Testing for co-adaptation of plants and arbuscular mycorrhizal fungi in a biodiversity experiment

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Summary

- Co-evolution between plants and arbuscular mycorrhizal fungi (AMF) may occur over short time spans. However, whether plants and AMF co-adapt and how this may be influenced by plant diversity has never been addressed.
- We carried out a plant–AMF experiment using five plant species and AMF selected over 11 years in plant monocultures or mixtures. Single plants were grown in sterile soil or soil inoculated with monoculture or mixture AMF or with a positive control (*Rhizoglomus irregulare*). We measured plant biomass, plant functional traits and AMF colonization.
- Mixture AMF were more beneficial than monoculture AMF for two plant species. Of the other three species, *Veronica chamaedrys* showed reduced performance with all tested AMF.
- Mixture-type plants generally grew better than monoculture-type plants, which suffered less leaf damage by pathogens. This suggests that plant mixtures were selected for increased growth at the expense of reduced defence and the opposite happened in plant monocultures.
- Cases of co-adaptation between AMF and plants from mixtures or AMF and plants form monocultures were rare and mostly in the direction of increased parasitism of the AMF, suggesting an arms-race leading to reduced performance of plant partners.

- ***Key-words***: biodiversity experiment, growth–defence trade-off, plant–AMF co-adaptation, plant–soil feedbacks, rapid evolution

Introduction

Associations between plants and soil organisms have received much attention in the past decades (e.g. Bever *et al.*, 1997; Klironomos, 2002; van der Heijden *et al.*, 2006; Petermann *et al.*, 2008; Van Nuland *et al.*, 2016), but only recently studies considered that interactions between plants and such organisms may change over ecological time-scales through adaptation (Lau & Lennon, 2011, 2012; terHorst *et al.*, 2014; Zuppinger-Dingley *et al.*, 2016). We tested whether beneficial interactions between plants and arbuscular mycorrhizal fungi (AMF) are affected by selection in plant communities characterized by different diversity levels in the so-called Jena Experiment (Roscher *et al.*, 2004).

AMF are soil-borne fungi, which form symbiotic relationships with most land plants. The fungus extracts plant-derived carbohydrates and in exchange provides mineral nutrients to the host (Gianinazzi-Pearson, 1996; van der Heijden *et al.*, 2006). AMF can improve plant survival and growth by increasing nutrient uptake of the host plant (Jones & Smith, 2004; van der Heijden *et al.*, 2006) or by protecting the plant from detrimental effects of both above- and belowground enemies (Newsham *et al.*, 1995; Azcón-Aguilar & Barea, 1997; Rodriguez & Redman, 2008; Vannette *et al.*, 2013). AMF have the potential for rapid adaptation due to their dependence on plant-derived carbon, their short generation time in comparison to the host plants and their limited dispersal (Rúa *et al.*, 2016).

AMF diversity has been shown to be a major driver of aboveground plant diversity and ecosystem functioning (van der Heijden *et al.*, 1998). It is furthermore known that different AMF isolates or species vary in their effect on plant performance and functional traits (Streitwolf-Engel *et al.*, 1997; Klironomos, 2003; Koch *et al.*, 2017), which may depend on co-infection with other AMF species (Argüello *et al.*, 2016). There is also evidence for local adaptation of AMF to above- and belowground conditions (Ji *et al*., 2010, Lambert *et al*., 2010). However, whether AMF selected in plant mixtures and AMF selected in plant monocultures would differentially affect plants in a homogenous environment is still largely unknown (but see Burrows & Pfleger, 2002). We hypothesized that AMF isolated from multi-species plant communities are generally more beneficial to plants than AMF isolated from plant monocultures (hypothesis 1).

Not only fungi, but also plants can adapt to short-term selection imposed by plant community diversity (Zuppinger-Dingley *et al.*, 2014; van Moorsel *et al.*, 2017). In one study, plants that had been selected for 8 years in mixtures (mixture-type plants) were more productive than plants selected for the same time in monocultures (monoculture-type plants) and this was due to evolutionary-increased combining ability (Zuppinger-Dingley *et al.*,

2014). These studies, however, have largely ignored the interactions of the plants with their associated soil microbiomes. In the present study we therefore analysed the responses of plants to selection in mixtures vs. monocultures in the context of different AMF treatments. Based on previous findings (Zuppinger-Dingley *et al.*, 2014 and 2016) we hypothesized that generally mixture-type plants grow faster whereas monoculture-type plants are better defended in mutual comparisons (hypothesis 2).

Plants with a selection history in monocultures (monoculture-type plants) were shown to evolve positive and mixture-type plants evolve negative plant–soil feedbacks after eight years of selection in respective communities (Zuppinger-Dingley *et al.*, 2016). In plant mixtures, co-evolution between plants and their associated AMF may have resulted in positive plant–soil feedbacks because of increased mutualistic interactions. In monocultures plants are exposed to a stronger accumulation of specialized pathogens than in diverse plant communities, thus potentially reducing productivity of monocultures over time (Kulmatiski *et al.*, 2012; van der Putten *et al.*, 2013; Marquard *et al.*, 2013). These results led us to hypothesize that co-adaption between plants and their associated AMF after a common selection history in the field of eleven years increases the plant–AMF mutualism (hypothesis 3).

