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RESEARCH ARTICLE

Counteracting effects of soil biota on emergence and growth of herbaceous plants

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Abstract

Background Plants condition the biotic composition of their rhizosphere. In turn, this plant legacy on the soil biota may affect the performance of plants recruiting in their vicinity. Unravelling how plant-soil legacies drive plant recruitment is key to understand vegetation dynamics and plant community assembly. Studies on the topic usually focus on the effects of soil microbiota as a whole, while the relative role of different guilds of soil organisms in the plant recruitment processes is not usually dissected.

Aims Here, we used soils of Mediterranean woody plant species to test whether arbuscular mycorrhizal fungi (AMF) and small-size microbiota (<50 μm) (MB) affect the germination success and growth of eight herbaceous plants.

Results We documented a significant increase in seedling emergence probability when small-sized MB was present and no effect of AMF. In contrast, the aboveground plant biomass decreased with the presence of MB and increased with that of AMF. Interestingly, those plants growing in the absence of MB and in soils from woody plants associated with higher AMF richness developed higher aboveground biomass.

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Conclusion This study brings new evidence on how soil microbial communities can determine the performance of their associated herb community, and also, how the effects of different microbial guilds may change across the plant ontogeny. Given these results, the differential effect of soil microbial functional guilds should be considered to better understand plant soil legacies and feedbacks, potentially driving plant recruitment and community assembly.

Keywords Arbuscular mycorrhiza · Rhizosphere · Community assembly · Ontogeny · Plant-plant interactions

Introduction

Soil constitutes a key driver of vegetation dynamics, affecting several processes related to the early life stages of plants and conditioning the relative abundance of species (Harper et al. 1965; Gómez-Aparicio 2008; Bever et al. 2010). Changes in soil properties may entail consequences for the performance of plants, strongly influencing plant diversity (Kulmatiski and Kardol 2008; Bardgett and Wardle 2010; van der Putten et al. 2013). For instance, a low soil water content can inhibit germination, while too waterlogged soils can impair plant performance and establishment (Dantas et al. 2020). However, the effects of soil properties are usually modulated by different factors, especially by the action of soil organisms (van de Voorde et al. 2011).

Plants modify the surrounding soil by producing and releasing chemical compounds, altering temperature, and/or modifying soil moisture, which shapes the composition of soil communities, giving way to a plant-soil legacy (Garbeva et al. 2008; Bardgett and Wardle 2010; van Dam et al. 2010; Doornbos et al. 2012; Aleklett and Hart 2013). Through direct and indirect pathways, up to 40% of photosynthesized fixed carbon is transferred from the plant to the rhizosphere, enabling higher microbial densities than in bulk soils (Berendsen et al. 2012; Philippot et al. 2013). Plant root exudates enhance or inhibit specific soil organisms inducing a selection that can impact their fitness (Garbeva et al. 2004; Rudrappa et al. 2008; Doornbos et al. 2012). Mycorrhizal plant species secrete chemicals to establish symbioses with mycorrhizal fungi to improve nutrient uptake and water acquisition, whereas they avoid plant antagonists like pathogens or nematodes (Bucher 2007; Sikes et al. 2009). The high specificity in plant-microbial interactions makes plant species identity a main driver of soil microbial community composition (Miethling et al. 2000; Garbeva et al. 2008).

Experimental approaches studying the effect of plant-soil legacies on plant recruitment are embedded in the theory of plant-soil feedback and constitute a solid background to study the reciprocal effects between soil organisms and plants (McCarthy-Neumann and Kobe 2010; van de Voorde et al. 2011). The soil community is composed of diverse microbial groups that can establish direct antagonistic or mutualistic relations with plant species. Microbial groups, such as decomposers, have a role in regulating soil nutrient availability for plants and hence can indirectly affect the balance between slow and fast-growing plant species (Zak et al. 2003; van der Heijden et al. 2008). However, in plant-soil legacy studies, soil communities are generally considered as a whole, and few works have considered functional differentiation across soil guilds (but see Klironomos 2002; Wang et al. 2019a, b; Martinović et al. 2021).

