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1 **Pearl millet genotype impacts microbial diversity and enzymatic activities in**
2 **relation to root-adhering soil aggregation**

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17 **ABSTRACT**

18 **Aims:** The interactions between plant roots and the associated microbiota impact soil aggregation, water retention
19 and plant nutrient availability. Thus, selection of plant genotypes that promote microbial species involved in root-
20 adhering soil aggregation and rhizosheath formation could help improve yield sustainably. Here, we tested pearl
21 millet genotypic variation in both root-adhering soil aggregation, microbiological and biochemical characteristic.

22 **Methods:** A collection of 181 pearl millet inbred lines was phenotyped for their rhizosheath size, and thirteen
23 contrasting genotypes were selected and grown under field conditions, and their root-adhering soil (RAS) was
24 sampled. Microbial biomass, pH, mineral N content and six enzymatic activities involved in main nutrients cycles
25 were analyzed, and metabarcoding of 16S rDNA and ITS were performed for bacterial and fungal diversity.

26 **Results:** Enzymatic activities (chitinase, acid phosphomonoesterase, FDA-hydrolysis and β -glucosidase) were higher
27 in RAS of larger rhizosheath lines than that of smaller rhizosheath one. Bacterial β -diversity showed a separation of
28 the most contrasting lines in the principal coordinate analysis performed with the Bray-Curtis distance. Some
29 bacteria from the *Gaiellaceae* and *Sphingomonadaceae* families and the *Bradyrhizobium* genus were associated with
30 the large rhizosheath phenotype. Concerning the fungal community, we noticed a negative correlation between the
31 specific richness and the rhizosheath size and *Trichoderma* genus was positively associated to the rhizosheath size.

32 **Conclusions:** This study demonstrates that in pearl millet, rhizosheath size is related to soil nutrient dynamics and
33 microbiota diversity. However, it also shows that other factors shape this trait and their relative importance must be
34 determined.

35 **KEYWORDS:**

36 Pearl millet; Rhizosphere microbial diversity; Rhizosphere enzymatic activities; Rhizosheath; Metabarcoding

DECLARATIONS:

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- 37 **ABBREVIATIONS:**
- 38 CCA: Canonical correspondence analysis
- 39 CNRA: Centre national de recherche agronomique
- 40 FDA: 3'6'-diacetyl-fluorescein
- 41 GLM: Generalized linear model
- 42 ICRISAT: international crops research institute for the semi-arid tropics
- 43 ITS: Internal transcribed spacer
- 44 OTU: operational taxonomic unit
- 45 PCA: Principal component analysis
- 46 PCoA: Principal coordinate analysis
- 47 PERMANOVA: Permutational analysis of variance
- 48 *p*-NP: *p*-nitrophenol
- 49 *p*-NPG: para-nitrophenyl β -D-glucopyranoside
- 50 *p*-NP-NAG: para-nitrophenyl-N-acetyl-glucosaminide
- 51 *p*-NPP: para-nitrophenyl phosphate
- 52 QIIME: Quantitative insight into microbial ecology
- 53 RAS: Root-adhering soil mass
- 54 rDNA: Ribosomal deoxyribonucleic acid
- 55 RDP: Ribosomal database project
- 56 RT: Root mass
- 57 THAM: tris-hydroxymethyl aminomethane
- 58

59 INTRODUCTION

60 Pearl millet is the sixth most cultivated cereal around the World. It is mainly cultivated in the semi-arid regions of
61 the Indian subcontinent and sub-Saharan Africa (Hausmann et al. 2012). In the latter, subsistence farmers recently
62 privileged short cycle varieties that give optimal yield to cope with the reduction of the rainy season (Vigouroux et
63 al. 2011). Despite this adaptation, pearl millet yields remain very low (less than 1 ton/ha) compared to those of other
64 cereals. Besides water limitation, the factors explaining pearl millet low productivity in the Sahel region are soil
65 acidity and low nutrient content (Brück et al. 2003). The latter is one of the main factors as a result of exportation of
66 harvest residue and low input of fertilizer and manure due to the low financial capacity of farmers. Therefore, new
67 strategies need to be developed to improve pearl millet productivity in low fertility soils together with soil resilience.
68 This can be elaborated through the combination of plant breeding and rhizosphere soil microbial ecology
69 management, as the interactions between root, soil and microbiota can impact both soil aggregation (water
70 availability) and nutrient cycling (mineral nutrition). These interactions in the rhizosphere are in large part driven by
71 root carbon deposition.

72 The profile of root exudates may vary depending on the plant genotype (Aulakh et al. 2001; Czarnota et al.
73 2003; Micallef et al. 2009; Mönchgesang et al. 2016). In addition, a significant influence of plant genotype on
74 rhizosphere soil microbial diversity has been reported in different species including *Arabidopsis thaliana*, maize
75 (*Zea mays*), barley (*Hordeum vulgare*) and pear millet (*Pennisetum glaucum*) (Lundberg et al. 2012; Peiffer et al.
76 2013; Bulgarelli et al. 2015; Ndour et al. 2017). Indeed, the impact of plant genetic traits on microbial communities
77 was attributed to quantitative or qualitative variation in root exudation which constitute an important source of
78 carbon for rhizosphere microbiota (Micallef et al. 2009; Neumann et al. 2014; Schreiter et al. 2014; Carvalhais et al.
79 2015). This effect on microbial diversity could also have an impact on soil enzymatic activities and then on nutrient
80 cycling if the microorganisms involved in such processes are impacted.

81 Plant rhizosphere is known to be one of the most dynamic microbial and enzymatic hotspots (Kuzyakov and
82 Blagodatskaya 2015; Kuzyakov and Razavi 2019). This is related to rhizodeposition and root exudation of easily
83 degradable organic substances that stimulate the microbial extracellular enzyme activities (Ge et al. 2017; Ma et al.
84 2018), but also to the high density of active microorganisms that have an important role in biogeochemical processes
85 such as organic matter mineralization, pH setup and nutrient availability (Allison and Vitousek 2005; Blagodatskaya
86 et al. 2014). For pearl millet, microbial activity and some enzyme activities (acid phosphomonoesterase and
87 arylsulfatase activities) have been shown to be greater in the rhizosphere than in bulk soil (Diakhaté et al. 2016).
88 Moreover, plants can modify directly the availability of nutrients such as phosphorus (Lambers et al. 2006), zinc
89 (Hoffland et al. 2006) and iron (Ishimaru et al. 2007) through root exudation. These plant driven nutrient dynamics
90 depend on enzymes production and distribution in the rhizosphere, which are related to root morphology, root
91 exudation and rhizodeposition (Kuzyakov 2002; Ma et al. 2018). Thus, plant genetic control of carbon deposition
92 could impact diversity and activity of the microbiota and therefore could modify nutrients cycling and availability in
93 soil. For instance, two maize lines with contrasting nutrient use efficiency showed significant difference in their

94 rhizosphere microbial biomass, beta-glucosidase and urease activities in parallel with significant difference in the
95 composition of their rhizosphere microbiota (Pathan et al. 2015).

