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# Mycorrhiza

## Native arbuscular mycorrhizal symbiosis alters foliar bacterial community composition

--Manuscript Draft--

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<b>Abstract:</b>	<p>The effects of arbuscular mycorrhizal (AM) fungi on plant-associated microbes are poorly known. We tested the hypothesis that colonization by an AM fungus affects microbial species richness and microbial community composition of host plant tissues. We grew the grass <i>Deschampsia flexuosa</i> in a greenhouse with or without the native AM fungus, <i>Claroideoglossum etunicatum</i>. We divided clonally produced tillers into two parts: one inoculated with AM fungus spores and one without AM fungus inoculation (non-mycorrhizal, NM). We characterized bacterial (16S rRNA genes) and fungal communities (internal transcribed spacer region) in surface-sterilized leaf and root plant compartments. AM fungus inoculation did not affect microbial species richness or diversity indices in leaves or roots, but the AM fungus inoculation significantly affected bacterial community composition in leaves. A total of three OTUs in leaves belonging to the phylum Firmicutes positively responded to the presence of the AM fungus in roots. Another six OTUs belonging to the Proteobacteria (Alpha, Beta and Gamma) and Bacteroidetes were significantly more abundant in NM plants when compared to AM fungus-inoculated plants. Further, there was a significant correlation between plant dry weight and leaf microbial community compositional shift. Also, there was a significant correlation between leaf bacterial community compositional shift and foliar nitrogen content changes due to AM fungus inoculation. The results suggest that AM fungus colonization in roots has a profound effect on plant physiology that is reflected</p>	

in leaf bacterial community composition.

Dear Editor,

Please find attached our second revised version of the manuscript titled "Native arbuscular mycorrhizal symbiosis alters foliar bacterial community composition" by Anbu Poosakkannu, Riitta Nissinen, and Minna-Maarit Kytöviita. We thank you for your valuable comments and time. I carefully looked at your comments and I accepted almost all of them. Wherever applicable we used the term AM fungus to make sure we studied only one species. The primer name fITS7 is correct (We used same as in original reference). I have also modified the table legends and figures according to the suggestions. We hope the current version can be accepted for the publication.

Thanking you.

Sincerely yours,

Anbu Poosakkannu

[Click here to view linked References](#)

1

1 **Native arbuscular mycorrhizal symbiosis alters foliar bacterial community**

2 **composition**

3

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21

**22 Abstract**

23 The effects of arbuscular mycorrhizal (AM) fungi on plant-associated microbes are  
24 poorly known. We tested the hypothesis that colonization by an AM fungus affects  
25 microbial species richness and microbial community composition of host plant tissues.  
26 We grew the grass *Deschampsia flexuosa* in a greenhouse with or without the native  
27 AM fungus, *Claroideoglossum etunicatum*. We divided clonally produced tillers into two  
28 parts: one inoculated with AM fungus spores and one without AM fungus inoculation  
29 (non-mycorrhizal, NM). We characterized bacterial (16S rRNA genes) and fungal  
30 communities (internal transcribed spacer region) in surface-sterilized leaf and root plant  
31 compartments. AM fungus inoculation did not affect microbial species richness or  
32 diversity indices in leaves or roots, but the AM fungus inoculation significantly affected  
33 bacterial community composition in leaves. A total of three OTUs in leaves belonging  
34 to the phylum Firmicutes positively responded to the presence of the AM fungus in  
35 roots. Another six OTUs belonging to the Proteobacteria (Alpha, Beta and Gamma) and  
36 Bacteroidetes were significantly more abundant in NM plants when compared to AM  
37 fungus-inoculated plants. Further, there was a significant correlation between plant dry  
38 weight and leaf microbial community compositional shift. Also, there was a significant  
39 correlation between leaf bacterial community compositional shift and foliar nitrogen  
40 content changes due to AM fungus inoculation. The results suggest that AM fungus  
41 colonization in roots has a profound effect on plant physiology that is reflected in leaf  
42 bacterial community composition.

43

**44 Keywords**

45 Arbuscular mycorrhizal fungi; plant-associated microbes; high-throughput sequencing;  
46 foliar nitrogen

## 47 **Introduction**

48 All plants are associated with microbes, and today the importance of microbes for plant  
49 performance is well acknowledged. Mycorrhizal symbiosis, nodule forming bacteria  
50 and pathogens affect diverse plant species, and these symbiotic systems are relatively  
51 well studied. Recently, however, it has become clear that the symbiotic systems are  
52 complex in nature and multiple microbial interactions shape plant performance (Faust  
53 and Raes 2012). For instance, taxonomically diverse bacteria affect mycorrhizal fungal  
54 colonization of host plants and are known as ‘mycorrhiza helper bacteria’ (Frey-Klett et  
55 al. 2007; Bonfante and Anca 2009; Xavier and Germida 2003; Duponnois and  
56 Plenchette 2003).

57

58 The microbes that live in close association with plants are known as plant-associated  
59 microbes. The major plant-associated microbes are present in the phyllosphere (leaf  
60 surface), rhizosphere (root-attached soil) and endosphere (inside plant). Plant-associated  
61 microbes either are acquired from the environment or are passed from one generation to  
62 the next through propagules (Hardoim et al. 2008; Ferreira et al. 2008; Hardoim et al.  
63 2012). Many of these microbes have neutral or positive impacts on host plants (Hansen  
64 and Moran 2014; Haney et al. 2015, Ludwig-Müller 2015, Panke-Buisse et al. 2015).

65 Distinct factors such as plant part inhabited, host genotype and soil type affect the plant-  
66 associated microbial assemblage (Lundberg et al. 2012; Edwards et al. 2015; Coleman-  
67 Derr et al. 2016; Robinson et al. 2016; Poosakkannu et al. 2015; Poosakkannu et al.  
68 2017).

69

70 Arbuscular mycorrhizal fungi (AMF) inhabit the plant root endosphere and rhizosphere.  
71 The ecological influence of AMF is partly due to their hyphal network that connects the

72 inside of the host plants to the soil (Miller et al. 1995). AMF may improve host plant  
73 nutrient and water uptake (Finlay 2008). In return, AMF utilize photosynthetically  
74 assimilated carbohydrates from the host plants (Bago et al. 2000). AMF symbiosis is  
75 accompanied by several alterations in gene expression in the roots and shoots of  
76 mycorrhizal plants which are related to a variety of plant physiological functions (Liu et  
77 al. 2007).

78

79 As AMF have fundamental effects on both shoot and root physiology, it is conceivable  
80 that the symbiosis with AMF may affect other host-associated fungal and bacterial  
81 communities. Nevertheless, the effect of AMF on plant-associated microbial  
82 communities is poorly studied. It has been shown that AMF inoculation can positively  
83 or negatively affect leaf and root endophytic fungal community composition (Eschen et  
84 al. 2010; Wearn et al. 2012; Larimer et al. 2012). Also, in return, endophytic fungi  
85 could affect AMF colonization rate (Larimer et al. 2012). In contrast to interactions  
86 between AMF and endophytic fungi, thus far AMF effects have been verified on  
87 bacterial communities in soil (Scheublin et al. 2010, Wamberg et al. 2003; Gryndler  
88 2000; Jeffries et al. 2003) and in the rhizosphere (Marschner et al. 2001; Scheffknecht et  
89 al. 2006; Gupta 2003; Vigo et al. 2000).

90

91 Plant physiological status is one of the main factors determining the colonization and  
92 compatibility of plant-associated microbes (Gaiero et al. 2013). It is very well known  
93 that the presence of AMF increases host phosphorous and nitrogen contents  
94 (Karandashov and Bucher 2005; Fellbaum et al. 2012; Hodge and Fitter 2010).