Analysing the relative effect of different soil microbial functional groups on plant recruitment requires their isolation. Arbuscular mycorrhizal fungi (phylum Glomeromycota) (AMF, hereafter) constitute a key group for the success and performance of plants, especially in stressful environments (Nadeem et al. 2014). They establish one of the most common mutualistic symbiosis in natural systems, appearing in up to 70% of land plants (van der Heijden et al. 2015). Moreover, it can be easily separated from the rest of the members of the soil microbiota based on its size (see e.g. Wagg et al. 2014). Smaller soil microorganisms (ca. < 50 µm) (small-size MB, hereafter) comprise mainly bacteria and other fungi (non-Glomeromycotan), including mutualistic, antagonistic (pathogenic), and decomposers, but not AMF (Wagg et al. 2014). Some authors have studied the role of soil microorganisms associated with plants on facilitation (Rodríguez-Echeverría et al. 2016), whereas others have considered the relative importance of AMF and different size-classes of microbiota on several ecological processes such as plant community assembly (Klironomos 2002), litter decomposition (Li et al. 2020), or plant chemistry (Wang et al. 2019b). However, as far as we know, nobody has dissected the relative effect of AMF and small-sized MB on plant recruitment, from seedling emergence to vegetative growth.

Another classical feature of plant-plant interaction is that studies dealing with soil organisms are often based on advanced plant stages (Hayman 1980; Klironomos 2002; Hamel and Strullu 2006; Noceto et al. 2021). However, seeds and seedlings have reduced capacities to respond to antagonisms, so they encompass the most critical survival stages, and they sometimes require the presence of third organisms to survive (Barton and Koricheva 2010; Hardoim 2019). Approaching early life stages in this field would improve the knowledge of how the rhizosphere affects plant recruitment dynamics (Alcántara and Rey 2012; Montesinos-Navarro et al. 2019). This work aims to dissect the effect of different soil functional guilds during plant ontogeny, from seed emergence to vegetative growth. For that, we used the microbiota of natural soils modified by different arbuscular mycorrhizal long-lived woody plant species to test the establishment and growth of a set of fast-growing herbs (including non- and mycorrhizal species). This selection responds to difficulties of using woody plant species in this kind of experiments: they take longer times to develop and they have specific needs for space (Lekberg et al. 2018). Besides, woody plant species are less sensitive to belowground organisms than herbaceous plants (Kulmatiski et al. 2008).

Specifically, we tested (i) whether the presence of AMF and/or small-size MB affects the emergence and performance of herbaceous plant species; and (ii) whether AMF richness, instead of their mere presence, may also affect the emergence and performance of herbaceous plant species. We hypothesized that 1) plant performance should be higher in soils with small-sized MB due to the potential induction of germination caused by bacteria and the positive effects that have been reported in previous studies (Baskin and Baskin 2000; Rodríguez-Echeverría et al. 2013). Also, 2) we expect that AMF should not affect seed germination since they establish once roots have grown. Finally, 3) an increasing AMF richness should benefit plant growth (Maherali and Klironomos 2007).

Materials and methods

Experimental design

We conducted a greenhouse experiment to test the effect of the presence of two soil microbial groups that harbour different functional guilds, AMF and small-size MB, on plant performance of different herbaceous species. Specifically, we measured seedling emergence, plant growth (measured as above- and belowground biomass), and resource allocation (measured as the aboveground/belowground biomass ratio). For this, we collected rhizosphere soils associated with nine woody plant species (canopy soils, hereafter) in which eight herbaceous species (response species, hereafter) were grown following a full factorial (AMF and small-size MB presence/absence) design with four replicates (see scheme in Fig. 1). The experiment comprised a total of 1152 pots: Canopy soil (9 species) × Response species

