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Diverse plant mixtures sustain a greater arbuscular mycorrhizal fungi spore viability than monocultures after 12 years

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Abstract

Aims: Intensive land management practices can compromise soil biodiversity, thus jeopardizing long-term soil productivity. Arbuscular mycorrhizal fungi (AMF) play a pivotal role in promoting soil productivity through obligate symbiotic associations with plants. However, it is not clear how properties of plant communities, especially species richness and composition influence the viability of AMF populations in soils.

Methods: Here we test whether monocultures of eight plant species from different plant functional groups, or a diverse mixture of plant species, maintain more viable AMF propagules. To address this question, we extracted AMF spores from 12-year old plant monocultures and mixtures and paired single AMF spores with single plants in a factorial design crossing AMF spore origin with plant species identity.

Important Findings: AMF spores from diverse plant mixtures were more successful at colonizing multiple plant species and plant individuals than AMF spores from plant monocultures. Furthermore, we found evidence that AMF spores originating from diverse mixtures more strongly increased biomass than AMF from monocultures in the legume *Trifolium repens* L. AMF viability and ability to interact with many plant species was greater when AMF spores originated from 12-year old mixtures than monocultures. Our results show for the first time that diverse plant communities can sustain AMF viability in soils and demonstrate the potential of diverse plant communities to maintain viable AMF propagules that are a key component to soil health and productivity.

Keywords: Aboveground–belowground interactions, biodiversity, biomass production, mutualism, plant–AMF interaction, root colonization

摘要：密集的土地管理可能损害土壤生物多样性，从而危害长期的土壤生产力。丛枝菌根真菌(AMF)通过与植物专一性的共生关系，在促进土壤生产力方面发挥着关键作用。然而

，目前尚不清楚植物群落的性质，特别是植物物种丰富度和组成如何影响土壤中AMF种群的生存能力。本研究中，我们测试了来自不同植物功能类群的8种植物的单一栽培和不同植物的混合种植，是否可以维持更有活力的AMF繁殖体。为了回答这个问题，我们从12年的单种植物和混种植物中提取AMF孢子，并通过因子设计将单株AMF孢子与单株植物配对，并考察与AMF孢子来源和植物物种特性的交互作用。研究结果表明，不同植物混合种植的AMF孢子比单一栽培的AMF孢子更能成功地定植于多种植物和植株个体中。此外，我们还发现，来自不同混合种植的AMF孢子比来自单一栽培的AMF更能显著提高白车轴草(*Trifolium repens* L.)的生物量。与单一栽培相比，起源于不同植物混合种植的AMF孢子的生存能力和与多种植物相互作用的能力更强。我们的研究结果首次表明，多样的植物群落可以维持土壤中AMF的活力，也证明了多样的植物群落有潜力维持AMF繁殖体的活力对土壤健康和生产力至关重要。

关键词：地上-地下相互作用，生物多样性，生物量生产，互惠共生，植物-丛枝菌根真菌相互作用，根系定植

Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the majority of terrestrial plants and influence plant community composition and productivity by enhancing the nutrient acquisition of plants in exchange for photosynthetically derived carbon (Smith and Read 2010; Van Der Heijden et al. 1998). However, it is now apparent that intensive land management practices such as frequent tilling, synthetic fertilizer, mono-cropping, and pesticide use can reduce the diversity and abundance of AMF within soils (Oehl et al. 2004; Verbruggen et al. 2010; Verbruggen and Kiers 2010). This is concerning as it has been shown that a greater diversity of AMF can enhance plant productivity, mediate plant–plant competition, and promote plant diversity (Montesinos-Navarro et al. 2019; Vogelsang et al. 2006; Wagg et al. 2011a, b). As a result, there is currently great interest in how AMF abundance and diversity may be promoted in soils to improve soil productivity. A growing number of studies have assessed whether inoculating soils directly with AMF spores can help re-establish AMF communities and promote plant growth. These studies have produced context-dependent results ranging from growth promotion to inhibition (Bender et al. 2016; Hart et al. 2015; Hijri 2016; Köhl et al. 2016). However, growing evidence suggests that the identity and diversity of plant species itself can also alter the composition of arbuscular mycorrhizal fungi in soils (Burrows and Pfleger 2002; Johnson et al. 2004; Mummey and Rillig 2006). Therefore, considering the intimate and long evolutionary relationship between AMF and their plant hosts (Bonneville et al. 2020), it seems likely that AMF abundance and diversity in soils could be promoted by particular plant species and plant diversity. However, whether particular species and plant diversity can increase the viability of AMF within soil has not been assessed.

Many biodiversity experiments have shown that plant communities are more productive with higher plant species richness (e.g. Balvanera et al. 2006; Hector et al. 1999; Marquard et al. 2009). Moreover, diverse mixtures maintain greater productivity over many years compared

87 to monocultures resulting in a strengthening of plant diversity–productivity relationships over
88 time (Cardinale et al. 2007; Meyer et al. 2018; Reich et al. 2012; Tilman et al. 2006). The
89 decline in monoculture productivity has been proposed to result from negative plant–soil
90 feedbacks, where plant monocultures accumulate specialist pathogens over time (Guerrero-
91 Ramírez et al. 2017; Kulmatiski et al. 2008; Meyer et al. 2018; Petermann et al. 2008; Schnitzer
92 et al. 2011). Conversely, diverse plant species mixtures are able to maintain greater
93 productivity because of their ability to dilute effects of plant species-specific pathogens,
94 enhance soil biodiversity, and promote beneficial soil organisms such as AMF (Eisenhauer et
95 al. 2012; Hiiesalu et al. 2014). However, some studies have shown no significant relationship
96 between plant species richness and soil biodiversity and in particular AMF communities
97 (Dassen et al. 2017; Schlatter et al. 2015). Therefore, the effects of plants on some soil
98 organisms like AMF may not be driven by diversity, but rather by the identity of the plant
99 species and their functional characteristics (De Deyn et al. 2011; Milcu et al. 2008; Scheublin
100 et al. 2004). For example, plant species with a taproot (Yang et al. 2015) or greater specific
101 root length (Cortois et al. 2016) exhibit a greater dependency on AMF associations for growth.
102 Moreover, aboveground characteristics, such as plant height, may play an important role: small
103 plant species in diverse plant communities may be strongly mycorrhizal dependent to avoid
104 being outcompeted for resources by larger plant species (Grime et al. 1987). Thus, the identity
105 of plant species associated with particular functional characteristics may also be fundamental
106 for promoting AMF. In summary, it is unclear if the viability of the soil AMF community can
107 be enhanced by greater plant diversity or the identity of particular plant hosts.
108 Here, we investigated whether different plant species monocultures or a diverse plant
109 mixture (sown with 60 plant species) differ in their ability to maintain a greater AMF potential
110 in their soils after 12 years of development. In the first phase of the experiment, we isolated
111 single spores of AMF from beneath eight plant monocultures (two legumes, two grasses, two

small herbs, and two tall herbs) and four 60-species plant communities, that occur within a long-term grassland biodiversity experiment (Jena Experiment) (Roscher et al. 2004; Weisser et al. 2017). In a climate chamber experiment we inoculated single plantlets of the same eight grassland species with single AMF spores extracted from soil of their own monoculture, monocultures of the other plant species, or the 60-species mixtures. In a second phase of the experiment, plants and their AMF were then allowed to establish in larger pots for a total of five months of growth. This approach allowed us to assess the viability of AMF (health of the AMF potential in soil) as well as the selectiveness of plants for forming associations with AMF originating from their own monoculture soil or soil from more diverse plant communities. We tested this by quantifying the establishment success of AMF spores to successfully establish colonization of plant roots (yes/no), akin to germination trials for assessing the viability of the seed bank in plant communities. We also determined if the inoculation of the single AMF spore originating from a monoculture or diverse plant community differed in its effect on seedling growth to test whether after 12 years of development different populations of AMF would have exerted varying effects on the biomass production of the plant communities.

