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RESEARCH ARTICLE

The plant–mycorrhizal fungi collaboration gradient depends on plant functional group

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Abstract

1. Plant colonization by arbuscular mycorrhizal fungi (AMF) is widespread and can offer considerable benefits in terms of growth, nutrient uptake and plant yield. However, it is still unresolved how different plant species and plant functional groups respond to AMF and to different AMF taxa.
2. Here we established 336 grassland microcosms to determine the response of 14 plant species displaying contrasting functional groups (grasses, legumes and non-leguminous forbs) for the presence of three different AMF taxa. For each plant species, we calculated the degree to which plant growth depended on AMF colonization (i.e. mycorrhizal dependency [MD]). We also determined the degree to which each plant species relied on specific AMF taxa for optimal growth (i.e. mycorrhizal species sensitivity [MSS]). Additionally, we determined whether MD and MSS correlated to specific plant traits (i.e. specific root length [SRL], specific leaf area [SLA]).
3. The plant growth response to AMF ranged from −84.9% for a non-mycorrhizal plant (*Luzula campestris*) to +94.0% for a legume (*Trifolium arvensis*). The MD was systematically higher in legumes (91.9% ± 2.4%), followed by non-leguminous forbs (77.1% ± 11.06) and grasses (42.1% ± 15.73%). MSS was less variable (8.9%–37.7%); it was independent of plant functional group and did not correlate with MD. MD was linked to various mycorrhizal plant parameters, including AMF colonization ($R^2 = +0.80$) and total dry biomass ($R^2 = +0.32$). Moreover, among mycorrhizal plants ($n = 12$), MD negatively correlated with SRL ($R^2 = -0.24$) and positively with SLA ($R^2 = +0.24$).
4. *Synthesis*. This study shows that plants relying on AMF for biomass production also show higher root colonization, lower SRL, higher SLA and that different plant traits are interlinked with the way how plants respond to AMF. Overall, this study further demonstrates that different plant functional groups vary in their response to AMF.

KEYWORDS
arbuscular mycorrhizal fungi, leaf area, mycorrhizal dependency, plant traits, root length, symbiosis

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1 | INTRODUCTION

The symbiosis formed between arbuscular mycorrhizal fungi (AMF; Glomeromycota) and plant roots is one of the most widespread and ancient symbiosis on Earth (Smith & Read, 2008). The fossil register indicates that Glomeromycota were crucial in the colonization of land by plants 400 million years ago (Selosse & le Tacon, 1998; van der Heijden et al., 2015). This monophyletic group of soil-borne fungi associates with at least 60% of vascular plants, and is especially abundant in grasses, forbs, tropical trees and a wide range of cash crops including legumes, cereals, vegetables and horticultural plants, where it plays a role in a variety of processes including nutrient uptake (Basu et al., 2018; Soudzilovskaia et al., 2020). Resource uptake facilitation by AMF markedly differs among plant–mycorrhizal fungi combinations, leading to the so-called fungal collaboration gradient (Bergmann et al., 2022).

A relatively low number of AMF species have been described (ca. 340), compared to the high number of known and described mycorrhizal plant species (Oehl et al., 2011; Öpik & Davison, 2016; Öpik, Zobel, et al., 2013). Thus, individual AMF are expected to associate with many different host plants (Bonfante & Genre, 2008; Christenhusz & Byng, 2016; Öpik & Davison, 2016). Although many AMF lack specificity, experimental evidence supports the idea that the association between AMF species and their host plants is established non-randomly, and different plant species are colonized by different AMF communities (Bainard et al., 2014; Davison et al., 2016; Scheublin et al., 2004; Sepp et al., 2019). The fact that AMF–plant interactions are established non-randomly, and that plant species respond differently to different AMF taxa has led to the development and application of the *mycorrhizal dependency* (MD) and the *mycorrhizal species sensitivity* (MSS) concepts. MD, also named mycorrhizal responsiveness (Janos, 2007), refers to the responsiveness of plants to AMF colonization, and is calculated as the ratio between the difference on plant dry biomass with and without the presence of AMF, and the dry biomass of the AMF-colonized plant (Moora, 2014; Plenchette et al., 1983; van der Heijden, 2003). In line with this, previous work has shown that different plant species (i.e. forbs, wild grasses, trees) show contrasting MD (Tawaraya, 2003). Studies to date including plant functional group (e.g. forb vs. grass) in their assessments have mostly built upon non-co-occurring (and spatially distant) plant species, limiting our understanding of how plant species from the same location (e.g. co-occurring in the same grassland) respond to AMF colonization. These studies, however, have also demonstrated that certain plant traits (e.g. root fibrousness) are good predictors of MD, yet the role of other traits (e.g. root length, specific leaf area [SLA]) is less known. In line with this, leaf thickening as a response to nutrient-poor conditions can maximize leaf surface area and increase nutrient storage, although species-specific variability exists (Poorter et al., 2009; Poorter & Bongers, 2006). Plants growing in nutrient-poor conditions are known to rely more on AMF, yet the role that leaf architecture (e.g. thickness) might play in modulating MD in still not fully resolved (Bergmann et al., 2022; Chaudhary et al., 2016, 2022; Hoeksema et al., 2010). Importantly, the MD is not a fixed value because plant growth response to AMF depends on plant genotype, climate, soil type, soil fertility and the AMF taxa colonizing the roots. The identity of the AMF taxa colonizing a root is especially important if plant growth response to different AMF taxa is highly variable, that is if plant species have a high MSS (Klironomos, 2003). Plant species with high MSS benefit from associating with a particular AMF taxon but show neutral or even negative growth response when exposed to other AMF. This is because AMF might benefit plants through nutrient facilitation and disease suppression, but also compete with plants for resources (Berger & Gutjahr, 2021; Klironomos, 2003). The mechanisms driving MSS and their relationship with plant traits are still not fully resolved.

