*

172 Thirteen months after plantation, three jujube tree root systems for each treatment per
173 replicated block were sampled and pooled (a total of 32 root samples). Each composite root
174 sample was wrapped in tissue paper and placed in a plastic bag containing silica gel and

stored air-tight at room temperature. DNA was extracted from 40-50 mg of dried fine roots using a FastPrep-24 homogenizer (MP biomedicals Europe, Illkirch, France) and the FastDNA® SPIN kit (MP biomedicals, Europe) according to manufacturer's instructions. DNA extracts were then loaded onto PVPP (polyvinylpolypyrrolidone) Micro Bio-Spin® Columns (Bio-Rad, Marnes-la-Coquette, France) and eluted by centrifugation to improve DNA purity and avoid PCR inhibitors. Two replicates were done per composite root sample. The same approach was used to extract DNA from the AM fungal inoculum (20 g). DNA integrity was checked on 1.5 % agarose gel and stored at -20°C until used in the steps of gene amplification.

Molecular diversity of AM fungi (Glomeromycota) from plant DNA was assessed by 18S rRNA gene amplification with the primers NS31 and AML2 (Simon et al. 1992; Lee et al. 2008) according to Davison et al. (2012). PCR round was carried out in a final volume of 50 µl with NS31 and AML2 primers (0.6 µM each), 2 µl DNA (2 extracted DNA replicates per sample), 200 µM of each dNTP, 200 ng/ml BSA, GoTaq® DNA Polymerase (2 units) and 1X Green GoTaq® Reaction Buffer (Promega, Charbonnieres, France), with the following cycling conditions: 94°C for 3min; 30 cycles of 94°C for 30 s, 58°C for 90 s, 72°C for 80 s; a final elongation step at 72°C for 10 min. After PCR, the amplification products (pools of PCR: 2 × 50µl) were purified by using illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Velizy-Villacoublay, France) following manufacturer's guidelines. Then, DNA concentration of PCR products were quantified by using a Qubit fluorometer (Qubit Fluorometric quantitation, Invitrogen) and the Qubit dsDNA HS Assay Kit. PCR product concentration was adjusted to 10ng/µl and subjected to paired-end Illumina MiSeq sequencing (2×300 bp) by Molecular Research LP (MR DNA, TX, USA). The 18S rRNA gene from the AM fungal inoculum has been amplified with the primers AML1 and AML2 according Lee et al. (2008) and sequenced (Genoscreen, Lille, France). The sequence has been submitted to the NCBI database under accession number MH571752.

2.5. Bioinformatic data processing

MiSeq Illumina sequencing data were analysed by using Mothur software according the standard operating procedure (http://www.mothur.org/wiki/MiSeq_SOP) proposed in Kozich et al. (2013), except that only forward reads were analysed because of the length of PCR products not suitable for paired reads and of a higher quality of forwards reads compared to

reverse reads. All sequences were depleted of barcodes and primers and a quality cutoff of Q30 was selected. The sequences < 150 bp or with ambiguous base calls or with homopolymer runs exceeding 8bp were removed. A pre-clustering step (Huse et al. 2010) was also performed to remove sequences still likely due to illumina sequencing errors. Chimera were checked by Uchime (Edgar et al. 2011) implemented in Mothur software because it showed improved performance over the Chimera Slayer algorithm (Schloss et al. 2011). All sequences were first classified by using classify.seqs and a SILVA-compatible alignment database (Eukarya) to remove all no Glomeromycota sequences. Secondly, a preliminary clustering of sequences in OTUs with a 3 % divergence threshold was performed by using dist.seqs and cluster commands in Mothur, and all singleton OTUs were removed. The representative sequences of each OTU were then compared with a broader nucleotide database (Genbank database, BLASTN program) (http://www.ncbi.nlm.nih.gov/genbank), and all OTUs for which the representative sequence presented a similarity score < 95 % (100 % coverage) with the reference sequences were excluded of the data set. The number of sequences between each sample was then normalized with sub.sample command. This sub-sampling step allows reducing the number of spurious OTUs and is widely used to obtain robust estimation of alpha and beta diversity (Gihring et al. 2012). Finally, taxonomic affiliation of OTUs was done using classify.otu and the Glomeromycota-based 18S rDNA sequence database from Krüger et al. (2012). The taxonomic affiliation of OTUs was considered significantly robust for a given taxonomic level when the confidence threshold was superior to 50 % (https://rdp.cme.msu.edu/wiki/index.php/Classifier_Help). Raw data are available under the BioPproject ID PRJNA479949 (https://www.ncbi.nlm.nih.gov/bioproject).

2.6. Statistics

The AMF percentage colonization data were (arcsine x)$^{1/2}$ transformed to achieve homogeneity of variances. Means among all treatments (jujube cultivars, AM fungal inoculation or not, RP fertilization or not) were compared with three-way analysis of variance (ANOVA) followed by Tukey's HSD (Honestly significant differences (P < 0.05) using XLSTAT software (version 2010, Addinsoft).

Diversity (Shannon, inverse Simpson [1/D], coverage), richness (number of OTUs, Chao) and evenness indexes (Shannon index-based measure) were estimated. The sequencing effort was evaluated by using Boneh calculator (Boneh et al. 1998) implemented in Mothur. All indexes

were compared among all treatments using R version 3.3.1 (R Core Team , 2017) by three-way ANOVA followed by post-hoc Tukey test, as implemented in *aov*() and *TukeyHSD*() functions. AM fungal community membership among treatments (jujube cultivars, inoculation or not) was assessed using the *venn.diagram*() function from the R package VennDiagram version 1.6.17 (Chen, 2016). The differences in the AM fungal community structures among all treatments were based on the Bray-Curtis dissimilarity matrix and assessed using non-parametric permutational multivariate analysis of variance (PERMANOVA) as implemented in the *adonis*() function from the R package vegan version 2.4-3 (Oksanen et al. 2016). Multivariate dispersion was estimated for each treatment using the *betadisper*() function and *permutest*() as it can affect PERMANOVA results. The significance of AM fungal OTUs with respect to the jujube cultivar or the jujube status (inoculated or not, fertilized or not) was determined using the indicator value (IndVal) index, as implemented in *multipatt*() function from the R package indicspecies (De Cáceres and Legendre, 2009). Two different probabilities were calculated, *i.e.* A (specificity), representing the probability of a sample to be defined by a group (i.e., jujube cultivar, AM inoculation status, fertilization status), given that the OTU has been detected, and B (sensitivity) representing the probability of finding the OTU in different samples characterized by a given group. Only the OTUs present in more than half of samples for a given group are considered, i.e. B superior to 0.5. Table transformations in R were performed with the tidyverse packages version 1.1.1 (Wickham, 2017).

3. Results

3.1. Tree nursery – Growth, mineral nutrition and mycorrhizal colonization of jujube trees

Tree growth and nutrition of both cultivars were significantly improved by mycorrhizal inoculation with *Rhizophagus irregularis* IR27 (Table 1), with relatively stronger effects on the Gola cultivar compared to the Tasset cultivar. By contrast, fertilization with rock phosphate (RP) showed no effect on tree growth and nutrition, excepted regarding N nutrition for Gola cultivar, and no additional effect was observed when mycorrhizal inoculation was combined with RP fertilization compared to mycorrhizal inoculation only. A similar percentage of mycorrhizal infection was observed for both Tasset and Gola cultivars, reaching 65.8 % to 68.9 %, respectively. However, RP fertilization significantly decreased the mycorrhizal infection of both cultivars (Table 1).

3.2. Field monitoring – Survival, growth and mycorrhizal colonization of jujube trees

Beneficial effects (tree height and collar diameter) of mycorrhizal inoculation were observed on both jujube cultivars preliminary subjected to different mycorrhiza-based engineering strategies in tree nursery (mycorrhizal inoculation combined or not with RP fertilization), until the 13 months after outplanting. As observed in tree nursery, jujubes only fertilized with RP showed characteristics similar to controls during time.

Three months after planting, the rate of survival did not differ significantly between inoculated and non-inoculated plants, ranging from 75 % to 96 %. After 8 and 13 months, there was a significant increase in the rate of survival mediated by the mycorrhizal inoculation and mycorrhizal-fertilized treatments, notably for Tasset. The 13-month-old non-inoculated jujube trees showed low percentage of survival, 41.6 % and 45.8 % for Gola and Tasset respectively, whereas these percentage reach more than 70 % for the 13-month-old inoculated jujube trees, 70.8 % and 75 % for Gola and Tasset, respectively (Table 2). The height of jujube trees was the only parameter significantly different between 13-month-old inoculated jujube Gola (> 80 cm) and Tasset (< 75 cm) cultivars. In the mycorrhizal treatments, the highest values for height (81.2 cm) and collar diameter (24.8 mm) were recorded for Gola. The estimation of height and diameter evolution during 13 months after transplanting showed a stability of nursery-driven impacts, with substantial higher rates (slope value of linear regression) notably for the height of inoculated trees (Fig. S1).