95 Recently, it has been shown that the rhizosphere bacterial community composition  
96 changes induced by AMF inoculation are related to changes in plant physiology, for



97 instance, changes in plant phosphorus content (Rodríguez et al. 2017). Similarly plant  
98 physiological changes due to AMF inoculation could affect other plant-associated  
99 microbes.

100

101 It has been shown that AMF hyphae and spores harbor diverse microbial species  
102 (Bonfante and Anca 2009; Selvakumar et al. 2016). Also, it is known that different  
103 bacterial and fungal species co-occur with AMF (Mansfeld-Giese et al. 2002).

104 Therefore, AMF-associated microbes could be a source for plant-associated microbial  
105 consortia and subsequently might affect the microbial species richness of host plants. In  
106 order to gain insight into the interactions between AMF and other plant-associated  
107 microbes, we asked the following research questions: (i) does inoculation with an  
108 arbuscular mycorrhizal (AM) fungus affect leaf and root microbial community  
109 composition; (ii) does foliar nitrogen content correlate with microbial community  
110 composition; and (iii) do AM fungus-colonized plants harbour more numerous  
111 microbial species in leaves and roots than non-colonized plants? We examined  
112 microbial community composition by high-throughput sequencing of 16S rRNA  
113 (bacteria) genes and internal transcribed spacer (ITS) regions (fungi). We manipulated  
114 AM fungus presence in host plants grown in sterilized substrate under greenhouse  
115 conditions. As model species, we used the wild circumpolar grass, *Deschampsia*  
116 *flexuosa* which is considered to be a keystone pioneer plant species in inland sand dune  
117 ecosystems in northern Finland (Poosakkannu et al. 2015; Poosakkannu et al. 2016) and  
118 a native AM fungus species, *Claroideoglomus etunicatum*.

119

## 120 **Materials and methods**

### 121 **Study organisms**

122 *Deschampsia flexuosa* is commonly known as wavy hair grass, a cosmopolitan species  
123 of temperate and subarctic regions (Scurfield 1954). Seeds of *D. flexuosa* were collected  
124 from inland sand dunes in Northern Fennoscandia (68° 29' 16" N, 24° 42' 13" E) in  
125 summer, 2012. The seeds were germinated in sterile sand and grown under greenhouse  
126 conditions by providing water and Ingestad nutrient solution (Ingestad 1979).

127

128 *Claroideoglo mus etunicatum* isolate was prepared from arctic sand dune soil by first  
129 proliferating it in pot culture among mixed AMF species, followed by growing a hand-  
130 picked single spore and later a mass inoculum in sterilized growth substrate with  
131 *Plantago lanceolata* as the host. *C. etunicatum* has been isolated previously in different  
132 ecosystems from Arctic tundra to tropical regions ([http://invam.wvu.edu/the-](http://invam.wvu.edu/the-fungi/classification/claroideoglomeraceae/claroideoglo mus/etunicatum)  
133 [fungal/classification/claroideoglomeraceae/claroideoglo mus/etunicatum](http://invam.wvu.edu/the-fungi/classification/claroideoglomeraceae/claroideoglo mus/etunicatum); Toomer et al.  
134 2015; Gupta et al. 2014), suggesting that it is a widely distributed species.

135

### 136 **Mycorrhizal inoculation and sampling**

137 *Deschampsia flexuosa* grows by clonally reproduced tillers and therefore the plants are  
138 easily divided into genetically identical parts. In our experiment, each of the six  
139 different pre-grown plants was divided into three parts. The fresh weights of all three  
140 parts were measured (initial value). One part was planted into pots filled with substrate  
141 (A 9:1 mixture of sterile sand and perlite with 1 g/L dolomite and 1 g/L bone meal) and  
142 inoculated with 250 spores of *C. etunicatum*. The second part was planted in the same  
143 substrate and mock inoculated with filtered AM fungus inoculation solution containing  
144 no AM fungus spores as a control (NM, non-mycorrhizal plants). The third part was  
145 used to determine the dry weights of the initial plant samples.

146

147 The AM fungus inoculum for the present experiment was prepared by soaking the  
148 substrate containing spores in water for 2 hours, agitating the suspension vigorously,  
149 letting the solution sediment for approximately 5 seconds followed by decanting. The  
150 decanted solution containing the spores was used as the AM fungus inoculum. The NM  
151 control solution was prepared by additionally filtering the decanted AM fungus  
152 inoculum through a regular coffee filter. The filtrate was left to sediment for 2 minutes  
153 and decanted. The decanted solution was checked to confirm the absence of any AM  
154 fungus spores or hyphae, but was assumed to contain representatives of other microbial  
155 community members which accompanied the inoculum. The AM fungus and NM  
156 inoculations were applied as 6.4 ml pipetted suspensions onto the growth substrate and  
157 plant roots. The experiment was started on 1 May, 2014, and a total of six replicates of  
158 NM and AM fungus inoculation treatments each were maintained during the experiment  
159 under greenhouse conditions. The plants were watered once a day and fertilized with  
160 Ingestad nutrient solution containing 0.9 mM nitrogen and 0.06 mM phosphorous twice  
161 a week (Ingestad 1979).

162

163 The plants were harvested on 15 October, 2014. The shoot and root dry mass (final  
164 values) were measured. Leaves (250 mg) and roots (250 mg) from each plant (3 to 4  
165 representative leaves or roots for each plant) were surface sterilized following the  
166 method described in Poosakkannu et al. (2015) for molecular analysis. In brief, leaves  
167 and roots of each plant were surface sterilized by soaking in 70 % ethanol for 1 min, in  
168 3 % sodium hypochlorite for 3 minutes, and in 1 % sodium thiosulphate for 3 minutes,  
169 followed by a series of three washes with sterile deionized water for 3 minutes each. For  
170 determination of AM fungus colonization, a portion of roots was stored in 50 % ethanol  
171 until examined for colonization.

172

**173 Fungal colonization measurements**

174 Roots from each individual were stained after clearing by incubation in 5 % KOH  
175 overnight. After 2 h in 1 % HCl, the roots were stained with 0.02 % trypan blue solution  
176 for 2 h at 80 °C. The AM fungal root colonization was measured at ×100 magnification  
177 from 10 root segments of 1.5 cm length using the cross-hair intersection method  
178 (McGonigle et al. 1990). A total of 100 intersections were assessed per root sample.

179

**180 Foliar nitrogen content analyses**

181 Oven dried (65°C, 24h) leaf samples were ground to powder and samples (1.8 mg each)  
182 were measured for total nitrogen using an elemental analyzer (Flash EA1112, Carlo  
183 Erba) connected to a Finnigan Deltaplus Advantage (Thermo Electron Corp., Waltham,  
184 USA) continuous flow isotope ratio mass spectrometer (CFIRMS). We calculated the  
185 nitrogen content of the plants using following formula: mg nitrogen/plant shoot=  
186 (percentage nitrogen shoot /100) × dry weight mg shoot.

