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

Babacar Thioye, Hervé Sanguin, Aboubacry Kane, Sergio Mania de Faria, Dioumacor Fall, Yves Prin, Diaminatou Sanogo, Cheikh Ndiaye, Robin Duponnois, Samba Ndao Sylla, et al.

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

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17 **Abstract**

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

41

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

44

45

## 46 1. Introduction

47 The jujube tree (*Ziziphus mauritiana* Lam.) is a multipurpose fruit tree commonly used in  
48 Sahelian and Sudanian areas in West Africa (Okafor, 1991). It is an important source of  
49 income for rural communities and contributes to overcome nutritional problems (Arbonnier,  
50 2000). For these reasons, the jujube tree is one of tree species selected by the pan-African  
51 Great Green Wall (GGW) project to « green » and fight against the poverty, degradation of  
52 soils and desertification (Dia and Niang, 2010). In Senegal, the GGW project promotes tree  
53 planting and economically interesting drought-tolerant plant species, water retention ponds,  
54 agricultural production systems and other income-generating activities, as well as basic social  
55 infrastructures. However, fruit tree planting programs in such environmental conditions (i.e  
56 drought, degraded land) are generally subjected to a low tree growth and high tree mortality  
57 rates due to transplant shocks from tree nursery to field (Close et al. 2013). Different  
58 strategies were proposed to improve the growth and survival of fruit trees, e.g. inoculation  
59 with arbuscular mycorrhizal (AM) fungi, fertilization with rock phosphate (RP), and plant  
60 propagation by micrografting (Reena and Bagyaraj, 1990; Guissou et al. 1998; Bâ et al. 2000;  
61 Mathur and Vyas, 2000; Danthu et al. 2002, 2004; Bâ et al. 2003; Guissou, 2009; Sidibé et al.  
62 2012).

63 The jujube tree is highly dependent on AM symbiosis (Bâ et al. 2001; Thioye et al. 2007) and  
64 it has been suggested than AM fungal root colonization of jujube seedlings in a nursery was  
65 an essential prerequisite to limit the mortality of outplanted jujube trees in the field (Bâ et al.  
66 2001). AM fungi are known for their ability to improve plant growth and notably to  
67 efficiently scavenge for soil phosphorus (P) resources (Smith and Read 2008), one of most the  
68 limiting resources in West African soils for the establishment of tree plantations and  
69 agriculture crops (Friesen et al. 1997). Paradoxically, important resources in phosphate rocks  
70 (RP) are available in West Africa, and their use could provide an alternative to soluble P  
71 fertilizers that are poorly accessible to rural communities due to their high containable  
72 (Nziguheba et al. 2015).

73 Previous studies have demonstrated that jujube trees associated with AM fungi showed a  
74 better growth and mineral nutrition than non AM-associated jujube trees (Guissou et al. 1998;  
75 Bâ et al. 2000; Bâ et al. 2001; Sidibé et al. 2012; Guissou et al. 2016), for example by using  
76 more efficiently soluble P from RP (Bâ et al. 2001). However, the beneficial effects of AM  
77 fungal association were dependent on jujube species and AM fungal species (Thioye et al.  
78 2017).

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

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

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

105

## 106 **2. Materials and methods**

### 107 *2.1. Mycorrhizal inoculum, fertilizer and plant material*

108 The *Rhizophagus irregularis* isolate IR27 (syn. *Glomus aggregatum* IR27; Bâ et al. 1996)  
109 originated from an *Acacia holosericea* planting in the North of Burkina Faso, and provided by  
110 the LCM laboratory (IRD, Dakar, Senegal, certified ISO 9001, version 2000), was used as  
111 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<sub>2</sub>O<sub>5</sub>) 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<sub>3</sub>. 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<sup>-1</sup>; 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.