96 Moreover, several studies have evidenced a positive impact of a higher rhizosheath (synonym of root-
97 adhering soil aggregation; Ndour et al. 2020) on plant tolerance to abiotic stresses including drought (Shane et al.
98 2009; Benard et al. 2016; Liu et al. 2018, 2019; Basirat et al. 2019), phosphorus (George et al. 2014) or nitrogen
99 deficiency (Wullstein 1991; Othman et al. 2004), and soil acidity (Haling et al. 2010; Delhaize et al. 2012).
100 Importantly, numerous studies reported a plant genotypic control on this rhizosheath size in many cereals crop
101 including barley, wheat, maize and pearl millet (George et al. 2014; Delhaize et al. 2015; Ndour et al. 2017; Brown
102 et al. 2017). Notably, George et al (2014) have demonstrated that the specific rhizosheath varied reproducibly
103 between barley lines in both greenhouse and field conditions, and that this variation had an impact on plant growth
104 under water and phosphate deficiency conditions. Therefore, the interactions between plant root and soil microbiota
105 is of great importance for soil carbon management, nutrient mobilization and soil water retention and could be
106 considered in plant breeding programs for sustainable crop production. In a recent study performed under greenhouse
107 conditions, we found that rhizosphere soil bacterial communities changed significantly from one pearl millet line to
108 the other, and this variation was related to intensity of root-adhering soil aggregation (Ndour et al. 2017). So this
109 rhizosphere aggregation, genetically driven in pearl millet, is reported in other plants to be particularly important for
110 plant nutrition and improving soil water retention (Amellal et al. 1998; Alami et al. 2000). However, the impact of
111 this pearl millet intraspecific variability on microbial diversity, soil activity and nutrient availability, has not yet been
112 demonstrated under field conditions. This is particularly important as root exudation which is reported as potential
113 driver, is reported to change with plant growth stage (Aulakh et al. 2001; Jones et al. 2004; Vives-Peris et al. 2020),
114 and genetic variations could influence microbial communities in the rhizosphere under field conditions. These
115 questions should be answered before the consideration of this trait as valuable criterion for plant breeding programs.

116 The aim of this study was to determine in a field experiment whether different pearl millet lines selected for
117 their contrasting rhizosheath size, present differences in their rhizosphere microbial activity and diversity and to
118 check if such differences would be related with the rhizosheath size. For that, we measured and compared the
119 microbial biomass and different enzymatic activities involved in major nutrients cycling in the root-adhering soil
120 sampled from 13 pearl millet lines. The bacterial and fungal diversity was analyzed using metabarcoding of
121 respectively 16S rDNA genes and ITS region. We hypothesized that (i) the different pearl millet genotypes would
122 present significant differences in their microbial diversity and activities, and chemical properties in the root-adhering
123 soil fraction when cultivated in field condition and (ii) the differences in these microbial and chemical characteristics
124 could be correlated to those of the rhizosheath size.

125 MATERIALS AND MEHTODS

126 *Root-adhering soil mass phenotyping and selection of pearl millet lines under greenhouse conditions*

127 We used a panel of 181 pearl millet lines developed at the International Crops Research Institute for the Semi-Arid
128 Tropics (ICRISAT) from landrace and improved open-pollinated cultivars of West African origin previously
129 described (Debieu et al. 2018). The soil used was sampled in an experimental field of the CNRA (Centre National de
130 Recherche Agronomique), Bambey, Senegal (14.42°N, 16.28°W) and sieved at 4 mm. These pearl millet lines were
131 evaluated for their rhizosheath size using the ratio between the root-adhering soil mass and the root tissue mass
132 (RAS/RT) according to Ndour et al. (2017). Briefly, 1.5 kg of soil was filled in “WM” shaped pots installed in
133 plastic crates. Pearl millet lines were sown according to a complete random block design with 7 repetitions was
134 performed (with the sowing of successive block separated by 15 days for the harvest purpose). Pearl millet lines were
135 cultivated in a greenhouse for 28 days under natural light and temperatures ranging from 23 to 35 °C (April to
136 September 2015) with measurement of the soil mass closely adhering to the roots (RAS) and the root tissue mass
137 (RT), and calculation of the rhizosheath size. From this screening, 13 contrasting lines (from very small to large
138 rhizosheath size) were selected, taking into account germplasm availability, rhizosheath size stability and plant
139 development. These 13 lines were sown in a second experiment to confirm again their phenotypes by using the same
140 experimental procedure. We investigated the genetic relationship between these selected lines using 268,321 SNPs
141 identified through Genotyping-by-Sequencing (GBS) (Debieu et al. 2018). SNPRelate (Zheng et al. 2012) was used
142 to calculate the Identity-by-State (IBS) pairwise distances between genotypes.

143 *Analyses of root-adhering soil chemical and biochemical properties*

144 The analyses of the microbial diversity and activities of the root-adhering soil were performed on the 13
145 pearl millet lines we selected in the first part of this study. Pearl millet lines were sown during the dry season of 2016
146 (planting: March 10th) in a field experiment with the permission of the ISRA (Institut Sénégalais de Recherches
147 Agricoles). The field experiment was located at the CNRA (Centre National de Recherche Agronomique), Bambey,
148 Senegal (14.42°N, 16.28°W). The soil was sandy and had a low organic matter content (0.4 %) and low level of silt
149 and clay (12%). As the experiment was conducted in the dry season, the plants were irrigated twice a week with 30
150 mm water. The root-adhering soil was sampled in a field trial comprising the 181 inbred lines of pearl millet set up
151 for the phenotyping agromorphological traits (Debieu et al. 2018). This trial was set up using an incomplete
152 randomized blocks design and we sampled the root-adhering soil fraction of the 13 lines we selected.

153 For each of these 13 lines, root-adhering soil samples were taken from four different plants 77 days after sowing
154 (after flowering stage of millet lines). The root-adhering soils were sampled in the horizon 0-20 cm at 10 cm distance
155 from the plant stem base. In addition, four replicates of bulk soil were sampled for control in the same horizon (0-20
156 cm) of an adjacent unplanted plot that had the same physico-chemical characteristics and similarly watered. All soil
157 samples were divided into two fractions: the first one was stored in freezer (-20 °C) and was used for the microbial
158 biomass and microbial diversity analyses; the second one was stored in refrigerator (4 °C) and was used for
159 microbial activity and soil chemical analyses.

