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Arbuscular mycorrhizal fungi and associated microbial communities from dry grassland do not improve plant growth on abandoned field soil

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¹ **Author Contributions:** HP, JR, ZM conceived and designed the experiments. HP, AV performed the experiments. CL, HP, TD, ZM analysed the data. MJ analysed and wrote the molecular part about AMF communities. CL, TD, ZM wrote the manuscript; all authors provided advice and comments on the manuscript.

1 **Abstract**

2 After abandonment of agricultural fields, some grassland plant species colonize these sites with
3 a frequency equivalent to dry grasslands (generalists) while others are missing or
4 underrepresented in abandoned fields (specialists). We aimed to understand the inability of
5 specialists to spread on abandoned fields by exploring whether performance of generalists and
6 specialists depended on soil abiotic and/or biotic legacy. We performed a greenhouse
7 experiment with 12 species, six specialists and six generalists. The plants were grown in sterile
8 soil from dry grassland or abandoned field inoculated with microbial communities from one or
9 the other site. Plant growth, abundance of mycorrhizal structures and plant response to
10 inoculation were evaluated. We focused on arbuscular mycorrhizal fungi (AMF), one of the
11 most important parts of soil communities affecting plant performance. The abandoned field soil
12 negatively affected plant growth but positively affected plant response to inoculation. The AMF
13 community from both sites differed in infectivity and taxa frequencies. The lower AMF taxa
14 frequency in the dry grassland soil suggested a lack of functional complementarity. Despite the
15 fact that dry grassland AMF produced more arbuscules, the dry grassland inoculum did not
16 improve phosphorus nutrition of specialists contrary to the abandoned field inoculum. Inoculum
17 origin did not affect phosphorus nutrition of generalists. The lower effectiveness of the dry
18 grassland microbial community toward plant performance excludes its inoculation in the
19 abandoned field soil as a solution to allow settlement of specialists. Still, the distinct response
20 of specialists and generalists to inoculation suggested that they differ in AMF responsiveness.

21

22 **Keywords**

23 Soil legacy, Fungal structures, Mycorrhizal response, Native AMF, Soil biota

24

25

26 **Introduction**

27 After abandonment of formerly intensively managed fields, plant species from neighbouring
28 dry grasslands establish at these sites. However, the abandoned fields are often characterized
29 by less diverse plant communities than the grasslands, and these differences can persist for more
30 than a century (Knappová et al. 2012; Hemrová and Münzbergová 2015). Indeed, some
31 grassland species cannot settle or persist on abandoned fields and are consequently absent or
32 underrepresented in these fields (further called specialists) while other species occur with
33 similar frequency in both habitats (further called generalists) (Münzbergová 2004; Knappová
34 et al. 2012). The inability of specialists to settle on abandoned fields could be due to their low
35 competitive ability determined by their functional traits, i.e. later flowering, lower seed bank
36 longevity (Tremlová and Münzbergová 2007; Knappová and Münzbergová 2015) and limited
37 dispersal ability compared to generalists (Münzbergová and Herben 2005).

38 However, the lower plant diversity on abandoned fields could also be related to soil
39 legacy resulting from previous agricultural practices. The altered abiotic and biotic soil
40 properties may lead to legacy effects in favour of generalists (Fry et al. 2017). For example,
41 abandoned fields generally contain more nutrients (McLauchlan 2006; Duchicela et al. 2013)
42 and the specialists with their low nutrient requirements (Hemrová and Münzbergová 2015) are
43 then not able to colonize abandoned fields as they cannot withstand the competition of the
44 generalists at these sites. Similarly, the changes at the level of soil communities, with arbuscular
45 mycorrhizal fungi (AMF) likely being one of the most important components interacting with
46 the plant community, may affect performance of the specialists. Indeed, AMF colonize about
47 80% of all terrestrial plant species (Wang and Qiu 2006) having strong effect on plant
48 productivity (Vogelsang et al. 2006) facilitating plant P uptake (Smith and Smith 2011) and
49 supporting plant establishment (Knappová et al. 2016) and their subsequent growth and
50 development (van der Heijden and Horton 2009; Derelle et al. 2015).

51 The AMF-plant associations are characterized by low specificity (Lekberg and Waller
52 2016). Despite this, some preferences were noticed in these interactions (Kivlin et al. 2011).
53 Previous studies suggested that host plants may preferentially allocate photosynthates towards
54 AMF species more beneficial for their growth (Fitter 2005; Bever et al. 2009; Werner and Kiers
55 2015). Moreover, different AMF taxa have diverse life-history strategies concerning root
56 colonization, extraradical mycelium (ERM) length, intraradical structures and sporulation
57 resulting in different effects on plant performance (van der Heijden and Scheublin 2007;
58 Sýkorová et al. 2007; Chagnon et al. 2013). Although these functional traits are
59 phylogenetically conserved (Koch et al. 2017), soil characteristics (moisture, temperature, pH,
60 etc., Kivlin et al. 2011; Jansa et al. 2014) and nutrient availability (Johnson et al. 2003) can
61 modulate the production of the different mycorrhizal structures and so affect the AMF (van der
62 Heyde et al. 2017). In line with this, Hart and Reader (2002) demonstrated that the same plant
63 species in low and high nutrient soils were associated with AMF taxa producing more ERM,
64 i.e. being more efficient in providing nutrients, and with AMF taxa involved in protection
65 against pathogens, respectively.

66 Due to the tight AMF-soil-plant community associations, soil modifications due to
67 chemical inputs of fertilizers (Gryndler et al. 2006), herbicides (Alguacil et al. 2014) and
68 fungicides (Helgason et al. 1998) caused by agricultural practices may change AMF community
69 composition, diversity and functioning (Tsiafouli et al. 2015; Voříšková et al. 2016). Similarly,
70 changes in soil physical properties may directly or indirectly through the modification of soil
71 structure disrupt the hyphal network of AMF (Duchicela et al. 2013). Moreover, the presence
72 of plant monocultures further reduces AMF diversity (Oehl et al. 2003) and the effectiveness
73 of the symbiosis on plant growth and reproduction (e.g., Derelle et al. 2015). Generalists are
74 known to interact with ubiquitous and abundant AMF taxa while specialists interact with more
75 specific and rare AMF taxa (Öpik et al. 2009; Davison et al. 2011). Thus, a decrease in AMF

76 diversity and/or abundance involves mainly AMF taxa interacting with specialists (Fry et al.
77 2017).