170

### 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

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

184 Molecular diversity of AM fungi (Glomeromycota) from plant DNA was assessed by 18S  
185 rRNA gene amplification with the primers NS31 and AML2 (Simon et al. 1992; Lee et al.  
186 2008) according to Davison et al. (2012). PCR round was carried out in a final volume of 50  
187 µl with NS31 and AML2 primers (0.6 µM each), 2 µl DNA (2 extracted DNA replicates per  
188 sample), 200 µM of each dNTP, 200 ng/ml BSA, GoTaq® DNA Polymerase (2 units) and 1X  
189 Green GoTaq® Reaction Buffer (Promega, Charbonnieres, France), with the following  
190 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  
191 final elongation step at 72°C for 10 min. After PCR, the amplification products (pools of  
192 PCR: 2 × 50µl) were purified by using illustra GFX PCR DNA and Gel Band Purification Kit  
193 (GE Healthcare Life Sciences, Velizy-Villacoublay, France) following manufacturer's  
194 guidelines. Then, DNA concentration of PCR products were quantified by using a Qubit  
195 fluorometer (Qubit Fluorometric quantitation, Invitrogen) and the Qubit dsDNA HS Assay  
196 Kit. PCR product concentration was adjusted to 10ng/µl and subjected to paired-end Illumina  
197 MiSeq sequencing (2×300 bp) by Molecular Research LP (MR DNA, TX, USA). The 18S  
198 rRNA gene from the AM fungal inoculum has been amplified with the primers AML1 and  
199 AML2 according Lee et al. (2008) and sequenced (Genoscreen, Lille, France). The sequence  
200 has been submitted to the NCBI database under accession number MH571752.

201

## 202 *2.5. Bioinformatic data processing*

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

207 reverse reads. All sequences were depleted of barcodes and primers and a quality cutoff of  
208 Q30 was selected. The sequences < 150 bp or with ambiguous base calls or with  
209 homopolymer runs exceeding 8bp were removed. A pre-clustering step (Huse et al. 2010) was  
210 also performed to remove sequences still likely due to illumina sequencing errors. Chimera  
211 were checked by Uchime (Edgar et al. 2011) implemented in Mothur software because it  
212 showed improved performance over the Chimera Slayer algorithm (Schloss et al. 2011). All  
213 sequences were first classified by using classify.seqs and a SILVA-compatible alignment  
214 database (Eukarya) to remove all no Glomeromycota sequences. Secondly, a preliminary  
215 clustering of sequences in OTUs with a 3 % divergence threshold was performed by using  
216 dist.seqs and cluster commands in Mothur, and all singleton OTUs were removed. The  
217 representative sequences of each OTU were then compared with a broader nucleotide  
218 database (Genbank database, BLASTN program) (<http://www.ncbi.nlm.nih.gov/genbank>),  
219 and all OTUs for which the representative sequence presented a similarity score < 95 % (100  
220 % coverage) with the reference sequences were excluded of the data set. The number of  
221 sequences between each sample was then normalized with sub.sample command. This sub-  
222 sampling step allows reducing the number of spurious OTUs and is widely used to obtain  
223 robust estimation of alpha and beta diversity (Gihring et al. 2012). Finally, taxonomic  
224 affiliation of OTUs was done using classify.otu and the Glomeromycota-based 18S rDNA  
225 sequence database from Krüger et al. (2012). The taxonomic affiliation of OTUs was  
226 considered significantly robust for a given taxonomic level when the confidence threshold  
227 was superior to 50 % ([https://rdp.cme.msu.edu/wiki/index.php/Classifier\\_Help](https://rdp.cme.msu.edu/wiki/index.php/Classifier_Help)). Raw data are  
228 available under the BioPproject ID PRJNA479949 (<https://www.ncbi.nlm.nih.gov/bioproject>).  
229