160 • *pH and inorganic N*

161 Soil pH was measured in 1:2.5 soil-to-water suspensions using a pH meter (Delta 320, Mettler Toledo, Switzerland).
162 Exchangeable ammonium and nitrate concentrations were determined by colorimetry (Technicon, Auto Analyzer III)
163 after extraction in 1 M KCl solution (Bremner 1965). Inorganic N was obtained by summing the exchangeable
164 ammonium and nitrate content of each soil sample.

165 • *Soil respiration*

166 Soil respiration was evaluated by measuring the amount of CO₂ released after humidification over a period of seven
167 days. Ten grams of air dried soil of each soil sample were placed in plasma bottle, moistened to 80% of their water
168 holding capacity and incubated at 28 °C for seven days. The amount of CO₂ released was measured daily using gas
169 chromatography with an Agilent 490 Micro GC (Agilent Technologies) by sampling and analysis of the ambient air
170 of the bottle. The average amount of CO₂ was calculated over the seven days and the results were expressed in µg C-
171 CO₂. g⁻¹ soil. day⁻¹.

172 • *Microbial biomass*

173 Microbial biomass estimation was carried out using the fumigation-extraction method (Amato and Ladd 1988). Ten
174 grams of control soil and root-adhering soil samples were fumigated (incubated for 10 days in a chloroform saturated
175 atmosphere) for microbial cells lysis. Then, the α-amino N was extracted from fumigated soil and from non-
176 fumigated samples using 2 M KCl. A reagent containing ninhydrin was then added to the solution and a colorimetric
177 determination of this nitrogen was carried out at 570 nm. The α-amino N originating from the microbial lysis was
178 determined by coloration difference between fumigated and non-fumigated soil sample using spectrophotometry.
179 Microbial-C biomass was estimated by multiplying the α-amino N concentration from microbial cells by the factor
180 21 (Amato and Ladd 1988), and expressed as µg C. g⁻¹ dry soil.

181 • *Enzyme activities*

182 The β-glucosidase activity was measured according to Hayano (1973) with some modifications. Three replicates and
183 one control tube containing 100 mg were prepared for each fresh soil sample. In the three replicate tubes, 0.1 mL of
184 phosphate citrate buffer at pH 5.8 and 100 µL of 5 mM para-nitrophenyl β-D-glucopyranoside (*p*-NPG, Sigma) were
185 added. In control tubes, the substrate (*p*-NPG) was replaced with distilled water. A blank was also carried out in
186 another tube by addition of all reagents without the soil. After incubation at 37 °C for 2 h, the reaction was stopped
187 by adding 3 mL of 0.2 M Na₂CO₃. Then the optical density of the supernatants was measured at 400 nm after a
188 centrifugation at 10,000 rpm for 5 min. The coloration due to the reaction was corrected for blank and control tube
189 coloration. The β-glucosidase activity was calculated using a calibration solution and expressed in µg *p*-NP.g⁻¹ fresh
190 soil. h⁻¹.

191 The acid phosphomonoesterase activity was measured according to Tabatabai and Bremner (1969) with
192 minor changes. For each fresh soil sample, three replicates of 100 mg were placed in tubes. In each tube we added
193 400 µL of phosphate citrate buffer (Mac Ilvain) at pH 5.8 and 100 µL of para-nitrophenyl phosphate (*p*-NPP). After

194 incubation at 37 °C during 1 h, the reaction was stopped by alkalization with 400 µL of NaOH 0.5 M and 100 µL
195 of CaCl₂ 0.5 M. The mixture was then centrifuged for 5 min at 10,000 rpm and the optical density of the supernatant
196 was measured using a spectrophotometer at 400 nm. The acid phosphomonoesterase activity was expressed in µg *p*-
197 NP.g⁻¹ fresh soil. h⁻¹ after calibration of the spectrometer using a known *p*-NPP concentration solution.

198 The hydrolysis of FDA (3'6'-diacetyl-fluorescein) gives an estimation of microbial activity as it is
199 performed by both fungal and bacterial enzymes and was determined by the method described by Adam and Duncan
200 (2001). Briefly, 1 g of soil, 15 mL of 60 mM phosphate buffer at pH 7, and 200 µL of 4.8 mM FDA were mixed in a
201 tube. After incubation at 30 °C for 1 h, the reaction is stopped by addition of acetone (10 mL). The mixture was then
202 centrifuged at 10,000 rpm for 5 min and the optical density of the supernatant measured at 490 nm. The results are
203 expressed in µg fluorescein. g⁻¹ fresh soil. h⁻¹, after calibration with a standard solution.

204 The urease activity was assayed according to Kandeler and Gerber (1988). One g soil, 1.4 mL of phosphate
205 buffer at pH 7, and 100 µL of 1.2 M urea were mixed. After incubation at 37 °C for 2 h, the reaction was stopped by
206 addition of 3 mL of 2 M KCl. The solution was then agitated for 30 min at room temperature before being
207 centrifuged at 10,000 rpm for 5 min. To reveal the ammonium production, 1 mL of the supernatant was mixed with 9
208 mL of deionized water, 5 mL of a mixture of sodium salicylate and sodium nitroprusside 1 M and 2 mL of 39 M
209 sodium dichloro-iso-cyanurate, then agitated during 30 min and the optical density measured at 660 nm. The results
210 are expressed in µg N-NH₄⁺. g⁻¹ fresh soil. h⁻¹ after calibration of the spectrophotometer with a standard N-
211 NH₄⁺ solution.

212 The chitinase activity was assayed according to Parham and Deng (2000). The reaction consists of the
213 hydrolysis of para-nitrophenyl-N-acetyl-glucosaminide (*p*-NP-NAG) that produces *p*-nitrophenol (*p*-NP). The *p*-NP
214 concentration was measured by colorimetry at 400 nm. To do this, 0.25 g of fresh soil, 1 mL of sodium acetate
215 trihydrate 0.1 M (CH₃CO₂Na·3H₂O) at pH 5.5 and 250 µL of the *p*-NP-NAG substrate were mixed. After incubation
216 at 37 °C for 1 h, the reaction was stopped by adding 250 µL of 0.5 M CaCl₂ and 1 mL of 0.5 M NaOH. The mixture
217 was centrifuged at 1000 rpm for 5 min. The optical density was measured at 400 nm and the quantity of *p*-NP was
218 calculated after calibration with a *p*-NP standard solution. The results were expressed in µg *p*-NP.g⁻¹ fresh soil.h⁻¹.

