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1 **FUNGI DIVERSITY IN THE RHIZOSPHERE OF *Aspilia pruliseta* Schweif. ext Schweif**
2 **IN THE SEMI-ARID EASTERN KENYA**

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29 **Abstract**

30 Semi-arid eastern Kenya is a fragile ecosystem with continuous cultivation of dryland pulses and
31 grains. Farmers use artificial fertilizers most of which are deleterious to the environment. Previous
32 studies have shown that soil microbes in the rhizosphere could be used to sustainably enhance
33 levels of soil mineral nutrients and soil health. However, few studies have examined fungal
34 diversity in the rhizosphere of wild and native *Aspilia pruliseta* shrub. In this study, amplicons of
35 Internal Transcribed Spacer (ITS) region on Total Community DNA using Illumina sequencing
36 were used to explore the fungal community composition within the rhizosphere. Operational
37 taxonomic units (OTUs) were analyzed using QIIME 1.8.0, taxonomy assigned via BLASTn
38 against SILVA 119 database. Hierarchical clustering was done using R programming software.
39 72,093, 50,539 and 43,506 sequence reads were obtained from samples $MC1_a$, $MC2_a$ and $MC3_a$
40 respectively representing rhizosphere depth 0-20 cm, 21-40 cm and 41-60 cm. A total of 373
41 OTUs were realized at 3% genetic distance. Taxonomic analysis revealed that the genera *Glomus*
42 was most prevalent in all soil depths with 85.60 % of the OTUs in depth 0-20 cm, 69.04 % in depth
43 21-40 cm and 48.45 % in depth 41-60 cm. The results revealed high levels of obligate arbuscular
44 mycorrhiza fungi that if commercially cultured could enhance phosphates uptake in crops.

45 Keywords: Fungi diversity, Rhizosphere, *Aspilia pruliseta,* OTUs, DNA

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53 **Introduction**

54 Microorganisms were used in early civilizations for agricultural and industrial processes long
55 before they were well known and documented. Recent advancements in understanding about the
56 genetics, physiology, and biochemistry of fungi, has led to the exploitation of fungi for different
57 purposes in agriculture and industrial products of economic importance. Application of chemical
58 fertilizers to crop plants negatively affects human health and environments (1) . Beneficial plant-
59 microbes interactions in the rhizosphere are determinants of plant health and soil fertility (2).
60 Recent studies have focused on identification of alternative methods to enhance plant productivity
61 and protect the soil. This can only be possible with thorough knowledge and information of
62 microbes along the plants' rhizosphere and fungal microbes is one category, that with this
63 understanding, could provide the link and alleviate challenges posed by poor land productivity.
64
65 Arbuscular mycorrhizal fungi (AMF) are below ground symbiotic associations between plant roots
66 and fungi (3). AMF can improve plant growth under low fertility conditions, improve water
67 balance of the plants and help plants to establish in new areas (4). These beneficial effects imply
68 that the plant community structure and productivity in ecosystems are influenced significantly by
69 the AM fungal diversity in the soil (5). However, the genetic diversity of fungi and other microbes
70 in the plants' rhizosphere is not yet fully comprehended (1).
71
72 Fungi dominate in low pH or slightly acidic soils where soils tend to be undisturbed (6). A good
73 undisturbed ecological niche would be a plants' rhizosphere. Over 80% of vascular or non-vascular
74 terrestrial plants form symbiotic mycorrhizae fungi relationships along the rhizosphere by forming
75 hyphae networks (7). Through mycorrhizae the plant obtains mainly phosphate and other minerals,
76 such as zinc and copper, from the soil. The fungus obtains nutrients, such as sugars, from the plant

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77 root. Mycorrhiza fungi interdependence with roots of the higher plants is not only beneficial to the
78 host plant but also play a role in the aggradative process of soil structure formation (8).
79 Furthermore, a well diversed rhizosphere fungal community is thought to play a role in the
80 suppression of pathogens (9) and (10). Knowledge of the structure and diversity of the fungal
81 community in the rhizosphere will lead to a better understanding of pathogen-antagonist
82 interactions (10).
83 Most past plant rhizosphere mycological studies have concentrated on cultivated crops (11) (12)
84 and (13). This has left a gap in understanding fungal diversity preexisting before planting field crops. In
85 this study, illumina sequencing molecular method was used to identify fungal species in the
86 rhizosphere of *Aspilia pruliseta* Schweif. *Aspilia pruliseta* is a flowering plant in the asteraceae
87 family and was hypothesized to grow in coexistence with the mycorrhiza fungi. It is hypothesized
88 that this complex association leads to availability of phosphorus in the soils in usable forms to
89 plants. The herbaceous plant is common and grows naturally in the open woodlands and grasslands
90 in western, southern, central and eastern Africa. Farmers in central eastern Kenya have reported
91 good crop yields, particularly cereals, in farms previously growing *Aspilia pruliseta*.
92
93 **MATERIALS AND METHODS**
94 **Study sites**
95 This study was carried out in the semi-arid eastern Kenya at Gakurungu, Tunyai and Kanyuambora
96 with coordinates 00^{0}21’00” S, 37^{0}28’30” E, 00^{0}10’00” S, 37^{0}50’00” E and 00^{0}12’00” S, 37^{0}51’00”
97 E respectively. Elevation for all the studied sites was below 1000 m above sea level. Rainfall in
98 the selected areas was scanty and below 700 mm/year with two distinct, but unreliable wet seasons
99 in the months of March to May and October to December. Dry spells were more prolonged with

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100 temperature mean of 26^{0}C. Natural trees and shrubs found in the area were *Aspilia pruliseta*,
101 *Cassia sp, Euphorbia sp, Acasia sp* and *Balanites aegyptiaca*. *Cenchrus ciliaris* and *Hyperrhenia*
102 *rufa* grasses were interspersed in trees and shrubs.

