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RESEARCH ARTICLE

The Influence of Beneficial Fungi on Plant-Enemy Interactions and Plant Community Structure

Mutualistic and antagonistic phyllosphere fungi contribute to plant recruitment in natural communities

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Abstract

1. Phyllosphere fungal communities participate in multiple ecological functions (litter decomposition, disease-causing, plant defence). However, there is a lack of knowledge on whether and how these functions contribute to plant community dynamics under natural conditions.
2. One of the aspects of plant dynamics in which these fungi can most clearly affect is recruitment, since the success of newly germinated plants can be seriously compromised by pathogenic activity or the absence of mutualistic interactions.
3. To determine the relationship between phyllosphere fungal communities and plant recruitment, we combined published information on the frequency of plant–plant recruitment interactions and phyllosphere fungal communities in 38 woody species from two mixed forests in southern Spain.
4. Our results indicate that phyllosphere pathogens and saprotrophs have a negative effect on canopy–recruit interactions, while epiphytic fungi have a positive effect.
5. Additionally, the presence of canopy species hosting high richness of epiphytes or counting with a high diversity of saprotrophic fungi favours the formation of an abundant sapling bank.
6. *Synthesis*. Our results suggest that phyllosphere fungi play a relevant role in the assembly of the sapling bank in forest communities, thus, potentially influencing plant community dynamics. Beyond the well-known negative effect of pathogenic fungi on recruitment, our results show the mutualistic effect of fungal epiphytes and a dual role of saprotrophs as antagonistic, decreasing recruitment of certain species, or mutualistic, enhancing recruitment in the sapling bank.

KEYWORDS
canopy-recruit interactions, community assembly, epiphytic fungi, pathogenic fungi, phyllosphere fungi, plant recruitment, plant-fungal interactions, plant-plant interactions, recruitment networks, saprotrophic fungi

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1 | INTRODUCTION

The identification of the mechanisms that structure terrestrial plant communities is a central question in ecology (Sutherland et al., 2013). For considerable time, natural enemies (i.e. pathogens, herbivores and parasites) have been postulated to play critical roles in determining the dynamics of plant communities (Bever, 1994; Packer & Clay, 2000). Their importance resides in their capacity to cause negative density-dependent mortality, whereby host-specific enemies severely limit plant recruitment close to conspecifics, thus indirectly favouring the recruitment of heterospecific seedlings and promoting species diversity (Janzen-Connell effect) (Connell, 1971; Janzen, 1970). However, many plant antagonists are to some extent generalists rather than specialists, so density-dependent recruitment cannot be fully understood only through the simple dichotomy between conspecifics and heterospecifics, since not all heterospecifics have the same effect on recruitment. For example, recruitment is enhanced in the proximity of distantly related species (Alcántara et al., 2018; Liu et al., 2012; Verdú & Valiente-Banuet, 2011). Multiple types of plant antagonists can contribute to variability in species-specific effects on recruitment. Among them, fungal pathogens have been found as the drivers of negative density-dependent mortality in plant populations, from tropical (Bagchi et al., 2010; Bell et al., 2006; Mangan et al., 2010) to temperate forests (Bayandala & Seiwa, 2016; Jia et al., 2020). Many pathogenic fungi have the ability to infect multiple co-occurring plant species (Chen et al., 2019; Gilbert & Webb, 2007; Hersh et al., 2012; Rizzo et al., 2005), so their influence on plant community dynamics should not be simply understood in reference to the density of conspecific plants, since the density of alternative hosts must be taken into account (Perea et al., 2021).

Although there is a recognition of the role pathogenic fungi play in the maintenance of forest diversity, the process of recruitment is affected by the complexity of interactions with other non-pathogenic fungi associated with the plants. First, mutualistic fungi (i.e. mycorrhizae, fungal endophytes) are widely reported as contributors to plant immunity or resource uptake (Arnold & Lutzoni, 2007; Bacon & White, 2016; Delavaux et al., 2023; Raghavendra & Newcombe, 2013; Rodriguez et al., 2009); however, their role on natural communities is starting to be addressed (Martín et al., 2015), and often with contrasting results. For example, some studies show that root-associated arbuscular mycorrhizal fungi can defend against pathogens and promote conspecific survival (Jiang et al., 2020; Liang et al., 2015) and recruitment (Garrido et al., 2023). Conversely, it has also been shown that they can induce negative plant–soil feedback (Bever, 2003) or decrease conspecific recruitment (Bennett et al., 2017). A less studied group of potential mutualistic fungi are those inhabiting the surface of leaves, loosely referred to as epiphytes (Vorholt, 2012). Even though some studies on leaf epiphytic bacteria show they benefit the plant by improving cuticle resistance or permeability (Ritpitakphong et al., 2016; Schreiber et al., 2005), fixing nitrogen (Fürnkranz et al., 2008; Hietz et al., 2002) or outcompeting plant pathogens (Edwards & Blakeman, 1984), the actual effect of most epiphytic fungi remains to be determined (Kembel & Mueller, 2014; Lindow & Brandl, 2003). Besides antagonistic and mutualistic associations, many fungi live as saprotrophic and are most often assumed to play a commensalistic role by breaking down dead tissues (Tanunchai et al., 2023; Voříšková & Baldrian, 2013). While their activity can be linked to pathogens damage (Chen et al., 2020), fungal decomposers can have an indirect beneficial effect on plants by enhancing decomposition of organic matter in the soil (Song et al., 2017). In addition to this complexity, some studies report fungi can shift their role depending on the host, environmental factors or changes in the plant metabolic conditions (Alvarez-Loayza et al., 2011; Barrett et al., 2009; Redman et al., 2001). Identifying the relative contributions of these different functional groups of fungi on structuring natural plant communities in the wild still remains a challenge.