Even though the symbiotic nature and underground lifestyle of AMF could hinder efficient dispersal, attempts to determine the global distribution of AMF taxa have revealed that most of them are found in multiple continents (Davison et al., 2015; Savary et al., 2018). However, local environmental conditions and the spatial distance between sites are also important drivers of AMF community structure (Rincón et al., 2021). In line with this, Hazard et al. (2013) demonstrated that landscape-scale distribution of AMF taxa is driven by the local environment, especially by abiotic factors including pH, rainfall and soil type (Hazard et al., 2013). Similarly, other studies have shown that AMF communities respond in a deterministic manner to local conditions such as soil fertility, agricultural management and temperature (Davison et al., 2021; Guo et al., 2020). Overall, variations in beta-diversity of AMF taxa make it important to address AMF–plant interactions both at the local and global scales, since not all AMF should be considered equally when the responsiveness to AMF is assessed. In line with this, Klironomos (2003) showed that the response (i.e. change in biomass relative to non-AMF controls) of 10 co-occurring plant species to 10 different AMF taxa ranged from beneficial to highly detrimental, and even the same AMF taxon stimulated the growth of certain plant species, while reduced the growth of others (Klironomos, 2003). In another study, authors demonstrated that a range of six different co-occurring AMF taxa can show contrasting protection capacity towards the fungal pathogen *Fusarium oxysporum*; while some AMF species conferred protection against the pathogen, others did not (Sikes et al., 2009). Altogether, this indicates that AMF can function along a continuum from parasitism to mutualism, and that extreme plant responses to the presence of AMF are more common when using co-occurring (i.e. locally adapted) plants and fungi (Johnson & Graham, 2013). In addition, as plant and AMF diversity vary between sites (the pool of plant and AMF species varies from site to site), it is ecologically relevant to perform experiments with co-occurring plant and AMF species, and test how plants and AMF respond to the presence of different hosts.

Meta-analyses using experimental data on plant response to AMF inoculation indicate that plant functional group (i.e. grasses, legumes), fertilization and single vs. multiple AMF inoculation are important factors determining MD (Chaudhary et al., 2016; Hoeksema

et al., 2010). However, these results are based on a very limited number of co-occurring plant species–AMF taxa combinations, and few studies have systematically evaluated the MD and MSS using multiple plant species at the same time. This limits our understanding regarding whether changes in the abundance and composition of AMF communities alter plant growth and plant community structure. Despite this, several studies have shown that co-occurring plant species respond differently to AMF and to different AMF taxa (Reynolds et al., 2006; van der Heijden et al., 1998; Zaller et al., 2011). More recently, Davison et al. (2020) re-analysed 14 high-throughput sequencing datasets describing AMF communities associating with 2427 plant individuals belonging to 297 species and showed that associated AMF taxa constitute an important component of plant ecological strategies (Davison et al., 2020). For instance, the authors showed that legumes display a significantly higher alpha- and beta-diversity of AMF taxa in their roots than woody plants, whereas no differences were observed when considering functional differences such as nitrogen-fixing status.

In this study, we grew 14 different plant species that coexist in Dutch dune grasslands in sterilized dune soils and tested their response to inoculation with three AMF taxa that inhabit dune grasslands. We recorded changes in plant biomass with and without mycorrhizal inoculation and calculated MD and MSS. We also measured specific plant traits (root length and leaf area). The 14 plant species used display contrasting ecological strategies (mycorrhizal, non-mycorrhizal [NM]) and plant functional group (grasses, non-leguminous forbs, and legumes). We hypothesize that plant species will differ in terms of growth response to AMF colonization (i.e. MD), and that plant traits (i.e. leaf area, root length) will explain variation in plant responsiveness to AMF (e.g. plants with less complex rooting systems will show higher MD). Moreover, we hypothesize that plant functional groups with high MD would be more sensitive to changes in particular AMF taxa colonizing their roots (i.e. MSS).

2 | MATERIALS AND METHODS

2.1 | Plant and AMF taxa employed

The plants and soil used in this study originated from a species-rich, mid-successional stage dry dune grassland located in The Netherlands; Noordhollands Duinreservaat, 52°40′N, 4°39′E. The AMF symbiosis is the dominant mycorrhizal symbiosis type in this dune grassland and AMF are abundantly present in the roots of most plant species from this ecosystem (Scheublin et al., 2004). The plant community is dominated by *Festuca ovina* and *Anthoxanthum odoratum*, and contains many subordinate species.

In all, 14 different plant species were used in this experiment, all of them co-occurring and abundantly present in the field study site (Table 1). Seeds from *A. odoratum*, *F. ovina*, *Koeleria macrantha*, *Achillea millefolium*, *Hieracium pilosella*, *Galium verum*, *Plantago lanceolata*, *Senecio jacobaea*, *Lotus corniculatus* and *Cerastium semidecandrum* were collected from the field, whereas seeds from *Trifolium arvensis*, *Trifolium dubium*, *Trifolium repens* and *Luzula campestris* were obtained from a local seed supplier (Cruydt-hoeck, Groningen, The Netherlands). Three different AMF isolates were used in this experiment; two of them (DD-1 and DD-3) belonged to the *Rhizoglomus intraradices* group (previously *Rhizophagus intraradices* or *Glomus intraradices*; Scheublin et al., 2004; Sieverding et al., 2014), and the third one (BEG21) belonged to the *Rhizoglomus irregulare* group. Isolate DD-1 (GenBank ID: DQ377988) and isolate DD-3 (GenBank ID: DQ377989) were isolated from *Trifolium repens* plants obtained from the same dune grassland, while the isolate BEG-21 (GenBank ID: DQ377990) originated from a Swiss calcareous grassland. The *Rhizoglomus irregulare* isolate used in this experiment (i.e. BEG-21) is widespread and detected in almost any ecosystem investigated, including the dune grassland under study, and is known to positively influence plant growth (Köhl et al., 2016; Scheublin et al., 2004).

TABLE 1 Plant species from the study site used in this experiment. All plants were classified according to their capacity to establish a symbiosis with AMF. Mycorrhizal plant species were further classified according to their functional group (i.e. grass, non-leguminous forb and legume).

