Jyväskylän yliopiston julkaisuarkisto
Jyväskylä University Digital Archive
UNIVERSITY OF JYVÄSKYLÄ

This is an electronic reprint of the original article.
This reprint *may differ* from the original in pagination and typographic detail.

Author(s): Poosakkannu, Anbu; Nissinen, Riitta; Kytöviita, Minna-Maarit

Title: Native arbuscular mycorrhizal symbiosis alters foliar bacterial community composition

Year: 2017

Version:

Please cite the original version:

Poosakkannu, A., Nissinen, R., & Kytöviita, M.-M. (2017). Native arbuscular mycorrhizal symbiosis alters foliar bacterial community composition. Mycorrhiza, 27(8), 801-810. https://doi.org/10.1007/s00572-017-0796-6

All material supplied via JYX is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.

Mycorrhiza

Native arbuscular mycorrhizal symbiosis alters foliar bacterial community composition

--Manuscript Draft--

Manuscript Number:	MCOR-D-17-00079R2
Full Title:	Native arbuscular mycorrhizal symbiosis alters foliar bacterial community composition
Article Type:	Original Article
Keywords:	Arbuscular mycorrhizal fungi; plant-associated microbes; high-throughput sequencing; foliar nitrogen
Corresponding Author:	ANBU POOSAKKANNU University of Jyväskylä FINLAND
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Jyväskylä
Corresponding Author's Secondary Institution:	
First Author:	ANBU POOSAKKANNU
First Author Secondary Information:	
Order of Authors:	ANBU POOSAKKANNU
	Riitta Nissinen
	Minna-Maarit Kytöviita
Order of Authors Secondary Information:	

Funding Information:

Funder	Recipient
Maj ja Tor Nesslingin Säätiö (201300249)	Dr. Minna-Maarit Kytöviita
Suomen Akatemia (259180)	Dr Riitta Nissinen
Suomen Akatemia (287545)	Dr. Minna-Maarit Kytöviita
Suomen Kulttuurirahasto	Dr. ANBU POOSAKKANNU
Kansainvälisen Liikkuvuuden ja Yhteistyön Keskus	Dr. ANBU POOSAKKANNU
Oskar Öflunds Stiftelse	Dr. ANBU POOSAKKANNU

Abstract:

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 Deschampsia flexuosa in a greenhouse with or without the native AM fungus, Claroideoglomus etunicatum. 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

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

	in leaf bacterial community composition.

Powered by Editorial Manager® and ProduXion Manager® from Aries Systems Corporation

Authors Click here to download Authors' Response to Reviewers' Comments Reply to editor.docx

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

Manuscript
Click here to download Manuscript Manuscript_01082017.docx
Click here to view linked References

1 **Native arbuscular mycorrhizal symbiosis alters foliar bacterial community**
2 **composition**
3
4 Anbu Poosakkannu*[1], Riitta Nissinen[1], Minna-Maarit Kytöviita[1]
5
6 1) Department of Biological and Environmental Science, PO Box 35, FI-40014
7 University of Jyväskylä, Finland.
8
9 *Corresponding author
10 Anbu Poosakkannu
11 Department of Biological and Environmental Science
12 PO Box 35, FI-40014 University of Jyväskylä
13 Finland
14 Tel: +358 (0) 466137555
15 Fax: +358 (0) 14 617 239
16 Email: anbu.a.poosakkannu@jyu.fi
17 ORCID ID: http://orcid.org/0000-0003-2579-150X
18
19
20
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, *Claroideoglomus 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 *Claroideoglomus 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-
133 fungi/classification/claroideoglomeraceae/claroideoglomus/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 ºC. 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´-AACMGGATTAGATACCCKG-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 ± 6 %.
248
249 The total plant dry weight was significantly ($p<0.05$) higher for AM fungus-colonized
250 plants (569.05 ± 195.71 mg) than for NM plants (211.33 ± 100.69 mg). Also, AM
251 fungus inoculation significantly increased the foliar nitrogen content of AM fungus-
252 colonized plants (7.6 ± 2.7 mg / plant shoot) versus NM plants (4.7 ± 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 *Unclassifed 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 (Bessems 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

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

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

Acknowledgement

The authors wish to acknowledge funding from the Maj and Tor Nessling foundation (grant # 201300249), Finnish cultural foundation, Centre for International Mobility, Oskar Öflunds Stiftelse and Department of Biological and Environmental Science, University of Jyväskylä and Academy of Finland (grant # 259180, #287545). We especially thank The Ella and Georg Ehrnrooth Foundation for awarding the sequencing cost.

411 **References**

412 Amora-Lazcano E, Vazquez M, Azcon R (1998) Response of nitrogen-transforming
413 microorganisms to arbuscular mycorrhizal fungi. Biol Fertility Soils 27:65-70
414

415 Anderson MJ (2005) Permutational multivariate analysis of variance. Department of
416 Statistics, University of Auckland, Auckland 26:32-46
417

418 Bessems EPM (1973) Nitrogen fixation in the phyllosphere of Gramineae. Centre for
419 Agricultural Publishing and Documentation
420

421 Bago B, Pfeffer PE, Shachar-Hill Y (2000) Carbon metabolism and transport in
422 arbuscular mycorrhizas. Plant Physiol 124:949-958
423

424 Bonfante P, Anca I (2009) Plants, mycorrhizal fungi, and bacteria: a network of
425 interactions. Annu Rev Microbiol 63:363-383
426