The development of high-throughput sequencing techniques has revolutionized our understanding of microbial ecology, allowing to explore the effects of plant-associated fungi on plant ecology and evolution (Laforest-Lapointe et al., 2017). While research has been intensively focused on below-ground plant–fungi associations (Bever et al., 2015; Mangan et al., 2010; Van Der Heijden, 2004), the role of above-ground, phyllosphere fungal communities on plant population dynamics is less known (Chen et al., 2020; Whitaker et al., 2017; Zhu et al., 2022). Leaves are inhabited by functionally diverse fungal communities (Pajares-Murgó et al., 2023) that contribute to plant productivity and fitness by conditioning metabolic functions such as leaf senescence (Stone et al., 2018), stomatal regulation (Zhu et al., 2023) or stress tolerance (Arnold & Lutzoni, 2007; Hubbard et al., 2014; Rodriguez et al., 2009), or conversely, can cause detrimental impacts on plant health (Newton et al., 2010; Whipps et al., 2008; Zhu et al., 2022). In spite of the increasing knowledge on the factors driving the assembly and diversity of phyllosphere fungal communities, evidence on their impact in ecosystem functions at community level is still limited. Specifically, the effect of the different leaf-inhabiting fungi on plant–plant interactions during recruitment has never been evaluated.

Here, we address this knowledge gap by exploring whether the communities of phyllosphere pathogenic, saprotrophic and epiphytic fungi of Mediterranean woody species influence plant–plant interactions during recruitment in natural communities. In the context of plant–plant interactions, the framework based on canopy–recruit interactions (sensu Alcántara, Garrido, Montesinos-Navarro, et al., 2019) is providing new insights (Alcántara et al., 2018; Garrido et al., 2023; Perea et al., 2021). Recruitment interactions are defined as those occurring between established (canopy) plants and those plants (recruits) recruiting in their vicinity. The outcome of canopy–recruit interactions is typically measured in terms of the frequency, density or probability of recruitment of a given species in the proximity of individuals of a canopy species. This outcome is the combined result of direct interactions between the plants (e.g. competition for light and nutrients) and indirect interactions mediated by other organisms that are more or less specialized in different plant species (e.g. mycorrhizal fungi, seed dispersers, seed predators, herbivores and pathogens). From the perspective of community ecology, two species interact, directly or indirectly, if the presence of one of them affects the population dynamics of the other (Abrams, 1987). Therefore, any effect of the canopy plant on the recruiting plants can be considered as an interaction since recruitment is a key demographic process.

In this study we address the following questions: (i) Does the similarity in leaf fungal guilds (pathogens, epiphytes and saprotrophs) between plants affect the outcome of canopy–recruit interactions? Based on current knowledge, we hypothesize that pathogenic fungi should have a negative effect on plant recruitment, epiphytic fungi should have a positive effect on recruitment and saprotrophic fungi would not have an effect on canopy–recruit interactions as their contribution to the outcome of plant recruitment should not be plant species specific. (ii) Do the richness and the diversity of phyllosphere fungi associated with a plant species condition its abundance in the sapling bank or its influence on the recruitment of other plants? We hypothesize that plant species associated with a higher richness or diversity of leaf pathogens should be scarce in the sapling bank, since they are exposed to many potential agents of mortality. Those plant species associated with higher richness or diversity of epiphytes should present a more abundant sapling bank if there is a mutualistic effect on the recruits. However, we would not expect an effect of the richness or diversity of saprotrophs on overall recruitment since these fungi are not expected to be species-specific. Finally, we hypothesize that plant species associated with a higher richness or diversity of leaf pathogens should have a negative impact of recruitment in their vicinity, since they can potentially increase seedling mortality of many species. On the contrary, we expect that plant species with associated with higher richness or diversity of leaf epiphytes and leaf saprotrophs should favour the recruitment of many others because they might provide defence against pathogens and/or increase sapling access to nutrients.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in two mixed pine-oak Mediterranean forest communities of southern Iberian Peninsula: Sierra de Segura (38°16′48″ N, 2°34′48″ E; Segura, hereafter) and Sierra Sur de Jaén (37°22′48″ N, 3°26′23.9994″ E; Jaén, hereafter). Jaén has mean annual temperatures of 14.1° while Segura has mean annual temperatures of 11.6°. The annual rainfall is concentrated in spring and autumn, reaching mean annual values of 535.4 mm in Jaén and 611.76 mm in Segura.

Segura is characterized by the mixed forests of *Pinus nigra* subsp. *salzmannii*, *Quercus faginea* and *Quercus pyrenaica*. Jaén is dominated by mixed forests of *Pinus halepensis*, *Quercus ilex* and *Q. faginea*. The canopy is enriched with small trees and tall shrubs (with species of the genera *Acer*, *Crataegus*, *Juniperus*, *Sorbus*, *Prunus*, *Phillyrea* and *Pistacia*) and the understorey is composed of small shrubs (with species of the genera *Cistus*, *Genista*, *Phlomis*, *Rosmarinus* and *Thymus*).