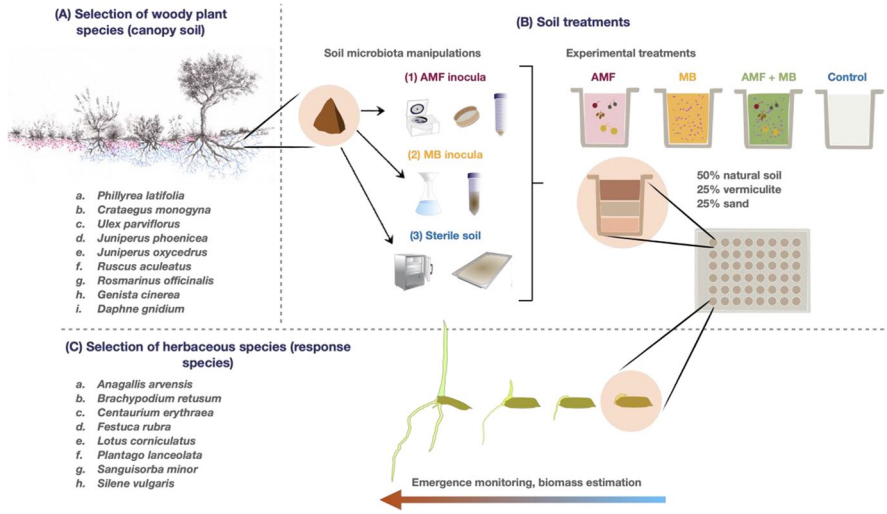

Fig. 1 (**A**) The experimental design included nine canopy soils (a-i) from woody plant species; (**B**) four soil treatments with experimentally controlled soil communities (Arbuscular mycorrhizal fungi (AMF); small-sized microbiota (MB); AMF + MB; and Control (sterile soil); (**C**) eight response species (1–8) with four replicates per canopy soil and treatment. This makes a total of 128 pots per type of soil and an experimental total of 1152 pots

(8 species) x AMF treatment (presence/absence) x small-sized MB treatment (presence/absence) × 4 replicates.

Canopy species selection and soil collection

The canopy soils were sampled from nine woody plant species typical of mixed Mediterranean forests of the south-eastern Iberian Peninsula, specifically from the Sierra Sur mountain range in Jaén province. The plant species identity is a major driver of the microbial community composition (López-García et al. 2017) independently from the type of soil, as plant species differently impact on soil properties as they grow. Canopy species may imprint a "legacy effect" to soils as the result of the action of their rhizosphere-associated microbiota (i.e. soils may be modify depending on the canopy species). Thus, differences in soil microbial community composition may be expected between species (Miethling et al. 2000; Garbeva et al. 2008). Hence, representing different soil sources, the selection of different plant species is an accurate way to get a wide representation of the microbial variability found in forest soils. Furthermore, the canopy species were selected based on their relevance for the assembly and stability of Mediterranean forest communities (Alcántara et al. 2019) and on the richness (number of virtual taxa sensu Öpik et al. 2010) of their root-associated AMF communities previously studied in the same sampled area (Garrido et al. under review).

The canopy species were: *Juniperus phoenicea* (average AMF richness per individual = 5.21), *Crataegus monogyna* (5.53), *Daphne gnidium* (6.86), *Genista cinerea* (7.55), *Ulex parviflorus* (8.45), *Juniperus oxycedrus* (9.00), *Phillyrea latifolia* (9.33), *Rosmarinus officinalis* (11.20), and *Ruscus aculeatus* (13.58). Soils were collected in April 2019 beneath three individuals located at three different locations (minimum 2 km apart) to capture the spatial variation between different plant populations (37.646035° N, 3.711252° W; 37.669268° N, 3.729562° W; 37.641203° N, 3.742311° W). Isolated individuals were selected, at least 5 m apart from another neighbourhood vegetation. The soil was excavated and collected discarding any potential soil affected by other plant roots, up to a volume of 5 L per individual (1.25 L in each cardinal direction), between 0–50 cm from the trunk and between 5–25 cm depth. Soils from the three individual plants of each species were homogenized to get a composite sample of 15 L per species. Samples were stored at 4 °C until the experimental setup a week later (Fig. 1, soil characterization is showed in table SI. 1).

Response plant species selection

As response species, we selected a set of herbaceous plants that can be naturally found in the soil sampling areas: *Anagallis arvensis*, *Brachypodium retusum*, *Festuca rubra, Lotus corniculatus, Plantago lanceolata, Sanguisorba minor, Silene vulgaris* and *Centaurium erythraea*. These species were selected to represent a wide diversity of plant families, including species that associate with AMF, non-mycorrhizal plant species and N-fixing plant species (see details in SI. 1). At the time of soil collection, these plant species were not found below sampled canopy plants.