Mycorrhizal colonization was observed at 13 months after transplanting in jujubes roots. Colonization levels were higher for all inoculated treatments of Gola (59.8 % inoculated and 50 % inoculated-fertilized) and Tasset (56.4 % inoculated and 46.4 % inoculated-fertilized) compared to non-inoculated controls and fertilized treatments (Table 2).

3.3. Field monitoring – Composition of the jujube root-associated AM fungal community

Overall, 285,783 sequences (forward reads) with a median length of 241 bp passed the initial quality assessment. Then, 166,737 sequences were retrieved after alignment denoising step, removal of chimera, non Glomeromycota sequences and singletons. In order to perform reliable comparison among samples, a normalization of sequence number was applied (number of sequence per sample set to 2,351), leading to a subset of 70,530 sequences. The clustering of final data revealed 239 AM fungal OTUs detected in a total of 30 composite root

samples. The majority of AM fungal OTUs belonged to Glomeraceae (94 % of total reads, 178 OTUs) (Table S1), and few OTUS to Diversisporaceae (3 %, 31 OTUs), Paraglomeraceae (2 %, 11 OTUs), Gigasporaceae (0.5 %, 8 OTUs), Acaulosporaceae (0.06 %, 6 OTUs), Geosiphonaceae (0.01 %, 3 OTUs), Claroideoglomeraceae (< 0.01 %, 1 OTU) and Pacisporaceae (<0.01 %, 1 OTUs). Glomeraceae OTUs belonged to *Sclerocystis* (28 % of Glomeraceae reads), *Rhizophagus* (27 %), Glomeraceae related to *Glomus* sensu lato (22 %), *Glomus* sensu stricto (20 %), and in a lesser extent to *Septoglomus* (4 %), *Funneliformis* (0.4 %) and unclassified Glomeraceae (0.02 %).

The native jujube root-associated AM fungal (untreated jujube trees) was composed of 85 (Gola) to 98 (Tasset) OTUs, with 80 % of sequences related to 15 known genera and 20 % only to Glomeraceae with uncertain position (unclassified Glomeraceae and *Glomus* sensu lato) (Table S2). A core AM fungal sub-community of 47 OTUs (93.4 % of sequences) (Fig. 1) was largely dominated by Glomeraceae, whose 26 % *Sclerocystis*, 18 % *Rhizophagus*, 28 % *Glomus* sensu stricto, 21 % *Glomus* sensu lato, 6 % *Septoglomus* and < 1 % *Funneliformis* (Fig. 1, Table S2). The cultivar Tasset presented the most diverse AM fungal community (Table 3), with a significant association of eight OTUs related to *Redeckera*, *Rhizophagus* and *Glomus* sensu stricto (Table S2). Three OTUs related to *Glomus* sensu stricto were significantly associated with the Gola cultivar but with a relatively low specificity (A < 0.7) (Table S2).

3.4. Field monitoring – Impact of ecological engineering strategies on the jujube root-associated AM fungal community

A robust diversity coverage was obtained for the AM fungal community associated with both jujube cultivars, independently of jujube status (inoculated or not, fertilized or not), reaching more than 99 %, and with Boneh estimates evaluated to less than seven OTUs (Table 3). After 13 months, AM fungal inoculation ($P < 0.001$) and the type of cultivar ($P < 0.05$) had significant effect on AM fungal community richness, whereas RP fertilization ($P < 0.05$) mostly impacted AM fungal community diversity (Table 3). However, results revealed a fertilization effect on AM fungal community richness ($P < 0.01$) and diversity ($P < 0.05$) highly dependent on AM fungal inoculation. No impact of ecological engineering strategies was observed on the evenness of AM fungal communities, but a relatively, low evenness was revealed, ranging from 0.4 to 0.6. Globally, mycorrhizal inoculation and RP fertilization

negatively impacted AM fungal richness and diversity, with the most significant impact observed for the local jujube cultivar Tasset when inoculated with *R. irregularis* IR 27. The use of RP fertilization in combination to mycorrhizal inoculation did not show a significant impact on AM fungal community richness and diversity compared to single treatments (inoculation or fertilization). Association analysis between each OTU and jujube cultivars or jujube status (inoculated or not, fertilized or not) revealed high significant associations for two OTUs related to *Rhizophagus* and *Glomus* sensu lato with the Tasset cultivar, independently of jujube status (Table 4), suggesting their stability through treatments. Fertilization status was characterized by the association with Glomeraceae OTUs, one with fertilized jujube trees and two with non-fertilize jujube trees independently of jujube cultivars and inoculation status (Table 4).

As observed for AM fungal community richness and diversity, jujube inoculation with *R. irregularis* IR27 appeared as the most significant treatment (P = 0.011) affecting the AM fungal community structure of jujube trees on the field (Table 5). Nevertheless, the type of cultivar and the RP fertilization significantly affected the inoculation impact on AM fungal community structure (P = 0.019). The analysis of AM fungal community membership among inoculated and non-inoculated jujube trees for the both cultivars Tasset and Gola (Fig. S2) revealed a predominant core AM fungal sub-community (97 % of sequences) composed of 26 OTUs, as well as rare 23 OTUs (0.1 %) only detected in jujube trees inoculated with *R. irregularis* IR27, and rare 66 OTUS (0.3 %) only in non-inoculated jujube trees. AM fungal inoculation of jujube trees negatively impacted (P < 0.05) the abundance of eight OTUs belonging to *Cetraspora* (OTU_31), *Gigaspora* (OTU_25), *Glomus* (OTU_07, OTU_39), *Redeckera* (OTU_08, OTU_34), *Rhizophagus* (OTU_04) and *Paraglomus* (OTU_12) for the Tasset cultivar, and eight OTUs belonging to *Glomus* sensu stricto (OTU_07, OTU_09, OTU_78), *Glomus* sensu lato (OTU_15, OTU_20), *Redeckera* (OTU_16, OTU_41), *Rhizophagus* (OTU_29) for the Gola cultivar (Fig. 2). However, two OTUs belonging to *Rhizophagus* (OTU_02, OTU_14) were positively impacted for the Gola cultivar (Fig. 2). It has to be noted that the comparison between the 18S rRNA gene sequence from the AM fungal inoculum and the representative sequence of each OTU revealed 100 % similarity with one the most dominant OTUs, i.e. OTU_2 related to *R. irregularis* (Table S1). Association analysis (Table 4) emphasized the global negative impact for five of these OTUs belonging to Glomeraceae and Gigasporaceae, independently of jujube cultivar and fertilization status.

4. Discussion

The improvement of plant growth through ecological engineering strategies, notably using mycorrhizal inoculants, constitute a sustainable approach for increased food security and ecosystem conservation (Rodriguez and Sanders, 2015; Hart et al. 2017). However, their long-term efficiency on field and their impact on native microbial communities remain critical issues for their adoption by national authorities and integration by end-users in agricultural and environmental practices. In this study, the efficiency and sustainability of two types of mycorrhiza-based ecological engineering strategies on jujubes were assessed from nursery to field.

4.1. Beneficial effects of mycorrhizal inoculation on jujube trees from nursery to field

The current nursery results emphasized the AM-mediated plant benefits previously observed on different jujube cultivars or provenances (Guissou et al. 1998, 2016; Sidibé et al. 2012; Thioye et al. 2017) in terms of growth and nutrient uptake (N, P, K). In addition, a higher P assimilation from RP of jujubes when inoculated by *R. irregularis* IR27 was confirmed compared to non-inoculated jujubes (Bâ et al. 1997). Nevertheless, no significant benefit was obtained when RP fertilization was used combined with mycorrhizal inoculation in comparison to mycorrhizal inoculation alone, as observed in Bâ et al. (2001). Some authors argue that mycorrhizal inoculation can be considered as a substitute of P fertilization in tree nursery management (Smith and Read 2008). In addition, RP fertilization negatively affected *R. irregularis* IR27 root colonization, which may suggest a non-optimum P-supply in the nursery conditions (Liu et al. 2016). The significance of increased P assimilation from RP through mycorrhiza has been already showed as unclear (Antunes et al. 2007), probably depending of biotic (mycorrhizal strain × host plant) and abiotic (soil or substrat P contents, provenance of RP) characteristics, and the duration of plant cultures (Bâ et al. 2001; Antunes et al. 2007; Khan et al. 2009). The mycorrhizal-mediated jujube nutritional benefit may explain the enhanced jujube growth performance, but non-nutritional benefits should also be investigated to fully decipher mycorrhizal-mediated plant fitness, notably on field (Delavaux et al. 2017; Lekberg and Koide, 2014).