2.2 | Data collection

2.2.1 | Sequencing data

To study the phyllosphere fungal guilds across plant species and sites, we used the dataset from Pajares-Murgó et al. (2023). Briefly, this dataset analysed phyllosphere fungal DNA from 276 leaf samples from 138 individuals belonging to 27 woody plant species in Jaén and 19 in Segura. DNA was sequenced with Illumina NovaSeq using specific fungal primers of ITS3 and ITS4 (ITS2 region, Tedersoo et al., 2014). The taxonomic assignment of amplicon sequencing variants into operational taxonomic units (OTUs) was determined against the UNITE database (Abarenkov et al., 2010) at 97% similarity cut-off (Tedersoo et al., 2012). The database contained a total of 1462 OTUs and 7173784 reads. The functional guild of each OTU was determined by matching OTUs' assigned genera and the genus-guild database FungalTraits (Põlme et al., 2020). It is worth noting that this database was additionally filtered for detecting legitimate interactions. Fungi classified as endophytes were discarded due to their low OTU abundance. The final dataset includes 381 OTUs of saprotrophs, 41 OTUs of epiphytes and 186 OTUs of plant pathogens (see Supporting Information S3 for further details).

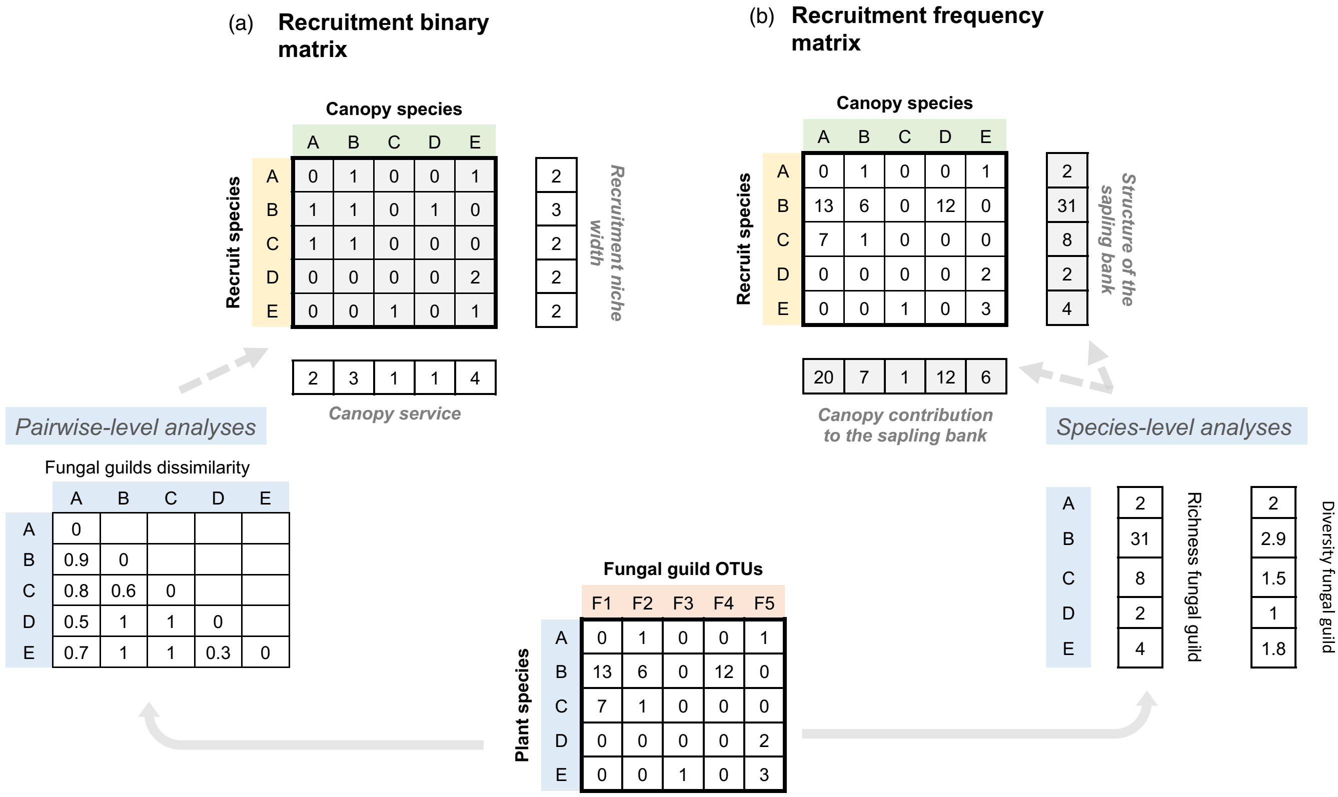
The studied plant species averaged 20.58 ± 13.8 OTUs of pathogenic fungi, with a range between 65 OTUs in *Juniperus oxycedrus* and 1 OTU in *Daphne laureola*. The mean richness was 37.35 ± 29.05 OTUs of saprotrophic fungi, with a range between 1 OTU in *D. laureola* and 138 OTUs in *J. oxycedrus*. The mean richness was 7.7 ± 4.12 OTUs of epiphytic fungi, with a range between 1 OTU in *Daphne gnidium*, *Acer monspessulanum*, *Pistacia therebintus* and *Prunus spinosa* and 19 OTUs in *Juniperus phoenicea* (Figure S1.1). Note that the PERMANOVA analyses previously computed in Pajares-Murgó et al. (2023) showed that phyllosphere fungal communities varied mostly between plant species for all functional guilds (saprotrophs: 33.44%; epiphytes: 42.64%; pathogens 33.73% of explained variance), while the study site accounted for a small proportion of the variation (<3% for all guilds; further details provided in Pajares-Murgó et al., 2023).