To test these three hypotheses, we conducted a factorial AMF-by-plant selection-history experiment. Our design corresponded to a reciprocal inoculation experiment (Klironomos, 2002) of plants selected in monocultures or mixtures with AMF selected in the same monocultures or mixtures. To increase generality, we used five different plant species, each selected for eleven years in monoculture (monoculture-type plants) or mixture (mixture-type plants) in the Jena Experiment. We combined them with AMF collected from the same plant communities (monoculture AMF and mixture AMF, where the two categories refer to the diversity of the plant community). In addition, we tested plant performance in soils lacking any AMF (control) or containing an external AMF species (*Rhizoglomus irregulare*), which did not share a common history with the studied plants.

Materials and Methods

Plant histories

We used five common perennial European grassland species from three different functional groups (Roscher *et al.*, 2004): three small herbs (*Plantago lanceolata* L., *Prunella vulgaris* L. and *Veronica chamaedrys* L.), one tall herb (*Galium mollugo* L.) and one legume (*Lathyrus pratensis* L.). Each of the studied plant species had undergone eleven years of

selection from 2002 until 2014 in either plant monocultures (monoculture-type plants) or species mixtures (mixture-type plants, Fig. 1). The five plant species are also known to enter symbioses with arbuscular mycorrhizal fungi (Harley & Harley, 1987).

First controlled seed production and "soil training"

In spring 2010, the entire plant communities of 48 plots (12 monocultures, 12 two-species mixtures, 12 four-species mixtures and 12 eight-species mixtures) of a biodiversity experiment in Jena, Germany (the Jena Experiment), were collected as cuttings and transplanted to an experimental garden in Zurich, Switzerland, in identical plant composition for a first controlled sexual reproduction among co-selected plants (Zuppinger-Dingley *et al.*, 2014). Additionally, the top 30 cm soil of the 48 plots was pooled together, mixed and placed back into the excavated locations in the Jena Experiment. In spring 2011, the seedlings produced from the seeds of the first controlled sexual reproduction in Zurich were transplanted back into the mixed soil in the same plots of the Jena Experiment from where the parents had originally been excavated. In these re-established plots, plant communities with identical composition as the original communities were maintained for three years until 2014 to allow them to become associated with their own microbial communities.

Second controlled seed production

The seeds were obtained from a second controlled sexual reproduction. In March 2014, entire plant communities from the re-established plots in the Jena Experiment were collected and planted in their respective communities in 1 x 1 m plots in the experimental garden in Zurich. The plots were filled with 30 cm of soil (1:1 mixture of garden compost and field soil, pH 7.4, Gartenhumus, RICOTER Erdaufbereitung AG, Aarberg, Switzerland), and fenced with netting to minimize cross-pollination with plants outside the plots. We collected seeds from five monoculture and five mixture plots (one four-species mixture and four eight-species mixtures). The seeds of the five plant species were stored at +4 ˚C for two months. Four weeks before the start of the experiment, the seeds were surface-sterilized with 7–14 % bleach for 10–45 min and subsequently germinated on 1% water-agar.

Soil collection and inoculum preparation

In March 2014 we collected rhizosphere soil samples attached to the roots of the plants collected in the Jena Experiment (Fig. 1). By then, the soil communities had undergone

three years of community re-assembly and eight plus three years of potential co-evolution with each of the five plant species in monocultures or mixtures.

To isolate AMF communities from the sampled rhizosphere soils, we passed deionized water and 25 g of soil sample through a series of sieves and isolated soil particles with a diameter of 32–500 µm using a sugar gradient-centrifugation method (Sieverding, 1991). The AMF spores were manually collected with a pipet under a microscope at 200-fold magnification. To accumulate the isolated AMF communities, we established trap cultures that consisted of 2 L of 4:1 sand-soil mixture, autoclaved at 120 °C for 99 min, and a monoculture of trap plants of one of each the five tested plant species (Fig. 1). All trap cultures received 300–400 AMF spores in 30 ml of deionized water, except for the negative control trap cultures, which received 30 ml of deionized water without AMF spores. We deliberately avoided that the trap plants shared a history of co-existence with the AMF spores collected from the rhizosphere of monoculture- or mixture-type plants of the same species. Therefore we used new seeds from a commercial seed supplier which provided the original seed material for the Jena Experiment (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). The seeds were surface-sterilized and pre-germinated on 1 % water agar. Each AMF trap culture was replicated twice. After ten months of growth in the glasshouse, we collected a root sample from each trap culture, fixed the root samples in 50 % ethanol, cleared them with 10 % KOH, and stained them with 5 % ink-vinegar (Vierheilig *et al.*, 1998). AMF colonization was quantified microscopically. For the trap cultures with fungal colonization, we further quantified the concentration of AMF spores. We isolated AMF spores from a 10-g soil sample with the same sieving and centrifugation methods used when setting up the AMF trap-culture pots. The AMF spores were then counted under a microscope. Trap-plant cultures that showed fungal root colonization were dried and the plants were harvested at ground level. The roots were harvested, cut into 3–5 cm fragments and the belowground content of the trap cultures was used as soil inoculum in the plant–soil feedback experiment described below.