The beneficial effects of mycorrhiza-based ecological engineering strategies used in nursery on jujube cultivars and their durability was evaluated on a field site characterized by degraded and arid conditions. The impacts of mycorrhizal inoculation in degraded or desertified

landscapes are expected to be highly significant because of low soil mycorrhizal potential (Hart et al. 2017), confirmed by current results. Consequently, a better establishment and growth of jujube trees were expected as for other plants in similar harsh conditions (Requena et al. 1996; Estaun et al. 1997; Duponnois et al. 2005; Bilgo et al. 2012). The most significant plant benefit was the rate of survival, which constitutes the primary target in horticulture, especially in such harsh environmental conditions as the ones encountered in the pan-African Great Green Wall (GGW) experimental sites. The benefit of mycorrhizal inoculation on jujube height was still significant after 13 months following outplanting but not on collar diameter. However, more plant parameters should be investigated in long term to fully evaluate the sustainability of mycorrhizal inoculation effects. A higher colonization rate was still observed between inoculated and non-inoculated jujubes in the 13-month-old orchard, but the differences observed in nursery between inoculated jujube seedlings with or without RP fertilization had disappeared likely due to native AM colonization. A two year-long field monitoring (Pellegrino et al. 2012) previously demonstrated the link between an increase colonization rate and yield increases, but a meta-analysis based on inoculation surveys between 1998 and 2003 confirmed this relationship for only 23 % of study sites (Lekberg and Koide, 2005). In addition, the benefit on field has to be put in perspective since jujube heights between untreated and treated (inoculation and/or fertilization) trees were different when transplanting. The monitoring of height-based or diameter-based growth rate tends to show a relative stability of pre-treatments in nursery, but more robust assessment of growth rates taking a higher number of plant parameters are needed. The better field survival of inoculated jujubes is evidently due to seedling status improvements (higher mycorrhizal infection rate, nutrition) in nursery and potentially to a residual effect of the AM inoculated strain.

4.2. Field environmental impacts of mycorrhizal inoculation on native AM fungal biodiversity

The range and sustainability of AM fungal-mediated plant benefits (biomass, yield, survival) are the most obvious concerns for end-users (Berruti et al. 2016), but the environmental impacts of AM fungal inoculant introduction in agroecosystems remains a critical issue. Three levels of environmental impacts were categorized (Rodriguez and Sanders, 2015), (i) alteration of composition and structure of native AM fungal population and/or community, (ii) exchange of genetic material with native AM fungal population and/or community, and (iii) persistence and/or spread of AM fungal inoculants, increasing consequently the first two impacts.

433 The native AM fungal community in jujube roots was targeted because considered as the
434 symbiotically AM fungal community (Chagnon et al. 2014; Hart et al. 2015), and differs
435 significantly from the soil (spore and extraradical mycelia) compartment (Varela-Cervero et
436 al. 2015). Although over-interpretations were suggested in experimental designs using
437 mycorrhizal-free plants as controls (Hart et al. 2017), the differences in mycorrhizal
438 infectivity between inoculated and non-inoculated jujube trees may indicate a higher
439 colonization of *R. irregularis* IR27 compared to the native AM fungal community because of
440 a priority effect due to pre-colonization of jujube roots in the nursery (Verbruggen et al. 2013;
441 Werner and Kiers, 2015). This hypothesis is emphasized by the predominance of OTU_2 in
442 roots of inoculated jujube trees, especially for the Gola cultivar. Indeed, this OTU may
443 indicate the persistence and abundance of *R. irregularis* IR27 since their 18S rRNA gene
444 fragment presented 100 % similarity. It has been demonstrated that the persistence and
445 abundance of an AM fungal strain could be promoted by the presence of other AM fungal
446 species (Hart et al. 2013), even if this AM fungal strain was not the most efficient one.
447 However, more informative methods should be used, notably because of the limited resolution
448 of 18S rRNA gene to distinguish certain AM fungal species (Hart et al. 2015). The new
449 advances in population genomic analysis (Savary et al. 2017) should be determinant to
450 evaluate not only the persistence of *R. irregularis*-based inocula but their spread, a major
451 environmental impact poorly investigated (Rodriguez and Sanders, 2015; Hart et al. 2017;
452 Janouskova et al. 2017). A second hypothesis may be the positive effect of *R. irregularis*
453 IR27 pre-colonization on the native AM fungal community colonization (Rodriguez and
454 sanders, 2015; Werner and Kiers, 2015). Beneficial interactions between AM fungal inocula
455 and the native AM fungal community have been suggested for field trials with *Olea europaea*
456 in semiarid, degraded land (Alguacil et al. 2011).

457 Few studies investigated in-depth the modifications of native AM fungal communities
458 following mycorrhizal inoculations, contrary to the impact of fertilization (Camenzind et al.
459 2014; Lin et al. 2012; Liu et al. 2016; Peyret-Guzzon et al. 2016; Williams et al. 2017). Most
460 of studies were based on low-throughput approaches and a limited number of community
461 characteristics (Pellegrino et al. 2012; Jin et al. 2013). The dominance of Glomeraceae and its
462 high frequency in the native AM fungal community of jujubes confirms the worldwide trend
463 described by Davison et al. (2015). These observations were hypothesized as a consequence
464 of its ruderal life strategy (Chagnon et al. 2013), i.e. early productions of spores, high growth
465 rates, higher intraradical host colonization, which is particularly adapted for early re-

colonization of host plants in degraded environments such as the ones encountered on the route of the GGW. The preferentially intraradical host colonization of Glomeraceae members may explained their predominance inside roots compared to others AM fungal families such as Pacisporaceae / Paraglomeraceae and Diversisporaceae / Gigasporaceae, which allocate their biomass mainly to the spores and the extraradical mycelium (ERM) (Goss et al. 2017). Glomeraceae members were also the main family allowing to differentiate the composition and response of native AM fungal community between both jujube cultivars, emphasizing the hypothesis that AM fungal species with a preferentially intraradical lifestyle are mostly affected by host characteristics (Sosa-Hernández et al. 2018). The native AM fungal community associated with jujube trees on the route of the GGW presented several particularities compared to the generally described AM fungal communities. First, *Rhizophagus* or *Funneliformis* or members of *Glomus sensu lato* generally constitute the most dominant genera in Glomeraceae in semiarid environments (Yamato et al. 2009; Alguacil et al. 2016; Torrecilas et al. 2012), but rarely *Glomus* sensu stricto or *Sclerocystis* as observed in the current study. Second, Paraglomeracae, a rare AM fungal family in AM fungal surveys (Davison et al. 2015), was the third most abundant family detected inside the roots, even if its abundance level remained low compared to Glomeraceae. This genus has been described as preferentially detected in soil compared to roots and ERM (Hempel et al. 2007; Varela-Cervero et al. 2015), probably due to its life strategy (see above). An in-depth AM survey in tropical African ecosystems revealed for the first time a high predominance of Paraglomeraceae in grasslands and open areas highlighting the existence of ecological specificity of AM fungi (Rodríguez-Echeverría et al. 2017).

An overall negative impact of the different treatments was observed on the native AM fungal community. The AM fungal richness was the characteristic the most affected by all treatments, notably inoculation. The pre-colonization of jujube roots in nursery by the exotic AM fungal strain was supposed to have a strong negative impact on the AM native fungal community colonization rate, but it was also supported by the persistence of the inoculum evidenced in the results (high level of OTU2). Richness is the main community characteristic assessed to monitor AM fungal community but a general trend remains difficult to define (Antunes et al. 2009; Mummey et al. 2009; Koch et al. 2011). For instance, whereas a negative impact was observed in the current study, a positive tendency had been observed in a 14-month-old olive orchard (Alguacil et al. 2011). When considering the global AM fungal community structure, only inoculation had a significant impact, leading to a negative effect on

abundance of few AM fungal OTUs. The importance to monitor multiple community characteristics appears essential, particularly given that it remains challenging to link a specific community characteristic to a beneficial or detrimental plant effect (Rodriguez and Sanders, 2015).

5. Conclusion

The current study constitutes an in-depth field investigation of mycorrhizal inoculation impact with an exotic isolate on native AM fungal community. Results clearly showed that ecological engineering strategies using *R. irregularis* significantly promote jujube performance (growth and nutrition) notably at very early stages in nursery, and highly improved the rate of survival on the field. In addition, a relative stability of nursery-driven plant benefits of inoculated jujube trees was observed. Nevertheless, the mycorrhizal field-observed benefits on jujube growth remain difficult to evaluate due to differences in jujube growth at outplanting. The comparison of a local (Tasset) and exotic (Gola) jujube cultivars pointed a potential higher persistence of AM fungal inoculum for the exotic and more limited disturbances of native AM fungal community. Results provide important insights to develop and improve the ecological management of jujube orchards on the route of the GGW (Senegal), but further investigations should be implemented to assess the long-term plant impact of such mycorrhiza-based ecological engineering strategies and to fully evaluate the persistence and spread of exotic mycorrhizal inocula *versus* native AM fungi. Further investigations are also required to evaluate the effect of inoculation with native AM fungi selected species or consortia of AM fungi. Understanding how introduced AM fungal strains interact and coexist with the native AM fungal community and whether this directly leads to changes in plant productivity is the key for an acceptation by stakeholders and national authorities of the use of AM fungi in agriculture, particularly in arid area where plant productivity sustainability is the major issue.

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References

Adelman, M.J., Morton, J.B., 1986. Infectivity of vesicular-arbuscular mycorrhizal fungi: Influence of host-soil diluent combinations on MPN estimates and percentage colonization. Soil Biol Biochem 18, 7–13. doi:10.1016/0038-0717(86)90106-9.

Alguacil, M.M., Torrecillas, E., Kohler, J., Roldan, A., 2011. A molecular approach to ascertain the success of “in situ” AM fungi inoculation in the revegetation of a semiarid, degraded land. Sci Total Environ 409, 2874–80.