103

104 **Measurements of physico-chemical information on soil rhizosphere depths in the study sites**

105 Physico-chemical analysis of the rhizosphere soil was done for factors that would influence fungal
106 populations and distribution (Table 1a, b, c). Latitude and longitude of the sampling sites were
107 taken using global positioning system (GARMIN eTrex 20). Soil pH for each rhizosphere depth
108 was taken with a portable pH meter (Oakton pH 110, Eutech Instruments Pty. Ltd) and confirmed
109 with indicator strips (Merck, range 5-10). *In situ* soil temperature was taken using an electrical
110 chemical analyzer (Jenway – 3405). 10 g of the composite soil from every rhizosphere depth
111 studied was analyzed in the laboratory for mycorrhiza fungi determination using (14) protocol.
112 The same soil sample was analysed for soil phosphorus in ppm using (15) protocol. Soil moisture
113 was determined using (16) method while soil nitrogen content was determined using (17) method.
114 The samples was also analysed for soil phosphates using (18) protocol and organic matter content
115 using the method by (19).

116

117 **Sample collection**

118 Soils were sampled from Gakũrũngũ, Tunyai and Kanyuambora field sites using a standard soil
119 auger (SOD-GP Dormer sampling equipment). A reconnaissance survey was initially carried out
120 in which natural seed-bearing *Aspilia pruliseta* plots were mapped out. Besides the seed bearing

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121 vegetation of interest, the selected areas of the survey for each of the three sites had to have three
122 soil textural types (sandy loam, silt loam and silty clay). In each site, a quadrant measuring one
123 metre by one metre was thrown at random in each sub-site (a sub-site consisted of an area within
124 the site with one soil textural type). In case the quadrant contained more than one *Aspilia pruliseta*
125 plant, the one closest to the centre of the quadrant was chosen for collection of rhizosphere soil
126 fungal spores. The quadrant was thrown five times in each sub-site and soil was sampled at $depth_1$,
127 0-20 cm; $depth_2$, 21-40 cm, $depth_3$, 41-60 cm using a soil auger with a scooping capacity of $40cm^3$
128 of soil. The sampled soil was put together for each rhizosphere depth from each sub-site and
129 homogenously mixed. 100 g of the mixture was put into khaki paper bags for soil and root DNA
130 analysis in the laboratory.

131

132 **Soil and rootlets total DNA extraction**

133 10 g of composite soil from the rhizosphere of *Aspilia pruliseta* comprising of the plant's rootlets
134 was weighed using an electronic balance. The weighed soil was put into 100 ml beaker and about
135 50 ml tap water was added. The mixture was placed and mixed on an electronic stirrer overnight.
136 After 12 hours the mixture was washed several times by passing it through a 710 µ sieve placed
137 on top of a 45 µ sieve (14). The 710 µ sieve collected the roots and course debris while the 45 µ
138 prevented the spores from passing through. The roots and course debris from the 710 µ sieve were
139 put into a mortar and air-dried in a hood while the process of sieving continued by collecting sieved
140 water and soil mixture in a 1-litre cylinder (14) . The washing and decanting process was done
141 several times until near-clear water was obtained. This was followed by filling the centrifuge tubes
142 with the sieved content. Centrifugation was done for 5 minutes at 1500 revolutions per minute
143 (rpm) and the filtrate was poured off while the supernatant remained at the bottom of the tube. 48

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144 % sucrose solution was added to the supernatant at equal volumes (50ml) and centrifuged for 1
145 minute at 1500 (rpm) (14). The filtrate was collected on the 45 μ sieve while the supernatant was
146 disposed off. The filtrate was then washed with slowly flowing tap water to wash off the sucrose.
147 The washed content was then collected in a 50ml plastic cylinder and the contents poured into a
148 filter paper. Using a fine pair of forceps, the contents were picked and transferred to eppendorf
149 tubes. The dried plant roots in the mortar were crushed into a fine powder using a pestle and the
150 contents added to fungal spore cells in the eppendorf (20) . The content in the eppendorf was re-
151 suspended in 100 μl of solution A {100mM Tris-HCL (pH 8.0), 100Mm EDTA (pH 8.0); added
152 to 5 μl of lysozyme (from a 20mg/ml solution) and incubated at 37^{0}C for 30 minutes in a water
153 bath}. 400 μl of lysis buffer (solution B) comprising 400 mM Tris-HCL (pH 8.0), 60 mM EDTA
154 (pH 8.0), 150 mM NaCl, 1% sodium dodycyl sulfate and the tube was left at room temperature for
155 10 minutes. 10 μl of Proteinase K (20mg/ml) was mixed gently and incubated at 65^{0}C for 1 hour
156 in a water bath. An equal volume of chloroform/ isoamyl alcohol was added and centrifuged at
157 13200 rpm for 5 minutes at 4^{0}C. The supernatant was transferred to new tubes. In the new tubes,
158 150 μl of sodium acetate (pH 5.2) and an equal volume of isopropanol alcohol was added
159 accordingly. The tubes were briefly mixed through inversion. The mixture was then incubated at
160 –20^{0}C overnight. The tubes were then spun at 13200 rpm for 30 minutes and the supernatant was
161 discarded. The resultant DNA pellets were washed in 300 μl of 70% ethanol. The pellets were then
162 spun at 10000 rpm for 1 minute and the supernatant discarded. The resultant DNA pellet was air
163 dried in the hood and dissolved in 50 ml of 13 Tris-EDTA. Genomic DNA (5–15 ng) in 10 μl of
164 ddH2O was used for RAPD amplification using 1.5% agarose gels and images obtained
165 confirming presence of DNA (20). 6 samples (3 samples each from depth$_1$, 0-20 cm; depth$_2$, 21-
166 40 cm and depth$_3$, 41-60 cm) were dried using LABCONCO machine. About 30 μl of the

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167 confirmed DNA was shipped to mrdnalabs (USA) for next generation sequencing with the primers
168 as diversity assay bTEFAP® average inhouse ITSwanda. Illumina was used as the sequencing
169 technology method.

