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1 **Soil properties and climate affect arbuscular mycorrhizal fungi and soil**
2 **microbial communities in Mediterranean rainfed cereal cropping systems**

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20 **ABSTRACT**

21 Soil microorganisms are generally considered sensitive indicators of soil health and
22 fertility. Understanding the role of environmental factors in driving soil microorganisms'
23 distributions is vital for sustainable crop production, particularly in vulnerable agricultural
24 systems. This study aims to investigate the effect of environmental factors on the variation of
25 soil microbial biomass and arbuscular mycorrhizal (AM) fungi in Mediterranean rainfed
26 cereal systems. To do so, plant roots and soil were sampled from three cereal crops: *Avena*
27 *sativa* L., *Hordeum vulgare* L. and *Triticum turgidum* L. growing in 24 rainfed sites from
28 three different bioclimatic zones (sub-humid, superior semi-arid and middle semi-arid) in

29 northern Tunisia. Soil microbial phospholipid fatty acids (PLFA) fingerprints, percentage of
30 root length colonized by AM fungi as well as AM fungal spore density were then assessed.
31 The redundancy analysis (RDA) revealed that soil organic matter (SOM), temperature,
32 altitude and soil sand content accounted for 22.7% of the variation of the microbial PLFA
33 profiles used to estimate both soil microbial biomass and their distribution across different
34 microbial groups. These environmental factors were the most important drivers affecting
35 bacterial PLFAs. However, no significant relationships were observed between fungal PLFAs
36 and environmental factors. Mycorrhizal colonization differed significantly among the
37 different host species and regions. Our RDA results indicated that environmental factors, in
38 particular mean annual precipitation (MAP), pH, available phosphorus (P) and carbon/
39 nitrogen ratio (C/N) explained 44.8% of AM fungal colonization and spore number variation.
40 Mycorrhizal colonization was positively correlated with pH but negatively with P, C/N and
41 MAP. Concerning AM fungal spore number, it was positively linked to P and MAP. In
42 contrast, it was inversely related to soil pH. Highlighting the main edaphic and climatic
43 factors disturbing soil microorganisms, especially AM fungi, helps to better understand how
44 environmental change will influence soil microbial communities in Mediterranean rainfed
45 cereal systems.

46 **Keywords:**

47 Soil properties, climatic variables, cereal crops, arbuscular mycorrhizal fungi, soil microbial
48 biomass.

49 **1. Introduction**

50 Soil microorganisms represent a major part of the earth's biota (Whitman et al., 1998)
51 and their number is estimated to be around $\sim 10^{29}$ in terrestrial ecosystems (Cavicchioli et al.,
52 2019). Soil microbial communities have key roles in ecosystem functioning as soil respiration

53 and denitrification (Bodelier, 2011; Philippot et al., 2013), C and N cycling processes and
54 other biogeochemical reactions (Delgado-Baquerizo et al., 2016; Falkowski et al., 2008;
55 Schimel and Schaeffer, 2012), decomposition and stability of organic matter (Cotrufo et al.,
56 2013; Nemergut et al., 2014), and plant nutrient acquisition (van der Heijden et al., 2008).
57 Hence, soil microbial communities are contributing to mitigate the impacts of the greenhouse
58 gases and climate change (Singh et al., 2010). Amongst the microbial communities,
59 arbuscular mycorrhizal (AM) fungi establish a beneficial association with the roots of more
60 than 85% of vascular plant species (Brundrett and Tedersoo, 2018) and are also known to
61 improve mineral nutrition and growth of the host plant (Smith and Read, 2008).

62 In microbial ecology, understanding the factors affecting the composition and the
63 abundance of bacterial and fungal communities represents an important issue. Many
64 environmental factors such as soil properties and climate characteristics were identified as
65 abiotic predictors of soil microbial communities (Graham et al., 2014). According to Brockett
66 et al. (2012) and Zheng et al. (2019), environmental factors can be divided into two
67 categories: (i) direct factors represented by edaphic variables which have a dominant
68 influence on microbial community structure and function, and (ii) indirect factors that are in
69 relation with the geographic site characteristics such as climatic conditions. It was proved that
70 soil microbial communities could be influenced by many edaphic variables such as pH (Rousk
71 et al., 2010; Wakelin et al., 2008; Zhalnina et al., 2015), soil organic carbon (Fierer et al.,
72 2009; Franklin, and Mills, 2009), nutrient elements including nitrogen and phosphorus (Dong
73 et al., 2015; Li et al., 2018; Ling et al., 2017; Wang et al., 2019), soil moisture (Brockett et al.,
74 2012) and texture (Hansel et al., 2008). In addition, several climatic conditions may determine
75 and/or alter the composition of fungal and bacterial communities such as temperature
76 (Bárcenas-Moreno et al., 2009; Braker et al., 2010), precipitation (Lau and Lennon, 2012;
77 Martiny et al., 2017) and altitude (Frindte et al., 2019; Lanzén et al., 2016).

78 In addition, climate change represents a major threat for biodiversity (Bálint et al.,
79 2011; Bellard et al., 2012), and thus for the functioning of the natural ecosystem (Cardinale et
80 al., 2012). Based on global scenarios of climate change, the Mediterranean area is expected to
81 be largely exposed to biodiversity (including microbial biodiversity) change and loss (Sala et
82 al., 2000). These changes also affect the microbial communities in agroecosystems, already
83 altered by anthropogenic activities (Cheng et al., 2011). Some of the most represented
84 agricultural systems across the Mediterranean area are grain systems (Gibbon, 1981). They
85 occupied about 46 million hectares in 2018 (FAOSTAT, 2018). Previous studies have
86 demonstrated that agricultural practices affect soil microorganism composition (Carbonetto et
87 al., 2014; García-Orenes et al., 2013; Wallis et al., 2010). Crop species also have an important
88 effect on the variation of soil microbial biomass. Host plant identity can have a highly
89 variable effect depending on the species, genotype and phenological stage (Maul and
90 Drinkwater, 2010; Qiao et al., 2017) and also depending on soil type and climate (Classen et
91 al., 2015). According to Innes et al. (2004), soil microbial biomass was constrained by host
92 plant species and varied significantly between three grasses (*Lolium perenne* L.,
93 *Anthoxanthum odoratum* L. and *Holcus lanatus* L.), and the three dicots (*Achillea millefolium*
94 L., *Trifolium pratense* L. and *Plantago lanceolata* L.). In addition, by studying the dynamics
95 of the microbial community in the rhizosphere of maize (*Zea mays* L.), oat (*Avena sativa* L.),
96 barley (*Hordeum vulgare* L.) and commercial grass mix, Garbeva et al. (2008) also revealed
97 that plant species had a strong effect on microbial community composition.

98 The interaction between environmental factors, host plants and the soil microbial
99 community have been investigated by several authors (Singh et al., 2009; Thakur et al., 2015).
100 However, there is still a lack of conclusive evidence of the main biotic or abiotic factors that
101 drive soil microbial communities. Previous studies have reported that soil microbial
102 communities, estimated by their specific phospholipid fatty acid (PLFA) profiles, was related

103 to environmental factors (e.g., altitude, temperature, rainfall and soil properties) in grasslands
104 on the Mongolian plateau in China (Chen et al., 2014) and in agricultural fields (wheat) and
105 natural ecosystems (forest and grasslands) in Australia (Xue et al., 2018). Other authors have
106 found that the microbial community biomass was more associated with the cropping system
107 (corn, switchgrass and prairies soils) compared to soil physico-chemical properties in
108 Wisconsin USA (Liang et al., 2012).

