

1 **Diversity loss selects for altered plant phenotypic responses to local arbuscular**  
2 **mycorrhizal communities**

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4 Terhi Hahl<sup>1</sup>§, Sofia J. van Moorsel<sup>1</sup>§, Marc W. Schmid<sup>1,2</sup>, Debra Zuppinger-Dingley<sup>1</sup>,  
5 Bernhard Schmid<sup>1</sup> and Cameron Wagg<sup>1\*</sup>

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7 <sup>1</sup>URPP Global Change and Biodiversity and Department of Evolutionary Biology and  
8 Environmental Studies, University of Zürich, Winterthurerstrasse 190, CH-8057, Switzerland

9 <sup>2</sup>MWSchmid GmbH, Möhrlistrasse 25, CH-8006 Zürich

10

11 §Equal contribution

12 \*Author for correspondence:

13 Cameron Wagg, [cameron.wagg@ieu.uzh.ch](mailto:cameron.wagg@ieu.uzh.ch)

14

## 15 **Summary**

16 **1.** Biodiversity loss not only impairs ecosystem functioning but can also alter the selection for  
17 traits in plant communities. At high diversity selection favours traits that allow for greater  
18 niche partitioning, whereas at low diversity selection may favour greater defence against  
19 pathogens. However, it is unknown whether changes in plant diversity also select for altered  
20 interactions with soil organisms.

21 **2.** We assessed whether the responses in plant growth and functional traits to their local  
22 arbuscular mycorrhizal fungal (AMF) communities have been altered by the diversity of the  
23 plant communities from which both plants and AMF communities were obtained. We grew  
24 plants with AMF communities that originated from either plant monocultures or mixtures in a  
25 fully factorial design that included both negative and positive controls, by inoculating no  
26 AMF or a foreign AMF respectively.

27 **3.** We found that AMF from plant mixtures were more beneficial than monoculture AMF for  
28 two out of five plant species. Plants from mixtures generally grew better than those from  
29 monocultures, but suffered greater damage by leaf pathogens. Although plant growth and  
30 phenotypic responses were dependent on the AMF communities with which they associated,  
31 we found little evidence for plant growth responses specific to their local AMF communities  
32 and results differed between species and traits.

33 **4.** Our results show that plants from mixtures were selected for increased growth at the  
34 expense of reduced defence and vice versa for plants from monocultures, providing evidence  
35 for plant diversity-dependent selection on competitive growth vs. defence. Furthermore, our  
36 study suggests that effects of a common history between plants and AMF do not follow a  
37 general pattern leading to increased or decreased mutualism.

38 **5. *Synthesis:*** Here we provide evidence that biodiversity loss can alter evolutionary  
39 trajectories of plant phenotypes and responses to their local AMF communities. However, the

40 selection for altered plant–AMF interactions differ between plant species. To understand how  
41 plant communities respond and evolve under a changing environment requires further  
42 knowledge about life strategies of plant species and their above–belowground interactions.

43

44 *Key-words:* biodiversity experiment, functional traits, growth–defence trade-off, mutualism–  
45 parasitism continuum, plant–AMF interactions, plant–soil feedbacks

## 46 **1 | Introduction**

47           How reciprocal interactions between plants and soil biota drive plant community  
48 structure and ecosystem functioning has been a focal point in ecology (Bever, 1994;  
49 Klironomos, 2002; van der Heijden et al., 2006; van der Putten et al., 2013). These plant–soil  
50 feedbacks (PSF) describe the relationships between plants and their associated soil pathogens  
51 and mutualists, which negatively or positively affect plant performance (Bever et al., 2010).  
52 Negative PSF are common and thought to be key to maintaining diversity in plant  
53 communities and to successional changes in plant community composition through time  
54 (Kardol, Martijn Bezemer, & van der Putten, 2006; Klironomos, 2002; Andrew Kulmatiski,  
55 Beard, Stevens, & Cobbold, 2008; Petermann, Fergus, Turnbull, & Schmid, 2008; van der  
56 Putten et al., 2013). Positive PSFs are known to improve plant performance by either  
57 enhancing pathogen defence (reducing negative PSF) or stimulating plant growth (van der  
58 Putten et al., 2013). Plants simultaneously interact with both pathogens and beneficial soil  
59 organisms that together determine plant performance. However, negative PSF and positive  
60 PSF have rarely been disentangled and only recently studies considered that both negative  
61 and positive PSF might change over ecological time-scales (van der Heijden, Bardgett, & van  
62 Straalen, 2008; terHorst et al. 2014). Here we assess the consequences of plant diversity loss  
63 on interactions between plants and associated communities of soil mutualists. We specifically  
64 tested whether changes in plant diversity result in intraspecific differences in plant  
65 phenotypic responses to their local arbuscular mycorrhizal fungi (AMF) communities over a  
66 short evolutionary time scale (11 years).

67           Arbuscular mycorrhizal fungi (AMF) are soil-borne fungi, which form symbiotic  
68 relationships with most land plants (Harley & Harley, 1987; Smith & Read, 1997; Wang &  
69 Qiu, 2006). These fungi are typically known for improving plant growth by providing  
70 enhanced soil nutrient uptake in exchange for plant photosynthates (Smith & Read 2008).

71 Furthermore, AMF diversity has been shown to be an important driver of aboveground plant  
72 diversity and ecosystem productivity (Francis & Read, 1994; van der Heijden et al., 1998;  
73 Wagg, Jansa, Stadler, Schmid, & van der Heijden., 2011a). Conversely, the composition of  
74 AMF communities can be strongly affected by the composition and diversity of plant  
75 communities, such that the diversity of plant communities is interlinked with the diversity of  
76 AMF communities (Burrows & Pflieger, 2002; Klironomos, McCune, Hart, & Neville, 2000;  
77 König et al., 2010; Milcu et al., 2013; Scherber et al., 2010). It is therefore conceivable that a  
78 more diverse plant community can sustain a more beneficial AMF community due to a  
79 positive effect of plant diversity on supporting a greater AMF diversity. On the other hand,  
80 plants growing in monocultures may also select for a more beneficial AMF community, as  
81 the greater abundance of a specific plant species could select for AMF that are particularly  
82 beneficial to that particular species (Arguello et al., 2016; Kiers et al., 2011; Werner & Kiers,  
83 2015). Although there is evidence indicating that plants growing in monoculture for a  
84 prolonged period of time had evolved altered phenotypic responses to competition and  
85 positive PSF (Zuppinger-Dingley et al., 2014; Zuppinger-Dingley, Flynn, De Deyn,  
86 Petermann, & Schmid, 2016), we currently lack evidence as to whether changes in plant  
87 diversity alter plant responses to their local AMF communities over time. We therefore test  
88 the hypothesis that AMF communities originating from plant communities differing in  
89 diversity (plant monocultures vs. plant mixtures) would differ in their influence on plant  
90 growth and phenotypic traits (H1).