170

171 **Amplicon library preparation and sequencing**

172 Amplification of the ITS region on PCR was done using ITS1 (TCCGTAGGTGAACCTGCGG)
173 and TS4 (TCCTCCGCTTATTGATATGC) primers with barcode according to (21). Amplification
174 proceeded in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with initial
175 heating at 94°C for 3 min, followed by 28 cycles of denaturation at 94°C for 30 s, annealing at
176 53°C for 40 s and extension at 72°C for 1 min, after which a final elongation step at 72°C for 5
177 min was performed. Polymerase chain reaction (PCR) products were visualized on 2% agarose gel
178 to determine the success of amplification and the relative intensity of bands. Multiple samples
179 were pooled together in equal proportions based on their DNA concentrations. Pooled samples
180 were purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA).
181 The pooled and purified PCR product was used to prepare DNA library according to Illumina
182 sequencing protocol (22). Sequencing was performed at Molecular Research DNA
183 (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq platform following the guidelines of
184 the manufacturer.

185

186 **Sequence analysis, taxonomic classification and data Submission**

187 Sequences obtained from the Illumina sequencing platform were depleted of barcodes and primers
188 using a proprietary pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX) developed at the

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189 service provider's laboratory. Low quality sequences were identified by denoising and filtered out
190 of the dataset according to (23). Sequences which were < 200 base pairs after phred20- based
191 quality trimming, sequences with ambiguous base calls, and those with homopolymer runs
192 exceeding 6bp were removed. Sequences were analyzed by a script optimized for high-throughput
193 data to identify potential chimeras in the sequence files, and all definite chimeras were depleted as
194 previously described (24). De novo OTU clustering was done with standard UCLUST method
195 using the default settings as implemented in QIIME pipeline Version 1.8.0 at 97% similarity level
196 (25) . Taxonomy was assigned to each OTU using BLASTn against SILVA SSU Reference 119
197 database at default e-value threshold of 0.001 in QIIME (26).
198

199 **Data analysis**

200 Shannon, Simpson and Evenness diversity indices were used for each sample and were calculated
201 using vegan package version 1.16-32 in R software version 4.0.2 (27). Community and
202 Environmental distances were compared using Analysis of similarity (ANOSIM) test, based upon
203 Bray-Curtis distance measurements with 999 permutations. Significance was determined at 95%
204 confidence interval ($p<0.05$). Calculation of Bray-Curtis dissimilarities between datasets and
205 hierarchical clustering were carried out using the R programming language (27) and the Vegan
206 package (28) . To support OTU-based analysis, taxonomic groups were derived from the number
207 of reads assigned to each taxon at all ranks from domain to genus using the taxa_summary.txt
208 output from QIIME pipeline Version 1.8.0. Obtained sequences were submitted to NCBI Sequence
209 Read Archive with SRP# Study accessions: SRP061806.
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212 **Results**

213 Soil pH for the three studied depths was slightly acidic with a range from 5.9-6.1 (Table 1a, 1b &
214 1c). The middle depth (21-40 cm, Table 1b) was more acidic with a pH of 5.9 compared to 6.1 and
215 6.0 in the first and third soil depth respectively. There was more organic matter content in depth
216 two at 9.1% compared to 8.8% and 8.6% for depth one and depth three respectively. Soil
217 temperatures declined with increasing rhizosphere depth from 25.5^{0}C in depth one to 25.4^{0}C and
218 25.2^{0}C in depth two and three respectively. Mycorrhiza fungi (MF) spore counts along the
219 rhizosphere of *Aspilia pruliseta* plant had an inverse relationship to soil depth with the top soil,
220 depth one having 624 spores per 10g of the sample soil tested compared to 325.3 spores in depth
221 two and 199.1 spores in depth three.

222 Table 1 (a). Soil rhizosphere depth$_1$ (0-20 cm) physico-chemical parameters in sampling sites

SITE CODE	pH	MF Counts /10 g soil sample	P_2O_4 (ppm)	SM %	N%	P (ppm)	OM%	Soil Temp^{0}C
SG1d1	6.4	720	48.09	2	0.17	21	5.96	25.8
LG1d1	5.9	960	57.25	3.7	0.04	25	6.9	25.5
CG1d1	6	640	120.21	2.3	0.15	52.5	10.84	25.4
ST1d1	6.2	688	41.22	2.97	0.15	18	8.62	25.7
LT1d1	6.3	368	177.48	3.86	0.22	77.5	12.37	25.4
CT1d1	6.7	640	65.07	5	0.11	20.5	13.22	25.3
SKId1	6.1	592	89.31	1.3	0.15	39	6.12	25.5
LK1d1	5.7	592	81.3	2.17	0.15	35.5	8.05	25.3
CK1d1	5.9	416	76.72	1.3	0.15	33.5	7.19	25.4
Average, d_1	6.1	624	84.1	2.7	0.1	35.8	8.8	25.5