From this dataset, we extracted the richness of each fungal guild for each plant species, and calculated the proportion of OTUs of each guild associated with each plant species and sites (fungal richness, hereafter) and the number of effective partners for each plant species and sites (fungal diversity, hereafter) by means of bipartite R package (Dormann et al., 2008). To obtain the dissimilarity of fungal communities between plant species, we first normalized each sample by its total reads and averaged the OTU proportions across plants of each species in each site. The dissimilarity matrices were computed for each guild and site using the Bray–Curtis index, by means of Betapart R package (Baselga & Orme, 2012).

2.2.2 | Recruitment data

The data on the frequency of canopy–recruit interactions of the same studied plant species and sites was obtained from Alcántara et al. (2018). The protocol for obtaining this data followed the standard published in Alcántara, Garrido, Montesinos-Navarro, et al. (2019). Briefly, the frequency of recruitment was sampled in five zones in Jaén (located between 0.78 and 3.69 km from each other) and four in Segura (1.10–3.58 km from each other). Each site consisted in a 50 × 50 m plot where the abundance of woody species and the frequency of canopy–recruit interactions was registered. Species abundance was assessed by quantifying the total cover of the canopy (the canopy projection in m^2) for each species in the plot. The frequency of canopy–recruit pairwise interactions was estimated by counting the number of saplings (recruits) of each species growing underneath each canopy species (including conspecific individuals). Recruits are defined as plants without symptoms of being reproductive (fruits and flowers), more than 1 cm of basal diameter, and a lower size than 25% of the typical adult of the species. Interactions were considered when a recruited species is located closer than 0.5 m from the canopy plant.

Here, we use the frequency of recruitment of species *i* underneath canopies of species *j* (F_{ij}) and the probability of recruitment (P_{ij}) as the presence or absence of recruitment of species *i* underneath canopies of species *j*. With this information, we assembled the recruitment matrix of each study site. First, we used the recruitment binary matrix (P_{ij}) to extract the recruitment niche width of each plant species (number of species under which each species recruits) as the sum of entries across the rows of the binary matrix, and the species canopy service (the number of species that recruit under a canopy species) as the sum across the columns. Second, we used the recruitment frequency matrix (F_{ij}) to extract the structure of the sapling bank as the sum of entries across the rows of the frequency matrix, and the contribution of the canopy species to the sapling bank as the sum across the columns (Figure 1).



(a) Recruitment binary matrix

	A	B	C	D	E	Recruitment niche width
A	0	1	0	0	1	2
B	1	1	0	1	0	3
C	1	1	0	0	0	2
D	0	0	0	0	2	2
E	0	0	1	0	1	2
Canopy service	2	3	1	1	4	

(b) Recruitment frequency matrix

	A	B	C	D	E	Structure of the sapling bank
A	0	1	0	0	1	2
B	13	6	0	12	0	31
C	7	1	0	0	0	8
D	0	0	0	0	2	2
E	0	0	1	0	3	4
Canopy contribution to the sapling bank	20	7	1	12	6	

Fungal guilds dissimilarity

	A	B	C	D	E
A	0				
B	0.9	0			
C	0.8	0.6	0		
D	0.5	1	1	0	
E	0.7	1	1	0.3	0

Fungal guild OTUs

Plant species	F1	F2	F3	F4	F5
A	0	1	0	0	1
B	13	6	0	12	0
C	7	1	0	0	0
D	0	0	0	0	2
E	0	0	1	0	3

	Richness fungal guild	Diversity fungal guild
A	2	2
B	31	2.9
C	8	1.5
D	2	1
E	4	1.8

FIGURE 1 Data analysis framework. (a) The binary matrix represents the existence of a recruitment interaction between individuals of the canopy species (columns of the matrix) and the recruit species (rows of the matrix). It allows to define the recruitment niche of a species (sum across rows) and the canopy service provided by a canopy species (sum across columns). (b) The frequency matrix contains the frequency (F_{ij}) of recruits under canopy species. It provides information of the structure of the sapling bank (sum across rows) and the canopy contribution to the sapling bank (sum across columns). Finally, the plant–fungi matrix represents the interactions between plant species and fungal OTUs pertaining to each fungal guild. We explored the role of phyllosphere fungi on recruitment at two levels. The pairwise-level analysis explored how the dissimilarity of each fungal guild between pairs of plant species influence their probability and frequency of recruitment. The species-level analysis explored whether the richness and the diversity of each fungal guild by plant species affect different aspects of recruitment (recruitment width, canopy service, structure of the sapling bank and canopy contribution to the sapling bank).

2.3 | Data analyses

2.3.1 | Effect of phyllosphere fungi on recruitment at pairwise level

We explored how the dissimilarity of phyllosphere fungal communities influence pairwise canopy–recruit interactions. We fitted two generalized linear mixed models (GLMMs), one for the frequency of recruitment (F_{ij}) and another for the probability of recruitment (P_{ij}) for each pair in each site. As predictors, we included the dissimilarity between canopy and recruit species for each fungal guild, the logarithms of the cover of canopy and recruit species (to control for different abundance of each species), the site and the square root of the phylogenetic distance between species pairs (phylogenetic distances were obtained from Alcántara et al. (2018)). As random effects, we included the random intercepts for the separate effects of canopy and the recruit species of each pair (to control for the non-independence of pairs with some species in common). The F_{ij} model was fitted using a zero-inflated negative binomial distribution that included the cover of the canopy and recruit species as zero-inflation terms (see Figure S1.2 for the data distribution). These zero-inflation terms account for the probability that rare species do not recruit under each other just by chance, what would explain the excess of observed zeroes in the dataset. The same model was fitted to P_{ij} using a binomial distribution (see Supporting Information S4 for further details). Note that we include the phylogenetic distance between plant species in the models since it has been found to affect both the canopy–recruit interactions in the study sites (Alcántara et al., 2018) and assembly of fungal communities in the studied plant species (Pajares-Murgó et al., 2023).