427 Chelius M, Triplett E (2001) The Diversity of Archaea and Bacteria in Association with
428 the Roots of *Zea mays* L. Microb Ecol 41:252-263
429

430 Clarke K, Warwick R (2001) Change in Marine Communities: An Approach to
431 Statistical Analysis and Interpretation (PRIMER-E). Plymouth Marine
432 Laboratory, Plymouth, UK.
433

434 Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T,
435 North G, Visel A, Partida-Martinez LP, Tringe SG (2016) Plant compartment

436 and biogeography affect microbiome composition in cultivated and native *Agave*
437 species. New Phytol 209:798-811
438
439 Duponnois R, Plenchette C (2003) A mycorrhiza helper bacterium enhances
440 ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species.
441 Mycorrhiza 13:85-91
442
443 Edwards J, Johnson C, Santos-Medellin C, Lurie E, Podishetty NK, Bhatnagar S, Eisen
444 JA, Sundaresan V (2015) Structure, variation, and assembly of the root-
445 associated microbiomes of rice. Proc Natl Acad Sci U S A 112:E911-20. doi:
446 10.1073/pnas.1414592112
447
448 Eschen R, Hunt S, Mykura C, Gange AC, Sutton BC (2010) The foliar endophytic
449 fungal community composition in *Cirsium arvense* is affected by mycorrhizal
450 colonization and soil nutrient content. Fungal Biology 114:991-998
451
452 Faust K, Raes J (2012) Microbial interactions: from networks to models. Nature
453 Reviews Microbiology 10:538-550
454
455 Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers
456 ET, Bucking H (2012) Carbon availability triggers fungal nitrogen uptake and
457 transport in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci U S A
458 109:2666-2671. doi: 10.1073/pnas.1118650109
459

460 Ferreira A, Quecine MC, Lacava PT, Oda S, Azevedo JL, Araujo WL (2008) Diversity
461 of endophytic bacteria from Eucalyptus species seeds and colonization of
462 seedlings by *Pantoea agglomerans*. FEMS Microbiol Lett 287:8-14
463
464 Finlay RD (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis
465 on the functional diversity of interactions involving the extraradical mycelium. J
466 Exp Bot 59:1115-1126. doi: 10.1093/jxb/ern059
467
468 Frey-Klett P, Garbaye Ja, Tarkka M (2007) The mycorrhiza helper bacteria revisited.
469 New Phytol 176:22-36
470
471 Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside
472 the root microbiome: bacterial root endophytes and plant growth promotion. Am
473 J Bot 100:1738-1750. doi: 10.3732/ajb.1200572
474
475 Groten K, Nawaz A, Nguyen NH, Santhanam R, Baldwin IT (2015) Silencing a key
476 gene of the common symbiosis pathway in *Nicotiana attenuata* specifically
477 impairs arbuscular mycorrhizal infection without influencing the root-associated
478 microbiome or plant growth. Plant, Cell Environ 38:2398-2416
479
480 Gryndler M (2000) Interactions of arbuscular mycorrhizal fungi with other soil
481 organisms. In: Anonymous Arbuscular mycorrhizas: Physiology and function.
482 Springer, pp. 239-262
483

484 Gupta Sood S (2003) Chemotactic response of plant-growth-promoting bacteria towards
485 roots of vesicular-arbuscular mycorrhizal tomato plants. FEMS Microbiol Ecol
486 45:219-227. doi: 10.1016/S0168-6496(03)00155-7
487
488 Gupta MM, Naqvi NS, Singh VK (2014) The state of arbuscular mycorrhizal fungal
489 diversity in India: an analysis. Sydowia 66:265-288
490
491 Haney CH, Samuel BS, Bush J, Ausubel FM (2015) Associations with rhizosphere
492 bacteria can confer an adaptive advantage to plants. Nat Plants 1:15051. Epub
493 2015 May 11. doi: 15051
494
495 Hansen AK, Moran NA (2014) The impact of microbial symbionts on host plant
496 utilization by herbivorous insects. Mol Ecol 23:1473-1496
497
498 Hardoim PR, Hardoim CC, Van Overbeek LS, Van Elsas JD (2012) Dynamics of seed-
499 borne rice endophytes on early plant growth stages. PLoS One 7:e30438
500
501 Hardoim PR, van Overbeek LS, Elsas JD (2008) Properties of bacterial endophytes and
502 their proposed role in plant growth. Trends Microbiol 16:463-471. doi:
503 10.1016/j.tim.2008.07.008; 10.1016/j.tim.2008.07.008
504
505 Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal
506 fungi from organic material has implications for N cycling. Proc Natl Acad Sci
507 U S A 107:13754-13759. doi: 10.1073/pnas.1005874107
508

509 Ihrmark K, Bodeker IT, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y,
510 Stenlid J, Brandstrom-Durling M, Clemmensen KE, Lindahl BD (2012) New
511 primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of
512 artificial and natural communities. FEMS Microbiol Ecol 82:666-677. doi:
513 10.1111/j.1574-6941.2012.01437.x
514
515 Ingestad T (1979) Mineral nutrient requirements of *Pinus silvestris* and *Picea abies*
516 seedlings. Physiol Plantarum 45:373-380
517
518 Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J (2003) The contribution of
519 arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil
520 fertility. Biol Fertility Soils 37:1-16
521
522 Karandashov V, Bucher M (2005) Symbiotic phosphate transport in arbuscular
523 mycorrhizas. Trends Plant Sci 10:22-29
524
525 Korkama T, Fritze H, Kiikkilä O, Pennanen T (2007) Do same-aged but different height
526 Norway spruce (*Picea abies*) clones affect soil microbial community? Soil Biol
527 Biochem 39:2420-2423
528
529 Larimer AL, Bever JD, Clay K (2012) Consequences of simultaneous interactions of
530 fungal endophytes and arbuscular mycorrhizal fungi with a shared host grass.
531 Oikos 121:2090-2096
532