## 230 2.6. Statistics

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

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

239 were compared among all treatments using R version 3.3.1 (R Core Team , 2017) by three-  
240 way ANOVA followed by post-hoc Tukey test, as implemented in *aov()* and *TukeyHSD()*  
241 functions. AM fungal community membership among treatments (jujube cultivars,  
242 inoculation or not) was assessed using the *venn.diagram()* function from the R package  
243 VennDiagram version 1.6.17 (Chen, 2016). The differences in the AM fungal community  
244 structures among all treatments were based on the Bray-Curtis dissimilarity matrix and  
245 assessed using non-parametric permutational multivariate analysis of variance  
246 (PERMANOVA) as implemented in the *adonis()* function from the R package *vegan* version  
247 2.4-3 (Oksanen et al. 2016). Multivariate dispersion was estimated for each treatment using  
248 the *betadisper()* function and *permutest()* as it can affect PERMANOVA results. The  
249 significance of AM fungal OTUs with respect to the jujube cultivar or the jujube status  
250 (inoculated or not, fertilized or not) was determined using the indicator value (IndVal) index,  
251 as implemented in *multipatt()* function from the R package *indicspecies* (De Cáceres and  
252 Legendre, 2009). Two different probabilities were calculated, *i.e.* A (specificity), representing  
253 the probability of a sample to be defined by a group (*i.e.*, jujube cultivar, AM inoculation  
254 status, fertilization status), given that the OTU has been detected, and B (sensitivity)  
255 representing the probability of finding the OTU in different samples characterized by a given  
256 group. Only the OTUs present in more than half of samples for a given group are considered,  
257 *i.e.* B superior to 0.5. Table transformations in R were performed with the tidyverse packages  
258 version 1.1.1 (Wickham, 2017).

259

### 260 **3. Results**

#### 261 *3.1. Tree nursery – Growth, mineral nutrition and mycorrhizal colonization of jujube trees*

262 Tree growth and nutrition of both cultivars were significantly improved by mycorrhizal  
263 inoculation with *Rhizophagus irregularis* IR27 (Table 1), with relatively stronger effects on  
264 the Gola cultivar compared to the Tasset cultivar. By contrast, fertilization with rock  
265 phosphate (RP) showed no effect on tree growth and nutrition, excepted regarding N nutrition  
266 for Gola cultivar, and no additional effect was observed when mycorrhizal inoculation was  
267 combined with RP fertilization compared to mycorrhizal inoculation only. A similar  
268 percentage of mycorrhizal infection was observed for both Tasset and Gola cultivars, reaching  
269 65.8 % to 68.9 %, respectively. However, RP fertilization significantly decreased the  
270 mycorrhizal infection of both cultivars (Table 1).

271

272 *3.2. Field monitoring – Survival, growth and mycorrhizal colonization of jujube trees*

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

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

291 Mycorrhizal colonization was observed at 13 months after transplanting in jujubes roots.  
292 Colonization levels were higher for all inoculated treatments of Gola (59.8 % inoculated and  
293 50 % inoculated-fertilized) and Tasset (56.4 % inoculated and 46.4 % inoculated-fertilized)  
294 compared to non-inoculated controls and fertilized treatments (Table 2).

295

296 *3.3. Field monitoring – Composition of the jujube root-associated AM fungal community*

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

303 samples. The majority of AM fungal OTUs belonged to Glomeraceae (94 % of total reads,  
304 178 OTUs) (Table S1), and few OTUs to Diversisporaceae (3 %, 31 OTUs), Paraglomeraceae  
305 (2 %, 11 OTUs), Gigasporaceae (0.5 %, 8 OTUs), Acaulosporaceae (0.06 %, 6 OTUs),  
306 Geosiphonaceae (0.01 %, 3 OTUs), Claroideoglomeraceae (< 0.01 %, 1 OTU) and  
307 Pacisporaceae (<0.01 %, 1 OTUs). Glomeraceae OTUs belonged to *Sclerocystis* (28 % of  
308 Glomeraceae reads), *Rhizophagus* (27 %), Glomeraceae related to *Glomus* sensu lato (22 %),  
309 *Glomus* sensu stricto (20 %), and in a lesser extent to *Septoglomus* (4 %), *Funneliformis* (0.4  
310 %) and unclassified Glomeraceae (0.02 %).