Since diverse plant communities produce greater aboveground biomass, they can allocate more carbon to belowground organs that may support greater viability of AMF propagules. Thus, we hypothesized (1) that AMF spore viability is greater in plant mixtures than in plant monocultures as represented by a greater establishment success of AMF spores with individual plants of different species and their ability to promote aboveground plant biomass production. If AMF spores from diverse plant mixtures are more viable than those from plant monocultures, because the plant mixtures are more productive and provide more resources belowground to support a greater AMF viability, we expect that (2) the viability of AMF spores should positively relate to the productivity of the plant communities under which the AMF spores originated.

Material and Methods

Field experiment and model plant species

The Jena Experiment is a long-term biodiversity experiment in which the role of plant species diversity for element cycling and trophic interaction in grassland communities is investigated (Roscher et al. 2004). The study site is located on the floodplain of the Saale river on the outskirts of the city of Jena (Thuringia, Germany, 50° 55′N, 11° 35′E, 130 m a.s.l.). The region around Jena has a mean annual air temperature of 9.9°C and annual precipitation of 660 mm (1980–2010) (Hoffmann et al. 2014). The Jena Experiment was established on a former agricultural field. The soil of the experimental site was classified as a Eutric Fluvisol developed from up to 2-m thick fluvial sediments. In 2002, 82 experimental communities were sown with different plant species richness (1, 2, 4, 8, 16, and 60) and plant functional group number (1, 2, 3, and 4) on plots of 20 x 20 m size (reduced to 6 x 6 m in 2010). The species in the pool of 60 native grassland plants was classified into small herbs (12 species), tall herbs (20 species), grasses (16 species), and legumes (12 species). These four functional groups were defined using a cluster analysis of functional traits (Roscher et al. 2004). Plots were mown and weeded twice a year to preserve the experimental species combinations. Plots did not receive any fertilization. Further information on design and setup of the Jena Experiment can be found in Roscher et al. (2004).

Eight plant species growing in monoculture and in 60-species mixtures were chosen for the inoculation experiment. These species were the grasses *Festuca pratensis* Huds. and *Poa pratensis* L., the legumes *Medicago x varia* Martyn and *Trifolium repens* L., the small herbs *Plantago lanceolata* L. and *Prunella vulgaris* L., and the tall herbs *Galium mollugo* ssp. *album* L., and *Crepis biennis* L. All used plant species commonly occur in Central European grassland and are facultative AMF host plants (Wang and Qiu 2006). Since plant-AMF establishment

may be species- and context-dependent, such that the mycorrhizal establishment in one plant species may occur but not in others, the use of eight different plant species in our experimental design also provides robustness to account for potential plant species-specific outcomes.

Soil and inoculum preparation

Starting in June 2014, three soil samples of each monoculture plot (N = 8) and replicates of the 60-species mixture (N = 4) were taken (one plot a day) using a soil corer (1 cm diameter, 10 cm depth). Soil samples were pooled per plot and stored at 4°C for a maximum of 12 h. Samples were sieved through a sieve with 5 mm mesh size to remove stones and coarse roots. Afterwards, 50 g soil of each sample was used to extract AMF spores following the centrifugation floatation method: samples were wet-sieved through a cascade of three sieves with 250, 100, and 32 µm mesh size. To isolate AMF spores from the residue of the sieves with 100 and 32 µm mesh size, a gradient-centrifugation method was used (Sieverding et al. 1991). Spores per plot were decanted in a 50 mL falcon tube, filled up with water and stored for a maximum of 12 h at 4°C (= 12 AMF samples). Background soil was prepared from a mix of soil from the Jena Experiment field site. Soil was sieved to 5 mm, mixed with sand and autoclaved for two hours at 120°C.

Climate chamber experiment

For the first phase of our experiment, seeds of the eight plant species were acquired from the same commercial supplier (Rieger-Hoffman GmBH, Blaufelden-Raboldshause, Germany), which also provided the seeds for original sowing of the Jena Experiment in 2002. Seeds were pre-germinated in petri dishes filled with moist sand at room temperature and natural light. To exclude contamination with AMF spores, seeds were surface-sterilized in a potassium hypochlorite solution (Eau de Javel composed of KClO mixed with KCl to water;

1:1) and washed with tap water before germination. AMF spores and seedlings were used for a two-phase climate chamber experiment as summarized in Figure 1. For the first phase, single seedlings at the stage of cotyledon emergence were transplanted into pipette tips (volume: 1 mL, length: 7 cm, upper diameter: 0.9 cm) filled to two-thirds with the background soil-sand (1:1) mixture. Then, a single AMF spore, randomly selected from one AMF sample, was manually collected with a pipet under a stereo microscope and transferred into the pipette tip near the root of the seedling. Afterwards, the remaining one-third soil-sand mixture was added. The origin of the single AMF spores was either from the respective monoculture plot of the conspecific plant species (MonoHome), from a different monoculture plot (MonoAway), or from a 60-species mixture plot (Mix). Per plant species, twelve seedlings were inoculated with a single MonoHome spore (N = 12), twelve seedlings with a single MonoAway spore from any other monoculture (N = 7 x 12 = 84), and twelve individuals with a single Mix spore from each of the four 60-species plots (N = 4 x 12 = 48). Seedlings inoculated with single AMF spores from the same field plot were collected in one pipette tip box (space for 96 pipette tips) to ensure that plants with AMF of same origin experienced similar environmental conditions. The design resulted in twelve boxes – eight monoculture boxes, each containing one plant species cultivated with its "home" AMF spores (MonoHome) and seven plant species cultivated with "away" AMF spores (MonoAway), and four mixture boxes containing the eight plant species cultivated with spores originated from one of the four 60-species mixture plots. For example, a box with AMF spores extracted from the *P. lanceolata* monoculture contained 12 MonoHome plants of *P. lanceolata* individuals, and 84 MonoAway plants of the other seven species (96 plants and 96 AMF spores); and a box with AMF spores from a plant mixture also contained 12 individuals (Mix plants) of each of the eight plant species, totaling 96 plants and 96 AMF spores. The boxes were cultivated in a climate chamber (with 15 h day at 23°C, and 9 h night at 15°C) for one month. Plants were watered every day by spraying with tap water.