533 Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ
534 (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic
535 alterations in gene expression and an increase in disease resistance in the shoots.
536 The Plant Journal 50:529-544
537
538 Ludwig-Müller J (2015) Plants and endophytes: equal partners in secondary metabolite
539 production? Biotechnol Lett 37:1325-1334
540
541 Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J,
542 Engelbrektson A, Kunin V, Del Rio TG (2012) Defining the core *Arabidopsis*
543 *thaliana* root microbiome. Nature 488:86-90
544
545 Mae T (1997) Physiological nitrogen efficiency in rice: nitrogen utilization,
546 photosynthesis, and yield potential. Plant Soil 196:201-210
547
548 Mäki A, Rissanen AJ, Tiirola M (2016) Benchmarks: A practical method for barcoding
549 and size-trimming PCR templates for amplicon sequencing. BioTechniques
550 60:88-90
551
552 Mansfeld-Giese K, Larsen J, Bødker L (2002) Bacterial populations associated with
553 mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. FEMS
554 Microbiol Ecol 41:133-140
555

556 Marschner P, Crowley D, Lieberei R (2001) Arbuscular mycorrhizal infection changes
557 the bacterial 16 S rDNA community composition in the rhizosphere of maize.
558 Mycorrhiza 11:297-302
559
560 McGonigle T, Miller M, Evans D, Fairchild G, Swan J (1990) A new method which
561 gives an objective measure of colonization of roots by vesicular—arbuscular
562 mycorrhizal fungi. New Phytol 115:495-501
563
564 Meyer JR, Linderman R (1986) Selective influence on populations of rhizosphere or
565 rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus*
566 *fasciculatum*. Soil Biol Biochem 18:191-196
567
568 Miller R, Jastrow J, Reinhardt D (1995) External hyphal production of vesicular-
569 arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities.
570 Oecologia 103:17-23
571
572 Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK (2013) An
573 arbuscular mycorrhizal fungus significantly modifies the soil bacterial
574 community and nitrogen cycling during litter decomposition. Environ Microbiol
575 15:1870-1881
576
577 Ordoñez YM, Fernandez BR, Lara LS, Rodriguez A, Uribe-Vélez D, Sanders IR (2016)
578 Bacteria with phosphate solubilizing capacity alter mycorrhizal fungal growth
579 both inside and outside the root and in the presence of native microbial
580 communities. PloS one 11:e0154438

581

582 Pimentel J, Olivares F, Pitard R, Urquiaga S, Akiba F, Döbereiner J (1991) Dinitrogen
583 fixation and infection of grass leaves by *Pseudomonas rubrisubalbicans* and
584 *Herbaspirillum seropedicae*. Plant Soil 137:61-65

585 Poosakkannu A, Nissinen R, Kytöviita M (2015) Culturable endophytic microbial
586 communities in the circumpolar grass, *Deschampsia flexuosa* in a sub-Arctic
587 inland primary succession are habitat and growth stage specific. Environmental
588 Microbiology Reports 7:111-122

589

590 Poosakkannu A, Nissinen R, Männistö M, Kytöviita M (2017) Microbial community
591 composition but not diversity changes along succession in arctic sand dunes.
592 Environ Microbiol 19:698-709

593

594 Ravnskov S, Larsen J, Olsson PA, Jakobsen I (1999) Effects of various organic
595 compounds on growth and phosphorus uptake of an arbuscular mycorrhizal
596 fungus. New Phytol 141:517-524

597

598 Robinson RJ, Fraaije BA, Clark IM, Jackson RW, Hirsch PR, Mauchline TH (2016)
599 Wheat seed embryo excision enables the creation of axenic seedlings and Koch's
600 postulates testing of putative bacterial endophytes. Sci Rep 6:25581. doi:
601 10.1038/srep25581

602

603 Rodríguez-Caballero G, Caravaca F, Fernández-González A, Alguacil M, Fernández-
604 López M, Roldán A (2017) Arbuscular mycorrhizal fungi inoculation mediated

605 changes in rhizosphere bacterial community structure while promoting
606 revegetation in a semiarid ecosystem. Sci Total Environ
607
608 Ruotsalainen AL, Kytöviita M (2004) Mycorrhiza does not alter low temperature impact
609 on *Gnaphalium norvegicum*. Oecologia 140:226-233
610
611 Scheffknecht S, Mammerler R, Steinkellner S, Vierheilig H (2006) Root exudates of
612 mycorrhizal tomato plants exhibit a different effect on microconidia germination
613 of *Fusarium oxysporum* f. sp. *lycopersici* than root exudates from non-
614 mycorrhizal tomato plants. Mycorrhiza 16:365-370
615
616 Scheublin TR, Sanders IR, Keel C, van der Meer, Jan Roelof (2010) Characterisation of
617 microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal
618 fungi. The ISME journal 4:752-763
619
620 Schloss PD, Gevers D, Westcott SL (2011) Reducing the effects of PCR amplification
621 and sequencing artifacts on 16S rRNA-based studies. PLoS One 6:e27310. doi:
622 10.1371/journal.pone.0027310]
623
624 Scurfield G (1954) *Deschampsia flexuosa* (L.) Trin. J Ecol 42:225-233
625
626 Selvakumar G, Krishnamoorthy R, Kim K, Sa T (2016) Genetic Diversity and
627 Association Characters of Bacteria Isolated from Arbuscular Mycorrhizal Fungal
628 Spore Walls. PloS one 11:e0160356
629