109 Understanding microbial interactions with soil and climate characteristics may help to
110 mitigate the negative effect of climate change through the development of sustainable crop
111 production in agroecosystems. Tunisia is considered among the most vulnerable countries to
112 climate change (IPCC, 2013). Most cereal cultivation lands are conducted under rainfed
113 conditions in the northern area. Soils in this region are low in soil organic matter due to the
114 harsh environmental conditions. It's necessary to determine the most important environmental
115 factors affecting soil biological fertility in cereals cropping systems which represent the most
116 important strategic crops in the country. Thus, the current work aims at (i) investigating the
117 influence of three important cereal crop species: *Avena sativa* L., *Hordeum vulgare* L. and
118 *Triticum turgidum* L., sampled from 24 sites located in different regions in sub-humid and
119 semi-arid areas of northern Tunisia, on soil microbial biomass (using PLFA fingerprints), and
120 AM fungi root colonization and spore number, and (ii) studying the relationships between
121 these soil microbial variables and environmental factors, in particular soil physico-chemical
122 properties and climatic characteristics.

123 **2. Material and methods**

124 *2.1. Field sites description and samples collection*

125 The study was conducted in rainfed cereal fields located in northern Tunisia (Fig. 1).
126 Twenty-four fields were selected randomly from 8 different regions (from 3 bioclimatic

127 zones: sub-humid, superior semi-arid and middle semi-arid), consisting of 7, 13 and 4 sites
128 where oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.) and durum wheat (*Triticum*
129 *turgidum* L.) were respectively, cultivated. For each site, latitude, longitude and altitude were
130 recorded. The sampling campaign was carried out during two cereal growth stages according
131 to Zadoks scale: tillering (Z21) in December 2015-January 2016 and heading (Z58) in April-
132 May 2016. During the 2015-2016 agricultural campaign, the maximum temperature of the
133 warmest month (M), the minimum temperature of the coldest month (m), and the mean annual
134 precipitation (MAP) were obtained from MRRA-2 application V5.12.4 (GMAO, 2015).

135 From each site and for each growth stage, three samples were taken following a
136 diagonal sampling pattern, each neighbouring sample separated by 50 m. Each sample was
137 composed of four plants, so twelve plants per site were collected. As for the soil, samples
138 were taken from the same place of sampled plants with an auger at a depth of 20 cm. The
139 three soil samples taken from each site were then sieved to 2 mm and air-dried for physico-
140 chemical analyses. Roots were collected from each of the three samples, washed with water,
141 kept in ethanol (50%) and conserved at 4 °C for the evaluation of arbuscular mycorrhizal
142 fungi colonization. Only at the heading growth stage, the three soil samples taken from each
143 site were divided into two subsamples. The first one was stored at -20°C for PLFA analysis
144 and the second subsample was kept in a refrigerator at 4°C for the counting of the indigenous
145 AM fungal spores.

146 2.2. *Physico-chemical soil analyses*

147 Twelve physico-chemical soil properties (supplementary Table S1, Table S2) were
148 assessed in this study. Soil moisture content (SMO) was determined by oven-drying (at 105
149 °C) the soil sample until the weight remained constant. The moisture content (%) was
150 calculated using the difference between soil fresh weight and dried one. Soil texture was
151 determined using the Robinson's pipette method (Naanaa and Susini, 1988). Soil pH on soil:

152 water (1: 2.5, v:v) suspension and soluble salt were determined by measuring the electrical
153 conductivity (EC) (dS/ m) on soil: water (1: 5, v:v) suspension at 25 °C with a pH meter
154 (Crison S.A, Barcelona, Spain) (Pauwels et al., 1992). Total calcium carbonates CaCO_3
155 (Total CaCO_3) were measured using the Bernard calcimeter method (Petard, 1993) and active
156 calcium carbonates CaCO_3 (Active CaCO_3) were determined according to the method
157 described by Drouineau (1942). Soil organic carbon was determined with the Walkley and
158 Black method using potassium dichromate oxidation (Pauwels et al., 1992) and soil organic
159 matter in the soil (SOM) was calculated by multiplying the organic carbon amount by 1.72.
160 The available (Olsen) phosphorus (ppm) (P) was extracted with NaHCO_3 (0.5 M) and then
161 measured by a spectrophotometer (OPTIZEN 3220UV, Daejeon, South Korea) (Olsen et al.,
162 1954). Soil organic nitrogen (mg/ g) (ON) was measured according to the Kjeldhal method
163 (Jones, 1987).

164 2.3. Microbial analyses

165 2.3.1. Soil phospholipid fatty acid (PLFA) analysis

166 Soil microbial composition was determined using a hybrid procedure of PLFA
167 analysis according to Frostegård et al. (1991) and Labidi et al. (2015). For each replicate,
168 lipids were extracted from 3 g of freeze lyophilized soils with methanol: chloroform: citrate
169 buffer (2:1:0.8, v:v:v) solution. The extracted lipids were fractionated into neutral lipids,
170 glycolipids and polar lipids on a silica acid column by successively eluting with chloroform,
171 acetone and methanol. The PLFA and the NLFA were transformed into free fatty acid methyl
172 esters by transesterification. The final extracts were then analyzed using a Perkin Elmer
173 Autosystem gas chromatograph (GC) equipped with a flame-ionization detector (Norwalk,
174 CT) with hydrogen as carrier gas (40 ml/ min). Fatty acids were characterized by gas
175 chromatography-mass spectrometry (GC-MS) with the use of a SHIMADZU 2010 Plus
176 system gas chromatograph equipped with an automatic injector AOC-20i+s and coupled to a

177 mass spectrometry SHIMADZU QP 2010 Ultra. The separation was performed with Zebron
178 Phenomenex ZB-Wax capillary column (length 10 m, diameter 0.1 mm, 0.1 μ m film
179 thickness). The GC instrument was operated in the split mode (200 as split ratio) and the
180 linear velocity (37 cm/ s) using helium as carrier gas.

181 Fatty acid quantification was made by using nonadecanoic acid methyl ester (C19:0)
182 as an internal standard. Their identification relied on the retention times of fatty acids standard
183 (Bacterial Acid Methyl Ester CP Mix in methyl coproate SUPELCO) and its mass analysis.
184 Fatty acids were identified by comparing their mass spectra with the standard mass spectra in
185 the NIST11s library. The amounts of the PLFA C16:1 ω 5 and NLFA C16:1 ω 5 representing
186 respectively viable fungal hyphal biomass and storage lipids of the AM fungi were used as
187 indicators of the AM fungal biomass (Frostegård et al., 1991). Since the PLFA C16:1 ω 5 is not
188 only specific for AM fungi but also for some Gram-negative bacteria, the ratio between
189 NLFA and PLFA C16:1 ω 5 amounts was also calculated. This ratio is high in AM fungi (1-
190 200) and low in bacteria (< 1) (Olsson, 1999). The PLFA C18:2 ω 6.9 was used as indicator for
191 saprotrophic fungi biomass (Frostegård et al., 1993). The fatty acids i15:0, a15:0, i16:0, i17:0,
192 a17:0 were used as indicators of Gram-positive bacteria PLFAs and cy17:0, C18:1 ω 7 and
193 cy19:0 as indicators of Gram-negative bacteria PLFAs in the soil (Frostegård et al., 1993;
194 Tunlid et al., 1989).