187

**188 DNA extraction, library preparation and sequencing**

189 The surface sterilized leaves and roots of each plant were frozen using liquid nitrogen,  
190 ground and homogenized. DNA was extracted from the homogenized leaf (100 mg) and  
191 root (100 mg) material from each plant using Invisorb Spin Plant Mini Kit (Stratec  
192 molecular). We used the M13 system (Mäki et al. 2016) for library preparation as  
193 described by Poosakkannu et al. (2017). In brief, a nested approach was used to amplify  
194 the partial 16S rRNA genes; the first round of 16S rRNA amplification was performed  
195 with primers 799F (5'-AACMGGATTAGATACCCCKG-3') and 1492R (5'-  
196 GGYTACTTGTTACGACTT-3') which excludes plant chloroplast amplification

197 (Chelius and Triplett 2001). The subsequent PCR was performed with M13-1062F (5'-  
198 FGTCAGCTCGTGYYGTGA-3') and P1-1390R (5'-ACGGGCGGTGTGTRCAA-3')  
199 primer pairs targeting the V7-V9 region. The ITS region was amplified using the fITS7  
200 (5'-GTGARTCATCGAATCTTTG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-  
201 3') primer pairs (Ihrmark et al. 2012). We followed Ion PGM Sequencing 400 Hi-Q Kit  
202 manufacturer's instructions to carry out the sequencing (Ion 314 chips; Life  
203 Technologies, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

204

### 205 **Bioinformatics and statistics**

206 The processing of bacterial 16SrRNA gene sequences was performed following a  
207 standard procedure in Mothur v.1.35.0 (Schloss et al. 2011). The parameters are  
208 minlength = 200; maxambigs = 0; maxhomop = 8; qwindowaverage = 25; qwindowsize  
209 = 50; and bdiffs = 1). The fungal sequences were processed as described by Tedersoo et  
210 al. (2015) using the same parameters as for the 16S rRNA genes. One of the AM  
211 fungus-inoculated root samples was not included in further downstream analyses  
212 because of not having enough good-quality sequences. The good-quality bacterial and  
213 fungal sequences were clustered at 97 % sequence similarity. Samples were normalized  
214 to their lowest number of reads for further statistical analyses. The observed species  
215 richness, estimated species richness (Chao1), Shannon and Inverse Simpson diversity  
216 indices were calculated using the normalized data. Raw data were submitted to the  
217 sequence read archive (SRA accession number: SRP087758).

218

219 The clustering pattern of bacterial and fungal community composition by treatment was  
220 visualized using unconstrained principal coordinate analysis (PCoAs) and the statistical  
221 significance of clustering patterns were estimated by Permutational Multivariate

222 Analysis of Variance (PERMANOVA; Anderson 2005). To test the correlation between  
223 microbial community composition and plant parameters (total, shoot and root dry  
224 weight and foliar nitrogen content), we performed distance-based linear model  
225 (DistLM) statistical analyses. All the above statistical analyses were performed in  
226 PRIMER software V6 (Clarke and Warwick 2001). Generalized linear model statistics  
227 were used to find the significance of diversity indices between treatments in R software  
228 (v 1.0.44). One-way analysis of variance was carried out for the plant biomass and foliar  
229 nitrogen content analyses after testing the homogeneity of variances using SPSS  
230 software (IBM SPSS 24).

231

## 232 **Results**

233 We obtained 94,836 and 45,628 good quality bacterial and fungal sequences,  
234 respectively. These sequences resulted in 2,300 bacterial OTUs and 1,460 fungal OTUs.  
235 We excluded OTUs with less than five sequences, which resulted in 487 (92,418  
236 sequences) bacterial OTUs and 418 (43,925 sequences) fungal OTUs. In one sample,  
237 there were between 1168 and 7,541 bacterial sequences and between 220 and 1,920  
238 fungal sequences. The normalization of samples resulted in 468 and 385 OTUs of  
239 bacteria and fungi. A total of 14 bacterial and 3 fungal phyla were found in the two  
240 compartments of the NM and AM fungus-inoculated plants (Figure 1). Because of the  
241 primers used, we were not able to detect the fungal phyla Glomeromycota and  
242 Zycomycota in this experiment (Figure 1B).

243

## 244 **Effect of AM fungus inoculation on plant growth and foliar nitrogen content**

245 At the end of the experiment, no AM fungal colonization was found in NM plants,  
246 while all the AM fungus-inoculated plants were colonized. The average hyphal  
247 colonization rate was  $42 \pm 6$  %.

248

249 The total plant dry weight was significantly ( $p < 0.05$ ) higher for AM fungus-colonized  
250 plants ( $569.05 \pm 195.71$  mg) than for NM plants ( $211.33 \pm 100.69$  mg). Also, AM  
251 fungus inoculation significantly increased the foliar nitrogen content of AM fungus-  
252 colonized plants ( $7.6 \pm 2.7$  mg / plant shoot) versus NM plants ( $4.7 \pm 1.7$  mg / plant  
253 shoot).

254

#### 255 **Effect of AM fungus inoculation on bacteria**

256 AM fungus inoculation did not affect the species richness (observed and estimated) or  
257 diversity indices (Shannon and Inverse Simpson) of bacteria in either leaf or root (Table  
258 1). AM fungus and NM plant leaf bacteria clustered strongly according to the AM  
259 fungus inoculation treatment, and the first axis of the PCoAs explained 18.1% of the  
260 variation (Figure 2). The PERMANOVA analysis confirmed the significance of the AM  
261 fungus treatment on leaf bacterial community composition (Figure 2). In contrast to leaf  
262 bacteria, AM fungus colonization did not affect the root bacterial community  
263 composition (Figure 2). A total of 90% bacterial sequences were shared between NM  
264 and AM fungus-inoculated plants (Supplementary figures: Supplementary figure 1).  
265 They accounted for 47 % of the total bacterial OTUs.

266

267 In order to gain insight into the effect of AM fungus treatment on the relative abundance  
268 of individual OTUs, we performed Kruskal -Wallis tests with log transformed (log  
269 [X+1]) relative abundance data to identify the OTUs that are responsible for community

270 separation between the NM and AM fungus treatments. We considered only OTUs with  
271 greater than 1% relative abundance in at least three samples of leaves or roots. There  
272 were three bacterial OTUs that were statistically significantly ( $p < 0.05$ ) more abundant  
273 in AM fungus-inoculated plant than in NM plant leaves (Figure 3). As an example,  
274 these included the OTU 00006 in the bacterial family “Bacillaceae” belonging to the  
275 Firmicutes phylum. There were six bacterial OTUs statistically significantly ( $p < 0.05$ )  
276 more abundant in NM than AM fungus-inoculated plant leaves (Figure 3). For instance,  
277 the OTU 00001 classified as the bacterial genus “*Pseudomonas*” belonging to the  
278 Proteobacteria phylum was significantly more abundant in NM than in AM fungus-  
279 inoculated leaves. In contrast, there was no significant difference in any root bacterial  
280 OTUs between NM and AM fungus-inoculated plants.

281

### 282 **Effect of AM fungus inoculation on fungi**

283 The AM fungus inoculation did not affect the species richness (observed and estimated),  
284 Shannon diversity and Inverse Simpson diversity of fungi in either leaves or roots  
285 (Table 1). Also, fungal community composition was not affected by the AM fungus  
286 inoculation (Figure 4). A total of 93 % fungal sequences were shared between NM and  
287 AM fungus-inoculated plants (Supplementary figures: Supplementary figure 1). They  
288 accounted for 55 % of total fungal OTUs. Furthermore, there were no significant  
289 differences in any leaf or root fungal OTUs between NM and AM fungus-inoculated  
290 plants.

291

### 292 **Correlation between microbial community composition and plant parameters**

293 Significant correlations were observed between the dry weights (Total, shoot and root)  
294 and leaf bacterial community composition ( $p < 0.05$ ; Table 2). Similarly, significant



295 correlations were observed between the plant dry weights (Total and shoot) and leaf  
296 fungal community composition ( $p < 0.05$ ; Table 2).

297

298 Foliar nitrogen content and leaf bacterial community composition also were correlated  
299 significantly ( $p < 0.05$ ; Table 2). The correlation explained 13% of the variation in leaf  
300 bacterial community (Table 2). In contrast, there was no significant correlation observed  
301 between foliar nitrogen content and leaf fungal community composition ( $p > 0.05$ ; Table  
302 2). Also, there was no significant correlation between foliar nitrogen content and root  
303 microbial community composition ( $p > 0.05$ ; Table 2).