For the second phase of our experiment, we transplanted the plants individually into pots (volume: 0.32 L, height: 7.2 cm, upper diameter: 9.0 cm) filled with 150 g of the sterile background soil-sand (1:3) mixture. However, because of the limited space in the climate chamber, we were not able to transplant all plants (N = 1,152). Therefore, we decided to transplant nine seedlings of each plant species inoculated with AMF spores of their own monoculture plots (MonoHome; N = 8 x 9 = 72) and twelve seedlings of each plant species inoculated with AMF spores originating from four replicates of the 60-species mixture plots (Mix; three individuals per 60-species plot; N = 8 x 4 x 3 = 96). Potted plants were grown for four additional months (five months in total) in the climate chamber (with 15 h day at 23°C, and 9 h night at 15°C), and the position of pots was randomized once a month. Plants were watered every day by spraying with tap water and were fertilized three times (8, 10, and 12 weeks after replanting). The fertilizer was a nutrient solution consisting of 0.066 g K_2SO_4, 0.021 g NH_4NO_3, 0.038 g $MgSO_4$, and 0.009 g $CaHPO_4$ per pot and fertilization event. Because of poor plant performance following transplanting, we repeated the second phase of the experiment in September 2014 for three plant species (*P. lanceolata* and both grasses) to compensate for those that did not initially establish well. For each plant species, ten seedlings inoculated with spores originating from their own monoculture plot (MonoHome) and eight seedlings inoculated with spores from a 60-species mixture plot (from two 60-species plots, respectively) were prepared in one pipette tip box and treated as explained above.

Data collection

Aboveground biomass was harvested after one month for plants in pipette tips that were not transplanted into larger pots ($N_{MonoAway}$ = 651; $N_{MonoHome}$ = 24; N_{Mix} = 284). The remaining plants that were transplanted to larger pots were harvested for aboveground biomass after an additional four months ($N_{MonoHome}$ = 73; N_{Mix} = 97). Shoots were dried for 48 h at 70°C and

237 weighed. Roots of plants were washed and stored in 70% ethanol. To assess the success of
238 AMF establishment, plant roots were stained following the methods of Vierheilig et al. (1998).
239 Roots were cleared by heating them in 10% KOH at 70°C for 90 to 180 min (times differed by
240 plant species) and then for 5 min at 70°C in an ink-vinegar solution (5% black ink: Parker
241 S0037460 Quink Black; 95% vinegar: white household vinegar, 5% acetic acid). Roots were
242 rinsed with water several times and stored in tap water with some vinegar to remove excess
243 stain. Roots were cut into smaller fragments and mounted on microscope slides to assess AMF
244 establishment success (yes/no colonization of plant roots).
245 To assess hypothesis (2), stating that the viability of AMF spores should be positively
246 related to the productivity of the plant communities under which the AMF spores originated,
247 aboveground biomass in the field was harvested in late May and late August 2014 using four
248 0.2 x 0.5 m randomly placed quadrats. Plants were harvested 3 cm above the soil surface.
249 Biomass was sorted into the focal plant species, dried at 70°C for 48 h and weighed. The annual
250 aboveground biomass production of focal plant species in 2014 was calculated as the sum of
251 the two biomass harvests per plant species (after averaging the four subsamples per plot). As
252 an additional metric of growth and productivity, we also recorded the height of each plant
253 species in the field using the average of measurements of five randomly chosen individuals in
254 two or three replicates of the 60-species mixture plots (Gubsch et al. 2011; Lipowsky et al.
255 2015; Roscher et al. 2011a).
256
257
258 *Statistical analysis*
259 In order to test our first hypothesis as to whether different plant species in monocultures
260 varied in their ability to maintain greater AMF spore viability and whether this differed from
261 the diverse plant species mixture, we used generalized linear mixed-effects models with a logit-

link function, and AMF establishment success (yes/no) modelled as a binary response variable. We compared the establishment success of Mix AMF with both MonoAway and MonoHome AMF in two separate models. Block (plot of AMF origin / box) was added as a random effect in both comparisons. Using the respective random-effects structure, we started with a null model with the random term only and compared it with models in which we added the following fixed effects in this order: plant species identity, AMF treatment (MonoAway vs. Mix or MonoHome vs. Mix), and the interaction of plant species identity and AMF treatment. Models were fitted with maximum likelihood (ML), and likelihood ratio tests were used to decide on the significance of the fixed effects. For both comparisons, we used AMF establishment success of the first and second phase of the climate chamber experiment; however, because of no colonization at all in grasses, we removed *P. pratensis* and *F. pratensis* from analyses.

For plant species-level analyses of AMF establishment success and to account for differences in sample size between monocultures and mixtures, we used a binomial null model to test for significant differences in AMF establishment success among treatments (i.e. probability analysis). This procedure was necessary because of the low sample size in some treatments (MonoHome), which precluded the use of more sophisticated methods. For monocultures, we calculated the cumulative probability of the observed number of successful colonization events, given the total number of monoculture plantlets and the fraction of colonization events that were successful in mixtures (i.e. pbinom (success of AMF_{mono}, total number of plant individuals with AMF_{mono}, $P_{mix}=k_{mix}/n_{mix}$)). Given a cumulative probability of less than 0.025, the test indicates that establishment success of monoculture AMF spores was significantly lower than that of mixture AMF spores; by contrast, if the cumulative probability was greater than 0.975, the test indicated that establishment success of monoculture AMF spores was significantly higher (i.e. a two-tailed test). One exception to this interpretation are

cases with zero colonization in mixtures, as under these circumstances the expected variance of a binomial distribution is zero. However, observed AMF establishment success in monocultures was also zero in these cases, suggesting no meaningful difference between treatments. Finally, to test whether mixture or monoculture AMF spores colonized more plant species, we used an independent samples t-test to compare average number of colonized plant species (independent of how many plant individuals were colonized) with monoculture spores and mixture spores from first and second phase.

To test whether plants inoculated with single AMF spores originating from a diverse plant mixture produced more biomass than plants inoculated with single monoculture spores, we again used linear mixed-effects models. Biomass production was square-root transformed to meet the assumptions of normality and variance homogeneity. Again, we compared plants with Mix AMF with plants with MonoAway and MonoHome AMF, respectively, and used block (plot of AMF origin / box) as random term (grasses were again excluded). However, unlike for our binomial models, we used only the plant biomass of the first phase of the experiment for the comparison of MonoAway vs. Mix (plants in pipette tip boxes), because we transplanted no MonoAway plants into pots, and only used the plant biomass of the second phase for the comparison of MonoHome vs. Mix (plants in pots), because only a few MonoHome plants per plant species (one to three individuals) were not transplanted. Further modeling of fixed effects was done as described for assessing AMF establishment success. For plant species-level analyses, we tested for differences in biomass of plants inoculated with mixture or monoculture spores, based on the effect size mean and standard error for each treatment and plant species estimated from the fitted models. A p-value less than 0.025 indicates that biomass production was significantly lower when inoculated with monoculture AMF than with mixture AMF, while a p-value higher than 0.975 indicates that biomass

311 production of plants with monoculture AMF spores was significantly higher than with mixture
312 AMF.