223 **Legend:** SG1-Sandy loam soil with *Aspilia pruliseta* vegetation at Gakurungu site; LG1-Silt loam
224 soil with *Aspilia pruliseta* vegetation at Gakurungu site; CG1-Silty clay soil with *Aspilia pruliseta*
225 vegetation at Gakurungu site; ST1- Sandy loam soil with *Aspilia pruliseta* vegetation at Tunyai
226 site; LT1-Silt loam soil with *Aspilia pruliseta* vegetation at Tunyai site; CT1- Silty clay soil with
227 *Aspilia pruliseta* vegetation at Tunyai site; SK1- Sandy loam soil with *Aspilia pruliseta* vegetation
228 at Kanyuambora site; LK1- Silt loam soil with *Aspilia pruliseta* vegetation at Kanyuambora site;
229 CK1- Silty clay soil with *Aspilia pruliseta* vegetation at Kanyuambora site; d_1-Depth one (0-20

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230 cm); MF-Mycorrhiza fungi; P_2O_5-Soil phosphates; SM-Soil moisture; N-Nitrogen; P-Phosphorus;
231 OM-Soil organic matter

232 Table 1(b). Soil rhizosphere depth$_2$ (21-40 cm) physico-chemical parameters in sampling sites

SITE CODE	pH	MF Counts /10 g soil sample	P_2O_4 (ppm)	SM %	N%	P (ppm)	OM%	Soil Temp0C
SG1d2	6.7	160	25.19	1.8	0.2	11	6.15	25.6
LG1d2	5.7	176	65.27	3.48	0.1	28.5	3.34	25.4
CG1d2	5	640	25.19	2.09	0.12	11	9.89	25.3
ST1d2	5.7	336	80.15	3.18	0.13	35	10.36	25.5
LT1d2	5.8	280	64.12	3.66	0.14	28	12.91	25.3
CT1d2	7.4	296	80.15	5.41	0.07	35	12.53	25.1
SK1d2	6.1	336	61.83	2.09	0.18	27	9.42	25.4
LK1d2	4.7	320	208.39	2.78	0.01	91	7.66	25.3
CK1d2	5.8	384	46.95	1.39	0.11	20.5	9.84	25.3
Average d2	5.9	325.3	73	2.8	0.1	31.9	9.1	25.4

233 **Legend:** SG1-Sandy loam soil with *Aspilia pruliseta* vegetation at Gakurungu site; LG1-Silt loam
234 soil with *Aspilia pruliseta* vegetation at Gakurungu site; CG1-Silty clay soil with *Aspilia pruliseta*
235 vegetation at Gakurungu site; ST1- Sandy loam soil with *Aspilia pruliseta* vegetation at Tunyai
236 site; LT1-Silt loam soil with *Aspilia pruliseta* vegetation at Tunyai site; CT1- Silty clay soil with
237 *Aspilia pruliseta* vegetation at Tunyai site; SK1- Sandy loam soil with *Aspilia pruliseta* vegetation
238 at Kanyuambora site; LK1- Silt loam soil with *Aspilia pruliseta* vegetation at Kanyuambora site;
239 CK1- Silty clay soil with *Aspilia pruliseta* vegetation at Kanyuambora site; d_2-Depth two (21-40
240 cm); MF-Mycorrhiza fungi; P_2O_5-Soil phosphates; SM-Soil moisture; N-Nitrogen; P-Phosphorus;
241 OM-Soil organic matter

242

243 Table 1(c). Soil rhizosphere depth$_2$ (41-60 cm) physico-chemical parameters in sampling sites

SITE CODE	pH	MF Counts /10 g soil sample	P_2O_4 (ppm)	SM %	N%	P (ppm)	OM%	Soil Temp0C
SG1d3	6.6	224	53.82	1.85	0.24	23.5	6.51	25.5
LG1d3	6	208	50.38	2.57	0.14	22	5.24	25.3
CG1d3	5	384	246.75	1.69	0.12	10.15	7.22	25.1
ST1d3	5.6	144	73.28	2.89	0.09	32	6.99	25.4

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LT1d3	5.9	200	95.04	4.76	0.04	41.5	15.82	25.2
CT1d3	8	160	44.66	5.25	0.05	19.5	12.58	25.0
SK1d3	6.6	184	90.46	1.3	0.04	39.5	7.17	25.3
LK1d3	5.4	208	19.47	3.06	0.1	8.5	7.52	25.2
CK1d3	4.8	80	81.3	1.89	0.13	35.5	8.1	25.1
Average, d3	6	199.1	83.9	2.8	0.1	25.8	8.6	25.2

244 **Legend:** SG1-Sandy loam soil with *Aspilia pruliseta* vegetation at Gakurungu site; LG1-Silt loam
245 soil with *Aspilia pruliseta* vegetation at Gakurungu site; CG1-Silty clay soil with *Aspilia pruliseta*
246 vegetation at Gakurungu site; ST1- Sandy loam soil with *Aspilia pruliseta* vegetation at Tunyai
247 site; LT1-Silt loam soil with *Aspilia pruliseta* vegetation at Tunyai site; CT1- Silty clay soil with
248 *Aspilia pruliseta* vegetation at Tunyai site; SK1- Sandy loam soil with *Aspilia pruliseta* vegetation
249 at Kanyuambora site; LK1- Silt loam soil with *Aspilia pruliseta* vegetation at Kanyuambora site;
250 CK1- Silty clay soil with *Aspilia pruliseta* vegetation at Kanyuambora site; d_3-Depth three (41-60
251 cm); MF-Mycorrhiza fungi; P_2O_5-Soil phosphates; SM-Soil moisture; N-Nitrogen; P-Phosphorus;
252 OM-Soil organic matter

253

254

255 **Sequence data**

256 Raw data consisted of *Aspilia pruliseta* rhizosphere soil samples taken in depth one (0-20 cm),
257 depth two (21-40 cm) and depth three (41-60 cm) consisting of 271582 sequences of which 175622
258 were retained after removing sequences with different tags at each end for quality filtering and
259 denoising. After removing singletons, chimeric sequences and OTUs of non-fungal organisms
260 (<200 base pairs after phred20- based quality trimming, sequences with ambiguous base calls, and
261 those with homopolymer runs exceeding 6 bp), a total of 373 OTUs were recovered at 3% genetic
262 distance. 330 OTUs were of fungal origin and were further analysed.