219 The dehydrogenase activity was assayed using the method of Mersi and Schinner (1991) as modified by
220 Prosser et al (2011). Five mL of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride (INT) and 3.5 mL
221 of 1 M tris-hydroxymethyl aminomethane (THAM) at pH 8.5 were added to 1 g of soil sample. After
222 homogenization and incubation of the mixture at 37 °C for 2 h, 10 mL of a mixture of ethanol and N, N-
223 dimethylformamide were added. Then, the optical density of the solution was measured at 464 nm. The results were
224 expressed in µg INTF.g⁻¹ fresh soil. h⁻¹.

225 ***Analyses of root-adhering soil microbial diversity***

226 DNA was extracted from 0.25 g of the root-adhering soil and of the control soil frozen samples using *FastDNA spin*
227 *kit for soil* (MP biomedical, USA) according to the supplier's recommendations. The extracted DNA was quantified
228 using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

229 The composition of bacterial and fungal communities was studied by sequencing the 16S r-RNA gene and
230 the ITS region from the DNA extracts. Sequencing was performed by MR DNA (503 Clovis Rd., Shallowater, TX,
231 USA) using the Ion Torrent PGM sequencer (Life Technology Corp) according to the supplier's instructions. For
232 bacteria, the V4 region of 16S ribosomal DNA was targeted using primers 515F/806R (Caporaso et al. 2011). The
233 diversity of fungal communities was studied by sequencing the ITS regions using the primers: ITS1F 5'-
234 CTTGGTCATTTAGAGGAAGTAA-3' / ITS2R: 5'-GCTGCGTTCTTCATCGATGC-3' (Orgiazzi et al. 2012). For
235 both 16S and ITS sequencing, PCR was performed using the HotStar Taq plus Master Mix reaction kit (Qiagen,
236 Germantown, MD, USA) under the following conditions: 94 °C for 3 min followed by 28 cycles consisting of a 94
237 °C step for 30 s, a step of 53 °C for 40 s and 72 °C for 1 min. A final elongation step of 72 °C for 5 min was added at
238 the end of these 28 cycles. We processed data using Quantitative Insight Into Microbial Ecology (QIIME) software
239 version 1.9 (Caporaso et al. 2010).

240 *16S rRNA amplicon sequence processing:* raw data were checked for quality, mismatches and
241 homopolymers. Sequences with a quality score average lower than 25, sequences with homopolymers, and
242 sequences shorter than 200 pb were removed from the analysis. Using an inhouse PHP script (LEMIRE/BIAM,
243 Cadarache, France), we conducted mitochondria and chloroplast sequences filtering after the denoising and chimera
244 check. The sequences were then demultiplexed according to their barcode. Sequences representing less than 0.1 % of
245 total sequence count were filtered. Thereafter the open reference operational taxonomic unit (OTU) picking method
246 from QIIME was used. It consists of the clustering of sequences against the Greengenes database at 97% threshold.
247 Sequences that did not match any entries in these references were then clustered using the *de novo* OTU picking at
248 97% similarity using Usearch. Taxonomic assignment of OTUs was performed using the RDP classifier and the
249 Greengenes database (DeSantis et al. 2006). The phylogenetic position of bacteria in the different treatment was then
250 determined using the QIIME pipeline script. To compare bacterial α -diversity of RAS fraction of the 13 pearl millet
251 lines, we used Shannon diversity index, Phylogenetic Diversity index (PD-Whole), Chao1 richness estimator and the
252 number of OTUs. To do this, we performed rarefaction of data by picking randomly 24839 sequences in which the
253 different α -diversity parameters were calculated.

254 *ITS amplicon sequence processing:* sequences with a quality score average lower than 25, sequences with
255 homopolymers were removed. As we have done for bacteria, mitochondria and chloroplasts sequences were filtered
256 after the denoising and chimera check. Therefore, a demultiplexing step was performed using barcodes to attribute
257 valid sequences to their corresponding treatments. The sequences representing less than 0.1 % of total count were
258 filtered from the dataset and the remaining sequences were then clustered into OTUs by the Usearch method at 97%
259 pairwise identity using the QIIME *de novo* picking strategy (Edgar 2010). The OTUs representative sequences were
260 classified using RDP and the non-redundant Unite sequence database at 97% identity. Using QIIME, fungal α -
261 diversity metrics was calculated using 19362 ITS sequences per sample to compare the different treatments and the
262 taxonomical composition was determined for each sample up to species level if possible. Sequence data from both
263 16S rDNA and ITS sequencing are available in NCBI under the SRA accession number PRJNA475215.

264 ***Statistical analysis***

265 Statistical analysis was performed using R statistical environment (version 3.3.3). The normality of different
266 variables was tested using the Shapiro test ($p < 0.05$). To test pearl-millet line effect on the different parameters
267 (RAS/RT ratio, microbial biomass, pH, mineral N, soil respiration, enzyme activities), we constructed general linear
268 models (GLM) using the “glm” function in R. Each model was fitted by considering data distribution and using the
269 corresponding link function *i.e.* Gaussian (link="identity") for normally distributed variables and Poisson
270 (link="log") for not normally distributed variables. Then, ANOVAs were fitted to these models using the “Chisq”
271 test, and Tukey HSD post-hoc tests (library multcomp) were performed ($p < 0.05$) to identify the significant
272 differences between treatments (13 lines + control soil). To compute bacterial and fungal beta-diversity of the soil
273 samples, Bray-Curtis distance matrices were generated from the OTU tables and Principal Coordinate Analyses
274 (PCoA) were performed. The significance of samples separation in the PCoA plot was tested using PERMANOVA
275 (Adonis, 999 permutations) of the Bray-Curtis distance matrixes. Correlation analyses were performed using the
276 Spearman method to test possible relation between the Bray-Curtis distance of the rhizobacterial communities of the
277 selected pearl millet lines and their phylogenetic distances. Moreover, a principal component analysis (PCA) was
278 performed on the enzyme activities to evaluate the relationship between root-adhering soil mass phenotype and
279 enzyme activities in the rhizosphere. To test the correlation of enzymatic activities, N content, and pH (constrained
280 variables) with microbial communities, canonical correspondence analyses (CCA) were performed using bacterial
281 and fungal genus matrices and the matrix of these chemical and biochemical data (*vegan package*). Then, the
282 *anova.cca* function was applied to assess the significance of the correlations ($p < 0.05$, 999 permutations). To detect
283 bacterial and fungal OTUs that were enriched or depleted in the rhizosphere of the most contrasting lines compared
284 to the control soil, the “group significance script” (available in QIIME) was performed with the *g*-test option. After
285 this, mean abundances of the discriminant taxa (genus or family) corresponding to these OTUs were compared in the
286 different lines libraries by carrying out a GLM and ANOVA.