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

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

Plant–soil feedback experiment

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

Data collection

We cut the plants to 4 cm aboveground three months after planting seedlings into the pots of the different soil treatments (referred to as first harvest). After five months of plant growth, maximum height and average leaf absorbance (SPAD-502Plus Chlorophyll Meter, KONICA MINOLTA, INC., Osaka, Japan) of three representative leaves of each plant were measured and the aboveground biomass was harvested at ground-level (referred to as second harvest). The biomass of each plant was dried at 70 °C for 48 h and then weighed. We assessed leaf mass per area (LMA) and leaf dry matter content (LDMC) at the second harvest by measuring the area of fresh leaves (LI-3100C Area Meter, LI-COR, Lincoln, USA) immediately after harvest and assessing the weight of the leaves before (fresh weight) and

after drying (dry weight). Finally, we estimated the degree of damage on plant aboveground tissues due to powdery mildew (family Erysiphaceae) and two-spotted spider mites (*Tetranychus urticae* Koch). To determine AMF colonization, roots and adhering rhizosphere soil were cut into small fragments and random subsamples of roots were then stored in 50 % ethanol for microscopic quantification of AMF using the same clearing and staining method as described above (Vierheilig *et al.* 1998). All measured traits are listed in Table 1.

Data analyses

Due to a contamination of control soil with AMF, one pot with *G. mollugo* was excluded from all analyses. The biomass data, morphological trait measurements, leaf damage estimates and AMF colonization were analysed using linear models. Plant survival and AMF presence/absence were analysed using analysis of deviance. The results were summarized in analysis of variance (ANOVA) and deviance (ANDEV) tables (McCullagh & Nelder, 1998; Schmid *et al.*, 2017). The explanatory terms of the models were block, plant history (monoculture-type vs. mixture-type), soil treatments (four soil treatments or sequence of the following three orthogonal contrasts: control vs. AMF treatments, *R. irregulare* vs. monoculture or mixture AMF and monoculture vs. mixture AMF), species and interactions of these. Statistical analyses were conducted using the software product R, version 3.0.2 (R Core Team, 2013).

Results

Influence of soil treatments on plant functional traits (hypothesis 1: AMF effects)

The four different soil treatments changed the performance and functional traits of the studied plant individuals but the response varied between the five studied species. We found significant effects of the soil treatment on five traits (Fig. 2, Table 2, interaction ST with Species): plant aboveground biomass at the first harvest, plant aboveground biomass at the second harvest, plant height, LDMC and AMF colonization.

The effects of the soil treatments on plant biomass were relatively consistent between the two harvests (Fig. 2). Biomass was highest in the treatment with *R. irregulare*, with the exception of *V. chamaedrys*, for which the control soil lacking any AMF resulted in the highest biomass. Soil containing AMF from mixture plots was the second best soil in terms of plant biomass, again with the exception of *V. chamaedrys* and also *P. lanceolata* exhibited higher biomass in soil containing AMF from monoculture plots. The effects of the soil treatments on plant height depended on the species. For example, in *G. mollugo*, soil

containing *R. irregulare* or soil containing mixture AMF resulted in the tallest plants. For three species, *L. pratensis*, *P. lanceolata* and *P. vulgaris*, the soil treatment did not have a strong effect on plant height, except that the control soil resulted in the shortest plants. In line with the biomass results, plant height was increased in control soil for the small herb *V. chamaedrys*. LDMC was strongly increased in control soil for *L. pratensis* and slightly increased for *G. mollugo*. For the three small herbs, LDMC was increased for the two soil treatments containing AMF from either mixture or monoculture plots as opposed to soil treatments without AMF or with the single species *R. irregulare*.

AMF colonization was highest in the *R. irregulare* treatment for all five studied species. For two species, *G. mollugo* and *L. pratensis*, AMF colonization was higher for soil containing monoculture AMF, whereas for two other species (*P. lanceolata* and *V. chamaedrys*) the opposite was the case and mixture AMF showed a higher AMF colonization. In *P. vulgaris*, AMF colonization was equally low for both treatments. Because the control soil did not contain AMF, this treatment was excluded from the analysis of AMF colonization. An increase in AMF colonization was positively correlated with an increase in individual plant biomass for all three AMF treatments (Fig. S1).

Influence of plant selection history on plant functional traits (hypothesis 2)

A selection history in either monoculture or mixture resulted in differential responses between individuals of the same species. However, these responses again varied between the five studied species. We found significant effects of plant selection history on seven traits (Fig. 3, Table 2, interaction PH with Species): plant survival, plant aboveground biomass at the first harvest, plant aboveground biomass at the second harvest, plant height, LMA, LDMC and leaf damage. Mixture-type plants survived better in the case of the three small herbs whereas for the legume *L. pratensis* and the tall herb *G. mollugo*, monoculture-type plants showed a higher survival (Fig. 3). Plant biomass was generally higher for mixture-type plants (Fig. 3), but *L. pratensis* and *G. mollugo* showed the opposite pattern with increased biomass for monoculture-type plants (for *L. pratensis* only at the first harvest). The difference in biomass production between monoculture-and mixture-type plants was smaller at the second harvest but still varied significantly among the different species (Fig. 3).