311 The native jujube root-associated AM fungal (untreated jujube trees) was composed of 85  
312 (Gola) to 98 (Tasset) OTUs, with 80 % of sequences related to 15 known genera and 20 %  
313 only to Glomeraceae with uncertain position (unclassified Glomeraceae and *Glomus* sensu  
314 lato) (Table S2). A core AM fungal sub-community of 47 OTUs (93.4 % of sequences) (Fig.  
315 1) was largely dominated by Glomeraceae, whose 26 % *Sclerocystis*, 18 % *Rhizophagus*, 28  
316 % *Glomus* sensu stricto, 21 % *Glomus* sensu lato, 6 % *Septoglomus* and < 1 % *Funneliformis*  
317 (Fig. 1, Table S2). The cultivar Tasset presented the most diverse AM fungal community  
318 (Table 3), with a significant association of eight OTUs related to *Redeckera*, *Rhizophagus* and  
319 *Glomus* sensu stricto (Table S2). Three OTUs related to *Glomus* sensu stricto were  
320 significantly associated with the Gola cultivar but with a relatively low specificity ( $A < 0.7$ )  
321 (Table S2).

322

### 323 3.4. Field monitoring – Impact of ecological engineering strategies on the jujube root- 324 associated AM fungal community

325 A robust diversity coverage was obtained for the AM fungal community associated with both  
326 jujube cultivars, independently of jujube status (inoculated or not, fertilized or not), reaching  
327 more than 99 %, and with Boneh estimates evaluated to less than seven OTUs (Table 3). After  
328 13 months, AM fungal inoculation ( $P < 0.001$ ) and the type of cultivar ( $P < 0.05$ ) had  
329 significant effect on AM fungal community richness, whereas RP fertilization ( $P < 0.05$ )  
330 mostly impacted AM fungal community diversity (Table 3). However, results revealed a  
331 fertilization effect on AM fungal community richness ( $P < 0.01$ ) and diversity ( $P < 0.05$ )  
332 highly dependent on AM fungal inoculation. No impact of ecological engineering strategies  
333 was observed on the evenness of AM fungal communities, but a relatively, low evenness was  
334 revealed, ranging from 0.4 to 0.6. Globally, mycorrhizal inoculation and RP fertilization

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

346 As observed for AM fungal community richness and diversity, jujube inoculation with *R.*  
347 *irregularis* IR27 appeared as the most significant treatment ( $P = 0.011$ ) affecting the AM  
348 fungal community structure of jujube trees on the field (Table 5). Nevertheless, the type of  
349 cultivar and the RP fertilization significantly affected the inoculation impact on AM fungal  
350 community structure ( $P = 0.019$ ). The analysis of AM fungal community membership among  
351 inoculated and non-inoculated jujube trees for the both cultivars Tasset and Gola (Fig. S2)  
352 revealed a predominant core AM fungal sub-community (97 % of sequences) composed of 26  
353 OTUs, as well as rare 23 OTUs (0.1 %) only detected in jujube trees inoculated with *R.*  
354 *irregularis* IR27, and rare 66 OTUS (0.3 %) only in non-inoculated jujube trees. AM fungal  
355 inoculation of jujube trees negatively impacted ( $P < 0.05$ ) the abundance of eight OTUs  
356 belonging to *Cetraspora* (OTU\_31), *Gigaspora* (OTU\_25), *Glomus* (OTU\_07, OTU\_39),  
357 *Redeckera* (OTU\_08, OTU\_34), *Rhizophagus* (OTU\_04) and *Paraglomus* (OTU\_12) for the  
358 Tasset cultivar, and eight OTUs belonging to *Glomus* sensu stricto (OTU\_07, OTU\_09,  
359 OTU\_78), *Glomus* sensu lato (OTU\_15, OTU\_20), *Redeckera* (OTU\_16, OTU\_41),  
360 *Rhizophagus* (OTU\_29) for the Gola cultivar (Fig. 2). However, two OTUs belonging to  
361 *Rhizophagus* (OTU\_02, OTU\_14) were positively impacted for the Gola cultivar (Fig. 2). It  
362 has to be noted that the comparison between the 18S rRNA gene sequence from the AM  
363 fungal inoculum and the representative sequence of each OTU revealed 100 % similarity with  
364 one the most dominant OTUs, i.e. OTU\_2 related to *R. irregularis* (Table S1). Association  
365 analysis (Table 4) emphasized the global negative impact for five of these OTUs belonging to  
366 Glomeraceae and Gigasporaceae, independently of jujube cultivar and fertilization status.