304

### 305 **Discussion**

306 To our knowledge, this is the first study to consider the effect of AM fungus  
307 colonization on foliar bacteria. In our study, the bacterial community composition in  
308 *Deschampsia flexuosa* leaves but not roots was altered by arbuscular mycorrhiza  
309 formation. Consistent with our study, Groten et al. (2015) showed that AM fungus  
310 colonization did not affect the root-associated bacterial community composition of  
311 *Nicotiana attenuata*.

312

313 In our study, phylogenetically diverse bacterial OTUs in the phyla Proteobacteria,  
314 Firmicutes and Bacteroidetes were differentially regulated in the leaves of NM and AM  
315 fungus-colonized plants. The presence of AMF has been shown to increase relative  
316 abundance of the members of the phylum Firmicutes in soil (Nuccio et al. 2013).  
317 Similar results for leaves were observed in this study and three OTUs classified as  
318 *Unclassified Bacillaceae* (OTU00006), *Paenibacillus* (OTU00020) and *Brevibacillus*  
319 (OTU00028) belonging to Firmicutes were significantly more abundant in AM fungus-

320 inoculated plant leaves. The bacterial genera *Paenibacillus* and *Brevibacillus* are  
321 described as mycorrhiza helper bacteria which increase mycorrhizal colonization of  
322 roots (Frey-Klett et al. 2007). Also, these bacterial genera are known to be closely  
323 associated with mycelia of AMF (Mansfeld-Giese et al. 2002). The present results  
324 warrant further targeted experiments to understand whether the bacterial genera  
325 *Paenibacillus* and *Brevibacillus* could have an ecological function in the leaves of  
326 arbuscular mycorrhizal plants.

327

328 A total of six OTUs belonging to Proteobacteria (Alpha, Beta and Gamma) and  
329 Bacteroidetes were significantly more abundant in NM than in AM fungus-inoculated  
330 plant leaves. Notably, the most abundant OTU in this study belonging to the genus  
331 *Pseudomonas* (OTU00001) was significantly more abundant in NM than AM fungus-  
332 inoculated plant leaves. The genus *Pseudomonas* is well-known for denitrifying  
333 capacity and responds negatively to AMF presence in the rhizosphere and soils  
334 (Ravnskov et al. 1999; Amora-Lazcano et al. 1998; Meyer and Linderman 1986). The  
335 OTUs belonging to potential nitrogen fixing genera such as *Pseudomonas* and  
336 *Bradyrhizobium* were significantly more abundant in NM than in AM fungus-inoculated  
337 plant leaves. It has been shown that different bacteria genera and *Pseudomonas* in  
338 particular may fix atmospheric nitrogen in grass leaves (Bessemers 1973; Pimentel et al.,  
339 1991). It also is known that many species of the bacterial genus *Pseudomonas* possess  
340 phosphate mobilization capacity in *D. flexuosa* plants (Poosakkannu et al. 2016). In  
341 contrast to our study, Ordoñez et al. (2016) showed that an AMF species (*Rhizophagus*  
342 *irregularis*) may have a synergistic effect with phosphate solubilizing *Pseudomonas*  
343 species. The more abundant *Pseudomonas* and other bacteria in NM than in AM fungus-

344 inoculated plants might fix atmospheric dinitrogen and mobilize phosphate for plant use  
345 in the absence of efficient AM fungus-mediated nutrient transfer from soil.

346

347 In this study, the fungal community composition of *D. flexuosa* leaves and roots was not  
348 altered by arbuscular mycorrhiza formation. This is in contrast with previous studies  
349 which have shown that AMF colonization can affect the fungal community composition  
350 of foliar and root fungi in different plant species such as *Cirsium arvense*, *Plantago*  
351 *lanceolata* and *Rumex acetosa* (Eschen et al. 2010; Wearn et al. 2012). It is worth  
352 noting that both NM and AM fungus-inoculated starting plant tillers in the present study  
353 were of the same clone at the same plant growth stage and most likely contained similar  
354 initial microbial communities which was not the case in previous, related studies  
355 (Eschen et al. 2010; Wearn et al. 2012; Larimer et al. 2012). Plant genets may harbour  
356 distinct microbial communities (Korkama et al. 2007), therefore the clone identity and  
357 growing history should be accounted. The effect of AMF on microbial community  
358 composition has been shown to be plant and AM fungus species dependent (Söderberg  
359 et al. 2002; Larimer et al. 2012). The host plant and AM fungus species of the present  
360 study are additional potential reasons for the difference in the effect of mycorrhizas on  
361 fungal community composition observed between our study and earlier studies. Also,  
362 the plants in this experiment were grown in sterilized substrate and the major source of  
363 microbes may have been seeds, AM fungus and NM inocula, water or air. Another  
364 explanation for differences may be the choice of methods. Eschen et al. (2010) and  
365 Wearn et al. (2012) relied on culture methods for microbe detection in contrast to our  
366 high-throughput sequencing. It is well known that culture methods and high-throughput  
367 sequencing give different results (Zhang and Xu 2008; Van Elsas and Boersma 2011).

368

369 Plant mass and microbial community composition correlated significantly in our  
370 experiment. This is in agreement with reports that have shown that rhizosphere bacterial  
371 community composition may correlate with plant biomass (Rodríguez et al. 2017). In  
372 the present study, a significant correlation also was observed between bacterial  
373 community composition and foliar nitrogen content. In contrast, shoot nitrogen content  
374 did not correlate with rhizosphere bacterial community composition in the study by  
375 Rodríguez et al. 2017. The difference between the studies could be due to the  
376 compartments studied (rhizosphere soil vs surface-sterilized tissues). We did not  
377 measure any other foliar elements than nitrogen in this study, and it is possible that  
378 other nutrients mediated by AM fungus affected the foliar bacterial community.  
379 Furthermore, we cannot rule out the possibility that AM fungus-inoculated plants  
380 possibly provided more carbon compounds to foliar bacteria because of high net  
381 photosynthesis. Although we did not measure photosynthesis rates, we previously have  
382 shown that AMF-inoculated arctic plants with higher foliar nitrogen content than NM  
383 plants also photosynthesize at higher rates (Ruotsalainen & Kytöviita 2004). The current  
384 study together with that by Rodríguez et al. (2017) shows that the mycorrhizal status of  
385 plants alters plant physiology and subsequently could change the community  
386 composition of bacteria intimately associated with plants.

387

388 In our study, bacterial and fungal species richness and diversity indices were not  
389 affected by the presence of an AM fungus in either leaves or roots of *D. flexuosa*. This  
390 is consistent with an earlier finding that non-mycorrhizal transgenic *Nicotiana attenuata*  
391 plants harbour similar root-associated fungal and bacterial species richness to that of  
392 mycorrhizal transgenic *N. attenuata* plants (Groten et al. 2015). Likewise, inoculation  
393 with AMF has been shown not to alter bacterial species richness in the rhizosphere

394 (Rodríguez et al. 2017). Furthermore, while AMF spores harbour microbes (Selvakumar  
395 et al. 2016), in our study, most microbial sequences were shared between NM and AM  
396 fungus-inoculated plants. This suggests that mock inoculation of spore-washing water to  
397 NM plants most likely transferred at least part of AM fungus-associated microbes.

398

399 In conclusion, this study demonstrates that AM fungus inoculation can modify leaf  
400 microbial composition under semi-sterile greenhouse conditions. Further targeted  
401 studies are needed to understand the mechanisms involved and the functional aspects in  
402 the interaction between leaf microbes and AM fungus.

