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REDES DE REEMPLAZAMIENTO EN BOSQUES:

INFLUENCIA DE LAS COMUNIDADES DE HONGOS DE LA FILOSFERA Y DE LAS INTERACCIONES PLANTA-SUELO

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REDES DE REEMPLAZAMIENTO EN BOSQUES: INFLUENCIA DE LAS COMUNIDADES DE HONGOS DE LA FILOSFERA Y DE LAS INTERACCIONES PLANTA-SUELO

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CERTIFICAN

Que los trabajos de investigación incluidos en la memoria titulada *Redes de reemplazamiento en bosques: Influencia de las comunidades de hongos de la filosfera y de las interacciones planta-suelo* han sido realizados por la doctoranda **Maria Pajares Murgó** y son aptos para ser presentados ante el tribunal para aspirar al grado de Doctor por la Universidad de Jaén.

Y para que así conste, y en cumplimiento de las disposiciones legales vigentes, extendemos el presente certificado a

2 de septiembre de 2024.

Fdo. Dr. Julio M. Alcántara

Fdo. Dr. José Luís Garrido

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Resumen

Las interacciones entre especies son un elemento fundamental de los ecosistemas, y su análisis bajo el prisma de redes ecológicas complejas puede aportar información sobre cómo se sostienen unas especies a otras y cómo cambian las comunidades con el tiempo. El objetivo principal de esta tesis ha consistido en demostrar el papel de las interacciones planta-hongo de la filosfera y planta-suelo en el resultado de las interacciones entre plantas durante el reclutamiento y su efecto en la coexistencia de especies y en el ensamblaje de la comunidad local. Para abordar este objetivo, se seleccionaron dos comunidades de bosques mixtos de pinos y quercíneas de la Sierra Sur de Jaén y la Sierra de Segura, localizados en el sur de la Península Ibérica. En total, se han analizado hasta 38 especies arbóreas y arbustivas.

Los tres capítulos principales de resultados abordan tres niveles de complejidad en las interacciones: las interacciones directas planta-hongo en la filosfera focalizadas a escala de centímetros en las hojas, las relaciones planta-planta durante el reclutamiento a escala de metros entre individuos y finalmente la agregación de múltiples interacciones planta-planta en redes de reclutamiento, reflejando la escala espacial de la comunidad local. Esta tesis integra el conocimiento a distintas escalas, aportando nuevas evidencias sobre los factores que influyen en la coexistencia y el funcionamiento de las comunidades vegetales naturales.

Nuestros resultados indican que los hongos de la filosfera pertenecientes a distintos gremios ecológicos (descomponedores, patógenos y epífitos) responden de forma común a un conjunto de rasgos foliares. Estos rasgos actúan como filtros bióticos sobre el conjunto de especies, limitando la colonización y el ensamblaje de la comunidad microbiana de las hojas. Aunque existan rasgos

funcionales tanto químicos como morfológicos que afectan de manera específica a cada gremio, el filtro biótico impuesto por las plantas genera estructuras modulares en la red planta-hongo que muestran una clara señal filogenética (**Capítulo 1**).

Los análisis sobre el papel de distintos grupos funcionales de hongos en las relaciones planta-planta durante el reclutamiento muestran su influencia en el ensamblaje y la estructuración del banco de reclutas en la comunidad. Los hongos patógenos y saprótrofos imponen un filtro específico en las interacciones adulto-recluta, potencialmente contrarrestado por un efecto positivo de los epífitos foliares. Además, la riqueza de epífitos y la diversidad de saprótrofos asociada a una especie de planta favorece la formación de un abundante banco de reclutas bajo su influencia (**Capítulo 2**).

Finalmente, los resultados experimentales con diez especies representativas del sistema de estudio demuestran la existencia de procesos de retroalimentación planta-suelo (PSFs, por las siglas en inglés de *plant-soil feedback*), y sugieren que el reclutamiento en la comunidad natural está influido por PSFs específicos para cada par de especies. En la comunidad estudiada, los PSFs tienden a generar diferencias de fitness entre pares de especies que superan los efectos (des)estabilizadores del suelo, impidiendo su coexistencia. Más allá del análisis por pares de especies, el análisis de la topología de la red en modelos de dinámica de comunidades demuestra que la intransitividad creada por los PSFs es poco frecuente, pero supone una condición suficiente para la coexistencia si todas las especies participan en el mismo ciclo de interacciones intransitivas (**Capítulo 3**).

Abstract

Species interactions are a fundamental element of ecosystems, and their analysis through the lens of complex ecological networks can provide information on how species support each other and how communities change over time. The main objective of the thesis was to demonstrate the role of phyllosphere plant-fungus and plant-soil interactions in the outcome of plant interactions during recruitment and their effect on species coexistence and local community assembly. To address this objective, we selected two mixed pine-oak forest communities from the Sierra Sur de Jaén and Sierra de Segura, located in the south of the Iberian Peninsula. In total, up to 38 species of tree and shrub species have been analysed.

The three main chapters of results address three levels of complexity in interactions: direct plant-fungus interactions in the phyllosphere, focused at the centimetre scale on leaves, plant-plant relationships during recruitment, at the scale of meters between individuals, and finally the aggregation of multiple plant-plant interactions in recruitment networks, reflecting the spatial scale of the local community. This thesis integrates knowledge at different scales, providing new evidence on the factors influencing the coexistence and functioning of natural plant communities.

Our results indicate that phyllosphere fungi belonging to different ecological guilds (decomposers, pathogens and epiphytes) respond to a common set of leaf traits. These functional traits act as biotic filters on the species pool, limiting colonisation and assembly of the leaf microbial community. Although there are both chemical and morphological functional traits that specifically affect each fungal guild, the biotic filter imposed by

plants generates modular structures in the plant-fungus network that show a clear phylogenetic signal (**Chapter 1**).

Analyses on the role of different fungal guilds in plant-plant interactions during recruitment show their influence on the plant species assembly and the structure of the sapling bank in the community. Pathogenic and saprotrophic fungi impose a specific filter on canopy-recruit interactions, potentially counteracted by a positive effect of foliar epiphytes. In addition, the richness of epiphytes and the diversity of saprotrophs associated with a plant favours the formation of an abundant bank of recruits under their influence (**Chapter 2**).

Finally, the experimental results with ten representative species of the study system demonstrate the existence of plant-soil feedback processes (PSFs), and suggests that recruitment in the natural community is influenced by specific PSFs for each pair of species. In the studied community, PSFs tend to generate fitness differences between pairs of species that can overcome soil (de)stabilising effects, preventing their coexistence. Beyond the pairwise analysis, the analysis of network topology in community dynamics models shows that intransitivity created by PSFs is rare, but is a sufficient condition for coexistence if all species are embedded in the same cycle of intransitive interactions (**Chapter 3**).

Introducción y objetivos

Introducción

Ensamblaje de comunidades y coexistencia de especies

Comprender los mecanismos que dirigen el ensamblaje y posibilitan la coexistencia de especies en una comunidad es uno de los grandes desafíos que afronta la ecología (Cody & Diamond 1975; Hutchinson 1961; Zobel 1992). Entender los mecanismos ecológicos que mantienen la diversidad local es clave para mejorar la gestión y conservación de los hábitats naturales en distintos escenarios ambientales presentes y futuros (p.ej. conservación de especies amenazadas, control de invasiones biológicas o mitigación del impacto del cambio climático).

El ensamblaje de comunidades es el proceso por el cual las especies de un conjunto regional colonizan e interactúan para formar comunidades o ensamblajes locales (HilleRisLambers *et al.* 2012). Este proceso se ve determinado por diversos filtros que condicionan el establecimiento de la comunidad y operan a distinta escala espacio-temporal. Por ejemplo, la probabilidad de que una especie alcance una comunidad local depende de su abundancia en el entorno geográfico, de su capacidad dispersiva y de diferentes procesos estocásticos (Vellend 2010). Seguidamente, deberá hacer frente a factores abióticos locales (i.e. temperatura, humedad, precipitación, propiedades del suelo) e integrarse en el entramado de factores bióticos característicos de la localidad (i.e. competición, depredación o herbivoría) (Thompson *et al.* 2020). Estos filtros abióticos y bióticos determinarán la composición y la estructura de la comunidad a escala local.

Las especies que son capaces de atravesar estos filtros deben poseer capacidades y requerimientos ecológicos similares. Por lo tanto, por definición, sus nichos ecológicos estarán solapados

en cierta medida, lo que implica la existencia de relaciones de competencia. Sin embargo, el principio de exclusión competitiva establece que especies que comparten el mismo nicho ecológico no pueden coexistir, ya que la competencia por un mismo recurso lleva a la especie más competitiva (por ejemplo, la que mejor persista en condiciones de baja disponibilidad de recursos) a prevalecer y excluir a las demás (Gause 1934; Hardin 1960). No obstante, esta predicción teórica basada exclusivamente en la capacidad competitiva de las especies contrasta con la realidad ecológica, donde múltiples especies coexisten en comunidades locales. Esta paradoja fue puesta de manifiesto por Hutchinson en su ensayo *Paradoja del plancton* de 1961, y desde entonces se han propuesto múltiples mecanismos que tratan de resolverla, como la limitación por diferentes recursos (Tilman 1982), diferentes consumidores (HilleRisLambers *et al.* 2010) o enemigos naturales (Allan *et al.* 2010), la segregación espacial o temporal (Abrams 1987), o la igualdad competitiva entre especies (Hubbell 2001).

En última instancia, la mayoría de los mecanismos teóricos de coexistencia propuestos se basan en la diferenciación de algún aspecto del nicho ecológico entre especies competidoras. Sin embargo, la teoría moderna de la coexistencia (MTC, en adelante) (Chesson 2000, 2003) sugiere que las diferencias de nicho no son suficientes para determinar la coexistencia entre especies. La MTC postula que la coexistencia de múltiples especies depende del balance entre mecanismos ecualizadores, que son aquellos que disminuyen las diferencias de fitness entre especies, y mecanismos estabilizadores, aquellos que surgen de las diferencias de nicho. Según la MCT, las diferencias de nicho son mecanismos estabilizadores porque promueven una coexistencia estable en el tiempo, por ejemplo, haciendo que las especies se limiten más a sí mismas de lo que limitan a sus competidoras. El

proceso de estabilización se ve reflejado en las tasas de crecimiento per cápita de las especies, que disminuyen cuando aumenta su abundancia en la comunidad. De esta manera, cuando una especie se vuelve escasa, su tasa de crecimiento poblacional puede ser positiva, evitando su extinción (i.e. criterio de invasibilidad). En ausencia de los procesos que dan ventaja a especies cuando son raras, las diferencias de fitness podrían provocar el crecimiento de especies competitivamente superiores y un decrecimiento de aquellas especies competitivamente inferiores. Por lo tanto, la coexistencia dependerá de que la magnitud de las diferencias de nicho supere las diferencias de fitness, de modo que incluso especies con desventajas competitivas puedan invadir y coexistir de forma estable en el sistema (Leibold & McPeek 2006).

Los mecanismos estabilizadores mejor estudiados son los que se basan en procesos de mortalidad negativamente denso-dependientes (Comita *et al.* 2010; Johnson *et al.* 2012; LaManna *et al.* 2016). Por ejemplo, la hipótesis de Janzen y Connell (Connell 1971; Janzen 1970) ha sido ampliamente estudiada en comunidades naturales vegetales de bosques tropicales (Augspurger 1983; Bagchi *et al.* 2010; Bell *et al.* 2006; Mangan *et al.* 2010), bosques templados (Jia *et al.* 2020) y pastizales (Petermann *et al.* 2008). Esta hipótesis propone que enemigos naturales especialistas pueden limitar el reclutamiento de una planta cerca de individuos conespecíficos o en lugares donde estos alcanzan altas densidades. De esta forma, los antagonistas pueden promover la diversidad al impedir la dominancia de sus especies hospedadoras. Sin embargo, para que dichos procesos de mortalidad denso-dependientes contribuyan a la coexistencia, el reclutamiento no sólo debería reducirse entre conespecíficos, sino que debe favorecerse o ser menos negativo en la proximidad de heterospecíficos (LaManna *et al.* 2016). En consecuencia, existe cada vez más evidencia de que las interacciones interespecíficas

planta-planta durante el reclutamiento desempeñan un papel clave en el ensamblaje de comunidades (Uriarte *et al.* 2004; Zhu *et al.* 2015).

Interacciones ecológicas a nivel de comunidad

Las interacciones entre múltiples especies son la base de una comunidad ecológica, y su estudio es fundamental para entender cómo se estructura espacialmente la diversidad y cómo persiste en el tiempo (Bimler *et al.* 2024). Sin embargo, la mayoría de estudios empíricos y teóricos se han centrado en mecanismos que operan entre pares de especies (p.ej. modelo clásico de competencia de Lotka-Volterra) (Gallien *et al.* 2017). Este enfoque no tiene en cuenta que, en comunidades naturales, las especies interactúan con muchas otras simultáneamente, lo que es fundamental para entender el efecto de las interacciones en el mantenimiento de la diversidad (Godoy *et al.* 2017; Miller *et al.* 2022). Para reflejar esta complejidad biológica, estudios recientes proponen estudiar las interacciones a nivel de comunidad mediante el uso de redes ecológicas.

La estructura de una red de interacciones puede aportar información sobre propiedades dinámicas de la comunidad, como la estabilidad ante perturbaciones. Una perturbación puede ser un cambio en la abundancia de las especies, por ejemplo, a causa de estocasticidad ambiental. En este contexto, se considera que una comunidad es estable si tiene la capacidad de regresar al punto de equilibrio original (Arnoldi *et al.* 2018; May & Allen 1973), y se considera que es factible (en inglés, *feasible*) si todas las especies tienen una abundancia positiva en ese punto de equilibrio (Cenci & Saavedra 2019; Saavedra *et al.* 2017). Todo modelo teórico de dinámica de comunidades incluye una matriz de interacciones, que describe las relaciones entre especies y la fuerza de cada

interacción. Los análisis clásicos de estabilidad tienen en cuenta la fuerza de las interacciones representadas en la matriz de la comunidad. Sin embargo, la incorporación de una perspectiva de red a la matriz de interacciones ha puesto de manifiesto la importancia de la estructura de interacciones directas e indirectas para comprender como se sostiene la diversidad de especies en una comunidad (Levine *et al.* 2017).

La intransitividad de las interacciones es uno de los fenómenos que mejor ponen en evidencia la relación entre la estabilidad y la coexistencia de especies en la comunidad. La competencia intransitiva ocurre cuando ninguna especie es capaz de ganar a todas las demás, lo que se suele ejemplificar a través del famoso juego de piedra-papel-tijera (Gilpin 1975; May & Leonard 1975). La intransitividad promueve la coexistencia porque actúa como un mecanismo estabilizador: un cambio en la abundancia de una de las especies se propagará a través de la red, creando un circuito de retroalimentación que contrarresta la perturbación inicial (Levine *et al.* 2017). Incluso, sería posible que pares de especies que se excluirían mutuamente, coexistan cuando se integran en una red de competencia intransitiva (Edwards & Schreiber 2010).

Varios estudios teóricos han explorado el efecto de la competencia intransitiva el mantenimiento de la diversidad (Allesina & Levine 2011; Barabás *et al.* 2016; Kandlikar *et al.* 2019; Laird & Schamp 2018). Sin embargo, aunque el fenómeno de la competencia intransitiva ha sido estudiado sobre todo en comunidades de plantas (Gallien *et al.* 2017), aún hay pocas evidencias empíricas sobre su papel en la coexistencia de las especies en comunidades vegetales naturales. Por ejemplo, Soliveres *et al.* (2015) sugieren que la competencia intransitiva está extendida en el medio natural. Por el contrario, Godoy et al. (2017) o Kinlock (2019) afirman que las interacciones intransitivas son infrecuentes en los sistemas naturales. Además,

las comunidades naturales ricas en especies pueden contener interacciones transitivas e intransitivas simultáneamente, complicando la inferencia de coexistencia y persistencia de la comunidad (Muyinda *et al.* 2020). En este contexto, Alcántara & Rey (2012) presentaron un marco analítico en el que se relaciona la topología de la red de interacciones con propiedades dinámicas de la comunidad. Este marco analítico está basado en el concepto de *Strongly Connected Components* (SCCs), procedente de la teoría de grafos, y permite inferir la persistencia de especies en redes de interacciones complejas más allá de la transitividad o la intransitividad de éstas.

De dinámicas de reemplazamiento a redes de reclutamiento

En el contexto de las redes de interacciones entre plantas, el proceso de reclutamiento de juveniles bajo plantas adultas permite adaptar datos observacionales para parametrizar modelos teóricos, aportando información sobre mecanismos que impulsan la estructura y la dinámica de comunidades naturales.

El reclutamiento entre plantas está vinculado al concepto del reemplazamiento como el impulsor de la dinámica de la comunidad. Este proceso se basa en la idea de que la muerte de un individuo (el adulto) libera un espacio donde otros individuos de su misma u otra especie (los reclutas) podrán reclutarse y crecer, ocupando su lugar (Grubb 1977; Hubbell 2001; Myster 2012). Los mecanismos involucrados en este proceso inducen cambios en el tiempo de la abundancia relativa de las especies, (i.e. en la dinámica de la comunidad). En el caso de especies de plantas leñosas, su longevidad impide observar directamente un proceso de reemplazamiento, por lo que es razonable asumir que cuando una planta muere será reemplazada por alguna de las plantas que se reclutaron bajo su copa (Alcántara *et al.* 2015; Horn 1975;

Siles *et al.* 2008). Por esta razón, analizar las interacciones planta-planta durante el reclutamiento puede proporcionar información sobre la persistencia de las especies en comunidades forestales (Alcántara & Rey 2012; Umaña *et al.* 2017; Valiente-Banuet *et al.* 2006). Cabe remarcar que estas interacciones pueden ser positivas, negativas o neutras, diferenciando las interacciones adulto-recluta de las interacciones nodriza-recluta resultantes de procesos de facilitación (Alcántara *et al.* 2019).

El conjunto de interacciones adulto-recluta de una comunidad local se puede representar como una red de reclutamiento, y su análisis aporta información sobre el ensamblaje, estructura y estabilidad de la comunidad (Alcántara et al., 2019). En primer lugar, se puede extraer información de la matriz de reclutamiento, que se construye a partir de datos de frecuencia (*Fij*) o probabilidad (*Pij*) de interacciones adulto-recluta. Uno de los aspectos que proporciona el análisis de la matriz de reclutamiento es el servicio de reclutamiento que provee una determinada especie, es decir, la densidad de individuos juveniles de diferentes especies reclutadas bajo los individuos adultos de dicha especie en la comunidad. El servicio de reclutamiento informa sobre qué especies de plantas refuerzan, debilitan o sesgan el banco de individuos juveniles en la comunidad. En segundo lugar, el análisis cualitativo de redes de reclutamiento permite evaluar las posibilidades de coexistencia a largo plazo de las distintas especies de la comunidad y el papel funcional que juega cada especie en el mantenimiento de la diversidad local (Alcántara y Rey 2012). Finalmente, la red de reclutamiento se puede incorporar a modelos de dinámica de comunidades para evaluar mecanismos de ensamblaje de la red en comunidades reales, infiriendo en la estabilidad de la comunidad y la coexistencia entre especies (Alcántara *et al.* 2015, 2017).

Factores que influyen en el reclutamiento

El proceso de reclutamiento engloba todas las etapas del ciclo vital de las plantas excepto el estadio adulto (Alcántara *et al.* 2018). En este proceso, distintos factores bióticos y abióticos actúan simultánea o secuencialmente para determinar la probabilidad de reclutamiento de un individuo, introduciendo estocasticidad (p.ej. el azar puede determinar donde cae una semilla al ser dispersada, si escapa o no a predación o si encuentra o no condiciones favorables para su germinación y supervivencia). Sin embargo, el resultado de los efectos de las plantas adultas sobre las que se reclutan bajo ellas puede destacar sobre esta estocasticidad (p.ej. semillas dispersadas por aves frugívoras tienen mayor probabilidad de ser depositadas bajo plantas de fruto carnoso) (González-Varo *et al.* 2014; González-Varo *et al.* 2022; Perea *et al.* 2021b). Hay modelos que sugieren que el ensamblaje de una comunidad se ve sujeto principalmente a mecanismos estocásticos (Adler *et al.* 2010; Marteinsdóttir *et al.* 2018). Si éste fuera el caso, el reclutamiento dependería principalmente de la abundancia de las especies en la comunidad. Sin embargo, varios estudios han demostrado que tanto las interacciones adulto-recluta como la estructura de la red están influenciadas también por mecanismos deterministas. Por ejemplo, la plantas más alejadas filogenéticamente tienen mayor probabilidad de reclutarse entre sí (Verdú *et al.* 2009; Verdú & Valiente-Banuet 2011). Otros factores relacionados con la identidad de la especie como los rasgos funcionales de la planta (Perea *et al.* 2021a) o las comunidades de microorganismos más o menos especializados con las que se asocian (p.ej. hongos micorrícicos o patógenos), también pueden tener un rol importante en el establecimiento de interacciones adulto-recluta (Garrido *et al.* 2023, Perea *et al.*

2020).

Los estudios sobre el papel de las interacciones planta-microorganismo en distintas funciones ecosistémicas y procesos ecológicos ha crecido enormemente a lo largo de la última década. Sutherland *et al.* (2013) plantearon la necesidad de mejorar nuestro conocimiento acerca de cómo la composición y diversidad de las comunidades de macroorganismos está determinada por interacciones con microorganismos. El desarrollo de técnicas de secuenciación masiva ha permitido identificar taxones microbianos no cultivables en laboratorio, incrementando el estudio de las comunidades microbianas en ecosistemas vegetales naturales (Laforest-Lapointe *et al.* 2017). Particularmente en especies leñosas no-comerciales, aún hay poca información sobre un grupo de organismos que interactúan íntimamente con las plantas: los hongos. Las plantas se asocian con una gran diversidad de hongos mutualistas (p.ej. hongos formadores de micorrizas arbusculares, hongos ectomicorrícicos y hongos endófitos foliares), comensales (p.ej. hongos saprótrofos) y antagonistas (p.ej. hongos patógenos). Estas interacciones que se establecen entre las plantas y sus comunidades de hongos asociadas, pueden afectar directa o indirectamente al desarrollo de los individuos, y por lo tanto a la dinámica poblacional y de comunidad.

La filosfera

La filosfera constituye toda la parte aérea de las plantas, pero su estudio está focalizado en las hojas (Vorholt 2012). Es un hábitat muy heterogéneo, que abarca un área estimada total de 10^9 km^2 en el planeta, considerando únicamente la superficie del haz y el envés de las hojas (Lindow & Brandl 2003; Peñuelas & Terradas 2014). Las hojas hospedan una gran diversidad de microorganismos, principalmente bacterias, hongos y protozoos que habitan tanto

dentro de los tejidos como en la superficie (Vorholt, 2012). El microbioma de la filosfera tiene el potencial de afectar al fitness de la planta y a las funciones ecosistémicas de la comunidad, pero los factores que condicionan su diversidad están aún poco investigados (Kembel & Mueller 2014; Koskella 2020).

Las hojas sirven como interfaz entre la planta y el entorno atmosférico, y su microbioma está relacionado con varias funciones clave relacionadas con la adquisición de recursos, la tolerancia al estrés ambiental o las defensas de la planta (Meyer & Leveau 2012). Algunos estudios han demostrado su participación en ciclos biogeoquímicos (Vorholt 2012), por ejemplo, reduciendo las emisiones de metanol a la atmosfera (Abanda-Nkpwatt *et al.* 2006), o en el ciclo de nutrientes (Perreault & Laforest-Lapointe 2022; Zhu *et al.* 2022), mediante la regulación de la fijación de nitrógeno (Chen *et al.* 2020), o fósforo (Richardson et al., 2011). También se ha demostrado su participación en el mantenimiento de la salud de la planta, previniendo de la colonización y el crecimiento de patógenos especializados en las hojas (Ritpitakphong *et al.* 2016).

Los microrganismos colonizan la superficie de las hojas mediante distintos vectores (p.ej. aire, lluvia o animales, particularmente insectos herbívoros) (Leveau & Lindow 2001). El proceso de ensamblaje de la comunidad microbiana en las hojas está muy condicionado por rasgos funcionales de la planta y como estos modulan las condiciones extremas de temperatura, exposición a radiación ultravioleta o desecación que se pueden experimentar en la superficie foliar. Varios estudios han identificado que la disponibilidad de nutrientes, el tamaño o el indumento de las hojas condiciona la diversidad o la abundancia de sus comunidades microbianas (Kembel *et al.* 2014; Wang *et al.* 2023; Yadav *et al.* 2005). Incluso rasgos más generales como la longevidad de la hoja pueden determinar la composición del

microbioma foliar (Flessa *et al.* 2012). Estos estudios sugieren que los rasgos funcionales de las hojas actúan como un filtro biótico en el ensamblaje de las comunidades microbianas de la filosfera.