195 2.3.2. *Isolation and quantification of AM fungal spores*

196 Indigenous AM fungal spores were extracted from 50 g of soil using the method of
197 wet-sieving (sieves of size 250, 125 and 40 μ m) and decanting described by Gerdemann and
198 Nicolson (1963), followed by the sucrose centrifugation method of Daniels and Skipper
199 (1982). The spores were collected from the different sieves and washed with distilled water.
200 Only intact and healthy spores were counted using a dissecting microscope. The AM fungal
201 spore number was evaluated by counting the number of spores per gram of soil.

202 2.3.3. *AM fungi root colonization*

203 In order to quantify arbuscular and vesicular AM fungal colonization, fresh and fine
204 roots were bleached in KOH (2.5%), then acidified in HCl (1%) and stained with trypan blue
205 (0.05%) (Phillips and Hayman, 1970). The percentage of mycorrhizal root length was
206 determined by microscopic examination ($\times 40$ magnification) of root fragments of 1 cm using
207 the method of McGonigle et al. (1990). From each sample, 225 observations (75 root
208 fragments with 3 intersections per root fragment) were realized in order to count specific
209 mycorrhizal structures (arbuscules and vesicles). In total, 675 observations per site were
210 analyzed.

211 2.4. *Data analysis*

212 All data analyses were conducted using R v.3.6.0 (R Core Team, 2019). The Shapiro
213 and Bartlett tests were used to assess for the normality and homogeneity assumptions of the
214 data. To satisfy the assumptions, data of available phosphorus (P) and soil organic nitrogen
215 (ON) were converted to natural logarithm and square root values, respectively. A one-way
216 ANOVA with Tukey's HSD (honestly significant difference) test (p -values < 0.05) was used
217 to compare the mean values of the soil microbial variables: soil microbial PLFA profiles
218 (PLFA C16:1 ω 5 for AM fungi, PLFA C18:2 ω 6.9 for saprotrophic fungi, Gram-positive and
219 Gram-negative PLFAs); percentage of total root colonization at the tillering and heading
220 stages; and AM fungal spores' number, among the different host plant species (*A. sativa*, *H.*
221 *vulgare* and *T. turgidum*). In addition, ANOVA was performed by host species as well as by
222 region of origin. Furthermore, a simple linear regression analysis was performed between AM
223 fungal spores' number and mycorrhizal root colonization in order to determine their specific
224 relationships. A PCA was also conducted to examine the variation between the 72 samples
225 based on the environmental variables (climatic and soil physico-chemical properties).
226 Canonical redundancy analysis (RDA) based on Hellinger-transformed data was used to

227 examine the relationships between the soil microbial and the environmental variables. Two
228 RDA were conducted, the first RDA was used to analyse the effect of the environmental
229 factors on the soil microbial biomass (using PLFAs profiles) and the second RDA, to analyse
230 the effect of the environmental factors on the AM fungal spores' number and root
231 colonization. This analysis was performed using the 'vegan' library in the R software package
232 (Oksanen et al., 2019). The proportion of explained variation was calculated using the
233 adjusted R^2 values (Borcard et al., 2011). The significance of the results was tested with a
234 Monte Carlo test (using 999 permutations). Backward selection of the explanatory variables
235 that were significantly correlated with these soil microbial variables was performed using the
236 stepwise regression and the Monte Carlo permutation test.

237 **3. Results**

238 *3.1. Soil microbial biomass*

239 In order to study the influence of host plant and the pedo-climatic conditions of the
240 cultivation sites, microbial biomasses were assessed using specific lipid biomarkers (PLFAs).
241 The amounts of PLFA C16:1 ω 5, used here to quantify the biomass of AM fungi in soils, were
242 not significantly different between the three studied host plant species ($p > 0.05$), while the
243 NLFA C16:1 ω 5 soil contents, which represent mainly storage lipid in spores, were
244 significantly different ($p < 0.05$) (Table 1, supplementary Table S4). However, whereas the
245 ratio NLFA /PLFA C16:1 ω 5 was inferior to 1 for *A. sativa*, indicating a predominant bacterial
246 contribution, it was superior to 1 for *H. vulgare* and *T. turgidum*, indicating AM fungi
247 predominance (Table 1).

248 There were no significant differences in saprotrophic fungi biomass, estimated by the
249 PLFA C18:2 ω 6.9 ($p = 0.99$). Gram-positive bacteria biomass (quantified by the sum of the
250 PLFAs i15:0, a15:0, i16:0, i17:0, a17:0) as well as Gram-negative bacteria (estimated by the

251 sum of the PLFAs cy17:0, C18:1 ω 7 and cy19:0) biomass did not significantly vary between
252 the different host plant species ($p > 0.05$) (Table 1, supplementary Table S4).

253 At a regional scale, no significant differences were observed between the studied
254 regions for the amounts of PLFA C16:1 ω 5, PLFA C18:2 ω 6.9, Gram-positive bacteria PLFAs
255 and Gram-negative bacteria PLFAs ($p > 0.05$) (Table S5, supplementary Table S6).

256 3.2. *Arbuscular mycorrhizal fungi*

257 There were no significant differences in the AM fungal spore number between the
258 three host plant species ($p > 0.05$, supplementary Table S4) (Table 2a). However, the AM
259 fungal spore number significantly varied among the different studied regions ($p < 0.0001$,
260 supplementary Table S6) (Table 2b).

261 Total AM fungi root colonization rates were significantly different between the
262 different host plant species (p -values < 0.01 , supplementary Table S4) (Table 2a). Compared
263 to *A. sativa*, total mycorrhizal rates for *H. vulgare* and *T. turgidum* were 1.9 and 2.3-fold
264 higher, respectively at tillering and 1.5 and 1.9-fold higher, respectively at heading (Table 2a).
265 Arbuscules were observed at both growth stages and they were significantly different among
266 the three studied host plant species ($p < 0.0001$ at tillering and $p < 0.01$ at heading,
267 supplementary Table S3a and Table S4). However, vesicle formations were observed only at
268 the heading stage for *H. vulgare* and *T. turgidum* and not for *A. sativa*. Vesicular colonization,
269 was two-times higher for *T. turgidum* as compared with *H. vulgare* (supplementary Table
270 S3a).

271 At the regional scale, total mycorrhizal colonization as well as arbuscular and
272 vesicular colonization rates differed significantly ($p < 0.001$, supplementary Table S6) (Table
273 2b, supplementary Table S3b). Arbuscular and vesicular colonization rates were 3 times
274 higher in the site of Tunis compared with Jendouba (Table S3b).