403

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670 **Figure legends**

671 Fig. 1. Phylum-level distribution based on (A) the bacterial 16S rRNA gene, and (B)  
672 fungal internal transcribed spacer region sequences of *Deschampsia flexuosa* leaf and  
673 root samples expressed as relative abundance in non-mycorrhizal and arbuscular  
674 mycorrhizal (AM) fungus-inoculated plants. Values mean of six (except for AM fungus-  
675 inoculated root fungi with five replicates) replicates from each sample. OTUs were  
676 clustered at 97 % similarity.

677

678 Fig. 2 Clustering pattern of bacterial (16S rRNA gene) community composition in  
679 *Deschampsia flexuosa* (A) leaves, and (B) roots in the non-mycorrhizal and arbuscular  
680 mycorrhizal fungus-inoculated plants. OTUs were clustered at 97 % similarity. The  
681 weighted and constrained principal-coordinate analysis (PCoA) based on Bray-Curtis  
682 dissimilarity on standardized and square-root transformed data was performed in  
683 PRIMER software v6. The pseudo-F and p values were obtained from PERMANOVA.

684

685 Fig. 3 Log-transformed relative abundances of the significantly different ( $p < 0.05$ )  
686 bacterial (16S rRNA gene) OTUs in arbuscular mycorrhizal (AM) *Deschampsia*  
687 *flexuosa* leaves compared to non-mycorrhizal (NM) plant leaves. The number of  
688 replicates is six. Only OTUs with a relative abundance more than 1 % in at least three  
689 samples were included in the analysis. Three bacterial OTUs (OTU 00006, 00020,  
690 00028 belonging to bacterial phylum, Firmicutes) were significantly more abundant in  
691 AM fungus-inoculated *D. flexuosa* leaves compared to NM *D. flexuosa* leaves. Another  
692 six bacterial OTUs (OTU 00001, 00010, 00017, 00033, 00035 belonging to phylum  
693 Proteobacteria and OTU 00046 belonging to bacterial phylum, Bacteroidetes) were

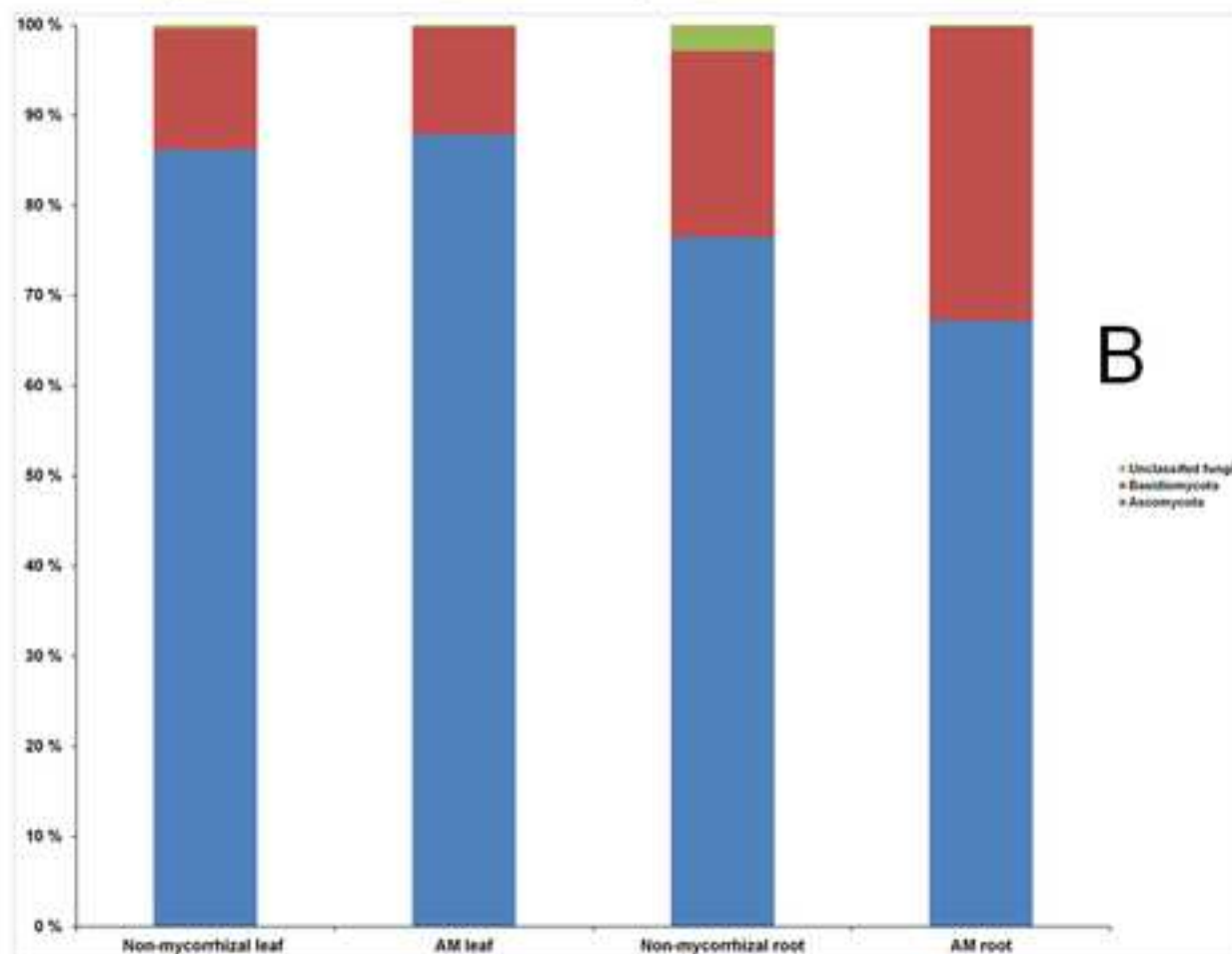
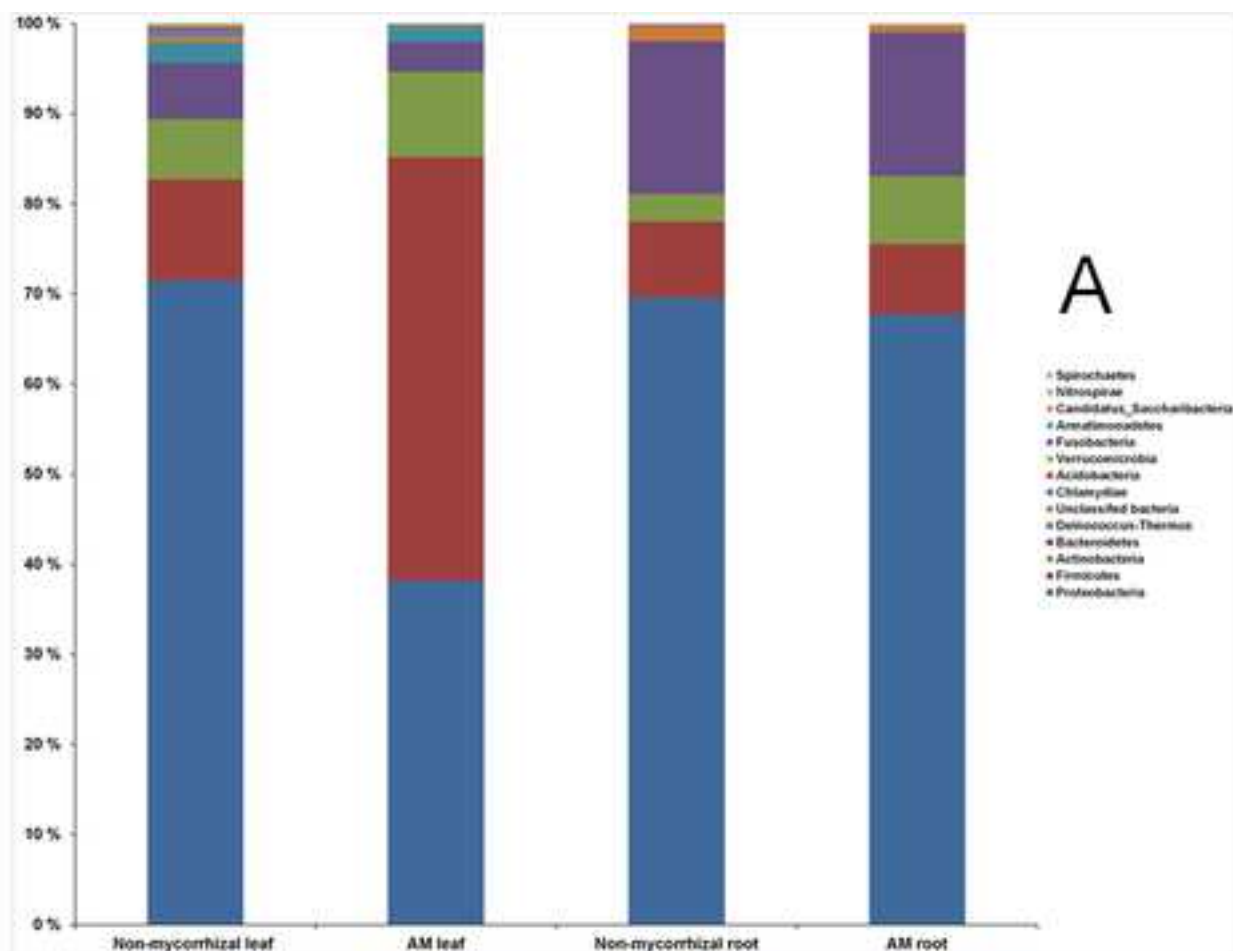
694 significantly more abundant in NM *D. flexuosa* leaves than in AM fungus-inoculated *D.*  
695 *flexuosa* leaves.