Las comunidades de hongos de la filosfera son taxonómica y funcionalmente muy diversas, y juegan un papel importante en el desarrollo de la planta (Almeida *et al.* 2024). Taxonómicamente, la mayoría de hongos que habitan las partes aéreas de las plantas pertenecen a los fila *Ascomycota* y *Basidiomycota* (Jumpponen & Jones 2009; Kembel & Mueller 2014). Dentro de *Ascomycota*, los géneros más comunes son *Aureobasidium*, *Clarospodium* y *Taphrina*. Por ejemplo, *Aureobasidium pullulans* es un hongo generalista muy abundante en la filosfera, que suele usarse como control biológico contra patógenos en especies comerciales (Cordier *et al.* 2012). Los hongos de la filosfera se suelen clasificar según su hábitat, como epífitos en caso de colonizar la superficie de las hojas, o como endófitos en el caso de habitar el interior de los tejidos. Desde un punto de vista funcional, se pueden clasificar según el tipo de interacción establecida con la planta huésped, que puede ser antagonista (patógenos), comensalista (saprótrofos) o mutualista (endófitos y epífitos) (Nguyen *et al.* 2016; Põlme *et al.* 2020). Los hongos patógenos se alimentan de tejidos vivos del huésped, causando lesiones en la hoja y consecuentemente un detrimento del fitness de la planta (Kirshner 2018). Los hongos saprótrofos viven como comensales en el huésped, y obtienen energía de degradar materia orgánica muerta (Osono 2006). Su actividad puede ser indirectamente beneficiosa para el huésped si se encuentran en el suelo, ya que pueden promover la descomposición de la hojarasca y el recambio de nutrientes (Voříšková, & Baldrian 2013). Por otro lado, también pueden estar ligados a la actividad patogénica (Chen *et al.* 2020). Los epífitos se alimentan de secreciones de la planta o substancias que se depositan en la superficie de la hoja. Suelen estar clasificados

como comensalistas, pero también pueden establecer relaciones mutualistas con su huésped produciendo metabolitos secundarios o compuestos antimicrobianos (p.ej. el caso de *Aureobasidium pullulans*). Finalmente, los endófitos se encuentran dentro de los tejidos, y también establecen relaciones mutualistas con la planta (Saikkonen *et al.* 2021), por ejemplo, incrementando su tolerancia al estrés o disminuyendo la infección patogénica (Arnold *et al.* 2003; Redman *et al.* 2001). Sin embargo, cabe mencionar que algunos hongos tienen la capacidad de cambiar su funcionalidad dependiendo de las condiciones ambientales o cambios en el metabolismo de la planta (Álvarez-Loayza *et al.* 2011; Barrett *et al.* 2009; Promputtha *et al.* 2007).

Explorar cómo los rasgos funcionales de las hojas condicionan el ensamblaje de los hongos de la filosfera en comunidades vegetales naturales es fundamental tanto para comprender el funcionamiento de las interacciones planta-hongo como para determinar su papel en la dinámica de la comunidad vegetal.

Retroalimentación planta-suelo - *Plant-soil feedbacks*

Cada vez hay más evidencia sobre el papel fundamental del microbioma del suelo en el desempeño de la planta (Bever *et al.* 2010; Reynolds *et al.* 2003; Van Der Heijden *et al.* 2008). En las dos últimas décadas, los ecólogos han empezado a cuantificar cómo estas interacciones influyen en la competencia entre las plantas y en la dinámica de las comunidades (Chesson 2003; Lanuza *et al.* 2018; Mordecai 2011). Las plantas establecidas en la comunidad modifican el microambiente del suelo en su entorno próximo (p.ej. mediante la liberación de exudados y materia orgánica muerta), condicionando la composición de la comunidad microbiana y las características abióticas del suelo. A su vez, esta modificación puede tener un efecto positivo o

negativo sobre el posterior desempeño de otras plantas que se reclutan en el entorno de las plantas establecidas, por ejemplo, mediante la acumulación de patógenos especialistas (Bever *et al.* 2015; Parker *et al.* 2015; Parker & Gilbert 2018) u organismos mutualistas como los hongos micorrícicos (Kadowaki 2024). Estos mecanismos de retroalimentación planta-suelo (*plant-soil feedback*, PSFs en adelante) condicionan distintos procesos en las comunidades de plantas (p.ej. dinámicas de sucesión o de invasión) así como la coexistencia de las especies (Bever 2003; van der Putten *et al.* 2013, van der Putten *et al.* 2016).

La base teórica para la mayoría de estudios sobre PSF se basa en los primeros modelos matemáticos propuestos por Bever et *al*., (1994; 1997) (Miller *et al.* 2022). La teoría de PSF proporciona una métrica (*Is*) que se puede cuantificar experimentalmente y que mide el desempeño relativo de las especies creciendo en suelo de otras especies frente a su desempeño cuando crecen en suelo propio (Bever *et al.* 1997; Crawford *et al.* 2019). Esta métrica informa sobre la capacidad del suelo para estabilizar o desestabilizar las interacciones entre especies dependiendo de si la plantas crecen mejor en el suelo ajeno (PSF negativo) o en suelo propio (PSF positivo). Un efecto de PSF negativo genera dinámicas negativamente dependientes de la densidad de conespecíficos, que estabilizan la coexistencia y ayudan a mantener la diversidad de la comunidad. Por el contrario, PSF positivos favorecen la dominancia de ciertas especies, reduciendo la diversidad en la comunidad.

Hay evidencia científica tanto experimental como observacional de la contribución de los PSFs a la coexistencia en bosques templados (Bennett *et al.* 2017), bosques tropicales (Jiang *et al.* 2022; Mangan *et al.* 2010), matorrales mediterráneos (Teste *et al.* 2017) y pastizales (Petermann *et al.* 2008). Además, los PSFs también pueden favorecer la invasión de especies que

no se vean afectadas por ellos (Klironomos 2002). Sin embargo, hay estudios que han encontrado resultados que contrastan con las predicciones de coexistencia por PSFs. Por ejemplo, se han encontrado dinámicas de dominancia a pesar de la existencia de PSFs negativos (Heinze *et al.* 2016) o PSFs positivos durante procesos de sucesión (Bauer *et al.* 2015). Una explicación a estas discrepancias podría residir en el diseño experimental. Así, algunos metaanálisis han encontrado que los efectos de PSF medidos en experimentos de invernadero suelen ser más fuertes que los obtenidos en el campo (Crawford *et al.* 2019; Kulmatiski *et al.* 2008). Por otro lado, las distintas posibilidades de cálculo de PSF pueden crear una desconexión entre los resultados empíricos y los fundamentos teóricos. Algunos estudios calculan el resultado del PSF estimando el éxito de la planta en suelo conespecífico comparado con suelo control (Crawford *et al.* 2019). Sin embargo, para predecir la dinámica de la comunidad se necesita medir el éxito relativo de las plantas en suelo conespecífico y heterospecífico de cada par de especies (Schroeder *et al.* 2019; Stump & Comita 2018). De hecho, varios estudios han encontrado experimentalmente que el resultado del PSF en árboles es específico para cada par de especies (Gómez-Aparicio *et al.* 2017; Mangan *et al.* 2010).

Usar la métrica de PSF "apareado" permite acercarse de una manera más correcta a los criterios de coexistencia establecidos por la teoría moderna de coexistencia (Crawford *et al.* 2019; Kandlikar *et al.* 2019; Ke & Wan 2020; Yan *et al.* 2022). Dos especies podrán coexistir si el efecto estabilizador del suelo es mayor que sus diferencias competitivas. Si los efectos desestabilizadores del PSF son más fuertes que las diferencias de fitness, no es posible la coexistencia entre las dos especies, dándose lugar efectos de prioridad. Finalmente, cuando las diferencias competitivas entre especies son más fuertes que los mecanismos estabilizadores o

desestabilizadores del suelo, la especie competitivamente inferior será excluida. Los estudios publicados hasta la fecha (Kandlikar *et al.* 2021; Yan *et al.* 2022) sugieren que el resultado más frecuente debería ser la exclusión competitiva, incluso entre pares de especies que coocurren en las mismas comunidades vegetales. Para resolver esta aparente paradoja, algunos estudios sugieren que hay que considerar los PSFs para el conjunto de la comunidad y no solamente por pares de especies (Barabás *et al.* 2016; Kandlikar *et al.* 2019; Levine *et al.* 2017; Mack *et al.* 2019). Kandlikar et al. (2019) propusieron que los PSFs pueden permitir la coexistencia si dan lugar a estructuras de interacciones intransitivas, aunque la coexistencia por pares no fuera teóricamente posible. En esta misma línea, Eppinga *et al.* (2018) mostraron que la coexistencia de múltiples especies se puede explicar por la estructura de la red de interacciones inducida por los PSFs.

Área de estudio: Sierra Sur de Jaén y Sierra de Segura

El sistema de estudio comprende dos comunidades de bosques mixtos de pinos y quercíneas de la Sierra Sur de Jaén y la Sierra de Segura, localizados en el sur de la Península Ibérica. Las comunidades estudiadas en la Sierra Sur de Jaén están dominadas por bosques mixtos de *Pinus halepensis*, *Quercus faginea* y *Q. ilex*, mientras que las de la Sierra de Segura conforman bosques mixtos de *P. nigra* subsp. *salzmannii*, *Q. faginea*, *Q. ilex* y *Q. pirenaica* (Figura 1, 2). Ambas comunidades presentan un rico sotobosque de árboles y arbustos altos compuesto principalmente por especies de los géneros *Acer*, *Crataegus*, *Juniperus*, *Sorbus*, *Prunus*, *Phillyrea* y *Pistacia*, junto con un estrato inferior dominado por pequeños arbustos de los géneros *Thymus*, *Cistus*, *Phlomis*, *Genista* y *Rosmarinus*. Cada comunidad se describió mediante muestreos realizados en cinco zonas en el caso de la Sierra Sur de

Jaén, y cuatro zonas en la Sierra de Segura, separadas entre 1.5 y 4.5 km (Figura 3). Todos los estudios presentados en esta tesis se realizaron en las mismas zonas.

En cada zona de estudio, se describieron las redes de reclutamiento entre plantas leñosas, estableciendo parcelas de 50 x 50 m y registrando la abundancia de las especies y la frecuencia de interacciones entre adultos y reclutas en cada parcela. Para el estudio de las comunidades de hongos de la filosfera, se muestrearon las hojas de 27 especies en la Sierra Sur de Jaén y 19 especies en la Sierra de Segura. Finalmente, para el estudio de los procesos de retroalimentación planta-suelo, se seleccionaron 10 especies representativas de ambas comunidades (*P. halepensis*, *Q. ilex, Q. faginea, J. phoenicea, J. oxycedrus, P. terebinthus, A. monspessulanum, C. atlantica, C. albidus* y *G. cinerea*).

Figura 1. Sierra Sur de Jaén

Figura 2. Sierra de Segura

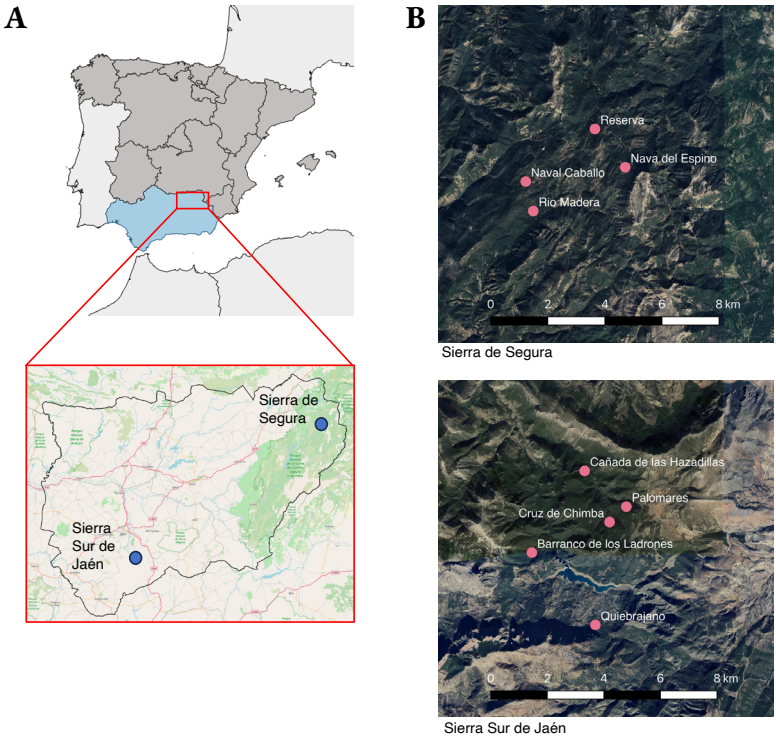


Figura 3. Ubicación del área de estudio: **A)** Ubicación de la Sierra Sur de Jaén y la Sierra de Segura, localizados en el sur de la Península Ibérica. **B)** Imágenes de satélite de cada comunidad, que muestra las cuatro zonas de estudio en el caso de la Sierra Sur de Jaén, y cuatro zonas en la Sierra de Segura.

Objetivos

El objetivo general de la tesis consiste en determinar cómo las interacciones planta-hongo y planta-suelo pueden mediar en las interacciones planta-planta que tienen lugar durante el reclutamiento. Este objetivo general se aborda mediante tres capítulos independientes (publicados como artículos científicos) pero interconectados entre sí que tienen como nexo conductor el proceso de reclutamiento (Figura 4). A continuación, se detalla el objetivo principal, junto con las hipótesis y enfoques usados en cada uno de ellos.

Capítulo 1. *Biotic filters driving the differentiation of decomposer, epiphytic and pathogenic phyllosphere fungi across plant species.* En este capítulo estudiamos el papel de los rasgos funcionales de las hojas y la filogenia de las plantas en el ensamblaje de los hongos de la filosfera en comunidades vegetales naturales. Los hongos establecen diferentes relaciones funcionales con la planta (patógenos, descomponedores y epífitos) y pueden responder de manera diferente a distintos rasgos funcionales de la planta que actúan como filtros bióticos, condicionando la colonización y ensamblaje de las comunidades fúngicas en las hojas. Para comprender mejor cómo se desarrollan estas relaciones en el medio natural, se han abordado las siguientes cuestiones: **a)** ¿Las comunidades de hongos de la filosfera varían según la especie de planta?; **b)** ¿Distintos grupos funcionales de hongos responden a un conjunto diferente de caracteres funcionales de las plantas?; **c)** ¿Cada grupo funcional de hongos de la filosfera varía independientemente de otros grupos funcionales?; **d)** ¿Los filtros bióticos impuestos por las plantas se ven reflejados en la estructura modular de la red planta-hongo?

Para abordar estas cuestiones *i*) describimos la composición taxonómica y funcional de los hongos de la filosfera de 38 especies de plantas leñosas y *ii*) analizamos su respuesta a distintos rasgos funcionales de las hojas y a la distancia filogenética entre plantas. Finalmente, *iii*) exploramos las propiedades de la red de interacciones planta-hongo para determinar qué aspectos están relacionados con los filtros bióticos impuestos por las plantas.

Capítulo 2. *Mutualistic and antagonistic phyllosphere fungi contribute to plant recruitment in natural communities.* Este capítulo trata de determinar el efecto de los distintos grupos funcionales de hongos de la filosfera en las interacciones de reclutamiento entre plantas de comunidades vegetales naturales. Para ello, se abordan las siguientes cuestiones: **a)** ¿La similitud de distintos grupos funcionales de hongos de la filosfera (patógenos, epífitos y saprótrofos) entre plantas influye en el resultado de las interacciones adulto-recluta?; **b)** ¿La riqueza y la diversidad de los hongos de la filosfera asociados a una especie vegetal condicionan su abundancia en el banco de reclutas o su influencia en el reclutamiento de otras plantas?

Para explorar estas cuestiones *i*) calculamos la riqueza y la diversidad de cada grupo funcional de hongos por cada especie de planta y la disimilitud de las comunidades fúngicas entre especies y *ii*) testamos su efecto en distintos aspectos del reclutamiento y en la frecuencia de interacciones adulto-recluta.

Capítulo 3. *Intransitivity in plant–soil feedbacks is rare but is associated with multispecies coexistence*. Avanzando en las interacciones que determinan el éxito de las plantas en el reclutamiento, el capítulo explora los mecanismos de retroalimentación planta-suelo (PSFs) y cómo las interacciones planta-microorganismo influyen en el reclutamiento de plantas y

la coexistencia de especies en comunidades vegetales naturales. Para ello, se plantearon distintas cuestiones: **a)** ¿El acondicionamiento del suelo por parte de las especies establecidas en la comunidad influye en el éxito de reclutamiento?; **b)** ¿Cuál es la frecuencia esperable de coexistencia, exclusión competitiva o efectos de prioridad generada por los PSFs entre pares de especies?; **c)** ¿Cuál es el papel de la intransitividad de las interacciones planta-planta mediadas por PSFs en la coexistencia de múltiples especies?

Para entender cómo los procesos de PSF influyen en la coexistencia de las especies *i*) determinamos experimentalmente el PSF de un subconjunto de 10 especies de plantas leñosas que coexisten en una comunidad natural y comparamos los resultados experimentales con las interacciones de reclutamiento observadas en el campo. Seguidamente, *ii*) estimamos la estabilización y las diferencias de fitness generadas por el PSF entre pares de especies y predecimos el resultado en términos de exclusión competitiva, coexistencia o efectos de prioridad usando el enfoque de la teoría de la coexistencia moderna. Finalmente, *iii*) utilizamos nuestros resultados experimentales para parametrizar el modelo de dinámica de comunidades de Eppinga *et al.* (2018) y estimamos la persistencia de todos los ensamblajes posibles obtenidos combinando las 10 especies estudiadas, y evaluamos el efecto de la intransitividad en la persistencia de las especies mediante el análisis topológico de la matriz de interacciones del modelo.

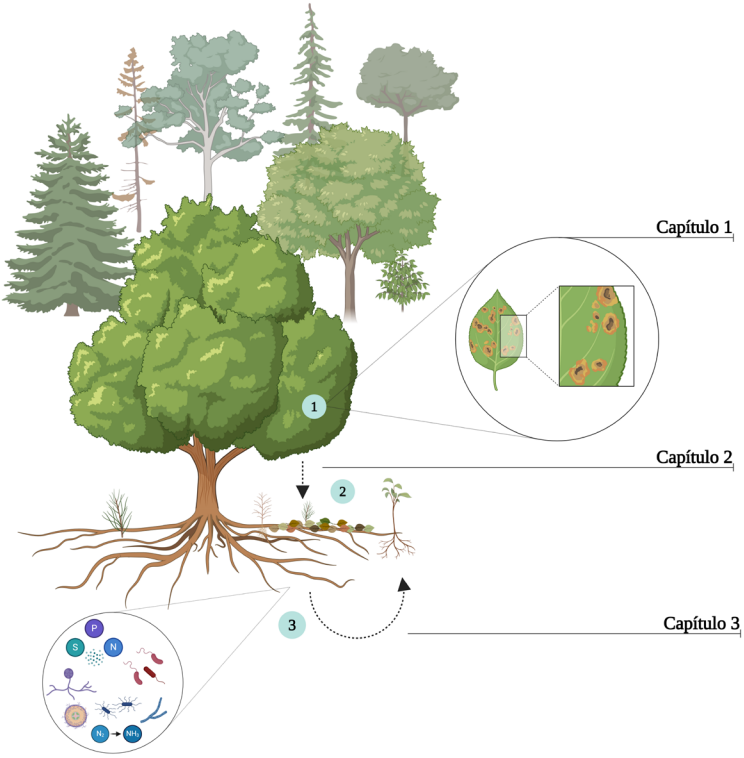


Figura 4. Ilustración esquemática de la organización de la tesis en tres capítulos principales que desglosan las interacciones entre plantas leñosas dentro de la comunidad vegetal. En el Capítulo 1, analizamos las interacciones entre las plantas y los hongos que habitan la filosfera. En el Capítulo 2, exploramos el efecto de los hongos de la filosfera en las interacciones planta-planta durante el reclutamiento. Finalmente, en el Capítulo 3 investigamos los mecanismos de retroalimentación planta-suelo y cómo las interacciones planta-microorganismo y las características abióticas del suelo influyen en el reclutamiento y en la coexistencia de las especies en comunidades naturales.

Capítulo 1

Biotic filters driving the differentiation of decomposer, epiphytic and pathogenic phyllosphere fungi across plant species

Pajares-Murgó, M., Garrido, J. L., Perea, A. J., López-García, Á., & Alcántara, J. M. (2023). Biotic filters driving the differentiation of decomposer, epiphytic and pathogenic phyllosphere fungi across plant species. *Oikos*, 2023(5), e09624.

(...) The gold and scarlet leaves that littered the countryside in great drifts whispered and chuckled among themselves, or took experimental runs from place to place, rolling like coloured hoops among the trees. It was as if they were practising something, preparing for something, and they would discuss it excitedly in rustly voices as they crowded round the tree trunks. (...)

Gerald Durrell, *My Family and Other Animals* (1956).

Abstract

The phyllosphere is a wide and complex ecosystem that provides a key support for microbial diversity. Fungal communities inhabiting the leaf are functionally variable and play important roles on plant performance. Factors conditioning the arrival and colonization of fungal communities will determine the phyllosphere fungal composition. Plant identity, leaf functional traits and host plant phylogeny have been shown to be regulators of the microbial colonization of the leaves, and can be considered as biotic filters determining the assembly of phyllosphere fungal communities. By high-throughput sequencing we analysed the phyllosphere fungal communities from 38 Mediterranean woody plant species in two forests of south-eastern Iberian Peninsula. We analysed the effect of plant species and site on fungal community composition. We also tested the effect of leaf functional traits and plant phylogeny on plant species differences in their fungal communities, and on the structure of the plant–fungus interaction network. Plant species account for a larger proportion than site in the variability of the composition of phyllosphere fungal communities. Leaf traits and host phylogeny influence the arrival and colonization of phyllosphere fungal communities across plant species. Plants with pubescent leaves and phylogenetically closer harbour more similar communities of decomposers, pathogens and epiphytes. Leaf habit (i.e. evergreen versus deciduous) also influences the community composition of decomposer and epiphytic fungi. Leaf carbon, leaf water content and leaf mass per area affect differentially each functional guild. Plant–fungus interaction networks present a modular structure in which plants belonging to the same module share more fungal species and are phylogenetically closer. We provide evidence that even though phyllosphere fungal communities are complex

ecosystems, fungi with contrasting relationships with the plant (decomposers, epiphytes and pathogens) respond similarly to a common subset of leaf traits that impose physical limitations to the assembly of phyllosphere fungal communities.

Keywords: community assembly, leaf functional traits, Mediterranean forests, phyllosphere fungi, plant epiphytic fungi, plant–fungus interaction network, plant pathogenic fungi.

Introduction

The phyllosphere, the aerial parts of plants mostly dominated by the leaves (Vorholt 2012), is a large and heterogeneous habitat for terrestrial microorganisms (Lindow & Brandl 2003, Bao *et al.* 2020). It has been estimated to be around 60% of the biomass across all taxa on Earth, harbouring hyper diverse microbial communities composed mostly of bacteria, fungi and protozoa (Meyer & Leveau 2012; Vacher *et al.* 2016; Koskella 2020).

Phyllosphere fungi are determinants of host-plant fitness and ecosystem functioning (Peñuelas & Terradas 2014; Qian *et al.* 2020). They play a variety of functions, some promoting plant growth (Peñuelas *et al.* 2012), other being pathogenic (Kembel & Mueller 2014), or degrading environmental pollutants or protecting the plant from environmental changes (Jia *et al.* 2020). Even, some of these fungi can condition population dynamics by altering the spatial distribution of plant recruitment (Perea *et al.* 2020).

Research on phyllosphere microbial communities focuses mainly on taxonomic descriptions (Laforest-Lapointe *et al.* 2016), physiological processes (Carvalho & Castillo 2018), crop productivity (Arya & Harel 2019) and species ecology (Osono 2008; Qian *et al.* 2020). The availability of massive sequencing

techniques has opened the possibility to test ecological concepts and theories in this environment (Meyer & Leveau 2012). These techniques have revolutionized microbial ecology (Bokulich *et al.* 2013; Zhu *et al.* 2021), allowing studies under complex natural settings such as diverse plant communities. At the plant community level, recent studies are exploring potential drivers of phyllosphere fungal communities, such as the influence of seasonality (Copeland *et al.* 2015), the spatial variability on a vertical canopy gradient (Izuno *et al.* 2016) or the relation with the soil microbiota (Beule *et al.* 2017). A functional approach to the study of phyllosphere fungi and their host plants could reveal key mechanisms to understand their interactions (Nguyen *et al.* 2016; Põlme *et al.* 2020; Bashir *et al.* 2022).

