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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)

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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 evaluate their sustainability in terms of plant growth and survival. In the current study, local 24 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.

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

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46 **1. Introduction**

47 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 48 income for rural communities and contributes to overcome nutritional problems (Arbonnier, 49 2000). For these reasons, the jujube tree is one of tree species selected by the pan-African 50 51 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 52 planting and economically interesting drought-tolerant plant species, water retention ponds, 53 54 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 55 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 with arbuscular mycorrhizal (AM) fungi, fertilization with rock phosphate (RP), and plant 59 propagation by micrografting (Reena and Bagyaraj, 1990; Guissou et al. 1998; Bâ et al. 2000; 60 61 Mathur and Vyas, 2000; Danthu et al. 2002, 2004; Bâ et al. 2003; Guissou, 2009; Sidibé et al. 62 2012).

The jujube tree is highly dependent on AM symbiosis (Bâ et al. 2001; Thioye et al. 2007) and 63 64 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. 65 2001). AM fungi are known for their ability to improve plant growth and notably to 66 efficiently scavenge for soil phosphorus (P) resources (Smith and Read 2008), one of most the 67 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).

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). 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 the most widely used AM fungal species in mycorrhiza-based ecological engineering 84 85 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 86 87 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

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 experimental field. The ecological impact of each practice will be assessed by the monitoring 98 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).

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- 106 2. Materials and methods
- 107 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

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sterilized sandy soil in a tree nursery. The sandy soil used in the experiment was collected 112 113 from Sangalkam (Senegal). It was a sandy soil with 88.8 % sand, 5.8 % silt, 5.4 % clay, 0.6 % organic matter, 0.3 % total C, 0.02 % total N, ratio C/N = 14, 333.5 ppm total K, 41.4 ppm 114 115 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 mm sieve, sterilized for four hours in an autoclave oven system at 180°C to eliminate native 117 AMF, and transferred into plastic bags (1.5 kg of soil per plastic bag). The AM fungal 118 119 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 (Adelman and Morton, 1986) as 1635 infective propagules per 20 g of inoculum. Non-121 122 inoculated controls also received 20 g of autoclaved crude AM fungal inoculum. The fertilizer 123 consisted of rock phosphate (RP, 30 % of P₂O₅) 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).

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

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132 2.2. Nursery experimental set up and plant growth measurements

Plants were grown in a tree nursery at research center ISRA / IRD (Bel Air, Dakar, Senegal) 133 134 (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 bag. The experiment was set up as a $2 \times 2 \times 2$ factorial design consisting of two jujube cultivars, 136 137 with AM fungal inoculation or not, and with or without RP fertilization. Experiment was arranged in a completely randomized design with 20 replicates per treatment combination. 138 139 Mycorrhizal inoculation and fertilization with RP were achieved by placing either 20 g portions of AM fungal and /or two different RP doses below the seeds during transplanting. 140

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

washed under tap water, bleached (KOH, 10 %) at 80°C during 30 min, and stained in 0.05 % 144 145 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 $\times 40$ 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 HNO₃. 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.

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156 2.3. Field experimental set up and plant growth measurements

The orchard (5 ha) was located near the village of Amally in the rural community of 157 158 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 Agricultural Chemistry Laboratory in Rio de Janeiro (Brazil) with means as following: pH: 163 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: 164 165 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

Thirteen months after plantation, three jujube tree root systems for each treatment per replicated block were sampled and pooled (a total of 32 root samples). Each composite root 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 175 176 using a FastPrep-24 homogenizer (MP biomedicals Europe, Illkirch, France) and the 177 FastDNA® SPIN kit (MP biomedicals, 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 sample), 200 µM of each dNTP, 200 ng/ml BSA, GoTaq® DNA Polymerase (2 units) and 1X 188 189 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: $2 \times 50 \mu$) 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 MiSeq sequencing (2×300 bp) by Molecular Research LP (MR DNA, TX, USA). The 18S 197 198 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.

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202 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 207 208 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 showed improved performance over the Chimera Slayer algorithm (Schloss et al. 2011). All 212 213 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 dist.seqs and cluster commands in Mothur, and all singleton OTUs were removed. The 216 217 representative sequences of each OTU were then compared with a broader nucleotide database (Genbank database, BLASTN program) (http://www.ncbi.nlm.nih.gov/genbank), 218 219 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 affiliation of OTUs was done using classify.otu and the Glomeromycota-based 18S rDNA 224 sequence database from Krüger et al. (2012). The taxonomic affiliation of OTUs was 225 considered significantly robust for a given taxonomic level when the confidence threshold 226 227 was superior to 50 % (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).