630 Soderberg KH, Olsson PA, Baath E (2002) Structure and activity of the bacterial
631 community in the rhizosphere of different plant species and the effect of
632 arbuscular mycorrhizal colonisation. FEMS Microbiol Ecol 40:223-231. doi:
633 10.1111/j.1574-6941.2002.tb00955.x]
634

635 Tedersoo L, Bahram M, Polme S, Koljalg U, Yorou NS, Wijesundera R, Villarreal Ruiz
636 L, Vasco-Palacios AM, Thu PQ, Suija A, Smith ME, Sharp C, Saluveer E, Saitta
637 A, Rosas M, Riit T, Ratkowsky D, Pritsch K, Poldmaa K, Piepenbring M, Phosri
638 C, Peterson M, Parts K, Partel K, Otsing E, Nouhra E, Njouonkou AL, Nilsson
639 RH, Morgado LN, Mayor J, May TW, Majuakim L, Lodge DJ, Lee SS, Larsson
640 KH, Kohout P, Hosaka K, Hiiesalu I, Henkel TW, Harend H, Guo LD, Greslebin
641 A, Grelet G, Geml J, Gates G, Dunstan W, Dunk C, Drenkhan R, Dearnaley J,
642 De Kesel A, Dang T, Chen X, Buegger F, Brearley FQ, Bonito G, Anslan S,
643 Abell S, Abarenkov K (2014) Fungal biogeography. Global diversity and
644 geography of soil fungi. Science 346:1256688. doi: 10.1126/science.1256688
645

646 Toomer KH, Chen X, Naito M, Mondo SJ, Bakker HC, VanKuren NW, Lekberg Y,
647 Morton JB, Pawlowska TE (2015) Molecular evolution patterns reveal life
648 history features of mycoplasma-related endobacteria associated with arbuscular
649 mycorrhizal fungi. Mol Ecol 24:3485-3500
650

651 Van Elsas J, Boersma F (2011) A review of molecular methods to study the microbiota
652 of soil and the mycosphere. Eur J Soil Biol 47:77-87
653

654 Vigo C, Norman J, Hooker J (2000) Biocontrol of the pathogen *Phytophthora*
655 *parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on
656 infection loci. Plant Pathol 49:509-514
657
658 Wamberg C, Christensen S, Jakobsen I, Müller A, Sørensen SJ (2003) The mycorrhizal
659 fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea
660 plants (*Pisum sativum*). Soil Biol Biochem 35:1349-1357
661
662 Wearn JA, Sutton BC, Morley NJ, Gange AC (2012) Species and organ specificity of
663 fungal endophytes in herbaceous grassland plants. J Ecol 100:1085-1092
664
665 Xavier LJ, Germida JJ (2003) Bacteria associated with *Glomus clarum* spores influence
666 mycorrhizal activity. Soil Biol Biochem 35:471-478
667
668 Zhang L, Xu Z (2008) Assessing bacterial diversity in soil. Journal of Soils and
669 Sediments 8:379-388

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.