91 Plants can adapt to short-term selection pressure imposed by plant community  
92 diversity (van Moorsel, Schmid, Hahl, Zuppinger-Dingley, & Schmid, 2017; Zuppinger-  
93 Dingley et al., 2014). This rapid evolution in plant communities can occur via a sorting-out  
94 from standing genetic variation (Fakheran et al., 2010). In monocultures, plants may  
95 accumulate more specialized pathogens than in diverse plant communities, which potentially

96 reduces plant productivity in monocultures over time (Kulmatiski, Beard, & Heavilin, 2012;  
97 Marquard et al., 2013; Schnitzer et al., 2011; van der Putten et al., 2013). At higher plant  
98 diversity levels, however, specialized pathogens may become diluted (Eisenhauer, 2012; Latz  
99 et al., 2012), thus conferring lower pathogen-based selection pressure than in monocultures.  
100 Consequently, in plant mixtures, plants would likely trade-off reduced defence for increased  
101 competitive growth, whereas in monocultures plants would trade-off reduced growth for  
102 increased defence (Herms & Mattson, 1992). If plants are differentially selected for pathogen  
103 defence or competitive growth traits depending upon their neighbourhood diversity from  
104 which they originate (e.g. Zuppinger-Dingley et al. 2014, Zuppinger-Dingley et al. 2016),  
105 then we hypothesize that plants with different selection backgrounds exhibit different  
106 phenotypic traits related to competition and pathogens (H2).

107         Furthermore, in plant monocultures under a high pathogen load (Schnitzer et al.,  
108 2011), it may be particularly important for plants to associate with AMF as these fungi are  
109 known to protect plants against pathogens (Newsham, Fitter, & Watkinson, 1995; Pozo &  
110 Azcón-Aguilar, 2007). The increasing positive PSF on monocultures through time (e.g.  
111 Zuppinger-Dingley et al. 2016) could thus be potentially reflecting co-selection of plant–  
112 AMF relationships. Considering the potential for plant diversity-driven trade-offs between  
113 competitive growth and plant defence, it is likely that over time plants and their associated  
114 soil biota, such as AMF, may have altered PSF depending on the diversity of the plant  
115 community from which they have originated. We therefore finally hypothesize that  
116 differences in plant diversity have differentially altered plant phenotypic responses to their  
117 local AMF communities resulting in specific ‘home’ vs. ‘away’ plant–AMF community  
118 interactions (H3). Home vs. away effects have been used in reciprocal transplant experiments  
119 (Joshi et al., 2001) and reciprocal inoculation experiments (Klironomos, 2002; Wagg,  
120 Husband, Massicotte, & Peterson, 2011b) to describe a matching of locally co-occurring

121 populations. In the present study, we compared home-combinations (same history for both  
122 AMF and plants) with away-combinations (different history for the two partners).

123 To address our three hypotheses, we used five plant species that occurred in plant  
124 mixtures and as monocultures within the Jena Biodiversity Experiment (Roscher et al., 2004)  
125 and the AMF spore communities collected from their rhizospheres. Plants and AMF had thus  
126 been co-occurring in natural field conditions for eleven years (van Moorsel et al., 2018). To  
127 specifically address the home and away plant–AMF interactions, we isolated AMF spore  
128 communities from the rhizosphere soil and propagated them in trap cultures. Our design was  
129 a reciprocal inoculation experiment where plants with a history of occurring in monocultures  
130 (monoculture-type plants) or diverse plant mixtures (mixture-type plants) were inoculated  
131 with AMF communities isolated from the same monocultures (monoculture AMF) or  
132 mixtures (mixture AMF). In addition, we included a negative AMF control treatment (no  
133 AMF present) as well as a positive control where plants were inoculated with an external  
134 AMF species (*Rhizoglyphus irregularis* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A.  
135 Silva & Oehl), which did not share any history with the plants.

136

## 137 **2 | Materials and methods**

138

### 139 **2.1 | PLANT SELECTION HISTORIES**

140 We used five common perennial European grassland species from three functional  
141 groups (Roscher et al., 2004): three small herbs (*Plantago lanceolata* L., *Prunella vulgaris* L.  
142 and *Veronica chamaedrys* L.), one tall herb (*Galium mollugo* L.) and one legume (*Lathyrus*  
143 *pratensis* L.). The plants had a selection history from the Jena Experiment (Roscher et al.,  
144 2004), where they were sown in either monoculture or mixture in 2002 (selection history  
145 “monoculture-type plants” and “mixture-type plants”, respectively). The species

146 compositions in the experimental plots in Jena were maintained by weeding three times per  
147 year in spring, summer and autumn and by mowing twice per year at peak biomass times in  
148 spring and summer. Each of the studied plant species had undergone eleven years of selection  
149 from 2002 until 2014 in either plant monocultures (monoculture-type plants) or mixtures  
150 (mixture-type plants, Fig. 1). The five plant species are known to associate with arbuscular  
151 mycorrhizal fungi (Harley & Harley, 1987).

152

## 153 2.2 | FIRST CONTROLLED SEED PRODUCTION

154 In spring 2010, entire plant communities of 48 plots (12 monocultures, 12 two-species  
155 mixtures, 12 four-species mixtures and 12 eight-species mixtures) of the biodiversity  
156 experiment in Jena, Germany (the Jena Experiment) were collected as cuttings. Additionally,  
157 the top 30 cm soil of the 48 plots was pooled together, mixed and placed back into the  
158 excavated locations at the Jena Experiment. The cuttings were then transplanted to an  
159 experimental garden in Zurich, Switzerland, in identical plant composition and seeds were  
160 collected over summer 2010. The plots were filled with 30 cm of soil (1:1 mixture of garden  
161 compost and agricultural field soil, pH 7.4, Gartenhumus, RICOTER Erdaufbereitung AG,  
162 Aarberg, Switzerland), and fenced with netting to minimize cross-pollination with plants  
163 outside the plots (for details see Zuppinger-Dingley et al. 2014). In spring 2011, seedlings  
164 were propagated from the collected seeds in a glasshouse in Zurich and then transplanted  
165 back into the mixed soil in the same plots of the Jena Experiment from where their parents  
166 had originally been excavated. In the re-established plots, these newly established plant  
167 communities were maintained for three years until 2014 to allow them to become associated  
168 with their own microbial communities (van Moorsel et al., 2018).

169



## 170 2.3 | SEED PRODUCTION AND SOIL COLLECTION

171 In March 2014, entire plant communities from the re-established plots at the Jena  
172 Experiment were collected and planted in their respective communities in 1 x 1 m plots in the  
173 experimental garden in Zurich. The plots were filled with 30 cm of soil (1:1 mixture of  
174 garden compost and agricultural field soil, pH 7.4, Gartenhumus, RICOTER Erdaufbereitung  
175 AG, Aarberg, Switzerland), and fenced with netting to minimize cross-pollination. During the  
176 relocation of the plant communities, we collected rhizosphere soil samples attached to the  
177 roots of the plants (Fig. 1). By then, the soil communities had undergone three years of  
178 establishment and eight plus three years of potential co-selection with each of the five plant  
179 species in monocultures or mixtures. Seeds were collected from five monocultures and five  
180 mixtures (one four-species mixture and four eight-species mixtures). The seeds of the five  
181 plant species were stored at +4 °C for two months. Four weeks before the start of the  
182 experiment, the seeds were surface-sterilized in 7–14 % bleach for 10–45 min and  
183 subsequently germinated on 1% water-agar.