78 Although the AMF play an important role in the plant performance, other microbial
79 functional groups may be involved in positive and negative plant responses to soil
80 modifications. Interactions between AMF and soil microbial communities, specifically
81 bacteria, may contribute to enhance plant performance and resistance to pathogens (Johansson
82 et al. 2004; Bidondo et al. 2016). In addition, these bacteria may increase AMF root
83 colonization and abundance of fungal propagules (Pivato et al. 2009) and decrease the soil-
84 mediated stresses on fungal hyphae (Revillini et al. 2016). Consequently, the AMF could be
85 indirectly affected by changes of soil microbial community composition caused by agricultural
86 practices. In addition, the soil pathogens are known to be an important soil component
87 regulating the abundance of rare species and thus the plant community diversity (Klironomos
88 2002; Mordecai 2011). Plant growth response to soil microbial community is thus a result of
89 balance between the negative effects of pathogens and the positive effects of AMF (Klironomos
90 2002; Mangan et al. 2010; van der Putten et al. 2013).

91 The AMF community composition of the typical dry grassland and abandoned field
92 chosen for this study was previously studied by Voříšková et al. (2016). They demonstrated
93 that the field soil abandoned several years ago had the same pool of AMF taxa as the dry
94 grassland soil but these AMF taxa were present in higher frequency than in the dry grassland
95 soil. Moreover, the abandoned field AMF produced lower number of propagules, but they
96 colonized plant roots more rapidly (Knappová et al. 2016; Voříšková et al. 2016). Thus, they
97 may be more competitive than grassland AMF to colonize host plant (Jansa et al. 2008) and
98 could be more aggressive (*sensu* Graham and Abbott 2000), i.e. negatively affect plant growth,
99 toward slow-growing specialists but more efficient for fast-growing generalists (Fry et al.
100 2017). We can thus ask if adding the dry grassland AMF community with associated soil biota

101 into the abandoned field soil could be used in species diversity restoration at former abandoned
102 fields (Knappová et al. 2012; Redhead et al. 2014).

103 Our objective was to find out if changes in the soil abiotic conditions or in the AMF
104 community composition resulting from agriculture in the past were responsible for the
105 incapability of specialists to settle at abandoned fields. We hypothesised that (i) the abandoned
106 field soil enhances plant growth, due to previous fertilization treatments, but also decreases
107 AMF root colonization and thus weakens the plant/AMF mutualism, as compared to the dry
108 grassland soil. Furthermore, we hypothesised that (ii) inoculation of the abandoned field soil
109 with the dry grassland microbial communities may improve the performance of specialists in
110 the abandoned field soil. Indeed, such a positive effect may be expected because specialists
111 should be preadapted to the dry grassland microbial communities (Klironomos 2003)
112 containing specific AMF taxa involved in the promotion of specialist's establishment.

113 To test these hypotheses, a greenhouse experiment was carried out by growing
114 specialists or generalists in sterile soil from the dry grassland or the abandoned field inoculated
115 with the native microbial community of the soil or that of the other soil. Plant growth and plant
116 response to inoculation were determined alongside with mycorrhizal parameters such as
117 infectivity, ERM length and community composition.

118

119 **Materials and methods**

120 *Study area and plant species*

121 Two distinct sites, an abandoned field and a directly neighbouring semi-natural dry grassland
122 located in northern Bohemia, Czech Republic (50°30'2.627''N, 14°18'57.45''E), were selected
123 for this study. These sites represent typical dry grassland and abandoned field localities in the
124 region, that has been previously extensively studied (e.g., Münzbergová 2004; Voříšková et al.
125 2016). The calcareous grassland has been continuous grassland for at least 50 years and hosts

126 a range of endangered plant species (Münzbergová 2004). The abandoned field soil was
127 intensively used for agriculture in the past and was abandoned 25 years ago. It is gradually
128 colonized by grassland plant species but remains with lower species diversity than the
129 neighbouring grassland (Knappová et al. 2012). The abandoned field has higher pH, P and K
130 contents but lower total N and C, Mg, organic C and carbonate contents than the dry grassland
131 soil (Voříšková et al. 2016; Online Resource 1).

132 Twelve plant species were selected according to their ability to grow on dry grasslands
133 or abandoned fields based on data collected by Knappová et al. (2012) from a large set of 339
134 dry grasslands and 49 abandoned fields in the region. The species were chosen to form pairs of
135 closely related species belonging to the same plant family to eliminate differences resulting
136 from different phylogenetic histories of species (Table 1). Each pair was constituted by one
137 specialist, i.e. missing or underrepresented in abandoned fields, and one generalist, i.e.
138 occurring with an equivalent frequency in both dry grasslands and abandoned fields.

139

140 *Pot experiment*

141 The soils were collected in June 2010, from one transect, perpendicular to the field edge, per
142 site, dry grassland and abandoned field, and consisted of 10 sampling points 5 m apart from
143 each other. For each sampling point, the soil was taken from the upper 20 cm after removing
144 the vegetation. After manual homogenization of soil from each site, the soils were sieved
145 through a 4-mm sieve. One part of each soil was sterilized with γ -irradiation (25kGy) to
146 eliminate soil biota and the other part was non-sterilized. We demonstrated in a previous study
147 that sterilisation of soil from the same region by γ -irradiation did not affect the soil nutrient
148 content (Pánková et al. 2008). Moreover, as the soil from each site was sterilized in the same
149 way, we consider that the eventual bias due to γ -irradiation was the same for all the soils.