Fungi can colonize and interact at the leaf surface (i.e. epiphytic microbiota) or internal tissues (i.e. endophytic microbiota) (Santamaría & Bayman 2005; Vorholt 2012; Zhu *et al.* 2021). This classification, based only on their location in the leaf, does not take into account the nature of their relationships with their host plants. For example, some fungi living inside the leaf tissues may be involved in mutualistic interactions with plants (Rodriguez *et al.* 2009), while some others may be pathogenic or asymptomatic and still others can even shift from mutualistic to antagonistic or commensalistic interactions (Beattie & Lindow 1995; Chaudhry *et al.* 2021). A functional perspective of the relationships between fungi and plants is being increasingly used in the study of phyllosphere fungi. Nguyen *et al.* (2016) and Põlme *et al.* (2020) classify fungi into several functional guilds, some of them clearly associated with plant leaves such as pathogens, decomposers (i.e. saprotrophs), or epiphytes. Pathogenic fungi feed on living leaf tissues causing various types of damage (Kirschner 2018). Decomposer fungi obtain the energy and nutrients by breaking down dead plant matter (Zanne *et al.*

2020; Tanunchai *et al.* 2022) and their activity can be linked to pathogens damage (Fokkema 1971; Chen *et al.* 2020). Epiphytic fungi are those that inhabit the leaf surface and feed mainly on deposited nutrients or leaf exudates, so they can be considered as commensalistic (Andrews & Harris 2000). However, they can be also mutualists since they can benefit the plant by producing microbial growth inhibitors, bioactive agents and secondary metabolites (Santamaría & Bayman 2005; Kharwar *et al.* 2010). There are extremely few studies that have explored simultaneously the multiple guilds present in plant phyllosphere (Nguyen *et al.* 2016). However, we need such integration to obtain a more complete picture of ecological processes driving the assembly of phyllosphere fungal communities.

The functional composition of phyllosphere fungal communities varies between plant species (Stone *et al.* 2018). Phylogenetically closer plant species share more similar pathogenic fungal communities (Gilbert & Webb 2007). It has also been reported that these communities depend on the host phenotype (Kembel & Mueller 2014; González-Teuber *et al.* 2021). For instance, Kembel & Muller (2014) showed that epiphytic fungal community composition depends on leaf nutrient content and leaf mass per area in tropical systems. Flessa *et al.* (2012) found that epiphytic fungal composition differed between perennial and deciduous trees in a temperate forest. In the case of phyllosphere bacterial communities the type of leaf indumentum also contributes to the differentiation of these communities between Mediterranean tree species (Yadav *et al.* 2005; Vokou *et al.* 2012), so it is possible that fungal communities may respond to this trait. When such relationships between leaf traits and fungal community structure occur, these traits can be considered as environmental filters conditioning the phyllosphere fungal community. Research on the links between phyllosphere

fungi and host plants in relation with their structural, functional and ecological properties can help to understand the mechanisms that underlie the maintenance and ecological role of microbial diversity in natural plant communities (Rodriguez *et al.* 2009; Friesen *et al.* 2011).

The main goal of our study is to understand the assembly of phyllosphere fungi in local communities of woody plant species of Mediterranean mixed forests. Community assembly rules aim to discern the processes that determine the composition of local communities (Diamond 1975; Mittelbach & Schemske 2015). The environmental filtering is one of the most ubiquitous concepts in the study of community assembly, which it is frequently approached based on functional traits and phylogenetic perspectives (Kraft *et al.* 2015). Considering that the ecological relationships between each guild and the plant are different, we predict that 1) phyllosphere fungal communities should vary between plant species and 2) each fungal guild should respond to a different set of biotic filters imposed by the plants. We expect epiphytic fungi to respond to leaf indumentum since it determines the microenvironment on the leaf surface. In turn, traits related to leaf nutrient composition and leaf habit (perennial versus deciduous leaves) should affect the differentiation of decomposer fungi, since these traits are related to the lability of leaf compounds. In the case of pathogens, several studies have reported the influence of leaf structural traits, nutrient availability or plant phylogenetic relatedness (Gilbert & Webb 2007; Toome *et al.* 2010; Engelsdorf *et al.* 2013; García-Guzmán & Heil 2014). If each guild responds to different sets of traits, we would expect that 3) each guild should vary across plant species independently of how the other guilds vary. Analysis of plant–fungal networks is being increasingly used to synthesize the complex structure of the relationships between these hyper diverse communities of

microorganisms and their host plants. Studies to date have found that plant–fungal networks are modular (Zhu *et al.* 2022), what means that certain groups of plants and fungi are associated with each other more than with species in other groups. If fungi assembly in response to particular plant traits, we hypothesise that 4) the modular structure of the association network between plants and their phyllosphere fungal communities should reflect the effect of the biotic filters imposed by the plants. We explore these hypotheses in two local plant communities so that we can assess whether the patterns detected may be context dependent.

Material and methods

Study system

The study was conducted in two pine-oak mixed Mediterranean forests of the SE Iberian Peninsula: Sierra de Segura (38.28ºN-2.58ºW; Segura, hereafter) and Sierra Sur de Jaén (37.38ºN-3.44ºW; Jaén, hereafter). In these forests summer seasons are dry and highest temperatures oscillate around 35 ºC. The average temperature of the coldest month (January) is 10ºC in Jaén and 6ºC in Segura. The annual rainfall is concentrated during spring and autumn, reaching mean annual values of 535.39 mm at Jaén and 611.76 mm at Segura.

The upper canopy of the forest in Segura is dominated by *Quercus faginea*, *Quercus pyrenaica* and *Pinus nigra* subsp. *Salzmanii*, and by *Quercus ilex*, *Quercus faginea* and *Pinus halepensis* in Jaén. However, the woody species richness of these forests is concentrated in a diverse understorey consisting of a subcanopy trees and tall scrubs, including species of *Crataegus*, *Juniperus*, *Phillyrea*, *Pistacia*, *Sorbus* or *Acer*, and short shrubs, including species of *Thymus*, *Cistus*, *Phlomis*, *Genista* and *Rosa*,

among others (Table S1).

Sample collection

The study was conducted in 2019 from September to October, since this is the season with highest fungal incidence in leaves (Pappas 1993; Materatski *et al.* 2019). We selected 27 representative woody species in Jaén and 19 in Segura (Table S1), and collected, from three individuals of each species, both healthy and damaged leaves trying to capture the highest diversity of fungal communities. Individuals were selected from different zones (five in Jaén and four in Segura, separated from 1.5 to 4.5 km) within each forest and leaves were collected from branches located in each cardinal direction of the canopy.

A total of 276 leaf samples from 138 individual plants were dried with sterilized silica gel and kept at 4ºC until processing. We did not sterilize the leaf surface because we were interested in detecting every fungus present on the leaf (Yang *et al.* 2016). This may lead to the presence of spores or hyphae belonging to fungi not necessarily interacting with the plant, like spores of specialist pathogens dispersed to the wrong host, or even fungi from functional guilds not living on the leaf (i.e. mycorrhizal, animal parasites, lichenized fungi or nectar saprotrophs). We used the presence of these misplaced fungi to develop a criterion for filtering our plant-fungal interactions database, specifically tailored to our system (see below).

DNA extraction and sequence analysis

Molecular characterization of fungal communities is fully explained in Suppl. Material S2. Briefly, extracted DNA were subjected to Illumina NovaSeq sequencing using the fungal

specific primers ITS3 and ITS4 (ITS2 region, Tedersoo *et al.* 2014). The library preparation and the Illumina NovaSeq run were carried out by AllGenetics and Biology SL (www.allgenetics.eu) (see Supplementary material S2 for further details). The bioinformatic analysis included removing sequencing primers and quality filtering of raw sequencing, estimating error rates and inferring of Amplicon Sequence Variants (ASVs). Taxonomic assignment was determined for each ASV against the UNITE database v. 8.2. (Abarenkov *et al.* 2020), complemented with representative sequences of the plant genera involved in the study. ASVs were clustered (97%) into Operational Taxonomic Units (OTUs) that resemble species level (see e.g. Tedersoo *et al.* 2012). Finally, 1462 OTUs and 7173784 reads were obtained (Table S2).

Fungal functional guilds were determined by matching OTUs' assigned genera and the genus-guild database FungalTraits (Põlme *et al.* 2020).

Sequencing depth was evaluated by representing the detected OTUs per number of reads. Additionally, to evaluate our sampling completeness we built sampling coverage curves by site and functional guild (Fig. S3). Sample coverage for incidence data can be interpreted as the proportion of the total number of individuals in the entire assemblage that belong to detected species (Chao *et al.* 2020). These analyses were conducted using *iNEXT* R package v. 2.0.20 (Hsieh *et al.* 2020).

Filtering for legitimate interactions

Bulk sequencing of all fungi present on non-surface sterilized leaves is likely to include DNA spores or hyphae fragments of non phyllosphere fungi that were haphazardly deposited on the leaves, or of phyllosphere fungi that were deposited on non-

legitimate host plants. Since we were interested only in fungi legitimately associated with each plant species, it was necessary to filter out those sequences in each sample that most likely represented contamination events. To this aim, we performed a general filtering to find a sequence number threshold from which an interaction can be considered as legitimate (see Drake *et al.* 2022 for a similar approach). Taxa belonging to guilds whose habitats do not correspond to the leaf (i.e. ectomycorrhizal and floral saprotrophs) or those that do not interact directly with plants (i.e. lichenized fungi and insect parasites) represent unequivocal events of contamination. On the other hand, epiphytes, decomposers, foliar endophytes and pathogens are, *a priori*, legitimate interacting guilds. Moreover, when a fungus occurs in a legitimate host plant it will grow actively, producing a larger number of reads than when it occurs in a non-legitimate host. We conducted a binomial generalized mixed model using presence type (legitimate *vs* contamination) as dependent variable and the number of reads per sample as predictor variable. We added plant species and zone within site as random effects. The model was fitted using binomial distribution, with *glmmTMB* R package (Brooks *et al.* 2017). The fitted model indicated that there is a probability of 0.97 that an OUT represented by at least 20 reads/sample belongs to a legitimate phyllosphere guild (See Table S4.1 and S4.2). Hence, the dataset was filtered to retain those OTUs having at least 20 reads per sample. After this filtering we conserved 86.59% of decomposers (381 OTUs), 78.85% of epiphytes (41 OTUs) and 84.93% plant pathogens (186 OTUs). Note that we could retain only seven OTUs of foliar endophytes thus this guild was excluded from the analyses. The following analyses are based on the filtered dataset.

Data analyses

Variation of phyllosphere fungal communities across plant species

We explored the variation of phyllosphere fungal communities composition across plant species and sites by means of a permutational multivariate analysis of variance (PERMANOVA) with the *adonis2* function of *vegan* R package (Oksanen *et al.* 2020). The analysis was performed separately for each guild (decomposers, pathogens and epiphytes). We used the zones within each site as strata, and Bray-Curtis dissimilarity. P-values were estimated by performing 999 permutations. The analysis was also performed using Jaccard distances with qualitatively the same results (not shown). To assess whether the contribution of site effect could be partly explained by differences between sites in plant species composition, we conducted a PERMANOVA including only the eight plant species that occurred in both sites, and adding the interaction term of plant species by site.

Effect of plant traits and phylogeny on phyllosphere fungal communities

We examined separately for each guild (decomposers, epiphytes and pathogens), the role of leaf functional traits and plants phylogeny as drivers of the assembly of fungal communities across plant species. To this aim, we first calculated compositional distances among fungal communities. We normalized each sample by its total reads, and then averaged the OTU proportions across samples of each plant species and sites. The compositional distances were calculated for each guild, both with Bray-Curtis and Jaccard distances, by means of the *Betapart* R package

(Baselga & Orme 2012). Phylogenetical distances for the plant species occurring in each site were obtained from Alcántara *et al.* (2019) (i.e. same study sites). Plant functional traits were obtained from Perea *et al.* (2021) and we took those traits related to leaves: leaf water content (LWC), leaf mass per area (LMA), leaf carbon content (LC), carbon nitrogen ratio (CN), and the categorical variables leaf habit (evergreen or deciduous) and leaf indumentum (predominantly glabrous or pubescent). For continuous variables we calculated plant traits phenotypical distances using Gower distance with *Betapart* R package (Baselga & Orme 2012). For categorical variables we used three categorical levels of similarity: for the leaf habit, both plants deciduous, both plants perennial or different; for the leaf indumentum, both plants glabrous, both plants pubescent or different. Thus, we fitted a generalized mixed model for each functional guild, with distances in fungal community composition between each pair of plant species as dependent variable, and leaf functional traits and phylogenetic distances as explanatory variables. Since each data in the analysis represents the distance between two plant species (compositional, phenotypical and phylogenetical), we included two random factors indicating the two compared plant species coded within site to control for the non-independence of comparisons involving the same plant species. The models were fitted with package *glmmTMB* R package (Brooks *et al.* 2017) using beta or Gaussian distribution, depending on which one provided a better fit to the model. Distributions of residuals were checked with the *DHARMa* R package (Hartig 2022). Data visualization was performed with the *ggplot2* R package (Wickham 2016).

Covariation between fungal guilds across plant species

To explore how the composition of fungal guild communities covaries across plant species we used Mantel tests between each pair of Bray-Curtis distance matrices with the *vegan* R package (Oksanen *et al.* 2020). Mantel tests were based on Spearman rank correlation. Tests were conducted separately for each study site.

Modular structure of plant-fungus interaction networks

Once tested that different plant species are associated with different fungal assemblages (i.e. they do not interact randomly) and fungal communities are affected by different biotic filters, we explored the properties of the plant-fungi interactions in each site. Thus, we use a binary matrix of plant species and OTUs of all guilds and build a species-level interactions network for each site and examined its modularity with the *cluster spinglass* algorithm of *iGRAPH* R package (Csardi & Nepusz 2006). To test for significance, we used 999 permutations of a non-sequential algorithm for binary matrices that preserves OTUs frequencies (Jonsson 2001), using the *c0* method option of the *NullMaker* function of *metacom* R package (Dallas 2020).

Finally, we tested whether the probability that plants belong to the same module (i.e. they share similar phyllosphere fungal communities) was related to plant phylogenetic distance and leaf traits. We fitted a generalized mixed model with a binary dependent variable coding whether the two plant nodes belonged to the same or different module (1 and 0 respectively), and leaf functional traits and phylogenetic distance as explanatory variables. We included plant species coded within site as random factor. The model was fitted with a binary distribution using the *glmmTMB* R package (Brooks *et al.* 2017). Distributions of

residuals were checked with the *DHARMa* R package (Hartig 2022). Data visualization was performed with the *ggplot2* R package (Wickham 2016).

Results

Rarefaction analysis showed the sequencing depth was enough to reveal nearly 100% of OTUs contained in the collected samples (Figure S3.1). A total of 811 OTUs were assigned to a functional guild, representing 55.47 % OTUs and 73.84% of fungal reads (Table S2). At site level, accumulation curves indicated that our sampling captured more than 80% of the fungal phyllosphere OTUs in the two sites (Figure S3.2). Results showed that the most abundant functional guilds in the phyllosphere were decomposers, epiphytes and pathogens (Table 1). Decomposer's guild coverage was 95.78 % in Jaén and 94.11 % in Segura, and the observed OTU species were 339 in Jaén and 249 in Segura. Epiphyte's guild coverage was 96.22% in Jaén and 95.67% in Segura, and the observed OTUs species were 40 in Jaén and 30 in Segura. Pathogen's guild coverage was 96.93 % in Jaén and 95.69 % in Segura, and the observed OTU species were 156 in Jaén and 139 in Segura (Figure S3.2).

Variation of phyllosphere fungal communities across plant species

Our dataset included 62.66% of decomposers, 6.74% of epiphytes, and 30.59% of pathogens (Figure 1). PERMANOVA analyses (Table 2) showed that phyllosphere fungal communities varied mostly between plant species for all functional guilds (decomposers: 33.44%; epiphytes: 42.64%; pathogens 33.73% of explained variance), while site accounted for a small proportion

of the variation (<3% for all guilds). The PERMANOVA analyses performed on the subset of species common to both sites confirmed that the contribution of site effect was not affected by plant species composition (Table S5).

The mean richness of decomposers per plant species in Jaén was 42.15, with a range between 2 In *Rosa* sp. and 134 fungal species in *Juniperus oxycedrus*, while in Segura the mean richness was 30.53, with a range between 1 in *Dapnhe laureola* and 121 fungal species in *Juniperus communis*. The mean richness of epiphytes in Jaén was 5.70, with a range between 1 (*Acer monspessulanum*, *Daphne gnidium*, *Pistacia terebinthus*, *Cytisus scoparius)* and 19 fungal species (*Juniperus phoenicea*), while in Segura the mean richness was 5.11 fungal species, with a range between 1 (*Prunus spinosa*) and 14 fungal species *(Juniperus communis*). The mean richness of pathogens in Jaén was 19.30, with a range between 2 in *Phillyrea angustifolia* and 65 fungal species in *Juniperus oxycedrus*, while in Segura the mean richness was 21.32, with a range between 1 in *Daphne laureola* and 64 fungal species in *Juniperus communis* (Table S6).

Pathogens and epiphytes were represented by the phyla *Ascomycota* and *Basidiomycota*, but decomposers had exclusively OTUs pertaining to the phylum *Mortierellomycota* and *Mucoromycota*. Representative classes of decomposers were *Dothideomycetes* (36.24% in Jaén and 36.06% in Segura), *Tremellomycetes* (15.44% in Jaén and 20.19% in Segura) and *Agaricomycetes* (12.75% in Jaén and 12.02% in Segura). Representative classes of epiphytes were *Lecanoromycetes* (64.29% in Jaén and 70% in Segura), *Cystobasidiomycetes* (28.13% in Jaén and 29.17% in Segura) and *Eurotiomycetes* (9.38% in Jaén and 4.17% in Segura). Representative classes of pathogens were *Dothideomycetes* (58.52% in Jaén and 48.28% in Segura), *Sordariomycetes* (11.85% in Jaén) and *Leotiomycetes* (16.38% in

Segura). Finally, the decomposer fungal genera associated with more host plants were represented by *Cladosporium* (26 in Jaén and 19 in Segura), *Phaeococcomyces* (26 in Jaén) and *Lapidomyces* (17 in Segura). The most represented epiphytic fungal genera were *Aureobasidium* (26 in Jaén and 18 in Segura) and *Buckleyzyma* (19 in Jaén and 16 in Segura). The most represented pathogenic genera in plant hosts were *Coniothyrium* (23 in Jaén and 18 in Segura), *Alternaria* (22 in Jaén) and *Naevala* (19 in Segura) (Figure 2).

Covariation between guilds across plant species

Mantel tests indicate significant correlations in the similarity of the decomposers, epiphytes and pathogenic communities across plant species in Jaén ($r > 0.3$, $P < 0.02$ in all cases). However, in Segura the correlations involving decomposers were significant ($r > 0.3$, $P < 0.03$) but not the correlation between pathogens and epiphytes (Table S7).

Effect of plant traits and phylogeny on phyllosphere fungal communities

GLMMs performed to assess the role of leaf functional traits and plants phylogeny as drivers of the fungal assemblages showed that, for all guilds, fungal communities were consistently influenced by phylogenetic distance and leaf indumentum (Table 3, Figure 4). Thus, plants with pubescent indumentum and less distantly related in the phylogeny tended to harbour more similar fungal communities. In the case of epiphytes and decomposers, fungal communities were also influenced by leaf habit, with deciduous plants showing more similar fungal communities. These results

were consistent both using Jaccard and Bray-Curtis distances. However, using Bray-Curtis distances we identified additional effects of different functional traits in each guild. Plant species differing in LWC were more different in their pathogen fungal communities, those differing in LC differed also in their decomposer fungal communities and plants differing in LMA differed in the epiphyte fungal communities.

Modular structure of plant-fungus interaction networks

Plant-fungus networks of Jaén and Segura had significantly modular structures (respectively: 0.287, null model ranging from 0.255 to 0.280; 0.319, null model ranging from 0.284 to 0.314) (Figure 3). GLMM testing the influence of leaf functional traits and phylogeny on the modular composition of the plant-fungus interaction network indicated that plants with the same leaf habit and less distantly related in the phylogeny were more likely to belong to the same module (Table 4).

Table 1. General dataset structured by functional guilds classified by FungalTraits database. It is shown for each guild, the number of OTUs (Nº OTUs), percentage of OTUs (% OTUs), number of reads (Nº reads), and percentage of reads (% reads).

Guild	Nº OTUs	% OTUs	Nº Reads	% Reads
Decomposers	440	30.10	2550259	35.55
Epiphytes	52	3.56	697437	9.72
Plant pathogens	219	14.98	1977093	27.56
Foliar endophytes	9	0.62	4785	0.07
Higher order parasites	59	4.04	57177	0.80

Guild	Nº OTUs	% OTUs	Nº Reads	% Reads
ECMs	12	0.82	8439	0.12
Floral saprotrophs	20	1.37	1756	0.02
NA	651	44.53	1876838	26.16

Table 2. Permutation multivariate analysis of variance performed to explore the variation of the phyllosphere fungal communities across plant species and sites (Jaén and Segura). The analysis was performed for each functional guild (decomposers, epiphytes and pathogens).

Guild	Effect	Df	R2	F	*p*
Decomposers	*Site*	1	0.00886	2.9823	0.532
	Plant species	37	0.33443	3.0427	**0.001**
	Residual	213	0.63274		
Epiphytes	*Site*	1	0.02759	9.2973	**0.002**
	Plant species	35	0.42642	4.1048	**0.001**
	Residual	183	0.54316		
Pathogens	*Site*	1	0.01926	6.3311	0.179
	Plant species	36	0.33738	3.0808	**0.001**
	Residual	198	0.60229		

Table 3. GLMM analysis performed to explore the effect of leaf functional traits and plant phylogeny on the composition (measured as Bray Curtis (**a**) and Jaccard (**b**) distances) of phyllosphere fungal communities of decomposers, epiphytes and pathogens. Significant effects are bold typed.

a)	**Decomposers**		**Epiphytes**		**Pathogens**	
	Estimate	***p***	**Estimate**	***p***	**Estimate**	***p***
Intercept	-0.047	0.778	-1.556	**<0.0001**	0.712	0.014
LWC	-0.064	0.480	0.014	0.945	0.348	**0.05**
LMA	0.020	0.268	0.109	**0.001**	0.013	0.641
LC	0.197	**0.039**	-0.023	0.470	0.023	0.408
CN	0.115	0.326	0.041	0.192	0.033	0.240
Leaf habit (both different)	0.482	**<0.0001**	0.536	**0.001**	0.226	0.122
Leaf habit (both perennial)	0.786	**<0.0001**	0.758	**0.002**	0.478	**0.049**
Leaf indumentum (both glabrous)	0.381	**<0.0001**	0.400	**0.004**	0.432	**0.003**
Leaf indumentum (both pubescent)	-0.481	**<0.0001**	-0.917	**<0.0001**	-0.466	**0.022**
Phylogeny	0.006	0.348	0.028	**0.006**	0.023	**0.017**

b)	Decomposers		Epiphytes		Pathogens	
	Estimate	***p***	**Estimate**	***p***	**Estimate**	***p***
Intercept	0.662	**<0.0001**	0.135	0.601	0.620	**<0.0001**
LWC	-0.007	0.602	-0.262	0.208	0.003	0.078
LMA	-0.003	0.352	0.013	0.668	-0.005	0.905
LC	0.017	0.260	-0.024	0.456	0.002	0.713
CN	-0.015	0.402	0.034	0.269	-0.002	0.200
Leaf habit (both different)	0.041	**0.006**	0.681	**<0.0001**	0.045	**0.004**
Leaf habit (both perennial)	0.047	**0.061**	0.557	**0.002**	0.056	**0.004**
Leaf indumentum (both glabrous)	0.053	**0.001**	0.121	0.271	0.046	**<0.0001**
Leaf indumentum (both pubescent)	-0.128	**<0.0001**	-0.487	**0.021**	-0.107	**<0.0001**
Phylogeny	0.003	**0.002**	0.011	0.247	0.004	**0.003**

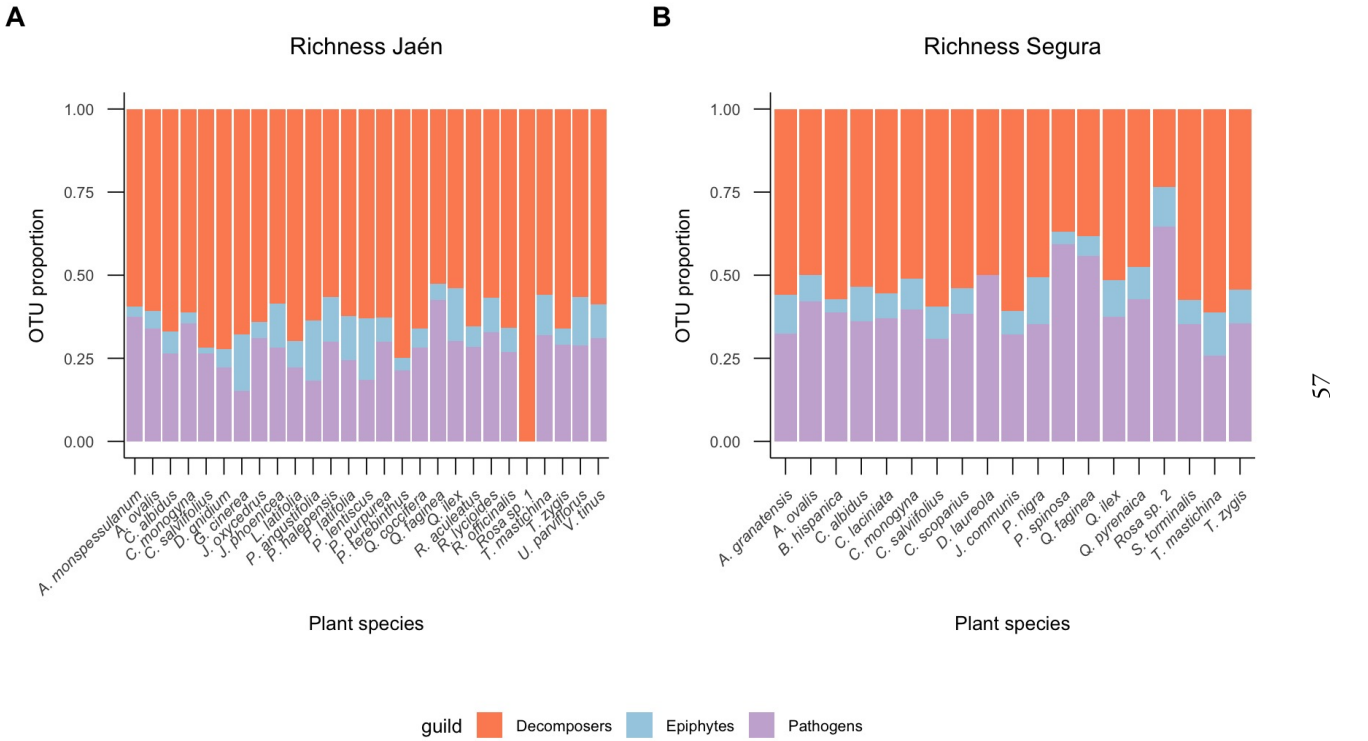

A
Richness Jaén
OTU proportion
1.00
0.75
0.50
0.25
0.00
A. monspessulanum
A. ovalis
C. albidus
C. monogyna
C. salvifolius
D. gnidium
G. cinerea
J. oxycedrus
J. phoenicea
L. latifolia
P. angustifolia
P. halepensis
P. latifolia
P. lentiscus
P. purpurea
P. terebinthus
Q. coccifera
Q. faginea
Q. ilex
R. aculeatus
R. lycioides
R. officinalis
Rosa sp. 1
T. masticina
T. zygis
U. parviflorus
V. tinus
Plant species
B
Richness Segura
OTU proportion
1.00
0.75
0.50
0.25
0.00
A. granatensis
A. ovalis
B. hispanica
C. albidus
C. lacciniata
C. monogyna
C. salvifolius
C. scoparius
D. laureola
J. communis
P. nigra
P. spinosa
Q. faginea
Q. ilex
Q. pyrenaica
Rosa sp. 2
S. torminalis
T. masticina
T. zygis
Plant species
guild
Decomposers
Epiphytes
Pathogens

Figure 1. OTU richness of each functional guild for each plant species. It is shown the proportion of OTUs of decomposers, epiphytes and pathogens occurring in each plant species at Jaén (**a**) and Segura (**b**).