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230 *2.6. Statistics*

The AMF percentage colonization data were $(\arcsin x)^{1/2}$ 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-239 240 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 non-parametric permutational multivariate analysis of variance assessed using 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*

Tree growth and nutrition of both cultivars were significantly improved by mycorrhizal 262 263 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 mycorrhizal infection of both cultivars (Table 1). 270

272 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.

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 jujube trees, 70.8 % and 75 % for Gola and Tasset, respectively (Table 2). The height of 284 jujube trees was the only parameter significantly different between 13-month-old inoculated 285 286 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 a stability of nursery-driven impacts, with substantial higher rates (slope value of linear 289 290 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).

295

296 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 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 (2 %, 11 OTUs), Gigasporaceae (0.5 %, 8 OTUs), Acaulosporaceae (0.06 %, 6 OTUs), 305 306 Geosiphonaceae (0.01 %, 3 OTUs), Claroideoglomeraceae (< 0.01 %, 1 OTU) and 307 Pacisporaceae (<0.01 %, 1 OTUs). Glomeraceae OTUs belonged to Sclerocystis (28 % of Glomeraceae reads), Rhizophagus (27 %), Glomeraceae related to Glomus sensu lato (22 %), 308 309 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) mostly impacted AM fungal community diversity (Table 3). However, results revealed a 330 331 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 332 333 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

negatively impacted AM fungal richness and diversity, with the most significant impact 335 336 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), Redeckera (OTU_08, OTU_34), Rhizophagus (OTU_04) and Paraglomus (OTU_12) for the 357 Tasset cultivar, and eight OTUs belonging to Glomus sensu stricto (OTU_07, OTU_09, 358 359 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 360 361 *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 one the most dominant OTUs, i.e. OTU_2 related to R. irregularis (Table S1). Association 364 365 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. 366

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 ecosystem conservation (Rodriguez and Sanders, 2015; Hart et al. 2017). However, their 371 long-term efficiency on field and their impact on native microbial communities remain critical 372 373 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*

The current nursery results emphasized the AM-mediated plant benefits previously observed 379 on different jujube cultivars or provenances (Guissou et al. 1998, 2016; Sidibé et al. 2012; 380 Thioye et al. 2017) in terms of growth and nutrient uptake (N, P, K). In addition, a higher P 381 assimilation from RP of jujubes when inoculated by R. irregularis IR27 was confirmed 382 383 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 argue that-mycorrhizal inoculation can be considered as a substitute of P fertilization in tree 386 nursery management (Smith and Read 2008). In addition, RP fertilization negatively affected 387 388 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 \times 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 investigated to fully decipher mycorrhizal-mediated plant fitness, notably on field (Delavaux 395 396 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 400 401 (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 Great Green Wall (GGW) experimental sites. The benefit of mycorrhizal inoculation on 406 407 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 relative stability of pre-treatments in nursery, but more robust assessment of growth rates 419 420 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 colonization of *R. irregularis* IR27 compared to the native AM fungal community because of 439 440 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; Janouskova et al. 2017). A second hypothesis may be the positive effect of R. irregularis 452 453 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 re466 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 and response of native AM fungal community between both jujube cultivars, emphasizing the 472 hypothesis that AM fungal species with a preferentially intraradical lifestyle are mostly 473 474 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, Rhizophagus or Funneliformis or members of Glomus sensu lato generally constitute the most 477 478 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, 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 481 482 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 tropical African ecosystems revealed for the first time a high predominance of 485 486 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 community. The AM fungal richness was the characteristic the most affected by all 489 490 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 491 492 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 493 assessed to monitor AM fungal community but a general trend remains difficult to define 494 (Antunes et al. 2009; Mummey et al. 2009; Koch et al. 2011). For instance, whereas a 495 496 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 497 498 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).