150 Then, 720 plastic pots of 500 mL were filled with sterile soil from the dry grassland or
151 the abandoned field. One third of the pots with each soil was inoculated by non-sterile soil from
152 the dry grassland and one third by non-sterile soil from the abandoned field. The inocula were
153 prepared from non-sterile soil from which roots were removed and returned back to the soil
154 after being cut into small fragments. Each inoculated pot received 8 g of such an inoculum that
155 contained the native AMF and the microbial communities of each soil. The remaining pots
156 served as non-inoculated controls and received the same amount of sterilized inoculum
157 corresponding to the soil in the pots. To maximize the fact that the main microbial parameter
158 that varied among the inoculation treatments was the AMF community, 5 mL of microbial
159 filtrate from the other soil than the origin of the inoculum were added. Thus, filtrate from dry
160 grassland was added to pots receiving inoculum from abandoned field and vice versa. Control
161 pots obtained microbial filtrates from both soils. The filtrates were prepared by shaking 30 g of
162 non-sterile soil with 300 mL of deionized water for 30 min and filtering twice through filter
163 paper with a 15 μm pore size to eliminate mostly mycorrhizal propagules but keeping most
164 members of bacterial communities (e.g., Doubková et al. 2013).

165 Seeds were collected in 2010 in the field and surface sterilized by 10% dilution of
166 sodium hypochlorite (SAVO Original) for 10 min. The seeds of specialists and generalists were
167 germinated in sterilized sand. Subsequently, for each of the 12 species, one 3-week old seedling
168 was transplanted into one pot. By transplanting seedlings, we delayed establishment of the AMF
169 symbiosis as AMF colonization is possible as soon as plant roots appear. However, as some
170 species were small and fragile, we decided to bring 3-week old seedlings to maximize the
171 survival of all species.

172 Each plant species was grown in six treatments resulting from the combination of two
173 soil types and three inoculation treatments: inocula of two origins and one non-inoculated
174 control. Ten replicate pots were established per treatment, each with one seedling. Thus, the

175 experiment comprised 60 pots per each of the 12 plant species (i.e. 720 pots in total). The
176 experiment was carried out in a greenhouse from June 2011 to May 2012 with no modifications
177 of the environmental conditions except during winter when the greenhouse was heated so that
178 the temperature did not drop below 5°C.

179

180 *Measuring plant growth*

181 Every month until flowering, plant growth parameters were measured except during winter
182 dormancy of some plants (from November to March). The determined parameters were the
183 number of leaves, ramets or branches and the length of the longest leaf or plant. The parameters
184 differed depending on plant species, but they all reflected plant growth. For each species, the
185 parameters measured were combined (number \times length) to obtain a composite parameter of
186 biomass reflecting the growth in term of biomass development in time (Online Resource 2).

187 The plants were harvested at the time of flowering. Species not flowering and thus not
188 harvested in the first year were kept during the winter season in a partly heated greenhouse (see
189 above) to facilitate flowering in the next vegetation season (Online Resource 2). At harvest,
190 shoots were cut and roots were washed and weighed. Then about one third of the root system
191 from its central part was sampled, and weighed, for the evaluation of mycorrhizal parameters.
192 One third provided large enough sample to properly evaluate the mycorrhizal parameters for
193 each species (personal observation). The rest of the roots and shoots were dried at 70°C for 72
194 h to determine aboveground and belowground dry biomass. The mass of the root sample used
195 to determine the mycorrhizal parameters was added to the root dry biomass thanks to the
196 dry/wet mass ratio based on the rest of the sample. The P concentration of aboveground biomass
197 was measured in all the species according to the method of Olsen and Sommers (1982). The P
198 content in aboveground biomass was then calculated by multiplying the P concentration by the
199 aboveground dry biomass.

200 To compare all plant species, the mycorrhizal response (MR) of the composite biomass
201 parameter (composite biomass MR), total biomass (total biomass MR) and aboveground P
202 content (aboveground phosphorus MR) were calculated as in Hetrick et al. (1993): $MR = (M -$
203 $NM_{\text{mean}}) / NM_{\text{mean}}$, where M is the given parameter recorded in one inoculated plant and NM_{mean}
204 is the mean value of this parameter in the control non-inoculated treatment.

205

206 *Mycorrhizal parameters*

207 The root samples (see above) were cut into segments of approximately 2 cm and stained with
208 0.05% Trypan blue in lactoglycerol (Koske and Gemma 1989). AMF root colonization,
209 representing the strength of the plant-AMF association through the intensity of root
210 colonization, was quantified by the method of Trouvelot et al. (1986) in 30 root segments under
211 a compound microscope at 200× magnification. In addition to scoring the frequency of root
212 colonization, this method allows calculation of the abundance of different morphological AMF
213 structures. The abundance of arbuscules (a%) and vesicles (v%) in the mycorrhizal parts of root
214 segments were calculated using the computer program Mycocalc
215 (<http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html>) as described by Trouvelot
216 et al. (1986). Arbuscules are the main sites of nutrient transfer from AMF to plant and indicate
217 the efficacy of the symbiosis (Smith and Smith 2011), while vesicles serve as fungal storage
218 structure and allow to evaluate their reproductive potential (Feddermann et al. 2010). These two
219 parameters bring complementary information because they are independent of the frequency of
220 AMF root colonization. They were determined in four species pairs (A1, A2, L and P) (Table
221 1) due to technical limitation.

222 Finally, growth of extraradical mycelium (ERM), AMF structures responsible for
223 nutrient uptake in soil (Smith and Smith 2011), was assessed using a membrane filtration
224 technique (Jakobsen et al. 1992). At harvest, a soil core (15 mL) was taken from the central part

225 of each pot and homogenized by hand. Then, 1 g of soil was blended in a household blender
226 with 500 mL of deionized water for 30 s. Two mL of the supernatant were pipetted onto a nitro-
227 cellulose membrane filter (24 mm diameter, 0.40 μ m pore size) and vacuum-filtered. The total
228 ERM length on the filter was measured under a compound microscope at 100 \times magnification
229 and calculated to m of hyphae per g of dry soil. This parameter was evaluated for the same four
230 species pairs as a% and v% (see above).

