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Papa Mamadou Sitor Ndour, Cheikh Mbacké Barry, Diamé Tine, Carla de La Fuente Cantó, Mariama Gueye, mohamed Barakat, Philippe Ortet, Wafa Achouak, Ibrahima Ndoeye, Bassirou Sine, et al.

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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  $\beta$ -glucosidase) were higher  
27 in RAS of larger rhizosheath lines than that of smaller rhizosheath one. Bacterial  $\beta$ -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  $\beta$ -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 10<sup>th</sup>) 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> was calculated over the seven days and the results were expressed in µg C-  
171 CO<sub>2</sub>. g<sup>-1</sup> soil. day<sup>-1</sup>.

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<sup>-1</sup> 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 Na<sub>2</sub>CO<sub>3</sub>. 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<sup>-1</sup> fresh  
190 soil. h<sup>-1</sup>.

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 CaCl<sub>2</sub> 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<sup>-1</sup> fresh soil. h<sup>-1</sup> 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<sup>-1</sup> fresh soil. h<sup>-1</sup>, 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-NH<sub>4</sub><sup>+</sup>. g<sup>-1</sup> fresh soil. h<sup>-1</sup> after calibration of the spectrophotometer with a standard N-  
211 NH<sub>4</sub><sup>+</sup> 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 (CH<sub>3</sub>CO<sub>2</sub>Na·3H<sub>2</sub>O) 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 CaCl<sub>2</sub> 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<sup>-1</sup> fresh soil.h<sup>-1</sup>.

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<sup>-1</sup> fresh soil. h<sup>-1</sup>.

## 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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -  
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<sup>-1</sup> 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  $\mu\text{g C-CO}_2 \text{ g}^{-1}$  dry soil  $\text{day}^{-1}$  (L3), always  
316 higher than that of the control soil (2.1  $\mu\text{g C-CO}_2 \text{ g}^{-1}$  dry soil.  $\text{day}^{-1}$ ) (**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 ( $\beta$ -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  $\beta$ -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  $2.06 \times 10^6$  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  $\alpha$ -diversity was observed in the  
342 control soil. The only significant decrease of  $\alpha$ -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  $\beta$ -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  $2.71 \times 10^6$  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  $\alpha$ -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  $\alpha$ -diversity in the RAS of large  
382 rhizosphere lines was confirmed by the negative correlations between the RAS/RT ratio and the different  $\alpha$ -diversity  
383 metrics (**Fig. 5**). The fungal  $\beta$ -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  $\alpha$ -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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