263

264 **Diversity and Composition of fungal communities in the rhizosphere of *Aspilia pruliseta***

265 Based on BLASTn searches in SILVA SSU Reference 119 database, 323 fungal OTUs were
266 identified, most of which had their best matches against accessions in SILVA database. These 324

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267 OTUs spanned 5 phyla namely; Glomeromycota, Basidiomycota, Chytridiomycota, Ascomycota
268 and unspecified phylum of fungi.

269 $MC2_a$ that consisted of rhizosphere soil depth of 21-40 cm had the highest overall number of OTUs
270 (283 OTUs) while MC1a (0-20 cm) and $MC3_a$ (41-60 cm) had 262 and 265 overall OTUs
271 respectively. 160 OTUs were shared among all sample types (Figure 1).
272 Fungal OTUs were distributed among the phyla as follows; Glomeromycota (90.7%),
273 Basidiomycota (3.7%), Ascomycota (3.4%), Chytridiomycota (1.5%), and unspecified phylum
274 fungi (0.7%). Fungal phylum Glomeromycota was more abundant in rhizosphere depth one (0-20
275 cm) with 232 OTUs compared to depth two (21-40 cm) and depth three (41-60 cm) which had 229
276 and 213 OTUs respectively. This phylum was represented by most genera as shown in figure 2.
277 The phylum Ascomycota had inverse OTU numbers to soil depth. At soil depth 0-20 cm, the
278 phylum had 2 OTUs. The phylum had 5 and 9 OTUs at soil depth 21-40 cm and 41-60 cm
279 respectively. Chytridiomycota and Basidiomycota fungal phylum had similar characteristics to
280 Ascomycota and tended to inhabit the lower rhizosphere echelons. At 0-20 cm soil depth, OTUs
281 were affiliated to the genus *Glomus* with a relative abundance of 85.3%, *Septoglomus* with 5.5%
282 and *Paraglomus* with 4.9% whereas at 21-40 cm, the dominant genus was *Glomus* with relative
283 abundance of 78.3% and *Rhizophagus* with a relative abundance of 15.8% while at soil depth 41-
284 60 cm the dominant genus was *Glomus* with a relative abundance of 50.9% and *Septoglomus* with
285 a relative abundance of 38.6% (Figure 2). The dominant species in the rhizosphere were *Glomus*
286 *sp* and *Paraglomus laccatum.* The soil sample collected at soil depth 0-20 cm (MC1a) was found
287 to harbor a higher diversity of fungi with low species richness as shown in Figure 2.
288 Hierarchical clustering between samples collected from the rhizosphere of *Aspilia pruliseta*
289 revealed samples from the second and third studied soil levels (21-60 cm) to be closer than from

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290 the sample in the first soil level, 0-20 cm (Figure 3). The dendogram shows relationships between
291 the three samples collected.
292

293 **Fungal richness and diversity indices**

294 Richness (S) estimated the rhizosphere depth MC3a (41-60 cm) to be the richest site, constituting
295 62 taxa. Soil samples from the three sites had Evenness (J') scores close to 0.1(0.0457 – 0.0978),
296 hence showing evenness in their number of taxa members than the soil sample (41-60 cm).
297 Simpson (1/D) also indicated the soil sample taken from depth 21-40 cm (MC2a) to harbor the
298 most diverse taxa (12.808). The Shannon's index (H' = 2.48–3.32) indicated low variation in the
299 level of diversity among the soil depth samples taken (Table 2).
300

301 Table 2. Diversity indices computed on all OTU-based fungal taxonomic units obtained from
302 samples collected from the rhizosphere of *Aspilia pruliseta*

Sample	Sequences after filtering	No. of OTUs	Richness (S)	Shannon (H)	Inverse Simpson (I/D)	Evenness (J)
MC1a	72,093	283	42	2.484	4.836	0.0457
MC2a	50,539	262	58	3.321	12.808	0.0978
MC3a	43,596	265	62	2.936	7.294	0.0711
Totals	**166,228**	**323**	**162**			

303
304

305 Analysis of similarity and distance based redundancy analysis at class (Figure 4) level showed
306 connectivity of distance matrix with threshold dissimilarity of 1 indicating that data of the three
307 samples are connected ([1] 1 1 1), hence there were no significant differences in community
308 structure in the samples at 95% level of confidence (P value=0.05).
309

310 **Discussion**

311 Soil microbial community is responsible for most nutrient transformations in soil, regenerating
312 minerals that limit plant productivity (29). Soil pH strongly influences fungal biomass composition
313 (30). In this experiment, moderately acidic and sandy loam textured soils tended to favour
314 proliferation of rhizosphere fungal growth (Table 1a, b &c). The level of soil organic matter was

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315 higher in the second rhizosphere layer (21-40 cm) but fungal microbe population was not
316 correspondingly high agreeing with the principal findings of (31) that soil has diverse elements
317 that contribute to its productivity and the proper balance between those elements is what actually
318 matters.
319 The high sensitivity of Illumina sequencing enabled detection of rare species, thus providing more
320 detailed information on fungal diversity in the rhizosphere of *Aspilia pruliseta* plant. The phylum,
321 *Glomeromycota* was more frequently identified in the plant's rhizosphere than those of
322 *Basidiomycota* and *Ascomycota* whereas members of *Chytridiomycota* were represented on a
323 smaller proportion of the rhizosphere fungal communities. The presence of unidentified fungal
324 phylum indicate that new and potentially useful fungal communities do exist. Results from most
325 rhizosphere mycological research findings indicate heavy presence of *Ascomycota* and
326 *Basidiomycota* phyla (11) (12) and (13) from cultivated crops. From this research, there is a clear
327 departure on the hierarchical fungal composition of the wild semi-arid shrub (*Aspilia pruliseta*)
328 that could prove beneficial to follower-cultivated crops.
329

330 **Conclusion**

331 This study presented fungal diversity analysis of rhizosphere soil samples collected from *Aspilia*
332 *pruliseta* in the semi-arid eastern Kenya using Illumina Sequencing Technology. The results
333 revealed heavy presence of phosphate solubilizing fungi suggesting the usefulness of the shrub for
334 use in improving fallows. Optimal physico-chemical properties for AMF proliferation include
335 sandy loam soils at 0-20 cm rhizosphere depth, warm and moderately acidic. The phylum,
336 *Glomeromycota* dominated the plant's rhizosphere depth.