Mixture-type plants grew taller than monoculture-type plants in three out of five species. Monoculture-type plants of the other two species, *L. pratensis* and of *P. lanceolata*, grew taller than their mixture-type counterparts. The leaf traits LDMC and LMA were generally increased in mixture-type plants, except for *V. chamaedrys*. However, mixture-type

plants had more damaged leaves than monoculture-type plants (in particular plant individuals of *L. pratensis* and *P. lanceolata*). For plant height, LMA, leaf damage and leaf absorbance the plant history effect was also visible across species (Table 2, main effect of PH).

Evidence for co-adaptation between AMF and plants (hypothesis 3)

To test for potential co-adaptation between AMF and plants, we compared "home"-combinations (same selection history for both partners) with "away"-combinations (different selection history for the two partners). We found such effects for two traits: LMA and leaf absorbance (Fig. 4). Again, these effects varied between the five species studied and, in addition, differed in direction.

The home-combination between mixture-type plants and mixture AMF (first bar in the panels in Fig. 4a) increased LMA in four out of five species, the exception being *V. chamaedrys*. The home-combination between monoculture-type plants and monoculture-AMF (fourth bar in Fig. 4a) only increased LMA in *P. vulgaris*. Both away-combinations increased LMA strongly in *V. chamaedrys* (second and third bar in Fig. 4a) and the away-combination between mixture-type plants and monoculture AMF increased LMA for *L. pratensis* (third bar in the panel in Fig. 4a). Both home-combinations (first and fourth bar in Fig. 4b) increased leaf absorbance in the small herbs *P. vulgaris* and *V. chamaedrys*. In contrast, both home-combinations reduced leaf absorbance for the tall herb *G. mollugo* and the legume *L. pratensis*.

Discussion

Our results provide mixed answers to our first hypothesis that AMF isolated from multi-species plant communities should be more beneficial to plants than AMF isolated from plant monocultures. As expected, we found significant effects of the soil treatments on plant performance and traits. However, mixture AMF were only more beneficial than monoculture AMF for the taller species *G. mollugo* and *L. pratensis* but not for the three shorter species *P. lanceolata*, *P. vulgaris* and *V. chamaedrys* (see Fig. 2). These different species-specific responses are in line with other studies showing context-dependent AMF effects on plants (Burrows & Pfleger, 2002; Hoeksema *et al.*, 2010,). All studied plant species except *V. chamaedrys* showed increased biomass production in the presence of AMF; and across species plant biomass generally increased with increasing AMF colonization (Fig. S1). Interestingly, however, mixture AMF showed lower colonization than monoculture AMF in the plant species that benefited from them and vice versa in the other three species. Although

AMF generally promote plant growth, the outcome of the interaction may vary from mutualism to parasitism (Johnson *et al.*, 1997; Klironomos, 2003; Kiers & Van Der Heijden, 2006; Argüello, 2013). That mixture AMF were more beneficial for species with low than with high colonization could indicate that a shift from mutualism to parasitism occurred at higher colonization. Colonization by the "control" AMF *R. irregulare*, which did not share a common history with the experimental plants, was greater than mixture or monoculture AMF and led to higher plant biomass especially at the first harvest. A high colonization ability of *R. irregulare* has previously been observed (Hart & Reader, 2002; Engelmoer *et al.*, 2014). The soil treatment with *R. irregulare* differed from the other AMF treatments also with respect to AMF diversity: inoculum with *R. irregulare* represented a single AMF species whereas monoculture and mixture AMF inocula likely included several AMF species. Their lower colonization could thus have been due to competition between AMF species for a single host plant (Engelmoer *et al.*, 2014).

Our second hypothesis, that mixture-type plants grow faster whereas monoculture-type plants are better defended was generally supported by our experiment (see significant main effects of plant history in Table 2). But here again the different plant species varied in their responses. Mixture-type plants of three species were more productive and mixture-type plants of three species grew taller than monoculture-type plants (see Fig. 3). Furthermore, three out of the five species showed higher LDMC and four species higher LMA for mixture-type plants in comparison with monoculture-type plants, indicating that mixture-type plants also invested more resources into leaf biomass production. In contrast, monoculture-type plants had less leaf damage, which confirmed the second part of our hypothesis 2, namely that monoculture-type plants evolved increased defence. We observed particularly severe infections by powdery mildew in mixture-type plants of *P. lanceolata*, suggesting, in agreement with Engelmoer et al. (2014), that monoculture-type plants of *P. lanceolata* may have been subjected to particularly strong selection pressure for pathogen defence in comparison with mixture-type plants. The increased resource investment into leaves in mixture-type plants thus may have been at the cost of lowered pathogen defence. Specialized pathogens have indeed been observed to become diluted at higher plant diversity levels in the Jena Experiment (Eisenhauer *et al.*, 2012; Rottstock *et al.*, 2014), thus conferring lower selection pressure in plant mixtures than in monocultures. To increase survival, plants in monocultures are expected to allocate more resources to defence (Bezemer & van Dam, 2005) or may enter more beneficial symbioses (Newsham *et al.*, 1995). Consequently,

survival in plant monocultures may depend on the ability of the plant to allocate resources to these interactions whereas plants in mixtures can allocate these resources to growth.