AMF inoculum extraction

The mycorrhizal fungal inoculum for the AMF treatments was obtained through an independent wet-sieving for each canopy soil (International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) protocol). The extraction came from 300 ml soil volume, equivalent to 5.2% of the natural soil volume used for filling the pots containing the same canopy soil. This proportion is equivalent to that used in previous studies inoculating soil microorganisms (see e.g. Wagg et al. 2014). Soils were suspended in tap water, shaken, and centrifuged at 2000 rpm for 2 min. The supernatant was decanted and discarded and the pellet was re-suspended in a 50% sucrose solution. The mix was centrifuged at 2000 rpm for 2 min. The supernatant was sieved through a mesh size of 50 µm to collect the AMF spores and hyphae of the mycorrhizal fungi present in the sample. The presence of AMF spores was checked visually by using a stereomicroscope (10x). The AMF inoculants were stored on falcon tubes at 4 °C for two days until inoculation. At the end of the experiment, a random subset of 25% of the AMF and small size MB (See next paragraph) treatments were stained and checked under microscope to verify the presence of AMF structures (Philips and Hayman 1970).

Small-size MB extraction

The small-sized MB inoculum (containing mainly bacterial and fungal -non-AMF- microbes) was extracted from 300 ml of each canopy soil. The soil was suspended in 3 L of distilled water and gently shacked overnight at 60 rpm at 20 °C. On the basis that AMF is largely excluded by 50 µm pore-size mesh (Wagg et al. 2014), the supernatant was then passed through a filter paper (30–50 µm pore size, according to manufacturer) that retained AMF propagules but allowed the collection of the bacteria and non-mycorrhizal fungi in the filtrate. Filtrates were kept at 4 °C for two days until inoculation.

Experimental setup and growing conditions

The experiment was arranged in trays of 6×8 pots. Pots (180 ml) were filled with a mix of sterile sand (25%), vermiculite (25%), and sterilized canopy soil (50%). Sand and vermiculite were sterilized through autoclaving (1 h, 120 ºC) whereas the natural soils were steam-sterilized (1 h, 100 ºC for three consecutive days). Each row in trays contained a single combination of canopy soil and inoculum treatment and the eight response plant species (separately in each pot) (see SI. 2 for details in experimental randomization).

Before filling pots, AMF inoculum corresponding to each canopy soil was added to by dragging down the spores with a small amount of water to the sterile canopy soil and then homogenized. An equivalent amount of water was added to non-AMF soils. Subsequently, the soils were mixed with sand and vermiculite and used to fill the corresponding pots depending on the treatment.

The small-size MB filtrates were incorporated to corresponding previously filled pots by watering with 25 mL filtrate in a single application. No loss by leaching was recorded. Non-MB-treated pots were watered with an equivalent amount of distilled water.

Five seeds from each response species were sown in each pot, except in the case of the tiny seeds of *Centaurium erythraea*, of which an average of 57 were sown. Seedling emergence was monitored three times a week throughout the 13 weeks the experiment lasted (April to mid-July 2019). At experiment completion, all plants present in the same pot were collected together, as a unique sample and weighted to obtain fresh above and belowground biomass per pot. Note that we did not remove any plant during the time course to obtain the overall effect of the treatment in the studied parameters. This may include intraspecific competition between plants, but will similarly affect all the species. After that, the plant material was dried in an air stove at 70 °C for 48 h, whereupon the dry weight was obtained.

The greenhouse experiment was conducted at the Estación Experimental del Zaidín (EEZ, CSIC, Granada, Spain). The temperature at the greenhouse was set to 24–18 °C (day-night). The experiment was exposed to natural light conditions. Seeds were watered with 30 ml of distilled water three days a week to favour seed germination. Once germination started, watering was gradually decreased until stabilized at 10 ml.

Data analyses

Seedling emergence was estimated as the proportion of seedlings emerged in each pot relative to the number of seeds sown, and treatment effects were tested through generalized linear mixed models (GLMMs), using beta-binomial family distribution. The models included AMF (presence/absence), small-sized MB (presence/absence), and their interaction as fixed factors, and canopy soil and response species independently as random factors (see SI.3, preliminary analyses for the effects of canopy and response species). The tray was also included as a random factor to control the spatial effect of tray location in the greenhouse. To control for pots with no emergence, this factor was included as a zero-inflated term. Due to the low emergence rate shown by *Centaurium erythraea*, this species was excluded from the analyses.

Aboveground biomass was estimated as the dry shoot biomass per emerged seedling in a pot, and analysed by fitting a linear mixed model, including the same fixed and random factors as in the previous case. The model was weighted by the maximum number of co-occurring seedlings per pot (i.e. at the same time) to control for possible competitive effects in biomass. The same model was also applied for belowground biomass and above/belowground biomass ratio (biomass allocation, hereafter).