184

## 185 2.4 | INOCULUM PREPARATION

186 We created trap cultures using AMF spore isolation to minimize the potential that  
187 other unintended fungi were also propagated. To isolate AMF spore communities from the  
188 sampled rhizosphere soils, we passed deionized water and 25 g of soil sample through a  
189 series of sieves from 500 µm to 32 µm using a sugar gradient-centrifugation method  
190 (Sieverding, 1991). The AMF spores were manually collected with a pipet under a  
191 microscope at 200-fold magnification. To bulk up the isolated AMF communities for use as  
192 inoculum, we established trap cultures that consisted of 2 L of 4:1 sand-soil mixture,  
193 autoclaved at 120 °C for 99 min, and a monoculture of trap plants of one the five tested plant  
194 species (Fig. 1). All trap cultures received approximately 400 AMF spores in 30 ml of

195 deionized water, except for the negative control trap cultures, which received 30 ml of  
196 deionized water without AMF spores. For the trap-culture plants we used new seeds from a  
197 commercial seed supplier that provided the original seed material for the Jena Experiment  
198 (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). The seeds were surface-  
199 sterilized in 7–14 % bleach for 10–45 min and pre-germinated on 1 % water agar. Each AMF  
200 trap culture was replicated twice. After ten months of growth in the glasshouse, we collected  
201 root samples from each trap culture that were then fixed in 50 % ethanol, cleared in 10 %  
202 KOH, and stained in 5 % ink-vinegar (Vierheilig, Coughlan, Wyss, & Piché, 1998).  
203 Colonization by AMF was microscopically assessed for successful mycorrhizal establishment  
204 by quantifying AMF root colonization following the transect-intersect method (McGonigle,  
205 Miller, Evans, Fairchild, & Swan, 1990). We further quantified the concentration of AMF  
206 spores in trap cultures with fungal colonization. We isolated AMF spores from a 10-g soil  
207 sample with the same sieving and centrifugation methods used when setting up the AMF  
208 trap-culture pots. Trap-plant cultures that exhibited fungal root colonization and spore  
209 production were dried and the plants were harvested at ground level. The roots were  
210 harvested, cut into 3–5 cm fragments and the belowground content of the trap cultures was  
211 used as soil inoculum in the PSF experiment described below.

212 For the positive control AMF treatment, we used a trap culture substrate containing  
213 *Rhizoglyphus irregularis* (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl as  
214 the inoculum. *Rhizoglyphus irregularis* (previous names *Glomus intraradices* and *R.*  
215 *irregularis*; (Sieverding, da Silva, Berndt, & Oehl, 2015) is an AMF taxon common in natural  
216 grasslands. The culture was developed for nine months in a substrate of 15 % soil, 65 % sand  
217 and 20 % oil binder with *P. lanceolata* plants, which had no shared selection history with  
218 plants or soils from the Jena Experiment. The *R. irregularis* material was obtained from

219 M.G.A. van der Heijden's Ecological Farming Group (Agroscope Reckenholz-Tänikon,  
220 Zurich, Switzerland).

221

## 222 2.5 | EXPERIMENTAL DESIGN

223 To establish the AMF treatments, we filled 1-L pots with gamma-radiated (27–54  
224 kGy) 1:1 (weight/weight) sand-soil mixture and added 9 % (volume/volume) of inoculum  
225 without AMF (negative control), inoculum of AMF isolated from plants grown in  
226 monoculture (monoculture AMF) or mixture (mixture AMF) or inoculum containing *R.*  
227 *irregular* (a positive control). One monoculture- or mixture-type plant of a single plant  
228 species was planted into each pot (Fig. 1, lower panel). To standardize the non-AMF  
229 microbial community within each pot, we created a microbial wash by filtering 1.2 L of a  
230 mixture of unsterilized field soil and the AMF trap culture substrates through a series of  
231 sieves and finally through filter paper (MN615, Macherey-Nagel GmbH & Co. KG) with 5 L  
232 of deionized water. Each pot received 10 ml of the microbial-wash filtrate. The experiment  
233 included four AMF treatments in total, two plant histories (monoculture- and mixture-type  
234 plants) and five plant species in a full factorial design (Table S1). Control and *R. irregular*  
235 AMF treatments were replicated five times and the two other AMF treatments were  
236 replicated ten times (five times per trap-culture replicate, Table S1). For the mixture-type  
237 plants of *G. mollugo*, we did not have sufficient seedlings for the full design and the AMF  
238 treatments were thus only replicated 9 and 8 times, respectively (Table S1). The 297 pots  
239 were randomly arranged within five experimental blocks in a glasshouse compartment with  
240 each particular treatment combination and trap-culture replicate occurring only once in each  
241 block.

242

## 243 2.6 | DATA COLLECTION

244 We cut the plants to 4 cm aboveground three months after planting seedlings into the  
245 pots of the AMF treatments (referred to as first harvest). After five months of plant growth,  
246 maximum height and average leaf absorbance (SPAD-502Plus Chlorophyll Meter, KONICA  
247 MINOLTA, INC., Osaka, Japan) of three representative leaves of each plant were measured  
248 and the aboveground biomass was harvested at ground-level (referred to as second harvest).  
249 The biomass of each plant was dried at 70 °C for 48 h and then weighed. We assessed leaf  
250 mass per area (LMA) and leaf dry matter content (LDMC) at the second harvest by  
251 measuring the area of fresh leaves (LI-3100C Area Meter, LI-COR, Lincoln, USA)  
252 immediately after harvest and assessing the weight of the leaves before (fresh weight) and  
253 after drying (dry weight). Finally, we estimated the degree of damage on plant aboveground  
254 tissues due to powdery mildew (family Erysiphaceae) and two-spotted spider mites  
255 (*Tetranychus urticae* Koch). To determine AMF colonization, roots were washed free of  
256 adhering rhizosphere soil and cut into small 1–5 cm fragments and a random subsample of  
257 roots were then stored in 50 % ethanol for microscopic quantification of AMF using the same  
258 clearing, staining and scoring methods as described above. All measured traits are listed in  
259 Table S2.

260

## 261 2.7 | DATA ANALYSES

262 Due to a contamination of control soil with AMF, one pot with *L. pratensis* was  
263 excluded from all analyses. The biomass data, morphological trait measurements, leaf  
264 damage estimates and AMF colonization were analysed using linear models. Because the first  
265 measure assessed growth and the second regrowth, the harvests were analyzed separately.  
266 Plant survival and AMF presence/absence were analysed using analysis of deviance. The  
267 results were summarized in analysis of variance (ANOVA) and deviance (ANDEV) tables

268 (McCullagh & Nelder, 1998; Schmid, Baruffol, Wang, & Niklaus, 2017). The explanatory  
269 terms of the models were block, plant history (monoculture-type vs. mixture-type), AMF  
270 treatments (four AMF treatments or sequence of the following three orthogonal contrasts:  
271 control vs. AMF treatments, *R. irregularare* vs. monoculture or mixture AMF and monoculture  
272 vs. mixture AMF), species and interactions of these. Statistical analyses were conducted  
273 using the software product R, version 3.0.2 (R Core Team, 2013).