Alguacil, M.M., Torres, M.P., Montesinos-Navarro, A., Roldán, A., 2016. Soil characteristics driving arbuscular mycorrhizal fungal communities in semiarid Mediterranean soils. Appl Environ Microbiol 82, 3348-56. doi:10.1128/AEM.03982-15.

Antunes, P.M., Koch, A.M., Dunfield, K.E., Hart, M., Downing, A., Rillig, M., 2009. Influence of commercial inoculation with Glomus intraradices on the structure and functioning of an AM fungal community from an agricultural site. Plant Soil 317:257. doi.org/10.1007/s11104-008-9806-y.

Antunes, P.M., Schneider, K., Hillis, D., Klironomos, J.N., 2007. Can the arbuscular mycorrhizal fungus *Glomus intraradices* actively mobilize P from rock phosphates? Pedobiologia 51, 281-286.

Arbonnier, M., 2000. Arbres, arbustes et lianes des zones sèches d’Afrique de l’Ouest. CIRAD, MNHN, UICN, Montpellier, France, pp. 438–442.

Bâ, A.M., Dalpé, Y., Guissou, T., 1996. Les Glomales d'*Acacia holosericea* A. Cunn. ex G. Don. et d'*Acacia mangium* Willd.: Diversité et abondance relative des champignons mycorhiziens à arbuscules dans deux types de sols de la zone Nord et Sud Soudanienne du Burkina Faso. Bois For Trop 250, 5–18.

Bâ, A.M., Duponnois, R., Guissou, T., Sanon, K.B., Dianou, D., 1997. Mobilization of rock phosphates by soil microorganisms associated with *Zizyphus mauritiana* Lam. and *Vigna unguiculata* (L.) Walp, in: Renard, G., Neef, A., Becker, K., von Oppen, M. (Eds), Soil Fertility Management in West African Land Use Systems, pp. 79–84.

Bâ, A.M., Plenchette, C., Danthu, P., Duponnois, R., Guissou, T., 2000. Functional compatibility of two arbuscular mycorrhizae with thirteen fruit trees in Senegal. Agrofor Syst 50, 95–105.

Bâ, A.M., Guissou, T., Duponnois, R., Plenchette, C., Danthu, P., Sacko, O., Sidibé, D., Sylla, K., Windou, B., 2001. Mycorhization contrôlée et fertilisation phosphatée: Application à la domestication du jujubier. Fruits 56, 261–269. doi:http://dx.doi.org/10.1051/fruits:2001128.

Bâ, A.M., Danthu, P., Duponnois, R., Soleviev, P., 2003. Domestication of jujube fruit trees (*Zizyphus mauritiana* Lam.), in : Dris, R., Niskanen, R., Jain, S.M. (Eds.), Crop Management and Postharvest Handling of Horticultural Crops, vol. 3, chap. 9, Crop fertilization, nutrition and growth, Science Publishers, Inc., pp. 255–279.

569 Bécard, G., Fortin, J.A., 1988. Early events of vesicular-arbuscular mycorrhiza formation on
570 Ri T-DNA transformed roots. New Phytol. 108, 211-218.

571 Bender, S.F., van der Heijden, M.G.A., 2015. Soil biota enhance agricultural sustainability by
572 improving crop yield nutrient uptake and reducing nitrogen leaching losses. J Appl Ecol
573 52, 228–239.

574 Berruti, A., Lumini, E., Balestrini, R., Bianciotto, V., 2016. Arbuscular Mycorrhizal Fungi as
575 Natural Biofertilizers: Let's Benefit from Past Successes. Front Microbiol 6:1559.

576 Boneh, S., Boneh, A., Caron, R.J., 1998. Estimating the prediction function and the number of
577 unseen species in sampling with replacement. J Am Stat Assoc 93, 372-379.

578 Bouffaud, M.L., Bernaud, E., Colombet, A., van Tuinen, D., Wipf, D., Redecker, D., 2016.
579 Regional-scale analysis of arbuscular mycorrhizal fungi: the case of Burgundy vineyards.
580 OENO One 50, 1-8. https://doi.org/https://doi.org/10.20870/oeno-one.2016.50.1.49.

581 Camenzind, T., Hempel, S., Homeier, J., Horn, S., Velescu, A., Wilcke, W., Rillig, M.C.,
582 2014. Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and
583 molecular diversity in a tropical montane forest. Glob Chang Biol 20, 3646-3659. doi:
584 10.1111/gcb.12618.

585 Ceballos, I., Ruiz, M., Fernández, C., Peña, R., Rodríguez, A., Sanders, I.R., 2013. The in
586 vitro mass-produced model mycorrhizal fungus, *Rhizophagus irregularis*, significantly
587 increases yields of the globally important food security crop cassava. PLoS One
588 8:e70633.

589 Chagnon, P.L., Bainard, L.D., 2014. Is root DNA a reliable proxy to assess arbuscular
590 mycorrhizal community structure? Can J Microbiol 60, 619-24. doi: 10.1139/cjm-2014-
591 0235.

592 Chen, H., 2016. VennDiagram: Generate High-Resolution Venn and Euler Plots. R package
593 version 1.6.17 [Internet]. Available: https://CRAN.R-
594 project.org/package=VennDiagram

595 Close DC, Beadle CL, Brown PH (2013) The physiological basis of containerised tree
596 seedling 'transplant shock': a review, Australian Forestry 68, 112-120. doi:
597 10.1080/00049158.2005.10674954.

598 Danthu, P., Soloviev, P., Touré, M.A., Gaye, A., 2002. Propagation végétative d'une variété
599 améliorée de jujubier (*Ziziphus mauritiana* Lam.) introduite au Sénégal. Bois et
600 Fruits des Tropiques 272, 93–96.

601 Danthu, P., Touré, M.A., Soloviev, P., Sagna, P., 2004. Vegetative propagation of *Ziziphus*
602 *mauritiana* var. Gola by micrografting and its potential for dissemination in the Sahelian
603 Zone. Agrofor Syst 60, 247–253.

604 Davison, J., Opik, M., Zobel, M., Vasar, M., Metsis, M., Moora, M., 2012. Communities of
605 arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not
606 vary throughout the growing season. PloS One 7:e41938.

607 Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Bâ, A., Burla, S., Diedhiou,
608 A.G., Hiiesalu, I., Jairus, T., Johnson, N.C., Kane, A., Koorem, K., Kochar, M., Ndiaye,
609 C., Pärtel, M., Reier, Ü., Saks, Ü., Singh, R., Vasar, M., Zobel, M., 2015. Global

assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349, 970-973.

De Cáceres, M., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566–3574. doi:10.1890/08-1823.1.

Delavaux, C.S., Smith-Ramesh, L.M., Kuebbing, S.E., 2017. Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. Ecology 98, 2111-2119.

Dia, A., Niang, A.M., 2010. Projet Majeur de la Grande Muraille Verte de l'Afrique : Concepts et mise en oeuvre. JOUVE (Eds.), pp. 11– 28.

Duponnois, R., Colombet, A., Hien, V., Thioulouse, J., 2005. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. Soil Biol Chem 37, 1460–1468.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194-2200.

Estaun, V., Save, R., Biel, C., 1997. AM inoculation as a biological tool to improve plant re-vegetation of a disturbed soil with *Rosmarinus officinalis* under semi-arid conditions. Applied Soil Ecology 6, 223–229.

Friesen, D.K., Rao, I.M., Thomas, R.J., Oberson, A., Sanz, J.I., 1997. Phosphorus acquisition and cycling in crop and pasture systems in low fertility tropical soils. Plant Soil 196, 289–294.

Garcia, K., Doidy, J., Zimmermann, S.D., Wipf, D., Courty, P.E., 2016. Take a Trip Through the Plant and Fungal Transportome of Mycorrhiza. Trends in Plant Science 21, 937-950.

Gihring, T.M., Green, S.J., Schadt, C.W., 2012. Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. Environ Microbiol 14, 285-290.

Goldbold, D.L., Sharrock, R., 2003. Mycorrhizas, in: Schroth, G., Sinclair, F.L. (Eds.), Trees, crops and soil fertility: concepts and research methods. CAB international, Wallingford, pp. 271–287.

Goss, M.J., Carvalho, M., Brito, I., 2017. Functional Diversity of Mycorrhiza and Sustainable Agriculture - Management to Overcome Biotic and Abiotic Stresses. Academic Press, Oxford, pp. 254, eBook ISBN: 9780128042861 Paperback ISBN: 9780128042441

Guissou, T., Bâ, A.M., Ouadba, J.M., Guinko, S., Duponnois, R., 1998. Responses of *Parkia biglobosa* (Jacq.) Benth., *Tamarindus indica* L. and *Ziziphus mauritiana* Lam. to arbuscular mycorrhizal fungi in a phosphorus-deficient sandy soil. Biol Fertil Soils 26, 194–198.

Guissou, T., 2009. Contribution of arbuscular mycorrhizal fungi to growth and nutrient uptake by jujube and tamarind seedlings in a phosphate (P)-deficient soil. Afr J Microbiol Res 3, 297–304.