To explore the potential effect of the AMF community associated with each canopy soil, we ran the same models, but using the AMF richness

associated to each canopy plant instead of their mere presence/absence.

All statistical analyses were conducted with the package glmmTMB v. 1.1.2.3 (Brooks et al. 2017) from R statistical software v. 4.1.2 (R Core Team, 2018) using the r-markdown v.2.16 (Allaire et al. 2018). Model fitting was checked by using the DHARMa R package (Hartig and Hartig 2017).

Results

From the 1152 pots, plants successfully established in 740. The success in establishment was variable across plant species. The two grasses (*B. retusum* and *F. rubra*), *P. lanceolata* and *S. vulgaris* reached in average ca. 3.6–3.8 individuals per pot (from five initial seeds), whereas *S. minor* (1.0 individuals per pot), *L. corniculatus* and *A. arvensis* (ca. 0.5) had much less success in establishing.

The effectiveness of the AMF treatment reached a 41% of pots with AMF structures, meanwhile less than 5% from non-AMF inoculated treatment showed signs of this symbiosis. These results suggest that, a part of AMF may pass the used filter or that some cross-contamination may exist. Nevertheless, our treatments were overall effective despite this noise.

Effects of MB and AMF on plant performance

Emergence probability increased significantly with the presence of small-sized MB (ca. 9% over the intercept estimate) (Table 1, Fig. 2), whereas the presence of AMF and the interaction AMF*small-sized MB did not have any effect. Regarding random effects, canopy soil and tray explained a low proportion of variance (0.2% and 1.5%, respectively), whereas most of the variance was explained by the identity of the response species (24.8%), i.e. the different response species showed contrasting emergence probability (SI. 3).

Regarding plant growth, MB affected negatively the aboveground biomass, whereas AMF presence had the opposite trend (Table 1, Fig. 3a and b, respectively). In this case, random factors (response species, canopy soil, and tray) explained a low variance (2.1%, 0.7%, and 0.1%, respectively). None of the fixed factors affected significantly the belowground biomass. Finally, the presence of AMF increased the biomass allocation towards aboveground (Fig. 4a, Table 1).

Effects of MB and AMF richness on plant performance

When including AMF richness instead of its presence/absence in the models, small-sized MB similarly affected the emergence and aboveground biomass

Table 1 Results of generalized linear mixed models testing for the effect of presence of small-sized microbiota (MB), arbuscular mycorrhizal fungi (AMF), AMF richness, and their interactions, on the success and performance of the response plant species. We show the Estimates, *Z*- and *p-values* obtained for each model tested. Emergence *estimates* shows a probability from 0 to 1, aboveground and belowground biomass show the *estimates* in mg, and biomass allocation *estimate* comprises the ratio between both biomass (aboveground/belowground). Significance of *p*-values is indicated as: *ns* ($p > 0.05$), * ($p < 0.05$); **($p < 0.01$); and *** ($p < 0.001$). The estimates for above and belowground biomass are expressed as miligrams

	Emergence			Aboveground biomass			Belowground biomass			Biomass allocation		
Fixed effects	*Est.*	*Z value*	*p*	*Est.*	*Z value*	*p*	*Est.*	*Z value*	*p*	*Est.*	*Z value*	*p*
Intercept	0.43	-0.45	ns	50.38	-14.99	***	69.83	6.72	***	0.92	-4.29	***
MB	0.52	2.37	*	46.43	-2.81	**	64.29	-1.78	ns	0.91	-1.65	ns
AMF	0.45	0.54	ns	56.64	4.42	***	74.55	1.55	ns	0.94	3.66	***
MB*AMF	0.41	-0.40	ns	49.87	-0.27	ns	72.85	0.70	ns	0.92	-0.43	ns
Fixed effects	*Est.*	*Z value*	*p*	*Est.*	*Z value*	*p*	*Est.*	*Z value*	*p*	*Est.*	*Z value*	*p*
Intercept	0.44	-0.37	ns	57.44	4.16	***	59.80	-10.71	***	0.78	13.98	***
MB	0.53	2.41	*	58.51	-3.56	***	60.24	1.78	ns	0.75	-1.43	ns
AMF richness	0.44	-0.22	ns	47.00	4.65	***	60.32	0.23	ns	0.78	4.43	***
MB*AMF richness	0.44	-0.32	ns	57.44	0.01	ns	59.73	-0.21	ns	0.77	-1.38	ns