274

### 275 **3 | Results**

#### 276 3.1 | ROOT COLONIZATION

277 The root colonization by AMF was highest in all five plant species with the  
278 inculcation of *R. irregularare* (the positive control). For two species, *G. mollugo* and *L.*  
279 *pratensis*, AMF colonization was higher in the treatment with AMF originating from the  
280 plant monocultures, whereas for two other species, *P. lanceolata* and *V. chamaedrys*, root  
281 colonization was greater with AMF originating from plant mixtures. In *P. vulgaris*, AMF  
282 colonization was equally low for both AMF community histories. An increase in AMF  
283 colonization was positively correlated with an increase in individual plant biomass for all  
284 three AMF treatments (Fig. S1).

285

#### 286 3.2 | EFFECTS OF AMF COMMUNITIES ON PLANT PHENOTYPIC RESPONSES (H1)

287 The four AMF treatments changed the phenotypes of the plants, but the response  
288 varied between the five species. We found significant effects of the AMF treatments on five  
289 traits: plant aboveground biomass at the first harvest, plant aboveground biomass at the  
290 second harvest, plant height, LDMC and AMF colonization (Fig. 2, Table 1). The effects of  
291 the AMF treatments on plant biomass were relatively consistent between the two harvests  
292 (Fig. 2). Biomass was generally highest in the treatment with the positive AMF control

293 (inoculation of *R. irregulare*), with the exception of *V. chamaedrys*, for which the negative  
294 control (no AMF inoculated) resulted in the highest biomass. Soil containing AMF from  
295 mixture plots was the overall second most productive AMF treatment in terms of plant  
296 biomass, but again with the exception of *V. chamaedrys* and also *P. lanceolata* which  
297 exhibited higher biomass when inoculated with AMF from monoculture plots. The effects of  
298 the AMF treatments on plant height also varied among plant species. For *G. mollugo*, soil  
299 containing *R. irregulare* or soil containing mixture AMF resulted in the tallest plants. For  
300 three species, *L. pratensis*, *P. lanceolata* and *P. vulgaris*, the AMF treatment did not have a  
301 strong effect on plant height, except that the control treatment lacking AMF resulted in the  
302 shortest plants. In line with the biomass results, plant height was increased in the control  
303 treatment for the small herb *V. chamaedrys*. The LDMC was strongly increased in the control  
304 treatment for *L. pratensis* and slightly increased for *G. mollugo*. For the three small herbs, *P.*  
305 *lanceolata*, *P. vulgaris* and *V. chamaedrys*, the LDMC was increased for the two AMF  
306 treatments containing AMF from either mixture or monoculture plots as opposed to  
307 treatments without AMF or with the external *R. irregulare*.

308

### 309 3.3 | INFLUENCE OF PLANT DIVERSITY HISTORY ON PLANT PHENOTYPES (H2)

310 Mixture-type plants grew taller than monoculture-type plants in three out of five  
311 species. Monoculture-type plants of the other two species, *L. pratensis* and of *P. lanceolata*,  
312 grew taller than their mixture-type counterparts. The leaf traits LDMC and LMA were  
313 generally increased in mixture-type plants, except for *V. chamaedrys*. For plant height, LMA,  
314 leaf damage and leaf absorbance the plant history effect was also visible across species  
315 (Table 1, e.g. the main effect of plant history).

316 As anticipated, mixture-type plants overall had more damaged leaves than  
317 monoculture-type plants, in particular for *L. pratensis* and *P. lanceolata* (Fig. 3, Table 1). The

318 monoculture-type or mixture-type plants resulted in differential phenotypes between  
319 individuals of the same species. However, the strength of the response to selection history  
320 varied greatly between the five studied species (Fig. 3, Table 1). We found significant overall  
321 effects of plant history on seven traits: plant survival, plant aboveground biomass at the first  
322 harvest, plant aboveground biomass at the second harvest, plant height, LMA, LDMC and  
323 leaf damage. Mixture-type plants had a greater survival in the case of the three small herbs,  
324 whereas for the legume *L. pratensis* and the tall herb *G. mollugo*, monoculture-type plants  
325 showed a higher survival (Fig. 3). Plant biomass was generally higher for mixture-type plants  
326 (Fig. 3), but *L. pratensis* and *G. mollugo* showed the opposite pattern with increased biomass  
327 for monoculture-type plants (for *L. pratensis* only at the first harvest). The difference in  
328 biomass production between monoculture-and mixture-type plants was smaller at the second  
329 harvest but still varied significantly among species (Fig. 3).

330

### 331 3.4 | EFFECTS OF MATCHING HOME AND AWAY PLANT AND AMF HISTORIES

332 (H3)

333 We did find overall home vs. away effects for LMA and leaf absorbance, but the  
334 effects varied among the five plant species studied and responses differed in direction (Fig.  
335 4). The home mixture combination (mixture-type plants and mixture AMF) resulted in  
336 increased LMA in four out of five species, the exception being *V. chamaedrys* (Fig. 4a). The  
337 home monoculture combination (monoculture-type plants and monoculture-AMF) only  
338 increased LMA in *P. vulgaris* (Fig. 4a). Both away-combinations (monoculture-plant type  
339 with mixture AMF and vice versa) increased LMA strongly in *V. chamaedrys* (Fig. 4a). The  
340 away-combination between mixture-type plants and monoculture AMF also increased LMA  
341 in *L. pratensis* (Fig. 4a). Both home-combinations increased leaf absorbance in the small

342 herbs *P. vulgaris* and *V. chamaedrys* (Fig. 4b). In contrast, both home-combinations reduced  
343 leaf absorbance for the tall herb *G. mollugo* and the legume *L. pratensis*.

344

## 345 **4 | Discussion**

### 346 4.1. | EFFECTS OF AMF COMMUNITIES ON PLANT PHENOTYPIC RESPONSES (H1)

347 Here we assessed whether plant diversity loss not only alters plant traits related to  
348 competition, but also the phenotypic responses of plants to their AMF communities.  
349 Specifically, we aimed to separate whether plant diversity loss alters the selection on plants  
350 for altered responses to local AMF communities (H1), traits related to competitive growth  
351 (H2) or both simultaneously (H3). For instance, although plants may be selected for altered  
352 competitive growth characteristics in a more species diverse community (Zuppinger-Dingley  
353 et al. 2014), it is also known that interactions with soil organisms alters how plants allocate  
354 resources to traits, which affects plant growth strategies (Dudenhöffer, Ebeling, Klein, &  
355 Wagg, 2018; Streitwolf-Engel et al., 1997). Conversely, plant traits have been thought to  
356 influence interactions between plants and AMF (Baxendale, Orwin, Poly, Pommier, &  
357 Bardgett, 2014). A recent study focusing on plant traits in relation to PSF effects found  
358 evidence that a lower specific root length and a greater percentage colonization of the root  
359 length by AMF resulted in more positive PSFs (Cortois et al. 2016). Furthermore,  
360 mycorrhizal associations have been known to alter leaf chemical properties and alter  
361 herbivore life-history traits via changes in leaf chemistry (Goverde, van der Heijden,  
362 Wiemken, Sanders, & Erhardt, 2000; Smith & Read, 1997).