367

#### 368 4. Discussion

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

377

##### 378 4.1. Beneficial effects of mycorrhizal inoculation on jujube trees from nursery to field

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

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

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

423

#### 424 4.2. Field environmental impacts of mycorrhizal inoculation on native AM fungal biodiversity

425 The range and sustainability of AM fungal-mediated plant benefits (biomass, yield, survival)  
426 are the most obvious concerns for end-users (Berruti et al. 2016), but the environmental  
427 impacts of AM fungal inoculant introduction in agroecosystems remains a critical issue.  
428 Three levels of environmental impacts were categorized (Rodriguez and Sanders, 2015), (i)  
429 alteration of composition and structure of native AM fungal population and/or community,  
430 (ii) exchange of genetic material with native AM fungal population and/or community, and  
431 (iii) persistence and/or spread of AM fungal inoculants, increasing consequently the first two  
432 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-

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

488 An overall negative impact of the different treatments was observed on the native AM fungal  
489 community. The AM fungal richness was the characteristic the most affected by all  
490 treatments, notably inoculation. The pre-colonization of jujube roots in nursery by the exotic  
491 AM fungal strain was supposed to have a strong negative impact on the AM native fungal  
492 community colonization rate, but it was also supported by the persistence of the inoculum  
493 evidenced in the results (high level of OTU2). Richness is the main community characteristic  
494 assessed to monitor AM fungal community but a general trend remains difficult to define  
495 (Antunes et al. 2009; Mummey et al. 2009; Koch et al. 2011). For instance, whereas a  
496 negative impact was observed in the current study, a positive tendency had been observed in a  
497 14-month-old olive orchard (Alguacil et al. 2011). When considering the global AM fungal  
498 community structure, only inoculation had a significant impact, leading to a negative effect on

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

503

## 504 **5. Conclusion**

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

524

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827

**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	ns
(C) × (I) × (F)	ns	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 <sup>1</sup>

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

<sup>1</sup> 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.

Treatment	Richness		Diversity		Evenness		Coverage (%)
	Number of OTUs	Chao	Shannon	Invsimpson	Shannoneven	Boneh	
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 assignment <sup>1</sup>	Abundance <sup>2</sup>	Jujube cultivar			Jujube status								
		Tasset			Non-inoculated			Fertilized			Non-fertilized		
		A	B	Index	A	B	Index	A	B	Index	A	B	Index
<b>Glomeraceae</b>													
OTU_33 – Un. Glomeraceae		1.00	0.60	0.775***									
OTU_35 - <i>Rhizophagus</i>		0.97	0.53	0.721**									
OTU_07 - <i>Glomus</i>					0.98	1.00	0.991***						
OTU_09 - <i>Glomus</i>					0.98	1.00	0.991**						
OTU_25 - <i>Gigaspora</i>					0.81	0.81	0.991*						
OTU_31 - <i>Cetraspora</i>					0.86	0.75	0.805**						
OTU_39 - <i>Glomus</i>					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 - <i>Glomus</i>											0.93	0.87	0.900***