Our third hypothesis was that co-adaption between plants and their associated AMF after a common selection history in the field strengthens the plant–AMF mutualism. For two plant traits, both related to leaf properties, we found some evidence of co-adaptation between monoculture-type plants and monoculture AMF and between mixture-type plants and mixture AMF. Namely, leaf absorbance and LMA were increased in the small herb *P. vulgaris* if the AMF were co-selected. Increased leaf absorbance and high LMA are related to higher area-based nitrogen content (Niinemets, 1997; Moran *et al.*, 2000), suggesting that co-selected AMF may have improved the nitrogen uptake of these plants. The opposite response was observed for leaf absorbance in *L. pratensis* and for LMA in *V. chamaedrys*. In these two cases, co-adaptation between plants and AMF was rather detrimental (see Fig 4), suggesting that the latter became more parasitic over the eleven years of co-selection history (see Methods). We speculate that this might have been due to faster evolution in the AMF than the plant. Comparative field studies that have examined evidence for AMF adaptation without selection imposed by an experimental setting yielded controversial results. Two studies focusing on the AMF found support for co-adaption (Weinbaum *et al.*, 1996; Pánková *et al.*, 2014a). However, other research (Pánková *et al.*, 2014b, Lambert *et al.*, 1980) did not find any evidence for co-adaptation, but rather an adaptation of the AMF or the plant to local soil conditions. Indeed, a reciprocal inoculation experiment (Johnson *et al.*, 2010) and a recent meta-analysis (Rúa *et al.*, 2016) suggested the variability in the outcomes of such studies was influenced by the soil in which the co-adaptation was tested. In line with these findings, our study showed that co-selection of plants and AMF did not generally result in more beneficial associations.

Conclusions

Here we developed an experimental procedure to test for co-adaptation of AMF and plants in plant monocultures and mixtures in a biodiversity experiment. We found that AMF selected in plant mixtures were more beneficial than AMF selected in plant monocultures for two out of five plant species. Furthermore, plants selected in mixtures generally grew better but suffered more leaf damage than plants selected in monocultures. However, cases of co-adaptation between AMF and plants from mixtures or AMF and plants form monocultures were rare and when they did occur they were generally in the direction of increased parasitism by the AMF, leading to reduced performance of the plant partner. This suggests

that co-adaptation between plants and AMF in plant biodiversity experiments does not follow a general pattern leading to increased mutualism but rather is context-dependent and more resembles an arms race in which sometimes the outcome may be reduced mutualism, depending on the plant functional group or species involved.

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Author contributions

T.H., C.W., S.J.V.M., D.Z.D and B.S. planned and designed the research, T.H. carried 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, T.H., C.W. and B.S. wrote the manuscript with the other authors contributing to revisions.

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Supporting information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

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

Fig. S1 Aboveground biomass production of monoculture- and mixture-type plants in control soil in dependence of different levels of AMF colonization.

Table S1. Experimental design.