275 Overall, negative correlations between mycorrhizal colonization and AM fungal spore
276 number were observed in particular at the heading growth stage ($R^2 = 0.55$, $p < 0.001$) (Fig.
277 2).

278 3.3. Variation of the environmental variables

279 The PCA analysis based on the 16 environmental variables showed differentiation
280 between the 72 studied samples. The first two dimensions of the PCA accounted for 47.5% of
281 the total variation in the environmental data (Fig. 3, supplementary Figure S1). The first
282 dimension which explained 29.7% of the environmental data variation, was strongly and
283 negatively correlated with the minimum temperature of the coldest month (m) ($r^2 = -0.85$, $p <$
284 0.0001), maximum temperature of the warmest month (M) ($r^2 = -0.44$, $p < 0.0001$), soil sand
285 content ($r^2 = -0.49$, $p < 0.0001$) and carbon/ nitrogen ratio (C/N) ($r^2 = -0.47$, $p < 0.0001$).
286 However, soil pH ($r^2 = 0.78$, $p < 0.0001$), soil silt content ($r^2 = 0.83$, $p < 0.0001$), total CaCO_3
287 ($r^2 = 0.74$, $p < 0.0001$) and active CaCO_3 ($r^2 = 0.77$, $p < 0.0001$) contents showed a high and
288 positive effect on the first dimension. The second dimension accounted for 17.8% of the
289 environmental data variation, to which soil moisture content (SMO) ($r^2 = 0.65$, $p < 0.0001$)
290 and soil sand content ($r^2 = -0.67$, $p < 0.0001$) contributed the most (Fig. 3, supplementary
291 Table S7). According to the PCA results, the region of Jendouba, Kef and Siliana were
292 separated from Tunis, Bizerte and Nabeul along the first dimension. However, the variability
293 within each region was mostly displayed along the second dimension of the PCA (Fig. 3).

294 3.4. Correlations between soil microbial and environmental variables

295 In order to determine which environmental factors affected soil microbial variables,
296 two redundancy analysis (RDA) were conducted. The first RDA was used to check the effect
297 of the environmental factors on the soil microbial PLFA profiles and the second RDA, to
298 analyse the effect of the environmental factors on AM fungal root colonization and spore
299 density. For soil microbial PLFA, 19.7 and 2.6% of the variation were explained by the first

300 two canonical axes (Fig. 4). The environmental factors (SOM, Sand, M, m and altitude) had a
301 significant effect on the variation of these microbial PLFA profiles ($F= 3.88$, $p < 0.001$,
302 supplementary Table S8) and they accounted for ($R^2= 22.3\%$) of the total variation. In
303 addition, the RDA revealed that Sand (score=-0.5, $p < 0.01$), M (score=-0.26, $p < 0.05$) and
304 altitude (score=-0.20, $p < 0.01$) were highly and positively correlated with BG-, while SOM
305 (score=0.51, $p < 0.01$) and m (score=0.05, $p < 0.01$) were correlated negatively with BG+.
306 (Fig.4, supplementary Table S9).

307 Similarly, there was a significant effect of the environmental variables on the
308 mycorrhizal colonization and AM fungal spore number ($F= 10.73$, $p < 0.01$) and they
309 explained 44.8% of the total variation (Fig. 5). According to the RDA analysis, only soil pH,
310 soil available phosphorus (P), C/N and mean annual precipitation (MAP) contributed
311 significantly to the variation of mycorrhizal colonization at both tillering and heading stages
312 and spore number of AM fungi (Table S9). The RDA1 axis which explained most of the
313 variation in mycorrhizal colonization and AM fungal spore number (44.35%, $p < 0.001$, Table
314 S9), was positively correlated with C/N (score= 0.3, $p < 0.01$), MAP (score=0.7, $p < 0.01$,
315 Table S8) and P (score= 0.53, $p < 0.01$) but negatively correlated with pH (score= -0.55, $p <$
316 0.01). Among these environmental factors, MC.Tillering and MC.Heading were positively
317 correlated with pH. However, the AM fungal spore number was positively correlated with
318 MAP, P and C/N but negatively with soil pH (Fig. 5, Table S8).

319 **4. Discussion**

320 The present study aims to determine which environmental factors influence soil
321 microbial PLFA profiles, AM fungal root colonization and spore density associated with the
322 rhizospheres of the main cereal crop species (*A. sativa*, *H. vulgare* and *T. turgidum*) located in
323 24 sites from 8 regions in sub-humid and semi-arid areas in northern Tunisia.

324 *Relationship between cereal species, soil microbial biomass, AM fungal root colonization*
325 *and spore density*

326 Using specific lipid biomarkers, we showed that there was no significant difference in
327 the microbial biomasses (AM fungi, saprotrophic fungi and bacteria) between the three
328 studied cereal crops (*A. sativa*, *H. vulgare* and *T. turgidum*) indicating that the host plant
329 species had no effect on soil microbial biomass. The influence of host plant species on
330 microbial biomass is still controversial. Some studies have suggested that the soil microbial
331 biomass did not differ significantly among several *Poaceae* species (*Briza media*, *Festuca*
332 *ovina* and *Bromus erectus*) (Bezemer et al., 2006). However, in other studies, the plant species
333 (*Arrhenatherum elatius*, *Holcus lanatus* and *Dactylis glomerata*) had a significant effect on
334 the variation of soil microbial PLFA (Patra et al., 2008).

335 In the present study, AM fungi root colonization rates were higher in *T. turgidum* and
336 *H. vulgare* than in *A. sativa*. This is in accordance with the work of Castillo et al. (2012), who
337 found that the AM fungi root colonization rate was lower in oats compared to barley and
338 wheat. The difference between the three cereal species could be linked to their carbohydrate
339 production. In fact, the quantity and quality of carbon resources produced by the plant vary
340 according to the plant species (Ngosong et al., 2012), resulting in a different response by the
341 mycorrhizal symbiosis (Jones et al., 2004). Moreover, root colonization percentages differed
342 between tillering and heading stages for the three cereal species. It has been reported in
343 previous studies that AM fungal colonization also changes across different phenological
344 stages of the host plant species (Mohammad et al., 1998; Schalamuk et al., 2006). AM fungal
345 root colonization increased with advancing plant growth (Mohammad et al., 1998; Panwar et
346 al., 2011). This may be explained by the fact that a low temperature during winter at the
347 tillering stage inhibited the germination of AM fungal spores and thus mycorrhizal root
348 colonization (Grey, 1991; Koske, 1981). Concerning AM fungal spore density, no significant

349 differences were observed between the studied cereal species. This could be explained by the
350 fact that sporulation depends more on AM fungi species physiology. There are AM fungi that
351 sporulate more (e.g., *Acaulospora* and *Glomus* species), while others sporulate less
352 (*Gigaspora* and *Scutellospora* species) (Jefwaa et al., 2012; Wang and Jiang, 2015).
353 Furthermore, the AM fungal spore number usually varies according to edaphic factors and
354 cropping systems.