231

232 *Analysis of AMF diversity*

233 AMF diversity was analysed in two species pairs, A1 and L (Table 1), for which the extended
234 root system allowed the DNA extraction. At harvest, subsamples of 100 mg root fresh weight
235 were taken from the same part of the root system as the samples for the evaluation of root
236 colonization. Five randomly selected pots were sampled per each treatment, 20 samples per
237 plant species in total (2 soils \times 2 inocula \times 5 samples). The root samples were flash-frozen in
238 liquid nitrogen and stored at -80 $^{\circ}$ C until use. DNA was extracted from the root samples using
239 DNeasy Plant Mini kit (QIAGEN) according to the manufacturer's protocol. Subsequently,
240 AMF diversity was analysed using terminal restriction fragment length polymorphism (T-
241 RFLP) as described in detail by Voříšková et al. (2016) (see Online Resource 3 for details). The
242 peaks obtained from a capillary sequencer (3130xl Genetic Analyzer, Applied Biosystems)
243 were matched against peaks derived from a library of AMF sequences from the studied sites.
244 The TRAMPR package (Fitzjohn and Dickie 2007) in R (version 3.0., R Development Core
245 Team, 2013) was used for the identification of AMF operational taxonomic units (OTUs) that
246 had been delimited by phylogenetic analysis of the sequence library. A presence/absence matrix
247 of OTUs in the samples was created.

248

249 *Statistical analysis*

250 Generalized linear models (GLM) with plant type (specialist or generalist), soil (from dry
251 grassland or abandoned field), inoculum (from dry grassland or abandoned field), species pair
252 (Table 1) and their interactions as independent variables were used at each month to test
253 composite biomass MR. Composite biomass MR were log-transformed to improve normality
254 of the residuals. At harvest, the plant parameters tested were total biomass, total biomass MR
255 and aboveground phosphorus MR used as dependent variables in GLM with the same
256 independent variables as described above. To improve normality of the residuals, total biomass
257 was square root transformed and total biomass MR and aboveground phosphorus MR were log-
258 transformed. The mycorrhizal parameters tested were AMF root colonization, ERM length, a%
259 and v% used as dependent variables. GLM were performed with the same independent variables
260 as for plant parameters. The data expressed in percentage as AMF root colonization, a% and
261 v% were arcsine-transformed. ERM length was corrected by subtracting the mean ERM of non-
262 mycorrhizal controls of each soil from the value obtained in each replicate from the same soil
263 (as in Olsson et al. 1997). This correction ensured that the estimated ERM length included only
264 AMF hyphae formed during the cultivation period and excluded dead fungal mycelium present
265 in the soils as background. ERM length was log-transformed. Finally, the links between
266 mycorrhizal (ERM length, AMF root colonization, a% and v%) and plant growth (total biomass,
267 total biomass MR and aboveground phosphorus MR) parameters were tested using GLM by
268 including plant type, species pair, soil, inoculum (without non-inoculated control) and their
269 interactions as covariates. Normality and equal variances of the dependent variables were tested
270 by Shapiro-Wilk and Bartlett tests, respectively. The statistical analyses were performed using
271 the software JMP 7 with an alpha level of 0.05.

272 The AMF community composition was analysed using multivariate analyses in
273 CANOCO (version 5.0., ter Braak and Šmilauer 2012). The input data were an OTU
274 presence/absence matrix. Canonical Correspondence Analysis (CCA) was used to evaluate the

275 effect of each factor (species pair, plant type, soil, inoculum) and their interactions on AMF
276 community composition. The significance of the effects was tested using a Monte Carlo test
277 with 499 permutations. Based on the significant effects (inoculum and plant type \times species pair
278 interaction), the factors plant species (representing the plant type \times species pair interaction) and
279 inoculum were combined in a final model with soil as covariate.

280

281 **Results**

282 *Plant growth parameters*

283 Concerning the effect of soil origin, composite biomass MR was higher in the abandoned field
284 soil than in the dry grassland soil over the plant growth (Online Resource 4a). Total biomass
285 was higher in the dry grassland soil than in the abandoned field soil (Table 2, Fig. 1a) while it
286 was the opposite for total biomass MR and aboveground phosphorus MR (Table 2, Fig. 1b, c).

287 Concerning the effect of inoculum origin, composite biomass MR, total biomass and
288 aboveground phosphorus MR were higher with the abandoned field inoculum than with the dry
289 grassland inoculum (Table 2, Fig. 1a, c, Online Resource 4b). Composite biomass MR was
290 mainly affected at the beginning of the growth (Online Resource 4b). Aboveground phosphorus
291 MR was significantly affected by the plant type \times inoculum interaction (Table 2). There was no
292 effect of inoculum origin in generalists contrary to specialists, which tended to have a higher
293 response with the abandoned field inoculum (Fig. 1c).

294 The plant type distinctly affected composite biomass MR over time (Online Resource
295 4c) because the composite parameters of biomass were differently affected by the plant type
296 according to the species pair considered (Online Resource 2). Total biomass was higher for
297 generalists than specialists and it was the opposite for total biomass MR (Table 2, Fig. 1a, b).

298

299 *Mycorrhizal parameters*

300 The soil origin significantly affected ERM length and AMF root colonization (Table 2) with
301 longer ERM and lower colonization in the dry grassland soil than in the abandoned field soil
302 (Fig. 2a, b). In addition, generalists were more colonized than specialists in the abandoned field
303 soil, while there was no difference between the plant types in the dry grassland soil (Fig. 2b).