2.3.2 | Effect of phyllosphere fungi on recruitment at species level

We explored how the fungal species richness or the fungal diversity of each plant species influenced the different aspects of the recruitment: canopy service, recruitment niche width, structure of the sapling bank and canopy contribution to the sapling bank. For each aspect, we fitted two GLMMs, one exploring the effect of the richness of each fungal guild and the other testing the effect of the diversity of each guild. The models testing the recruitment niche width and the structure of the sapling bank included also the logarithm of the cover of the recruit species as a covariate, while those testing the canopy service and the canopy contribution to the sapling bank included the logarithm of the cover of the canopy species. All models included site as a fixed effect (see Supporting Information S4 for further details). The models testing the structure of the sapling bank and the canopy contribution to the sapling bank were fitted using a negative binomial distribution, while those testing the recruitment niche width and canopy service was fitted using zero-inflated negative binomial distribution (as suggested by the function check distribution in the *performance* R package, Lüdecke et al., 2021).

All statistical analyses were performed in the R-environment 326 (R Development Core Team, 2020) by means of RStudio IDE (RStudio Team, 2020). GLMMs were fitted using *glmmTMB* R package (Brooks et al., 2017) and residual distributions were checked with *DHARMa* R package (Hartig & Lohse, 2022). Graphical representation was performed by *ggplot2* R package (Wickham, 2016).

3 | RESULTS

The mean proportion of OTUs of pathogens across plant species was 0.16 ± 0.11, with a range between 0.0086 in *D. laureola* and 0.55 in *J. oxycedrus*. In the case of saprotrophs, this proportion was 0.14 ± 0.11 with a range between 0.0048 in *D. laureola* and 0.58 in *Juniperus communis*, and for epiphytes it was 0.20 ± 0.15 of epiphytes, with a range of 0.031 in *D. gnidium*, *A. monspessulanum*, *P. therebintus* and *P. spinosa* and 0.59 in *J. phoenicea* (Table S1.1).

The mean number of effective partners of pathogens across plant species was 9.03 ± 4.32 OTUs, with a range between 1 in *D. laureola* and 22.27 in *J. oxycedrus*. In the case of saprotrophs, this mean was 12.26 ± 6.28 OTUs, with a range between 1 in *D. laureola* and 31.98 in *J. communis*, and in the case of epiphytes it was 3.07 ± 2.37, with a range of 1 in *D. gnidium*, *A. monspessulanum*, *P. terebinthus* and *P. spinosa* and 11.63 in *J. phoenicea* (Table S1.2).

The largest Bray–Curtis dissimilarity between pathogenic fungal communities was found between the pairs: *A. monspessulanum*–*Pistacia lentiscus*, *D. gnidium*–*P. lentiscus*, *Phillyrea latifolia*–*P. lentiscus*, *Phillyrea angustifolia*–*P. latifolia*, *P. angustifolia*–*D. gnidium* and *P. angustifolia*–*Acer monspessulanum*. The largest difference between saprotrophic fungal communities was found between *P. lentiscus* and *Rosa* sp. The largest difference between epiphytic fungal communities was found between *Amelanchier ovalis* and *J. phoenicea*. The average recruitment niche width was 9.11 ± 5.87 canopy species (range 0–23), canopy service was 9.40 ± 7.08 recruited species (range 0–26), the average density of the sapling bank of a recruit species was 0.03 ± 0.03 saplings/m^2 (range 0–0.14) and the mean density of saplings under canopy species was 1.40 ± 8.84 saplings/m^2 (range 0–58.04) (Table S1.3).

3.1 | Effect of phyllosphere fungi on recruitment at pairwise level

The GLMM analyses revealed that plants with similar pathogenic fungal communities had a lower frequency of recruitment (F_{ij}) (Table 1a; Figure 2a; Figure S4.1). Also, we found that plants with similar pathogenic and saprotroph fungal communities had lower probability of recruitment (P_{ij}); however, species sharing more similar epiphytic fungal communities had a higher probability to be recruited one under the other (Table 1b, Figure 2b; Figure S4.2). Additionally, canopy and recruit cover had a consistent positive effect on plant recruitment in both analyses; moreover, both had a clear negative contribution in the zero-inflation term (Table 1a) indicating that

TABLE 1 Generalized linear mixed model analyses performed to explore the effect of the phyllosphere fungal communities' compositional distance (measured as the Bray–Curtis distance), plant phylogenetic distance, site and cover of canopy and recruit on the probability (a) and the frequency (b) of plant species recruitment. Significant effects are bold typed.

Fixed effects	Estimate	SE	*Z*-value	*p*-Value
(a)				
Pathogen dissimilarity	1.548	0.502	3.083	**0.002**
Saprotroph dissimilarity	−0.087	0.583	−0.149	0.881
Epiphyte dissimilarity	−0.505	0.356	−1.417	0.156
Recruit cover	0.394	0.067	5.851	**<0.001**
Canopy cover	1.014	0.077	13.103	**<0.001**
Site	0.121	0.218	0.557	0.578
Phylogenetic distance	−0.009	0.011	−0.856	0.392
Zero-inflation terms				
Recruit cover	−0.054	0.384	−0.139	0.889
Canopy cover	−1.019	0.383	−2.661	0.008
	Variance	**SD**		
Random effects				
Canopy species	0.688	0.829		
Recruit species	0.233	0.483		
(b)				
Pathogen dissimilarity	1.953	0.955	2.046	**0.041**
Saprotroph dissimilarity	2.266	1.123	2.018	**0.044**
Epiphyte dissimilarity	−2.289	0.698	−3.28	**0.001**
Recruit cover	0.551	0.084	6.602	**<0.001**
Canopy cover	1.257	0.113	11.143	**<0.001**
Site	0.187	0.354	0.528	0.598
Phylogenetic distance	−0.025	0.021	−1.219	0.223
	Variance	**SD**		
Random effects				
Canopy species	0.679	0.824		
Recruit species	0.195	0.441		

interactions are less likely to occur between rare (low abundance) plants. Neither site nor phylogenetic distance showed consistent effects on F_{ij} or P_{ij}.