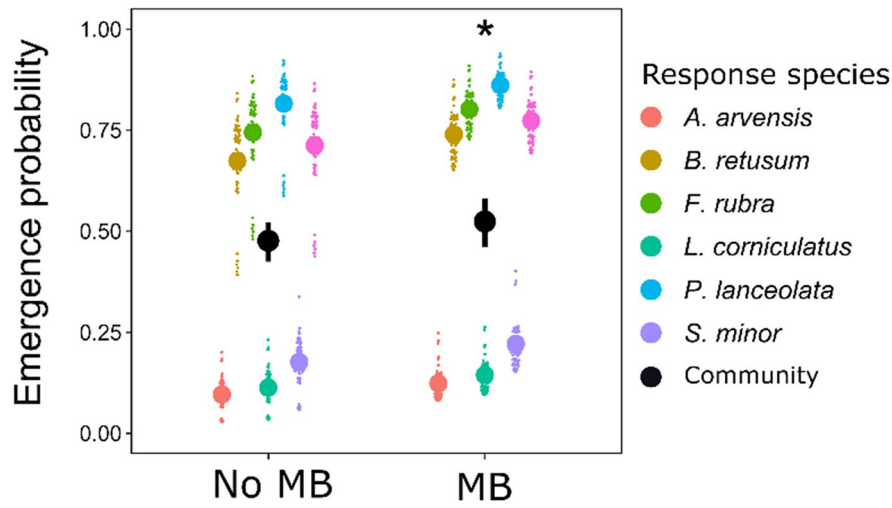

Fig. 2 Seedling emergence probability (y axis) in relation to the presence or absence of small-size MB (x-axis). Reported values are the fitted coefficient for the emergence probability model. The figure shows the community patterns (black circles) and the response species-level patterns (coloured circles). Significance levels are showed by *, ** and ***, meaning p values $< 0.05, < 0.01$ and < 0.001 respectively

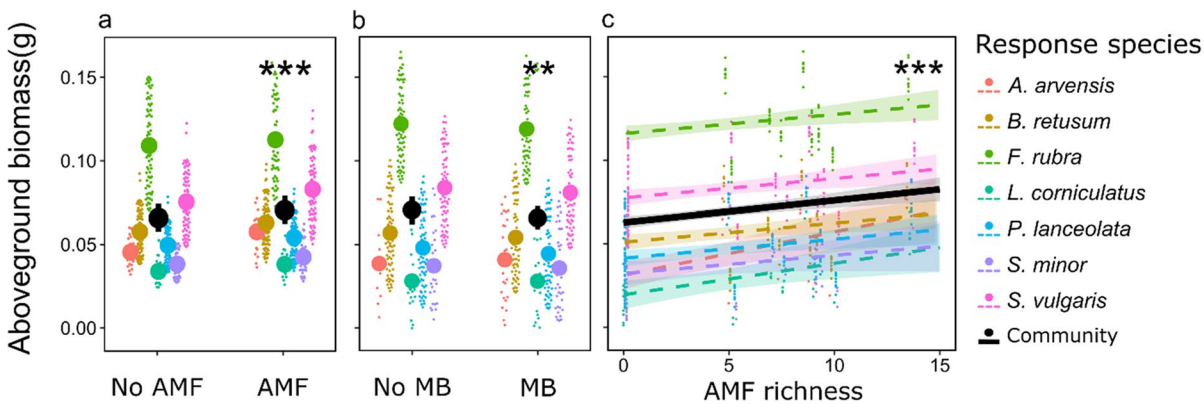

Fig. 3 Aboveground biomass (g) of herbaceous plants (y axis) depending on the presence or absence of AMF (**3a**) and small-size MB (**3b**), and on the AMF richness in the canopy soil (virtual taxa)(**3c**). Reported values are the fitted coefficient for the emergence probability model. All panels show the community patterns (black circles and solid lines) and the herbaceous species-level patterns (coloured circles and dashed lines). Significance levels are showed by *, ** and ***, meaning p values $< 0.05, < 0.01$ and < 0.001 respectively

(Table 1). AMF richness positively affected the aboveground biomass and biomass allocation to the upper parts (Figs. 3c and 4b). The interaction between MB and AMF richness had no significant effects on any of the studied parameters (Table 1).