363 We assessed whether plant diversity can act as a selective environment on how AMF  
364 communities influence plant growth and functional traits. In support of our first hypothesis  
365 (H1) we found significant effects of the AMF treatments on plant phenotypes. Mixture AMF  
366 were more beneficial than monoculture AMF for the taller species *G. mollugo* and *L.*



367 *pratensis*, but not for the three shorter species *P. lanceolata*, *P. vulgaris* and *V. chamaedrys*.  
368 All studied plant species except *V. chamaedrys* showed increased biomass production in the  
369 presence of AMF; and across species, plant biomass generally increased with increasing  
370 AMF colonization. Interestingly, mixture AMF showed lower colonization than monoculture  
371 AMF in the plant species that benefited from them and vice versa in the other three species.  
372 These species-specific responses are in line with other studies showing context-dependent  
373 AMF effects on plants (Burrows & Pfleger, 2002; Hoeksema et al., 2010). Although AMF  
374 generally promote plant growth, the outcome of the interaction may vary from beneficial to  
375 antagonistic for plant growth (Johnson, Graham, & Smith, 1997; Kiers & van der Heijden,  
376 2006; Klironomos, 2003). The positive effect of mixture AMF on species with a low  
377 colonization could indicate increased AMF efficiency in promoting plant growth. However,  
378 lower growth at high colonization could also indicate that a shift from mutualism to  
379 antagonism occurred in these species.

380 Colonization in our positive AMF control treatment of *R. irregulare*, which did not  
381 share a common history with the experimental plants, was greater than in mixture or  
382 monoculture AMF treatments and led to higher plant biomass especially at the first harvest. A  
383 high colonization ability of this taxon is typical and has been shown to be not as beneficial to  
384 plant hosts as less abundant colonization by other AMF taxa (Hart and Reader 2002, Wagg et  
385 al., 2011a, Engelmoer, Behm, & Kiers 2014). This particularly productive and cosmopolitan  
386 AMF taxon may have been more effective in colonizing plant roots and influencing plant  
387 responses compared to the inoculation of AMF spore communities native to the field site  
388 where a greater diversity of AMF taxa were likely present (Dassen et al., 2017). The co-  
389 inoculation of several AMF taxa could have resulted in competition among AMF taxa for a  
390 single host plant in our study, thus reducing their ability to colonize roots and influence plant  
391 responses (Bennett & Bever, 2009; Engelmoer et al., 2014). These results are in line with

392 previous findings reporting that different AMF isolates vary in their effect on plant  
393 phenotypes (Klironomos, 2003; Koch, Antunes, Maherali, Hart, & Klironomos, 2017;  
394 Streitwolf-Engel, Boller, Wiemken, & Sanders, 1997), which may depend on co-infection  
395 with other AMF species (Arguello et al., 2016; Bennett & Bever, 2009).

396

## 397 4.2 | INFLUENCE OF PLANT DIVERSITY HISTORY ON PLANT PHENOTYPES (H2)

398 Our second hypothesis, that mixture-type plants grow faster whereas monoculture-  
399 type plants are better defended was generally supported by our experiment (see significant  
400 main effects of plant history in Table 1). But here again the plant species varied in their  
401 responses. Mixture-type plants of three species were more productive and mixture-type plants  
402 of three species grew taller than monoculture-type plants. Furthermore, three out of the five  
403 species showed higher LDMC and four species higher LMA for mixture-type plants in  
404 comparison with monoculture-type plants, indicating that mixture-type plants also invested  
405 more resources into leaf biomass production. In contrast, monoculture-type plants had less  
406 leaf damage, which confirmed the second part of our hypothesis 2, namely that monoculture-  
407 type plants evolved increased defence. We observed particularly severe infection by powdery  
408 mildew in mixture-type plants of *P. lanceolata*, suggesting, in agreement with Engelmoer et  
409 al. (2014), that monoculture-type plants of *P. lanceolata* may have been subjected to  
410 particularly strong selection pressure for pathogen defence in comparison with mixture-type  
411 plants. Previously it has been shown that community diversity as selective environment can  
412 alter the selection on plant traits favouring greater character displacement in more diverse  
413 plant communities (Zuppinge-Dingley et al., 2014), with the assumption that competitive  
414 growth traits trade off with selection for pathogen defence in plant monocultures (Herms &  
415 Mattson, 1992). The increased resource investment into leaves in mixture-type plants thus  
416 may have been at the cost of lowered pathogen defence. Specialized pathogens have indeed

417 been observed to become diluted at higher plant diversity levels in the Jena Experiment  
418 (Eisenhauer, Reich, & Scheu, 2012; Rottstock, Joshi, Kummer, & Fischer, 2014), thus  
419 conferring lower selection pressure in mixtures than in monocultures. To increase survival,  
420 plants in monocultures are expected to allocate more resources to defence (Bezemer &  
421 Vandam, 2005) or may enter more beneficial symbioses (Newsham et al., 1995).  
422 Consequently, survival in plant monocultures may depend on the ability of plants to allocate  
423 resources to these interactions whereas plants in mixtures can allocate these resources to  
424 growth.

425

#### 426 4.3 | HOME VS. AWAY EFFECTS OF PLANT AND AMF HISTORIES (H3)

427 Our third hypothesis was that a common selection history of plants and their  
428 associated fungi in the field would lead to a matching between plants and their associated  
429 AMF communities, i.e. ‘home’ vs. ‘away’ AMF effects on plant phenotypic responses. We  
430 found limited evidence for this hypothesis. The main predictor for increased positive PSF,  
431 plant biomass, was not affected by home vs. away combination, which indicates that  
432 beneficial interactions did not strengthen over time. Instead, we found a home vs. away effect  
433 for the two functional traits LMA and leaf absorbance. For these two leaf properties, we  
434 found some evidence that monoculture-type plants were more influenced by monoculture  
435 AMF and mixture-type plants were more influenced by mixture AMF. Namely, leaf  
436 absorbance and LMA were increased in the small herb *P. vulgaris* if the AMF had a common  
437 selection history. Increased leaf absorbance and high LMA are related to higher area-based  
438 nitrogen content (Niinemets, 1997), suggesting that co-selected AMF may have improved the  
439 nitrogen uptake of these plants. The opposite response was observed for leaf absorbance in *L.*  
440 *pratensis* and for LMA in *V. chamaedrys*. Although for LMA and leaf absorbance the results  
441 would be in line with our hypothesis for a specific effect of ‘matching’ plant and AMF

442 community histories (indicating their co-selection), the common selection history between  
443 plants and AMF was rather detrimental to the plants' growth response. This would suggest  
444 that a common association history between plants and their AMF communities may have  
445 become more antagonistic, rather than mutualistic, over the eleven years of co-selection.