Species name	Acronym	Mycorrhizal	Functional group (only for mycorrhizal plants)
Cerastium semidecandrum	Ces	No	—
Luzula campestris	Luz	No	—
Anthoxanthum odoratum	Ao	Yes	Grass
Festuca ovina	Feo	Yes	Grass
Koeleria macrantha	Kom	Yes	Grass
Achillea millefolium	Acm	Yes	Non-leguminous forb
Hieracium pilosella	Hip	Yes	Non-leguminous forb
Galium verum	Gav	Yes	Non-leguminous forb
Plantago lanceolata	Pll	Yes	Non-leguminous forb
Senecio jacobaea	Sej	Yes	Non-leguminous forb
Lotus corniculatus	Loc	Yes	Legume
Trifolium arvensis	Tra	Yes	Legume
Trifolium dubium	Tdu	Yes	Legume
Trifolium repens	Trr	Yes	Legume

Additional information on the fungal isolates used is available in Scheublin et al. (2007). No special permission was required from the Dutch authorities to collect the seeds from the grassland under study.

2.2 | Growth system and experimental conditions

The experiment was performed in a non-climate-controlled greenhouse (Hortus Botanicus; Vrije Universiteit Amsterdam, The Netherlands). We filled 800-mL pots with 600 g of dune sterilized sand (sterilized by autoclaving at 110°C for 2 h). Each pot was inoculated either with 25 g of AMF inoculum (DD-3, DD-1, or BEG-21) or with an autoclaved mixture of the three inocula (i.e. the NM control). The inoculum was carefully mixed with the sterilized soil and finally 225 g of autoclaved sand was added to each pot on top of the inoculated soil. Seeds of each plant species (Table 1) were sterilized in 5% chloride for 10 min and thoroughly rinsed four times with demineralized water. Seeds were allowed to germinate in sterile sand, and four seedlings were transplanted into the pots, which were then covered with transparent plastic foil, to guarantee a moist atmosphere and promote growth. After 2 weeks, the plastic foils were removed, and dead seedlings were replaced by new ones.

To compensate for the differences in bacterial communities potentially present in each AMF inoculum, 5 mL of microbial wash was also added to all pots. This wash was prepared by mixing 1500 g of soil from the dune site and 1500 g of inoculum (i.e. 500 g of each) with 5 L of autoclaved deionized water. The mixture was then sieved through a 10-µm mesh size sieve, to ensure the removal of mycorrhizal fungi from the microbial wash and keep only the bacterial community. We also applied to each pot a 2-mL *Rhizobium* solution ($OD_{580} = 0.2$) to favour nodulation. Water content of each pot was kept between 10% and 20% of the water-holding capacity, and 35 g of sterile plastic pellets was deployed on the surface of each pot to further reduce desiccation. Plants were supplied with a modified Hoagland solution containing half of the normal phosphorus concentration (Hoagland & Arnon, 1950). Each pot received 5 mL of this nutrient solution before every weekly watering, to ensure mixing of the nutrients with the soil.

2.3 | Experimental design

The experiment was set up as a complete randomized block design with two factors. The first factor (i.e. plant species) contained 14 levels (Table 1) and the second factor (i.e. AMF taxa) contained 4 levels (i.e. DD-1, DD-3, BEG-21 and control), making 56 treatment combinations. Each treatment was replicated six times, giving 336 pots in total. Given the impossibility to prepare, plant, water or harvest all pots during the same day, each replicate was assigned to a block, making a total of six blocks.

2.4 | Harvest

Plants were harvested after 12 weeks, washed with deionized water and paper dried. Dried plants were then divided into roots and shoots. Roots were divided into two sub-samples; one was used for AMF colonization analyses, and the second for specific root length (SRL) and dry weight calculations (70°C, 3 days). The sum of root and shoot dry mass gave the total dry biomass.

2.4.1 | AMF colonization and calculation of MD and MSS

Dried roots were rehydrated, cleared with 10% KOH and stained with tryptan blue (0.05% in 2:1:1 lactic acid:glycerol:deionized water solution) in hot water (90°C) for 15 min, following the procedure detailed elsewhere (Phillips & Hayman, 1970). The modified line intersection method (McGonigle et al., 1990) was used to determine the percentage of root length colonized by AMF. For each sample, 50 line intersections per root sample were screened for the presence of hyphae, vesicles and arbuscules. From these measures, the total percentage of root length colonized by AMF was calculated (which equals the summed amount of root occupied by hyphae, vesicles and arbuscules). Finally, the MD was calculated according to the formula (1-[*b*/*a*]) * 100; (van der Heijden et al., 2003), where *b* is the mean plant dry biomass of non-AMF treatments and *a* the mean plant dry biomass in treatments with AMF presence. Therefore, a MD > 0 indicates plant biomass enhancement by AMF, while MD < 0 indicates the opposite. MSS refers to the variation in plant growth response when associated with different AMF species (van der Heijden et al., 2003), and is calculated as the coefficient of variation of total dry biomass in response to the three different treatments with AMF. For each plant species, MSS was calculated following the formula (s_a/a) * 100, where s_a is the standard deviation of the average total plant biomass in treatments containing AMF. Higher values of MSS indicate larger differences on the effects caused by the different AMF species on total plant biomass.

2.4.2 | SLA and root length

Eight leaves per pot were used to calculate SLA. The surface of the leaves was measured with a LI-3100 area meter (LI-COR Biosciences GmbH, Germany). Final SLA was obtained by dividing leaf area by the total dry weight of the measured leaves. Due to a data transcription error, SLA was not recorded in two replicates of *Plantago lanceolata* inoculated with DD-3; the number of replicates for this treatment is therefore four (instead of six). SRL was determined on fresh root samples following the gridline intersect method (Marsh, 1971). Briefly, total root length per unit area was manually calculated using a gridline. Then, SRL was determined by dividing total root length by total root dry biomass. The SLA and SRL parameters have been previously used to compare treatment effects independently of shoot and root biomass (Cheng et al., 2016; Tani et al., 2003).