355 *Soil characteristics, temperature and site altitude influence soil microbial biomass in rainfed*
356 *conditions*

357 According to the current data, the redundancy analysis (RDA) revealed a significant
358 and positive correlation between soil organic matter (SOM) and Gram-positive bacteria
359 PLFAs. This result was consistent with those of Liang et al. (2012) who found that Gram-
360 positive bacteria tend to correlate with SOM. Gram-positive and Gram-negative bacteria
361 depend on different carbon sources (Fanin et al., 2019). Compared with Gram-negative
362 bacteria, Gram-positive bacteria use carbon resources derived from SOM that are more
363 complex and recalcitrant (Bird et al., 2011; Kramer and Gleixner, 2006).

364 It has been reported in previous studies that soil texture had a strong influence on soil
365 microbial biomass (Mellado-Vázquez et al., 2019). In the present study, soil sand content was
366 positively correlated with Gram-negative bacteria but negatively with Gram-positive bacteria.
367 These results were in line with those of Hemkemeyer et al. (2018). Gram-positive bacteria
368 were more abundant in silt and clay soils, while the Gram-negative bacteria were found in the
369 larger fractions (> 200 µm) in sand particles (Zhang et al., 2015). These differences could be
370 related to the nutrient preference of Gram-positive and Gram-negative bacteria. In fact, the
371 nutrient concentration in the soil differs with soil particle size fractions, which have an
372 important influence on bacterial community structure (Carson et al., 2009; Nishiyama et al.,
373 2012).

374 For climatic characteristics, Gram-positive and Gram-negative bacteria showed a
375 different response to temperature. According to the current data, the maximum temperature of
376 the warmest month had a positive and significant effect on Gram-negative bacteria but a
377 negative effect on Gram-positive bacteria. These results are in contradiction with those of
378 Zhou et al. (2017) who worked on the effect of temperature on soil microbial PLFAs
379 signatures. These inconsistent results could be related to the differences in substrate
380 preferences and metabolic abilities between Gram-positive and Gram-negative bacteria (Ho et
381 al., 2017). Gram-positive and Gram-negative bacteria contain the characteristics of
382 oligotrophic (nutrient-poor) and copiotrophic (nutrient-rich) microorganisms, respectively
383 (Fierer et al., 2007; Naylor et al., 2017). Increasing temperature enhanced fast growers
384 (copiotrophic) bacteria that prefer high nutrient availability (Männistö et al., 2016).

385 In the present study and in line with previous studies, the altitude was positively
386 correlated with Gram-negative bacteria but negatively with Gram-positive (Margesin et al.,
387 2009; Siles and Margesin, 2016). Altitude had a significant effect on soil properties such as
388 pH, and nutrient availability (Wu et al., 2015). The low availability of nutrients such as
389 carbon and nitrogen at high altitude could negatively affect the growth of some bacterial
390 groups but select other adapted ones (Kumar et al., 2019).

391 *Soil properties and precipitation influence AM fungal root colonization and spore density in*
392 *rainfed conditions*

393 Based on the current data, chemical soil characteristics and climate had a significant
394 influence on both AM fungal root colonization and spore density.

395 Concerning the soil available phosphorus content, our results showed that this
396 parameter was negatively correlated with mycorrhizal colonization but positively with AM
397 fungal spore number. The inverse relationship between AM fungi root colonization and soil
398 phosphorus levels has been already described in several studies (Kluber et al., 2012; Liu et al.,

399 2016; Treseder, 2004). Increasing soil available phosphorus can cause depressed growth of
400 the extra-radical hyphae of the AM fungi, which reduce mycorrhizal root colonization
401 (Kahiluoto et al., 2001). From our data, the AM fungal spore number and available
402 phosphorus were positively correlated. This result is consistent with previous studies
403 (Welemariam et al., 2018; Wu et al., 2011) but contradicts the findings of Soka and Ritchie
404 (2018) who observed a significant decrease in AM fungal spore density with increasing soil
405 phosphorus. According to García et al. (2017), the increase in spore density with increasing
406 soil available phosphorus could be explained by a change in pattern of resource allocation
407 between the hyphal networks as functional structures that provide nutrients to host plants and
408 the spores as survival resting structures (Sheng et al., 2013).

409 Furthermore, the soil pH ranging between 6.6 and 8.6 had a positive influence on
410 mycorrhizal root colonization but a negative one on AM fungal spore density. It was reported
411 that AM fungi had a preference for a near neutral or alkaline soil pH (Marschner et al., 2005).
412 The negative effect on spore number as observed by Panwar et al. (2011), could be linked to
413 the presence of different AM fungi taxa in the rhizosphere that cannot tolerate neutral or
414 strongly alkaline soil pH.

415 Among the different soil properties, a negative correlation was found between soil
416 C/N ratio and mycorrhizal colonization could be due to the availability of nitrogen in the soil.
417 It has been reported by Atul-Nayyar et al. (2009) that C/N ratio decreased in the presence of
418 AM fungi (from 17.8 to less than 13), indicating that AM fungi enhance organic matter
419 decomposition.

420 For climate parameters, the negative correlation between precipitation and AM fungi
421 root colonization contrasted with previous finding showing a positive correlation between
422 mycorrhizal variables and precipitation (Lingfei et al., 2005; Oliveira and Oliveira, 2010;
423 Silvana et al., 2018) but was consistent with other studies (He et al., 2016; Muthukumar and

424 Udaiyan, 2002). The AM fungi are aerobic (Sylvia and Williams, 1992), soil water and
425 aeration have an important impact on their development and effectiveness. Increase in soil
426 moisture is associated with a decrease in oxygen level in soil, which could negatively affect
427 the spore germination and the hyphal growth of AM fungi (Hattori et al., 2014). For the AM
428 fungal spore number, our data showed a positive correlation between this parameter and
429 precipitation. The high sporulation is a stress response of AM fungi to adverse weather
430 conditions (Escudero and Mendoza, 2005). It has been suggested that when soil conditions are
431 suitable for spore germination, AM fungal root colonization increases and spore density
432 decreases and vice versa (Oliveira and Oliveira, 2005). The relationships between
433 precipitation and soil moisture are complex and irregular (Li et al., 2016) and depends on
434 others environmental conditions such as soil texture, infiltration and evapotranspiration (Yang
435 et al., 2018).

436 **5. Conclusion**

437 This research represents the first investigation on soil microbial communities and AM
438 fungi symbiosis of cereal crops in sub-humid and semi-arid regions in northern Tunisia. The
439 results obtained herein revealed that the microbial distribution in soils did not differ according
440 to the three studied crop species (*A. sativa*, *H. vulgare* and *T. turgidum*). However, there is a
441 complex interaction between the distribution of the soil microorganisms and the
442 environmental factors (climatic conditions and soil properties). In addition, Gram-positive and
443 Gram-negative bacterial biomass were mainly linked to SOM, soil texture, temperature and
444 altitude. This study shows that mycorrhizal root colonization varied significantly according to
445 the host species. It also highlighted that soil properties, particularly pH, soil available
446 phosphorus and precipitation, were all correlated with mycorrhizal root colonization status.
447 All these results demonstrate the effect of environmental factors on soil microorganisms'
448 distribution in rainfed Mediterranean agricultural systems. Further research should be

449 conducted to select suitable native microorganisms playing a crucial role in enhancing cereal
450 crop productivity and reducing the impact of conventional cultural practices in rainfed
451 agricultural systems.