446

## 447 **5 | Conclusions**

448 Here we present evidence that the loss of plant diversity differentially alters how  
449 AMF communities influence phenotypic responses in plants. We found that AMF  
450 communities selected in plant mixtures were more beneficial than AMF selected in plant  
451 monocultures for two out of five plant species. Furthermore, mixture-type plants generally  
452 grew better, but suffered more leaf damage than monoculture-type plants, providing evidence  
453 for the differential selection on competitive growth vs. defence depending on the interspecific  
454 diversity of neighbouring plants. However, home vs. away effects between AMF and plants  
455 from mixtures or AMF and plants from monocultures were rare. When they did occur they  
456 were generally in the direction of increased antagonism by the AMF, leading to a reduced  
457 performance of the plant partner. We assert that conducting reciprocal inoculation  
458 experiments over longer ecological time-scales could contribute to a better understanding of  
459 plant-AMF interactions (Lekberg & Koide, 2014). Our results suggest that biodiversity loss  
460 can alter evolutionary trajectories in the interactions between plants and AMF through  
461 differential selection pressures on both partners at high and low diversity. Furthermore, our  
462 data suggest that a shared history between plants and AMF does not follow a general pattern  
463 leading to increased mutualism. Rather, changes in these interactions are context-dependent  
464 and may strengthen over time, even switching directionality. Further investigating the  
465 sustainability of AMF-plant mutualisms in a world with changing species composition and

466 biodiversity will be important when searching for applications for AMF communities to  
467 support ecosystem functioning in the future.

468

#### 469 **Acknowledgements**

470 We thank M. Furler, D. Topalovic, D. Trujillo Villegas and T. Zwimpfer for technical  
471 assistance and A. Ferrari, S. Karbin, J. Moser Frias and Y. Xu for help with measurements.  
472 Thanks to M. van der Heijden for assistance and the AMF isolates. This study was supported  
473 by the Swiss National Science Foundation (grants number 147092 and 166457 to B. S.) and  
474 the University Research Priority Program Global Change and Biodiversity of the University  
475 of Zurich. The Jena Experiment is supported by the German Science Foundation (FOR 1451,  
476 SCHM 1628/5-2).

477

#### 478 **Authors' contributions**

479 T.H., C.W., S.J.V.M., D.Z.D and B.S. planned and designed the research, T.H. carried  
480 out the experiment and T.H., C.W., M.W.S., S.J.V.M and B.S. analyzed the data. S.J.V.M,  
481 T.H., C.W. and B.S. wrote the manuscript with the other authors contributing to revisions.

482

#### 483 **Data accessibility**

484 Data will be archived on Pangaea upon acceptance of this article.

485

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695

### 696 **Supporting information**

697 Additional Supporting Information may be found online in the Supporting Information tab for

698 this article:

699 **Methods S1.** Assessment of soil N and P content at the beginning of the experiment.

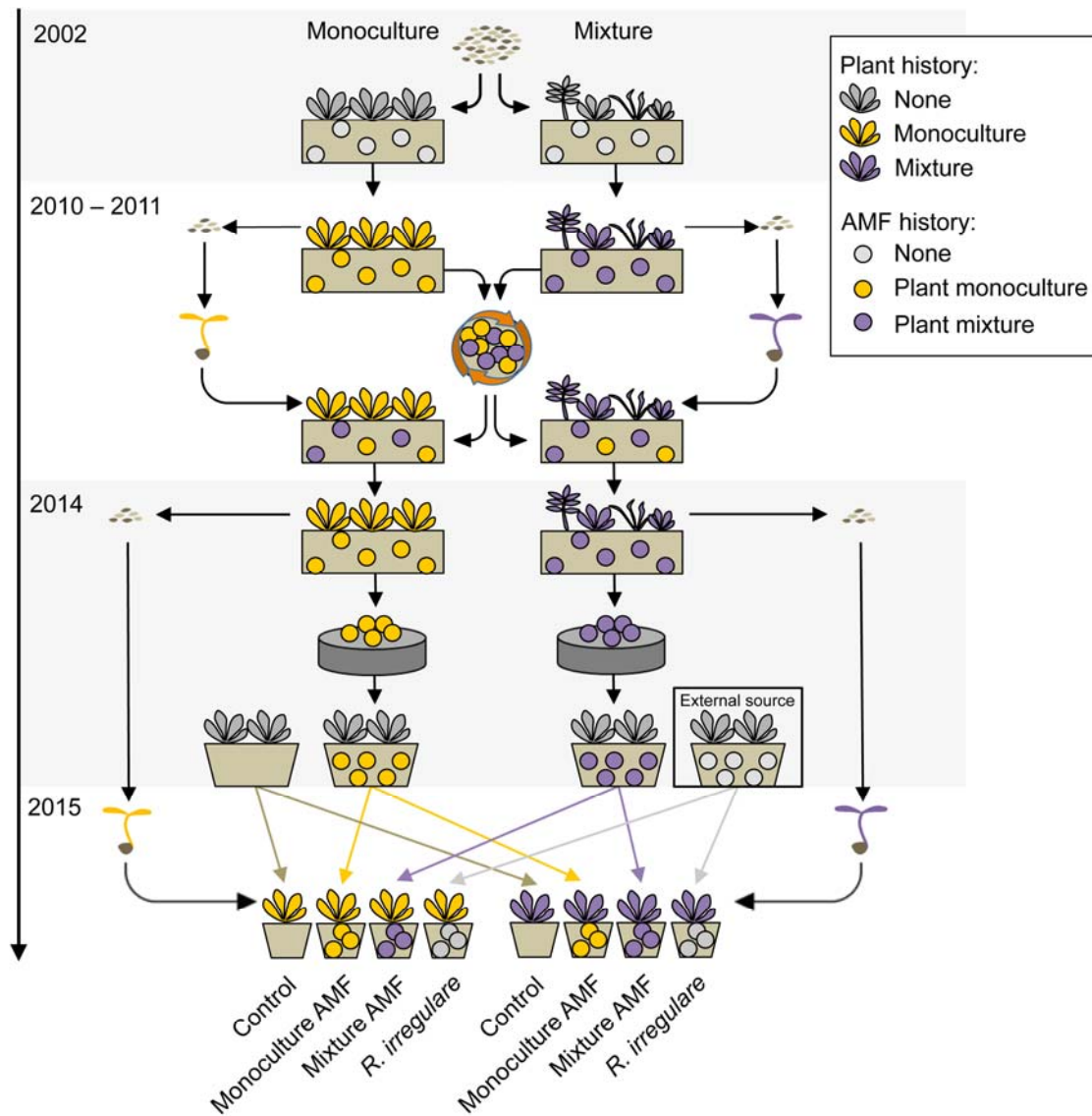
700 **Fig. S1** Aboveground biomass production of monoculture- and mixture-type plants in control

701 soil in dependence of different levels of AMF colonization.

702 **Table S1.** Experimental design.

703 **Table S2.** Plant traits measured at the experimental plant age.