2.5 | Statistical analyses

The effects of plant functional group (NM plants; NM, grasses, legumes and non-leguminous forbs) and AMF taxa (isolates DD-1, DD-3 and BEG-21) on different plant metrics were assessed by means of correlation tests and linear mixed-effects models implemented using the *lme* function of the NLME package in R version 4.0.5 (Pinheiro et al., 2007). The response variables in the model included plant dry biomass, SRL, SLA and percentage of roots colonized by AMF structures (i.e. arbuscules, vesicles and hyphae).

Correlation tests were performed to explore the strength and direction of the relationship between AMF colonization and plant parameters. The linear mixed-effects model employed in this study included plant functional group and AMF taxa as fixed factors. To account for potential variation across experimental blocks ($n=6$) and plant species ($n=14$), these factors were nested within the fixed factors and included in the model as random factors. Because highly correlated predictor variables might influence the estimation of fixed and random effects, we tested for multicollinearity (Pearson's coefficient > 0.9). None of our variables was excluded as the multicollinearity threshold was not surpassed.

Correlations between AMF colonization and plant parameters were performed using the *cor* function indicating 'Pearson' as an argument for 'method' in the STATS package (R 4.0.5). Significance of correlations was set at $\alpha=0.05$ using the function *mcor.test* in the SAME package. When available, Tukey post-hoc tests were performed using the HSD.test function within the AGRICOLAE R package (de Mendiburu, 2019). All figures in this manuscript have been performed using the *ggplot2* function in R 4.0.5 (R Core Team, 2013).

3 | RESULTS

3.1 | AMF colonization

All mycorrhizal plant species inoculated with AMF were successfully colonized and contained visible AMF structures (hyphae, vesicles or arbuscules) in their roots. Total AMF colonization levels in this experiment (excluding NM plants and non-inoculated controls) ranged from 63.7% to 97.0%. Specifically, AMF colonization levels ranged 11.0%–48.7% (hyphae), 8.0%–47.7% (vesicles) and 3.7%–37.3% (Arbuscules; Figure S1, Table S1). Controls of all plant species (i.e. non-inoculated with AMF) remained completely free of AMF (Table S1). NM plants (i.e. *C. semidecandrum* and *L. campestris*) showed very low levels of AMF colonization (0%–6% of root length colonized by hyphae, vesicles or arbuscules, Figure S1, Table S1). ANOVA results for linear mixed-effects models indicated that AMF inoculation had the largest overall effect on total AMF colonization ($F=5972.14$, $p<0.0001$), followed by the interaction between plant functional group and AMF colonization ($F=346.75$, $p<0.0001$; Table S2).

3.2 | MD and MSS

MD ranged from −84.9% (*L. campestris*) to +94.0% (*T. arvensis*) and responded significantly to plant functional group (Table 2). Importantly, the effect of plant functional group on MD was maintained when NM plants (*C. semidecandrum* and *L. campestris*) were removed from the linear mixed-effects model (Table S3) Among mycorrhizal plant species, MD was lowest for grasses (42.2% ± 15.7%), followed by non-leguminous forbs (77.1% ± 11.1%) and legumes (91.0% ± 2.4%; Figure 1, Table S4). A positive relationship ($R^2=0.80$) was observed between total AMF colonization and MD (Figure 2). This positive relationship was maintained ($R^2=0.46$) after removing the two NM plant species (*C. semidecandrum* and *L. campestris*) from the dataset (Figure S2). MSS ranged from 8.94% (*C. semidecandrum*) to 37.73% (*S. jacobaea*; Figure 3, Table S5). No correlation was found between MD and MSS (Figure 3). No clear pattern was observed relating plant functional group and MSS, although grasses averaged the highest MSS values (29.6% ± 6.4%), followed by non-leguminous forbs (22.7% ± 10.3%) and legumes (19.3% ± 6.0%; Figure 3, Table S5).

3.3 | Plant parameters

3.3.1 | Plant dry biomass

Total dry biomass ranged from 32.6 ± 14.2 mg (*T. arvensis*—control) to 839.2 ± 92.0 mg (*A. odoratum*—BEG-21) (Table S6). AMF inoculation

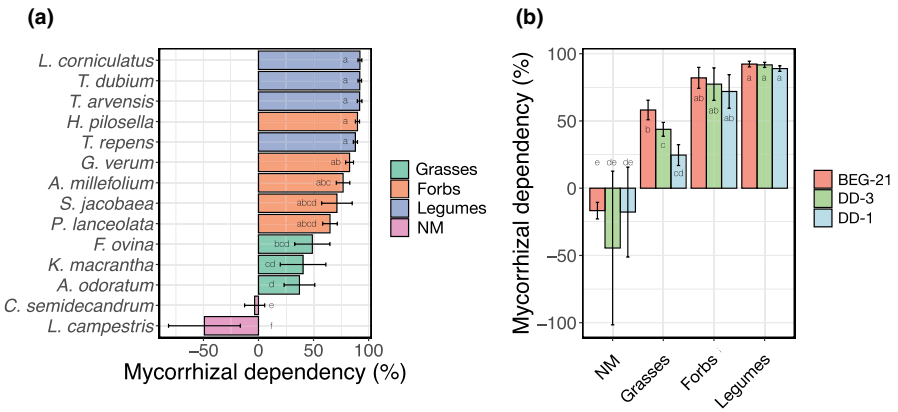


FIGURE 1 Mycorrhizal dependency (MD) across plant species and functional groups. MD across the 14 plant species used in this study (a). Plant functional group response to the different arbuscular mycorrhizal fungi (AMF) taxa (b). Individual MD values are available in Table S4. Different letters indicate significant differences ($p<0.05$) following Tukey's HSD post-hoc test. NM, non-mycorrhizal. Error bars indicate standard deviation.

TABLE 2 Model summary and ANOVA results for linear mixed-effects models (LMM) assessing the effects of fixed factors (i.e. plant functional group and arbuscular mycorrhizal fungi [AMF] inoculation) and their interaction on plant parameters (i.e. total dry biomass, specific root length and specific leaf area [SLA]) and mycorrhizal dependency. Plant species and experimental block are included in the LMM as random factors. Significance is highlighted in bold and indicated as follows: ***p-value < 0.0001; **p-value < 0.0010; *p-value < 0.050. *df*, degrees of freedom. For SLA, n is 334 instead of 336 because due to technical reasons SLA was not recorded on two replicates (see details in Section 2). LMMs were also run after filtering out non-mycorrhizal plant species from the dataset (see Table S3).