Guissou, T., Sanon, K.B., Babana, A., Bâ, A.M., 2016. Effect of of arbuscular mycorrhizae on growth and mineral nutrition of greenhouse propagated fruit trees from diverse geographic provenances. Biotech Agro Soc Environ 20, 417-426.

651 Hart, M.M., Aleklett. K., Chagnon, P.L., Egan, C., Ghignone, S., Helgason, T., Lekberg, Y.,
652 Öpik, M., Pickles, B.J., Waller, L., 2015. Navigating the labyrinth: a guide to sequence-
653 based, community ecology of arbuscular mycorrhizal fungi. New Phytol. 207, 235-47.
654 doi: 10.1111/nph.13340.

655 Hart, M.M., Antunes, P.M., Chaudhary, V.B., Abbott, L.K., 2017. Fungal inoculants in the
656 field: Is the reward greater than the risk? Funct Ecol. https://doi.org/10.1111/1365-
657 2435.12976.

658 Hart, M., Forsythe, J., Oshowski, B., Bücking, H., Jansa, J., Kiers, E.T., 2013. Hiding in a
659 crowd-does diversity facilitate persistence of a low-quality fungal partner in the
660 mycorrhizal symbiosis? Symbiosis 59, 47–56.

661 Helgason, T., Merryweather, J.W., Young, J.P.W., Fitter, A.H., 1998. Where have all the
662 fungi gone ? I. Soil disturbance alters the diversity of arbuscular mycorrhizas of
663 bluebell growing in semi-natural woodland. Journal of Experimental Botany 49:40.

664 Hempel, S., Renker, C., Buscot, F., 2007. Differences in the species composition of
665 arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland
666 ecosystem. Environ Microbiol 9, 1930–1938. doi:10.1111/j.1462-2920.2007.01309.x.

667 Huse, S.M., Welch, D.M., Morrison, H.G., Sogin, M.L., 2010. Ironing out the wrinkles in the
668 rare biosphere through improved OTU clustering. Environ Microbiol 12, 1889-1898.

669 Janoušková, M., Krak, K., Vosátka, M., Püschel, D., Štorchová, H., 2017. Inoculation effects
670 on root-colonizing arbuscular mycorrhizal fungal communities spread beyond directly
671 inoculated plants. PLoS One 12:e0181525. doi: 10.1371/journal.pone.0181525.

672 Jin, H., Germida, J.J., Walley, F.L., 2013. Impact of arbuscular mycorrhizal fungal inoculants
673 on subsequent arbuscular mycorrhizal fungi colonization in pot-cultured field pea
674 (*Pisum sativum* L.). Mycorrhiza 23, 45-59.

675 Khan, M.S., Zaidi, A., Ahemad, M., Oves, M., Wani, P.A., 2009. Plant growth promotion by
676 phosphate solubilizing fungi – current perspective, Archives of Agronomy and Soil
677 Science 56, 73-98. doi: 10.1080/03650340902806469.

678 Koide, R.T., Mosse, B., 2004. A history of research on arbuscular mycorrhiza. Mycorrhiza
679 14, 145–163. doi:10.1007/s00572-004-0307-4.

680 Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013.
681 Development of a dual-index sequencing strategy and curation pipeline for analyzing
682 amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ
683 Microbiol 79, 5112–5120.

684 Krüger, M., Krüger, C., Walker, C., Stockinger, H., Schüssler, A., 2012. Phylogenetic
685 reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from
686 phylum to species level. New phytol. 193, 970-984.

687 Lee, J., Lee, S., Young, J.P.W., 2008. Improved PCR primers for the detection and
688 identification of arbuscular mycorrhizal fungi. FEMS Microbiol Ecol 65, 339–349.

689 Lekberg, Y., Koide, R.T., 2005. Is plant performance limited by abundance of arbuscular
690 mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New
691 Phytol. 168, 189-204.
692 Lekberg, Y., Koide, R.T., 2014. Integrating physiological, community, and evolutionary
693 perspectives on the arbuscular mycorrhizal symbiosis. Botany 92, 241–251.
694 Lin, X., Feng, Y., Zhang, H., Chen, R., Wang, J., Zhang, J., Chu, H., 2012. Long-term
695 balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil
696 in north China revealed by 454 pyrosequencing. Environmental Science and
697 Technology 46, 5764-5771.
698 Liu, W., Zhang, Y., Jiang, S., Deng, Y., Christie, P., Murray, P.J., Li, X., Zhang, J., 2016.
699 Arbuscular mycorrhizal fungi in soil and roots respond differently to phosphorus inputs
700 in an intensively managed calcareous agricultural soil. Sci Rep 6:24902.
701 Mathur, N., Vyas, A., 2000. Influence of arbuscular mycorrhizae on biomass production,
702 nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water
703 stress. Journal of Arid Environments 45, 191-195.
704 Mummey, D.L., Antunes, P.M., Rillig, M.C., 2009. Arbuscular mycorrhizal fungi
705 preinoculant identity determines community composition in roots. Soil Biology and
706 Biochemistry 41, 1173–1179.
707 Nziguheba, G., Zingore, S., Kihara, J., Merckx, R., Njoroge, S., Otinga, A., Vandamme, E.,
708 Vanlauwe, B., 2015. Phosphorus in smallholder farming systems of sub-Sahara Africa:
709 implication for agricultural intensification. Nutrient Cycling in Agroecosystems.
710 http://dx.doi.org/10.1007/s10705-015-9729-y.
711 Okafor, J.C., 1991. Amélioration des essences forestières donnant des produits
712 comestibles. Unasylva 165, 17-23.
713 Öpik, M., Moora, M., Liira, J., Zobel, M., 2006. Composition of root-colonizing arbuscular
714 mycorrhizal fungal communities in different ecosystems around the globe. Journal of
715 Ecology 94, 778-790.
716 Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J.M., Reier, U., Zobel,
717 M., 2010. The online database MaarjAM reveals global and ecosystemic distribution
718 patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol. 188, 223–241.
719 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al., 2016.
720 vegan: Community Ecology Package. R package version 2.4-1 [Internet]. Available:
721 https://CRAN.R-project.org/package=vegan.
722 Öpik, M., Davison, J., Moora, M., Zobel, M., 2014. DNA-based detection and identification
723 of *Glomeromycota*: the virtual taxonomy of environmental sequences. Botanique 92,
724 135-147.
725 Parniske, M., 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev
726 Microbiol 6, 763–775. doi:10.1038/nrmicro1987.

727 Pellegrino, E., Turrini, A., Gamper, H.A., Cafà, G., Bonari, E., Young, J.P., et al., 2012.
728 Establishment, persistence and effectiveness of arbuscular mycorrhizal fungal
729 inoculants in the field revealed using molecular genetic tracing and measurement of
730 yield components. New Phytol. 194, 810–822.

731 Peyret-Guzzon, M., Stockinger, H., Bouffaud, M.L., Farcy, P., Wipf, D., Redecker, D., 2016.
732 Arbuscular mycorrhizal fungal communities and *Rhizophagus irregularis* populations
733 shift in response to short-term ploughing and fertilisation in a buffer strip. Mycorrhiza
734 26, 33-46. doi:10.1007/s00572-015-0644-5.

735 Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining
736 parasitic and Vesicular-Arbuscular Mycorrhizal fungi for rapid assessment of infection.
737 Trans Br Mycol Soc 55, 158–161.

738 Quince, C., Lanzen, A., Davenport, R.J., Turnbaugh, P.J., 2011. Removing noise from
739 pyrosequenced amplicons. BMC Bioinformatics 12:38.

740 R Core Team. R: A Language and Environment for Statistical Computing [Internet]. R
741 Foundation for Statistical Computing, Vienna, Austria; 2017. Available: https://www.R-
742 project.org.

743 Ræbild, A., 2012. Improved management of fruit trees in West African parklands. Agrofor
744 Syst 85, 425–430.

745 Redecker, D., 2000. Glomalean fungi from the ordovician. Science 289, 1920–1921.
746 doi:10.1126/science.289.5486.192.

747 Reena, J., Bagyaraj, D.J., 1990. Growth stimulation of *Tamarindus indica* by selected VA
748 mycorrhizal fungi. World J Microbiol Biotechnol 6, 59–63.

749 Requena, N., Jeffries, P., Barea, J.M., 1996. Assessment of natural mycorrhizal potential in a
750 desertified semiarid ecosystem. Appl Environ Microbiol. 62, 842-847.

751 Rodriguez, A., Sanders, I.R., 2015. The role of community and population ecology in
752 applying mycorrhizal fungi for improved food security. ISME J 9, 1053-1061.

753 Rodríguez-Echeverría, S., Teixeira, H., Correia, M., Timóteo, S., Heleno, R., Öpik, M.,
754 Moora, M., 2017. Arbuscular mycorrhizal fungi communities from tropical Africa
755 reveal strong ecological structure. New Phytol. 213, 380-390. doi: 10.1111/nph.14122.