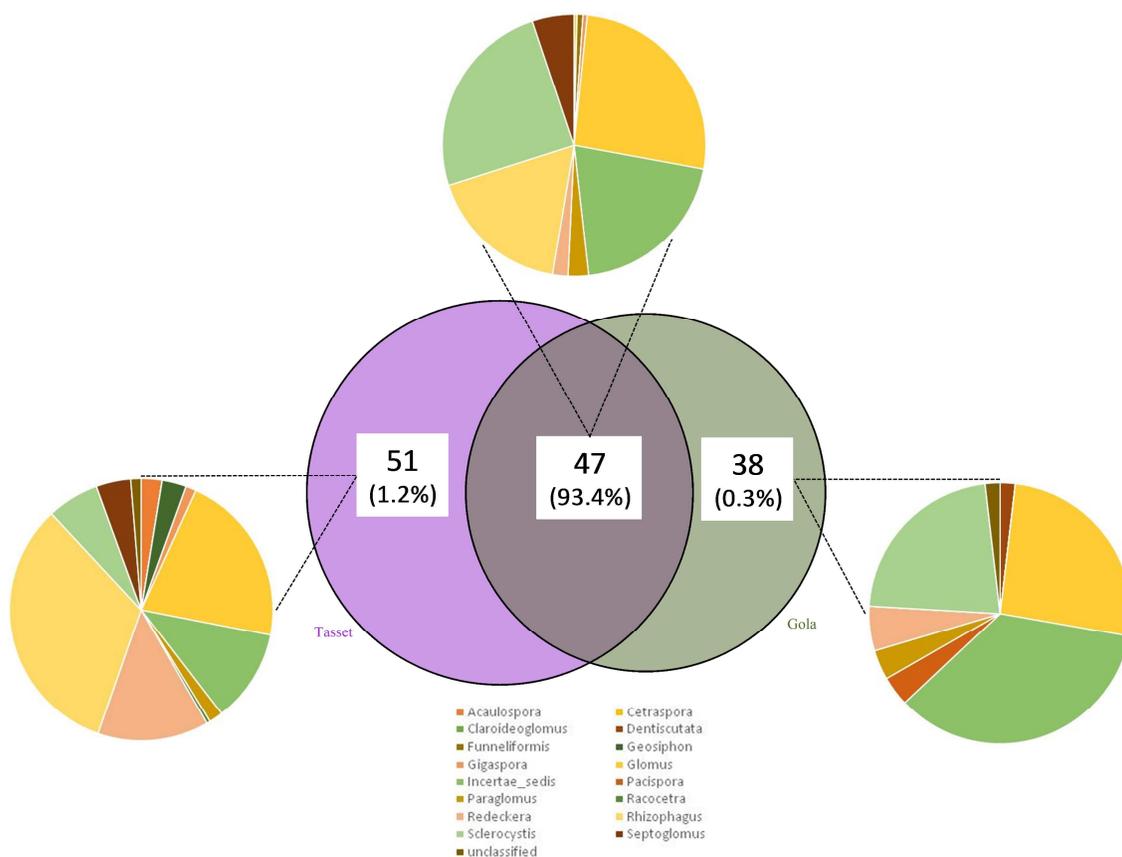
<sup>1</sup>Taxonomic affiliation was based on a k-nearest neighbor consensus and the Wang method used in Mothur (function classify.otu) using reference sequences from Krüger et al (2012). Genus level is indicated when confidence threshold is superior to 95, if not the higher taxonomic level is indicated.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

package *indicspecies* (De Cáceres and Legendre, 2009). <sup>3</sup>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.