		Plant functional group	AMF	Interaction
Total dry biomass	*F*	3.23	**544.74*** **	**62.95*** **
	df	3	3	**9**
Specific root length	*F*	2.05	**17.90*** **	**3.53****
	df	3	3	**9**
Specific leaf area	*F*	**4.57***	1.43	1.60
	df	3	3	9
Mycorrhizal dependency	*F*	**46.85*** **	**6.36****	**3.75***
	df	3	2	6

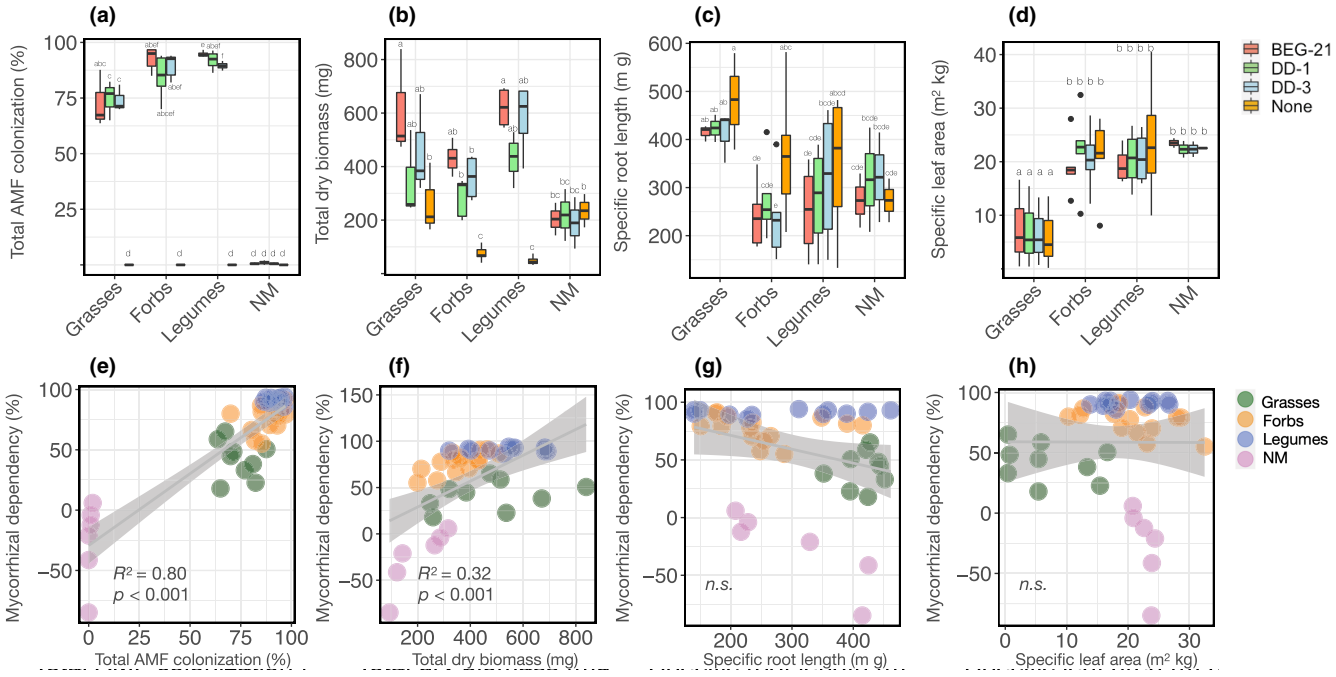


FIGURE 2 Relationship between plant parameters and mycorrhizal dependency (MD). Plant parameters (arbuscular mycorrhizal fungi [AMF] colonization, total dry biomass, specific root length and specific leaf area) and their response to the different AMF taxa (BEG-21, DD-1, DD-3) grouped by plant functional group (a–d). Relationship between plant parameters and MD (e–h). Fit statistics are displayed (linear model R^2 and p-value). Different letters indicate significant differences ($p < 0.05$) following Tukey's HSD post-hoc test. NM, non-mycorrhizal; n.s., non-significant at $p < 0.05$. The same relationships were investigated after removing the NM plants, and results are available in Figure S2.

showed the largest significant effect on total dry biomass (linear mixed-effects model; $F = 544.7$, $p < 0.0001$), and the interaction with plant functional group was significant (Table 2). These effects were maintained after filtering out NM plants (*C. semidecandrum* and *L. campestris*) from the linear mixed-effects model (Table S3). Among the three AMF species, BEG-21 showed the largest (positive) impact on plant dry biomass, followed by DD-3, and DD-1 (Figure 2, Table S5). Control (non-inoculated with AMF) mycorrhizal plants showed an average total dry biomass of 113.7 ± 109.3 mg, while plants inoculated with AMF reached a total dry biomass of 349.6 ± 115.3 mg (DD-1), 457.8 ± 153.6 mg (DD-3) and 539.1 ± 138.2 mg (BEG-21) (Table S6). Among plant functional groups, the highest impact of AMF inoculation on total dry biomass was observed in legumes, which increased from 48.6 ± 18.5 mg (control plants) to 544.3 ± 127.5 mg in

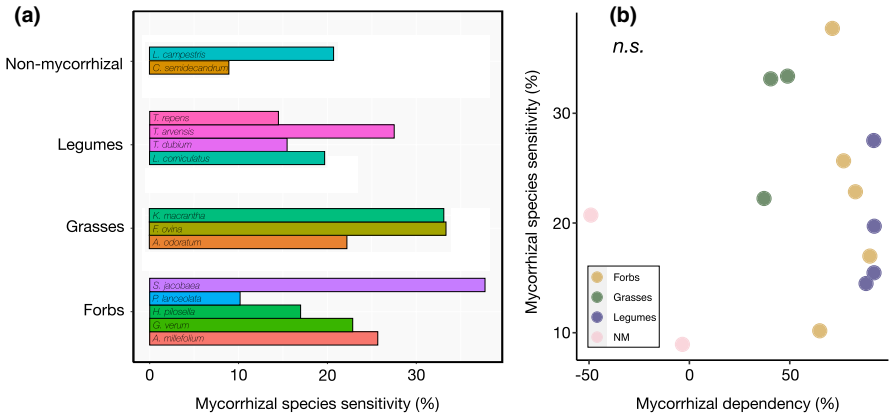


FIGURE 3 Mycorrhizal species sensitivity (MSS) across plant species by functional group. MSS across the 14 plant species used in this study (a). Relationship between MSS and mycorrhizal dependency (b). Individual values are available in Tables S4 and S5. NM, non-mycorrhizal; n.s., non-significant at $p < 0.05$.