756 Savary, R., Masclaux, F.G., Wyss, T., Droh, G., Cruz, Corella. J., Machado, A.P., Morton,
757 J.B., Sanders, I.R., 2018. A population genomics approach shows widespread
758 geographical distribution of cryptic genomic forms of the symbiotic fungus
759 *Rhizophagus irregularis*. ISME J 12, 17-30. doi: 10.1038/ismej.2017.153.

760 Schloss, P.D., Gevers, D., Westcott, S.L., 2011. Reducing the Effects of PCR Amplification
761 and Sequencing Artifacts on 16S rRNA-Based Studies. PLoS ONE 6:e27310.

762 Schnoor, T.K., Lekberg, Y., Rosendahl, S., Olsson, P.A., 2011. Mechanical soil disturbance
763 as a determinant of arbuscular mycorrhizal fungal communities in semi-natural
764 grassland. Mycorrhiza 21, 211-220.

765 Schüßler, A., Schwarzott, D., Walker, C., 2001. A new fungal phylum, the Glomeromycota:
766 phylogeny and evolution. Mycol Res 105, 1413–1421.
767 doi:http://dx.doi.org/10.1017/S0953756201005196.

768 Schüßler, A., Walker, C., 2010. The Glomeromycota: a species list with new families and
769 genera. Edinburgh & Kew, UK: The Royal Botanic Garden; Munich, Germany:
770 Botanische Staatssammlung Munich; Oregon, USA: Oregon State University. URL:
771 http://www.amfphylogeny.com. ISBN-13: 9781466388048; ISBN-10: 1466388048

772 Sidibé, D., Sanou, H., Teklehaimanot, Z., Mahamadi, D., Kone, S., 2012. The use of
773 mycorrhizal inoculation in the domestication of *Ziziphus mauritiana* and *Tamarindus*
774 *indica* in Mali (West Africa). Agrofor Syst 85, 519–528. doi:10.1007/s10457-0129486-
775 8.

776 Simon, L., Lalonde, M., Bruns, T.D., 1992. Specific amplification of 18S fungal ribosomal
777 genes from VA endomycorrhizal fungi colonizing roots. Appl Environ Microbiol 58,
778 291–295.

779 Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, third ed. Academic Press, London.

780 Sosa-Hernández, M.A., Roy, J., Hempel, S., Kautz, T., Köpke, U., Uksa, M., Schloter, M.,
781 Caruso, T., Rillig, M.C., 2018. Subsoil arbuscular mycorrhizal fungal communities in
782 arable soil differ from those in topsoil. Soil Biology and Biochemistry 117, 83-86.
783 https://doi.org/10.1016/j.soilbio.2017.11.009.

784 St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J.A., 1996. Enhanced hyphal
785 growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices*
786 in an in vitro system in the absence of host roots. Mycol Res 100, 328-332.

787 Thioye, B., Mania, S., Kane, A., Ndiaye, C., Soule, A.O., Fall, D., Duponnois, R., Sylla, S.N.,
788 Bâ, A.M., 2017. Growth response of different species of *Ziziphus* to inoculation with
789 arbuscular mycorrhizal fungi. Fruits 72, 174-181.

790 Tichý, L., Chytrý, M., 2006. Statistical determination of diagnostic species for site groups of
791 unequal size. J Veg Sci 17, 809–818.

792 Torrecillas, E., Alguacil, M.M., Roldán, A., 2012. Host preferences of arbuscular mycorrhizal
793 fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies.
794 Appl Environ Microbiol 78, 6180-6. doi: 10.1128/AEM.01287-12.

795 Trouvelot, A., Kough, J.L., Gianinazzi-Pearson, V., 1986. Mesure du taux de mycorhization
796 VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une
797 signification fonctionnelle, in: Gianinazi-Pearson, V., Gianinazzi, S., (Eds.), Physiology
798 and genetics aspects of mycorrhizae. INRA, Paris, pp. 217–221

799 Uhlmann, E., Görke, C., Petersen, A., Oberwinkler, F., 2004. Arbuscular mycorrhizae from
800 semiarid regions of Namibia. Canadian Journal of Botany 82, 645-653.
801 https://doi.org/10.1139/b04-039.

802 van der Heijden, M.G.A, Boller, T., Wiemken, A., Sanders, I.R., 1998. Different arbuscular
803 mycorrhizal fungal species are potential determinants of plant community structure.
804 Ecology 79, 2082–2091.

805 Varela-Cervero, S., Vasar, M., Davison, J., Barea, J.M., Öpik, M., Azcón-Aguilar, C., 2015.
806 The composition of arbuscular mycorrhizal fungal communities differs among the roots,

spores and extraradical mycelia associated with five Mediterranean plant species. Environ Microbiol 17, 2882-95. https://doi.org/10.1111/1462-2920.12810.

Vashishtha, B.B., 1997. *Ziziphus* for drylands – a perennial crop solving perennial problems. Agroforestry Today 9, 10–12.

Verbruggen, E., van der Heijden, M.G., Rillig, M.C., Kiers, E.T., 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. New Phytol. 197, 1104–1109. https://doi.org/10.1111/j.1469-8137.2012.04348.x.

Williams, A., Manoharan, L., Rosenstock, N.P., Olsson, P.A., Hedlund, K., 2017. Long-term agricultural fertilization alters arbuscular mycorrhizal fungal community composition and barley (*Hordeum vulgare*) mycorrhizal carbon and phosphorus exchange. New Phytol 213, 874-885. doi: 10.1111/nph.14196.

Willis, A., Rodrigues, B.F., Harris, P.J.C., 2013. The ecology of arbuscular mycorrhizal fungi. Crit Rev Plant Sci 32, 1–20.

Wickham, H., 2017. Tidyverse: Easily Install and Load "Tidyverse" Packages. R package version 1.1.1. [Internet]. Available: https://CRAN.R-project.org/package=tidyverse.

Werner, G.D., Kiers, E.T., 2015. Order of arrival structures arbuscular mycorrhizal colonization of plants. New Phytol 205, 1515-24.

Yamato, M., Ikeda, S., Iwase, K., 2009. Community of arbuscular mycorrhizal fungi in drought-resistant plants, *Moringa* spp., in semiarid regions in Madagascar and Uganda. Mycoscience 50, 100-105. https://doi.org/10.1007/S10267-008-0459-8.

Table 1 Effect of *Rhizophagus irregularis* IR27 inoculation and rock phosphate fertilization on growth, mycorrhizal colonization and mineral nutrition of 4-month-old jujube (*Z. mauritiana*) tree cultivars (Tasset, Gola) (tree nursery).

Treatment	Height (cm)	Collar diameter (mm)	Total dry biomass (g)	Mycorrhizal colonization (%)	N (%)	P (%)	K (%)
Gola	19.7 ± 2.3 c	2.8 ± 0.4 cd	1.4 ± 0.1 cd	0.0 d	1.3 ± 0.1 b	1.3 ± 0.7 d	08.4 ± 1.0 d
Gola+RP	22.6 ± 4.4 c	3.2 ± 0.3 c	1.6 ± 0.1 c	0.0 d	2.4 ± 0.4 a	1.5 ± 0.6 cd	10.0 ± 2.0 bcd
Gola+Ri	54.3 ± 8.6 a	4.8 ± 0.6 a	3.6 ± 0.5 a	68.9 ± 14.1 a	2.4 ± 0.2 a	2.7 ± 0.2 a	14.6 ± 0.9 a
Gola+Ri+RP	50.2 ± 9.6 a	4.5 ± 0.4 ab	3.5 ± 0.5 a	51.4 ± 14.9 b	2.5 ± 0.4 a	2.6 ± 0.1 ab	14.3 ± 0.5 a
Tasset	15.9 ± 2,4 d	2.5 ± 0.3 c	1.3 ± 0.1 d	0.0 d	1.3 ± 0.1 b	1.2 ± 0.1 d	09.4 ± 0.7 cd
Tasset+RP	17.8 ± 4.0 cd	3.1 ± 0.4 c	1.3 ± 0.1 cd	0.0 d	1.6 ± 0.2 b	1.2 ± 0.2 d	08.7 ± 1.1 d
Tasset+Ri	35.9 ± 6.9 b	4.1 ± 0.9 b	2.6 ± 0.4 b	65.8 ± 11.5 a	2.1 ± 0.0 a	2.1 ± 0.0 bc	11.0 ± 0.6 bc
Tasset+Ri+RP	37.7 ± 7.5 b	4.1 ± 0.9 b	2.7 ± 0.5 b	38.7 ± 15.6 c	2.1 ± 0.1 a	2.3 ± 0.0 ab	11.3 ± 0.4 b
All treatments							
Cultivar (C)	***	**	***	ns	**	*	**
Inoculation (I)	***	***	***	***	***	***	***
Fertilization (F)	ns	ns	ns	ns	**	ns	ns
(C) × (I)	***	ns	***	ns	ns	ns	***
(I) × (F)	ns	**	ns	ns	**	ns	ns

(C) × (F)	ns	ns	ns	ns	ns	ns	ns
(C) × (I) × (F)	ns	ns	ns	***	ns	ns	ns

Values in columns followed by the same letter do not differ significantly ($P < 0.05$) according to Tukey's HSD. Significant levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant; Ri: *Rhizophagus irregularis* IR27; RP: Rock phosphate.