287 RESULTS

288 *Plant phenotyping and lines selection for microbiological analyses*

289 The phenotyping of the 181 pearl millet lines revealed a large variation on the rhizosheath size (RAS/RT ratio),
290 ranging from 7.4 (L220) to 26.3 (L132) (**Fig. 1**). The results of the second phenotyping experiment confirmed
291 significant differences of the rhizosheath size, ranging from 7.8 (L220) to 25.6 (L24) (**Table 1**). The four lines (L24,
292 L64, L132 and L253) with significantly higher RAS/RT ratios were named "*large rhizosheath lines*", and L220 with
293 significantly lower RAS/RT ratio was named "*small rhizosheath line*". Other lines with RAS/RT ratio not
294 significantly different from the mean value (L3, L55, L62, L71, L137, L126, L177 and L216) were considered as
295 "*intermediary rhizosheath lines*".

296 *pH and inorganic N*

297 The pH values of RAS fraction from the different millet lines ranged from 6.6 (L132) to 8.4 (L177). Compared to
298 control soil, there was a significant decrease of pH in the RAS fraction of 3 lines, two large rhizosheath lines (L132,
299 L253) and one intermediary rhizosheath line L71. There was no significant change in root-adhering soil pH for other
300 lines compared to the control (**Fig.2A**).

301 Mineral N (including the exchangeable ammonium and nitrate content) varied significantly ($p < 0.0001$)
302 between 2.5 (L177) and 16.3 $\mu\text{g N.g}^{-1}$ dry soil (L253) in the RAS (**Fig.2B**). Even if the line L253 showed a mineral
303 N content in root-adhering soil not significantly different from those of the control soil, it showed much more
304 mineral N compared to most of the intermediary lines and the small rhizosheath line L220 (more than two-fold
305 increase). This increased mineral N content was due to nitrate content in RAS of L253 pearl millet line (significantly
306 higher than the control soil and most of the other pearl millet lines, **Supplementary file 1: Fig. S1**) but not to the
307 ammonium content (**Supplementary file 1: Fig. S2**).

308 *Microbial biomass*

309 The microbial biomass was 18.5 $\mu\text{g C.g}^{-1}$ dry soil⁻¹ in the control soil and varied from 5.5 (L220) to 35 $\mu\text{g C.g}^{-1}$ dry
310 soil (L71) in the RAS fraction of the 13 pearl millet lines (**Fig. 2C**). None of the microbial biomass C values of these
311 RAS fractions were significantly different compared to the control soil. The main significant differences were
312 observed between the small rhizosheath line (L220) and one intermediary rhizosheath line (L3) and on the other side
313 the large rhizosheath line (L64) and two intermediary rhizosheath lines (L71 and L62) (4-fold average increase).

314 *Soil respiration*

315 Respiratory activity of RAS samples ranged from 2.6 (L253, L132) to 3.8 $\mu\text{g C-CO}_2 \text{ g}^{-1} \text{ dry soil day}^{-1}$ (L3), always
316 higher than that of the control soil (2.1 $\mu\text{g C-CO}_2 \text{ g}^{-1} \text{ dry soil. day}^{-1}$) (**Fig. 2D**). However, only the RAS of the large
317 rhizosheath line L64, and two intermediary rhizosheath lines (L3, L126) showed a significant increase of respiratory
318 activity compared to the control soil ($p < 0.05$).

319 *Enzymatic activities*

320 Six enzymatic activities (β -glucosidase, acid phosphomonoesterase, chitinase, FDA hydrolysis, dehydrogenase and
321 urease) were determined on the RAS fraction of the 13 pearl millet lines and on the control soil (**Table 2**). The
322 Principal Component Analysis (PCA) performed on these enzymatic activities showed a total inertia percentage of
323 62.8 % by combining the components F1 and F2. The biplot of the samples from pearl millet lines and the different
324 variables in the F1 * F2 plane is shown in **Fig. 3**. The F1 component (dim1: 48 % of the variability) was mainly
325 explained by four enzymatic activities: chitinase, acid phosphomonoesterase, FDA hydrolysis and β -glucosidase.
326 The F2 component (dim 2: 14 % of the variability) was explained by dehydrogenase and urease (**Supplementary file**
327 **1: Fig. S3 and S4**). We found that the enzymatic activities of RAS samples from three of the four large rhizosheath
328 lines (L64, L132, L253) were clearly separated from those of the small rhizosheath line (L220) and of the control
329 soil. The fourth large rhizosheath line (L24) was grouped with the small rhizosheath line L220 and the control soil
330 (**Fig. 3**). Due to high variability of the enzymatic activities data, very few significant differences were evidenced
331 (**Table 2**). However, some enzymatic activities measured in the RAS of large rhizosheath lines were significantly
332 higher ($p < 0.05$) than the ones of the control soil and of the small rhizosheath line (L220). Acid
333 phosphomonoesterase and chitinase were, respectively, 8-fold and 3-fold higher in the RAS fraction of L253 than in
334 control soil. Compared to the small rhizosheath line (L220), these two activities were, respectively, 6-fold and 5-fold
335 higher in RAS fraction of this large rhizosheath line (L253). Chitinase activity was 3-fold and 4-fold higher in the
336 RAS of L64 than in, respectively, control soil and RAS of the small rhizosheath line (L220).

337 *Bacterial diversity*

338 The sequencing of 16S rRNA gene in root-adhering soil samples produced 2.06×10^6 sequences (min=26449, max=
339 53890 sequences per sample). The results showed that the Shannon diversity index calculated from the abundances
340 of the defined OTUs did not show any significant variability between the different treatments (including control soil)
341 (**Table 3**). However, the general trend is that the highest value for these estimators of α -diversity was observed in the
342 control soil. The only significant decrease of α -diversity in RAS of pearl millet lines was observed with the
343 intermediary rhizosheath line L71 (PD-Whole and OTU richness). We used Bray-Curtis distance matrix and a PCoA
344 to assess the β -diversity of bacterial community in RAS fractions of pearl millet lines and in the control soil (**Fig.**
345 **4A**). The projection of the individuals showed that the soil sampled from three large rhizosheath lines (L64, L132,
346 L253) were distantly separated from the one of small rhizosheath line (L220) and of the control soil ($p < 0.001$;
347 F.Model = 2.93; PERMANOVA, 999 permutations). Again, the fourth large rhizosheath line (L24) is closer to the
348 control soil and the small rhizosheath line (L220) (**Fig. 4A**). Moreover, the CCA performed revealed significant
349 relations between bacterial community and N content, pH and enzymes activities we measured in the soil samples (p
350 < 0.05). These variables used as constraints explained 21 % of the community distribution (**Supplementary file 2**).