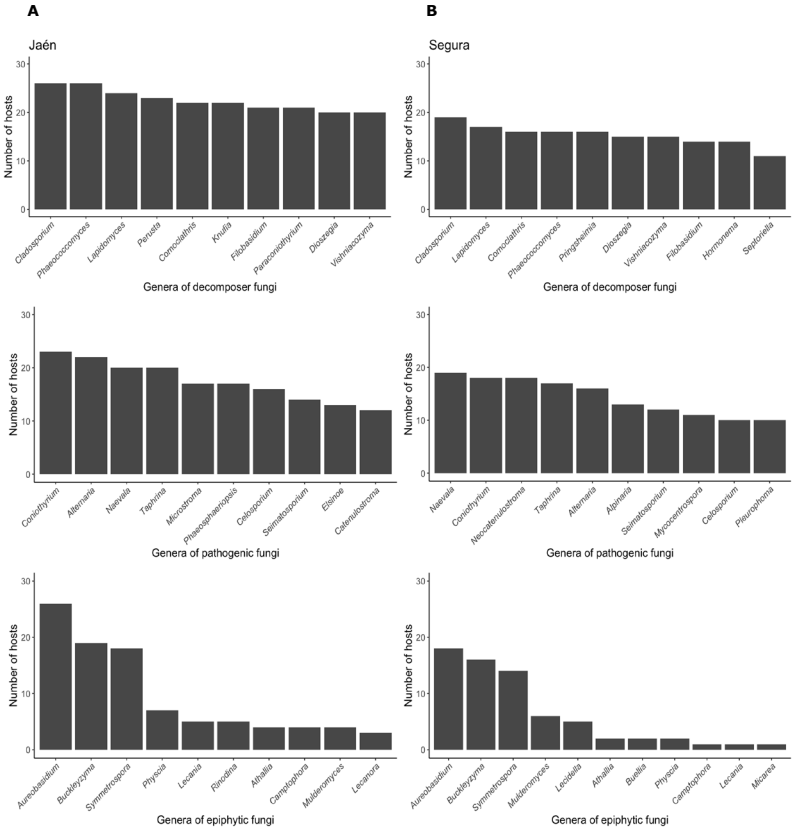


Figure 2. Fungal genera which are associated with the larger number of host plants divided by decomposers, epiphytes and pathogens at Jaén (**a**) and Segura (**b**).

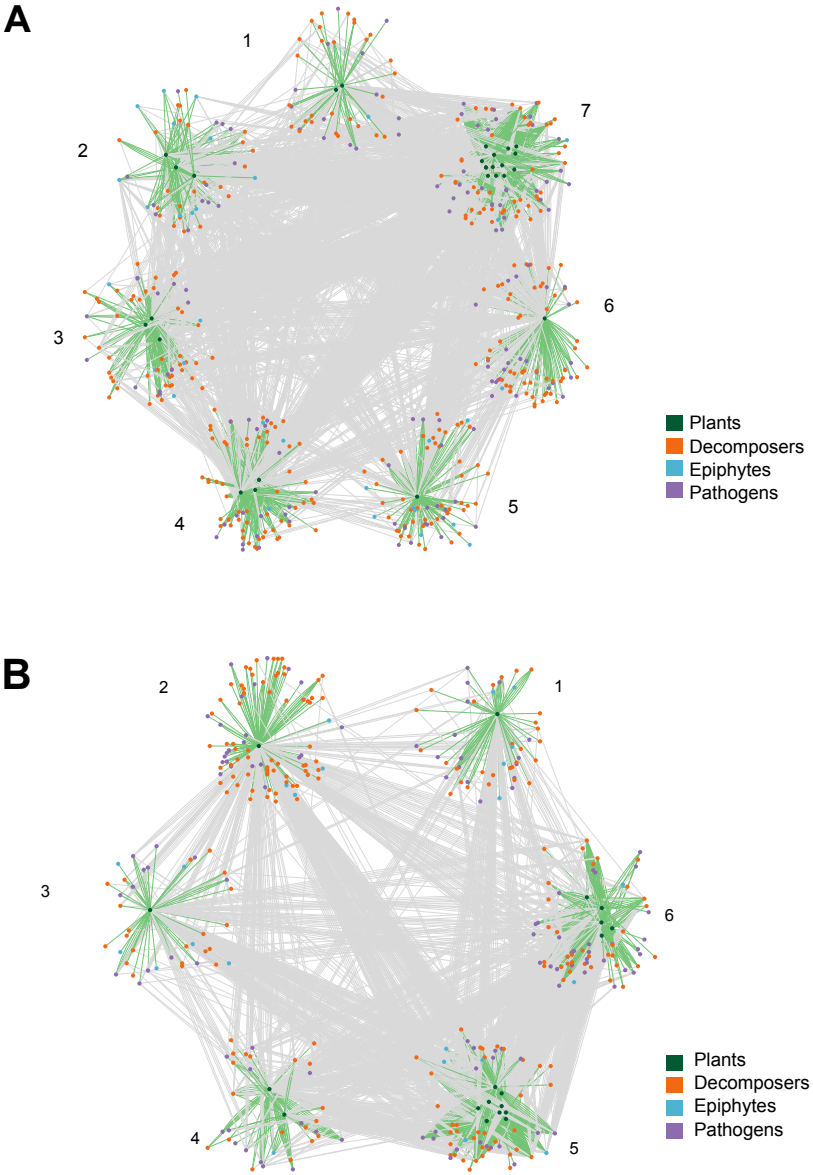
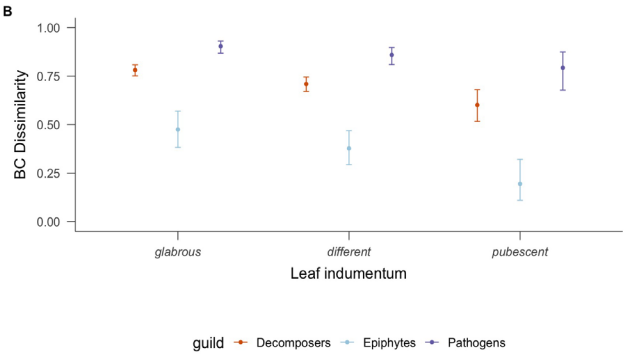

A
1
2
3
4
5
6
7
Plants
Decomposers
Epiphytes
Pathogens
B
1
2
3
4
5
6
Plants
Decomposers
Epiphytes
Pathogens

Figure 3. Plant-fungus interaction networks showing the modular structure of the interaction with seven statistically significant modules in Jaén (**a**) and nine in Segura (**b**). Node colour indicates the type of organism: decomposer (orange), epiphyte (blue), pathogen (purple) and plant species (green). Edge colour indicates whether links are established between (grey) or within (blue) modules. The modules of the Jaén network are configured by *Daphne gnidium* and *Phlomis purpurea* (**1**), *Genista cinerea*, *Quercus ilex* and *Ulex parviflorus* (**2**), *Cistus albidus*, *Lavandula latifolia* and *Thymus zygis* (**3**), *Phillyrea latifolia*, *Ruscus aculeatus* and *Viburnum tinus* (**4**), *Juniperus phoenicea* (**5**), *Juniperus oxycedrus* (**6**) and *Acer montpessulanum*, *Amelanchier ovalis*, *Cistus salvifolius*, *Crataegus monogyna*, *Phillyrea angustifolia*, *Pinus halepensis, Pistacia lentiscus*, *Pistacia terebinthus*, *Quercus coccifera*, *Quercus faginea*, *Rhamnus lycioides*, *Rosa sp*., *Rosmarinus officinalis and Thymus masticchina* (**7**). The modules of the Segura network are configured by *Thymus zygis* (**1**), *Juniperus communis* (**2**), *Pinus nigra* (**3**), *Amelanchier ovalis* and *Crataegus laciniata* (**4**), *Acer granatensis*, *Cistus salvifolius*, *Crataegus monogyna*, *Cystus scoparius*, *Daphne laureola*, *Prunus spinosa*, *Rosa sp.*, *Sorbus torminalis* and *Thymus mastichina* (**5**) and *Berberis hispanica*, *Cistus albidus*, *Quercus faginea*, *Quercus ilex* and *Quercus pyrenaica* (**6**).


A
1.00
0.75
0.50
0.25
0.00
BC Dissimilarity
perenne
different
caduca
Leaf habit
guild
Decomposers
Epiphytes


B
1.00
0.75
0.50
0.25
0.00
BC Dissimilarity
glabrous
different
pubescent
Leaf indumentum
guild
Decomposers
Epiphytes
Pathogens

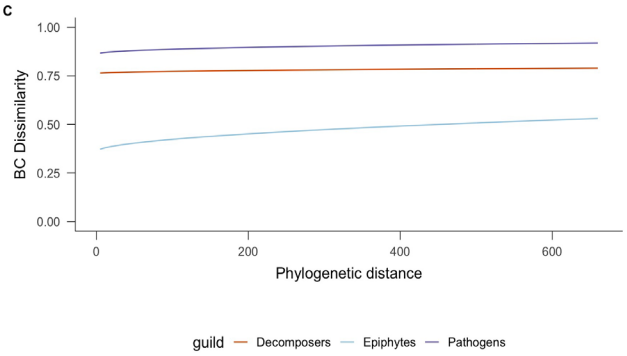

C
1.00
0.75
0.50
0.25
0.00
BC Dissimilarity
0
200
400
600
Phylogenetic distance
guild
Decomposers
Epiphytes
Pathogens

Figure 4. Results of the GLMM analysis performed to explore the effect of leaf functional traits and plant phylogeny on the composition of phyllosphere fungal communities of decomposers, epiphytes and pathogens. It is shown the effect of leaf indumentum (**a**), leaf habit (**b**) and plant phylogeny (**c**) on fungal composition dissimilarities between plants measured as Bray-Curtis distances.

Discussion

Phyllosphere fungal communities are highly diverse. A single plant may contain hundreds of fungal OTUs, and studies at plant community level often find several thousands of them both on tropical and temperate forests (Kembel & Mueller 2014; González-Teuber *et al.* 2020). Although comparisons of OTU richness between studies must be taken with caution due to methodological differences, compared with the cited studies, our results suggest that Mediterranean mixed forests harbour relatively lower OTU richness, since we found a total of 1462 fungal OTUs distributed across 38 plant species with a mean of 161 ± 18.51 OTUs per plant species (ranging 7 to 474). The few studies that have been conducted to date on Mediterranean plants seem to agree with our results in terms of OTU richness. For example, studies of cultivated *Olea europaea* from Italy and Portugal found 195 and 290 OTUs respectively (Abdelfattah *et al.* 2015; Gomes *et al.* 2018), and Lazarević & Menkis (2020) found between 104 and 192 OTUs in different populations of *Pinus heldreichii* from Montenegro.

Despite the relatively low species richness, the studied phyllosphere fungal communities are still complex systems. From a taxonomic point of view, the studied phyllosphere communities comprise a large taxonomic diversity that includes

157 fungal families from 21 fungal classes. From a functional perspective, this diversity involves the cooccurrence of multiple fungal lifestyles (endophytes and epiphytes), fungi with different relationships with the plant (pathogens, decomposers and commensalistic epiphytes), and likely with different ecological requirements, different life cycles, and different degree of specialization on their hosts. At first sight, this complexity, observed even within a single host species or leaf, would suggest an absence of general factors driving the assembly of phyllosphere fungal communities. However, it is becoming clear that phyllosphere fungal communities differ between plant species (Chen *et al.* 2020; Qian *et al.* 2020). Accordingly, the phyllosphere fungal communities in our study sites were clearly differentiated between plant species, even taking into account large scale spatial variation within plant species.

Recent studies provide evidence that leaf traits or plant phylogeny can influence the assembly of certain fungal guilds (González-Teuber *et al.* 2021; Kembel & Mueller 2014). We hypothesised that the contrasting ecological relationships between plants and each fungal guild should imply that each guild would respond to different sets of functional traits. Our results partially support this hypothesis but, interestingly, indicate that plant phylogeny and certain leaf traits can represent biotic filters that operate on all fungi regardless of their ecological guild. To understand how these biotic filters drive the assembly of phyllosphere fungal communities, we can use the complementary information provided by Jaccard and Bray-Curtis dissimilarities. Differences between presence-absence and abundance data, as captured by these indices, can be interpreted in the sense of fungi having the capacity to reach and stay on the leaf (presence), vs. really thriving on it (abundance). The assembly of phyllosphere fungal communities is driven by the differentiation of all guilds

across plant species in response to the same set of plant properties. The assembly is related to the leaf indumentum in all guilds and also to the leaf habit in epiphytes and decomposers. These traits can be interpreted as physical and temporal constrains to the arrival and colonization of fungal species. Plants with pubescent leaves host more similar fungal communities of all guilds than plants with glabrous leaf surfaces. The set of plant species that we have studied have predominantly non-glandular leaf trichomes. The intricate physical structure that trichomes build on the leaf may impose a strong filter for the arrival of spores and early hyphal growth (Allen *et al.* 1991). Further, trichomes has also been recently shown to act as an early layer of the plant immune system: by reacting to mechanical stimuli, like raindrops, trichomes can induce the immune response against pathogens (Matsumura *et al.* 2022). However, the fungi that are able to overcome those barriers reach a tempered microenvironment with lower radiation, moderated temperature and higher relative humidity which is particularly well suited for microbial colonization (Yadav *et al.* 2005; Damián *et al.* 2020).

In turn, the epiphytic and decomposer fungal communities of deciduous plants tend to be more similar with each other than those of plants with evergreen leaves. Accordingly, several studies have found differences between deciduous and evergreen plants in their phyllosphere fungal communities (Flessa *et al.* 2012; Millberg *et al.* 2015). Like plants, fungal life-cycles show seasonality based on the development of specific vegetative and reproductive structures (Rodriguez & Redman 1997; Rayment *et al.* 2020). Deciduousness imposes a clear constraint to the seasonal rhythm of fungal life-cycles, which must adapt the length and the timing of its phases to the plant phenology. Instead, evergreen leaves do not impose such strong constraint, so they may allow the establishment of a more diverse fungal

community. For example, the evergreens *Juniperus oxycedrus* hosted 134 decomposers and *J. phoenicea* hosted 19 epiphytes, while the most species-rich deciduous was *Crataegus laciniata* with 45 decomposers and 6 epiphytes. Evergreen and glabrous species present more interspecific dissimilarity than deciduous and pubescent ones, which can indicate that they provide more niche diversity in which fungal species can distribute. Indeed, the leaf functional traits that we have identified as related to differences between plants on their phyllosphere fungal communities (LC, LWC, LMA) showed higher variance among evergreen than deciduous plants (mean ratio of variances and SE: 9.26 ± 7.54), and among glabrous than pubescent plants (mean ratio of variances and SE: 8.34 ± 6.53).

Even though the assembly of phyllosphere fungal communities is based on a common response to certain leaf traits (i.e. in a biotic filtering mediated by the plant host), other traits involving leaf chemical and morphological characteristics have particular effects on the colonization by each functional guild. Water availability in the leaf apoplast determines the colonization success of fungi in general (Chaundry *et al.* 2021). However, we only found a significant relationship with LWC in the case of pathogens. It is possible that the magnitude of the response of other guilds to the gradient of variation in LWC between plant species in the studied communities was much weaker. The infection process of a pathogen involves a strong selection filter (Rutten *et al.* 2021), in which humidity can control the colonization of pathogens in the apoplast and be an initial determinant of leaf colonization (Chaudhry *et al.* 2021). Various studies have reported that many foliar pathogens are benefitted by a high leaf water content because it suppresses the effector-triggered defences of the plant (Beattie & Lindow, 1999). Consequently, some plant species also promote localized desiccation at the local infection and restrict

pathogen growth as a defence mechanism. As a limited resource, water is subjected to manipulation by both plants and pathogens (Beattie 2011).

Decomposers are specialized on the leaf carbon content, which can be explained by their involvement on the early stages of leaf degradation. The structural carbon of the cell wall on leaves is contained on lignin and several polysaccharides such as cellulose, hemicellulose and pectin (Zeisler-Diehl *et al.* 2018), but the cell walls are heterogeneous structures highly variable among plant species (Walton, 1994). For example, coniferous leaves are rich in waxes, resins, and lignin, which makes them more resistant to decomposition (Gołębiewski *et al.* 2019), while deciduous leaves are reported to be more easily degraded (Aerts 1995). Phyllosphere decomposers produce a wide range of extracellular enzymes to break down the dead matter (Voriskova & Baldrian, 2013). Leaf diverse composition may select for fungal decomposers which are specialized on degrading specific leaf components of different plant species (Osono & Takeda 1999; Fanin *et al.* 2021).

The communities of epiphytic fungi present in each plant species are clearly differentiated (mean and SD of Jaccard index: 0.67 ± 0.2). However, when the relative abundances of the OTUs are taken into account, this differentiation diminishes substantially (mean and SD of Bray-Curtis index: 0.37 ± 0.21). This suggests that the same epiphytic fungi become dominant in the phyllosphere of most plant species. For example, it is well known that some epiphytic fungi such as *Aureobasidium pullulans* can colonize and dominate the community of a large diversity of plant species (Osono 2008; Bashir *et al.* 2022). In fact, in our study *A. pullullans* represents 81 ± 27.73 % of the reads of epiphytic fungi per sample. The differentiation between plants in epiphytic fungal communities is related to variation in leaf mass per area. This trait has been found to affect the

phyllosphere microbial composition in other studies (Kembel & Mueller 2014). However, the mechanisms that may explain this relationship are not clear. After all, LMA is correlated with other structural and physiological characteristics of the leaves and is a key component of the leaf economics spectrum (Wright *et al.* 2004). For example, some studies suggest that plants with high LMA might limit nutrient leach to the surface (Yadav *et al.* 2005; Lajoie *et al.* 2020), what could contribute to the filtering of the epiphytic communities in different plant species since epiphytic microbes exploit such nutrients (Lindow & Leveau 2002; Schimann *et al.* 2022).

As we hypothesised, the studied phyllosphere fungal communities are structured into modules that can be partly consequence of processes of biotic habitat filtering mediated by plant host traits (Vacher *et al.* 2016). Besides specific functional traits, we found an effect of phylogenetic distance between plants on the phyllosphere community composition, as has been reported for other organisms inhabiting the phyllosphere such as bacteria, herbivorous insects and also fungal pathogens (Redford *et al.* 2010; Gilbert & Webb 2007; Lajoie & Kembel, 2021). In the present study, the phylogenetic signal was detected across the three functional guilds, which indicates that not only fungal pathogens but also decomposers and epiphytes show certain degree of specialization related to the phylogeny of the host plant. Moreover, clustering of plant species into modules with similar fungal community composition also shows the signal of the phylogenetic distance between the plant species. The phylogenetic distance effect may be indicating two non-mutually exclusive processes contributing to the assembly of phyllosphere fungal communities. On the one hand, it seems likely that phylogenetically conserved traits not included in this study (e.g. secondary metabolites, anatomical structures) are contributing

to the assembly of the studied communities (Parker *et al.* 2015; Lajoie & Kembel, 2021). On the other hand, it is also possible that the evolution of fungal lineages has occurred in concert with the diversification of plant lineages (Arya & Harel 2019; Rutten *et al.* 2021).

The phyllosphere is a heterogeneous environment (Massoni *et al.* 2020) and a key support for microbial diversity (Vorholt 2012), which constitutes a well-suited model system for the study of ecological concepts and theories (Meyer & Leveau, 2012). The results of the present study indicate that part of the complexity of phyllosphere fungal communities can be interpreted in terms of a hierarchical structure that emerges from biotic filters operating on the species pool (Mittelbach & Schemske, 2015). The differentiation of fungi from all guilds filtered by a common set of functional leaf traits gives rise to modules of plants where more closely related plants tend to share more fungi with each other, than with distantly related plants from different modules. Exploring the consequences of the integration of multiple functional guilds in the phyllosphere may provide new insights to understand their role on the multiple ecosystem functions played by these microorganisms.

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.98sf7m0nh (Pajares-Murgó *et al.* 2022). Raw sequences have been deposited in the NCBI Sequence Read Archieve under Bioproject PRJNA909185.

Supporting information

Appendix S1 (List of species); Appendix S2 (DNA extraction and sequencing analyses); Appendix S3 (Rarefaction curves and sample coverage); Appendix S4 (Contamination filtering); Appendix S5 (PERMANOVA results); Appendix S6 (Richness, Simpson and Shannon indices); Appendix S7 (Mantel tests).

Capítulo 2

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

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If one wishes to succeed in producing a forest, it is necessary to imitate nature, and to plant shrubs and bushes which can break the force of the wind, diminish that of frost, and moderate the inclemency of the seasons. These bushes are the shelter which guards the young trees, and protects them against heat and cold. (...) The best shelter in wet soil is popular or aspen, and in dry soil Rhus, for the growth of oak. One need not fear that the sumac, aspen or popular can injure the oak or birch. After the latter have passed the first few years in the shade and shelter of the others, they quickly stretch up, and suppress all the surrounding plants.

Buffon 1742, *Mémoire sur la culture des forêts*, citado por Egerton 2015:428.

Abstract

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. 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. 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. Our results indicate that phyllosphere pathogens and saprotrophs have a negative effect on canopy-recruit interactions, while epiphytic fungi have a positive effect. 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.

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.

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, 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 (Verdú & Valiente-Banuet 2011; Liu *et al.* 2012; Alcántara *et al.* 2018). Multiple types of plant antagonists can contribute to variability in species-specific effects on recruitment. Among them, fungal pathogens have been found as 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 *et al.* 2016; Jia *et al.* 2020). Many pathogenic fungi have the ability to infect multiple cooccurring 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 (Barrett *et al.* 2009; Redman *et al.* 2001, Alvarez-Loayza *et al.* 2011). 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-fungal 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 *et al.* 2019a) 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 specialised 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 hypothesise 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*)

Does the richness and 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 hypothesise 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 hypothesise 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.

Material and methods

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 14.1º while Segura has mean annual temperatures of 11.6º. The annual rainfall is concentrated in spring and autumn, reaching mean annual values of 535.4 mm in Jaén and 611.76 mm at Segura.

Segura is characterized by mixed forests of *Pinus nigra* subsp. *salzmanii*, *Quercus faginea* and *Quercus pyrenaica*. Jaén is dominated by mixed forests of *Pinus halepensis*, *Quercus ilex* and *Quercus 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 understory is composed by small shrubs (with species of the genera *Cistus*, *Genista*, *Phlomis*, *Rosmarinus* and *Thymus*).

Data collection

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 OUT 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 OUT abundance. The final dataset includes 381 OTUs of saprotrophs, 41 OTUs of epiphytes and 186 OTUs of plant pathogens (see Supplementary Material S3

for further details).