3.2 | Effect of phyllosphere fungi on recruitment at species level

The GLMM analyses indicated that the canopy contribution to the sapling bank (sum across columns in the F_{ij} matrix) of a given plant species was enhanced by the richness of epiphytic fungi and by the diversity of saprotroph fungi (Table 2b,d; Figure 3a,b). We did not find any relationships between fungal richness and diversity and the plant species canopy service (number of species that recruit under canopy species) or recruitment niche width (number of species under which each species recruits; Table S2).

4 | DISCUSSION

Leaf fungal communities associated with plant species are fundamental to promote multiple ecosystem functions (Laforest-Lapointe & Whitaker, 2019; Peñuelas & Terradas, 2014). Research on their potential effects on the host performance is largely based on detailed laboratory studies of metabolic activities or pathogenic effects on plant species with agronomic and forestry interests (Parker & Gilbert, 2004). However, thanks to the development of high-throughput sequencing technologies that allow the identification of large numbers of microbial taxa that cannot be cultivated in the laboratory, the study of their implications in the functioning of ecosystems and plant communities under natural conditions has recently increased (Laforest-Lapointe et al., 2017). Here, we take advantage of these advances to provide evidence on the multiple roles that phyllosphere fungi play in the recruitment process of plant species in natural communities.

Our results suggest that phyllosphere pathogenic communities impose a pairwise-specific filter on recruitment: when two plant species hosted similar pathogen communities, it was less likely that they recruited under each other and, even when recruitment occurred, the density of recruitment decreased. However, at species level, we did not find any effect of the richness and the diversity of leaf pathogens associated with a plant species on their abundance as saplings or on their contribution as canopy plants to the sapling bank. This suggests that phyllosphere fungal pathogens in our study system may play a role in regulating the dynamics of the plant community by modulating the structure of the sapling bank at the pairwise scale. The role of pathogens as drivers of plant species diversity and community dynamics is classically framed in terms of negative conspecific density-dependent mortality caused by the interaction between plants and host-specialist pathogens in early stages of recruitment (Janzen-Connell mechanism; Comita et al., 2010; Jia et al., 2020; Song et al., 2017). However, the generality of this hypothesis has been increasingly called into question (Barrett et al., 2009; Parker & Gilbert, 2018; Song et al., 2017). Along this line, the community of fungal pathogens in our study system shows low levels of specialization (Pajares-Murgó et al., 2023). In fact, it is well known that many fungal pathogens are able, or obligated, to infect multiple hosts with differential impact depending on the plant identity (Gilbert & Webb, 2007; Hersh et al., 2012; Perea et al., 2020; Spear & Mordecai, 2018). For example, Spear and Broders (2021) show that generalist pathogens are the main drivers of seedling death and disease, with differences in pathogenic susceptibility among woody species of tropical forests. Our results suggest that negative density-dependent mortality may not depend solely on the density of conspecific individuals but also on the density of individuals of plant species that host similar pathogen communities.

Although interactions between plants and pathogenic fungi have received most of the attention, they are not the only guild that can play a relevant role on plant community dynamics (Delavaux et al., 2023; Liang et al., 2015). Our study allows inferring the differential effects of fungal guilds on plant recruitment.