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870 **Figures**

871 **Figure 1.** Map showing the location of the 24 studied sites in northern Tunisia. *Avena sativa*,
872 *Hordeum vulgare* and *Triticum turgidum* sites are represented in blue, yellow and red,
873 respectively.

874 **Figure 2.** Simple regression analysis between AM fungal spore number and mycorrhizal root
875 colonization at tillering and heading stages for all the sampled plants (from all studied sites).

876 **Figure 3.** Principal component analysis of the 72 samples collected from the 24 studied sites,
877 based on the environmental variables. Samples were color-coded according to the region of
878 origin. Ellipses are drawn at 95% confidence intervals for each region of origin and they
879 describe one standard deviation around the centroid position of each region. Mean annual
880 precipitation (MAP); maximum temperature of the warmest month (M); minimum
881 temperature of the coldest month (m); altitude; soil pH; soil electrical conductivity (EC); total
882 calcium carbonates content (TotalCaCO₃); active calcium carbonates content (ActiveCaCO₃);
883 soil organic matter (SOM); soil organic nitrogen (ON); carbon/ nitrogen ratio (C/N); available
884 phosphorus (P); soil moisture (SMO), soil clay content (Clay); soil silt content (Silt) and soil
885 sand content (Sand).

886 **Figure 4.** Redundancy Analysis (RDA) triplots showing the significant relationship between
887 the environmental factors and the soil microbial composition based on the PLFA profiles
888 using the Hellinger distance. Scaling type = 2. RDA1 (19.66%) and RDA2 (2.63%). The

889 PLFA profiles are shown by filled orange triangles “▲”: PLFA C16:1 ω 5 for AM fungi
890 (AMF); ratio between NLFA and PLFA C16:1 ω 5 (NL/PL C16:1 ω 5); PLFA C18:2 ω 6.9 for
891 saprotrophic fungi (SF); Gram-positive bacteria PLFAs (BG⁺); Gram-negative bacteria
892 PLFAs (BG⁻) and the significant environmental variables by blue arrows: maximum
893 temperature of the warmest month (M); minimum temperature of the coldest month (m); soil
894 organic matter (SOM); soil sand content (Sand) and altitude. “+” represent the different
895 studied samples.

896 **Figure 5.** Redundancy Analysis (RDA) triplots showing the significant relationship between
897 the environmental factors and the root colonization and the spore number of AM fungi using
898 the Hellinger distance. Scaling type = 2. RDA1 (44.35%) and RDA2 (0.46%). The
899 mycorrhizal colonization and spore number are shown by filled orange triangles “▲”:
900 mycorrhizal colonization at tillering (MC.Tillering); mycorrhizal colonization at heading
901 (MC. Heading); AM fungal spore number (Spore) and the significant environmental variables
902 by blue arrows: mean annual precipitation (MAP); pH; carbon/ nitrogen ratio (C/N); available
903 phosphorus (P) and altitude. “+” represent the different studied samples.

904 **Tables**

905 **Table 1.** Fungal and bacterial biomass estimated by PLFA ($\mu\text{g/ g}$ dry soil) analysis of 72 soil samples collected from the different host plant
 906 species Data were reported as means and standard deviations.

Host plant	PLFA C16:1 ω 5	NLFA C16:1 ω 5	NLFA /PLFA C16:1 ω 5	PLFA C18:2 ω 6.9	Gram-positive bacteria PLFAs	Gram-negative bacteria PLFAs	Total microbial PLFA
<i>A. sativa</i> (n= 21)	0.97 \pm 0.12	0.94 \pm 0.524 ab	0.90 \pm 0.49	0.90 \pm 0.15	2.42 \pm 0.80	1.27 \pm 0.22	5.56 \pm 0.99
<i>H. vulgare</i> (n=39)	0.93 \pm 0.10	1.49 \pm 1.04 a	1.37 \pm 0.90	0.90 \pm 0.24	2.21 \pm 0.81	1.22 \pm 0.22	5.27 \pm 1.02
<i>T. turgidum</i> (n=12)	1.01 \pm 0.11	1.01 \pm 0.54 a	1.01 \pm 0.55	0.89 \pm 0.18	2.43 \pm 1.10	1.32 \pm 0.28	5.66 \pm 1.27
ANOVA							
<i>P</i> -value	0.103	0.0407	0.0615	0.997	0.569	0.363	0.412
<i>Significance</i>	Ns	*	Ns	ns	ns	ns	ns
907	Different letters in the same row indicate significant differences at 0.05 by Tukey's HSD test. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p <$						
908	0.001, **** $p < 0.0001$.						

909 **Table 2.** Arbuscular Mycorrhizal (AM) fungi colonization across the 72 samples collected from the different studied sites. Means and standard
 910 deviations were reported by (a) host plant species and by (b) sites region.

911 **(a)**

Host plant	Total mycorrhizal colonization (%) at tillering	Total mycorrhizal colonization (%) at heading	AM fungal spore number (per 50 g of soil)
<i>A. sativa</i> (n= 21)	5.14 ± 3.18 b	21.11 ± 14.41 b	124.71 ± 56.50
<i>H. vulgare</i> (n=39)	9.68 ± 4.03 a	31.11 ± 14.82 a	104.23 ± 55.23
<i>T. turgidum</i> (n=12)	12.04 ± 3.33 a	39.77 ± 11.52 a	86.00 ± 36.13
ANOVA			
<i>P</i> -value	1.96e ⁻⁶	0.00166	0.123
<i>Significance</i>	****	**	ns

912 (b)

Region	Total mycorrhizal colonization (%) at tillering	Total mycorrhizal colonization (%) at heading	AM fungal spore number (per 50 g of soil)
Bizerte (<i>n</i> = 9)	8.0 ± 2.5 bc	26.4 ± 11.6 abc	109.0 ± 43.6 bc
Tunis (<i>n</i> = 9)	13.5 ± 5.8 a	44.2 ± 19.0 a	77.3 ± 44.8 c
Nabeul (<i>n</i> =12)	8.4 ± 3.8 abc	31.7 ± 13.9 ab	70.7 ± 45.9 c
Zaghouan (<i>n</i> = 6)	9.3 ± 2.7 abc	30.0 ± 10.3 abc	173.5 ± 57.5 ab
Beja (<i>n</i> = 9)	10.4 ± 1.3 ab	27.7 ± 13.7 abc	110.0 ± 29.3 bc
Jendouba (<i>n</i> = 9)	3.5 ± 2.6 c	11.7 ± 8.9 c	175.7 ± 22.3 a
Kef (<i>n</i> = 12)	7.8 ± 2.9 bc	25.9 ± 9.4 bc	94.8 ± 37.7 c
Siliana (<i>n</i> = 9)	9.6 ± 5.5 ab	40.9 ± 13.1 ab	72.7 ± 41.6 c
ANOVA			
<i>P</i> -value	8.00 e ⁻⁵	6.22 e ⁻⁵	1.49 e ⁻⁷
<i>Significance</i>	****	****	****

913 Different letters in the same row indicate significant differences at 0.05 by Tukey's HSD test. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p <$
 914 0.001, **** $p < 0.0001$.