337 **ACKNOWLEDGEMENT**

338 Authors acknowledge funding in carrying out this research by National Research Fund (NRF) from
339 the Kenya government.

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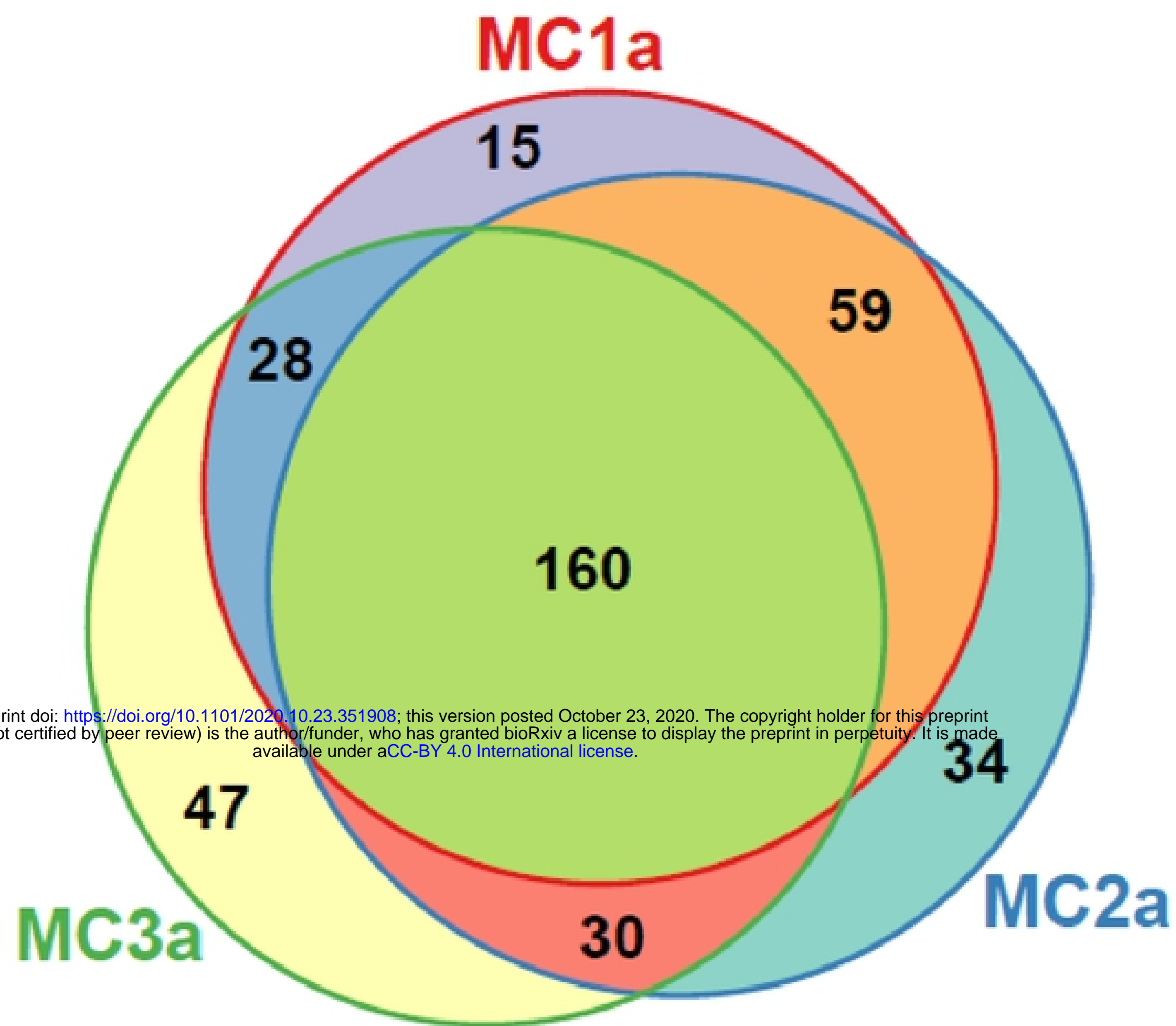

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Figure 1. Venn diagram showing the distribution of unique and shared OTUs within various sample types in the three sampling sites. The number of OTUs in each rhizosphere depth is indicated in the respective circle.