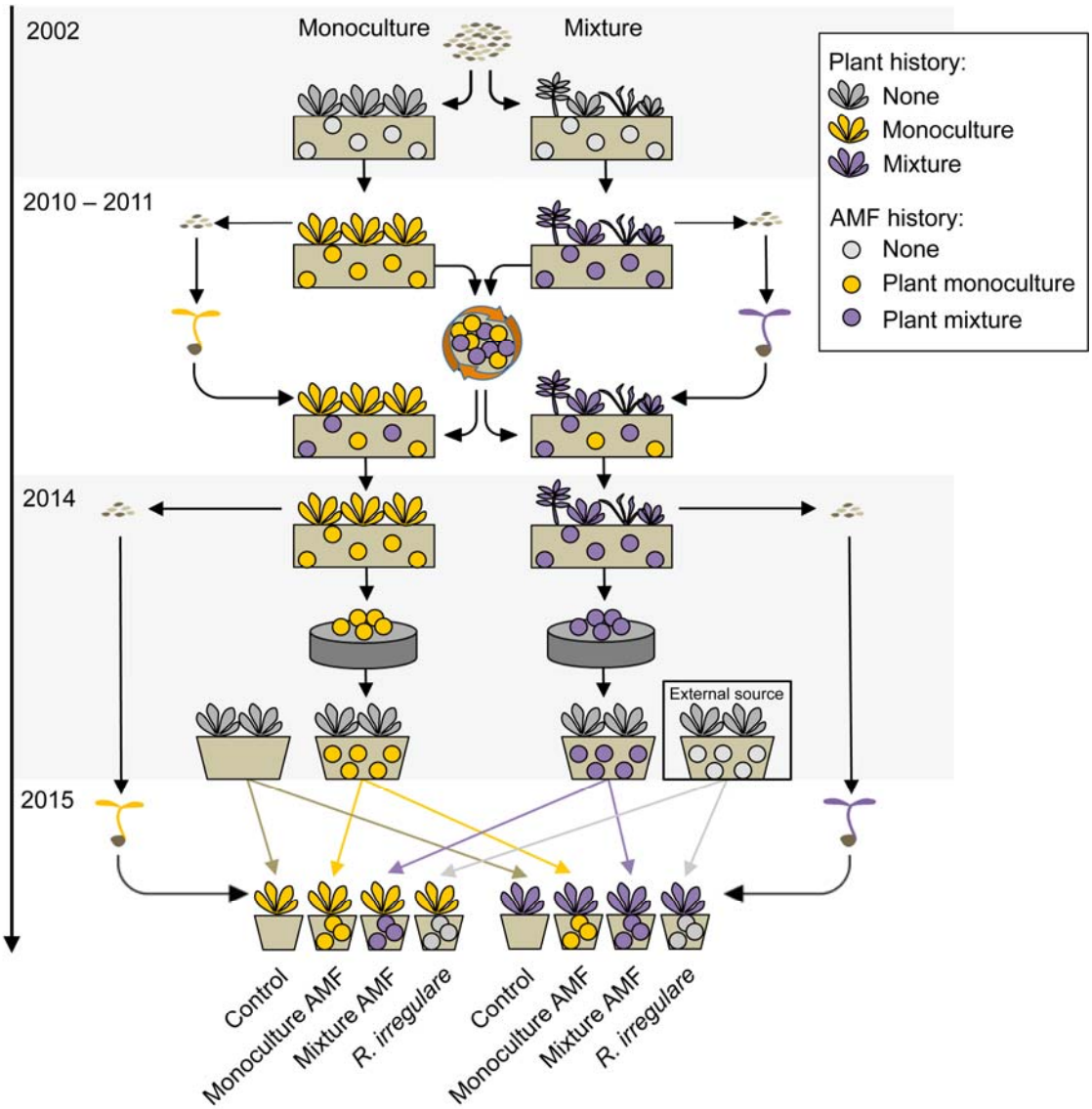

Fig. 1 Experimental design. Plant monocultures and mixtures in the Jena Experiment were sown in 2002 and maintained until 2010. In 2010, the plants of 48 plots underwent a first controlled seed production event and the soil of the plots was pooled, mixed and placed back to the excavated locations. In spring 2011, the seedlings produced were transplanted back to the mixed soil in the same plots from which their parents were excavated. The plant communities could then again associate with their own microbial communities potentially co-assembling and co-evolving until 2014. In spring 2014, the plants underwent a second controlled seed production event, and the AMF spores from their rhizosphere soil were isolated. The isolated AMF communities accumulated in trap-cultures for ten months with trap plants lacking a common selection history with the AMF spores. Control trap-cultures without AMF spores were established as negative control. Four soil treatments were used: 1) pots with sterile soil and 9 % inoculum without AMF, 2) inoculum of AMF isolated from

plants grown in monoculture, 3) inoculum of AMF isolated from plants grown in mixture and 4) inoculum containing *Rhizoglomus irregulare*. Finally, the plants with a selection history in either monoculture (monoculture-type plants) or mixture (mixture-type plants) were planted individually into the prepared pots.