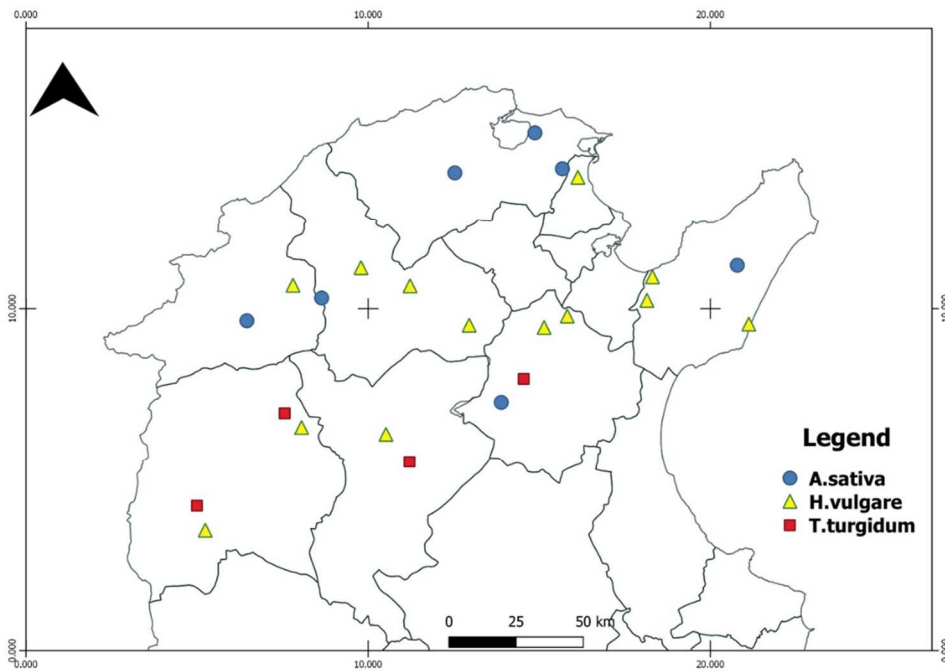


Figure 1.

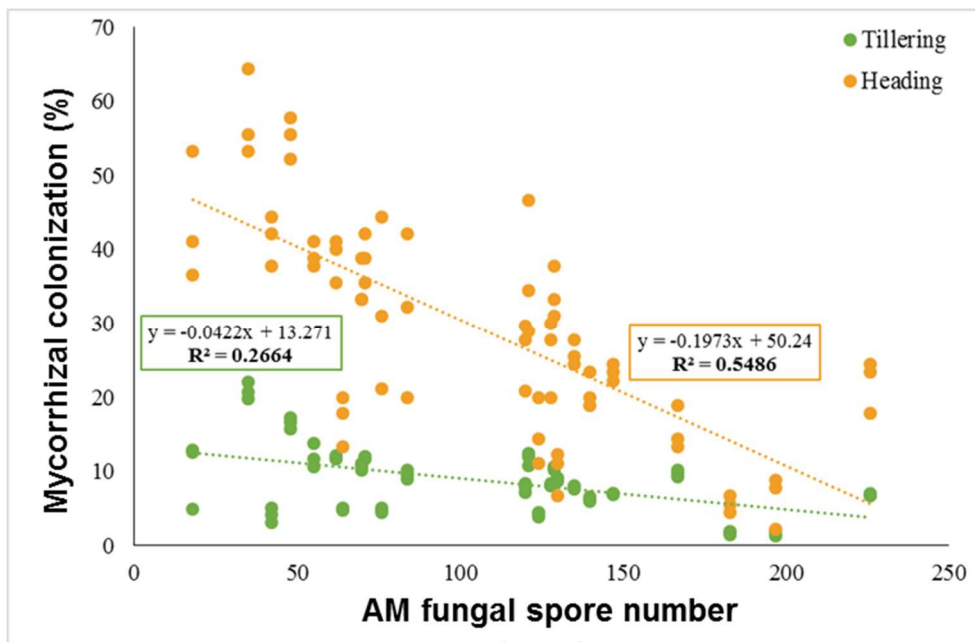


Figure 2.

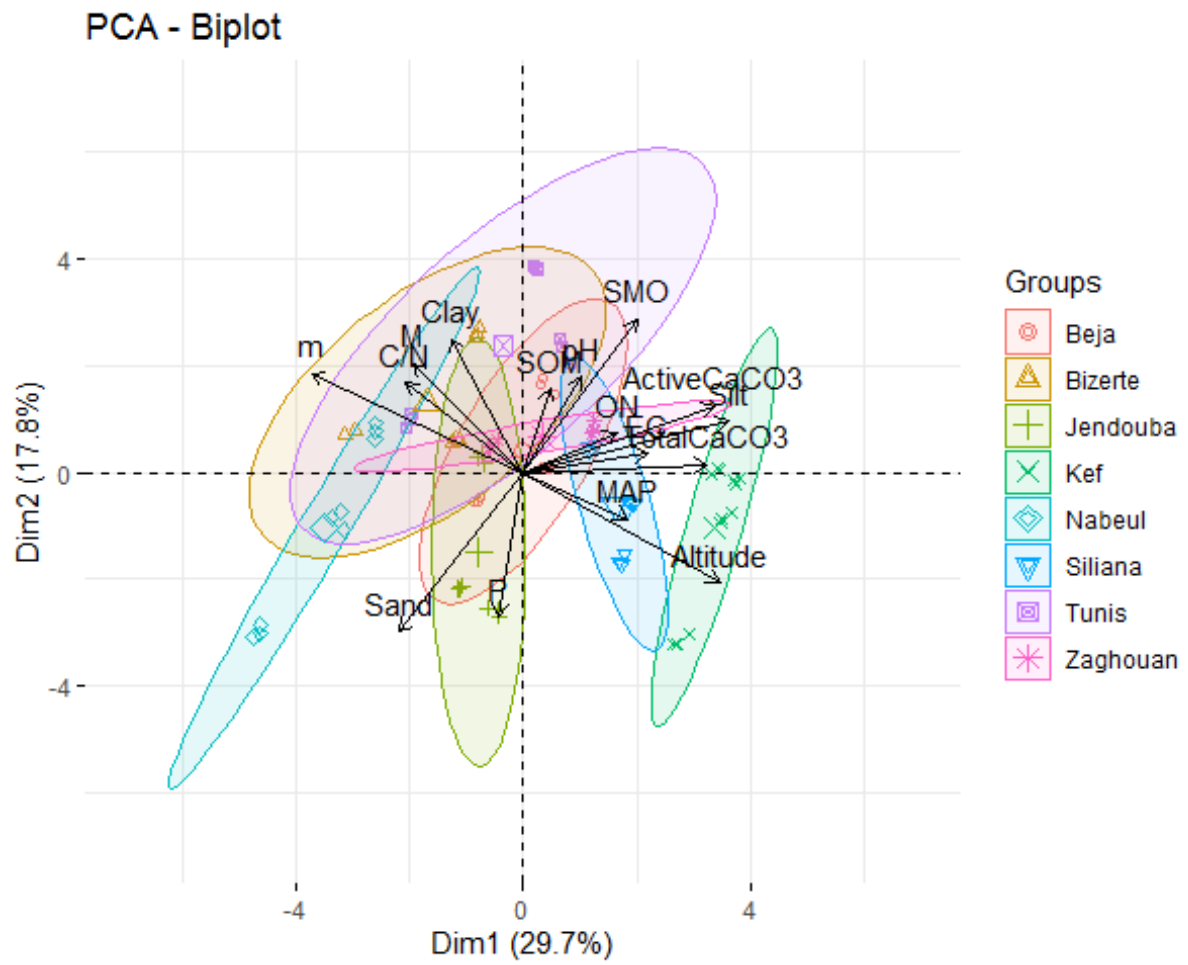


Figure 3.