Figure 1

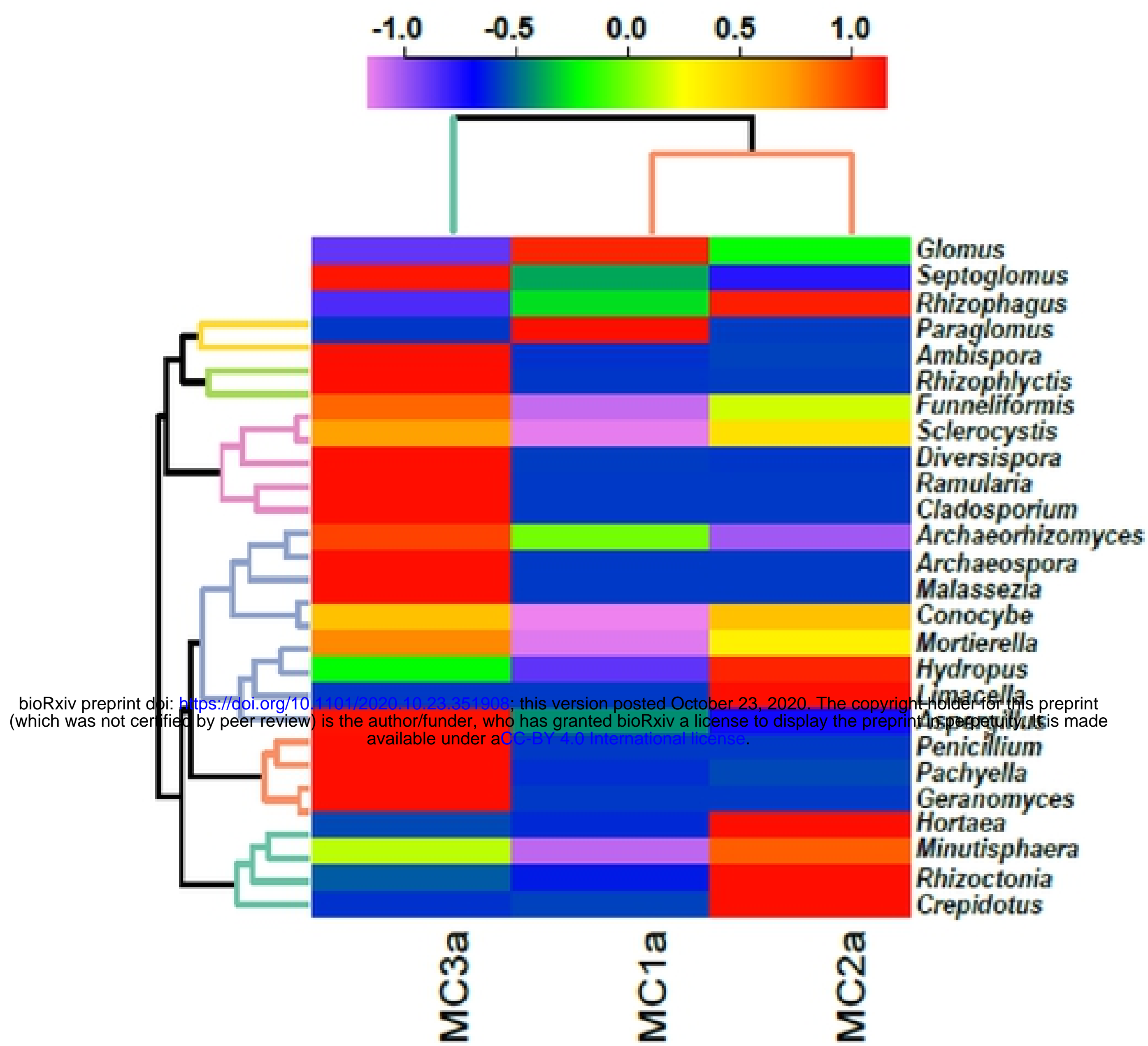

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Figure 2. Heat map showing relative abundance of the most predominant fungal genera in various samples collected from *Aspilia pruliseta* rhizosphere.