351 The projection of soil samples and these variables in the two first axes confirmed the separation between the control
352 soil and the small rhizosphere line (L220) and the 3 large rhizosphere lines (L132, L64 and L253), as we observed in
353 the PCA of enzyme activities and the PCoA of bacterial communities (**Supplementary file 1 Fig S5**). However, we
354 did not find a significant correlation between the Bray-Curtis distances based on rhizobacterial communities of the
355 RAS of pearl millet lines and the phylogenetic distances of these lines ($p > 0.05$, **Supplementary file 1: Fig S6**)
356 based on genotyping-by-sequencing data.

357 At the phylum level, the two dominant phyla in this soil and in the RAS of the 13 pearl millet lines tested
358 were *Proteobacteria* and *Firmicutes*, which constituted together more than 50% of the total bacterial phyla. These
359 two dominant phyla were followed by *Actinobacteria* (from 12 to 20%) and *Acidobacteria* (4 and 8%) according to
360 the treatments (**Supplementary file 1: Fig. S7**). At this level of taxonomic analysis of the microbiota composition in
361 the RAS fraction, there was no difference between large and small rhizosphere lines, and between each of them
362 compared to the control soil (**Supplementary file 1: Fig. S7**).

363 At the OTU level, using the QIIME *g-test* script we detected the dominant OTUs differentially abundant in
364 the RAS fraction of the large rhizosphere lines (L24, L64, L132, L253) compared to the control soil. The most
365 discriminant OTUs showing significant increase compared to control soil ($p < e-12$) were affiliated to the following
366 taxa: *Bradyrhizobium*, *Mesorhizobium* and *Kaistobacter* (*Alphaproteobacteria*), and *Gaiellaceae* (*Actinobacteria*).
367 The most discriminant OTUs showing significant decrease compared to control soil were affiliated to *Ammoniphilus*.
368 To compare the abundance of these taxa in the RAS samples of the different pearl millet lines, we used a GLM to fit
369 an ANOVA and used the Tukey HSD post-hoc test. Their differential distribution on the bacterial sequence libraries
370 can be observed in **Table 4**. The abundance of *Bradyrhizobium*, *Mesorhizobium*, *Kaistobacter* and *Gaiellaceae* was
371 increased in the RAS of the four large rhizosphere lines (L24, L64, L132, L253) when compared to that of the small
372 rhizosphere line (L220), with only one exception (abundance of *Kaistobacter* decreased in RAS of L24).
373 Concomitantly the abundance of *Ammoniphilus* was decreased in the rhizosphere of the large rhizosphere lines (L24,
374 L64, L132, L253).

375 **Fungal diversity**

376 After the sequencing of ITS gene in root-adhering soil of the 13 pearl millet lines, we obtained 2.71×10^6 sequences
377 (min =19816, max= 105293 sequences per samples). Using the three diversity indices (Shannon index, Chao1 and
378 OTU number), there was no significant difference of fungal α -diversity between RAS fractions of three large
379 rhizosphere pearl millet lines (L24, L64, L253) and that of the control soil (**Table 3**). However, for these three lines,
380 the fungal specific richness (OTUs number and Chao1 estimator) of the RAS fraction was significantly lower than
381 that of the small rhizosphere line (L220). This tendency of a reduced fungal α -diversity in the RAS of large
382 rhizosphere lines was confirmed by the negative correlations between the RAS/RT ratio and the different α -diversity
383 metrics (**Fig. 5**). The fungal β -diversity was evaluated by a PCoA performed on the Bray-Curtis distance matrix. The
384 projection of the individuals (soil samples) in the two first components PC1 and PC2 explained only 21 % of the
385 distribution. In this projection, there was no separation between the RAS sampled from the different pearl millet lines
386 in relation to their rhizosphere size (**Fig. 4B**). The CCA performed did not reveal any significant relation between

387 fungal community and N content, pH and enzymes activities we measured in RAS sample ($p > 0.05$, **Supplementary**
388 **file 2**).

389 The taxonomical composition of the soil samples from the different pearl millet lines was determined at the
390 phylum level (**Supplementary file 1: Fig. S8**). Overall, there was no relationship between the RAS/RT ratio and the
391 abundance of fungal groups in the RAS fraction of the different pearl millet lines. We noticed that 35 to 57% of the
392 fungal sequences (depending on the treatments) could not be affiliated. The *Ascomycota* and a non-identified phylum
393 constituted the most represented phyla with abundances ranging, respectively, from 38 to 62% and from 29 to 58%
394 (**Supplementary file 1: Fig. S8**), followed by *Basidiomycota* and *Glomeromycota* phyla for which relative
395 abundances ranged respectively from 1.6% to 19% and from 0.1% (for L64) to 2.8% (L177).

396 The group significance test performed using the QIIME *g-test* script gives fungal OTUs that have differential
397 abundances between the rhizosphere of pearl millet lines and the control soil. However, due to so many non-
398 identified OTUs, only the genus *Trichoderma* has been clearly identified to be differentially distributed in the RAS
399 of large rhizosheath lines compared to control soil and the small rhizosheath line. Indeed, *Trichoderma* sequences
400 were significantly more abundant ($p < 0.05$) in the rhizosphere of two large rhizosheath lines (L132: n=77 sequences
401 [0.11% of sequence count]; L253: n=87 sequences [0.30 % of sequence count]) compared to one of the small
402 rhizosheath line (L220: n=10 sequences [0.02% of sequence count]) and the control soil (n=25 sequences [0.02% of
403 sequence count]).

404 DISCUSSION

405 In a previous study, Ndour et al. (2017) found a 2.9-fold variation in the rhizosheath size using a subset of 9 pearl
406 millet lines selected for bacterial diversity study. The rhizosheath size we measured in the present work are
407 consistent with these previous results, as we found a 2.85-fold variation in the rhizosheath size in 13 different pearl
408 millet lines that we selected for microbial diversity and activities analyses. Moreover, in both studies (pot and field
409 experiment), the same dominant bacterial phyla were found in the rhizosphere of pearl millet lines (*Proteobacteria*,
410 *Firmicutes* and *Actinobacteria*). This is not surprising since the soil used in the pot experiment of this previous work
411 was sampled in the same area that the field experiment of this present work. However, the relative importance of
412 such dominant phyla varied according to the millet lines in the two studies. Since the pearl millet lines used in these
413 two studies are different, it was not possible to make further comparisons of the composition of microbial taxa in
414 RAS between these two studies.

415 *pH and microbial biomass in the RAS of the contrasting pearl millet lines*

416 We noticed for three of the large rhizosheath lines (L64 L132, L253,) a global reduction of the pH compared to the
417 control soil (significant differences for L253 and L132, pH 6.6-6.7) in contrast with the small rhizosheath line (L220)
418 and the control soil value (respectively pH= 8.1 and 7.9). This reduction of pH in the RAS of the large rhizosheath
419 lines could be related to a higher amount of root exudates deposited in the rhizosphere of these lines compared to the
420 small rhizosheath one. Indeed, root exudates include many organics compounds such as amino acids, organic acids
421 that could modify the pH and then impact the microbial diversity (Li et al., 2016; Rousk et al., 2010; Zhahnina et al.,
422 2015) and activities in the rhizosphere. Importantly, it has been shown a positive correlation between root exudates
423 amount and the stability of soil aggregates (Traoré et al. 2000) or the rhizosheath size (Liu et al. 2019).