The studied plant species averaged 20.58 ± 13.8 OUTs of pathogenic fungi, with a range between 65 OTUs in *Juniperus oxycedrus* and 1 OUT in *Daphne laureola*. The mean richness was 37.35 ± 29.05 OTUs of saprotrophic fungi, with a range between 1 OUT in *Daphne laureola* and 138 OTUs in *Juniperus oxycedrus*. The mean richness was 7.7 ± 4.12 OTUs of epiphytic fungi, with a range between 1 OUT 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 OUT 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).

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 *et al.* (2019a). Briefly, the frequency of recruitment was sampled in 5 zones in Jaén (located between 0.78 and 3.69 km from each other) and 4 in Segura (1.10-3.58 km from each other). Each site consisted in a 50 x 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* (*Fij*), and the probability of recruitment (*Pij*) 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 (*Pij*) 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 (*Fij*) 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).

Data analyses

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 (*Fij*) and another for the probability of recruitment (*Pij*) for each pair in each site. As predictors, we included the dissimilarity between canopy-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 *Fij* 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 *Pij* using a binomial distribution (see Supplementary 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 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).

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 different aspects of the recruitment: canopy service, recruitment niche width, structure of the sapling bank, 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 another 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 Supplementary 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.* 2023) and residual distributions were checked with *DHARMa* R package (Hartig & Lohse 2022). Graphical representation was performed by *ggplot2* R package

(Wickham 2016).

Results

The mean proportion of OTUs of pathogens across plant species was 0.16 ± 0.11, with a range between 0.0086 in *Daphne laureola* and 0.55 in *Juniperus oxycedrus*. In the case of saprotrophs, this proportion was 0.14 ± 0.11 with a range between 0.0048 in *Daphne 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 *Daphne gnidium, Acer monspessulanum, Pistacia therebintus* and *Prunus spinosa* and 0.59 in *Juniperus phoenicea* (Supplementary Material 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 *Daphne laureola* and 22.27 in *Juniperus oxycedrus*. In the case of saprotrophs, this mean was 12.26 ± 6.28 OTUs, with a range between 1 in *Daphne laureola* and 31.98 in *Juniperus communis*, and in the case of epiphytes it was 3.07 ± 2.37, with a range of 1 in *Daphne gnidium, Acer monspessulanum, Pistacia terebinthus* and *Prunus spinosa*, and 11.63 in *Juniperus phoenicea* (Supplementary Material Table S1.2).

The largest Bray-Curtis dissimilarity between pathogenic fungal communities was found between the pairs: *Acer monspessulanum-Pistacia lentiscus*, *Daphne gnidium-Pistacia lentiscus*, *Phillyrea latifolia-Pistacia lentiscus*, *Phillyrea angustifolia-Phillyrea latifolia, Phillyrea angustifolia-Daphne gnidium* and *Phillyrea angustifolia-Acer monspessulanum*. The largest difference between saprotrophic fungal communities was found between *Pistacia lentiscus* and *Rosa* sp. The largest difference between epiphytic fungal communities was found between *Amelanchier ovalis* and *Juniperus phoenicea*. The average recruitment niche width was 9.11 ± 5.87 canopy species (range 0

to 23), canopy service was 9.40 ± 7.08 recruited species (range 0 to 26), the average density of the sapling bank of a recruit species was 0.03 ± 0.03 saplings/m^2 (range 0 to 0.14) and the mean density of saplings under canopy species was 1.40 ± 8.84 saplings/m^2 (range 0 to 58.04) (Supplementary Material Table S1.3).

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 (*Fij*) (Table 1a, Figure 2a, Figure S4.1). Also, we found that plants with similar pathogenic and saprotroph fungal communities had lower probability of recruitment (*Pij*), 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 interactions are less likely to occur between rare (low abundance) plants. Neither site nor phylogenetic distance showed consistent effects on *Fij* or *Pij*.

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 *Fij* matrix) of a given plant species was enhanced by the richness of epiphytic fungi and by the diversity of saprotroph fungi (Table 2b, 2d, Figure 3a, 3b). We did not find any relationships between fungal richness and diversity and the plant species canopy service (number of species thar recruit under canopy species) or recruitment niche width (number of species under which each species recruits) (Table S2).

Table 1. GLMM analyses performed to explore the effect of the phyllosphere fungal communities' compositional distance (measured as 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.

a)

Fixed effects	**Estimate**	**Std. error**	***Z* value**	***p***
Pathogens dissimilarity	1.548	0.502	3.083	**0.002**
Saprotrophs dissimilarity	-0.087	0.583	-0.149	0.881
Epiphytes 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
Random effects	**Variance**	**Std. dev**		
Canopy species	0.688	0.829		
Recruit species	0.233	0.483		

b)

Fixed effects	**Estimate**	**Std. error**	***Z value***	***p***
Pathogens dissimilarity	1.953	0.955	2.046	**0.041**
Saprotrophs dissimilarity	2.266	1.123	2.018	**0.044**
Epiphytes 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
Random effects	**Vatiance**	**Std. dev**		
Canopy species	0.679	0.824		
Recruit species	0.195	0.441		

Table 2. GLMM analyses exploring the effect of fungal richness (OTU 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.

a) Structure of the sapling bank

Fixed effects	Estimate	Std. error	*Z* value	*p*
Pathogens richness	6.097	4.198	1.452	0.146
Saprotrophs richness	-8.107	4.368	-1.856	0.063
Epiphytes 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

Fixed effects	Estimate	Std. error	*Z* value	*p*
Pathogens richness	-0.019	2.956	-0.006	0.995
Saprotrophs richness	-1.096	2.916	-0.376	0.707
Epiphytes 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

Fixed effects	Estimate	Std. error	*Z* value	*p*
Pathogens diversity	0.003	0.043	0.077	0.939
Saprotrophs diversity	-0.035	0.031	-1.128	0.259
Epiphytes 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

Fixed effects	Estimate	Std. error	*Z* value	*p*
Pathogens diversity	-0.042	0.029	-1.454	0.146
Saprotrophs diversity	0.065	0.021	3.127	**0.002**
Epiphytes 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**

a) Recruitment binary matrix
Recruit species
Canopy species
A B C D E
A 0 1 0 0 1
B 1 1 0 1 0
C 1 1 0 0 0
D 0 0 0 0 2
E 0 0 1 0 1
Recruitment niche width
2 3 2 2 2
Canopy service
2 3 1 1 4
Pairwise-level analyses
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
b) Recruitment frequency matrix
Recruit species
Canopy species
A B C D E
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
Structure of the sapling bank
2 31 8 2 4
Canopy contribution to the sapling bank
20 7 1 12 6
Species-level analyses
Plant species
Fungal guild OTUs
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
Diversity fungal guild
2 2.9 1.5 1 1.8
Richness fungal guild
2 31 8 2 4
A B C D E

Figure 1. Data analysis framework. 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). The frequency matrix contains the frequency (*Fij*) 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 affects different aspects of recruitment (recruitment width, canopy service, structure of the sapling bank, canopy contribution to the sapling bank).

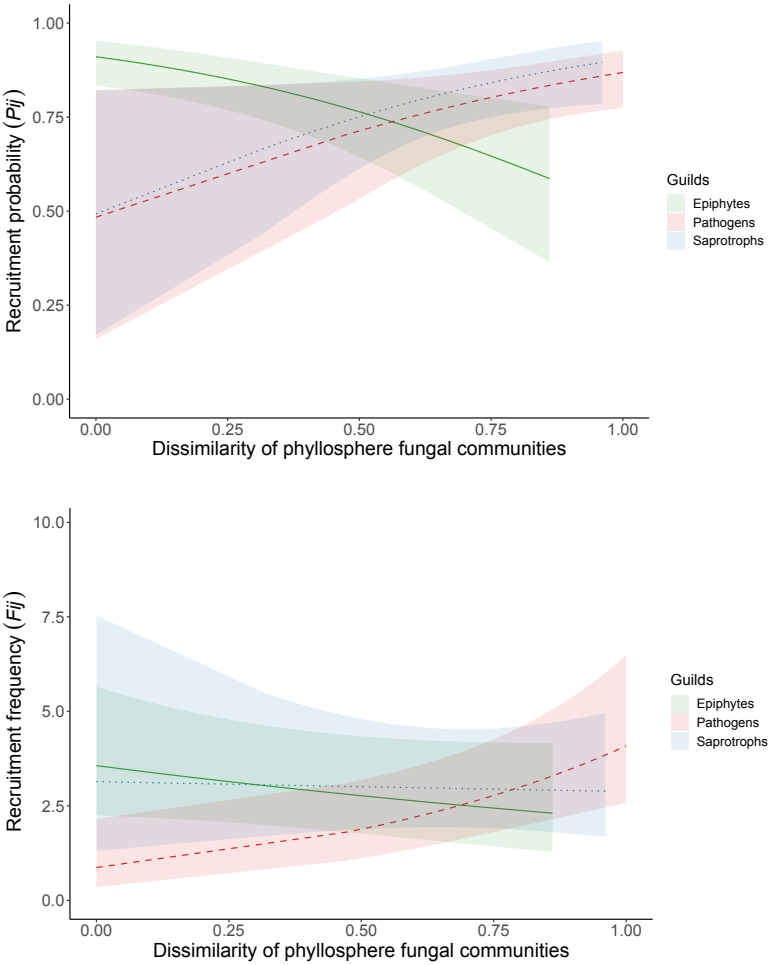

Figure 2. Predictions of the GLMM analysis performed to explore the effect of the dissimilarity of phyllosphere fungal communities of pathogens, saprotrophs and epiphytes measured as Bray-Curtis distance on (**a**) recruitment frequency and (**b**) recruitment probability between pairs of plant species.

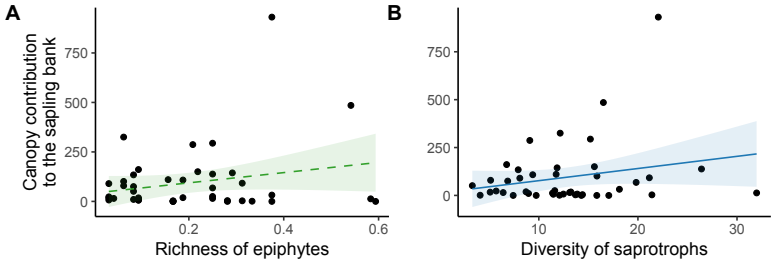

Figure 3. Predictions of the GLMM analysis performed to explore the effects of (**a**) the richness (OTU proportion) of fungal epiphytes on the canopy contribution to the sapling bank, (**b**) the diversity (effective number of partners) of fungal saprotrophs to the canopy contribution to the sapling bank.

Discussion

Leaf fungal communities associated with plant species are fundamental to promote multiple ecosystem functions (Peñuelas & Terradas 2014, Laforest-Lapointe & Whitaker 2019). 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 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 & 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. 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 shows 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, that 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.* 2021). 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 in 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 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.

Conclusions

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 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 *et al.* 2019b), 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.

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).

Supporting information

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).

Capítulo 3

Intransitivity in plant–soil feedbacks is rare but is associated with multispecies coexistence

Pajares-Murgó, M., Garrido, J. L., Perea, A. J., López-García, Á., Bastida, J. M., Prieto-Rubio, J., Lendínez, S., Azcón-Aguilar, C., & Alcántara, J. M. (2024). Intransitivity in plant–soil feedbacks is rare but is associated with multispecies coexistence. *Ecology Letters*, 27(3), e14408.

The environment is not a structure imposed on living beings from the outside but is in fact a creation of those beings. Just as there is no organism without an environment, so there is no environment without an organism.

Richard Lewontin (1985). The Organism as the Subject and Object of Evolution. *The Dialectical Biologist*, p. 99.

Abstract

Although plant-soil feedback (PSF) is being recognized as an important driver of plant recruitment, our understanding of its role on species coexistence in natural communities remains limited by the scarcity of experimental studies on multispecies assemblages. Here, we experimentally estimated PSFs affecting seedling recruitment in 10 co-occurring Mediterranean woody species. We estimated weak but significant species-specific feedbacks. Pairwise PSFs impose similarly strong fitness differences and stabilizing-destabilizing forces, most often impeding species coexistence. Moreover, a model of community dynamics driven exclusively by PSFs suggests that few species would coexist stably, the largest assemblage with no more than six species. Thus, PSFs alone do not suffice to explain coexistence in the studied community. A topological analysis of all subcommunities in the interaction network shows that full intransitivity (with all species involved in an intransitive loop) would be rare but it would lead to species coexistence through either stable or cyclic dynamics.

Keywords: plant-soil feedback, intransitivity, interactions network, plant recruitment, fitness differences, niche differences, equalizing mechanisms, stabilizing mechanisms, strongly connected components.

Introduction

Recruitment is the part of the plant life cycle that spans from the seed to juvenile stages, involving different demographic processes (germination, emergence, seedling survival and sapling growth and survival) (Herrera *et al.* 1994; Merges *et al.* 2020). These processes are modulated by direct or indirect plant-plant

interactions where the success of a recruiting plant may depend on the identity of the established plants around it (Rey & Alcántara 2000; Gómez-Aparicio *et al.* 2004; Landero & Valiente-Banuet 2010; Alcántara *et al.* 2018). Understanding the mechanisms that condition the specificity of such plant-plant interactions is of major importance in the study of species coexistence (Alcántara *et al.* 2019). Among these mechanisms, plant-soil feedbacks have garnered increasing interest (Lekberg *et al.* 2018; Crawford *et al.* 2019). Plant-soil feedback (PSF, hereafter) is the process by which plants exert an influence on soil properties that affect the performance of individuals of their own or other plant species (Bever *et al.* 1997). In the last decade, it is being widely recognized that feedbacks operate in structuring the composition and dynamics of plant communities (Klironomos 2002; Eppinga *et al.* 2018; Miller *et al.* 2022). A decrease of conspecific relative to heterospecific performance (i.e. a negative PSF) can prevent dominance and drive species coexistence in forests (Mangan *et al.* 2010; Jiang *et al.* 2022) and grasslands (Petermann *et al.* 2008) but it can also favour invasiveness of alien species not affected by such feedback (Klironomos 2002). Conversely, positive PSFs may lead to the destabilization of diverse communities by enhancing the performance of conspecifics, thus increasing their dominance (Kulmatiski *et al.* 2011; Suding *et al.* 2013). However, some studies have found contrasting results, such as dominance dynamics or invader species success in spite of the existence of negative PSFs (Nijjer *et al.* 2007; Heinze *et al.* 2016), or the absence of relationship between the species abundance and positive PSFs (Reinhart *et al.* 2021). This suggests that there are knowledge gaps in the factors influencing plant-plant interaction outcomes (Lekberg *et al.* 2018), so determining the impact of PSFs on plant species diversity and dynamics in natural communities still remains a challenge (Kandlikar *et al.* 2019).

The mathematical models of PSFs by Bever and colleagues (Bever 1994, 2003; Bever *et al.* 1997) provided the basis for the theoretical framework that has guided most empirical research in the field (Miller *et al.* 2022). The pairwise feedback metric (I_{ij}) proposed by Bever can be quantified experimentally and informs on the strength of stabilization or destabilization of species coexistence (Bever *et al.* 1997; Crawford *et al.* 2019). However, it does not take into account the importance of interspecific fitness differences when analysing plant coexistence (Ke & Wan 2020). To disentangle the role of PSFs in the outcome of plant-plant interactions, recent studies (Kandlikar *et al.* 2019; Ke & Wan 2020; Yan *et al.* 2022) have applied modern coexistence theory (Chesson 2000). This approach dissects the competitive outcome between a pair of species into stabilizing niche differences that favour the recovery of rare species, and fitness differences that determine the relative competitive ability of plant species (Ke & Wan 2020). This allows determining whether PSFs, when isolated from other plant-plant interaction mechanisms, promote or prevent coexistence between species pairs, and whether this is mediated by niche differences, fitness differences or both (Kandlikar *et al.* 2019). The balance between both parameters indicates whether the interaction between two species would allow their coexistence or would lead one of them to extinction through competitive exclusion or priority effects. Kandlikar *et al.* (2021) and Yan *et al.* (2022) found that PSFs tend to generate stronger fitness differences than stabilizing or destabilizing effects, consequently predicting a preponderance of species exclusion if competition were driven exclusively by PSFs.

In natural communities, plant species interact with many others, so analysing coexistence through pairwise interactions in isolation may not suffice to discern the actual role of interactions between competing species in the maintenance of diversity

(Barabás *et al.* 2016; Miller *et al.* 2022). For example, Kandlikar *et al.* (2021) predicts a prevalence of competitive exclusion among six plant species which, however, are known to co-occur in Southern California grasslands. To address these problems, we need to scale the study of PSFs from pairwise to community-level (Eppstein & Molofsky 2007; Eppinga *et al.* 2018; Miller & Allesina 2021). The model proposed by Eppinga *et al.* (2018) is a community-level extension of the classic PSF model of Bever *et al.* (1997) and is particularly well suited to explore the role of complex interaction network structures on species coexistence.

When considering competition between more than two species, several studies have shown that intransitivity can promote coexistence (Barabás *et al.* 2016; Levine *et al.* 2017; Kandlikar *et al.* 2019). Intransitive interactions imply that none of the species is able to outcompete all the others, as in the popular game of "rock-paper-scissors", so all species can coexist (Gilpin 1975; May & Leonard 1975). Nevertheless, it is still controversial to what extent intransitive interactions actually contribute to coexistence in natural plant communities. While Soliveres *et al.* (2015) suggested intransitive interactions are widespread, Godoy *et al.* (2017) and Kinlock (2019) concluded that intransitive competition is infrequent. In the context of multispecies PSFs, the network approach has been proposed (Eppinga *et al.* 2018; Kandlinkar *et al.* 2019; Mack *et al.* 2019) but it has not been applied to experimental results yet, so we lack evidence on whether intransitive PSFs may contribute to coexistence. To advance in this direction, we apply to the model of Eppinga *et al.* (2018) the approach proposed by Alcántara & Rey (2012) that makes it possible to qualitatively infer the potential persistence of species through the analysis of strongly connected components in the network structure.

The main aim of this study is understanding how PSFs

influence coexistence in woody plant communities. We experimentally studied the PSFs among ten species co-occurring in Mediterranean-mixed forests and compared the results with data on recruitment interactions in natural communities. We estimated the stabilization and fitness differences generated by PSFs and predicted their outcome in terms of competitive exclusion, priority effects or coexistence. We used our experimental results to parameterise the community dynamics model of Eppinga *et al.* (2018) and estimate the persistence of all the possible assemblages obtained by combining the ten studied species. Finally, we evaluated the effect of intransitivity on species persistence through the topological analysis of the model's interactions matrix. Specifically, we aimed to assess the following questions: (1) Is recruitment success influenced by the canopy species conditioning the soil? (2) what is the frequency of predicted coexistence, competitive exclusion or priority effects across species pairs driven by PSFs? And (3) what is the role of intransitivity in species coexistence in multispecies plant assemblages driven by PSFs?

Material and methods

Experimental design

The study focuses on the woody species assemblages of Mediterranean mixed pine-oak forest in the SE Iberian Peninsula. We selected 10 woody plant species co-occurring in the area: *Pinus halepensis*, *Quercus ilex*, *Quercus faginea*, *Juniperus phoenicea*, *Juniperus oxycedrus*, *Pistacia terebinthus*, *Acer monspessulanum*, *Colutea atlantica*, *Cistus albidus* and *Genista cinerea*.

Soil and seed sources

We collected seeds during 2020 from 5 individuals of each species (individual seed sources or ISEs, hereafter). Their seeds were processed following recommendations in Navarro & Gálvez (2002) and stored at 4°C until the start of the experiment. In October 2020, we collected soil beneath 6 adult individuals of each species (individual soil sources or ISOs, hereafter). Soil samples were collected below the canopy of the shrubs and less than 50 cm from the trunk of the trees. We removed the surface litter and extracted soil up to a depth of 30 cm. ISOs of the same species were chosen at least 10 m from each other. Under each ISO, we collected 4 soil samples (approximately 8 kg of soil per sample), one in each cardinal direction. After collecting the soil from each ISO, the tools used were cleaned with a 10% bleach solution to prevent cross-contamination. We stored the soil at room temperature.

Experimental setting

Our experimental design produced PSF estimates of recruitment success in 100 canopy–recruit pairs. The experiment started in October 2020 in a shade-house at Estación Experimental del Zaidín (EEZ-CSIC, Granada, Spain). We used 15-cell plastic trays of 0.41 L per cell. Each cell was filled with soil from one ISO and sowed with 10 seeds from one ISE (only two seeds in the case of *Quercus* species due to their large size). The experimental design consisted in 10 soil-source species x 6 ISOs/species x 10 recruit species x 5 ISEs/species, totalling 3000 cells. The combinations of soil and seed species were randomized across the cells. Germination and seedling survival were monitored weekly until October 2021. Only the first seedling emerged in each cell

was allowed to grow to avoid competition for space.

Data analyses

Testing for species-specific PSF

The existence of species-specific PSFs can be determined by comparing the success of a plant in soil from conspecifics against its success in soil from a different species (Brinkman *et al.* 2010; Lekberg *et al.* 2018). We tested species-specific PSFs for each species separately. We used generalized linear mixed models (GLMMs) with the mean recruitment success of each ISEs of species *i* averaged across the ISOs of species *j* as dependent variable, and the soil-source species as predictor variable. All models were fitted assuming beta family distribution. We also added the ISE as random effect. Significant terms indicate that PSFs are species-specific.

To assess whether the experimental results reflect the efficiency of canopy-recruit interactions occurring in the field, we used data from Alcántara *et al.* (2018) obtained in the same study site as the experimental seeds and soils. We estimated the efficiency of recruitment for the 10 studied species under the canopy of the soil-source species (*Eij*) as the number of saplings of species *i* recruiting under species *j* divided by the cover of the canopy and recruit species. We fitted a GLMM with *Eij* as dependent variable and the experimental recruitment success of each canopy-recruit pair as the predictor variable, and the recruit and canopy species as random effects. Note that many field estimates were zero, so we fitted the model using Tweedie distribution. Further details are provided in Supplementary methods S1.1.

Pairwise-level feedback

We estimated recruitment success (s_{ij}) as the proportion of experimental cells with soil of species *j* containing a seedling of species *i* at the end of the experiment. Although biomass is most frequently used as performance measure in PSF studies (Kandlikar *et al.* 2021), using biomass in our experiment would discard information from all the replicates that lacked seedling at the end of the experiment (52%). s_{ij} can differ between recruit species due to intrinsic differences in seed viability not related with PSFs. To account for these differences, we rescaled s_{ij} to remove the effect of seed viability. We divided the recruitment success of species *i* in soil from species j (s_{ij}) by its success in reference soil (s'_{ij}). As a reference, we used the average recruitment success of species *i* in soils from species other than *j*:

$$s'_{ij} = \frac{1}{n-1}\sum_{k \neq j} s_{ik} \qquad (1)$$

In this way, we obtained rescaled recruitment success values, σ_{ij}, which can be more appropriately compared across species. Values of σ_{ij} between 0 and 1 indicate a negative effect of species *j* soil on species *i* performance, while values >1 indicate a positive effect. By relativizing to the reference soil, our estimate of σ_{ij} controls for the confounding effect of seed viability. From an ecological point of view, this represents the relative advantage or disadvantage in recruitment that species *i* obtains by dispersing seeds to soils conditioned by species *j* or to soils conditioned by any of the other species present in the community.

To evaluate the possibility of coexistence of pairs of species, we followed Kandlikar *et al.* (2019) and Yan *et al.* (2022). This approach calculates the (de)stabilizing niche difference effects

and fitness differences (SD and FD, hereafter) between each species pair as follows:

$$SD = -\frac{1}{2}[\sigma_{11} - \sigma_{12} - \sigma_{21} + \sigma_{22}] \quad (2)$$

$$FD = \frac{1}{2}[\sigma_{11} + \sigma_{12} - \sigma_{21} - \sigma_{22}] \quad (3)$$

We compared the absolute magnitude of SD and FD by fitting a GLMM with the difference in the absolute values of SD and FD as a predictor variable, and the recruit and soil-source species as random variables.

Comparing the values of SD and FD for a given pair of species allows determining whether they could coexist or one of them would exclude the other through competitive exclusion or priority effects. Specifically, the following relationships can be assessed for each species pair:

SD > 0 and SD > abs (FD): Coexistence
SD < 0 and abs (SD) > abs (FD): Priority effects
abs (SD) < abs (FD): Competitive exclusion

To assess the robustness of these comparisons, we obtained 1000 matrices of randomized s_{ij} values from a binomial distribution with probability equal to s_{ij} (see details in Supplementary methods S1.2). From these matrices we obtained the SD and FD and the frequency of each outcome for each randomization.