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 management of jujube orchards on the route of the GGW (Senegal), but further investigations 515 516 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 517 mycorrhizal inocula versus native AM fungi. Further investigations are also required to 518 evaluate the effect of inoculation with native AM fungi selected species or consortia of AM 519 520 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 acceptation 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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| Treatment | Height | Collar diameter | Total dry biomass | Mycorrhizal | N | Р | К |
|-------------------|---------------------------|-----------------------|--------------------------|---------------------------|-------------------------|--------------------------|---------------------------|
| | (cm) | (mm) | (g) | colonization (%) | (%) | (%) | (%) |
| Gola | 19.7 ± 2.3 c | $2.8\pm0.4~\text{cd}$ | $1.4\pm0.1~\text{cd}$ | <mark>0.0 d</mark> | $1.3 \pm 0.1 \text{ b}$ | $1.3 \pm 0.7 \text{ d}$ | $08.4 \pm 1.0 \text{ d}$ |
| Gola+RP | $22.6\pm4.4~c$ | 3.2 ± 0.3 c | $1.6 \pm 0.1 \text{ c}$ | <mark>0.0 d</mark> | 2.4 ± 0.4 a | 1.5 ± 0.6 cd | 10.0 ± 2.0 bcd |
| Gola+Ri | 54.3 ± 8.6 a | $4.8 \pm 0.6 a$ | 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 | $51.4 \pm 14.9 \text{ b}$ | 2.5 ± 0.4 a | $2.6 \pm 0.1 \text{ ab}$ | $14.3 \pm 0.5 a$ |
| Tasset | $15.9 \pm 2,4$ d | $2.5\pm0.3\;c$ | $1.3\pm0.1~\text{d}$ | <mark>0.0 d</mark> | $1.3\pm0.1\;b$ | $1.2 \pm 0.1 \text{ d}$ | $09.4 \pm 0.7 \text{ cd}$ |
| Tasset+RP | $17.8 \pm 4.0 \text{ cd}$ | 3.1 ± 0.4 c | $1.3 \pm 0.1 \text{ cd}$ | <mark>0.0 d</mark> | $1.6\pm0.2\;b$ | $1.2 \pm 0.2 \text{ d}$ | $08.7 \pm 1.1 \text{ d}$ |
| Tasset+Ri | $35.9\pm6.9~b$ | $4.1\pm0.9~b$ | $2.6\pm0.4\ b$ | 65.8 ± 11.5 a | 2.1 ± 0.0 a | 2.1 ± 0.0 bc | 11.0 ± 0.6 bc |
| Tasset+Ri+RP | $37.7\pm7.5~b$ | $4.1\pm0.9~b$ | $2.7\pm0.5~\text{b}$ | 38.7 ± 15.6 c | 2.1 ± 0.1 a | 2.3 ± 0.0 ab | $11.3\pm0.4~b$ |
| All treatments | | | | | | | |
| Cultivar (C) | *** | ** | *** | ns | ** | * | ** |
| Inoculation (I) | *** | *** | *** | *** | *** | *** | *** |
| Fertilization (F) | ns | <mark>ns</mark> | ns | ns | ** | ns | <mark>ns</mark> |
| $(C) \times (I)$ | *** | <mark>ns</mark> | *** | ns | ns | ns | *** |
| $(I) \times (F)$ | ns | ** | ns | ns | ** | <mark>ns</mark> | <mark>ns</mark> |

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).

| $(\mathbf{C}) \times (\mathbf{F})$ | <mark>ns</mark> | <mark>ns</mark> | ns | ns | <mark>ns</mark> | <mark>ns</mark> | <mark>ns</mark> |
|--|-----------------|-----------------|-----------------|-----|-----------------|-----------------|-----------------|
| $(\mathbf{C})\times(\mathbf{I})\times(\mathbf{F})$ | ns | ns | <mark>ns</mark> | *** | ns | <mark>ns</mark> | <mark>ns</mark> |

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 | r planting | | 13 months after planting | | | | |
|--------------|-------------------------|-------------|--------------|----------------|---------------|-------------------|--------------------------|--------------|---------------|--------------|--|
| | Height | Collar | Rate of | Height | Collar | Rate of | Height | Collar | Rate of | Mycorrhizal | |
| | diameter Sur (cm) | | Survival | (cm) | diameter | liameter Survival | | diameter | survival | colonization | |
| | | (mm) | (%) | | (mm) | (%) | | (mm) | (%) | (%) | |
| 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 | |

| Cultivar (C) | ** | *** | ns | ** | * | ns | ns | ns | ns | ns |
|-----------------------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Inoculation (I) | *** | *** | <mark>ns</mark> | *** | *** | *** | ** | * | ** | *** |
| Fertilization (F) | ns | ns | ns | ns | <mark>ns</mark> | ns | <mark>ns</mark> | <mark>ns</mark> | <mark>ns</mark> | <mark>ns</mark> |
| $(C) \times (I)$ | ** | <mark>ns</mark> | <mark>ns</mark> | ** | <mark>ns</mark> | ** | ** | <mark>ns</mark> | * | ** |
| $(C) \times (F)$ | ns | * | <mark>ns</mark> | <mark>ns</mark> | * | ns | ns | ns | ns | ns |
| $(I) \times (F)$ | ns | <mark>ns</mark> | <mark>ns</mark> | <mark>ns</mark> | ns | ns | ns | ns | ns | <mark>ns</mark> |
| $(C)\times(I)\times(F)$ | ns | <mark>ns</mark> | ns | <mark>ns</mark> | <mark>ns</mark> | <mark>ns</mark> | <mark>ns</mark> | <mark>ns</mark> | ns | ns |
| ¹ The block fact | or was not sig | nificant $(P >$ | 0.05) | | | | | | | |