AMF-inoculated plants (Figure 2). NM plants showed higher total dry biomass in control (non-inoculated) pots (235.0 ± 87.9 mg) compared to AMF-inoculated pots (219.1 ± 137.0 mg; DD-1, 189.6 ± 135.9 mg; DD-3, and 203.7 ± 86.1 mg; DD-3). A significant correlation between the percentage of root colonization and plant dry biomass was observed; this correlation was strongest (spearman's correlation coefficient of 0.61, $p < 0.001$) for grasses dry biomass and the percentage of root length colonized by AMF hyphae (Figure S3).

3.3.2 | Specific root length

SRL ranged from 133.0 ± 19.0 $m\,g^{-1}$ (*L. corniculatus*—control) to 581.8 ± 151.9 $m\,g^{-1}$ (*S. jacobaea*—control). SRL depended on AMF treatment and its interaction with plant functional group (Table 2), and these effects were maintained after filtering out NM plants (*C. semidecandrum* and *L. campestris*) from the linear mixed-effects model (Table S3). Among plant functional group, the highest SRL values were observed for grasses (432.7 ± 9.3 $m\,g^{-1}$), followed by legumes (297.8 ± 130.9 $m\,g^{-1}$), NM plants (296.1 ± 107.2 $m\,g^{-1}$) and non-leguminous forbs (282.2 ± 62.3 $m\,g^{-1}$; Figure 2, Table S6). Inoculation with AMF had little (negative) impact on SRL (Figure 2); control plants averaged 372.7 ± 149.4 $m\,g^{-1}$, while plants inoculated with AMF averaged SRL values of 314.0 ± 114.3 $m\,g^{-1}$ (DD-3), 310.4 ± 128.5 $m\,g^{-1}$ (DD-1) and 286.4 ± 103.7 $m\,g^{-1}$ (BEG-21). SRL of mycorrhizal plants (i.e. grasses, non-leguminous forbs and legumes) negatively correlated with MD ($R^2 = 0.24$, $p = 0.023$, Figure S2). This correlation became non-significant ($p > 0.05$) when NM plants (*C. semidecandrum* and *L. campestris*) were included in the regression (Figure 2).

3.3.3 | Specific leaf area

SLA ranged from 0.18 ± 0.09 $m^2\,kg^{-1}$ (*F. ovina*—control) to 40.5 ± 15.4 $m^2\,kg^{-1}$ (*T. dubium*—control). These values were in line with previous studies employing the same plant species (Bourdot et al., 1985; Carter et al., 1997; Poorter & de Jong, 1999). Only plant functional group significantly affected SLA according to linear mixed-effects model results (Table 2). This effect was maintained after filtering out NM plants (*C. semidecandrum* and *L. campestris*) from the linear mixed-effects model (Table S3). AMF inoculation and the interaction term did not affect SLA ($p > 0.05$). Among plant functional groups, the highest SLA values were observed for NM plants (22.7 ± 1.4 $m^2\,kg^{-1}$), followed by legumes (21.2 ± 6.6 $m^2\,kg^{-1}$), non-leguminous forbs (20.6 ± 6.6 $m^2\,kg^{-1}$) and grasses (6.8 ± 7.3 $m^2\,kg^{-1}$; Figure 2, Table S6). Inoculation with AMF had no impact on SLA (Table 2); control plants averaged 18.8 ± 11.4 $m^2\,kg^{-1}$, while plants inoculated with AMF averaged SLA values of 18.5 ± 9.2 $m^2\,kg^{-1}$ (DD-1), 17.7 ± 8.8 $m^2\,kg^{-1}$ (DD-3) and 17.4 ± 7.8 $m^2\,kg^{-1}$ (BEG-21) (Table S6). SLA of mycorrhizal plants (i.e. grasses, non-leguminous forbs and legumes) positively correlated with MD ($R^2 = 0.24$, $p = 0.026$, Figure S2). This correlation became non-significant ($p > 0.05$) when NM plants (*C. semidecandrum* and *L. campestris*) were included in the regression (Figure 2).

4 | DISCUSSION

This study demonstrates that the MD(i.e. the degree to which plant growth relies on the presence of AMF) is related to plant functional group in co-occurring plant species. We further show that not only functional group but also plant traits, including root and leaf architecture, are indicators of MD. In this study, MD was highest in legumes, followed by non-leguminous forbs and grasses. Importantly, previous manipulative studies on the factors shaping MD have built their conclusions from a few plant species, usually from the same functional group (Malicka et al., 2021; Reynolds et al., 2006). Only a few studies have included plant functional groups in their assessments, and they have shown the importance of considering these traits to get a reliable picture of the interaction between AMF and host plants (Davison et al., 2020; Gui et al., 2018; Sepp et al., 2019). In addition, we observed that MD was negatively linked to SRL, and positively linked to SLA. Thus, our work supports the idea that the ability to benefit from AMF is strongly linked to plant functional group and is intrinsically associated with plant traits (Bergmann et al., 2022).