Community-level effects of plant-soil feedbacks

To explore the consequences of PSFs on coexistence at community level, we parameterised the model of Eppinga *et al.*

(2018) using our experimental results (E-model, hereafter). The E-model is an example of the replication equation originating from evolutionary game theory (Hofbauer & Sigmund 1998) and describes the dynamics of species under the influence of frequency-dependent PSFs assuming that the plants are equally competitive in any other respects. These assumptions allow exploring the potential effects of PSFs if these were the only drivers of community dynamics, disregarding other properties of the species (e.g. life span, dispersal and competitive ability). The dynamics of the E-model are described by the following system of equations:

$$\frac{dP_i}{dt} = P_i(w_i - \sum_{j=1}^{n} w_j P_j) \qquad (4)$$

Where P_i is the vector of relative frequencies of the n species in the community (i, j: 1 ... n) and w_i is:

$$w_i = \sum_{j=1}^{n} \sigma_{ij} P_j \qquad (5)$$

The only parameters needed in the model are σ_{ij}. The set of all σ_{ij} form the matrix $\mathbf{A}$ (Fig. 1a). The relative abundance of each species at equilibrium can be estimated as:

$$P_i^* = \frac{det(\boldsymbol{A_i})}{\sum_{j=1}^{n} det\,(\boldsymbol{A_j})} \qquad (6)$$

Where $\mathbf{A}_i$ is the $\mathbf{A}$ matrix with the column corresponding to species i replaced by a column of ones, and *det* indicates the determinant of the matrix. The community has a feasible

equilibrium if $0 < P_i^* < 1$ for all species. If a feasible equilibrium does not exist, at least one of the species will become extinct. Even if a feasible equilibrium exists, it may not be stable so some species may become extinct. Stability can be estimated through standard eigenvalue analysis of the system's Jacobian matrix at equilibrium. If the largest real part across all eigenvalues (λ) is negative, then the equilibrium is stable. If a community is feasible and stable according to the E-model, then we will say that the community is E-persistent. The E-model can allow unstable coexistence through cyclic dynamics when λ is positive but sufficiently small and there is negative community-level feedback (I_c):

$$I_c = (-1)^n \sum_{j=1}^{n} \det A_j \qquad (7)$$

Negative I_c is also a necessary but not sufficient condition for E-persistence. It is not possible to determine which values of I_c and λ would lead to such cyclic dynamics, so it must be determined through simulations of the community dynamics. We conducted such simulations using Runge-Kutta 4th and 5th order integration implemented in R-package *deSolve* (Soetaert *et al.* 2010).

To assess the robustness of the results, we parameterized the E-model with 1000 random **A** matrices obtained as described in the pairwise analyses. Moreover, our experiment allows to parameterise the E-model for any combination of the ten species. This provides the opportunity to explore which subcommunities would be able to persist. Thus, we recalculated the **A** matrix and applied the E-model to each of the 1013 subcommunities that can result from combining between two and ten species. The **A** matrix of each subcommunity was built by rescaling the corresponding σ_{ij} values considering in Eq. 1 only the species in

the subcommunity.

Topological analysis of intransitivity

We used matrix **A** to extract the network of interactions between species caused by PSFs following the approach of Eppinga *et al.* (2018). The largest value in a column of matrix **A** indicates the species that would be most benefited in this type of soil. The adjacency matrix corresponding to **A** is constructed by transforming these largest values into ones and the rest into zeroes (Fig. 1a, b). The corresponding networks (Fig. 1c, d, e) have species as nodes and arrows pointing from the soil conditioning species to the species that is most benefited in this soil.

We used this binary adjacency matrix for the qualitative estimate of persistence following the approach of Alcántara and Rey (2012). This approach is based in the analysis of strongly connected components (SCCs) in the network structure (Fig. 1c, d). An SCC can consist of a single species (in which case it is called a trivial SCC) or by more than one species (a non-trivial SCC). The largest SCC is named the core. Multiple trivial and/or non-trivial SCCs may form chains of network elements connected transitively (Fig. 1d, e). Thus, a complex network may contain simultaneously sets of species interacting transitively and intransitively (Alcántara *et al.* 2017). A network is fully intransitive when all the nodes belong to the core (Fig. 1c).

Assuming linear time-invariant dynamics, Alcántara *et al.* (2012) showed that species in the core SCC and those benefited by some core species (called satellite species) can persist in the long term, while species that are not benefited by any core or satellite species will become extinct (called transient species). In the case of non-linear time invariant models (like the E-model) these relationships between network structure and species persistence

are not mathematically guaranteed, but simulations showed that core and satellite species are less likely to become extinct and have longer time to extinction than transient species (Alcántara *et al.* 2017). As defined in Eppinga *et al.* (2018), the network derived from the **A** matrix cannot contain satellite species, so we will say that an assemblage is SCC-persistent under the E-model if all the species belong to the same non-trivial SCC, what amounts to say that it is fully intransitive.

Our objective by comparing the E-persistence and SCC persistence is to demonstrate the influence of the subjacent topology of the **A** matrix on the feasibility and stability properties of the E-model. This influence is one of the basic tenets of ecological network studies (Poisot *et al.* 2016), but has not been previously demonstrated in the context of PSF theory. To assess the agreement between subcommunities persistence according to the E-model and the SCC analysis, we used a confusion matrix that synthetises the number of subcommunities in which both approaches agree or disagree. From this matrix, one can use a Chi-Square test to determine whether both approaches agree more often than expected by chance. It is important to note that the agreement between E-persistence and SCC-persistence can depend on the way **A** matrix is binarized. We performed these analyses using another two binarization methods and found that the one proposed by Eppinga *et al.* (2018) provided the better agreement according to Cohens' kappa statistic (Cohen 1960) (Supplementary Methods S1.3).

The list of software used in this study is provided in the Supplementary methods S1.

Results

Testing for species-specific PSF

The estimated recruitment success in the experiment for each species pair was positively related with the efficiency of recruitment observed for the same species pairs in the field (slope ± SE: 3.375 ± 1.548, p = 0.029; Fig. 2). Experimental estimates explained 16% of the variance in field estimates. We found evidence that recruitment success in heterospecific soil differed from success in conspecific soil across plant species (Fig. 3, Table S1). Overall, fourteen of eighty-nine species pairs (15.7%) produced statistically significant species-specific PSFs. There were 35 species pairs with negative species-specific PSF and 54 with positive species-specific PSFs with an average close to 0 (log-ratio between conspecific and heterospecific soil effects: 0.016 ± 0.117; range -0.302 to 0.298).

Pairwise-level feedback

The absolute magnitude of FD did not differ from the absolute value of SD (estimate ± SE: -0.006 ± 0.044, p = 0.895). Competitive exclusion was the most frequently predicted outcome of pairwise competition, followed by priority effects and coexistence (40%, 35.56% and 24.4% respectively). Specifically, coexistence was predicted in 11 out of 45 species pairs, 6 of which included *A. monspessulanum* and 4 included *J. oxycedrus* (Table S2). The randomization of the outcome for each species pair verified the robustness of our results indicating pairwise competitive exclusion as the dominant outcome (Fig. 4).

Community-level dynamics

The 10-species assemblage has a feasible but unstable equilibrium, with a positive I_c, so it is not E-persistent. This conclusion was repeated in all of the 1000 simulated plausible matrices ($\lambda > 0.01$ in all cases). Although many simulations (47.1%) had a negative I_c, none showed stable cyclic dynamics. The 10-species network had very low intransitivity, with just one non-trivial (core) SCC formed by the pair *C. albidus* - *G. cinerea*, and the rest of species as transients relative to this small core (Fig. 5a). In addition, *P. terebinthus* was disconnected from the rest of the network. The very small core with many transient species and a disconnected SCC makes this assemblage not SCC-persistent.

The analysis of the 1013 subcommunities (Table S3, Fig. S1) shows that only 157 were not feasible, thus leading some species to extinction (Fig. 5b). However, only 54 of the 856 feasible assemblages were E-persistent, many of them (20 out of 54) with just two species and the largest one consisting in 6 species (Fig. 5c). In total 959 assemblages (94.66%) were not E-persistent.

Most assemblages (68.11%) contained some intransitive group (i.e. at least one non-trivial SCC) which involved on average 52.21% of the species. The largest intransitive groups were formed by up to 5 species. The probability that an assemblage contained at least one transient species (and thus, some level of transitivity) increased sharply with the number of species (binomial regression: intercept = 9.91 ± 1.77, slope = 3.55 ± 0.58, $p < 0.001$, $df = 688$), to the point that transients occurred in all assemblages with 5 or more species. The qualitative analysis indicates that 41 assemblages are SCC-persistent.

The association between the predictions of E-persistence and SCC-persistence is highly significant (Chi square = 447.73, $p < 0.001$; Table S4). The SCC approach classifies correctly as not-

persistent 950 of the 959 assemblages identified as such by the E-model. Thus, the SCC approach has a specificity of 99.06%. On the other hand, the SCC approach classifies correctly as persistent 32 out of the 54 assemblages identified as E-persistent (Fig. 5d), so the SCC approach has a sensitivity of 59.26%. When the SCC approach predicts that an assemblage is not E-persistent, it succeeds in 950 out of 972 cases (97.74%), and it succeeds 32 out of 41 times (78.05%) when predicting that an assemblage is E-persistent. All 9 assemblages classified as persistent by the SCC approach but as unstable by the E-model allow non-equilibrium coexistence through heteroclinic cycles (Fig. 5e, f). Thus, all of the 41 assemblages with full intransitivity allow species coexistence, even the one that combines full intransitivity with positive I_c (Fig. 5f). Since the SCC approach applied to the E-model predicts persistence only when the community is fully intransitive, our results indicate that full intransitivity always allows species coexistence, and the lack of full intransitivity is almost always associated with the lack of persistence of at least one species.

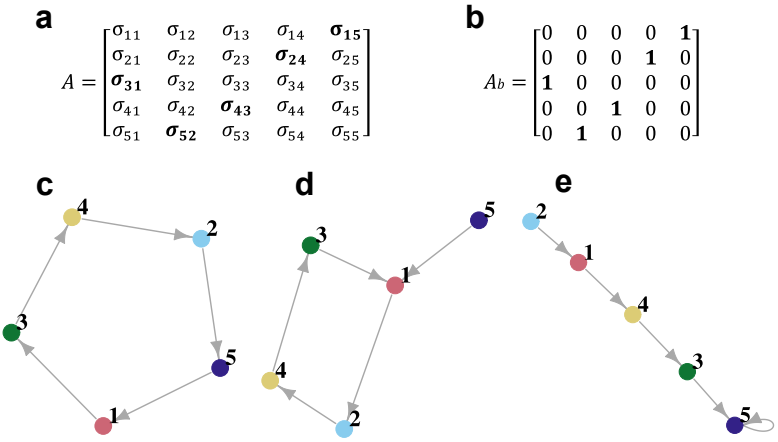

Figure 1. Relationships between the E-model and the topology of the corresponding network. **A**) Matrix **A** of the E-model, containing the information on the frequency-dependent effect (σ_{ij}) of species *j* (soil source species in columns) on the performance of species *i* (recruit species in rows). The largest σ_{ij} in each column are indicated in bold type. **B**) Matrix **A** transformed in the adjacency (binary) matrix of the E-model. ($\mathbf{A}_b$) by transforming the largest σ_{ij} from each column into 1 and the rest of values into 0. **C**) The adjacency matrix corresponds to a directed graph where species are nodes and arrows point from the soil source species to the recruit species. Strongly Connected Components (SCCs) are the maximal groups of nodes so that there is a route starting and ending in any node after passing through all the rest of nodes of the SCC. In this example there is a single non-trivial SCC that contains all species. **D**) Example of a network with a non-trivial SCCs and one trivial SCC (species 5 in the example is a transient species). **E**) A totally transitive network would contain only trivial SCCs, implying a hierarchical system of interactions (note that species 5 has a self-loop, indicating that it benefits conspecific more than heterospecific plants (i.e. it has positive PSF). The species within a non-trivial SCC form an intransitive group. The network depicted in panel C) is fully intransitive while the one in panel D) contains both transitive and intransitive relationships. Note that method used in the binarization of the **A** matrix does not allow the existence of satellite SCCs.

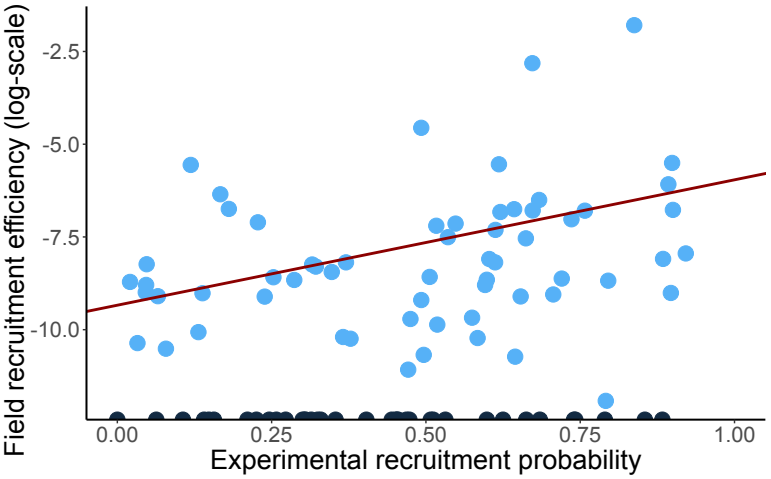

Figure 2. Relationship between experimental estimates of the mean recruitment probability of species *i* in soil from species *j* (s_{ij}) and the efficiency of recruitment of species *i* in the close proximity of species *j* in the field (E_{ij}). Experimental estimates explain 16% of the variance in field estimates.

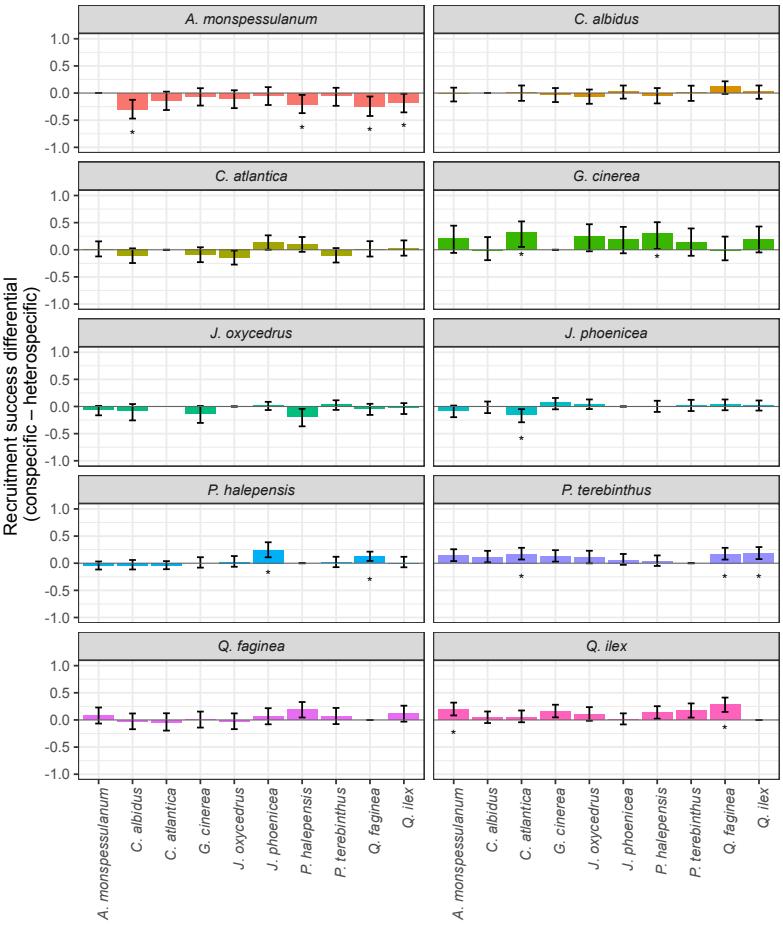

Figure 3. Species-specific feedback effects of recruitment success across plant species in each conspecific or heterospecific soil. Each panel contains results for the recruitment success of the 10 studied species which is indicated by the x-axis label. PSFs (y-axis) were calculated as the difference in recruitment success between

conspecific and each heterospecific soil. Positive values indicate positive PSF effects (higher recruitment success in conspecific soil compared to the soil of other plant species) and negative values indicate negative PSF effects (lower recruitment success in conspecific soil compared to the soil of other plant species). Error bars show the standard error. Asterisk indicates statistically significant species-specific PSFs ($p < 0.05$) from the generalized mixed models for each recruit species (Table S1).

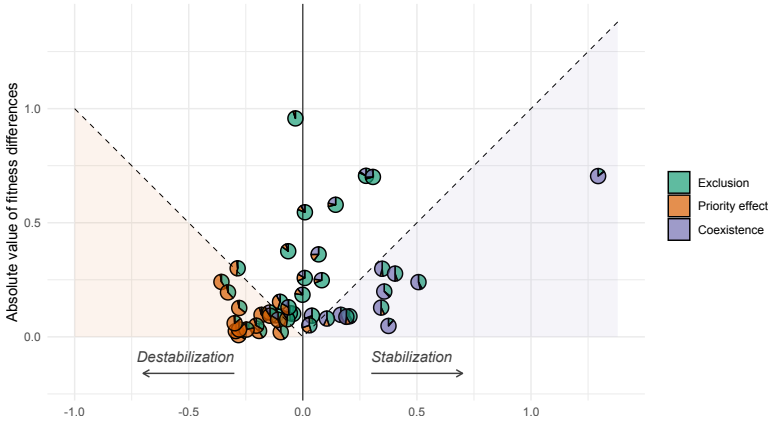

Figure 4. Outcome of pairwise plant-plant interactions on recruitment success. The coordinates show the mean strength of (de)stabilization effects and the absolute value of fitness differences. Each species pair is represented by a pie chart with colours indicating the proportion of random sampling draws that resulted in each coexistence outcome (competitive exclusion, priority effects or coexistence).

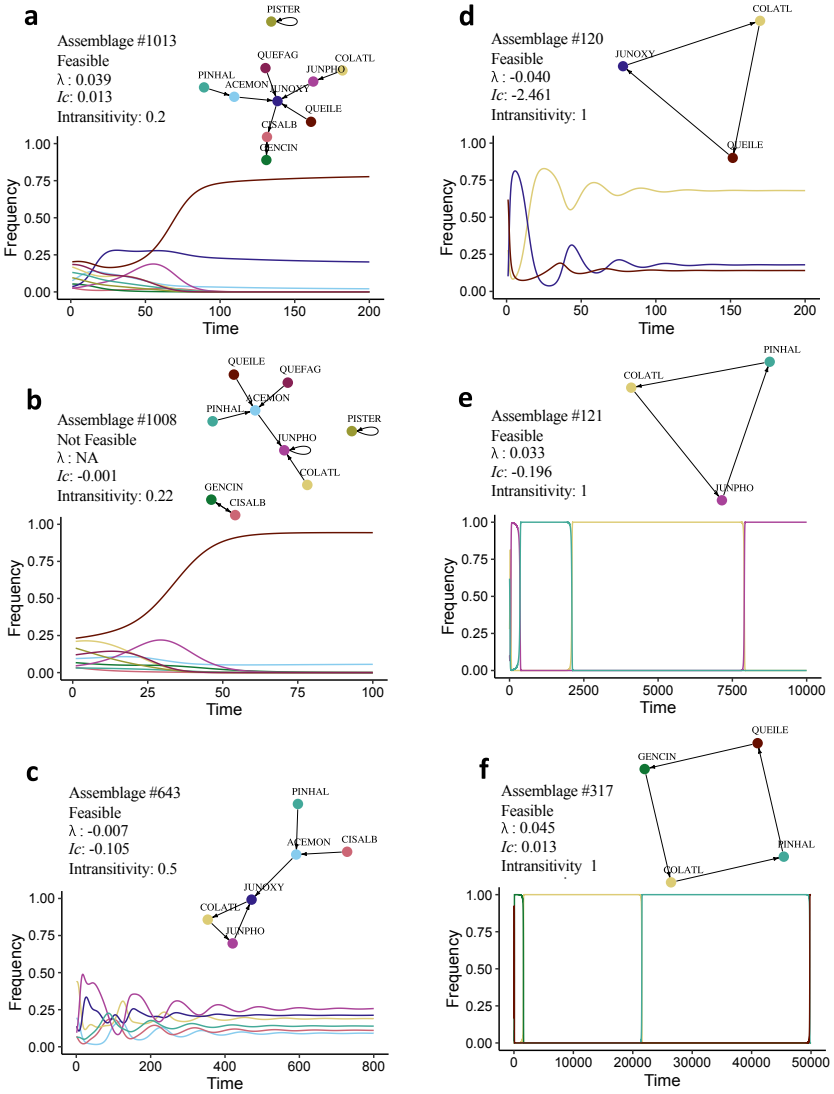
a
Assemblage #1013
Feasible
λ : 0.039
Ic: 0.013
Intransitivity: 0.2
PISTER
QUEFAG
COLATL
JUNPHO
PINHAL
ACEMON
JUNOXY
QUEILE
CISALB
GENCIN
Frequency
Time
b
Assemblage #1008
Not Feasible
λ : NA
Ic: -0.001
Intransitivity: 0.22
QUEILE
QUEFAG
ACEMON
PINHAL
PISTER
JUNPHO
COLATL
GENCIN
CISALB
Frequency
Time
c
Assemblage #643
Feasible
λ : -0.007
Ic: -0.105
Intransitivity: 0.5
PINHAL
ACEMON
CISALB
JUNOXY
COLATL
JUNPHO
Frequency
Time
d
Assemblage #120
Feasible
λ : -0.040
Ic: -2.461
Intransitivity: 1
COLATL
JUNOXY
QUEILE
Frequency
Time
e
Assemblage #121
Feasible
λ : 0.033
Ic: -0.196
Intransitivity: 1
PINHAL
COLATL
JUNPHO
Frequency
Time
f
Assemblage #317
Feasible
λ : 0.045
Ic: 0.013
Intransitivity 1
QUEILE
GENCIN
PINHAL
COLATL
Frequency
Time

Figure 5. Examples of the dynamics and network structures generated by different assemblages of species. Each panel shows (*i*) a simulated run of the E-model including only the species of the assemblage, with lines representing the temporal trajectories of the species, (*ii*) information on the identification code of the assemblage, whether it is feasible or not, the leading eigenvalue of its Jacobian matrix evaluated at equilibrium (if it was feasible), the community-level feedback (I_c), the level of intransitivity (proportion of species in the largest SCC) and (*iii*) a representation of the interactions network (species acronyms formed by first three letters of genus and species). **A**) Assemblage formed by the 10 studied species is feasible but not stable. *Pistacia terebinthus* is disconnected from the rest of species. The core is formed by *C. albidus* and *G. cinerea*, with the rest of species interacting transitively. **B**) Not-feasible assemblage of 9 species forming three disconnected subnetworks and a single non-trivial SCC (*C. albidus* and *G. cinerea*). **C**) The largest persistent assemblage in this study, formed by 6 species. It had a core formed by three species that interact intransitively (*C. atlantica, J. oxycedrus* and *J. phoenicea*). It has intermediate intransitivity as 50% of the species interact transitively. **D**) Example of persistent assemblage with full intransitivity. **E**) One of the eight assemblages that are feasible but not stable according to the eigenvalue analysis, but nevertheless allow the persistence of all species through a successional cycle (note the longer time axis required to evidence the cycles). **F**) The only assemblage that is I_c positive but full intransitive and allows species persistence through heteroclinic cycles.

Discussion

Basic evidence for a role of PSF on plant community dynamics involves demonstrating that plants perform differently in soil conditioned by conspecifics than in soil conditioned by other species (Bever *et al.* 1997; Van der Putten *et al.* 2013). Our results show that recruitment success varied depending on the soil-conditioning species, reflecting the existence of species-specific PSFs in the studied community. Nevertheless, we found species-specific PSFs of moderate strength between the ten studied species, with an average strength close to zero. This agrees with the findings of weaker PSFs in woody than in herb species in Kulmatiski *et al.* (2008) and Lekberg *et al.* (2018). Moreover, experiments conducted with naturally-conditioned soils tend to obtain weaker PSFs than those using experimentally-trained soils (Brandt *et al.* 2013; Lekberg *et al.* 2018; Klinerová & Dostál 2020). In any case, it is not known how large, small or variable PSFs must be to actually have an impact on real plant communities. One way to discern the participation of PSFs in the dynamics of natural plant communities consists in contrasting experimental results against patterns observed in the field. A correspondence between experimental estimates of PSFs and observed demographic properties has been found in several studies (Klironomos 2002; Mangan *et al.* 2010; Bennet *et al.* 2017). In woody plants, Mangan *et al.* (2010) found a negative correlation between their experimental estimates of PSFs and the observed abundance of tree species in a tropical forest; similarly, Bennett *et al.* (2017) found that their experimental estimates of PSFs were positively related with the strength of negative density-dependent effects in temperate trees. We found that recruitment success in pairwise experiments was positively related with the recruitment efficiency of the same pairs of woody

species observed in the field, clearly suggesting that recruitment in Mediterranean forests is influenced by species-specific PSFs. Thus, our study provides further evidence that PSFs measured under controlled experimental conditions contribute to explain demographic processes that occur under natural conditions in forest ecosystems.