Figure 2

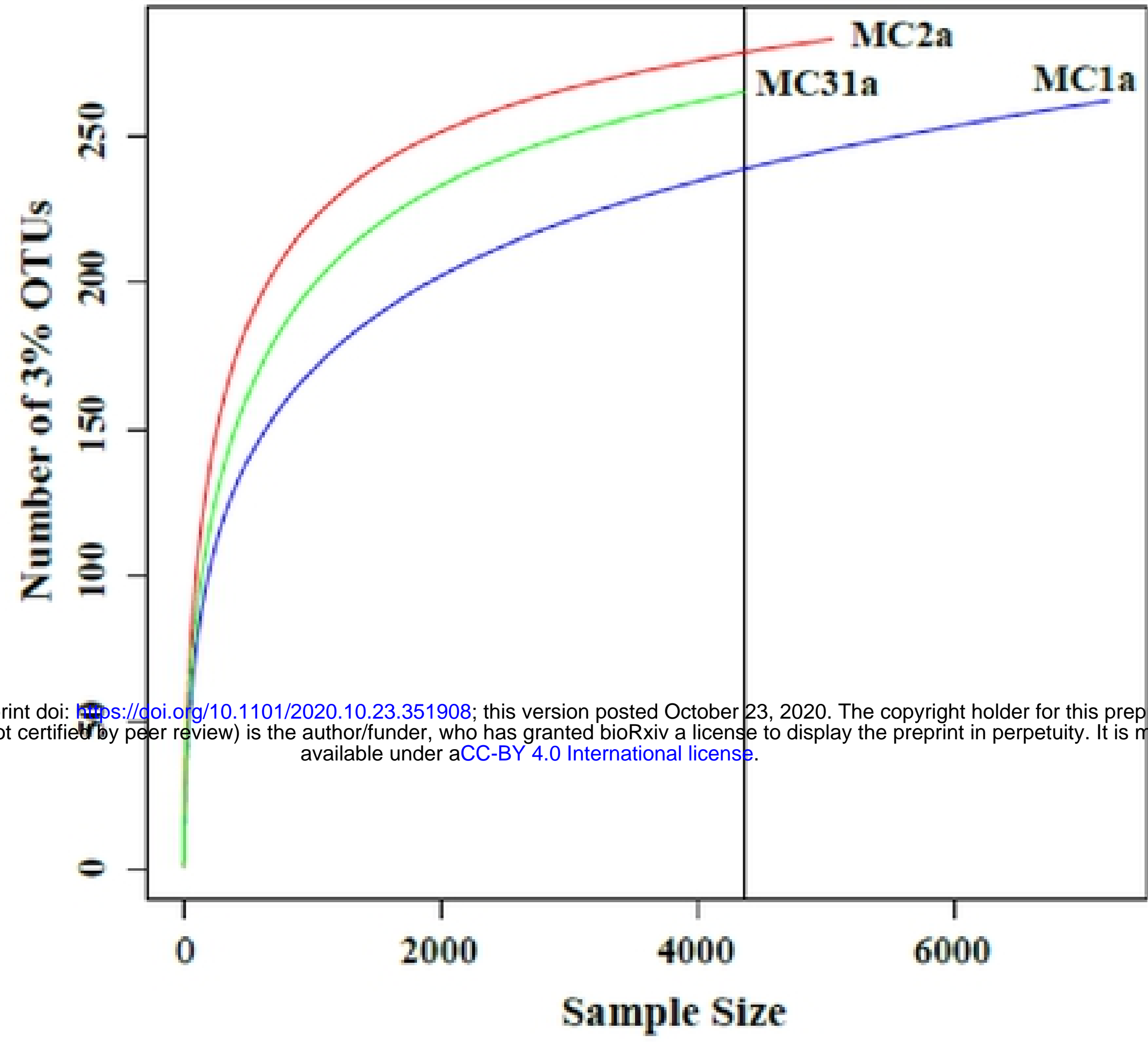

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Figure 3. Relationships between the sample size sequenced and OTUs in the tested samples

Figure 3

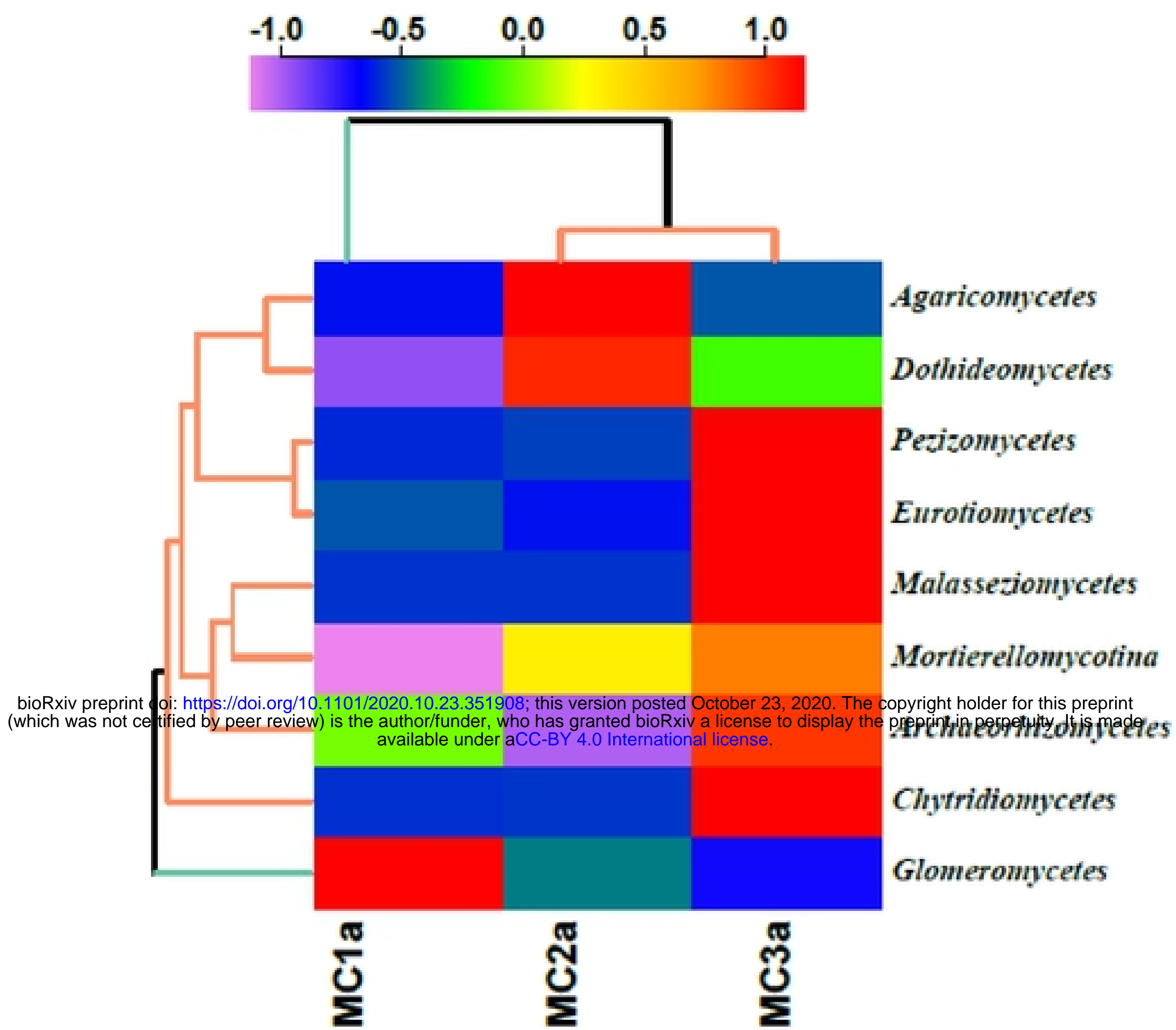

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Figure 4. Hierarchical clustering of DNA samples collected from the studied soil rhizosphere depth. Class level was chosen to be used in hierarchical clustering to assess the relationships between samples and taxa.

Figure 4