313 One important note regarding these analyses is that our study followed a blocked
314 treatment design. In most cases, we replicated the treatment across multiple blocks, allowing
315 separation of block-level vs. treatment-level effects (i.e. confounding effects caused by
316 differences among boxes, vs. ecologically meaningful effects of the monoculture vs. mixture
317 treatment). However, because each block represented a single origin soil type (research plot),
318 we were only able to test the 'home' effect on monocultures of individual plant species within
319 individual blocks, meaning that the 'block' level effect is potentially confounded with the
320 treatment effect for these tests. However, because blocks were randomized with respect to soil
321 origin, and because the boxes were all presumably identical prior to soil application, and were
322 held within the same, controlled conditions for the duration of the experiment, there is no a
323 priori reason to believe that this caused a bias in our estimates, although it does reduce the
324 power of our analyses.

325 To test our hypothesis (2) concerning the positive correlation between viability of AMF
326 spores and the total aboveground biomass produced by the plant communities from which the
327 AMF spores originated, we used correlation analyses comparing AMF establishment success
328 (colonization percentage) against community biomass production in the field. Additionally we
329 assessed whether the establishment success of AMF spores was related to individual plant
330 species shoot biomass and height in mixtures by correlating the establishment success of Mix
331 AMF spores (colonization percentage) and plant species-level biomass production in the field
332 (averaged over the four 60-species mixtures) as well as the average height of the respective
333 plant species in the 60-species mixture plots (excluding grasses, because of no colonization
334 occurred in our climate chamber experiment and no biomass was produced by these grasses in
335 the 60-species mixtures in the field). All calculations and statistical analyses were done in R

(version 3.6.1, R Development Core Team, http://www.R-project.org) including the package *lme4* (glmer and lmer) (Bates et al. 2013).

Results

AMF spore establishment success

In general, the establishment success of single AMF spores was low (Table 1), but nonetheless more plant species were colonized when the spores originated from diverse plant species mixtures (4.0 ± 0.4 (SE) plant species) than from plant monocultures (1.6 ± 0.5 plant species; t-test: t = -2.2439, df = 10, p-value = 0.049). Establishment success of AMF spores strongly differed among plant species. We did not find any successful colonization with single AMF spores in the two grass species (*F. pratensis, P. pratensis*). Both small herbs (*P. lanceolata*, *P. vulgaris*) and the legume *T. repens* showed relatively high colonization with AMF spores (10–14%), while the number of colonized plants was low for both tall herbs (*C. biennis*, *G. mollugo*) and the legume *M. x varia* (2–5%; Table 1).

On AMF treatment level, establishment success of AMF spores originating from mixtures varied between 4% (*C. biennis*) and 21% (*T. repens*; Table 1). When seedlings were inoculated with AMF spores from their own monoculture (MonoHome), colonization was not successful in five out of six plant species, but reached 40% in *P. lanceolata* (Table 1). When seedlings were inoculated with AMF spores from a different monoculture (MonoAway), all herb and legume species showed successful colonization. AMF establishment success varied between 1% in *G. mollugo* and *C. biennis*, respectively, and 8% in *P. lanceolata* (Table 1).

The establishment success of MonoAway and Mix AMF spores were significantly different among plant species and AMF treatments (Table 2) – AMF spores from diverse mixtures (Mix) had a higher success in colonizing plant individuals than AMF spores from a non-conspecific monoculture (MonoAway). The plant species-level analysis of MonoAway vs.

361 Mix comparisons (probability analysis) showed the same pattern in *P. vulgaris* and *T. repens*
362 (significant) and in *G. mollugo, M. x varia,* and *P. lanceolata* (marginally significant; Table 3).
363 In both tall herbs, we found no significant difference of establishment success between
364 MonoAway and Mix AMF spores (low success in both groups; grasses had no colonization at
365 all).

366 The establishment success of MonoHome vs. Mix AMF spores was significantly
367 different among plant species; however, in contrast to MonoAway vs. Mix comparison, we
368 found no significant influence of AMF treatments, but a significant influence of the interaction
369 of plant species identity and AMF treatments (Table 2). Results for the plant species-level
370 comparison of MonoHome vs. Mix (probability analysis) supported this by showing on the one
371 hand higher establishment success of Mix AMF spores in *T. repens* and *P. vulgaris* (marginally
372 significant), and on the other hand higher establishment success of MonoHome AMF spores in
373 *P. lanceolata* (Table 3). In three out of six plant species (the two tall herbs and *M. x varia*), we
374 found no difference in establishment success of Mix and MonoHome AMF spores (low success
375 in both groups; Table 3).

376

377 *Effects of AMF on shoot biomass*

378 Both comparisons, MonoAway vs. Mix and MonoHome vs. Mix, revealed that plant
379 species identity and the interaction of plant species identity and AMF treatment significantly
380 influenced the biomass performance of the plants (Table 2). Three out of six plant species
381 differed in biomass production when treated with MonoAway or Mix AMF spores – both
382 legumes (*T. repens, M. x varia*) tended to produce more biomass with AMF spores from diverse
383 mixtures, while the small herb species *P. lanceolata* tended to produce more biomass with
384 AMF spores from other monocultures (Table 3; Figure 2a). Both legumes also differed in
385 biomass production, when treated with MonoHome or Mix AMF spores – *T. repens* produced

more biomass when inoculated with AMF spores from diverse mixtures, while *M. x varia* had higher biomass production with AMF spores from the own monoculture (Table 3; Figure 2b). Other plant species (tall herbs, small herbs) showed no significant difference in biomass production when treated with MonoHome or Mix AMF spores (Table 3; Figure 2b).

AMF spore viability and associations with plant community and species productivity from which they originated

The establishment success of AMF spores was positively correlated with biomass production of the plant community from under which the AMF spores originated (Fig. 3a). However, the success of AMF spores from the diverse plant mixture was negatively correlated with shoot biomass and the average height of the respective plant species in these mixtures in the field experiment (Figs. 3b and 3c).

Discussion

There is a growing need to understand how to maintain and promote soil health, for which AMF are of key importance. Here we tested the hypothesis that (1) AMF spore viability is greater in plant mixtures than in plant monocultures, as represented by a greater establishment success of AMF spores with individual plants of different species and their ability to promote aboveground plant biomass production. In support of this hypothesis, we found that although AMF spore colonization of plants varied among species, AMF spores from diverse plant mixtures were more likely to establish with a greater number of plant individuals from a higher number of plant species than AMF spores from plant monocultures. In addition, we also found that the ability for the AMF to not only establish colonization but also promote plant growth was species specific. Our results show strong evidence that the legume *T. repens* was able to establish associations more frequently with AMF spores from plant mixtures that

411 also promoted its ability to produce aboveground biomass than if AMF spores originated from
412 plant monocultures, including its own and different species monocultures. The other five plant
413 species showed no or inconsistent results. These findings indicate that diverse plant
414 communities are better able to support a more viable population of AMF spores than plant
415 monocultures. However, whether these AMF from diverse plant mixtures promote plant
416 productivity was species-specific and was only highly beneficial for the legume *T. repens*. This
417 confirms previous studies that found plant diversity can support various soil organisms
418 (Eisenhauer et al. 2013; Lange et al. 2015; Mellado-Vázquez et al. 2016; Scherber et al. 2010)
419 and that, although plants form AMF associations, the ability of the plant to benefit from AMF
420 associations is species specific (Cortois et al. 2016; Klironomos 2002) and may change over
421 time (Sendek et al. 2019).