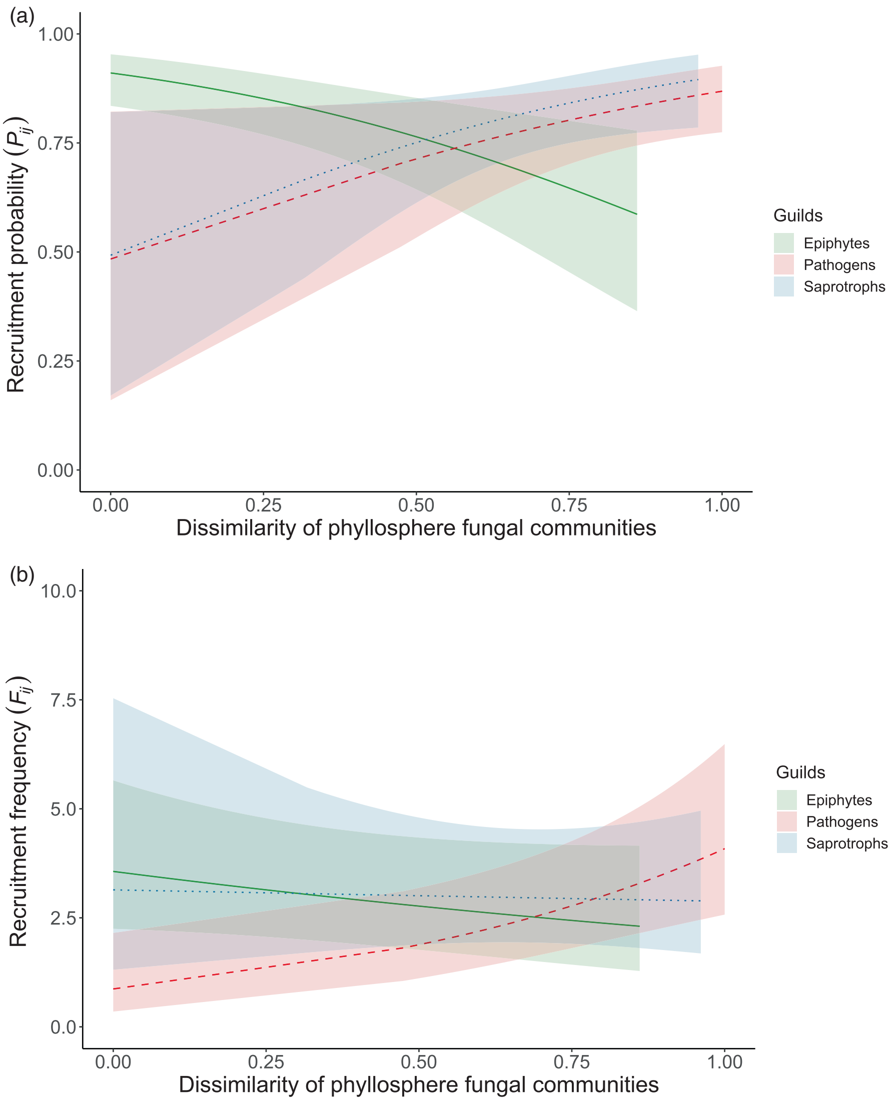


FIGURE 2 Predictions of the generalized linear mixed model analysis performed to explore the effect of the dissimilarity of phyllosphere fungal communities of pathogens, saprotrophs and epiphytes measured as the Bray–Curtis distance on (a) recruitment frequency and (b) recruitment probability between pairs of plant species.

We found that plant species with similar saprotrophic fungal communities have lower probability to recruit one under the other. Therefore, leaf saprotrophs add to the negative effect exerted by plant pathogens and contribute to filter the recruitment of species with similar fungal communities. This result was against our predictions, since we did not expect saprotrophs to increase or decrease seedling establishment as they feed on dead tissues. It is possible that phyllosphere saprotrophs may benefit from the dead plant tissues caused by pathogen damage, possibly acting synergistically with them, contributing to increase the extension of leaf damage beyond that inflicted by the pathogen, hence increasing seedling mortality. This hypothesis may be supported by Mantel tests that show plant species with similar fungal communities of saprotrophs also present similar communities of pathogens, suggesting a potential link between these guilds (Pajares-Murgó et al., 2023). Indeed, saprotrophic fungi can accelerate leaf senescence (Bertelsen et al., 2001) or shift into a pathogenic secondary lifestyle (Newton et al., 2010; Petrini, 1991). Interestingly, while we found that leaf saprotrophs have a negative pairwise-specific effect on plant recruitment, our species-level analyses show that canopy plants with diverse saprotrophic fungal communities facilitate abundant sapling recruitment. This suggests that a high diversity of saprotrophic fungi could promote the decomposition of soil organic matter and benefit those saplings that are not affected by the pathogenic filtering. Upon leaf decay, the early stage of decomposition typically entails the exudation of significant quantities of easily decomposable and nutrient-rich compounds (Voříšková & Baldrian, 2013). Some studies have found that leaf saprotrophic fungi participate in litter decomposition on the soil, making accessible more recalcitrant matter compounds in the initial stages of the process (Fanin et al., 2021; Promputtha et al., 2007; Voříšková & Baldrian, 2013). The production of litter and exudates richer in nutrients and labile carbon can enhance seedling performance and survival (Berg & McClaugherty, 2008; Deniau et al., 2018). Therefore, a higher diversity of saprotrophic fungi could improve decomposition rates (Cox et al., 2001; Setälä & McLean, 2004) due to niche differentiation or facilitation (Hättenschwiler et al., 2005), indirectly benefiting recruitment.

Finally, our findings suggest there is a beneficial effect of epiphytic fungi on recruitment, which counters the detrimental effect of plant pathogens, in which shared epiphytes contribute to enhance canopy–recruit interactions. Furthermore, we found that canopy plants hosting higher richness of epiphytic fungi contribute positively to the sapling bank, increasing recruitment density under their canopy. These results provide evidence on the mutualistic role of these fungi by promoting recruitment and thus affecting plant community dynamics. This adds to recent studies that show the capacity of some foliar fungi to regulate plant disease severity (Arnold et al., 2003) or enhance disease resistance (Ganley et al., 2008). However, the precise mechanisms remain unclear and may result from direct protection from antagonists or indirect positive effects on different components of plant fitness (Vorholt, 2012). For example, some studies report that endophytic fungi can be used as biocontrol agents inducing host local or systemic resistance (Andrews, 1992; Bailey et al., 2006; Raghavendra & Newcombe, 2013) or outcompeting plant pathogens by impeding their colonization of the leaf (Arnold et al., 2003; Saikkonen et al., 2015). However, although epiphytic fungal communities have been found to be more abundant compared to endophytic fungi (Gomes et al., 2018; Yao et al., 2019), and several studies prove the protective role of epiphytic bacteria in leaf surfaces (Aragón et al., 2017; Innerebner et al., 2011; Ritpitakphong et al., 2016; Wei et al., 2016), few experimental studies have particularly addressed the potential mutualistic role of fungal epiphytes (Kembel & Mueller, 2014). Research on epiphytic fungi is mostly based on seedlings of cultivated plants (Kembel & Mueller, 2014; Warren, 1972; Widmer & Dodge, 2013). For example, research on *Aureobasidium pullulans*, a dominant epiphyte used in biocontrol for agricultural and commercial forestry (Bashir et al., 2022; Kharwar et al., 2010; Osono, 2008). Yet, the role of these fungi in wild plant species, where environmental conditions and density of host plants are much more variable, still remains unexplored.