The varying MD of plant functional group is one of the main factors that have been proposed to explain AMF effects on plant communities (van der Heijden et al., 1998). Accordingly, plant species dependency on AMF ranges from parasitic (i.e. negative impact

of AMF presence in plant growth), to neutral (no effect), to obligate symbionts (Johnson et al., 1997; Johnson & Graham, 2013). Especially legumes and forbs have a high MD as shown in this study. Consequently, the presence of AMF can enhance plant diversity in grassland communities with many legume and forb species (Grime et al., 1987; van der Heijden et al., 2016). Note that in this study we used three phylogenetically similar AMF taxa (all *Rhizoglomus*), and this might explain why we did not detect larger differences in MD across plant species, as previous research has demonstrated (Klironomos, 2003). Future research should experimentally address MD across plant species using phylogenetically distant AMF taxa. One of the key factors shaping plant–AMF interactions is believed to lie in the plant root system (Comas et al., 2014). The roots of plant species with a high MD, such as legumes, are often thick and unbranched, with few root hairs. This hinders roots to acquire nutrients, especially if these are present at low concentration in the soil, as it was the case for many nutrients in this experiment, including phosphorus (van der Heijden et al., 2006). Therefore, such plant species rely on the presence of AMF to establish within plant communities (van der Heijden et al., 2016). On the other hand, plant species that have a low MD (e.g. many grasses) have a well-developed, fibrous root system consisting of many fine roots adapted for nutrient uptake even in adverse conditions (Brundrett, 2002). As such, these plant species do not rely on the presence of AMF to acquire nutrients and grow. In line with our results (excluding NM plants), several studies have shown that root traits (e.g. fibrousness) are good predictors of MD (Comas et al., 2014; Hetrick et al., 1992).

Here we show that plant species displaying high MD (e.g. legumes) also showed the highest rates of AMF root colonization (ca. 90%). supporting the evidence that high root colonization is often (but not always) beneficial for plants (Bergmann et al., 2022; Treseder, 2013). Despite this, we here acknowledge that some plant species are colonized (sometimes heavily) by AMF but perceive AMF as antagonistic (Francis & Read, 1994; Veiga et al., 2013). Importantly, AMF not only acquire nutrients for plant growth, but can also provide protection to fungal diseases; it has, for instance, been shown that humid conditions favour plant disease (Romero et al., 2022), and that AMF colonization can provide protection towards disease by enhancing the plant immune system (Martinez-Medina et al., 2016; Pozo et al., 2009).

Using 14 plant species (of which, 12 mycorrhizal plants) from contrasting functional groups, our results indicate that mycorrhizal plants display a negative correlation between SRL and MD. We systematically observed the highest dependency on AMF (MD > 80%) in plants with thick roots and a lower SRL (SRL < 200 $m\,g^{-1}$). Among the plant functional groups studied here, non-leguminous forbs showed the strongest negative correlation between SRL and MD. In line with previous studies, we observed that inoculation of mycorrhizal plants with AMF resulted in a reduction of SRL (Liu, 2009). Conversely, we observed a positive correlation between SLA and MD (when excluding NM plants). Since the earliest reports of AMF-induced changes in gas exchange within host plants, it has been demonstrated that photosynthetic efficiency, stomatal conductance and transpiration rates are often higher in mycorrhizal plants relative to their non-colonized controls (Augé et al., 2016). Our results add to previous evidence that plants relying on AMF for growth also show thinner leaves. We argue that more research is needed to fully understand how mycorrhizal symbiosis modulates plant traits, as alternative (i.e. non-AMF dependent) strategies to optimize physiological processes can also play a role, including root clustering and the synthesis of chelating compounds (Rauser, 1999; Shane & Lambers, 2005).

Despite the overall effect of SRL and SLA on MD, no correlation was found between MD and total dry biomass. However, closer inspection of the data reveals that total dry biomass and MD were significantly correlated for non-leguminous forbs. This suggests that, whereas plant productivity in some species (i.e. legumes) shows a switch-on/switch-off response to AMF colonization, others (i.e. non-leguminous forbs) allow for quantitative prediction of biomass content based on their MD. Finally, we observed an antagonistic effect on *L. campestris* (AMF inoculation reduced plant biomass compared to non-inoculated controls). *L. campestris* is a slow-growing, NM plant species commonly found in nutrient-poor sites. Accordingly, we found no root colonization on *L. campestris* neither in controls nor in individuals inoculated with AMF. Previous studies have shown that AMF can reduce plant biomass of non-hosts, and this is especially true if the non-host is growing together with a host plant (Veiga et al., 2013).

In this study, we also analysed the degree to which co-occurring plant species are dependent on specific mycorrhizal fungi for optimal growth (i.e. the MSS). We indeed found that different plant species can show contrasting growth responses when exposed to different AMF taxa, that was particularly the case of the forb *S. jacobaea* and the grasses *K. macrantha* and *F. ovina*. However, our results did not point to functional group as a predictor of MSS. In line with our results, other studies have found that the effects of AMF colonization on plant biomass vary as a single plant species is grown together with different AMF taxa (Klironomos, 2003; van der Heijden et al., 2003). Moreover, other studies have confirmed that high variation in MSS is not only found in greenhouse manipulative experiments, but also when these plant–AMF symbioses are established in the field (Pringle & Bever, 2008). It has been suggested that variation in MSS can be explained by the successional status of the plant species employed; late-successional plant species show greater MSS than early-successional plants (Koziol & Bever, 2016).

On average, grasses showed the highest MSS, followed by non-leguminous forbs and legumes, although variation was found across species within the same functional group. We observed that the three grass species used here (i.e. *A. odoratum*, *K. macrantha* and *F. ovina*) systematically showed higher biomass when grown together with the *Rhizoglomus* isolate BEG-21, compared to the isolates DD-1 and DD-3. Other studies have shown that certain *Rhizoglomus* isolates can colonize plant roots to a greater degree and be more effective in promoting growth than other isolates (Munkvold et al., 2004; Paul Schreiner, 2007). However, roots of all plant species investigated here were equally colonized by the three AMF isolates tested, suggesting that no selectivity for colonization existed as found

elsewhere (Helgason et al., 2002). Therefore, we assume that differences in plant biomass resulted from higher nutrient acquisition capacity in the BEG-21 isolate. Another potential explanation for increased biomass after BEG-21 inoculation could be an enhanced capacity of BEG-21 to protect plants from opportunistic pathogens. However, visual observation of plant individuals at the end of the experiment revealed no evidence of disease neither in control nor in inoculated plants. On the other hand, forbs and legumes displayed lower MSS, thus equally benefiting from the three *Rhizoglomus* isolates used in this experiment. In this study, we only assessed the plant growth response to three AMF isolates. Thus, the MSS of a plant species should only be considered and estimate and to obtain a thorough understanding of the MSS of specific plant species, further isolates, ideally from different AMF genera and families need to be tested in future studies (Marro et al., 2022). For instance, a recent study demonstrated that ancient AMF families are less beneficial for plant growth than more recently evolved taxa (Säle et al., 2021). Including broader ranges of AMF taxa in future studies will provide a better picture of the plant responsiveness to AMF colonization (Sikes et al., 2009).