Factors tested

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**, **n**ot 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.

| | Rich | nness | Diversity | | Evenness | | |
|-------------------|-----------------------|-----------------------|--------------------------|----------------------|-------------------------|-------------|--------------|
| Treatment | Number of OTUs | Chao | Shannon | Invsimpson | Shannoneven | Boneh | Coverage (%) |
| Gola | $46 \pm 1 \text{ b}$ | 76 ± 6 a | $1.9 \pm 0.1 \text{ ab}$ | $4.4\pm0.5~b$ | 0.5 ± 0.0 a | 7 ± 0.3 | 99.2 |
| Gola+RP | 34 ± 5 cd | $50 \pm 7 \text{ bc}$ | 1.4 ± 0.5 bc | $2.7\pm1.0\ b$ | 0.4 ± 0.1 a | 4 ± 1.0 | 99.5 |
| Gola+Ri | $33 \pm 6 \text{ cd}$ | $51 \pm 6 bc$ | $1.7\pm0.2\;b$ | $3.9\pm0.6\;b$ | $0.5\pm0.0\ a$ | 6 ± 0.5 | 99.4 |
| Gola+Ri+RP | $34 \pm 2 \text{ cd}$ | 53 ± 5 bc | $1.5 \pm 0.4 \text{ bc}$ | $3.9\pm1.2\ b$ | 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 | $1.7\pm0.1\;b$ | $3.8\pm0.9\;b$ | $0.5\pm0.0\ a$ | 7 ± 1.0 | 99.2 |
| Tasset+Ri | $30\pm 6 d$ | $46 \pm 1 c$ | $1.2\pm0.7~\mathrm{c}$ | $3.1\pm2.0\ b$ | 0.4 ± 0.2 a | 5 ± 0.4 | 99.5 |
| Tasset+Ri+RP | 38 ± 5 bcd | $61 \pm 8 b$ | 1.6 ± 0.2 bc | $3.4\pm1.0\text{ b}$ | $0.4 \pm 0.0 \text{ a}$ | 6 ± 1.5 | 99.3 |
| All treatments | | | | | | | |
| Cultivar (C) | * | ** | <mark>ns</mark> | <mark>ns</mark> | <mark>ns</mark> | - | - |
| Inoculation (I) | *** | *** | * | ns | ns | - | - |
| Fertilization (F) | * | <mark>ns</mark> | * | ** | <mark>ns</mark> | - | - |
| $(C) \times (I)$ | ns | ns | ns | ns | ns | - | - |

| $(C) \times (F)$ | <mark>ns</mark> | *** | ns | ns | <mark>ns</mark> | - | - |
|-----------------------------|-----------------|-----|----|----|-----------------|---|---|
| $(I) \times (F)$ | *** | *** | * | * | ns | - | - |
| $(C) \times (I) \times (F)$ | 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.

| OTU label | | | ujube cult | | Jujube status | | | | | | | | |
|----------------------------------|-----------------------|--------|------------|----------|---------------|-----------|----------|------------|-------|-------|----------------|------|----------|
| taxonomic assigment ¹ | bundance ² | Tasset | | | N | on-inocul | ated | Fertilized | | | Non-fertilized | | |
| | ₹. | A | В | Index | A | В | Index | A | В | Index | A | В | 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*** |

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

¹Taxonomic 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. ²OTU abundance corresponds to the number of reads. ³Indicator OTUs were obtained using indicator value (IndVal.g) index as implemented in *multipatt*() function from R

package indicspecies (De Cáceres and Legendre, 2009). ³Statistics 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.

| 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} |
| $(\mathbf{C}) \times (\mathbf{I})$ | 1 | 0.135 | 0.135 | 0.655 | 0.020 | 0.722 ^{ns} |
| $(C) \times (F)$ | 1 | 0.343 | 0.343 | 1.660 | 0.051 | 0.113 ^{ns} |
| $(I) \times (F)$ | 1 | 0.130 | 0.130 | 0.630 | 0.019 | 0.767 ⁿ |
| $(\mathbf{C}) \times (\mathbf{I}) \times (\mathbf{F})$ | 1 | 0.496 | 0.496 | 2.400 | 0.074 | 0.019* |
| Residuals | 22 | 4.546 | 0.206 | | 0.681 | |
| Total | 29 | 6.668 | | | 1 | |

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

¹PERMANOVA 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, -- \blacksquare --; inoculated, -- \blacklozenge --; fertilized, -- \bullet --) or untreated (-- \blacktriangle --) 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 R² 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.