304 The inoculum origin in interaction with soil and with the plant type \times soil interaction
305 had an effect on ERM length (Table 2). In the abandoned field soil, the dry grassland inoculum
306 led to longer ERM than the abandoned field inoculum. Conversely, in the dry grassland soil,
307 the abandoned field inoculum led to longer ERM. These trends were stronger in specialists than
308 generalists (Fig. 2a). Arbuscules and vesicles were more abundant with the dry grassland
309 inoculum than the abandoned field inoculum (Table 2, Fig. 2c, d). For abundance of arbuscules,
310 the soil and inoculum origin also interacted. Thus, plants growing in the dry grassland soil had
311 more arbuscules when they were inoculated with the inoculum of the same origin than with the
312 abandoned field inoculum (Fig. 2c).

313 Specialists had longer ERM while generalists were more colonized by AMF (Table 2,
314 Fig. 2a, b). The AMF colonization ranged between 28% and 90% for specialists and 31% and
315 99% for generalists (Table 1). There were more arbuscules in specialists than generalists (Table
316 2, Fig. 2c). These differences were detected in both soils, but were stronger in the abandoned
317 field soil (Fig. 2c).

318

319 *Composition of AMF communities*

320 Altogether, 12 AMF OTUs were detected in the root samples with frequency ranging between
321 10 and 79 (out of 80 analysed samples) (see Voříšková et al. (2016) for GenBank accession
322 numbers of the original sequences and taxonomical identification of the OTUs). CCA analyses
323 showed that the AMF community composition was significantly influenced by inoculum origin
324 (adjusted explained variation 5.7%), species pair (2.8%), plant type (1.4%) and the species pair

325 × plant type interaction (2.0%) (Table 2). The root-colonizing AMF communities were more
326 similar between phylogenetically related species than between species of the same plant type
327 (Online Resource 5).

328

329 *Link between plant and mycorrhizal parameters*

330 According to GLM, we found a positive correlation between AMF root colonization and
331 aboveground phosphorus MR ($\chi^2 = 8.58$, $P = 0.003$) as well as between ERM length and total
332 biomass ($\chi^2 = 4.90$, $P = 0.027$). The abundance of arbuscules and vesicles were not significantly
333 related to any of the plant growth parameters ($P > 0.06$ in all cases).

334

335 *Effect of species pair*

336 The 12 species were grown separately in each pot and, as they were grouped by pairs belonging
337 to the same family, we also tested the effect of the family, i.e. the species pair. However, the
338 differences between species pairs were not the main purpose of our study, consequently we
339 added these data in supplementary material (Online Resources 6-12). Briefly, the species pair
340 affected all the plant growth and mycorrhizal parameters alone and in interaction with the other
341 variables.

342

343 **Discussion**

344 We aimed to understand why specialists are not able to settle or persist on abandoned fields.
345 We observed legacy effects of former agricultural practices on the abiotic conditions and biotic
346 component of the abandoned field soil. These legacy effects distinctly affected plant growth,
347 mycorrhizal parameters and plant response to inoculation and this depended also on the plant
348 type. On the other hand, the AMF community composition in plant roots depended on the

349 inoculum origin, plant species pair and ecological specificity, i.e. the plant type, specialist or
350 generalist, of host plant.

351

352 *Abiotic legacy effects of abandoned field soil*

353 In contrast to our hypothesis, the abiotic legacy effects of the abandoned field soil negatively
354 affected total biomass in our experiment. The abandoned field soil was richer in P but poorer
355 in N and C than the dry grassland soil (Voříšková et al. 2016, see Online Resource 1). The
356 negative effect of the abandoned field soil on plant growth could be therefore explained by the
357 fact that N was more limiting for plant growth than P in our system, as previously reported from
358 a range of other systems (Güsewell et al. 2003; LeBauer and Treseder 2008; Johnson et al.
359 2010). In addition, the lower organic C content in the abandoned field soil may affect the
360 microbial community structure (Spohn et al. 2016) and limit the microbial activity (Kuzyakov
361 et al. 2009) supporting plant growth. Morriën et al. (2017) found that interaction strength
362 between AMF and bacteria was strongly dependent on C availability.

363 The higher P availability in the abandoned field soil could have decreased the role of
364 AMF in the P nutrition of their host plants compared to the dry grassland soil thus reducing the
365 strength of plant-AMF interactions (Collins and Foster 2009). Surprisingly, we observed higher
366 MRs in the abandoned field soil than in the dry grassland soil. Furthermore, the aboveground
367 phosphorus MR was higher than the total biomass MR. This suggests that the positive effect of
368 inoculation in this soil could be mainly due to better P nutrition. One explanatory hypothesis
369 could be the greater need of plants for AMF in the less suitable abiotic conditions of the
370 abandoned field soil. Moreover, this effect of the abandoned field soil on the MRs was more
371 important for specialists. This is in line with the fact that the specialists are more dependent on
372 AMF (Dostálek et al. 2013; Knappová et al. 2016).

373 These positive effects of the abandoned field soil on plant growth could be partly
374 explained by the higher AMF root colonization in this soil suggesting a stronger plant/AMF
375 association. It was also consistent with the fact that AMF colonization was positively correlated
376 with aboveground phosphorus MR. In contrast, the negative soil abiotic legacy effects on plant
377 growth in abandoned field soil could be mediated by the lower ERM length in this soil. Indeed,
378 we found a positive correlation between total biomass and ERM length. The decrease in ERM
379 length can be related to the former fertilization (Gryndler et al. 2006) and tillage practices
380 (Kabir et al. 1998) that is known to affect ERM growth. However, although ERM length was
381 lower in the abandoned field soil, P uptake by the plant was higher. In line with this, Hart and
382 Reader (2002) demonstrated that ERM was less important than frequency of internal
383 mycorrhizal structures for plant P concentration. Moreover, in the abandoned field soil,
384 generalists were more colonized than specialists. This could explain their capacity to settle also
385 on abandoned fields as high level of AMF root colonization may ensure better competitive
386 ability (e.g., Lekberg et al. 2013). Finally, in the abandoned field soil, the higher abundance of
387 arbuscules and total biomass MR for specialists compared to generalists did not ensure a
388 significantly better P nutrition than generalists. This may be caused by lower use of resources
389 by specialists compared to generalist in this soil.