Table 2 Effect of *Rhizophagus irregularis* IR27 inoculation and rock phosphate fertilization on growth, rate of survival and mycorrhizal colonization of jujube tree cultivars after transplanting.

Treatment	3 months after planting			8 months after planting			13 months after planting			
	Height (cm)	Collar diameter (mm)	Rate of Survival (%)	Height (cm)	Collar diameter (mm)	Rate of Survival (%)	Height (cm)	Collar diameter (mm)	Rate of survival (%)	Mycorrhizal colonization (%)
Gola	24.5±3.1 de	07.2±1.2 de	83.3±38.7 ab	29.1±3.3 cd	13.8±2.2 de	50.0±51.0 cd	38.5±8.7 d	17.9±1.8 bc	41.6±50.3 d	21.4±4.8 c
Gola+RP	27.2±3.3 cd	08.0±1.6 cd	95.8±20.4 a	31.4±3.5 c	16.5±3.5 abcd	66.6±48.1 bc	39.2±3.8 d	21.8±2.0 ab	54.1±50.8 c	21.8±4.2 c
Gola+Ri	59.9± 8.8 a	09.3±1.9 ab	91.6±28.2 ab	66.3±12.9 a	16.9±2.0 abc	83.3±38.0 ab	81.2±13.8 a	20.6±3.5 abc	70.8±46.4 ab	59.8±5.7 a
Gola+Ri+RP	55.6±11.1 ab	10.2±1.8 a	95.8±20.0 a	63.4±9.8 a	19.3±4.9 a	91.6±28.2 a	79.5±11.1 ab	24.8±3.8 a	66.6±48.1 abc	50.0±8.1 ab
Tasset	18.9±5.2 de	06.7±1.7 e	79.1±41.4 ab	22.7±3.4 d	13.1±1.2 bcde	62.5±49.4 bcd	34.6±3.5 d	18.1±1.6 bc	45.8±50.8 d	26.0±3.7 c
Tasset+RP	20.4±6.9 e	07.2±1.8 de	75.0±44.2 b	26.1±6.7cd	12.8±2.1 e	41.6±50.3 d	37.6±4.6 d	15.3±1.3 c	41.6±50.3 d	21.0±4.7 c
Tasset+Ri	40.1±6.8 bc	09.2±1.9 ab	91.6±28.2 ab	51.2±9.6 b	17.6±2.6 ab	83.3±38.0 ab	74.9±15.7 c	20.9±2.3 abc	75.0±44.2 a	56.4±7.3 ab
Tasset+Ri+RP	42.1±11.2 bc	08.6±2.8 bc	83.3±38.0 ab	53.1±6.6 ab	16.7±2.4 cde	70.8±46.4 abc	77.2±38.3 abc	19.7±2.4 abc	62.5±49.4 bc	46.4±5.2 b

Factors tested [1]										
Cultivar (C)	**	***	ns	**	*	ns	ns	ns	ns	ns
Inoculation (I)	***	***	ns	***	***	***	**	*	**	***
Fertilization (F)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(C) × (I)	**	ns	ns	**	ns	**	**	ns	*	**
(C) × (F)	ns	*	ns	ns	*	ns	ns	ns	ns	ns
(I) × (F)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
(C) × (I) × (F)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1] The block factor was not significant ($P > 0.05$)

Values in columns followed by the same letter do not differ significantly according to Tukey's HSD. Significant levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant; Ri: *Rhizophagus irregularis*; RP: rock phosphate; Mycorrhizal infection was only assessed at 13 months after planting.

Table 3 Effect of jujube cultivar, mycorrhizal inoculation and rock phosphate fertilization on richness, diversity and evenness of the jujube root-associated AM fungal community.

	Richness		Diversity		Evenness		
Treatment	Number of OTUs	Chao	Shannon	Invsimpson	Shannoneven	Boneh	Coverage (%)
Gola	46 ± 1 b	76 ± 6 a	1.9 ± 0.1 ab	4.4 ± 0.5 b	0.5 ± 0.0 a	7 ± 0.3	99.2
Gola+RP	34 ± 5 cd	50 ± 7 bc	1.4 ± 0.5 bc	2.7 ± 1.0 b	0.4 ± 0.1 a	4 ± 1.0	99.5
Gola+Ri	33 ± 6 cd	51 ± 6 bc	1.7 ± 0.2 b	3.9 ± 0.6 b	0.5 ± 0.0 a	6 ± 0.5	99.4
Gola+Ri+RP	34 ± 2 cd	53 ± 5 bc	1.5 ± 0.4 bc	3.9 ± 1.2 b	0.4 ± 0.1 a	6 ± 0.5	99.4
Tasset	53 ± 5 a	75 ± 8 a	2.3 ± 0.1 a	6.5 ± 1.0 a	0.6 ± 0.0 a	7 ± 1.1	99.3
Tasset+RP	42 ± 3 bc	72 ± 7 a	1.7 ± 0.1 b	3.8 ± 0.9 b	0.5 ± 0.0 a	7 ± 1.0	99.2
Tasset+Ri	30 ± 6 d	46 ± 1 c	1.2 ± 0.7 c	3.1 ± 2.0 b	0.4 ± 0.2 a	5 ± 0.4	99.5
Tasset+Ri+RP	38 ± 5 bcd	61 ± 8 b	1.6 ± 0.2 bc	3.4 ± 1.0 b	0.4 ± 0.0 a	6 ± 1.5	99.3
All treatments							
Cultivar (C)	*	**	ns	ns	ns	-	-
Inoculation (I)	***	***	*	ns	ns	-	-
Fertilization (F)	*	ns	*	**	ns	-	-
(C) × (I)	ns	ns	ns	ns	ns	-	-

(C) × (F)	ns	***	ns	ns	ns	-	-
(I) × (F)	***	***	*	*	ns	-	-
(C) × (I) × (F)	ns	ns	ns	ns	ns	-	-

Values in columns followed by the same letter do not differ significantly according to Tukey's HSD. Significant levels: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant. Ri: *Rhizophagus irregularis* IR27; RP: rock phosphate.

Table 4 Characterization of AM fungal indicator of jujube cultivars and jujube status (inoculated or not, fertilized or not).

OTU label taxonomic assigment [1]	Abundance [2]	Jujube cultivar			Jujube status								
		Tasset			Non-inoculated			Fertilized			Non-fertilized		
		A	B	Index	A	B	Index	A	B	Index	A	B	Index
Glomeraceae													
OTU_33 – Un. Glomeraceae		1.00	0.60	0.775***									
OTU_35 - *Rhizophagus*		0.97	0.53	0.721**									
OTU_07 - *Glomus*					0.98	1.00	0.991***						
OTU_09 - *Glomus*					0.98	1.00	0.991**						
OTU_25 - *Gigaspora*					0.81	0.81	0.991*						
OTU_31 - *Cetraspora*					0.86	0.75	0.805**						
OTU_39 - *Glomus*					0.92	0.56	0.719*						
OTU_20 - Un. Glomeraceae								0.90	0.800	0.84*			
OTU_15 - Un. Glomeraceae											0.90	1.00	0.954*
OTU_22 - *Glomus*											0.93	0.87	0.900***

[1]Taxonomic affiliation was based on a k-nearest neighbor consensus and the Wang method used in Mothur (function classify.otu) using reference sequences from Krüger et al (2012). Genus level is indicated when confidence threshold is superior to 95, if not the higher taxonomic level is indicated. [2]OTU abundance corresponds to the number of reads. [3]Indicator OTUs were obtained using indicator value (IndVal.g) index as implemented in *multipatt*() function from R

package indicspecies (De Cáceres and Legendre, 2009). [3]Statistics were obtained using 9,999 permutations. Significance code: '***' $P<0.001$; '**' $P<0.01$; '*' $P<0.05$. A and B correspond to specificity and sensibility. Only the OTUs present in more than half of samples for a given group are considered, i.e. B superior to 0.5. "Un. Glomeraceae" indicates affiliation to references belonging to *Glomus* sensu lato, for which uncertain position in Glomeraceae has been described.

Table 5 Impact of jujube cultivar, AM fungal inoculation and RP fertilization on the jujube root-associated AM fungal community structure (field experiment).

Treatments	*df*	SS	MS	*F* model	R^2	*P* value[1]
Cultivar (C)	1	0.253	0.253	1.224	0.038	0.268^{ns}
Inoculation (I)	1	0.563	0.563	2.726	0.084	0.011*
Fertilization (F)	1	0.199	0.199	0.967	0.029	0.470^{ns}
(C) × (I)	1	0.135	0.135	0.655	0.020	0.722^{ns}
(C) × (F)	1	0.343	0.343	1.660	0.051	0.113^{ns}
(I) × (F)	1	0.130	0.130	0.630	0.019	0.767^{n}
(C) × (I) × (F)	1	0.496	0.496	2.400	0.074	0.019*
Residuals	22	4.546	0.206		0.681	
Total	29	6.668			1	

[1]PERMANOVA was based Bray-Curtis dissimilarity matrix and assessed using *adonis*() function (iterations = 9,999 permutations). '*' $P < 0.05$; '^{ns}' $P > 0.05$. Multivariate dispersion was tested using the *betadisper*() and *permutest*() functions (iterations = 9,999 permutations; alpha = 0.05) revealing a significant homogeneity of group dispersions. *df* = degrees of freedom; SS = sum of squares; MS = mean sum of squares; *F* model = *F* statistics; R^2= partial R-squared.