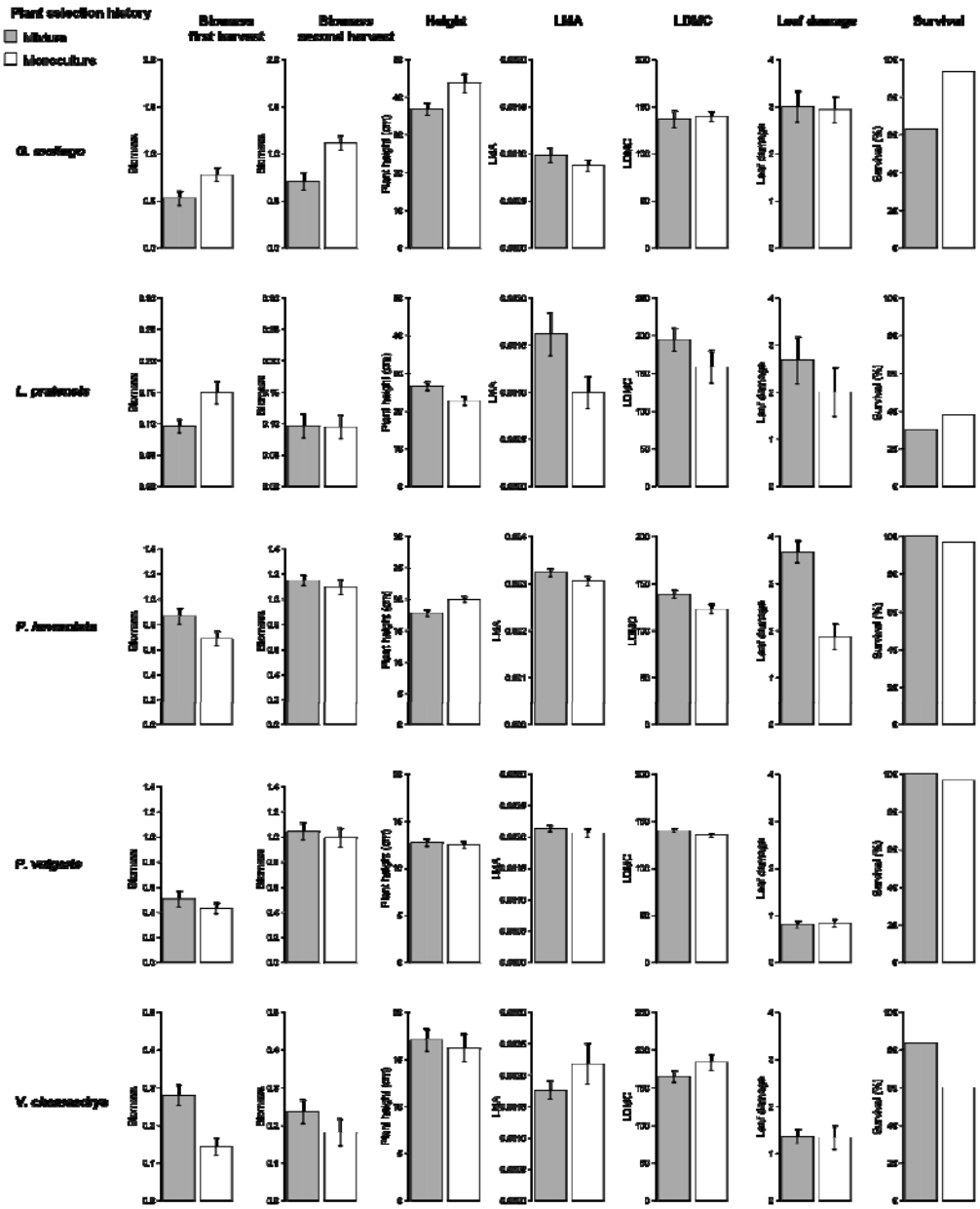

Fig. 2 Functional traits in response to the soil treatments for five species. Shown are means and standard errors calculated from raw data. See Table 2 for significance of effects.