390

391 *Biotic legacy effects of abandoned field soil*

392 The soil inocula contained the whole microbial community of the native soil, i.e. bacteria and
393 fungi (AMF and potential pathogens). The filtrates contained bacteria of the opposite soil but
394 could also contain potential pathogens that passed the filter (Beakes et al. 2012). Nevertheless,
395 comparison with the negative control containing the filtrates of both soils, i.e. bacteria and
396 potential pathogens of both soils, allowed us to consider that the effects observed on the plant
397 growth were mainly due to the AMF community contained in the inoculum. We also excluded

398 the presence of fine AMF that could pass through the filter as our control plants did not have
399 any fungi in their roots (personal observation).

400 Contrary to our expectations, the plant growth was better with the abandoned field
401 inoculum. This can be linked to the observations of Knappová et al. (2016) and Voříšková et
402 al. (2016), who demonstrated that the abandoned field AMF colonized roots more rapidly than
403 the dry grassland AMF. Thus, the abandoned field AMF had an early positive effect on plant
404 growth, which was consistent with the effect of inoculum origin noticed at the beginning of
405 plant development. This positive effect persisted up to harvest as higher total biomass with the
406 abandoned field inoculum. It is important to notice that our late inoculation, on 3-week old
407 seedlings, could have slightly reduced the success of AMF colonization as the beginning of
408 growth may be determinant for AMF establishment (Verbruggen et al. 2013).

409 The positive effect of the abandoned field inoculum on plant growth was also reflected
410 by higher MRs of composite biomass and aboveground phosphorus content. However, total
411 biomass MR was not affected by the inoculum origin. We can suppose that the abandoned field
412 AMF, by providing more P to the plant, caused a higher demand for N. This could have led to
413 competition for N between the plant and its AMF limiting plant growth (Hodge and Storer
414 2015). The effect of AMF could also be indirect via its interaction with the rest of the microbial
415 community. Indeed, the significant effect of inoculum origin on total biomass was not reflected
416 in total biomass MR. This suggested a beneficial role of the interaction between AMF and soil
417 microbes on total biomass. It was also interesting to see that specialists displayed higher
418 aboveground phosphorus MR than generalists when inoculated with the abandoned field
419 inoculum. However, generalists benefited from inoculum whatever its origin. This could be one
420 reason of their success in colonizing both sites.

421 Concerning the mycorrhizal parameters, however, we showed that plants inoculated
422 with the dry grassland inoculum had more arbuscules and vesicles than with the abandoned

423 field inoculum. Despite such a difference, it was surprising to find that the dry grassland
424 inoculum was less efficient to supply P to the plant. These differences in the root colonization
425 structures could be related to the frequencies of fungal taxa in the two AMF communities
426 (Voříšková et al. 2016). Indeed, the lower frequency of AMF taxa in the dry grassland inoculum
427 could suggest a lack of functional complementarity explaining that some taxa are too rare to
428 compete with other AMF taxa (Koide 2000; Sýkorová et al. 2007; Jansa et al. 2008). Thus, we
429 could hypothesise that the more competitive AMF taxa within the dry grassland inoculum could
430 have spread, reflected by the high percentage of arbuscules, and outcompeted the less
431 competitive AMF taxa that the plants need (Öpik et al. 2009; Davison et al. 2011). This could
432 lead to the loss of functionality toward plants, reflected by the lower MR of aboveground P
433 content with the dry grassland inoculum. We could also hypothesise that the more numerous
434 internal fungal structures formed by the dry grassland AMF, particularly vesicles, could have
435 accumulated more P to the detriment of the plant. Indeed, the intraradical structures are known
436 to be highly costly for the plant (Feddermann et al. 2010).

437

438 *Inoculation of the microbial community from dry grassland in the abandoned field soil*

439 In our experiment, the dry grassland inoculum did not support the growth of specialists in the
440 abandoned field soil. It only slightly modified the mycorrhizal parameters especially increased
441 ERM length. This was not consistent with Johnson et al. (2010) who showed that ERM length
442 was higher when AMF were grown in their native soil. In their study, ERM length was
443 positively correlated with the plant's concentration of that mineral element that was most
444 limiting in the given soil. Given the strong soil \times inoculum interaction we observed on ERM
445 length, both factors played an important role. We may assume for our system that in the
446 abandoned field soil, which contained more P and less N, AMF adapted to this soil may provide
447 more P to the plant with lower ERM length than the dry grassland AMF.

448 The absence of positive effect of the dry grassland inoculum on the specialist
449 performance in the abandoned field soil could have several explanations. Firstly, the dry
450 grassland AMF may have another function than to ensure plant growth such as effects on their
451 competitive ability. Indeed, van der Heijden et al. (1998) demonstrated that dry grassland AMF
452 determined the plant species composition and its effect could be visible only in situation of
453 plant competition. Secondly, some AMF are difficult or impossible to cultivate in greenhouse
454 conditions (Sýkorová et al. 2007). Thus, we cannot be sure that our inoculum contained all the
455 specific and less abundant AMF fungi required by the specialists (Öpik et al. 2009). Among the
456 17 OTUs delimited from field samples by Voříšková et al. (2016), 5 were missing in our
457 greenhouse experiment. However, among these 5 OTUs, 4 were very rare in the field samples
458 which could explain why they were not identified in our pot experiment. Nevertheless, we
459 assume that this bias was not so important as we used an inoculation process commonly used
460 (Pánková et al. 2008, 2011). Finally, we assumed that the main difference between microbial
461 communities was the AMF. However, the microbial community composition in the inocula and
462 the filtrates was not studied, and it is possible that some other microbial groups contributed
463 to plant responses. In the inoculum, some pathogens may negatively affect the host plant (Bever
464 et al. 1997; Pánková et al. 2014) and mask the positive effect of the dry grassland AMF. On the
465 other hand, the pathogens from the opposite soil potentially present in the filtrate may disrupt
466 the plant growth (Gandon et al. 1996). However, such a role of pathogens is unlikely because
467 we observed mostly positive growth responses with both soil inocula. Thus, although we did
468 not analyse the pathogenic fraction, we assumed that fungal and bacterial pathogens are not
469 functionally important in the microbial communities of both soils. Furthermore, the microbial
470 communities in our inoculum probably differed from those in the field and some microbes could
471 have been removed or brought in too low abundance, during the inoculation process, to ensure
472 their functional cooperation with the AMF (Paluch et al. 2013; Emam 2016; Wubs et al. 2016).