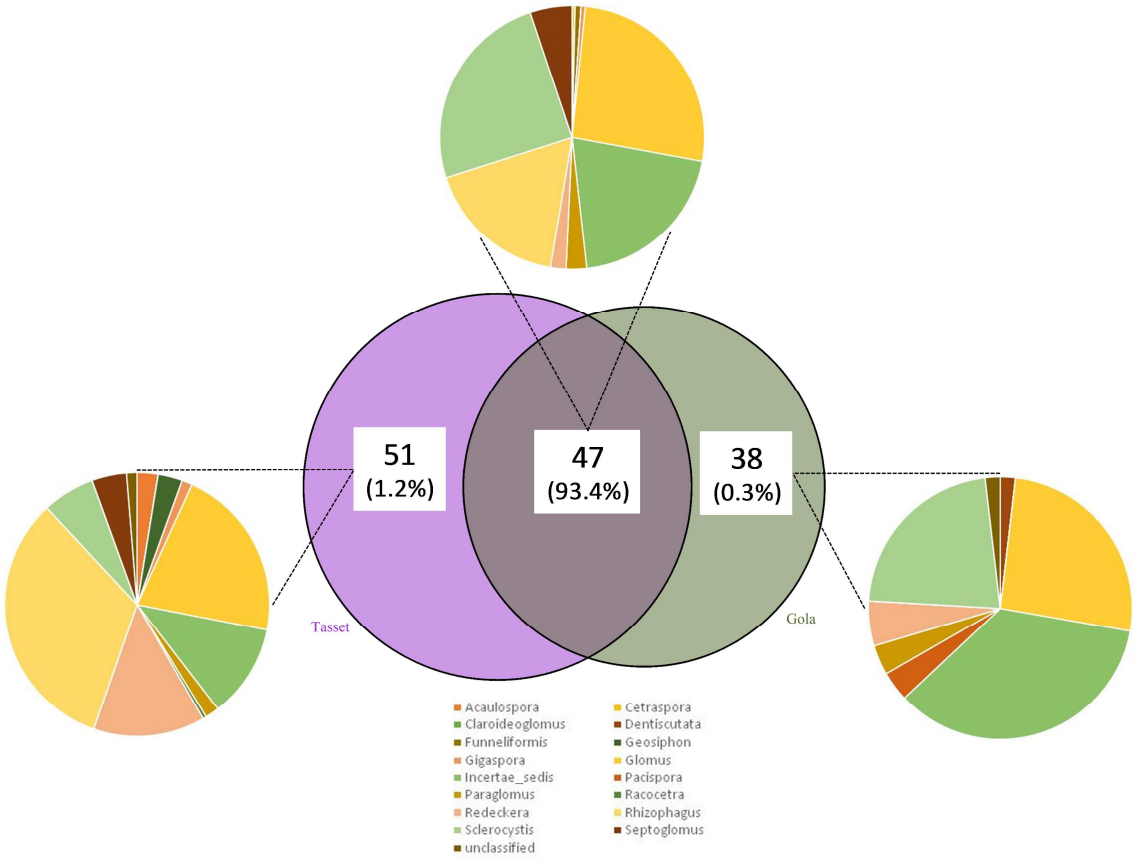

Fig. 1. Comparison of AM fungal community membership (Venn diagram analysis) between non-inoculated jujube trees from the two cultivars Tasset and Gola. All sequences were clustered in OTUs (97 % similarity). For each Venn category, the number of OTU and the relative abundance (% of sequences) are indicated. Color pie charts represent the abundance of OTUs shared between the two cultivars and specific to each. “incertae sedis” represents OTUs related to *Glomus* sensu lato for which uncertain position in Glomeraceae has been described, and “unclassified” OTUs affiliated only to Glomeraceae level.

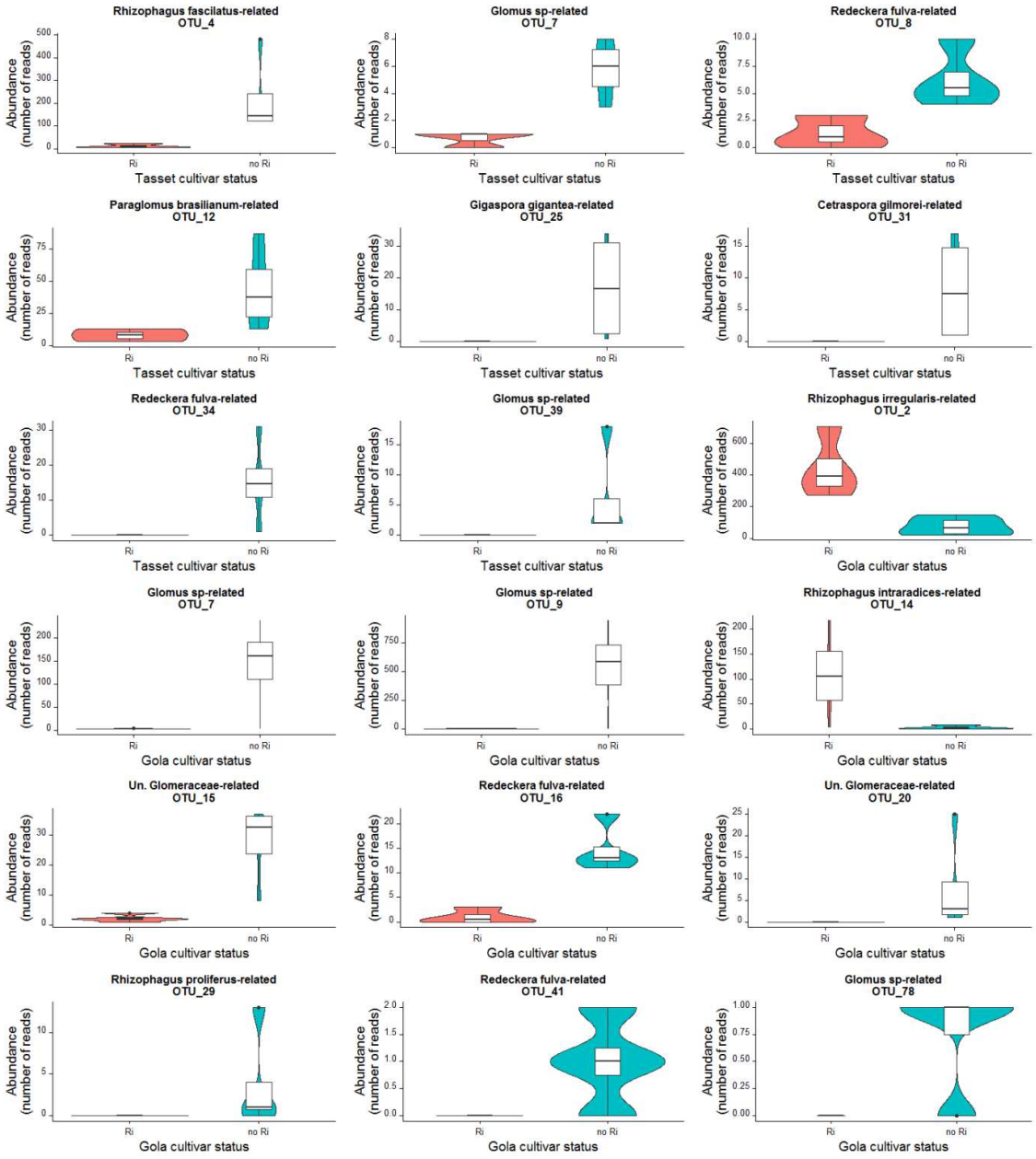

Fig. 2. Changes in abundance of AM fungal OTUs among inoculated and non-inoculated jujube Tasset and Gola cultivars. Only OTUs with significant differences ($P < 0.05$) are shown. Statistics were performed using Kruskal–Wallis' test. "Un. Glomeraceae" indicates affiliation to references belonging to *Glomus* sensu lato, for which uncertain position in Glomeraceae has been described.

Supplementary material

Table S1. Taxonomic affiliation of AM fungal OTUs

Table S2. Native AM Fungal OTU indicators with respect to jujube cultivars

Fig. S1. Monitoring of jujube height and diameter evolution during 13 months after outplanting of jujube seedlings pre-treated (inoculated and fertilized, --■--; inoculated, --♦--; fertilized, --●--) or untreated (--▲--) in nursery. Mean values are indicated for each treatment and four sampling time (0, 3, 8, 13 months) and error bars correspond to standard deviations. Formula and R^2 for each linear regression are indicated on the right of each panel. The regression slope represents the relative height-based or diameter-based growth rate.

Fig. S2. Comparison of AM fungal community membership (Venn diagram analysis) between inoculated and non-inoculated jujube trees from the two cultivars Tasset and Gola. Ri, *R. irregularis* IR27.