Discussion

This study has evaluated whether AMF *versus* small-size MB condition the performance of recruiting plants. We showed that a part of the soil microbiota, represented by the small-sized MB, improved the emergence of plants but depressed their vegetative growth, as expected in hypothesis 1. On the other hand, AMF did not influence emergence but increased their aboveground biomass, neutralizing small-sized MB's negative effects once plants were established (hypothesis 2). This pattern was consistent across the studied response plant species and for all canopy soils, but the variance explained by the response species was larger, suggesting that canopy plant identity had little effect on herb recruitment. However, we found that soils from canopy species with richer AMF communities enhanced significantly the aboveground biomass of the response species, likely improving

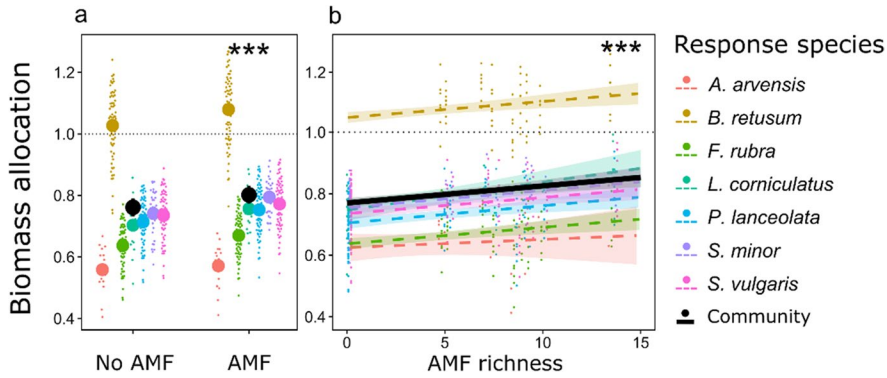

Fig. 4 Biomass allocation of herbaceous plants (y axis) in relation to the presence or absence of AMF (4a), and on the AMF richness in the canopy soil (virtual taxa) (**3c**). Reported values are the fitted coefficient for the emergence probability model. The panel 4a contains the results for both herbaceous species (coloured circles) and community levels (black circles), which additionally shows the predicted confidence intervals at 95%. The panel 4b shows this relationship for both species (coloured-dashed lines) and community (black-solid lines). Shaded areas around the black line indicate the predicted confidence intervals by the biomass allocation model for the community, whereas coloured shaded areas indicate the SE for the species. Both panels also show a pointed line in value (y) = 1. Values upper exceeding this line indicate that plants allocate more resources aboveground. Significance levels are shown by *, ** and ***, meaning p values $< 0.05, < 0.01$ and < 0.001 respectively

their establishment in these soils and suggesting the action of associated microorganisms (hypothesis 3). The magnitude of the effect for the studied plant parameters was not as large as expected, likely due to the high stochasticity found in natural study systems. Nevertheless, our results bring new evidence of the important influence of the canopy plants on the recruiting community by dissecting the role of the small-sized MB and AMF communities.

Effects of the presence of AMF and small-sized MB

Seeds interact locally with a wide range of microbes, which potentially alter the germination, performance, and demography of plant species (Wagner and Mitschunas 2008; Chee-Sanford and Fu 2010; Nelson 2018). As expected, emergence was higher in soils with small-size MB. Out of all microbes present in soil, emergence probability may be influenced by seed pathogens that can impede germination, by mutualistic organisms that protect seeds against pathogens, and by saprotrophs that can degrade the seed coat (Pérez et al. 2016). We found a general trend evidencing that small-sized MB promote seedling emergence, and this effect was independent of the soil origin (i.e. canopy soil). This points out to generalist small-size MB guilds that can digest the seed coat, thus enhancing water permeability, seed imbibition and, consequently, seeding emergence (Guttridge et al. 1984; but see Baskin and Baskin 2000). As another option, fungi or bacteria may stimulate germination by releasing chemicals, or by preventing pathogenic attacks, as it has been reported in other systems (Gallery et al. 2007; Dalling et al. 2011). As a counterpart, growing with small-size MB may entail negative consequences due to pathogenic activity or competition for nutrients (see e.g. Klironomos 2002).