Studies on coexistence mediated by PSFs have focused on the stabilizing effect of negative PSFs that prevent dominance of abundant species while favouring rare ones (Bever *et al.* 1997; Crawford *et al.* 2019). However, modern coexistence theory applied to PSFs (Kandlikar *et al.* 2019) indicates the need to check the magnitude of SD against FD to predict how PSFs would affect the outcome of competition between pairs of species in the absence of other interaction mechanisms (e.g. facilitation). We found similar magnitudes of SD and FD, consistent with a predominance of competitive exclusion, followed by priority effects and coexistence. Similar magnitude of FD and SD were found in the meta-analysis of Yan *et al.* (2022) for those studies that, like ours, used a live reference soil and field-trained soil sources. Moreover, a predominance of pairwise competitive exclusion was predicted in empirical studies with co-occurring annual plants in central European grasslands (Klinerová & Dostál 2020) and southern California grasslands (Kandlikar *et al.* 2021). This suggests that the fitness advantages generated by PSFs should most often overcome their (de)stabilizing effect, preventing species coexistence and thus, decreasing diversity (Chu & Adler 2015).

However, predictions at pairwise-level do not necessarily explain the coexistence of species in natural ecosystems (Barabás *et al.* 2016). Recently, Miller *et al.* (2022) showed that coexistence in the classic PSF model becomes unstable in systems including more than two species. Eppinga *et al.* (2018) developed a

community-level extension of Bever's model that allows the stable coexistence of multiple species. Moreover, even when the coexistence equilibrium is unstable, negative community-level feedback may enable the persistence of all species through persistent cycles (e.g. their SI Fig S4). However, although the E-model allows for the existence of stable cycles, the parameter space of σ values that lead to such cycles is likely very restricted, and our results show that coexistence in our study system more likely involves heteroclinic than stable cycles (May & Leonard 1975). Our results add evidence on this possibility since we found that some fully intransitive subcommunities may coexist through heteroclinic cycles, even when the eigenvalue analysis deemed them as unstable, and even when the community-level feedback was positive. Still, the proportion of subcommunities that could persist in this way was small and most often involved a small number of species. This suggests that PSFs may impede the building up of highly diverse communities due to the high probability that newcoming species will join the network as transients. In this way, it is possible that PSFs could be acting as strong filters against the invasion of local assemblages by new species (Inderjit & Cahill, 2015).

In any case, our results indicate that, although dynamics driven exclusively by PSFs have the potential to reach feasible equilibrium (84% assemblages are feasible), this would seldom happen because such equilibrium is most often unstable. The final state in most assemblages would involve some extinctions depending on the initial state, in a multispecies version of priority effects. This general conclusion is at odds with the observation that the ten studied species actually co-occur very often in natural habitats. Therefore, although PSFs are at play in determining the outcome of plant-plant interactions during recruitment, other mechanisms must be considered to understand species

coexistence, like trade-offs between colonization and competition (Kisdi & Geritz 2003), facilitation between competitors (Gross 2008) or the action of specialist aerial antagonists (Janzen, 1970). Nevertheless, the role of PSFs could still be important in combination with some of these mechanisms. For example, facilitation between plants can result in a positive PSF between them (Ashton *et al.* 2008). On the other hand, since PSFs seem to favour priority effects, a strong spatial structuring of the communities in the landscape could allow the persistence of diverse species if different patches were colonized by different sets of species, or if these arrived in different sequences (Fukami 2015; Song *et al.* 2021). Studies are needed exploring the spatial environmental gradients and landscape-scale patterns structuring the biotic communities involved in PSFs, and addressing the possibility that PSFs could give rise to multiple alternative states, thus favouring the persistence of plant species diversity at ecosystem level (Van Nuland *et al.* 2017, Senthilnathan, A. & D'Andrea, R., 2023).

Besides exploring the possible roles of PSFs on multispecies coexistence, our study provides an example of how an approach to network topology can help to understand species persistence in dynamic models. By applying this approach to the results of the E-model, we found that PSFs would often give rise to moderate levels of intransitivity. Full intransitivity was rare in the studied community and would occur mainly in the smallest subcommunities. Nevertheless, full intransitivity is not a necessary condition for persistence in this community since 2.26% (22 out of 972) of subcommunities that were not fully intransitive were persistent. On the other hand, all fully intransitive subcommunities were persistent through either stable or heteroclinic dynamics. Therefore, although PSFs do not seem to promote it, full intransitivity is a sufficient condition for

species coexistence.

Data accessibility statement

The dataset and code have been deposited at Zenodo Repository. (https://doi.org/10.5281/zenodo.10519824)

Supporting information

Appendix S1 Expanded data analysis and methods: Appendix S1.1 (species-specific feedbacks); Appendix S1.2 (Pairwise-level feedback); Appendix S1.3 (Community-level effects of plant-soil feedbacks); Appendix S1.4 (List of software used in the study).

Resultados generales

Resultados generales

Los resultados correspondientes al estudio de las interacciones entre las plantas y los hongos de la filosfera y sus efectos en el reclutamiento. Este bloque aborda los factores que condicionan la colonización y el ensamblaje de las comunidades de hongos de la filosfera (**Capítulo 1**) y explora el efecto de los distintos grupos funcionales de hongos en las interacciones de reclutamiento en las comunidades naturales (**Capítulo 2**). El segundo bloque incluye los resultados del experimento de retroalimentación planta-suelo (PSF) y sus efectos en el reclutamiento y la coexistencia de las especies en comunidades naturales. Este bloque explora el efecto de las interacciones planta-suelo en el reclutamiento y aborda diferentes enfoques para predecir el resultado de las interacciones mediadas por PSFs en la coexistencia de las especies (**Capítulo 3**).

La filosfera

Los resultados indican que las comunidades de hongos se diferencian entre especies de planta en todos los grupos funcionales (descomponedores, epífitos y patógenos), incluso teniendo en cuenta la variación espacial entre comunidades de plantas (Sierra Sur de Jaén o Sierra de Segura). Hay evidencia científica sobre la influencia de los rasgos funcionales de las hojas o la distancia filogenética entre plantas en el ensamblaje de determinados grupos funcionales (Kembel & Mueller 2014; González-Teuber *et al.* 2021). Nuestros resultados sugieren que la distancia filogenética y un conjunto de rasgos foliares comunes actúan como filtros bióticos que operan sobre todos los hongos, independientemente de su gremio ecológico. Plantas cuyas

hojas tienen el mismo tipo de indumento foliar y más cercanas filogenéticamente tienden a hospedar comunidades de hongos más parecidas entre sí. Además, tanto los epífitos como los descomponedores también se ven afectados por la longevidad de las hojas.

Aunque el ensamblaje de las comunidades fúngicas de la filosfera se base en una respuesta común a ciertos rasgos de la hoja, otros rasgos que implican características químicas y morfológicas de la hoja tienen efectos particulares en la colonización de cada gremio funcional. Especies de plantas que difieren en el contenido de agua en las hojas hospedan comunidades de patógenos distintas, mientras que plantas con distinto contenido de carbono en la hoja difieren en su comunidad de descomponedores. Además, la diferenciación entre plantas en las comunidades de hongos epífitos está relacionada con la variación en la masa foliar por unidad de área. Finalmente, los resultados indican que las comunidades de hongos se estructuran modularmente, con una señal filogenética en los tres grupos funcionales. La agrupación de especies vegetales en módulos con una composición similar de las comunidades fúngicas muestra la señal de la distancia filogenética entre las especies de plantas (**Capítulo 1**).

Los análisis sobre el papel de distintos grupos funcionales de hongos en el reclutamiento revelaron que tanto saprótrofos, epífitos o patógenos pueden modular las interacciones adulto-recluta. Los resultados indican que tanto los patógenos como los saprótrofos foliares tienen un efecto negativo en las interacciones planta-planta durante el reclutamiento, de forma que plantas con comunidades fúngicas de estos grupos funcionales más parecidas entre sí, se reclutan con menor frecuencia una junto a la otra o tienen menor probabilidad de interactuar. Por el contrario, los hongos epífitos influyen positivamente en el reclutamiento, ya que las plantas que comparten comunidades fúngicas epífitas

más similares tienen una mayor probabilidad de ser reclutadas unas bajo otras.

Adicionalmente, los análisis sobre el efecto de los hongos de la filosfera en distintos aspectos del reclutamiento indican que la contribución de las plantas adultas al banco de reclutas es mayor en especies con más riqueza de epífitos y más diversidad de saprótrofos foliares. En general, los resultados sugieren que los hongos de la filosfera juegan un papel relevante en el ensamblaje del banco de reclutas en comunidades de leñosas mediterráneas, influyendo potencialmente en la dinámica de la comunidad vegetal. Los patógenos foliares presentan un efecto negativo sobre el reclutamiento, los epífitos actúan como mutualistas, y los saprótrofos tienen un rol dual disminuyendo las interacciones adulto-recluta, pero también potenciando la contribución al banco de reclutas (**Capítulo 2**).

Retroalimentación planta-suelo - *Plant-soil feedbacks*

Los resultados del experimento de PSF demostraron que las plantas se desempeñan de manera diferente en suelos condicionados por conespecíficos comparado con su desempeño en suelos de otras especies, indicando la existencia de PSF "apareados". Además, el éxito de reclutamiento obtenido experimentalmente se relacionó con la eficiencia de reclutamiento de los mismos pares de especies observada en el medio natural, evidenciando el rol de los PSFs en el proceso de reclutamiento del bosque mediterráneo.

Para los análisis del PSF a nivel de pares de especies, la aplicación de la teoría de la coexistencia moderna permitió determinar los efectos estabilizadores y ecualizadores impulsados por el PSF. La exclusión competitiva fue el resultado predicho más frecuente, seguido por los efectos de prioridad, siendo la coexistencia entre pares de especies el resultado menos frecuente (40%, 35.56% y

24.4% respectivamente).

Para los análisis del PSF a nivel de comunidad, usamos la extensión del modelo de Bever *et al*. (1997) desarrollado por Eppinga *et al.* (2018) (*E-model*). El *E-model* indicó que la comunidad formada por las diez especies estudiadas era factible pero no estable en el tiempo. Además, los análisis de la estructura topológica de la red aplicados al *E-model* revelaron que la comunidad de las 10 especies estudiadas presentaba una baja intransitividad, y por lo tanto no era persistente en el tiempo. Los análisis de las subcomunidades obtenidas combinando las 10 especies estudiadas indicaron que los PSFs daban lugar a niveles moderados de intransitividad, y que la intransitividad total era infrecuente y focalizada en subcomunidades de pocas especies. Además, los resultados sugieren que la intransitividad no parece ser una condición necesaria para la coexistencia, ya que subcomunidades parcialmente intransitivas persistían según la parametrización del *E-model*. Sin embargo, a pesar de que las comunidades totalmente intransitivas fueran escasas y compuestas de pocas especies, todos los ensamblajes totalmente intransitivos persistieron a través de dinámicas estables o heterociclos, permitiendo la coexistencia. Así, nuestros resultados indican que una intransitividad total siempre permite la coexistencia de especies, y la falta de intransitividad total casi siempre está asociada a la falta de persistencia de al menos una especie.

Discusión y conclusiones

Discusión general

Las interacciones entre especies son uno de los principales motores de la dinámica de la comunidad, y su estudio mediante redes ecológicas complejas puede aportar información sobre cómo se dan soporte unas especies a otras y cómo cambian las comunidades con el tiempo. El resultado de las interacciones planta-planta durante el reclutamiento depende a su vez de múltiples interacciones, tanto directas (p.ej. competencia por recursos) como indirectas (dispersión o depredación de semillas, herbivoría, organismos antagonistas y mutualistas). Los tres capítulos principales de esta tesis reflejan tres niveles distintos de la complejidad de las interacciones:

1. El nivel inferior corresponde a las interacciones directas planta-hongo de la filosfera, que tienen lugar en una escala espacial de pocos centímetros en las hojas.

2. El ensamblado de múltiples interacciones directas e indirectas da lugar a un nivel superior de complejidad que caracteriza las relaciones planta-planta durante el reclutamiento y que se desarrolla en una escala espacial de pocos metros entre individuos.

3. Por último, la agregación de múltiples interacciones planta-planta da lugar a las redes de reclutamiento que conforman el nivel superior de complejidad y que refleja procesos que ocurren a escala espacial de la comunidad local.

La integración del conocimiento a estas tres escalas aporta nuevas evidencias sobre los factores que contribuyen a la coexistencia y el funcionamiento de las comunidades vegetales naturales.

¿Retroalimentación filosfera-microbioma?

Hay cada vez más evidencias sobre el papel que desempeñan los microorganismos que habitan la filosfera en el fitness de la planta huésped (Ritpitakphong *et al.* 2016, Fürnkranz *et al.* 2008) y en diversas funciones ecosistémicas (Rodríguez *et al.* 2009, Laforest-Lapointe *et al.* 2017, Chen *et al.* 2020, Perreault & Laforest-Lapointe 2022). Sin embargo, la microbiota de la filosfera apenas se ha integrado en los estudios de ecología de comunidades (Vatcher *et al.* 2016). En esta tesis, se ha demostrado su función potencial en la dinámica de la comunidad (**Capítulo 2**), pero también se ha proporcionado una descripción general integral de las comunidades de hongos que habitan las hojas desde una perspectiva ecológica (**Capítulo 1**).

Algunos estudios afirman que la filosfera es un sistema particularmente adecuado para testar conceptos ecológicos (Meyer & Leveau 2012, Nix-Sthor *et al.* 2008, Redford *et al.* 2010). Por ejemplo, es un hábitat con una alta heterogeneidad espacio-temporal en cuanto a la disponibilidad y concentración de nutrientes (Leveau & Lindow 2001) y en su topografía (Mechaber *et al.* 1996). Esta variabilidad depende de diversos factores como la especie de planta o distintos rasgos funcionales de las hojas, y condicionará el proceso de colonización microbiana (Andrews & Harris 2000). El antiguo principio en microbiología *everything is everywhere, but the environment selects* (todo está en todas partes, pero el ambiente selecciona) (Becking 1934) ha impulsado el uso teorías ecológicas como la teoría de biogeografía de islas de MacArthur & Wilson (1967) para describir el proceso de ensamblaje del microbioma foliar. Por ejemplo, Kinkel *et al.* (1987) usaron este enfoque para describir la estabilidad de las comunidades microbianas de la filosfera, aunque no encontraron relación con el tamaño de la hoja. Sin

embargo, con el desarrollo de nuevas técnicas de secuenciación masiva, otros estudios han encontrado correlaciones entre la estructura de la comunidad microbiana y los rasgos funcionales de las hojas. Por ejemplo, Kembel & Mueller (2014) encontraron varios rasgos morfológicos y químicos de las hojas asociados a la estructura del microbioma fúngico en árboles de bosque tropical y Flessa *et al.* (2012) encontraron distintas comunidades epifitas en hojas caducas y hojas perennes en árboles de bosque templado. Los resultados obtenidos en el **Capítulo 1** muestran rasgos funcionales de las hojas asociados a cada gremio de hongo específicamente, pero también un conjunto de rasgos que afectan a toda la comunidad fúngica. Desde un punto de vista ecológico, se puede interpretar los rasgos funcionales de las hojas como filtros bióticos que seleccionan ciertos gremios o taxones, condicionando la colonización y ensamblaje de las comunidades microbianas de la filosfera (Meyer & Leveau 2012). Estudiar estos filtros puede permitir, por ejemplo, determinar estructuras que resistan la invasión de patógenos vegetales en un entorno tan variable como la filosfera.

Hay estudios que proponen otros marcos conceptuales, como investigar la potencial alteración de los rasgos funcionales de la planta por parte del microbioma (*microbially-mediated functional traits*, Friesen *et al.* 2011; Rosado *et al.* 2018) y su consecuente efecto en funciones ecosistémicas. Los rasgos funcionales pueden influir en distintos procesos ecosistémicos, como por ejemplo en la descomposición de la hojarasca según el nitrógeno de las hojas (Cornwell *et al.* 2008) o en el reclutamiento según la similitud de rasgos funcionales entre adultos y reclutas (Navarro-Cano *et al.* 2020). En nuestro sistema de estudio, Perea *et al.* (2021a) demostraron que, aunque la complementariedad de rasgos funcionales no estaba relacionada con la frecuencia de interacciones adulto-recluta, plantas con más masa foliar por

unidad de área o con hojas más longevas tenían un mayor banco de reclutas. Los resultados obtenidos en el **Capítulo 2** muestran la influencia de las comunidades de hongos de la filosfera sobre las interacciones adulto-recluta, pero también en aspectos del reclutamiento como la abundancia del banco de reclutas. Por ejemplo, árboles con más diversidad de saprótrofos en sus hojas facilitan un banco de reclutas abundante, lo que sugiere que los saprótrofos podrían promover el proceso de descomposición una vez las hojas forman parte de del suelo. Sería interesante estudiar si los efectos del microbioma foliar en la función ecosistémica del reclutamiento se retroalimentan a través de la modificación previa de los rasgos foliares de las plantas. Sin embargo, el primer paso es estudiar el efecto potencial de los microrganismos sobre los rasgos de las plantas tanto a escala ecológica como evolutiva (Friesen *et al.* 2011).

¿Quién dirige el reclutamiento?

Comprender los mecanismos que mantienen la biodiversidad y las funciones ecosistémicas de los bosques presenta importantes retos, como la falta de información sobre múltiples aspectos de la historia vital de la mayoría de especies, particularmente las no comerciales, o la complejidad inherente a los modelos empleados para estimar la dinámica de especies longevas en comunidades naturales. Aunque se suele destacar la importancia de la supervivencia (mortalidad) o el crecimiento (Pfister 1998; Franco & Silvertown 2000), los estudios a nivel de comunidad remarcan la contribución del reclutamiento en la coexistencia de las especies (Grubb 1977, Pacala *et al.* 1996, Adler *et al.* 2010, Rüger *et al.* 2020). El reclutamiento es un proceso demográfico que abarca distintas etapas del ciclo vital de las plantas, desde la producción y dispersión de semillas hasta la supervivencia de las plántulas

y el establecimiento de los juveniles. Dado que en cada estadio vital pueden operar distintos mecanismos estabilizadores, el reclutamiento puede contribuir incluso más que la supervivencia o el crecimiento a la coexistencia (Moll & Brown 2008, Chu & Adler *et al.* 2015). Por ejemplo, varios estudios han demostrado la contribución de procesos focalizados en el reclutamiento como la retroalimentación planta-suelo (Mangan *et al.* 2010; Brandt *et al.* 2013; Dudenhöffer *et al.* 2018) o el efecto de patógenos especializados (Janzen-Connell effects; Freckleton & Lewis 2006; Comita *et al.* 2010, Bagchi *et al.* 2014) en la estructura de la comunidad y la coexistencia de las especies.

Todos estos mecanismos se basan en procesos de mortalidad negativamente denso-dependientes, pero la mayoría de estudios solamente tienen en cuenta el efecto de la densidad de conespecíficos. Sin embargo, la diversidad inherente a las comunidades naturales hace inevitable la existencia de multitud de interacciones interespecíficas entre plantas, que han de tenerse en cuenta para comprender mejor el funcionamiento de estas comunidades. Los resultados de esta tesis demuestran que tanto el microbioma fúngico de las hojas como los procesos de retroalimentación planta-suelo, participan en la determinación del resultado de las interacciones interespecíficas planta-planta (**Capítulo 2 y 3**). En el **Capítulo 2** mostramos que existe un efecto negativo de los hongos patógenos de las hojas variable entre pares de especies. Aunque los patógenos de la filosfera presentan una baja especialización, estudios recientes apuntan que incluso enemigos generalistas pueden tener efectos específicos dependientes del contexto en cada huésped (Shemenko *et al.* 2022). Aun así, nuestros resultados sugieren que los efectos denso-dependientes de los patógenos podrían deberse no solo a la densidad de individuos de una misma especie de planta, sino también a la de individuos de plantas que hospedan comunidades

patogénicas similares. También en este capítulo observamos un efecto mutualista de los epífitos foliares contribuyendo al ensamblaje de interacciones adulto-recluta. Más aún, los resultados del experimento de retroalimentación planta-suelo en el **Capítulo 3** indican que el éxito de reclutamiento depende de la especie de planta que condiciona el suelo y de la especie que se recluta en el suelo modificado. En conclusión, esta tesis aporta evidencias sobre cómo la especificidad de las interacciones planta-planta, mediada por similitud de los hongos antagonistas de la filosfera y procesos de retroalimentación planta-suelo, puede contribuir a la demografía de los ecosistemas forestales mediterráneos al afectar a la dinámica de reclutamiento en la comunidad. En el futuro, será interesante investigar sobre marcos teóricos que tengan en cuenta el efecto conjunto de todas las interacciones (antagonistas y mutualistas) en la dinámica de la comunidad y la coexistencia de las especies (Bachelot *et al.* 2015).

¿La complejidad de las interacciones puede dar lugar a estados alternativos?

Los estudios sobre coexistencia suelen desarrollarse en comunidades diversas, pero las predicciones tienden a evaluarse entre pares de especies (Kandlikar 2024). Si bien los análisis por pares proporcionan información relevante, interpretar los resultados en sistemas de múltiples especies puede llevar a conclusiones erróneas (Levine *et al.* 2017, Barabás *et al.* 2016). Los resultados del **Capítulo 3** muestran que los PSFs frecuentemente generan diferencias de fitness que superan los efectos (des)estabilizadores, impidiendo la coexistencia entre pares de especies. En consonancia con otros estudios que aplican el mismo procedimiento analítico (Kandlikar *et al.* 2021, Yan *et al.* 2022), la prevalencia de exclusión competitiva contrasta con

la aparente coexistencia de especies en ecosistemas naturales. De hecho, Miller *et al.* (2022) demostró que el modelo clásico de PSF formulado por Bever *et al.* (1997) imposibilitaba la coexistencia estable en sistemas de más de dos especies. Así, estudios recientes han propuesto distintos marcos analíticos que pueden modificar los resultados obtenidos para pares de especies por separado (Eppinga *et al.* 2018, Ke & Wan 2020, 2023, Kandlikar *et al.* 2021). En el **Capítulo 3** observamos que las dinámicas derivadas de los PSFs pueden dar lugar a distintos ensamblajes dependiendo de las condiciones iniciales de abundancia de las especies. Esto puede interpretarse en términos de efectos de prioridad que permiten la persistencia de conjuntos de especies reclutadas en distintos parches espaciales o que colonizan el espacio secuencialmente (Fukami *et al.* 2015). La intransitividad de las interacciones planta-planta generadas por PSFs es un factor importante en la determinación de cuales de esos subconjuntos de especies son estables. En un futuro sería interesante investigar cómo distintas características del paisaje interactúan con los PSFs y condicionan la persistencia o la diversidad de la comunidad mediante la generación de múltiples estados alternativos (Senthilnathan & D'Andrea, 2023).

Conclusions

1. Leaf-inhabiting fungi with contrasting relationships with the plant (decomposers, epiphytes and pathogens) respond similarly to a common set of leaf traits that impose physical limitations to their assembly, which can be interpreted as biotic filters operating on the species pool. Plants with similar indumentum tend to host similar fungal communities of decomposers, epiphytes and pathogens. Furthermore, both epiphytes and decomposers are also affected by leaf habit.

2. Different leaf functional traits related to their morphological and chemical characteristics have specific effects on the colonization of each functional guild. Saprotrophic fungi were conditioned by leaf carbon content, pathogenic fungi were related with leaf water content and epiphytes with leaf mass per area.

3. The biotic filter imposed by the plants gives rise to a modular structure in the plant-fungus network, where phylogenetically close plants tend to share more fungi compared to distantly related plants belonging to different modules.

4. Phyllosphere fungi play a relevant role in the assembly of canopy-recruit interactions and contribute to structure the forest sapling bank in natural communities, thus, having the potential to influence plant community dynamics.

5. Pathogenic and saprotrophic phyllosphere fungi impose a pairwise-specific filter on recruitment that can be potentially counteracted by a pairwise mutualistic effect of epiphytic

fungi. Furthermore, the presence of canopy species hosting high richness of epiphytes or high diversity of saprotrophs favours the formation of an abundant sapling bank.

6. The recruitment success of plants varies depending on the soil-conditioning species, which demonstrates the existence of species-specific plant-soil feedbacks (PSFs) in the studied community. Furthermore, the positive correlation between the experimental and the observed recruitment success of the same species pairs in the wild suggest that recruitment in Mediterranean forests is influenced by species-specific PSFs.

7. Pairwise PSFs generate fitness advantages that tend to overcome their (des)stabilizing effects, ultimately preventing coexistence and decreasing species diversity.

8. The approach to network topology in dynamic models demonstrates that full intransitivity driven by PSFs is rare but a sufficient condition for species coexistence.

Información Suplementaria

Información suplementaria Capítulo 1

Pajares-Murgó, M., Garrido, J. L., Perea, A. J., López-García, Á., & Alcántara, J. M. (2023). Biotic filters driving the differentiation of decomposer, epiphytic and pathogenic phyllosphere fungi across plant species. *Oikos*, 2023(5), e09624.