We had expected that plants with a high MD would also show high MSS, as a high degree of plant benefit from AMF associations could translate into a high potential for variation in plant growth response to different AMF taxa, and potentially even evolutionary adaptation to the most beneficial AMF (van der Heijden et al., 2003). However, high MD can also translate into low MSS, if plants show a negative response to some AMF species and positive response to others (Reynolds et al., 2006). Here all mycorrhizal plant species showed a positive effect to AMF inoculation. Therefore, we argue that the lack of MD-MSS correlation could derive from a relatively low range of MSS; our mycorrhizal plant species showed MSS ranging ~10%–30%. Future studies should include more AMF isolates and contrasting environmental conditions, to derive firm conclusions on the mycorrhizal sensitivity of co-occurring plant species. In line with this, future studies should explore how MD and MSS drive carbon cycling in soils and overall ecosystem multifunctionality (Emilia Hannula & Morriën, 2022).

In conclusion, this study demonstrates that contrasting plant functional groups respond differently to AMF exposure. Legumes and forbs showed the highest MD, followed by grasses and NM plants. This study also shows that, among mycorrhizal plants, plant traits (i.e. SRL, SLA) correlate to MD. Accordingly, our results demonstrate that plants relying on AMF for their growth tend to have thicker roots, but thinner leaves. Further studies with a wider range of plant species from different environments are needed to verify that plant traits are linked to MD. Overall, this study provides empirical evidence linking the impact of mycorrhizal symbiosis on plant yield and plant functional groups.

AUTHOR CONTRIBUTIONS

Ferran Romero formal analysis, data curation, visualization, writing—original draft, writing—review & editing. Alicia Argüello conceptualization, methodology, investigation, formal analysis, data curation, writing—review & editing. Susanne de Bruin conceptualization, validation, writing—review & editing, supervision. Marcel G. A. van der Heijden initiation, conceptualization, validation, writing—review & editing, supervision, project administration, funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

Data used to generate all figures and tables in this article are deposited in Figshare https://doi.org/10.6084/m9.figshare.23540577.v2 (Romero et al., 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Percentage of root colonized (arbuscules, hyphae, vesicles, and total colonization) across all plant species employed in this study grouped by growth form (NM; non-mycorrhizal, grasses, non-leguminous forbs, and legumes). Boxplots are color-coded according to AMF taxa (blue; control, yellow; DD-3, red; DD-1, green; BEG-21).

Figure S2. Relationship between plant parameters (total AMF colonization, total dry biomass, specific root length, and specific leaf area) and mycorrhizal dependency after removing from the analyses the two non-mycorrhizal plants (*C. semidecandrum* and *L. campestris*). Fit statistics are displayed (linear model R^2 and *p*-value).

Figure S3. Correlations between plant parameters (total dry biomass, specific root length, and specific leaf area) and total colonization (percentage of root length) by arbuscular mycorrhizal fungi (AMF). Spearman's correlation coefficient for each plant functional group and significance (*p*-value) are indicated. Note that non-mycorrhizal plants (*C. semidecandrum* and *L. campestris*) and non-inoculated pots (i.e. controls) were not included in these correlations. Shape indicates AMF taxa (circle; BEG-21, triangle; DD-1, square; DD-3), and color indicates plant functional group (red; grasses, green; non-leguminous forbs, blue; legumes).

Table S1. Root colonization (as percentage of colonized root length) by arbuscular mycorrhizal fungi (AMF) and its main structures (arbuscules, vesicles, hyphae, and corpuscles) across all plant species ($n = 14$) and AMF taxa (DD-1, DD-3, and BEG-21) used in this study. Values are the average of six independent replicates ± the standard deviation.

Table S2. Model summary and ANOVA results for linear mixed-effects models (LMM) on the effects of plant functional group (non-mycorrhizal, grasses, non-leguminous forbs, and legumes) and AMF taxa (DD-1, DD-3, BEG-21) on root colonization by AMF and its structures (vesicles, arbuscules, and hyphae). Degrees of freedom are indicated as "df". Plant species and experimental blocks were included in the LMM as random factors.

Table S3. Model summary and ANOVA results for linear mixed-effects models (LMM) assessing the effects of fixed factors (i.e. plant functional group and AMF inoculation) and their interaction on plant parameters (i.e. total dry biomass, specific root length, and specific leaf area) and mycorrhizal dependency. Here LMM were run after filtering out non-mycorrhizal plants (*C. semidecandrum* and *P. lanceolata*). Plant species and experimental block are included in the LMM as random factors. N.s.; non-significant at $p < 0.05$; df: degrees of freedom. For specific leaf area (SLA) n is 334 instead of 336 because due to technical reasons SLA was not recorded on 2 replicates.

Table S4. Mycorrhizal dependency (MD) across all plant species ($n = 14$) and AMF taxa (DD-1, DD-3, and BEG-21) used in this study.

Table S5. Mycorrhizal species sensitivity (MSS) and best-performing

AMF taxa across all plant species ($n = 14$) used in this study.

Table S6. Plant parameters (shoot dry biomass, root dry biomass, total dry biomass, specific root length, and specific leaf area) across all plant species ($n = 14$) and AMF taxa (DD-1, DD-3, and BEG-21) used in this study. Values are the average of six independent replicates ± the standard deviation.

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