TABLE 2 Generalized linear mixed model analyses exploring the effect of fungal richness (operational taxonomic unit proportion) and the diversity (number of effective partners), the site and the cover of canopy and recruit on the structure of the sapling bank (a, c) and the canopy contribution to the sapling bank (b, d) of plant species on the frequency of canopy–recruit interactions. Significant effects are bold typed.

Fixed effects	Estimate	SE	*Z*-value	*p*-Value
(a) Structure of the sapling bank				
Pathogen richness	6.097	4.198	1.452	0.146
Saprotroph richness	−8.107	4.368	−1.856	0.063
Epiphyte richness	−0.049	1.304	−0.037	0.970
Site	−0.558	0.361	−1.544	0.123
Cover of recruit species	0.505	0.054	9.338	**<0.001**
(b) Canopy contribution to the sapling bank				
Pathogen richness	−0.019	2.956	−0.006	0.995
Saprotroph richness	−1.096	2.916	−0.376	0.707
Epiphyte richness	2.078	0.986	2.107	**0.035**
Site	0.081	0.270	0.302	0.763
Cover of canopy species	1.075	0.075	14.304	**<0.001**
(c) Structure of the sapling bank				
Pathogen diversity	0.003	0.043	0.077	0.939
Saprotroph diversity	−0.035	0.031	−1.128	0.259
Epiphyte diversity	−0.023	0.057	−0.411	0.681
Site	−0.323	0.347	−0.932	0.351
Cover of recruit species	0.536	0.053	10.055	**<0.001**
(d) Canopy contribution to the sapling bank				
Pathogen diversity	−0.042	0.029	−1.454	0.146
Saprotroph diversity	0.065	0.021	3.127	**0.002**
Epiphyte diversity	−0.017	0.044	−0.385	0.701
Site	0.609	0.247	2.464	**0.014**
Cover of canopy species	1.110	0.071	15.558	**<0.001**

5 | CONCLUSION

Taken together, our results suggest that phyllosphere pathogens and saprotrophs impose a pairwise-specific filter on recruitment in natural plant communities, which can be counteracted, to some extent, by a pairwise-specific mutualistic effect of leaf epiphytic fungi. The present study did not allow to explore the interactions between different fungal guilds that may explain their counteracting effects. Future experimental studies could allow to understand, for example, how often and in which species the beneficial effect of epiphytes is due to protection against pathogens or to nutrient

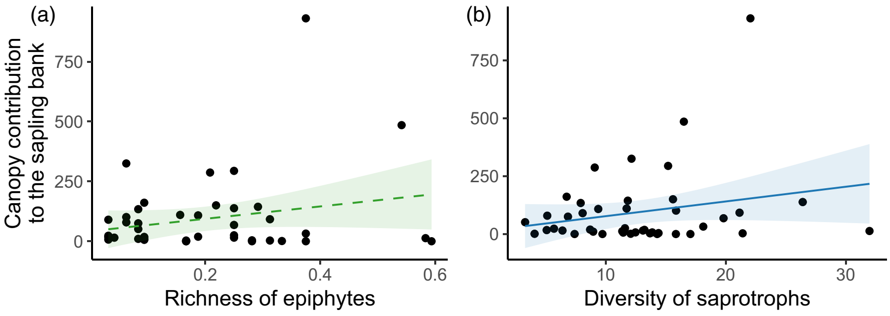


FIGURE 3 Predictions of the generalized linear mixed model analysis performed to explore the effects of (a) the richness (operational taxonomic unit proportion) of fungal epiphytes on the canopy contribution to the sapling bank, (b) the diversity (effective number of partners) of fungal saprotrophs to the canopy contribution to the sapling bank.

deposition on leaf surfaces. Although the mechanisms involved in this counteracting effect remain to be disclosed, our study clearly suggests that phyllosphere fungi play a relevant role in the assembly of canopy–recruit interactions in plant communities (Alcántara, Garrido, & Rey, 2019), with potential consequences for plant species coexistence (Alcántara & Rey, 2012). Phyllosphere fungi also contribute to structure forest sapling banks. The presence of canopy species hosting high richness of epiphytes or high diversity of saprotrophs favours the formation of an abundant sapling bank. These results provide a new example on the relationship between diversity and a key ecological function such as plant recruitment. In order to determine whether these relationships are widespread, similar studies should be conducted in multiple biogeographic regions and ecosystems.

AUTHOR CONTRIBUTIONS

Julio M. Alcántara and Jose L. Garrido designed the study. Julio M. Alcántara and Mariona Pajares-Murgó lead the writing and conducted the statistical analyses. Álvaro López-García conducted the bioinformatic analyses. All authors collected field samples. All authors contributed critically to the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data on the phyllosphere fungal communities are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.98sf7m0nh (Pajares-Murgó et al., 2022). Raw sequences are deposited in the NCBI Sequence Read Archive under BioProject PRJNA909185. Data on the frequency of canopy–recruit interactions are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.bh8n5j8 (Alcántara et al., 2018).

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SUPPORTING INFORMATION

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

Appendix S1. Data description tables and figures.

Appendix S2. Pairwise analyses tables.

Appendix S3. DNA extraction and sequencing analyses.

Appendix S4. Generalized linear mixed model formulas and figures.

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