704

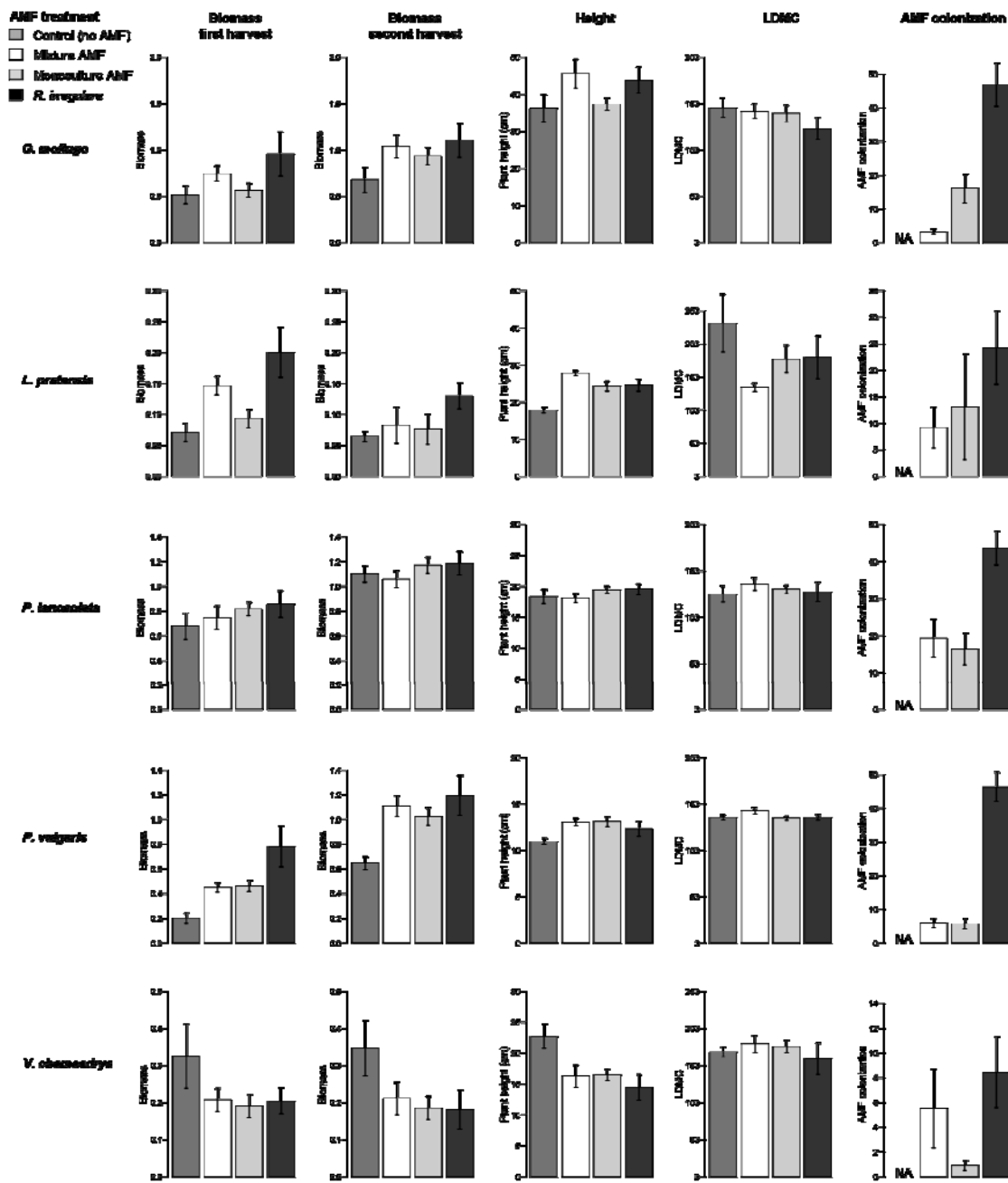


705  
 706 **Figure 1.** Experimental design. Plant monocultures and mixtures in the Jena Experiment  
 707 were sown in 2002 and maintained until 2010. In 2010, the plants of 48 plots underwent a  
 708 first controlled seed production event and the soil of the plots was pooled, mixed and placed  
 709 back to the excavated locations. In spring 2011, the seedlings produced were transplanted  
 710 back to the mixed soil in the same plots from which their parents were excavated. The plant  
 711 communities could then again associate with their own microbial communities potentially co-  
 712 assembling and co-evolving until 2014. In spring 2014, the plants underwent a second  
 713 controlled seed production event, and the AMF spores from their rhizosphere soil were  
 714 isolated. The isolated AMF communities accumulated in trap-cultures for ten months with

715 trap plants lacking a common selection history with the AMF spores. Control trap-cultures  
716 without AMF spores were established as negative control. Four treatments were created: pots  
717 with sterile soil and 1) 9 % inoculum without AMF, 2) inoculum of AMF isolated from plants  
718 grown in monoculture, 3) inoculum of AMF isolated from plants grown in mixture and 4)  
719 inoculum containing *Rhizoglonus irregulare*. Finally, the plants with a selection history in  
720 either monoculture (monoculture-type plants) or mixture (mixture-type plants) were planted  
721 individually into the prepared pots.  
722



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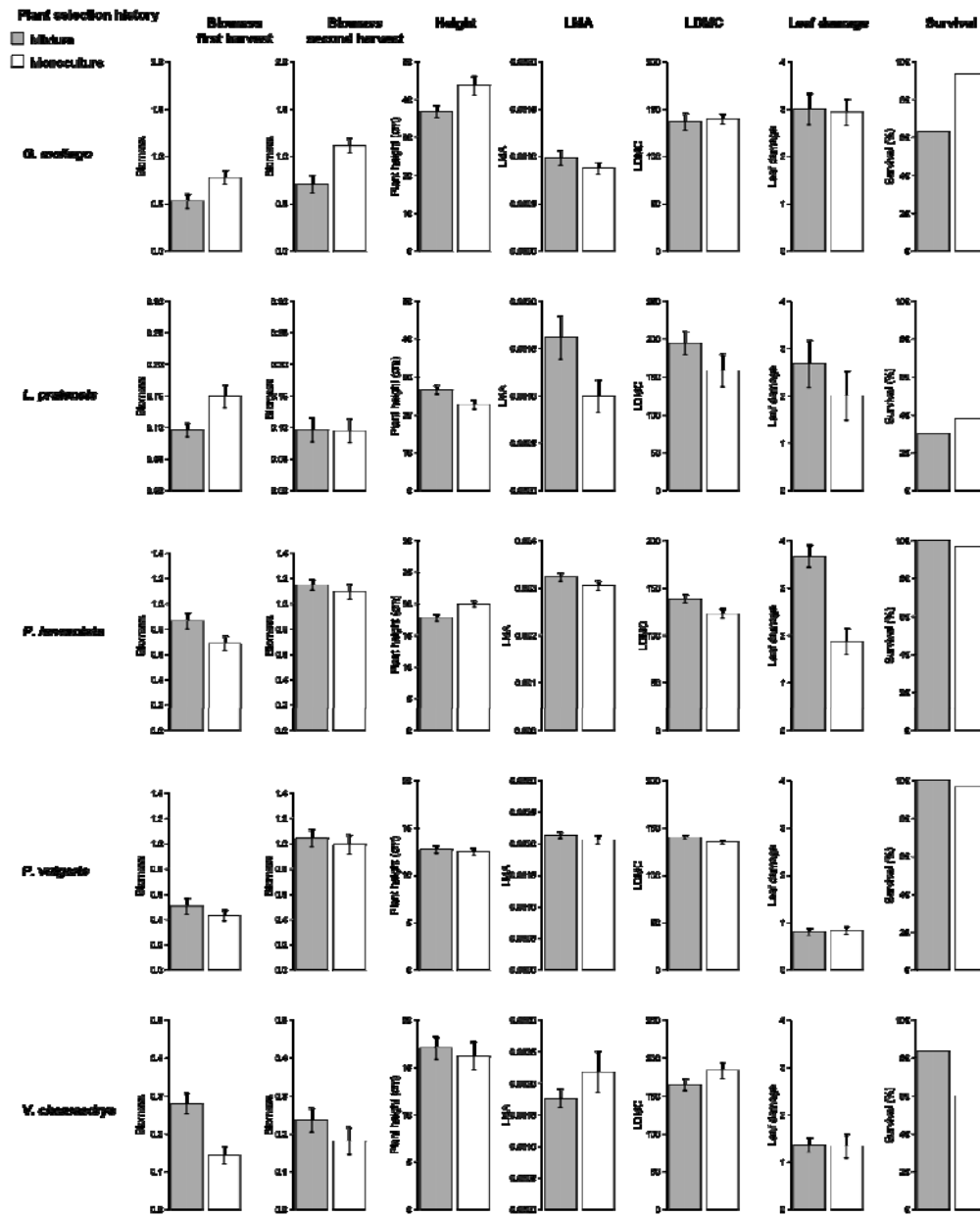
724