473 In addition, the bacteria from the other soil may have detrimental effect on the AMF and so the
474 plant response to AMF (Rudgers and Orr 2009; Revillini et al. 2016).

475 The differences in plant growth and mycorrhizal parameters observed between species
476 pairs and plant type could result from distinct rhythms of development among species and AMF
477 taxa selected as confirmed by the molecular data. Moreover, some plant families in our study
478 area are known to be more AMF-dependent and therefore become more colonized (see Dostálek
479 et al. 2013). The difference of AMF species selected by specialists and generalists was
480 consistent with a slight difference in AMF infectivity: specialists were less colonized by AMF,
481 had more arbuscules and longer ERM than generalists. Such a finding was in accordance with
482 Öpik et al. (2009) who demonstrated that the plant ecological specificity had a key role in the
483 specificity of plant-AMF associations. This may be due to their tight co-evolution (Brundrett
484 2002; Wang and Qiu 2006).

485 Our greenhouse experiment demonstrated negative abiotic legacy effects in an
486 abandoned field soil on plant growth and ERM length even after 25 years without intensive
487 agricultural management. Nevertheless, the plant response to inoculation was better in the
488 abandoned field soil. Concerning biotic legacy effects, the lower frequency of AMF taxa with
489 the dry grassland inoculum suggested a lack of functional complementarity. This may lead to
490 AMF competition in the root system explaining the higher arbuscule abundance but lower
491 efficiency of the dry grassland inoculum to support plant P nutrition. Consequently, contrary to
492 what we expected, in our study, specialists performed better with the abandoned field inoculum
493 than with the dry grassland inoculum while generalists were not affected by the inoculum
494 origin. Thus, inoculation of the abandoned field soil with the microbial communities from dry
495 grassland does not seem to be the solution for supporting the settlement of specialists on
496 abandoned fields. Further research should test several plant assemblages, in competition
497 experiments between generalists and specialists, to evaluate if a positive plant response to the

498 dry grassland microbial community could be visible for specialists when they have to compete
499 with generalists.

500

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506

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508

509

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759 **Table 1** Plant species selected for the experiment with their percentage of AMF root
760 colonization in our greenhouse experiment. The plant types are grassland specialists (S) or
761 generalists (G) that are able to colonize both dry grassland and abandoned field. The root
762 colonization, given for plants inoculated with the native microbial community of the given soil,
763 is expressed as mean percentage of frequency of root colonization (\pm standard error)

Plant species	Abbr. species	Family	Abbr. species pair	Plant type	Root colonization (%)	
					Dry grassland	Abandoned field
<i>Aster amellus</i>	AstAme	Asteraceae	A1	S	86 (\pm 1.95)	85 (\pm 2.01)
<i>Inula salicina</i>	InuSal			G	82 (\pm 1.44)	83 (\pm 1.40)
<i>Centaurea scabiosa</i>	CenSca	Asteraceae	A2	S	48 (\pm 4.44)	48 (\pm 2.56)
<i>Centaurea jacea</i>	CenJac			G	71 (\pm 3.25)	69 (\pm 3.58)
<i>Prunella grandiflora</i>	PruGra	Lamiaceae	L	S	63 (\pm 5.36)	66 (\pm 3.37)
<i>Stachys recta</i>	StaRec			G	73 (\pm 5.20)	69 (\pm 4.79)
<i>Briza media</i>	BriMed	Poaceae	P	S	37 (\pm 3.45)	28 (\pm 2.94)
<i>Brachypodium pinnatum</i>	BraPin			G	31 (\pm 2.66)	32 (\pm 5.15)
<i>Sanguisorba minor</i>	SanMin	Rosaceae	R	S	85 (\pm 2.54)	90 (\pm 2.42)
<i>Agrimonia eupatoria</i>	AgrEup			G	88 (\pm 9.25)	99 (\pm 0.51)
<i>Asperula cynanchica</i>	AspCyn	Rubiaceae	Ru	S	32 (\pm 5.80)	47 (\pm 7.55)
<i>Galium verum</i>	GalVer			G	36 (\pm 8.50)	50 (\pm 7.14)

764

765 **Table 2** Generalized linear models explaining the plant growth and mycorrhizal parameters,
766 and canonical correspondence analysis (CCA) explaining arbuscular mycorrhizal fungi (AMF)
767 community composition. The plant growth parameters are the total biomass and the mycorrhizal
768 response (MR) of total biomass and aboveground phosphorus content. The mycorrhizal
769 parameters are the length of extraradical mycelium (ERM), the AMF root colonization and the
770 abundance of arbuscules (a%) and vesicles (v%). Plant type (specialist or generalist), soil (from
771 dry grassland or abandoned field), inoculum (from dry grassland or abandoned field), species
772 pair and their interactions were used as independent explanatory variables. n.s. non-significant
773 with $P > 0.05$, values in bold are significant at $P \leq 0.05$