422 Secondly, we hypothesized (2) that the viability of AMF spores should relate to the
423 attributes of the plant communities under which they have been conditioned for over a decade.
424 We found that the viability of AMF spores was positively related to the productivity of the
425 plant communities from under which they originated (i.e. more productive plant mixtures were
426 associated with a greater viability of AMF spores). However, at the plant species-level,
427 productivity and average height of the plants within mixtures was negatively associated to the
428 establishment success of AMF spores. This provides intriguing insights into the plant
429 community *versus* species-level effects on supporting a more viable AMF spore population.
430 The positive relationship would support the hypothesis that because diverse plant communities
431 are more productive, there is a greater allocation of carbon compounds belowground
432 (Eisenhauer et al. 2017; Lange et al. 2015; Mellado-Vázquez et al. 2016) that may support
433 greater AMF spore viability. However, at the species level, shorter plants that produce less
434 aboveground biomass were more related to AMF viability than taller, more productive plants.
435 This could suggest that these smaller plant species are more dependent on supporting their

436 AMF partners for soil resource competition with larger more productive plant species (Lin et
437 al. 2015).
438
439 *Plant species specific effects on AMF spore viability*
440 In this study, we showed for the first time that a more diverse plant community can
441 maintain greater AMF viability in soils in comparison to plant species monocultures, which
442 supports our first hypothesis, yet there was also strong evidence of species-specific effects.
443 Intriguingly, AMF spores originating from the monoculture of *P. lanceolata*, however, showed
444 highest AMF establishment success with *P. lanceolata* (40%). Based on the observed patterns,
445 we assume that plant species differently influence AMF spore viability and therefore can
446 influence the AMF potential in soils. The relatively high success of AMF spores from *P.*
447 *lanceolata* monocultures to establish with *P. lanceolata* is possibly due to the well-known
448 ability of this plant species to readily form AMF associations from which it benefits in the
449 ability to produce greater shoot biomass (Orłowska et al. 2012; Smith and Read 2010). The
450 lack of success of these spores to successfully establish with other plant species suggests that,
451 after 12 years, the AMF community conditioned by the *P. lanceolata* monoculture has become
452 strongly co-adapted to *P. lanceolata.* This co-adaptation between plant species and AMF was
453 also shown in previous studies (Johnson et al. 2010; Wagg et al. 2015).
454 In contrast to the high success of *P. lanceolata* AMF spores with *P. lanceolata*,
455 MonoHome spores of other plant species showed no colonization at all with their conspecific
456 plant species, suggesting that these plant species do not strongly interact with AMF when
457 growing in monoculture. Furthermore, both grasses, both tall herbs and the legume *M. x varia*
458 also showed no or low success in establishing associations with single AMF spores originating
459 from mixtures or other monocultures (MonoAway), suggesting that these plant species
460 generally did not strongly interact with AMF. The strong differences between the legumes *M.*

461 *x varia* (poor host) and *T. repens* (good host) indicate that plant species within functional
462 groups can strongly differ in AMF association with respect to specific characteristics of the
463 plants (e.g. growth height; will be discussed in the hypothesis 4 paragraph; Hahl et al. 2020).
464 The low AMF association of *P. pratensis* and *F. pratensis* is in line with several studies
465 showing that grasses are commonly poor hosts for AMF (Eisenhauer et al. 2009; Hokka et al.
466 2004; Scheublin et al. 2004, 2007).

467 Interestingly, *T. repens* and *P. vulgaris* were not able to successfully establish
468 associations with AMF spores from their own monocultures (MonoHome), although these
469 plant species showed the highest success rates of establishing associations with spores that
470 originated from the diverse plant mixtures. It is well known that both plant species associate
471 with and strongly positively respond to AMF (Benabdellah et al. 2011; Streitwolf-Engel et al.
472 1997; Van Der Heijden et al. 1998; Wagg et al. 2011b). Nevertheless, it is well known that
473 monocultures decline in productivity over time, which could lead to a continuous loss of
474 resource availability for AMF and thus AMF viability, explaining the low establishment
475 success of monoculture AMF even with mycorrhizal-dependent plant species (such as *T. repens*
476 and *P. vulgaris*). This is also supported by our finding of a positive association between the
477 establishment success of AMF spores and the aboveground biomass produced by the plant
478 communities from under which they originated.

479

480 *AMF spore effects on plant shoot biomass*

481 We found that one out of six plant species (*T. repens*) produced more biomass when
482 inoculated with AMF spores from diverse mixtures than from monocultures. This finding may
483 indicate that the strengthening positive diversity-productivity relationship over time in long-
484 term biodiversity studies (Reich et al. 2012; Tilman et al. 2006) is not only induced by an
485 accumulation of mutualists (Eisenhauer et al. 2012) but could also be fostered by an increased

viability. Furthermore, our results suggest that specific plant species (*T. repens*) are responsible for this increased viability, while for example grasses and tall herbs showed no or little interaction with AMF and thus may have limited influence on AMF spore viability.

A possible explanation for finding no difference of biomass production in *P. vulgaris* (and *P. lanceolata* plants in the second phase), despite high AMF establishment success, could be that our approach of inoculating only one single spore and thus the use of only one AMF individual of a specific AMF species caused this result. For instance, previous experiments have shown that the interaction of two or more AMF species increased plant productivity more than a single AMF species alone, which may be due to the insurance of greater species richness increasing the likelihood that some AMF species will establish and promote plant productivity (Van Der Heijden et al. 1998; Wagg et al. 2011a).

Contrary to our hypothesis, we also found some evidence for a greater promotion of biomass production by monoculture AMF spores than AMF spores from plant mixtures, but effects were also species specific. In the first phase of our experiment (MonoAway vs. Mix), *P. lanceolata* tended to produce more biomass with MonoAway spores, which could be further evidence that this plant species is specialized to AMF communities selected in monocultures. In the second phase of our experiment (MonoHome vs. Mix), *M. x varia* produced more biomass, when inoculated with AMF spores from its own monoculture. Thus, none of the MonoHome AMF spores successfully colonized *M. x varia* and only one out of 12 Mix AMF spores. Therefore, and because we found no consistent patterns in both plant species (and even opposing results for *M. x varia*), we think that differences in biomass performance are not induced by monoculture or mixture AMF; in contrast to *T. repens*, which was strongly colonized by AMF and showed a consistent pattern in both phases of the experiment.

Effects of plant community and species level productivity on AMF spore viability

We found strong evidence for our second hypothesis stating that there should be a positive association between the establishment success of AMF spores and the biomass production of the plant communities from which the AMF spores had originated. This strongly suggests that plant biomass is an important resource for maintaining the viability of AMF spores, perhaps due to an elevated availability of photosynthates and root exudates associated with increase biomass production (Eisenhauer et al. 2017; Lange et al. 2015).