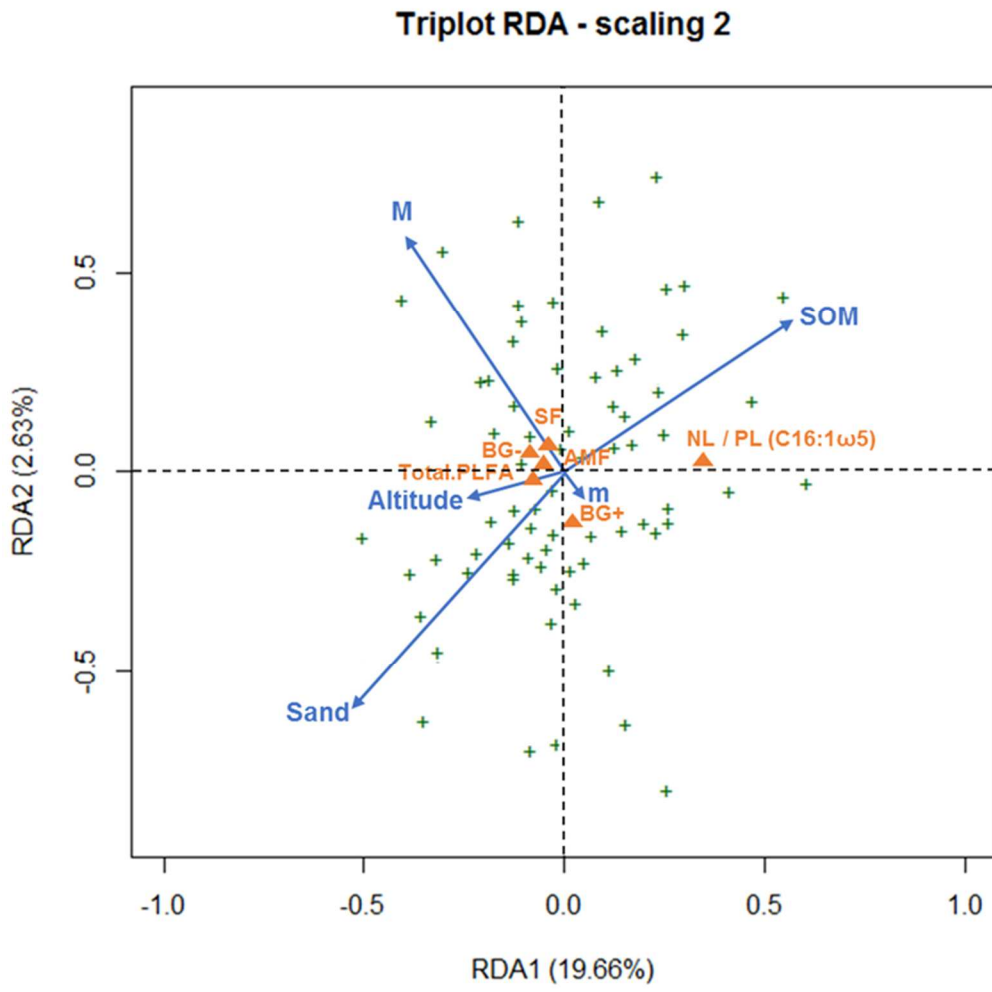


Figure 4.

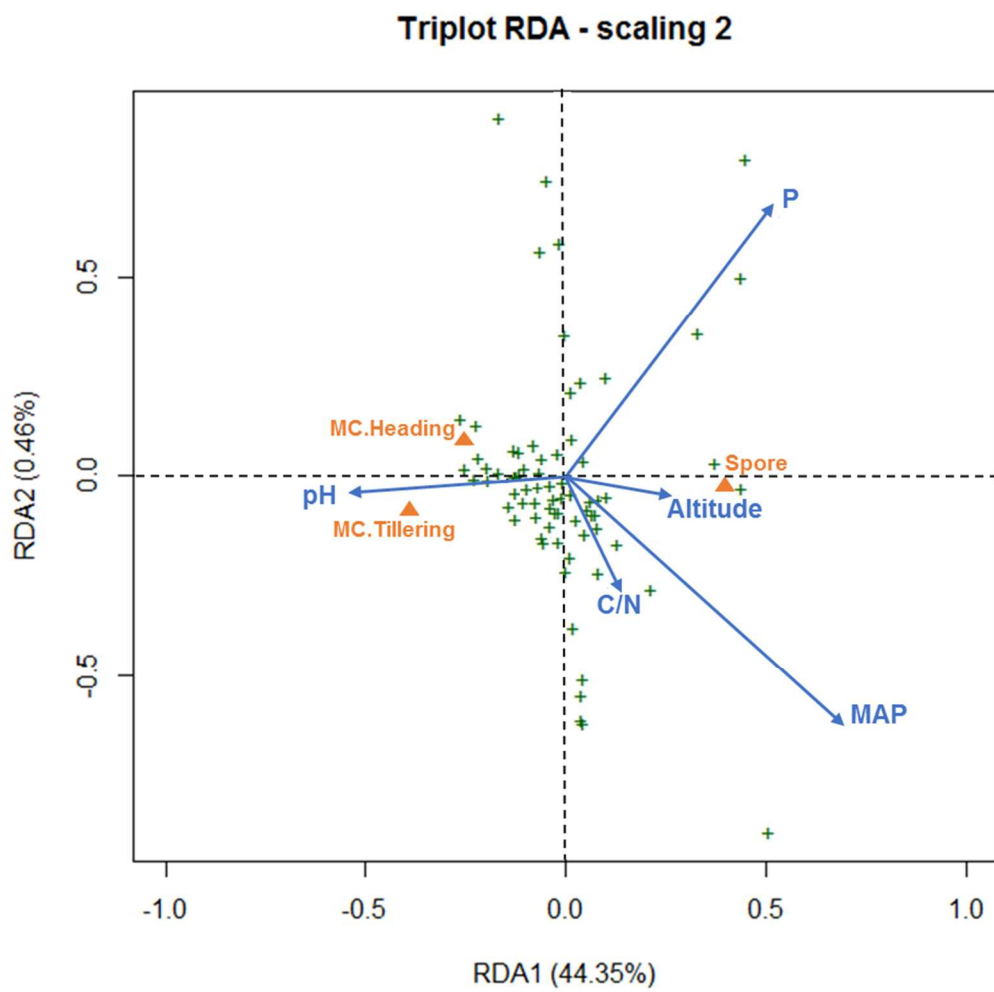


Figure 5.