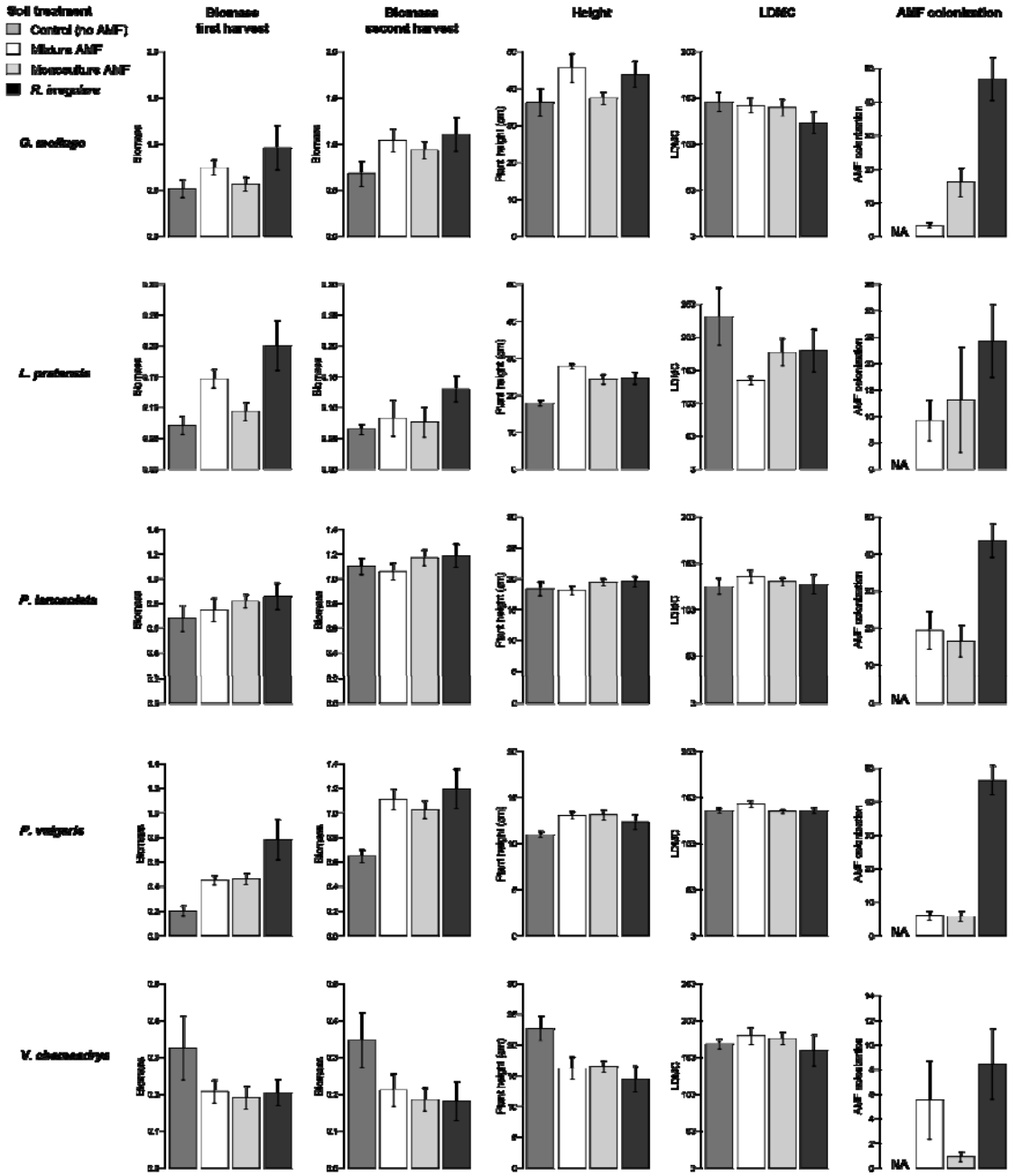

Fig. 3 Functional traits in response to plant selection history for five species. Grey bars refer to plants with a history of growing in species mixtures in the Jena Experiment, white bars refers to plants with a history of growing in monoculture at the same field site. Shown are means and standard errors calculated from raw data. See Table 2 for significance of effects.

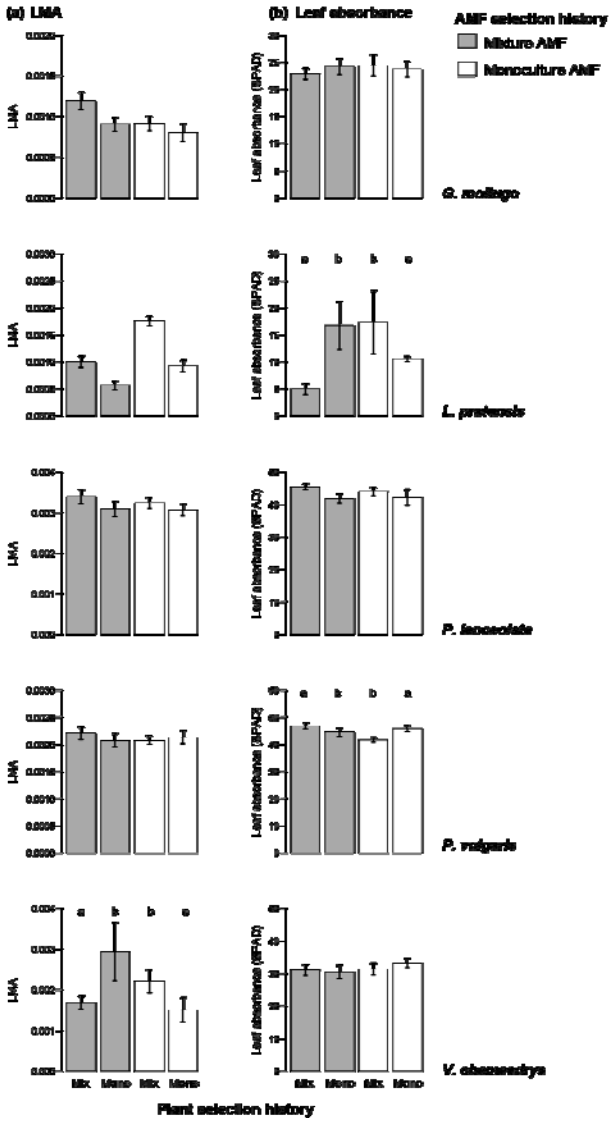

Fig. 4 (a) LMA and (b) leaf absorbance in response to home and away combinations of plant selection history and AMF selection history for five species. Grey bars refer to AMF from mixed plant communities; white bars refer to AMF from monocultures. The first and last bar of each plot corresponds to home combinations; the two middle bars correspond to away combinations. Shown are means and standard errors calculated from raw data. Significant home effects are indicated by letters "a" and "b" on top of bars: $P < 0.01$ for leaf absorbance in *L. pratense*, $P > 0.05$ for leaf absorbance in *P. vulgaris* and $P > 0.05$ for LMA in *V. chamaedrys*.

Table 1. Plant traits measured at the experimental plant age.

Measured plant trait	Unit	Plant age (weeks)
Survival	%	20
Aboveground biomass, first harvest	g dry weight/pot	12
Aboveground biomass, second harvest	g dry weight/pot	20
Maximum height	cm	19
Leaf damage	severity 0–5 (none to high)	20
Leaf absorbance	SPAD (0–50)	19
Leaf dry matter content (LDMC)	mg dry weight g^{-1} fresh weight	20
Leaf mass per area (LMA)	g dry weight/cm^2	20
AMF presence/absence	presence/absence	20
AMF colonization	%	20

Note: SPAD values are index values, defined by the manufacturer of the chlorophyll content measuring device, that indicate the relative amount of chlorophyll present in the leaf.

Table 2. ANOVA results for eight traits and ANDEV results for two traits (plant survival and AMF presence/absence data) in response to the design variables.

	Plant survival			Biomass first harvest			Biomass second harvest			Plant height			LMA			LDMC			Leaf damage			Leaf absorbance			AMF colonization			AMF presence		
Source of variation	***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***	**Df**	**% Dev**	***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	4	0.35	0.844
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	1	0.14	0.449
Soil 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	3	39.74	< 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	4	4.46	0.002
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	3	1.76	0.074
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	4	1.78	0.135
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	12	2.68	0.559
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	12	1.95	0.798
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		188	47.13	

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