In addition, we found that the number of plant individuals with successful colonization by mixture AMF was higher when biomass production of the plant species in mixture was low and their stature was small. This finding suggests that small-growing subordinate plant species interact more strongly with AMF in diverse mixtures than tall-growing dominant plant species. We discuss two possible mechanisms, which could be responsible for this phenomenon. First, small plant species could benefit more from the interaction with AMF than taller species that leads to their coexistence in plant mixtures (Grime et al. 1987; Lin et al. 2015). At the same time, AMF can be costly in nutrient-rich soils, which could lead, *inter alia*, to lower abundance of the small-growing plant species in mixtures. It has been shown that small-growing plant species change their growth strategy when growing in mixture by forming longer shoots, increasing biomass allocation to supporting tissue, and producing leaves with higher specific leaf area and higher chlorophyll concentrations to optimize carbon gain and tolerate shading by taller plants (Roscher et al. 2011a, b; Lipowsky et al. 2015). It is possible that smaller plant species interact more strongly with AMF in plant mixtures to facilitate this strategy. A study by Streitwolf-Engel et al. (1997) showed that mean total leaf area of *P. vulgaris* plants was increased from 0.48 cm^2 without AMF to 160.16 cm^2 with AMF after growing 115 days in a greenhouse. Therefore, the greater interaction of small plant species and AMF in mixtures could be explained by strategies avoiding the outcompeting by taller plant species. On the other side, several studies have shown that plant species richness has positive impacts on soil nutrient

levels (Lange et al. 2019; Oelmann et al. 2011). Consequently, plant species may, on average, be less dependent on the interaction with AMF in diverse mixtures, so that the mutualistic relationship could turn into an antagonistic one (Johnson et al. 1997; Johnson & Graham 2013). AMF-dependent small-growing plant species would then have a disadvantage in mixtures. Probably it is a combination of both mechanisms, allowing small subordinate plant species to exist in diverse mixtures, albeit with low abundance. This is partly in line with a meta-analysis by Lin et al. (2015) showing that subordinate species were relieved from competitive suppression in mixtures, when the dominant species were not AMF-dependent.

The negative relationship of AMF establishment success and height of the plants in the field could also explain the unexpected low interaction of the legume *M. x varia* with AMF found in the present study. *Medicago x varia* is, in contrast to *T. repens*, a tall-growing legume, which has a high competitiveness in mixture due to tall stature, the legume-specific interaction with rhizobacteria, and thus probably does not depend on the interaction with AMF in mixture as much relative to the small and non-legume species. Tall herb species (*G. mollugo*, *C. biennis*) also have a tall stature and reach the upper canopy levels, which enables a high competitiveness for light and probably no strong interaction with AMF as mutualists. While we found a clear negative correlation between plant stature and number of colonized individuals by mixture AMF, the correlation between successful colonization and biomass production in the 12-year old mixture plots was inconsistent for *M. x varia*, which showed lower biomass production in mixture than expected (as tall-stature plant species). This can be explained by the fact that this plant species strongly decreased in productivity after several highly productive years in mixture plots (Roscher et al. 2011c).

To address these assumptive mechanisms, we propose regarding our hypothesis that more studies are needed to compare small- vs. tall-growing life strategies in their competitive interactions when associated with AMF communities previously conditioned by a plant from

monoculture or mixture. In mixtures, AMF interact with several neighboring plant species, while cooperation is only possible for those plants, which can provide high concentrations of carbohydrates (Argüello et al. 2016; Kiers et al. 2011). Therefore, the beneficial cooperation of AMF originating from the mixture and single individuals of small-growing plant species could be lowered or disappear when a tall-growing plant species is present, because taller plants produce more carbohydrates that can be allocated to the AMF symbiosis and thus the taller plants may benefit more from the AMF association than smaller plants.

Our study shows that the viability of AMF spores is greater and will likely establish with a greater diversity of plant species if they originated from soils that had been conditioned by a diverse plant species mixture over the last decade than by a plant monoculture. Thereby, the different establishment success of AMF spores with plant species in our experiments suggests that plant species differ in their effect on the viability of the AMF community in monocultures and plant species-rich plant communities. This in turn may also contribute to explaining the coexistence of plant species in diverse species mixtures. However, it should be noted that different plant species acquire and support a different community composition of AMF taxa (Becklin et al. 2012; Dassen et al. 2017; Martínez-García and Pugnaire 2011; Scheublin et al. 2004; Yang et al. 2012). Thus, it should be considered that the greater viability of AMF spores from diverse plant mixtures may be due to the fact that diverse plant communities are able preferentially select AMF taxa that have particular life strategies that resulted in more viable spore population, which we observed in diverse plant mixtures. Further investigation is needed to assess the underlying mechanisms by which diverse plant communities produce more viable AMF spores than plant monocultures and whether the beneficial cooperation of these AMF and plants change, or disappear, when several plant species with different growth strategies co-occur together. Overall, our results provide some of

585 the first evidence that diverse plant communities can sustain AMF spore viability in soils,
586 which is a key component to soil health and productivity.
587
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803 **Figure captions**

804

805 **Fig. 1** Overview of the experiment. Details are provided in the Materials and methods section.
806 Plant monocultures (N = 8; different species) and plant mixtures (N = 4; 60-species
807 communities) were established in 2002. In 2014, AMF spores were extracted from soil of these
808 plots. AMF spores and seedlings of 8 plant species were used for a two-phase climate chamber
809 experiment. For the first phase, single seedlings were transplanted into pipette tips. Then, a
810 single AMF spore was transferred into the pipette tip. The origin of the single AMF spores was
811 either from the respective monoculture plot of the focal species (MonoHome), from a different
812 monoculture plot (MonoAway), or from a 60-species mixture plot (Mix). The plants were
813 cultivated in a climate chamber for one month. For the second phase, seedlings inoculated with
814 MonoHome AMF spores and Mix AMF spores were transplanted in pots. Potted plants were
815 grown for four additional months in a climate chamber.

816

817 **Fig. 2** Species-level results from the climate chamber experiment assessing growth-promoting
818 effects of AMF spores from plant monocultures or diverse plant mixtures. Shown is the relative
819 response in plant aboveground biomass when inoculated with a spore from a diverse plant
820 mixture relative to inoculation with a spore from a monoculture of either the plant species own
821 home monoculture (a) or the monoculture of different plant species (b). Points are means and
822 error bars are the 95% confidence intervals. Stars indicate significant differences among plants
823 with mixture or monoculture spores, dots indicate marginal significant differences.

824

825 **Fig. 3** Associations between the viability of AMF spores obtained in the climate chamber
826 experiment and the attributes of the plant communities, by which they had been conditioned
827 for over a decade in the field experiment. Shown are AMF establishment success (regardless
828 of species identity of host plant; colonization percentage) in relation to the community-level
829 aboveground biomass production in the plots of origin of the AMF spores (a), the establishment
830 success (colonization percentage) of AMF spores originating from the diverse plant mixture
831 with six plant species in the climate chamber experiment with aboveground biomass production
832 of these plant species in the diverse plant mixtures (b), and the establishment success
833 (colonization percentage) of AMF spores originating from the diverse mixtures and height of
834 the respective species in these mixtures (c). Note that no AMF spores were able to establish an
835 association with either of the two grass species. Therefore, we removed *P. pratensis* and *F.*

836 *pratensis* from mixture analyses (b, c). *R* indicates the Pearson's correlation coefficient and
837 associated level of significance (*p*).