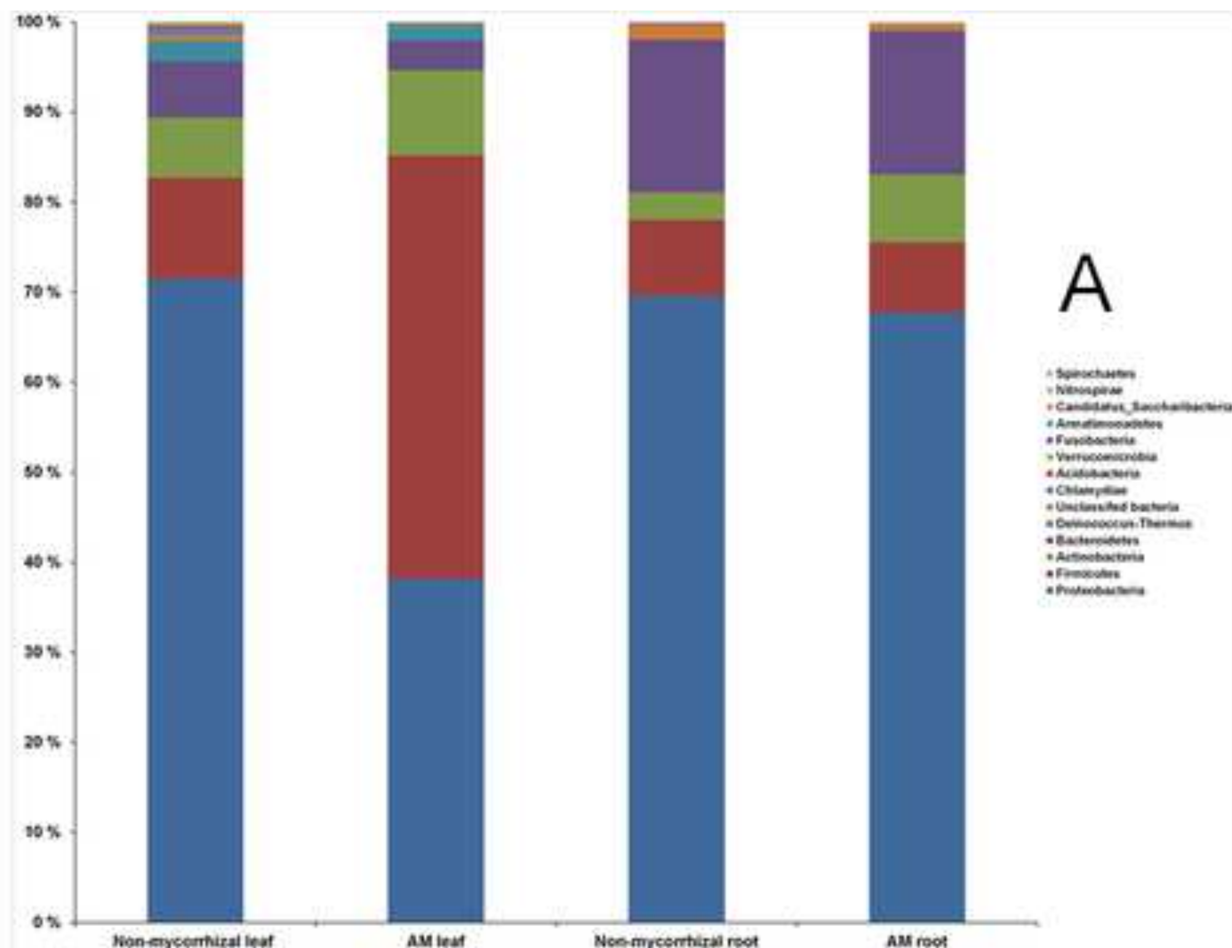
684

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.
696
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