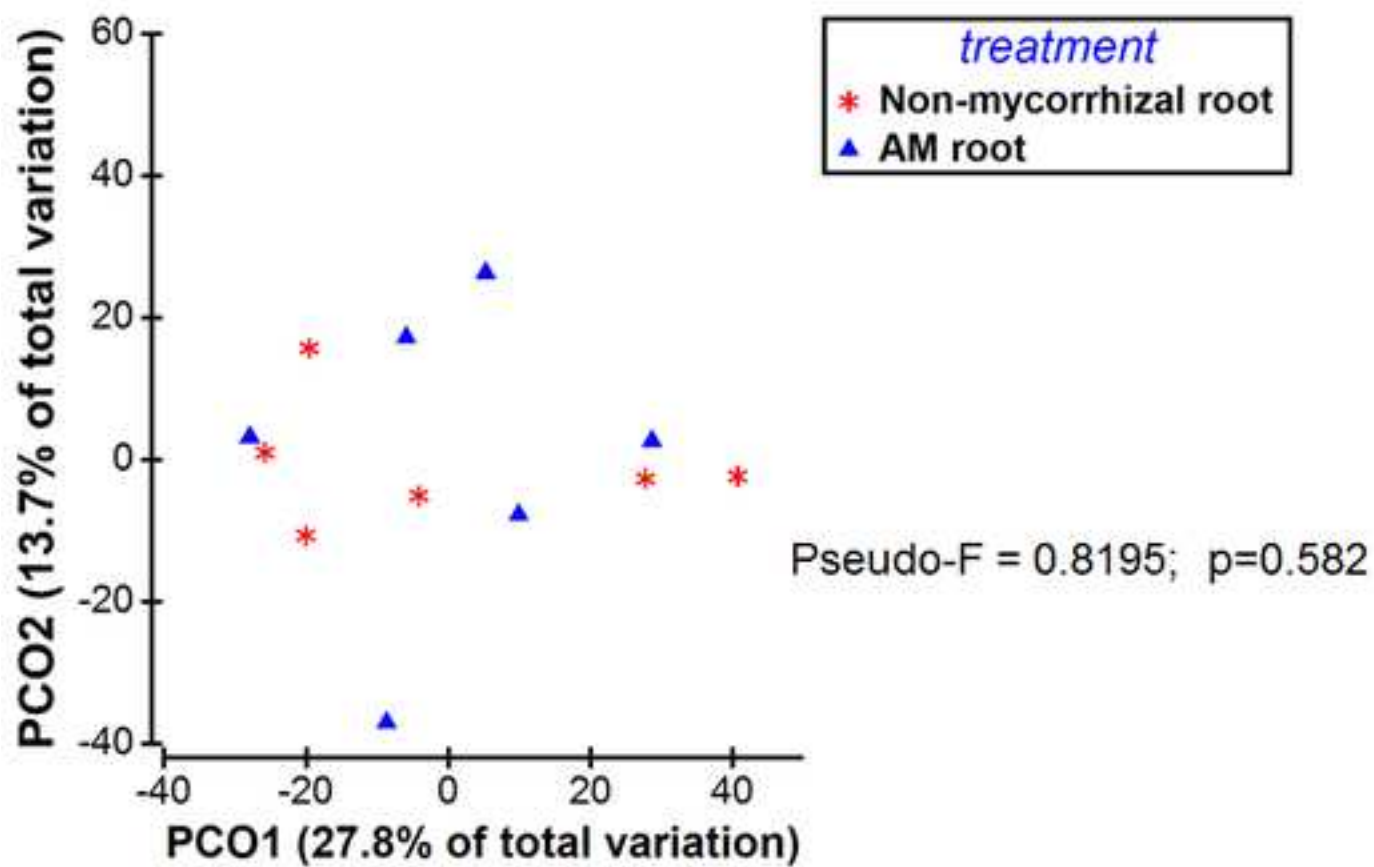
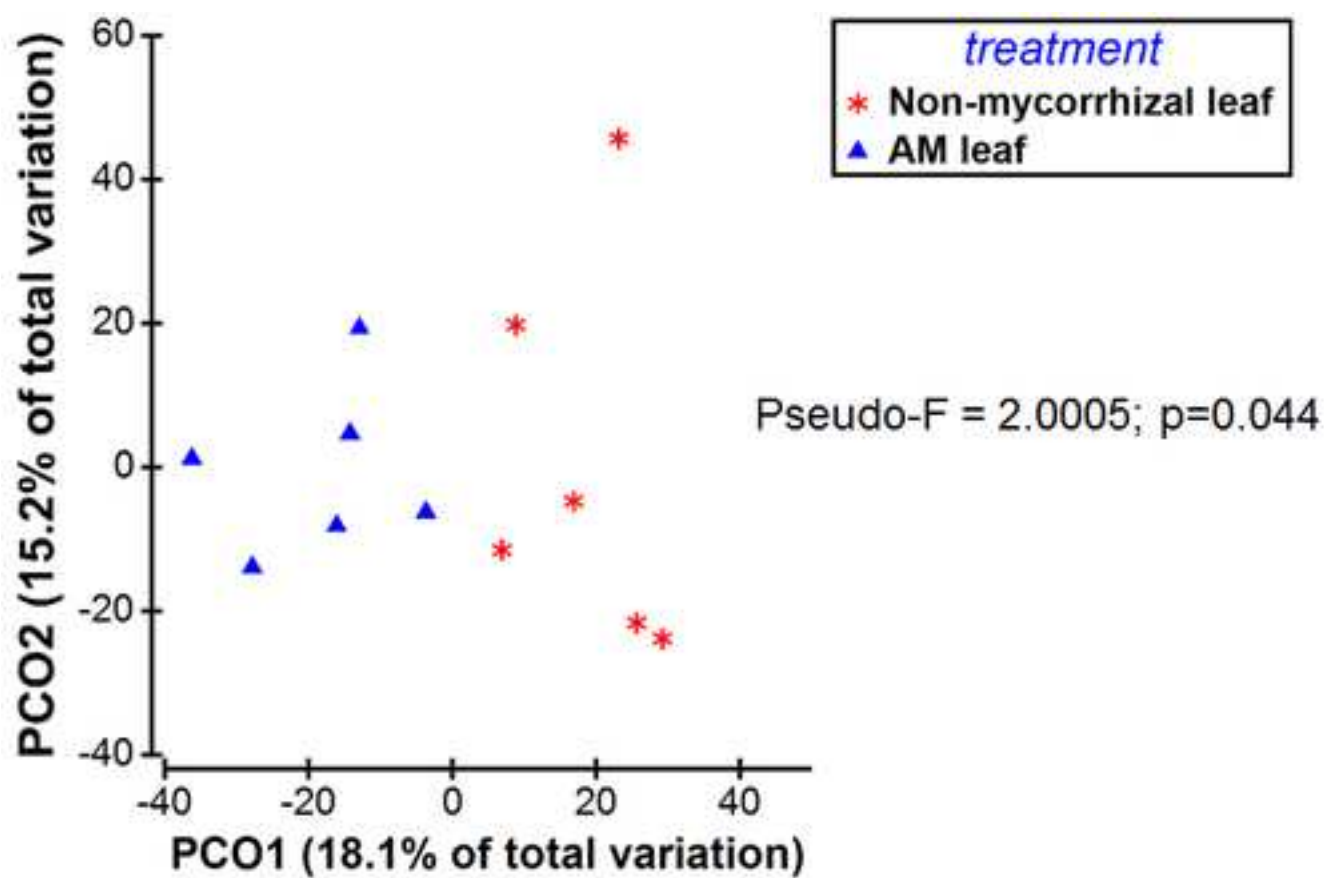
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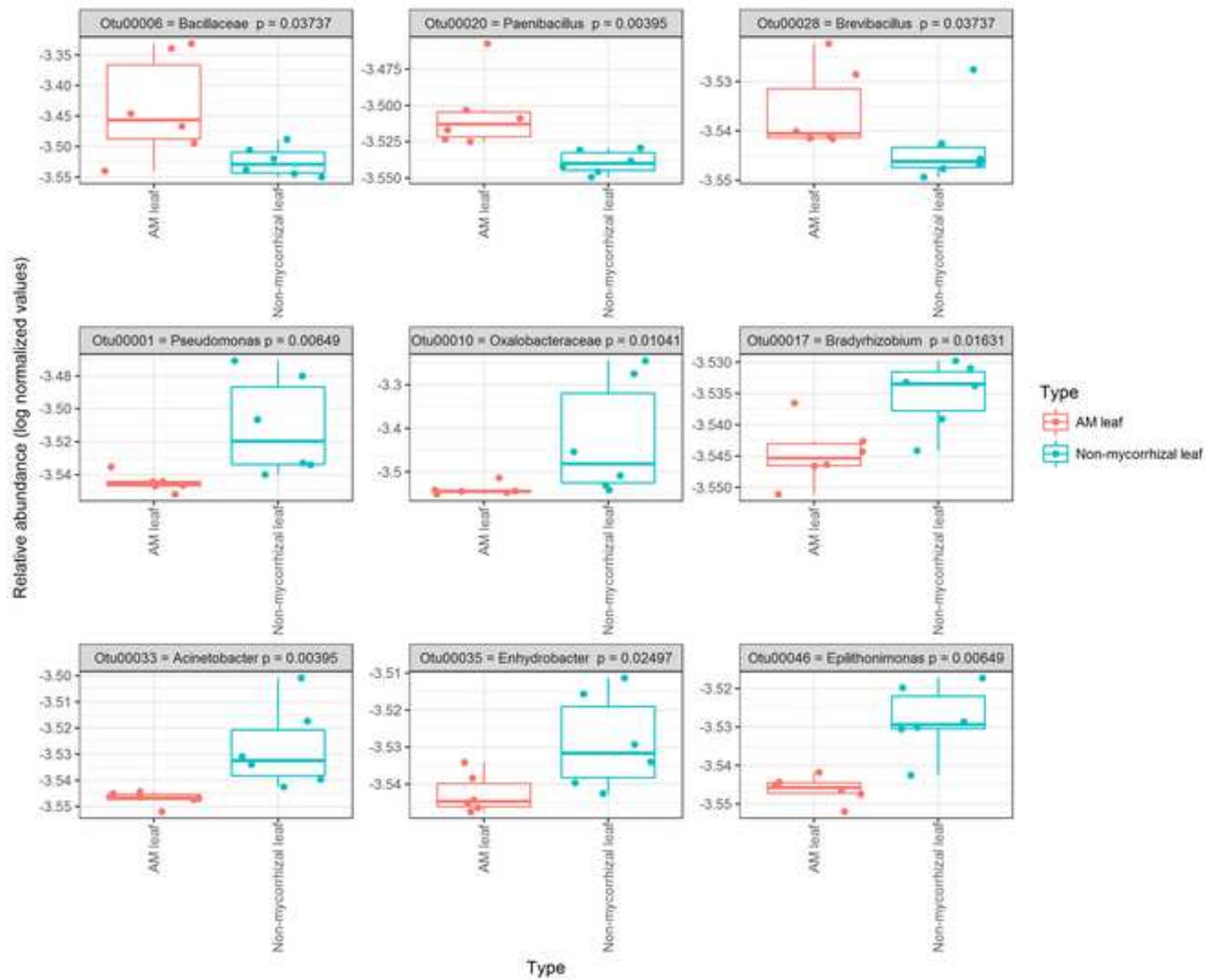
697 Fig. 4 Clustering pattern of fungal (internal transcribed spacer region) community  
698 composition in *Deschampsia flexuosa* (A) leaves, and (B) roots in the non-mycorrhizal  
699 and arbuscular mycorrhizal fungus-inoculated plants. OTUs were clustered at 97 %  
700 similarity. The weighted and constrained principal-coordinate analysis (PCoA) based on  
701 Bray-Curtis dissimilarity on standardized and square-root transformed data was  
702 performed in PRIMER software v6. The pseudo-F and p values were obtained from  
703 PERMANOVA.