838 **Tables**

839

840 **Table 1** Summary of AMF establishment success with eight plant species in four functional
841 groups (FG). Shown are number of replicates per experimental phase (Phase 1, Phase 2), AMF
842 establishment success (colonization percentage) for the different AMF spore treatments
843 (MonoHome, MonoAway, Mix) and total AMF establishment success (regardless of AMF
844 spore origin; colonization percentage).

845

		MonoHome spores			MonoAway spores			Mix spores			Total
FG	Species	Phase 1	Phase 2	%	Phase 1	Phase 2	%	Phase 1	Phase2	%	%
Grasses	*F. pratensis*	7	13	0	83	-	0	38	15	0	0
	P. pratensis	3	9	0	83	-	0	35	12	0	0
Legumes	*M. x varia*	3	8	0	76	-	3	34	12	9	5
	T. repens	3	8	0	82	-	5	36	11	21	10
Small herbs	*P. lanceolata*	4	11	40	84	-	8	34	14	15	14
	P. vulgaris	3	9	0	83	-	6	36	12	19	10
Tall herbs	*C. biennis*	3	9	0	79	-	1	36	10	4	2
	G. mollugo	1	6	0	81	-	1	35	11	7	3

846

847 **Table 2** Summary of mixed-effect model analyses testing the effects of species identity (N =
848 6), AMF treatment (origin of AMF, i.e. diverse mixture (Mix) or monoculture (MonoAway;
849 MonoHome)) and their interaction on AMF establishment success and biomass production of
850 plants. Fixed effects were added stepwise to an initial null model with block (plot of origin /
851 pipette tip box) as random effect. Likelihood ratio test (Chi^2) were used to decide on the
852 significance of the fixed effects. Shown are degrees of freedom (Df), Chi^2 and p-values (P).
853 Significant factors and interactions are given in bold.
854

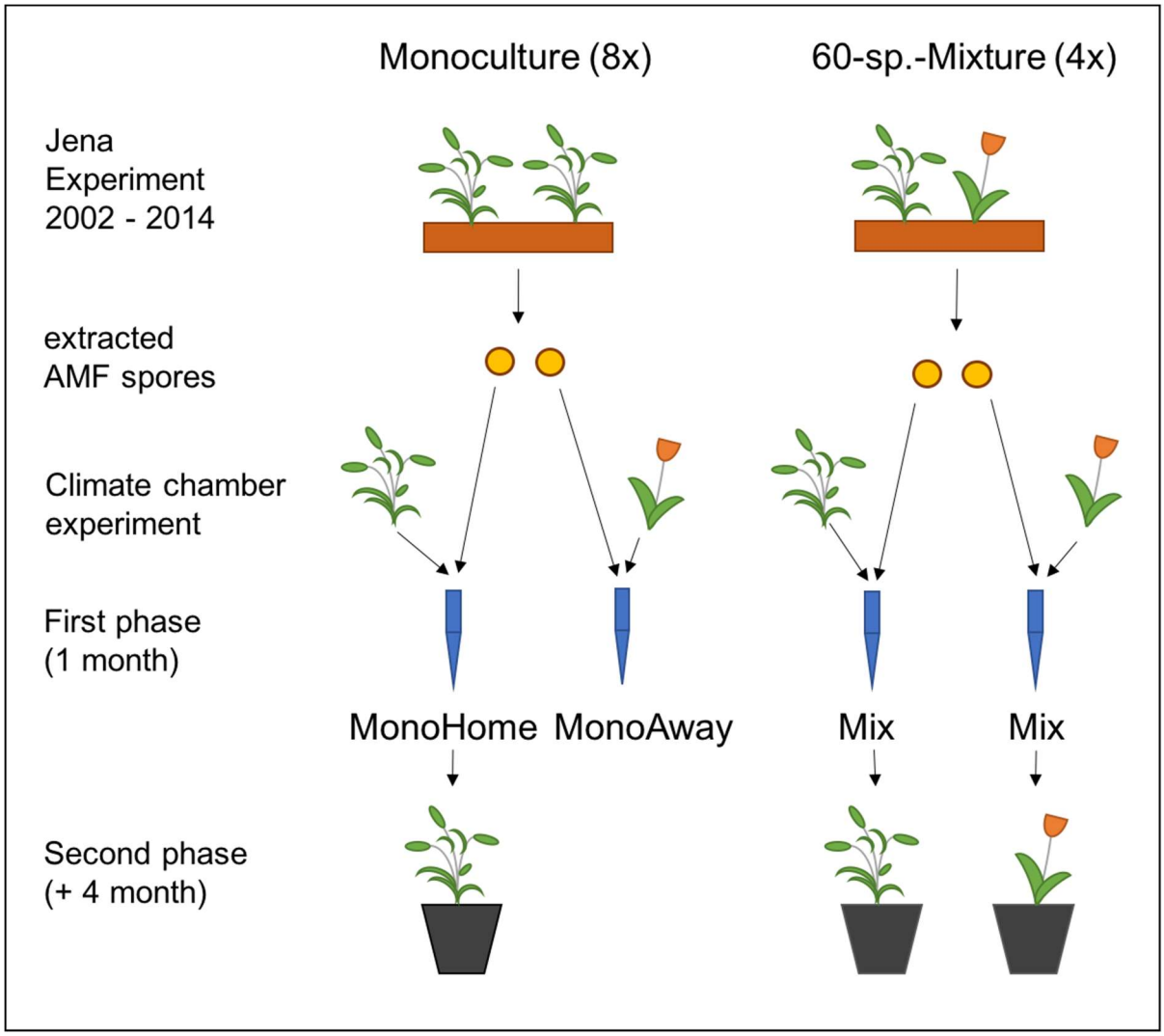
Response variable	Establishment success						Aboveground biomass production					
Comparison	MonoAway vs. Mix			MonoHome vs. Mix			MonoAway vs. Mix			MonoHome vs. Mix		
	Df	Chi^2	P	Df	Chi^2	P	Df	Chi^2	P	Df	Chi^2	P
Species identity	7	17.98	**0.003**	7	12.18	**0.032**	8	230.29	**<0.001**	8	76.35	**<0.001**
AMF treatment	8	6.07	**0.014**	8	0.43	0.512	9	0.41	0.522	9	0.02	0.886
Species x treatment	13	1.80	0.876	13	12.10	**0.034**	14	32.63	**<0.001**	14	16.06	**0.007**

855

Table 3 Species-level results from the climate chamber experiment assessing viability and growth-promoting effects of AMF spores. Shown are establishment success (colonization percentage) of AMF originated either from diverse mixture (Mix) or from monoculture (MonoHome = monoculture of the respective plant species; MonoAway = monoculture of other plant species) with different plant species as well as biomass production of individual plant species inoculated with AMF spores with different origin. We compared effects of Mix AMF with MonoAway and MonoHome AMF, respectively. P-values lower than 0.025 indicate significant higher effects of Mix AMF, while p-values higher than 0.975 indicate significant higher effects of monoculture AMF. Significant differences are given in bold and marginal significant differences in italics.