725 **Figure 2** Functional traits in responses to the AMF treatments are shown separately for each

726 of the five plant species assessed. Bars are means with standard errors calculated from raw

727 data. See Table 1 for significance of effects.

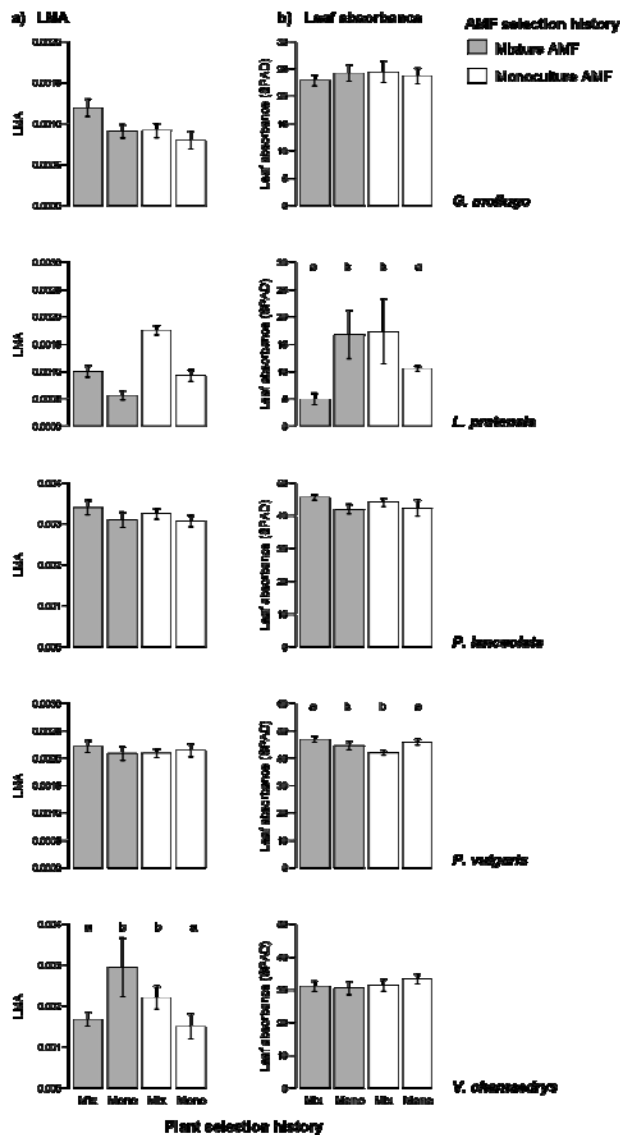
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729

730 **Figure 3.** Functional traits in response to plant selection history for five species. Grey bars  
 731 refer to plants with a history of growing in species mixtures at the Jena Experiment, white  
 732 bars refers to plants with a history of growing in monoculture at the same field site. Shown  
 733 are means and standard errors calculated from raw data. See Table 1 for significance of  
 734 effects.

735



736

737 **Figure 4** (a) LMA and (b) leaf absorbance in response to home and away combinations of

738 plant selection history and AMF selection history for five species. Grey bars refer to AMF

739 from mixed plant communities; white bars refer to AMF from monocultures. The first and

740 last bar of each plot corresponds to home combinations; the two middle bars correspond to

741 away combinations. Shown are means and standard errors calculated from raw data.

742 Significant home effects are indicated by letters “a” and “b” on top of bars:  $P < 0.01$  for leaf

743 absorbance in *L. pratense*,  $P < 0.05$  for leaf absorbance in *P. vulgaris* and  $P < 0.05$  for LMA

744 in *V. chamaedrys*.

**Table 1.** ANOVA results for eight traits and ANDEV results for plant survival in response to the design variables.

Source of variation	Plant survival			Biomass first harvest			Biomass second harvest			Plant height			LMA			LDMC			Leaf damage			Leaf absorbance			AMF colonization		
	Df	% Dev	P	Df	% SS	P	Df	% SS	P	Df	% SS	P	Df	% SS	P	Df	% SS	P	Df	% SS	P	Df	% SS	P	Df	% SS	P
Block	4	0.81	0.448	4	6.25	<0.001	4	6.63	<0.001	4	1.31	0.018	4	4.85	<0.001	4	5.58	0.002	4	5.68	<0.001	4	3.40	<0.001	4	1.70	0.130
Plant history (PH)	1	0.03	0.689	1	0.03	0.656	1	0.47	0.050	1	2.26	<0.001	1	0.88	0.014	1	0.63	0.164	1	1.43	0.016	1	0.39	0.032	1	0.10	0.510
AMF treatment (ST)	3	1.44	0.088	3	3.52	<0.001	3	0.67	0.139	3	0.44	0.258	3	0.95	0.090	3	0.25	0.852	3	0.57	0.503	3	1.58	0.000	3	38.21	<0.001
Species (Sp)	4	31.48	<0.001	4	45.11	<0.001	4	62.74	<0.001	4	69.90	<0.001	4	59.82	<0.001	4	20.53	<0.001	4	35.70	<0.001	4	75.64	<0.001	4	7.21	<0.001
PH x ST	3	0.34	0.668	3	0.06	0.935	3	0.20	0.646	3	0.38	0.315	3	0.47	0.357	3	0.72	0.524	3	0.04	0.982	3	0.04	0.916	3	0.11	0.923
PH x Sp	4	5.06	<0.001	4	3.58	<0.001	4	2.55	<0.001	4	1.67	0.005	4	1.60	0.028	4	3.70	0.024	4	6.70	<0.001	4	0.31	0.446	4	0.56	0.665
ST x Sp	12	3.85	0.131	12	4.44	0.005	12	2.94	0.024	12	3.37	0.003	12	2.18	0.245	12	7.09	0.045	12	3.07	0.396	12	1.46	0.147	12	9.02	<0.001
PH x ST x Sp	12	2.35	0.546	12	3.00	0.081	11	1.73	0.224	11	1.03	0.568	11	3.30	0.024	11	3.61	0.429	11	2.59	0.470	11	1.78	0.038	11	2.00	0.671
Residuals	252	54.65		224	34.01		183	22.06		183	19.63		180	25.94		180	57.88		183	44.21		183	15.38		174	41.08	

746