As plant ontogeny advances, so does the net balance of the interaction between the plant and small-size MB turning from positive to negative in certain aspects of plant development, in our case plant biomass production (Rodríguez-Echeverría et al. 2013; Chaparro et al. 2014). This aligns with other studies that proved that once plants emerge, MB composition changes (Hortal et al. 2013; Barret et al. 2015). The negative effect of small-size MB on seedling biomass could be attributed to the accumulation of plant pathogens and root antagonists that may alter the nutrient uptake (Jonhson and Riegler 2013; Liang et al. 2021). Contrary to our expectations, the presence of small-size MB did not stimulate plant performance. This contrasts with results found by Rodríguez-Echeverría et al. (2013), however, in this study different microbial guilds were not considered.

AMF presence did not affect seedling emergence, as expected because these organisms need plant roots to interact. By contrast, AMF enhanced the aboveground biomass, providing more evidence to the increasing literature dealing with the beneficial effects of AMF on plant performance (e.g. Varga 2015; Bowles et al. 2018; Montesinos-Navarro et al. 2019). Nevertheless, this trend was not found at the belowground biomass level, pointing towards the fact that AMF colonized plants tend to increase the ratio aboveground/belowground biomass (Veresoglou et al. 2012). This effect must be a consequence of increased nutrient availability provided by the fungi that is primarily invest in aboveground biomass. In agreement, O'Brien et al. (2018) evidenced that plants grown in a medium with high nutrient availability, invest straight to the aboveground biomass.

Effects of AMF richness

Dealing exclusively with the absence or presence of AMF provided a significant result, but a partial view. Including AMF richness complemented the found pattern. Our study revealed that plants grown in soils of canopy plants associated with higher AMF richness produced more aboveground biomass than plants grown in soils of canopies with low AMF richness. Likely, woody plants with a higher range of associated AMF species provide recruiting plants with more chances to find their best symbiotic fungal partners. In this context, Van der Heijden et al. (2006) pointed out that plants respond to inoculation with AMF depending on the identity of both partners. Moreover, high AMF richness encompasses more functional diversity (attributed to different families by Powell et al. 2009), providing a large variety of services to the plants: from different nutrient absorption capacities to different protection abilities against stresses that should enhance plant performance (van der Heijden et al. 1998; Maherali and Klironomos 2007). Therefore, the AMF richness associated to the different woody species may help to predict which plant species perform better underneath different canopy plants.

Conclusion

From a purely ecological perspective, this study suggests that the facilitative service provided by the canopy plants is related to their associated organisms, and that it is important to consider the soil legacies left by woody species, since they condition the soil microbial communities (Aleklett and Hart 2013; Sasse et al. 2018). Whereas small-size MB enhance the emergence, richer AMF communities make the plants perform better and counteract the negative effects of the former on later life-cycle stages. The successive and counteracting action of both guilds, suggests ontogenetic shifts in the net balance of plant-soil interactions. This may have relevant implications for plant recruitment and facilitation processes, as has been suggested by several studies (Navarro-Cano et al. 2015; Dayrell et al. 2018; Perea et al. 2022).

There is abundant literature dealing with the effect of soil microorganisms on plant-plant interactions. However, most of these studies have considered these microorganisms as a black box, ignoring the relative role of species and functional guilds. The present study has dissected how different functional groups of soil microorganisms influence plant recruitment. Our results bring new evidence to the understanding of how plant soil legacies condition the response of herbaceous plants. Moreover, we have revealed an ontogenetic change in the relationships between plants and small-sized MB. These microorganisms promoted seedling emergence but depressed vegetative growth, suggesting a shift from mutualistic to antagonistic overall effect. On the other hand, AMF improved plant vegetative growth and could potentially counteract the negative effect of antagonistic small-sized MB. Finally, the influence of AMF richness should be studied further to better account for the effect of microbial diversity on plant-soil legacy and the processes governing plant recruitment.

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Author contribution ALG, CAG, JMA, and JLG conceived the experiment. ALG, JLG, JMA, AJP, JPR, BMM, and MMA carried out the experiment. AJP, ALG, JMA, JLG, and BMM analysed the data. AJP, ALG, and BMM wrote the initial draft. Every author contributed to the final version and approved it for publication.

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Data availability The experimental data and analytical scripts that support the findings of this study are available in Zenodo with the identifier https://doi.org/10.5281/zenodo.7972878.

Declarations

Competing interests Authors declare not conflict of interest.

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