424 The microbial biomass of the large rhizosheath lines (L64, L132, L253) was globally higher than the one of
425 the small rhizosheath line (L220) even if the differences were significant only with the L64 line. In a previous study,
426 using quantitative PCR of 16S rRNA gene, we found that different pearl millet lines with contrasting RAS/RT ratios
427 did not show any significant difference in bacterial community density when grown in greenhouse conditions (Ndour
428 et al. 2017). An element of explanation may reside in the difference in plant age that can influence the
429 rhizodeposition and then impact soil microbiota as sampling of RAS was performed 28 days after sowing in the first
430 study (Ndour et al. 2017) and 77 days after sowing in the present work. Accordingly, Pausch et al. (2016) reported
431 significant variations of microbial biomass in barley rhizosphere between two different sampling times (tillering vs
432 head emergence). On the other hand, Pausch et al. (2016) reported that different barley genotypes did not show any
433 significant difference in the microbial biomasses measured in their rhizosphere. This could be related to the
434 difference in genetic distances between barley genotypes and between pearl millet genotypes used in these studies.
435 Even if significant only for the line L64, the microbial biomass was more important in the RAS of large rhizosheath
436 lines (L24, L64, L253, L132) compared to those of the small rhizosheath line (L220; two to five-fold variation). This
437 suggests that the size of the microbial community may contribute to the determination of the RAS/RT ratio in pearl
438 millet. It is important to note that our microbial biomass values are low compared to those founded by Diakhaté et al.

439 (2016). This could be related to the lower organic carbon content of the soil (0.4 % vs 0.52 %) and the different
440 protocol of the estimation as we used the fumigation-extraction method whereas, in this comparative study, the
441 authors used the respiratory rate of glucose to estimate soil microbial biomass.

442 ***Microbial activity in the RAS of the contrasting pearl millet lines***

443 Several enzymatic activities have been reported to be elevated in the rhizosphere of plants compared to unplanted
444 control soil (Pathan et al. 2015; Giagnoni et al. 2016). Particularly, acid phosphomonoesterase activity was
445 significantly higher in soybean rhizosphere than in control soil (Fraser et al. 2017). In our study, the different
446 enzymatic activities measured in the RAS of pearl millet lines and in control soil showed very different patterns.
447 Chitinase, acid phosphomonoesterase and to a lesser extent FDA activities were generally stimulated in the RAS of
448 pearl millet lines compared to control soil. On the other hand, beta-glucosidase, urease and dehydrogenase activities
449 were not significantly different between the RAS of pearl millet lines and the control soil. The differential responses
450 between FDA and dehydrogenase activity, which both indicate general microbial activity, could be explained by the
451 differences in pH in which they were tested. Indeed, the former was measured at pH 7, which is closer to the pH
452 measured in the RAS than the latter measured at pH 8.5. In addition, dehydrogenase is an indicator of viable
453 microbial community activity (active microbiota) whereas the FDA gives an estimation of both viable and dead
454 microbial community activity (intracellular and extracellular enzymes). These variations of enzymatic activities
455 could be explained by a potential variation in root exudation patterns of the different pearl millet lines as reported
456 earlier on different pearl millet varieties (Venkateswarlu and Rao 1985). Indeed, root exudates shape rhizosphere
457 microbial diversity (Paterson et al. 2007; Haichar et al. 2008; Micallef et al. 2009; Turner et al. 2013) and then could
458 select or deplete some microbial populations that could be differentially competent for different soil functions. For
459 instance, Pathan et al. (2015) and Giagnoni et al. (2016) reported concomitantly variations in beta-glucosidase and
460 urease activities, and variations in the bacterial and fungal communities structure in the rhizosphere of two maize
461 lines. Moreover, it is important to note that this influence of root exudates on enzymatic activities could also be
462 direct as it was reported that plants can also release phosphatases directly into soil to hydrolyze soil phytates in
463 phosphorus deficient conditions (Hammond et al. 2004; Rengel and Marschner 2005).

464 The principal component analysis carried out on these enzymatic activities showed differences between
465 pearl millet lines according to their root-adhering soil mass (rhizosheath size) phenotype. Chitinase, acid
466 phosphomonoesterase, beta-glucosidase and FDA enzymatic activities appear globally higher in the rhizosphere of
467 lines with high RAS/RT ratio (L64, L132, L253). In barley, it was shown that the RAS/RT ratio varied significantly
468 between different lines (George et al., 2014) and the increase of this ratio was associated with an improvement of
469 plant growth in phosphorus and water deficit conditions (Brown et al., 2012; George et al., 2014). Thus, this growth
470 improvement could be explained by a greater phosphatase activity in the rhizosphere of lines with high RAS/RT ratio
471 (large rhizosheath line). We therefore propose two hypotheses to explain this convergence between some enzymatic
472 activities and the rhizosheath size. The first one is that pearl millet root exudation could be variable between the
473 different lines, as reported in numerous other cereal species (Aulakh et al., 2001; Czarnota et al., 2003; Micallef et
474 al., 2009; Mönchgesang et al., 2016). Thus, a more important root exudation in the rhizosphere of the large

475 rhizosheath lines could lead to a decrease of the pH (that potentially stimulated some enzymatic activities: chitinase,
476 beta-glucosidase, and acid phosphomonoesterase, that have their optimal potential under acid pH conditions), as well
477 as an increase of aggregates stability and then a larger rhizosheath as reported (Traoré et al. 2000; Liu et al. 2019).
478 Moreover, a variation in root exudate amount could also affect rhizosphere microbial populations involved in root-
479 adhering soil aggregation and rhizosheath formation such as EPS-producing bacteria as reported in several studies
480 (Alami et al. 2000; Bezzate et al. 2000; Kaci et al. 2005; Berge et al. 2009). The second hypothesis is a genotypic
481 variation of root hair characteristics (density and length) between the different millet lines as reported in others cereal
482 species (Delhaize et al. 2015; James et al. 2016; Adu et al. 2017). It has been shown that root hair density and length
483 had an impact on several root traits such as exudation (Yan et al., 2004), microbial diversity (Robertson-Albertyn et
484 al., 2017), rhizosheath formation (Delhaize et al., 2012; George et al., 2014) as well as leaf phosphorus
485 accumulation, plant biomass and phosphorus deficiency tolerance (Brown et al., 2012). Importantly, these different
486 processes could have an impact in nutrient recycling in the rhizosphere.