Click here to download Figure Fig1.tif

Relative abundance (%)



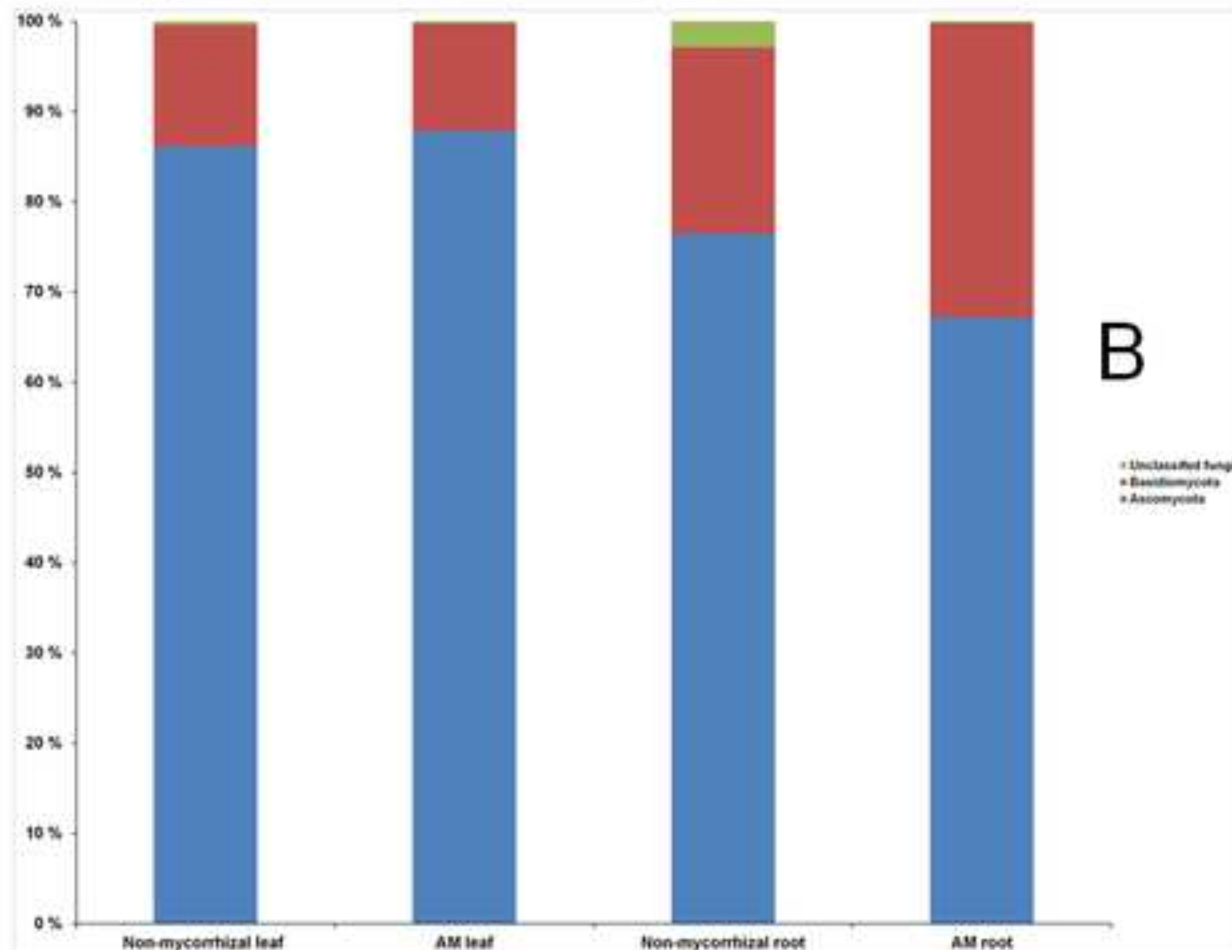
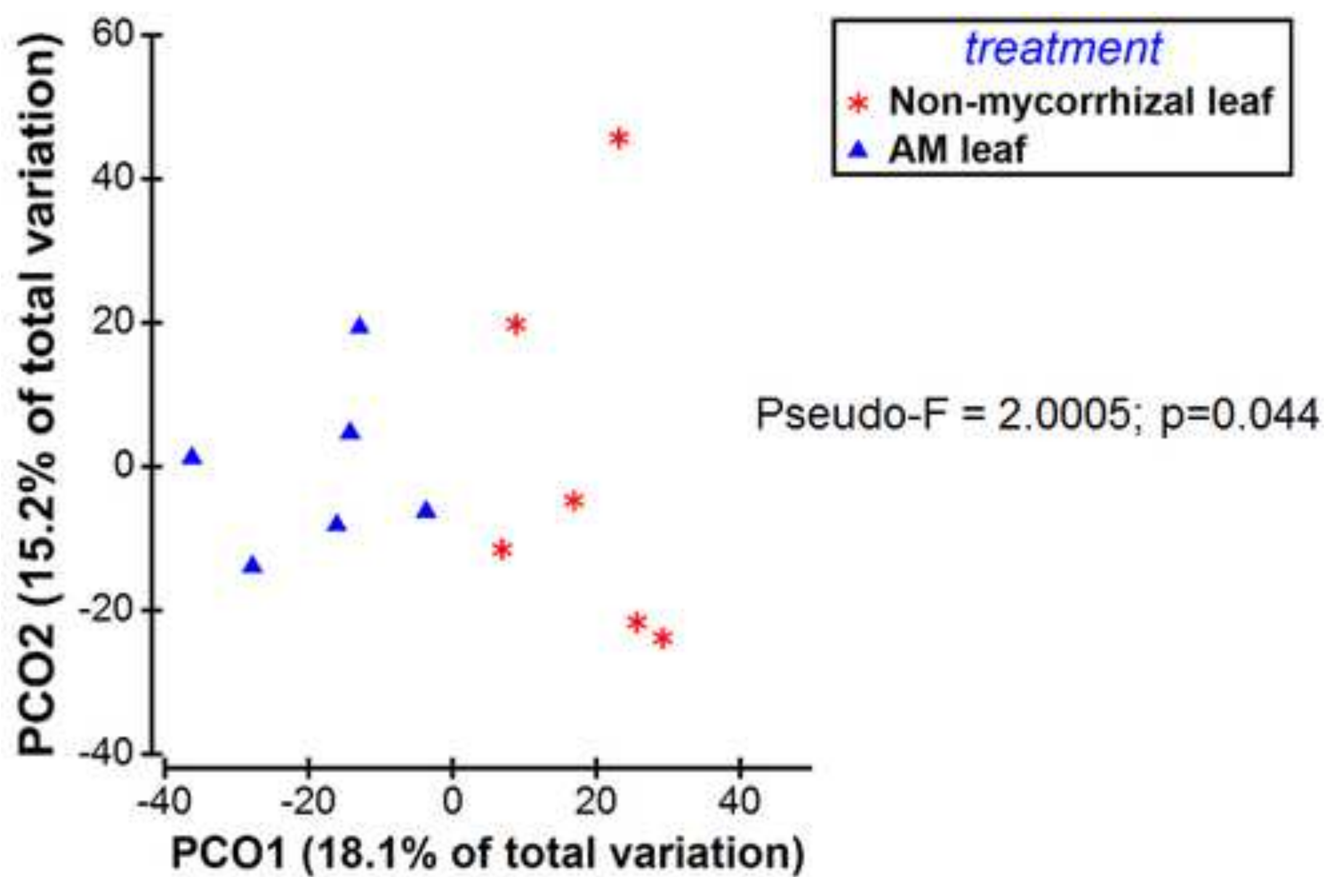