MonoAway vs. Mix		Establishment success (%)			Average biomass (mg) ± SD				
FG	Species	MonoAway	Mix	p-value	MonoAway		Mix		p-value
Legumes	*M. x varia*	3	9	*0.034*	4.74	± 2.36	5.78	± 2.06	*0.084*
	T. repens	5	21	**<0.001**	4.39	± 2.27	5.75	± 2.65	*0.032*
Small herbs	*P. lanceolata*	8	15	*0.063*	7.22	± 2.33	5.85	± 2.39	*0.922*
	P. vulgaris	6	19	**<0.001**	4.39	± 1.70	5.87	± 1.69	0.126
Tall herbs	*C. biennis*	1	4	0.146	4.81	± 1.82	5.35	± 2.56	0.301
	G. mollugo	1	7	*0.030*	2.93	± 1.06	2.77	± 1.06	0.816
MonoHome vs. Mix		Establishment success (%)			Average biomass (g) ± SD				
FG	Species	MonoHome	Mix	p-value	MonoHome		Mix		p-value
Legumes	*M. x varia*	0	9	0.367	2.77	± 1.04	2.09	± 0.95	**0.975**
	T. repens	0	21	*0.072*	4.61	± 1.86	6.13	± 1.75	**0.024**
Small herbs	*P. lanceolata*	40	15	**0.997**	2.18	± 0.56	2.51	± 0.48	0.150
	P. vulgaris	0	19	*0.082*	1.62	± 0.46	1.63	± 0.71	0.697
Tall herbs	*C. biennis*	0	4	0.590	1.11	± 0.34	1.14	± 0.23	0.384
	G. mollugo	0	7	0.625	1.37	± 0.25	1.57	± 0.39	0.263

869 Figure 1



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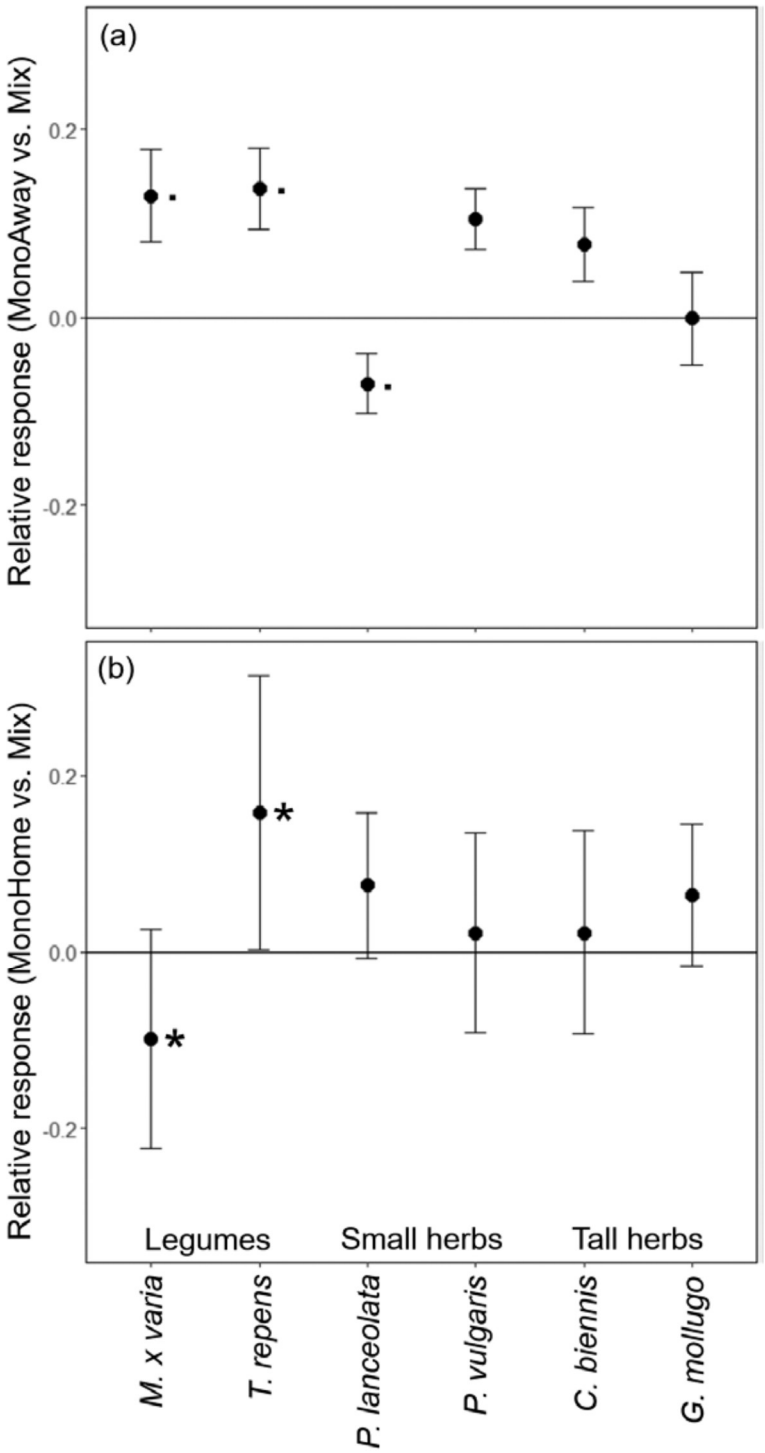
872 Figure 2



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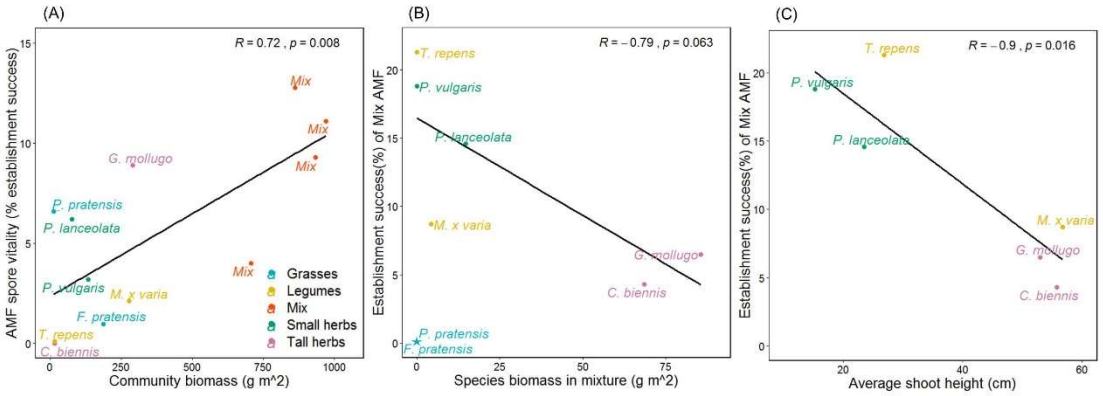
875 Figure 3



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