487 ***Bacterial diversity in the RAS of the contrasting pearl millet lines***

488 The analysis of the bacterial α -diversity that refers to the diversity within a sample (Jurasinski et al. 2009) showed
489 very few significant differences between samples from the RAS of different pearl millet lines and control soil: only
490 one exception for L71 line which was different from L3, L55, L137 and the control soil for PD-whole and OTU
491 richness (**Table 4**). This result is quite different from that obtained by Peiffer et al. (2013), which showed high
492 variability on specific richness (OTU richness) between the rhizosphere samples from different maize lines. They
493 found that plant genetic effect explained 19 % of the variability of OTUs number. This discrepancy may be
494 explained by many factors such as the intraspecific genetic distance, which may be greater in their panel than in ours
495 but also by difference in soil physicochemical characteristics that could have a stronger influence on bacterial
496 diversity. The analysis of beta-diversity or “differential diversity” (Jurasinski et al. 2009) between the rhizosphere
497 samples of the pearl millet lines, using the Principal Coordinate Analyses of Bray-Curtis distance matrix, evidenced
498 a separation between millet lines. As reported by Peiffer et al. (2013), we could not relate the kinship matrix for the
499 pearl millet lines to the rhizobacteria beta-diversity distances matrix. This may suggest a control of the microbial
500 community by a few major localized alleles of the genome or an indirect control of host plant on rhizosphere
501 microbiota. Previous studies also showed that the plant genotype explains a small but significant part of the beta-
502 diversity variation in the rhizosphere (Lundberg et al. 2012; Peiffer et al. 2013; Ndour et al. 2017; Robertson-
503 Albertyn et al. 2017). Interestingly, we found that the three large rhizosheath lines (L64, L132 and L253) are clearly
504 different from the small rhizosheath line (L220) and the control soil (**Fig. 4A**) and that this separation of pearl millet
505 lines within the PCoA plot was similar to that we observed in the PCA plot performed with the enzymatic activities.
506 This suggests that some bacterial taxa that were differentially distributed in RAS of contrasting rhizosheath lines
507 (species belonging to *Gaiellaceae* and *Sphingomonadaceae* families and *Bradyrhizobium* genus) could play specific
508 role in both soil chitinase, acid phosphomonoesterase and beta-glucosidase activities, and in RAS aggregation
509 (rhizosheath formation).

510 However, it remains very challenging to highlight bacterial species responsible for rhizosheath formation by
511 plant using metabarcoding approach because (i) most of the time attribution cannot be made at the species level and,
512 (ii) the rhizosheath size is a very integrative phenotype which responds to various biotic and abiotic drivers (Brown
513 et al. 2017; Pang et al. 2017; Ndour et al. 2020). Concerning the bacteria component, several studies have reported
514 (i) an increase of the rhizosheath size after inoculation of plant with an exopolysaccharides (EPS)-producing
515 bacterial strains (Alami et al. 2000; Kaci et al. 2005; Sandhya et al. 2009), (ii) a correlation of some rhizobacterial
516 taxa abundances with the rhizosheath size (Ndour et al. 2017; Zhang et al. 2020). However these bacteria belong to
517 different phylogenetic groups: *Rhizobiaceae/Alphaproteobacteria* (Alami et al. 2000; Berge et al. 2009),
518 *Enterobacteriaceae/Gammaproteobacteria* (Amellal et al. 1998; Zhang et al. 2020), *Paenibacillaceae and*
519 *Bacillaceae/Firmicutes* (Bezzate et al. 2000; Fernández Bidondo et al. 2012) as well as their mechanisms used to
520 shape the rhizosphere are very diversified: production of EPS (for instance levane; Bezzate et al. 2000) , ethylene
521 production (1-aminocyclopropane-1-carboxylic acid [ACC] deaminase activity) and plant hormonal balance
522 resulting in root hairs formation (Zhang et al. 2020).

523 ***Fungal diversity in the RAS of the contrasting pearl millet lines***

524 The impact of plant genotype on the diversity of rhizosphere fungal populations was already demonstrated (Yao and
525 Wu 2010; Yu et al. 2016). Our study confirms these results and further shows a significant decrease in the specific
526 richness of the fungal community in the RAS of two large rhizosheath lines (L24 and L64) compared to small
527 rhizosheath line (L220) and one intermediary rhizosheath line (L137). Interestingly, negative correlation was found
528 between RAS/RT ratio (rhizosheath size) and fungal specific richness. This could mean that the large rhizosheath
529 pearl millet lines could be associated to the selection of specific fungi leading to a decrease in global fungal
530 diversity. It has been demonstrated a positive impact of arbuscular mycorrhiza on soil aggregation in several studies
531 (Wu et al. 2014, 2015; Rillig et al. 2015). However, the g-test we have performed did not highlight any OTU
532 matching for arbuscular mycorrhiza to differentiate RAS from large and small rhizosheath line. We evidenced an
533 enrichment of the *Trichoderma* genus in the RAS of two large rhizosheath lines among the four. Interestingly, some
534 recent studies reported an increase of polysaccharides amount in the rhizosphere and a PGPR effect after inoculation
535 of plants (including chickpea, wheat and cotton) with *Trichoderma* species (Velmourougane et al. 2017, 2019). So
536 we can hypothesize that some species belonging to this genus could contribute to root-adhering soil aggregation
537 depending on pearl millet line.

538 **CONCLUSION**

539 The data presented show that, in pearl millet, the RAS/RT ratio phenotype (rhizosheath size) could be correlated with
540 different rhizosphere microbial parameters (bacterial and fungal diversity, enzymatic activities) which may act
541 concomitantly or independently, depending on the genotype. We identified some bacterial and fungal taxa associated
542 with a large rhizosheath phenotype and noticed a decrease of the pH in the rhizosphere of some large rhizosheath
543 lines indicating a role of root exudation as a potential driver. Moreover, our study highlighted that, in pearl millet,
544 the root-adhering soil aggregation process (rhizosheath formation) is very complex and could potentially involve
545 other mechanisms which were identified as drivers such as root architecture (Adu et al. 2017; Burak et al. 2018), root
546 hair characteristics (length and density; Delhaize et al. 2012, 2015; George et al. 2014), and the composition of root
547 exudates (Traoré et al. 2000; Liu et al. 2019). The relative importance of these different drivers will be investigated
548 in future studies. Nevertheless, rhizosheath size is a promising trait to cope with abiotic stress and identification of
549 plant genes controlling this trait could contribute to sustainable agriculture particularly in arid and semi-arid regions
550 where this cereal is cultivated.

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