Figure2

Click here to download Figure Fig2.tif



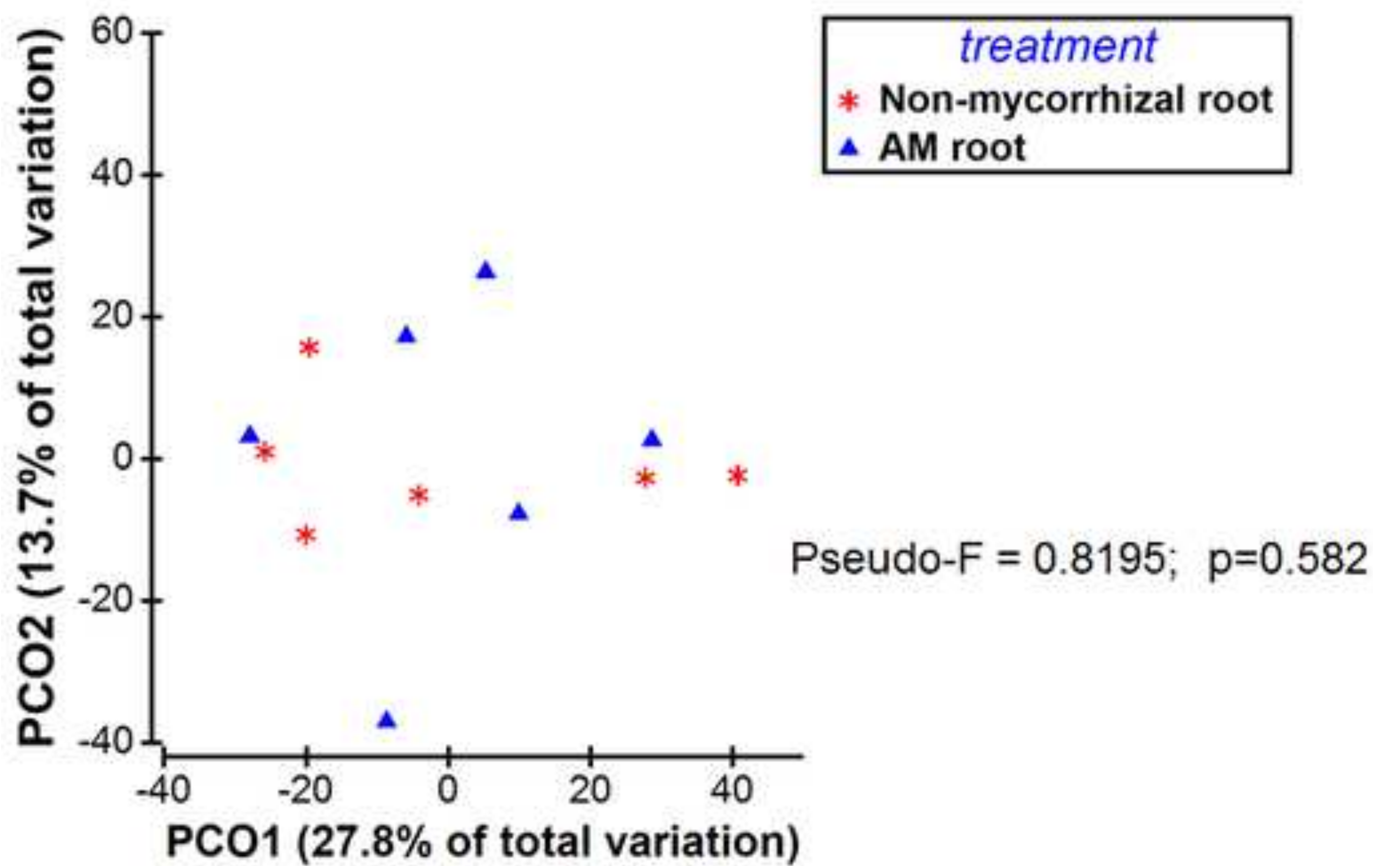


Figure3

Click here to download Figure Fig3.tif

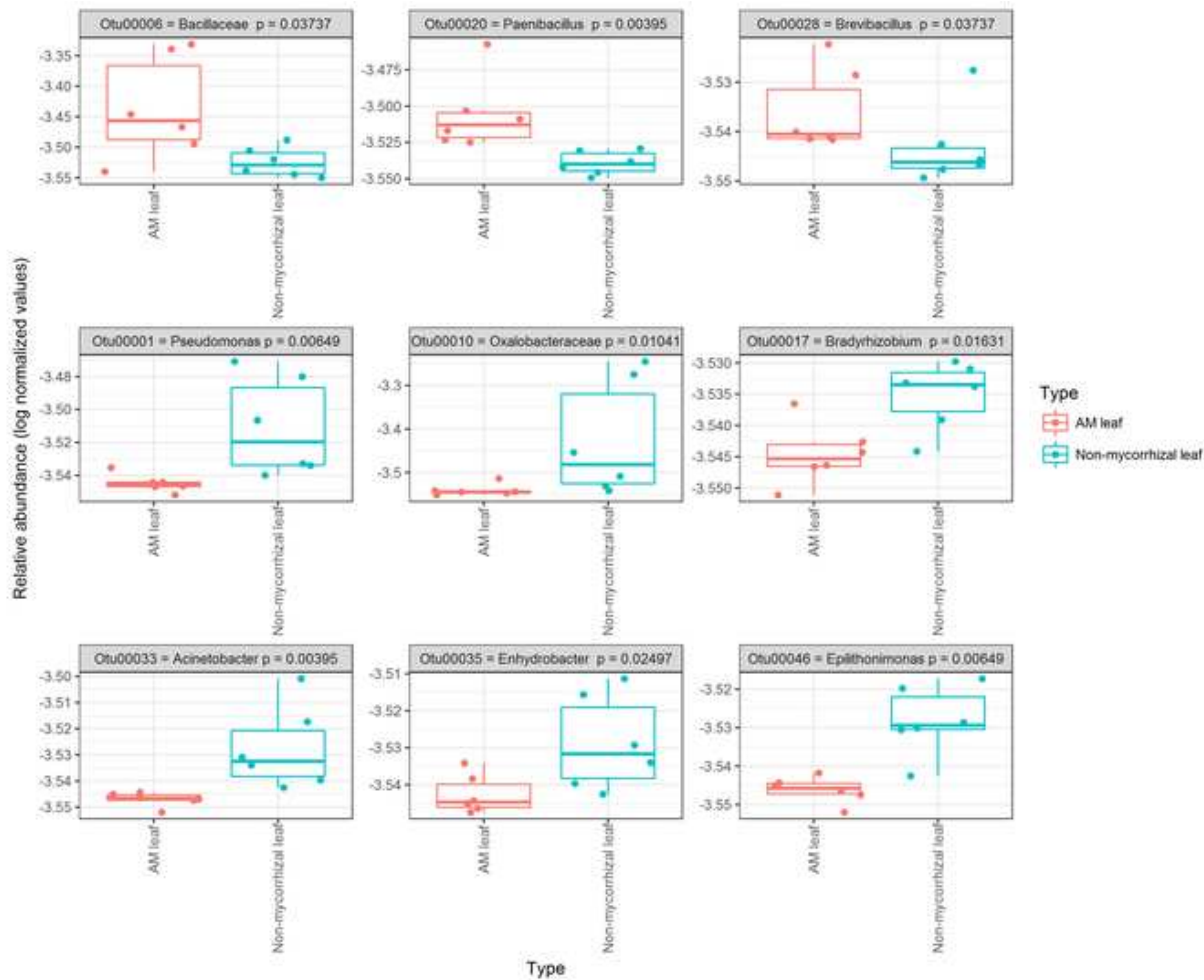
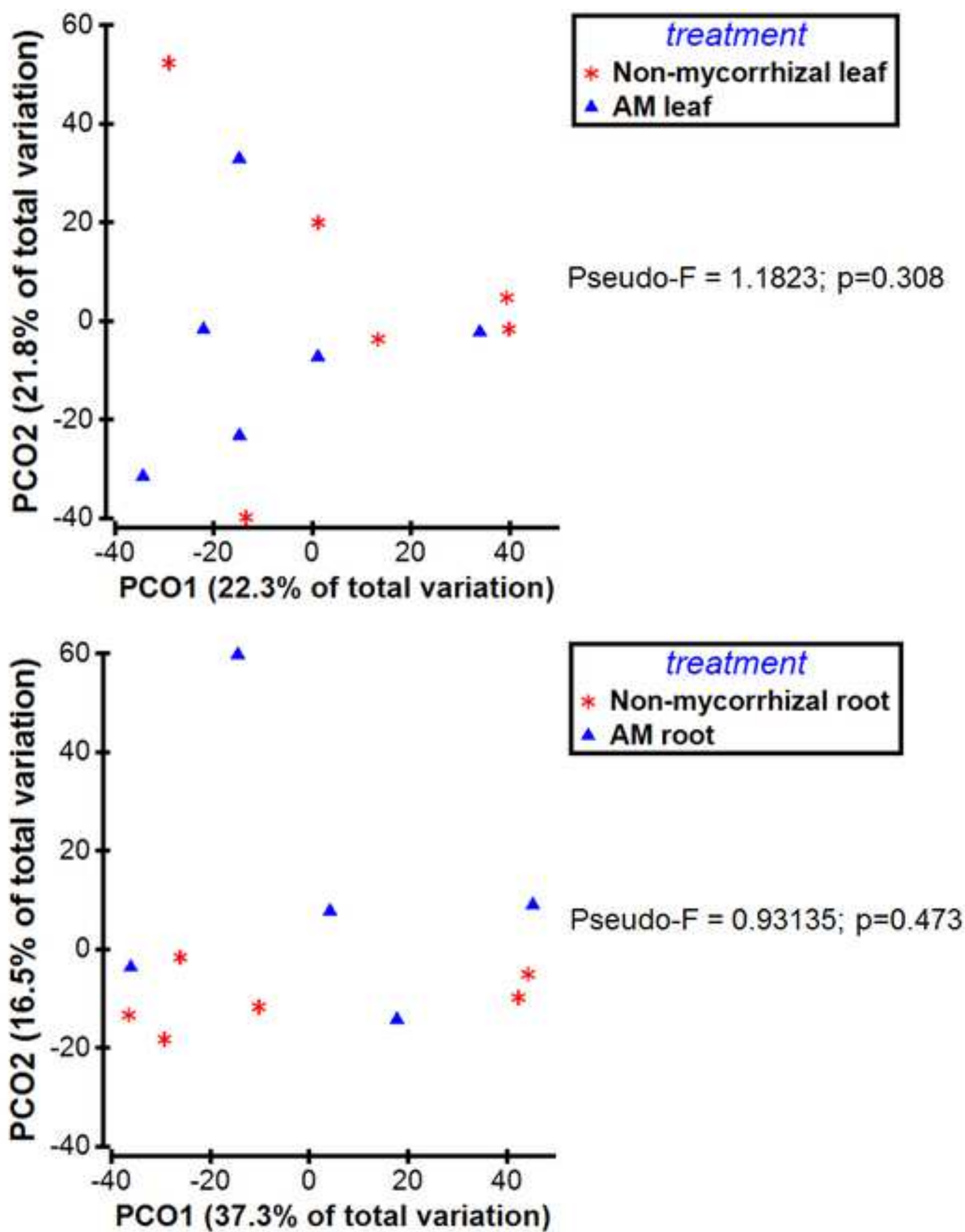


Figure4

Click here to download Figure Fig4.tif



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

Table2

(A) Leaf bacteria				
Source of variation	**SS(trace)**	**Pseudo-F**	**P**	**Proportion**
Total weight	**4419.7**	**1.8177**	**0.002**	**0.15381**
Shoot weight	**4179**	**1.7018**	**0.006**	**0.14543**
Root weight	**4654**	**1.9327**	**0.001**	**0.16197**
Foliar nitrogen	**3821.5**	**1.5339**	**0.021**	**0.13299**
(B) Root bacteria				
Source of variation	**SS(trace)**	**Pseudo-F**	**P**	**Proportion**
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
(C) Leaf Fungi				
Source of variation	**SS(trace)**	**Pseudo-F**	**P**	**Proportion**
Total weight	**5464**	**1.9097**	**0.018**	**0.16035**
Shoot weight	**5465.8**	**1.9104**	**0.018**	**0.1604**
Root weight	4676	1.5905	0.092	0.13722
Foliar nitrogen	4841.9	1.6563	0.066	0.14209
(D) Root fungi				
Source of variation	**SS(trace)**	**Pseudo-F**	**P**	**Proportion**
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 ($p<0.05$).