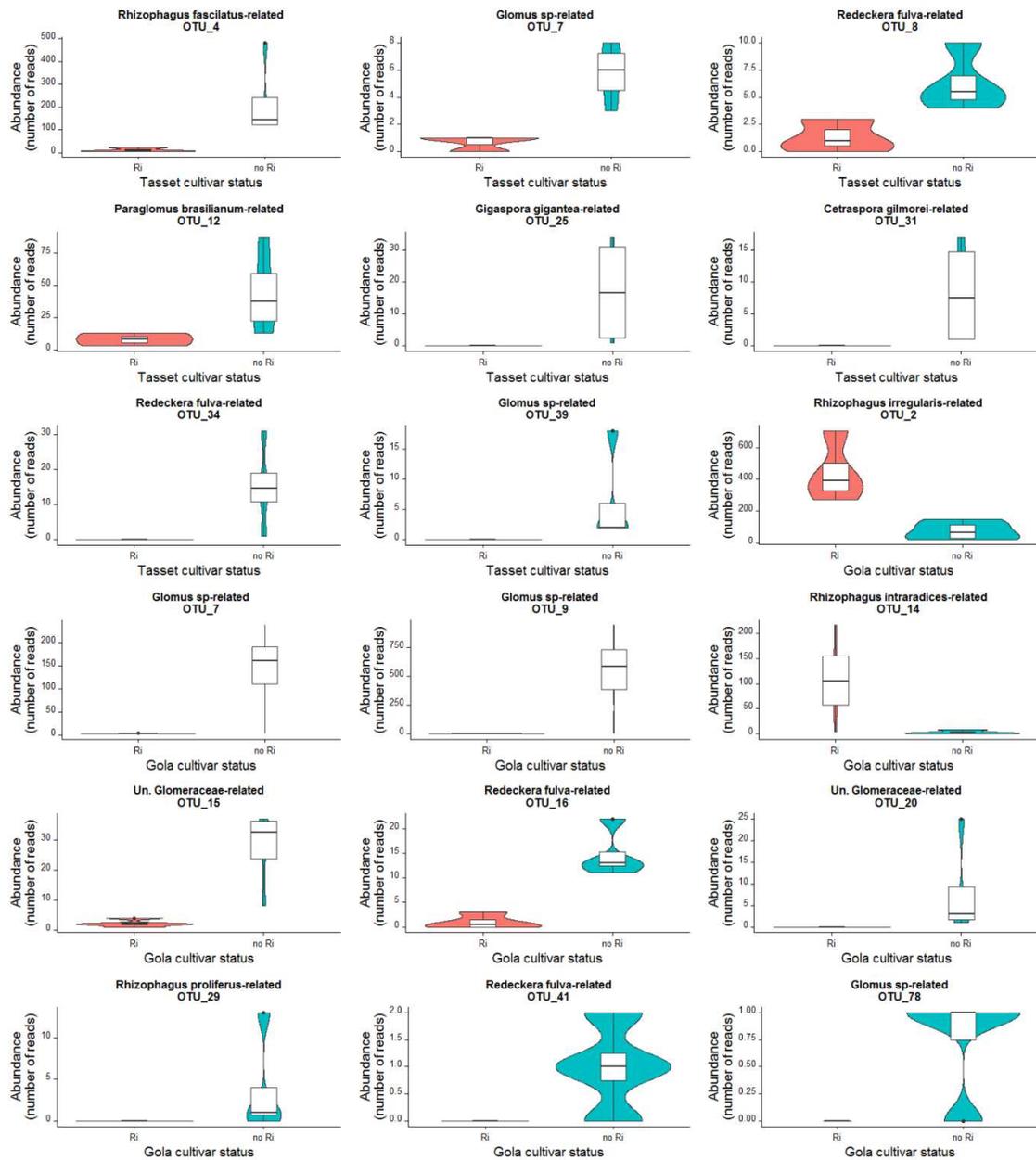
**Table 5** Impact of jujube cultivar, AM fungal inoculation and RP fertilization on the jujube root-associated AM fungal community structure (field experiment).

Treatments	<i>df</i>	SS	MS	<i>F</i> model	<i>R</i> <sup>2</sup>	<i>P</i> value <sup>1</sup>
Cultivar (C)	1	0.253	0.253	1.224	0.038	0.268 <sup>ns</sup>
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 <sup>ns</sup>
(C) × (I)	1	0.135	0.135	0.655	0.020	0.722 <sup>ns</sup>
(C) × (F)	1	0.343	0.343	1.660	0.051	0.113 <sup>ns</sup>
(I) × (F)	1	0.130	0.130	0.630	0.019	0.767 <sup>n</sup>
<b>(C) × (I) × (F)</b>	1	0.496	0.496	2.400	0.074	0.019*
Residuals	22	4.546	0.206		0.681	
Total	29	6.668			1	

<sup>1</sup>PERMANOVA was based Bray-Curtis dissimilarity matrix and assessed using *adonis()* function (iterations = 9,999 permutations). ‘\*’ *P* < 0.05; ‘<sup>ns</sup>’ *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*<sup>2</sup> = 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.