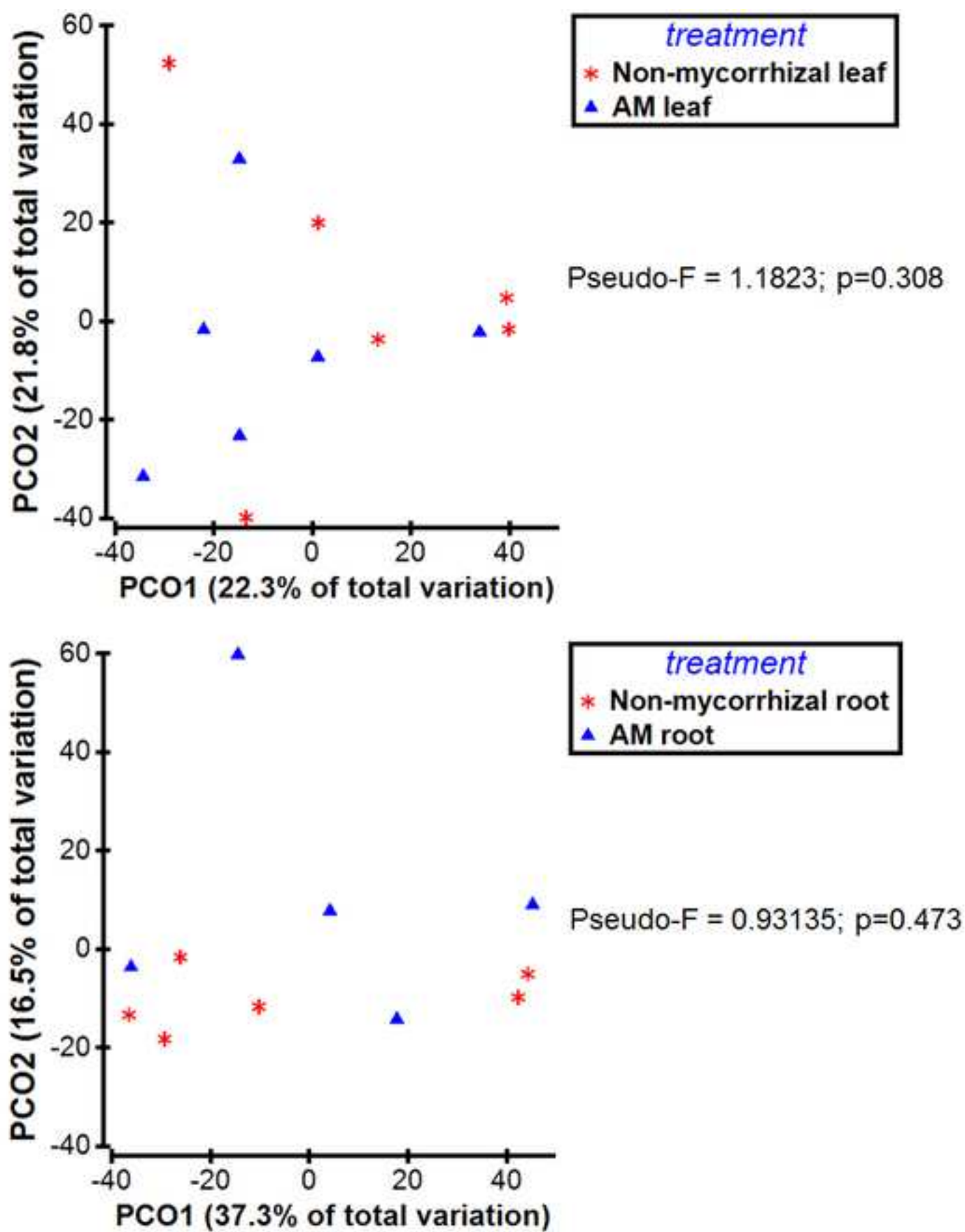
704

Relative abundance (%)









<b>Taxa</b>	<b>Diversity indices</b>	<b>Treatment</b>	<b>Leaf (mean <math>\pm</math> SE)</b>	<b>Root (mean <math>\pm</math> SE)</b>
Bacteria	Observed OTU richness	NM	62.50 $\pm$ 7.01	87.67 $\pm$ 11.57
		AM	59.17 $\pm$ 5.70	75.67 $\pm$ 5.38
	Estimated OTU richness	NM	81.03 $\pm$ 14.86	122.15 $\pm$ 15.90
		AM	76.37 $\pm$ 8.24	107.56 $\pm$ 5.31
	Shannon diversity index	NM	2.80 $\pm$ 0.32	2.50 $\pm$ 0.47
		AM	2.77 $\pm$ 0.21	2.55 $\pm$ 0.36
Inverse Simpson index	NM	10.74 $\pm$ 2.58	8.16 $\pm$ 2.33	
	AM	9.12 $\pm$ 2.30	8.65 $\pm$ 2.84	
Fungi	Observed OTU richness	NM	42.33 $\pm$ 7.49	30.17 $\pm$ 2.57
		AM	38.50 $\pm$ 6.21	36.20 $\pm$ 6.70
	Estimated OTU richness	NM	62.44 $\pm$ 8.32	46.39 $\pm$ 6.12
		AM	75.62 $\pm$ 11.53	57.48 $\pm$ 15.27
	Shannon diversity index	NM	2.51 $\pm$ 0.38	2.23 $\pm$ 0.09
		AM	2.16 $\pm$ 0.29	2.28 $\pm$ 0.30
	Inverse Simpson index	NM	10.79 $\pm$ 4.51	5.73 $\pm$ 0.74
		AM	4.72 $\pm$ 0.95	6.06 $\pm$ 1.43

Table 1. Observed OTU richness, estimated OTU richness, Shannon-wiener index and Inverse Simpson index of bacterial OTUs (16S rRNA gene) and fungal OTUs (internal transcribed spacer region) in *Deschampsia flexuosa* leaf and root in non-mycorrhizal (NM) and arbuscular mycorrhizal (AM) fungus-colonized plants. OTUs were clustered at 97 % similarity level. Generalized linear model analyses showed that none of the differences are statistically significant between NM and AM fungus-colonized plants.

<b>(A) Leaf bacteria</b>				
<b>Source of variation</b>	<b>SS(trace)</b>	<b>Pseudo-F</b>	<b>P</b>	<b>Proportion</b>
<b>Total weight</b>	<b>4419.7</b>	<b>1.8177</b>	<b>0.002</b>	<b>0.15381</b>
<b>Shoot weight</b>	<b>4179</b>	<b>1.7018</b>	<b>0.006</b>	<b>0.14543</b>
<b>Root weight</b>	<b>4654</b>	<b>1.9327</b>	<b>0.001</b>	<b>0.16197</b>
<b>Foliar nitrogen</b>	<b>3821.5</b>	<b>1.5339</b>	<b>0.021</b>	<b>0.13299</b>
<b>(B) Root bacteria</b>				
<b>Source of variation</b>	<b>SS(trace)</b>	<b>Pseudo-F</b>	<b>P</b>	<b>Proportion</b>
Total weight	1480.5	0.77018	0.764	7.15E-02
Shoot weight	1521.4	0.79316	0.742	7.35E-02
Root weight	1493.8	0.77765	0.745	7.22E-02
Foliar nitrogen	2406.2	1.3151	0.156	0.11622
<b>(C) Leaf Fungi</b>				
<b>Source of variation</b>	<b>SS(trace)</b>	<b>Pseudo-F</b>	<b>P</b>	<b>Proportion</b>
<b>Total weight</b>	<b>5464</b>	<b>1.9097</b>	<b>0.018</b>	<b>0.16035</b>
<b>Shoot weight</b>	<b>5465.8</b>	<b>1.9104</b>	<b>0.018</b>	<b>0.1604</b>
Root weight	4676	1.5905	0.092	0.13722
Foliar nitrogen	4841.9	1.6563	0.066	0.14209
<b>(D) Root fungi</b>				
<b>Source of variation</b>	<b>SS(trace)</b>	<b>Pseudo-F</b>	<b>P</b>	<b>Proportion</b>
Total weight	4081.1	1.5041	0.136	0.14319
Shoot weight	4097.4	1.5111	0.151	0.14376
Root weight	3605	1.3032	0.246	0.12649
Foliar nitrogen	4632.6	1.7468	0.088	0.16254

Table 2. Distance based linear model statistical test (DistLM) for correlation between microbial community composition and plant parameters (total, shoot, root dry weight and foliar nitrogen content) in *Deschampsia flexuosa* leaves (A and C) and roots (B and D). Bold letters indicate the significant correlation ( $p < 0.05$ ).