Source of variation	df	Plant growth parameters						Mycorrhizal parameters									
		Total biomass (g)		Total biomass MR		Aboveground phosphorus MR		ERM length (m.g ⁻¹ of soil)		AMF root colonization (%)		a (%)		v (%)		Community composition (CCA)	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	pseudo F	P
Plant type	1	5.28	0.022	15.84	<0.001	0.72	n.s.	7.27	0.007	30.49	<0.001	50.19	<0.001	1.22	n.s.	2.75	0.028
Soil	1	145.86	<0.001	45.46	<0.001	146.22	<0.001	8.19	0.004	12.02	<0.001	1.63	n.s.	0.06	n.s.	0.5	n.s.
Inoculum	1	14.59	<0.001	3.43	n.s.	6.49	0.011	2.18	n.s.	0.50	n.s.	19.49	<0.001	58.26	<0.001	5.6	0.002
Soil × inoculum	1	1.48	n.s.	0.16	n.s.	0.97	n.s.	215.15	<0.001	0.89	n.s.	7.56	0.006	1.31	n.s.	1.2	n.s.
Plant type × soil	1	0.21	n.s.	0.17	n.s.	0.87	n.s.	1.22	n.s.	4.22	0.040	10.14	0.001	0.23	n.s.	1.5	n.s.
Plant type × inoculum	1	2.86	n.s.	2.65	n.s.	8.83	0.003	3.43	n.s.	2.17	n.s.	2.34	n.s.	0.00	n.s.	0.9	n.s.
Plant type × soil × inoculum	1	7.91	0.005	2.13	n.s.	1.14	n.s.	37.97	<0.001	0.94	n.s.	0.97	n.s.	0.02	n.s.	0.4	n.s.

775 **Figure Legends:**

776 **Fig. 1** The effect of inoculum and soil origin [from dry grassland (DG), abandoned field (AF)
777 or control (C)] on a) the total biomass and the mycorrhizal response (MR) of b) total biomass
778 and c) aboveground phosphorus content, as depending on the plant type (generalist or
779 specialist). Error bars represent means \pm SE ($46 \leq n \leq 57$). Columns connected by distinct
780 lowercase or uppercase letters indicate values significantly different for generalists and
781 specialists, respectively, after Tukey-Kramer HSD tests performed independently for each plant
782 type ($P \leq 0.05$)

783

784 **Fig. 2** The effect of inoculum and soil origin [dry grassland (DG) or abandoned field (AF)] on
785 mycorrhizal parameters, as depending on the plant type. Plant types are generalists (light bar)
786 or specialists (dark bar). Error bars represent means \pm SE ($38 \leq n \leq 57$). Asterisks indicate that
787 the two columns are significantly different after non-parametric Mann-Whitney tests ($P \leq 0.05$).
788 Columns connected by distinct lowercase or uppercase letters indicate values significantly
789 different for generalists and specialists, respectively, after Tukey-Kramer HSD tests performed
790 independently for each plant type ($P \leq 0.05$)

Fig. 1

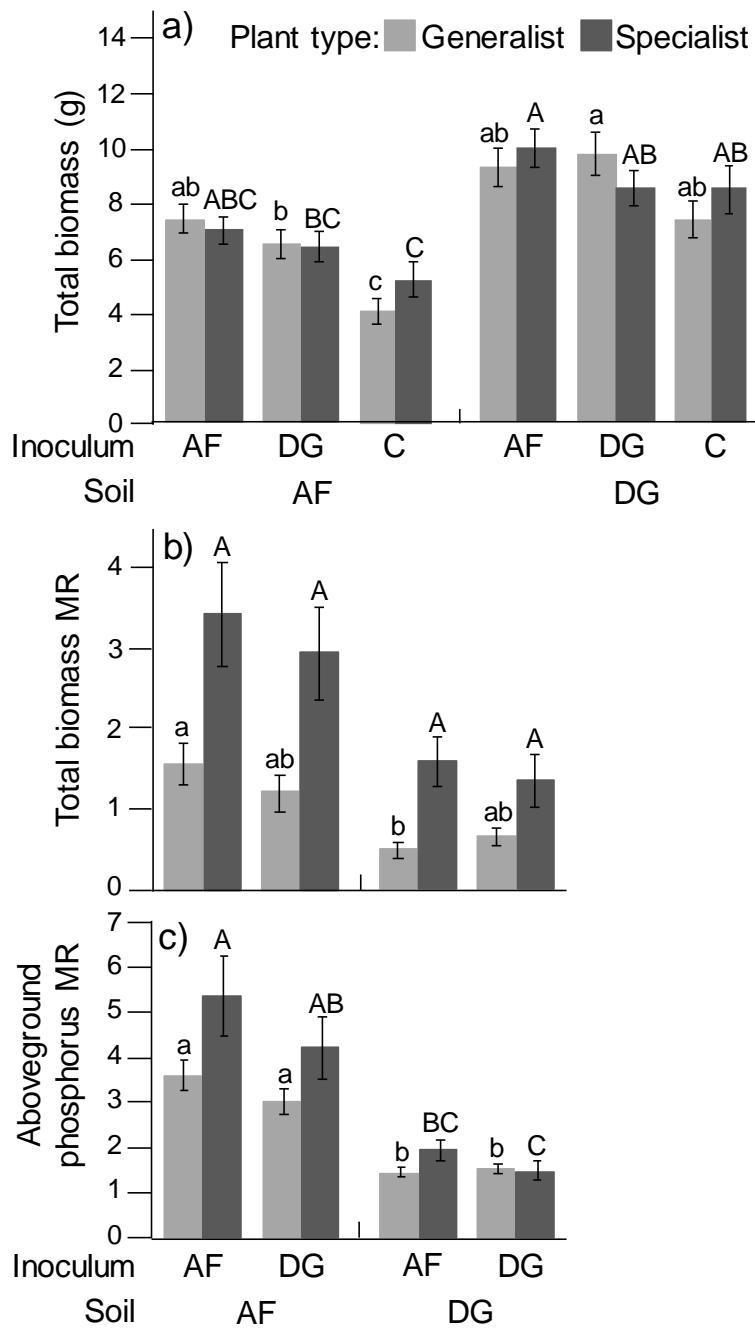


Fig. 2