Appendix S1 List of species

Plant species	Jaén	Segura
Acer granatensis		X
Acer monspessulanum	X	
Amelanchier ovalis	X	X
Berberis hispanica		X
Cistus albidus	X	X
Cistus salvifolius	X	X
Crataegus laciniata		X
Crataegus monogyna	X	X
Cytisus scoparius		X
Daphne gnidium	X	
Daphne laureola		X
Genista cinerea	X	
Juniperus communis		X
Juniperus oxycedrus	X	
Juniperus phoenicea	X	
Lavandula latifolia	X	

Phillyrea angustifolia	X	
Phillyrea latifolia	X	
Phlomis purpurea	X	
Pinus halepensis	X	
Pinus nigra		X
Pistacia lentiscus	X	
Pistacia terebinthus	X	
Prunus spinosa		X
Quercus coccifera	X	
Quercus faginea	X	X
Quercus ilex	X	X
Quercus pyrenaica		X
Rhamnus lycioides	X	
Rosa sp. Jaén	X	
Rosa sp. Segura		X
Rosmarinus officinalis		
Ruscus aculeatus	X	
Sorbus torminalis	X	
Thymus mastichina		X
Thymus zygis	X	X
Ulex parviflorus	X	X
Viburnum tinus	X	

Appendix S2 DNA extraction and sequencing analyses

DNA isolation

DNA was isolated from the dry leaf material using the ISOLATE II Plant DNA kit (BioLine), strictly following the manufacturer's instructions. 25 mg of dry leaf tissue from healthy and infected individuals was put into a tube along with stainless-steel beads, and ground into a fine powder using the TissueLyser (Qiagen). That powder was used as input for the ISOLATE II Plant DNA kit. The DNA was finally eluted in a volume of 50 µL. Negative controls that contained no tissue were included in every DNA isolation round to check for cross-contamination. DNA was quantified using the Qubit High Sensitivity dsDNA Assay (Thermo Fisher Scientific).

Library preparation and sequencing

For library preparation, a 250 bp fragment of the ITS genomic region was amplified using the primers ITS3 (5' CAHCGATGAAGAACGYRG 3') and ITS4 (5' TCCTSCGCTTATTGATATGC 3') (Tedersoo *et al.* 2014). These primers included the Illumina sequencing primer sequences attached to their 5' ends. PCRs were carried out in a final volume of 25 µL, containing 2.5 µL of template DNA, 0.5 µM of the primers, 12.5 µL of Supreme NZYTaq 2x Green Master Mix (NZYTech), and ultrapure water up to 25 µL. The reaction mixture was incubated as follows: an initial denaturation step at 95 ºC for 5 min, followed by 35 cycles of 95 ºC for 30 s, 49 ºC for 45 s, 72 ºC for 30 s, and a final extension step at 72 ºC for 10 min. Negative controls that contained no DNA (BPCR) were included in every PCR round to check for contamination

during library preparation. The oligonucleotide indices which are required for multiplexing different libraries in the same sequencing pool were attached in a second PCR round with identical conditions but only 5 cycles and 60 ºC as the annealing temperature. For a schematic overview of the library preparation process, please see Figure 1 in Vierna *et al.* (2017). The libraries were run on 2 % agarose gels stained with GreenSafe (NZYTech), and imaged under UV light to verify the library size. Libraries were purified using the Mag-Bind RXNPure Plus magnetic beads (Omega Biotek), following the instructions provided by the manufacturer. Then, they were pooled in equimolar amounts according to the quantification data provided by the Qubit dsDNA HS Assay (Thermo Fisher Scientific). This pool also contained a testimonial amount (1 µL) of each of the negative controls (both the extraction negative controls and the PCR negative controls). The pool was sequenced in a fraction of a NovaSeq PE250 run (Illumina) aiming for a total output of 20 gigabases. Illumina paired-end raw files consist of forward (R1) and reverse (R2) reads sorted by library and their quality scores. The indices and sequencing primers are trimmed during the demultiplexing step.

Bioinformatic analysis

Briefly, forward and reverse primers were removed using cutadapt. Forward and reverse sequences were quality filtered to allow as maximum two expected errors per read. Sequences were dereplicated and the rate error model was inferred and used to implement the sample inference algorithm. Forward and reverse reads were merged and chimeric sequences removed, resulting in 18668 ASVs from 32317404 reads. Afterwards, LULU curation tool (Frøslev *et al.* 2017) was used to remove further sequencing

errors. To correct possible mistagging, ASVs present in less than 0.01% of reads in a sequenced sample were discarded (Bokulich *et al.* 2013).

Taxonomic assignment was determined for each ASV against the UNITE database v. 8.2. (Abarenkov *et al.* 2020), complemented with representative sequences of the plant genera involved in the study, using the RDP algorithm implemented in DADA2 pipeline. ASVs non assigned at a fungal phylum level were discarded. Then, 2968 ASVs were retained representing 7173784 reads.

In order to get Operational Taxonomic Units (OTUs) that resemble species level (see, e.g. Tedersoo *et al.* 2012), ASVs were clustered into 97% sequence similarity using VSEARCH v. 2.8.1 (Rognes *et al.* 2016) to finally obtain 1462 OTUs (Table S2).

Fungal functional guilds were determined by matching OTUs' assigned genera and the genus-guild database FungalTraits (Põlme *et al.* 2020). A recent comparison by Tanunchai *et al.* (2022) found it better than FUNGuild (Nguyen *et al.* 2016) in assigning a higher quantity and quality of OTUs. We assigned each OTU to a primary functional guild at genus level. From this analysis, 812 OTUs (73.8 % of reads) were assigned to a primary-lifestyle (Põlme *et al.* 2020).

Sequencing depth was evaluated by representing the detected OTUs per number of reads. Additionally, to evaluate our sampling completeness we built sampling effort curves (i.e. OTUs detected per number of samples by site). This analysis was also performed for each fungal guild (Fig. S3). These analyses were conducted using *iNEXT* R package v. 2.0.20 (Hsieh *et al.* 2020).

Table S2. Bioinformatic analyses obtained from the sequencing.

	Reads	% Reads	OTUs	% OTUs
Total	7173784		1462	
Identified to species	4029093	56.16	480	32.83
Identified to genus	5296971	73.84	812	55.54
Identified to family	5491723	76.55	992	67.85
Identified to order	5601623	78.08	1185	81.05
Identified guild	5296946	73.84	811	55.47

References

Abarenkov, K., *et al.* 2020. UNITE general FASTA release for Fungi. Version 04.02.2020. UNITE Community.

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Rognes, T. *et al.* 2016. VSEARCH: A versatile open-source tool for metagenomics. *PeerJ,* 2016(10).

Põlme, S. *et al.* 2020. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers., 105*(1).

Hsieh T. H. *et al.* 2020. iNEXT: iNterpolation and EXTrapolation for species diversity. R package version 2.0.20.

Tanunchai, B. *et al.* 2022. FungalTraits vs. FUNGuild: Comparison of Ecological Functional Assignments of Leaf-

and Needle-Associated Fungi Across 12 Temperate Tree Species. *Microb. Ecol.*, 1-18.

Tedersoo, L. *et al.* 2014. Global diversity and geography of soil fungi. *Science, 346*(6213).

Appendix S3 Rarefaction curves and sample coverage

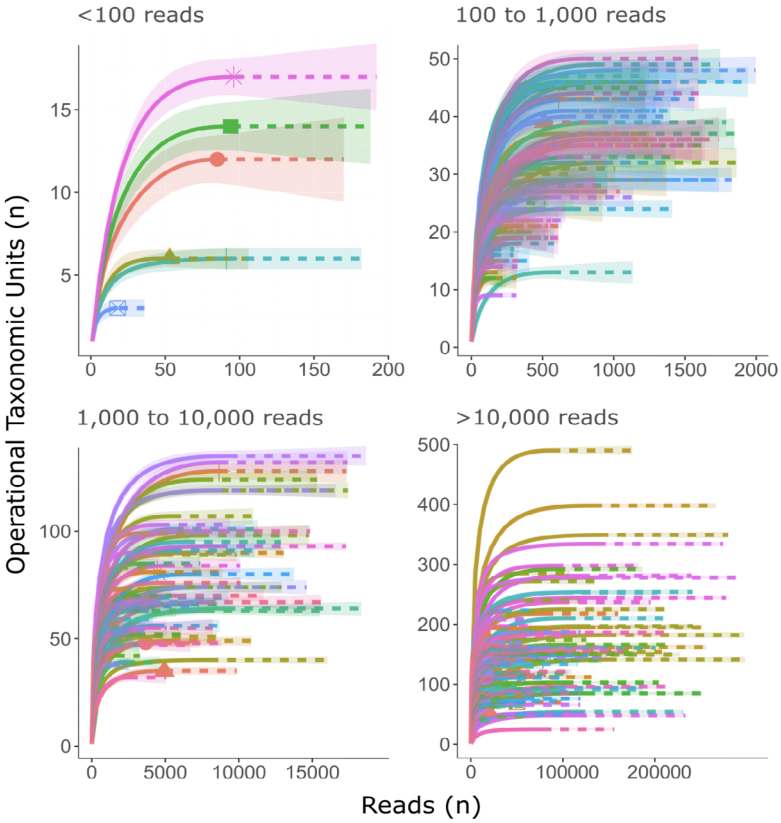

Figure S3.1 Rarefaction analysis of the number of fungal Operational Taxonomic Units (OTUs) by number of reads per sequenced sample. Rarefaction curves were obtained with *iNEXT* R package. Continuous lines indicate the reached sequencing depth and dashed lines indicate the extrapolated values of OTU number. Confidence intervals are indicated as coloured shadows. The analysis is plotted by four different sequencing depths (indicated in each panel) to improve readability. Every extrapolation indicated, at least, 99.9% of sample coverage.

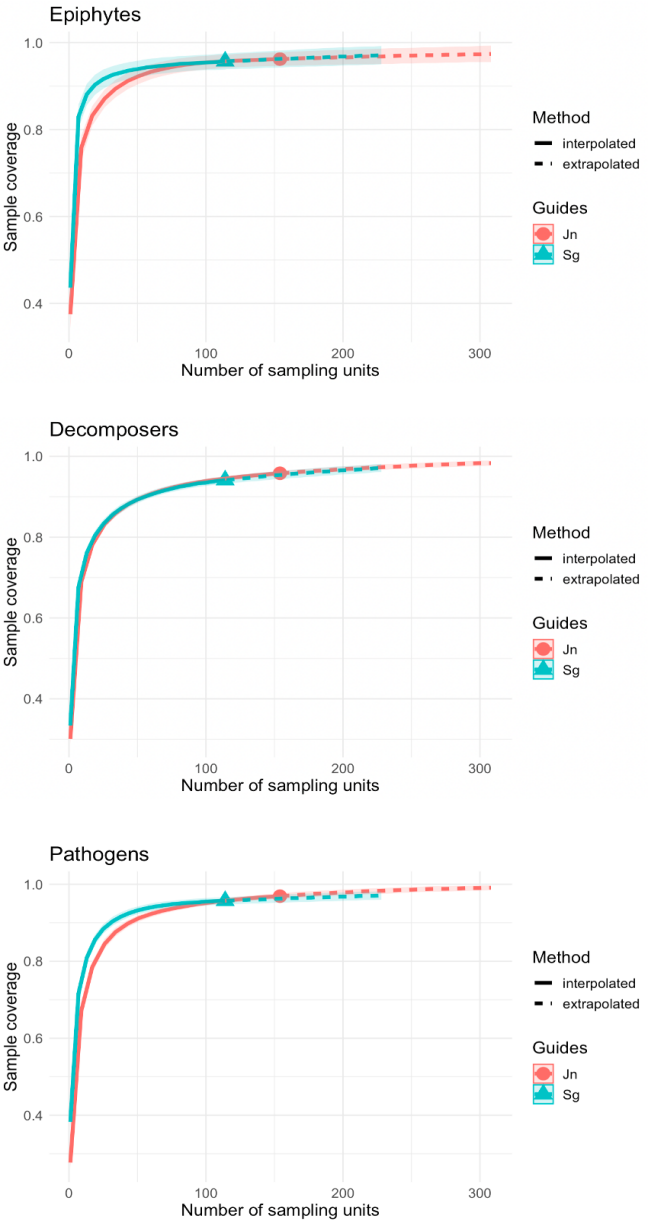
Epiphytes
Sample coverage
1.0
0.8
0.6
0.4
0
100
200
300
Number of sampling units
Method
interpolated
extrapolated
Guides
Jn
Sg
Decomposers
Sample coverage
1.0
0.8
0.6
0.4
0
100
200
300
Number of sampling units
Method
interpolated
extrapolated
Guides
Jn
Sg
Pathogens
Sample coverage
1.0
0.8
0.6
0.4
0
100
200
300
Number of sampling units
Method
interpolated
extrapolated
Guides
Jn
Sg

Figure S3.2 Sample coverage curve of the samples divided by guild (Epiphytes, Decomposers and Pathogens) and site (Jn = Sierra de Jaén, Sg = Sierra de Segura).

Appendix S4 Contamination filtering

Table S4.1. Analysis of general dataset contamination filtering. Results from Generalized Mixed Model testing whether the number of reads determined the legitimacy of an interaction with the plant. The contamination punctuation corresponds to the response variable. Estimates of the fixed effects are the value, plant species and the site with the variable zone as nested

Effects		Std. Error	Estimate	*Z* value	*p*
Fixed	*Intercept*	0.1204	-2.8855	-23.971	< 2e-16
	Nº reads	0.0001	-0.0010	-9.573	< 2e-16
Random	*Plant species*	0.3938	0.1551		
	Site	0.1391	0.0194		
	Zone: site	0.1079	0.0116		

Table S4.2. Probability of the number of reads in which a decomposer, epiphyte or pathogenic OTU is active on the plant host

Nº reads	Probability	SE
0	0.03648	0.00678
1	0.03647	0.00678
10	0.03637	0.00676

20	0.03626	0.00674
100	0.03538	0.00658
1000	0.02683	0.00505
2000	0.01968	0.00379
5000	0.00769	0.00169
10000	0.00158	0.00047

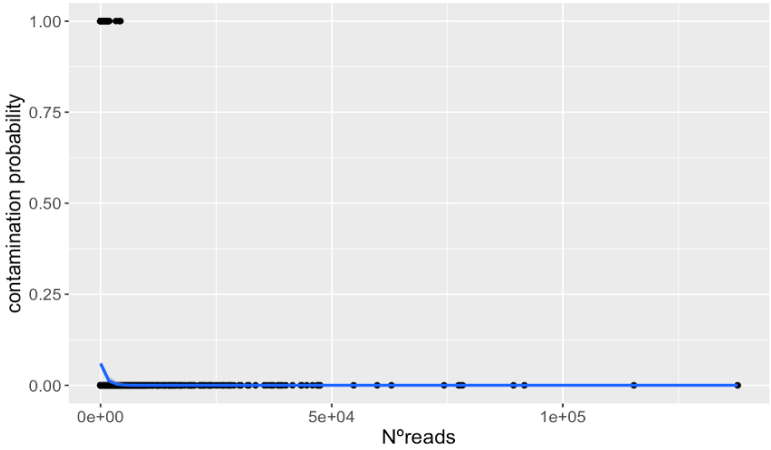

Figure S4.1. The probability of a particular OTU to be contamination event (decomposers, epiphytes and pathogens) depending on the number of reads.

Appendix S5 PERMANOVA results

Table S5. PERMANOVA with the common plant species occurring in both Jaén and Segura with the interaction of the terms plant species and site.

	Effects	Df	SS	R2	F	*p*
Decomposers	*Site*	1	0.964	0.0272	2.7999	0.001
	Plant species	7	6.227	0.17568	2.583	0.001
	Site:Plant species	7	2.081	0.05871	0.8632	0.964
	Residual	76	26.172	0.73841		
	Total	91	35.444	1		
Epiphytes	*Site*	1	0.825	0.03322	3.2082	0.079
	Plant species	7	4.0207	0.16193	2.2337	0.002
	Site:Plant species	7	2.7559	0.11099	1.5311	0.048
	Residual	67	17.2286	0.69386		
	Total	82	24.8301	1		
Pathogens	*Site*	1	1.359	0.03768	3.9204	0.001
	Plant species	7	6.884	0.19081	2.8357	0.001
	Site:Plant species	7	3.212	0.08903	1.3231	0.02
	Residual	71	24.621	0.68248		
	Total	86	36.076	1		

Appendix S6 Richness, Simpson and Shannon indices

Table S6. Mean richness of each functional guild and site

Plant species	Site	Richness decomposers	Richness epiphytes	Richness pathogens
Acer monspessulanum	Jn	19	1	12
Amelanchier ovalis	Jn	34	3	19
Cistus albidus	Jn	71	7	28
Cistus salvifolius	Jn	38	1	14
Crataegus monogyna	Jn	38	2	22
Daphne gnidium	Jn	13	1	4
Genista cinerea	Jn	36	9	8
Juniperus oxycedrus	Jn	134	10	65
Juniperus phoenicea	Jn	85	19	41
Lavandula latifolia	Jn	72	8	23
Phillyrea angustifolia	Jn	7	2	2
Phillyrea latifolia	Jn	28	6	11
Phlomis purpurea	Jn	69	8	33
Pinus halepensis	Jn	34	8	18
Pistacia lentiscus	Jn	17	5	5
Pistacia terebinthus	Jn	21	1	6
Quercus coccifera	Jn	35	3	15
Quercus faginea	Jn	21	2	17
Quercus ilex	Jn	41	12	23
Rhamnus lycioides	Jn	33	6	19
Rosa sp. Jaén	Jn	2	*NA*	*NA*

Rosmarinus officinalis	Jn	27	3	11
Ruscus aculeatus	Jn	108	10	47
Thymus mastichina	Jn	14	3	8
Thymus zygis	Jn	41	3	18
Ulex parviflorus	Jn	47	12	24
Viburnum tinus	Jn	53	9	28
Acer granatensis	Sg	19	4	11
Amelanchier ovalis	Sg	25	4	21
Berberis hispanica	Sg	28	2	19
Cistus albidus	Sg	46	9	31
Cistus salvifolius	Sg	25	4	13
Crataegus laciniata	Sg	45	6	30
Crataegus monogyna	Sg	27	5	21
Cystus scoparius	Sg	14	2	10
Daphne laureola	Sg	1	*NA*	1
Juniperus communis	Sg	121	14	64
Pinus nigra	Sg	46	13	32
Prunus spinosa	Sg	10	1	16
Quercus faginea	Sg	13	2	19
Quercus ilex	Sg	33	7	24
Quercus pyrenaica	Sg	30	6	27
Rosa sp. Segura	Sg	4	2	11
Sorbus torminalis	Sg	31	4	19
Thymus mastichina	Sg	19	4	8
Thymus zygis	Sg	43	8	28

Table S6B. Mean Simpson (Si) and Shannon (H) indices for each functional guild and site

Plant species	Site	Si dec	H dec	Si pat	H pat	Si epi	H epi
Acer monspessulanum	Jn	0.356	0.762	0.346	0.685	0.000	0.000
Amelanchier ovalis	Jn	0.498	1.086	0.340	0.717	0.139	0.212
Cistus albidus	Jn	0.728	1.825	0.591	1.333	0.028	0.077
Cistus salvifolius	Jn	0.557	1.322	0.435	0.888	0.000	0.000
Crataegus monogyna	Jn	0.690	1.642	0.516	1.153	0.014	0.031
Daphne gnidium	Jn	0.408	0.706	0.540	0.813	0.000	0.000
Genista cinerea	Jn	0.472	0.983	0.298	0.576	0.418	0.782
Juniperus oxycedrus	Jn	0.599	1.613	0.726	1.904	0.376	0.719
Juniperus phoenicea	Jn	0.631	1.636	0.493	1.091	0.670	1.382
Lavandula latifolia	Jn	0.808	2.183	0.584	1.170	0.066	0.163
Phillyrea angustifolia	Jn	0.250	0.416	0.161	0.225	0.101	0.178
Phillyrea latifolia	Jn	0.435	0.878	0.216	0.444	0.350	0.557
Phlomis purpurea	Jn	0.725	1.878	0.479	1.016	0.037	0.087
Pinus halepensis	Jn	0.686	1.663	0.386	0.788	0.270	0.531
Pistacia lentiscus	Jn	0.511	1.085	0.361	0.618	0.430	0.757
Pistacia terebinthus	Jn	0.539	1.092	0.097	0.163	0.000	0.000
Quercus coccifera	Jn	0.516	1.198	0.356	0.676	0.000	0.002
Quercus faginea	Jn	0.520	1.066	0.551	1.115	0.010	0.024
Quercus ilex	Jn	0.765	1.828	0.588	1.248	0.228	0.496
Rhamnus lycioides	Jn	0.438	1.027	0.474	0.912	0.166	0.334
Rosa sp. Jaén	Jn	0.000	0.000	*NA*	*NA*	*NA*	*NA*
Rosmarinus officinalis	Jn	0.372	0.780	0.334	0.737	0.065	0.124

Ruscus aculeatus	Jn	0.625	1.564	0.349	0.881	0.353	0.697
Thymus mastichina	Jn	0.675	1.346	0.539	0.901	0.220	0.345
Thymus zygis	Jn	0.534	1.157	0.403	0.917	0.020	0.046
Ulex parviflorus	Jn	0.578	1.460	0.612	1.369	0.404	0.923
Viburnum tinus	Jn	0.739	1.726	0.626	1.302	0.286	0.575
Acer granatensis	Sg	0.229	0.439	0.457	0.858	0.003	0.011
Amelanchier ovalis	Sg	0.527	1.131	0.338	0.746	0.339	0.521
Berberis hispanica	Sg	0.513	1.068	0.679	1.337	0.120	0.183
Cistus albidus	Sg	0.718	1.838	0.782	1.919	0.134	0.296
Cistus salvifolius	Sg	0.665	1.521	0.564	1.138	0.070	0.152
Crataegus laciniata	Sg	0.594	1.577	0.762	1.901	0.465	0.884
Crataegus monogyna	Sg	0.465	1.095	0.502	1.090	0.536	0.944
Cystus scoparius	Sg	0.410	0.668	0.127	0.215	0.017	0.043
Daphne laureola	Sg	0.000	0.000	0.000	0.000	*NA*	*NA*
Juniperus communis	Sg	0.825	2.270	0.581	1.482	0.592	1.218
Pinus nigra	Sg	0.630	1.532	0.588	1.366	0.544	1.024
Prunus spinosa	Sg	0.383	0.683	0.339	0.687	0.000	0.000
Quercus faginea	Sg	0.561	1.087	0.565	1.127	0.047	0.096
Quercus ilex	Sg	0.676	1.483	0.572	1.307	0.389	0.655
Quercus pyrenaica	Sg	0.630	1.489	0.625	1.521	0.156	0.336
Rosa sp. Segura	Sg	0.221	0.368	0.610	1.121	0.119	0.201
Sorbus torminalis	Sg	0.709	1.548	0.637	1.398	0.286	0.491
Thymus mastichina	Sg	0.748	1.599	0.507	0.974	0.359	0.563
Thymus zygis	Sg	0.627	1.565	0.681	1.522	0.209	0.365

Appendix S7 Mantel Tests

Table S7. Test de Mantel

		R	*p*	R	*p*
Jaén		*Epiphytes*		*Pathogens*	
	Decomposers	0.4196	0.001	0.4216	0.001
	Pathogens	0.3262	0.015		
Segura		*Epiphytes*		*Pathogens*	
	Decomposers	0.5456	0.002	0.348	0.025
	Pathogens	0.1113	0.25		

Información suplementaria Capítulo 2

Pajares-Murgó, M., Garrido, J. L., Perea, A. J., López-García, Á., Bastida, J. M., & Alcántara, J. M. (2024). Mutualistic and antagonistic phyllosphere fungi contribute to plant recruitment in natural communities. *Journal of Ecology*.

Appendix S1 Data description tables and figures

Table S1.1 Richness (OTU proportion) of each fungal functional guild and site (Jn = Jaén, Sg = Segura) in each plant species.

Plant species	Site	Richness saprotrophs	Richness epiphytes	Richness pathogens
Acer monspessulanum	Jn	0.064	0.031	0.089
Amelanchier ovalis	Jn	0.114	0.094	0.141
Cistus albidus	Jn	0.238	0.219	0.207
Cistus salvifolius	Jn	0.128	0.031	0.104
Crataegus monogyna	Jn	0.128	0.063	0.163
Daphne gnidium	Jn	0.044	0.031	0.030
Genista cinerea	Jn	0.121	0.281	0.059
Juniperus oxycedrus	Jn	0.450	0.313	0.481
Juniperus phoenicea	Jn	0.285	0.594	0.304
Lavandula latifolia	Jn	0.242	0.250	0.170
Phillyrea angustifolia	Jn	0.023	0.063	0.015
Phillyrea latifolia	Jn	0.094	0.188	0.081
Phlomis purpurea	Jn	0.232	0.250	0.244

Pinus halepensis	Jn	0.114	0.250	0.133
Pistacia lentiscus	Jn	0.057	0.156	0.037
Pistacia terebinthus	Jn	0.070	0.031	0.044
Quercus coccifera	Jn	0.117	0.094	0.111
Quercus faginea	Jn	0.070	0.063	0.126
Quercus ilex	Jn	0.138	0.375	0.170
Rhamnus lycioides	Jn	0.111	0.188	0.141
Rosa sp. Jaén	Jn	0.007	*NA*	*NA*
Rosmarinus officinalis	Jn	0.091	0.094	0.081
Ruscus aculeatus	Jn	0.362	0.313	0.348
Thymus mastichina	Jn	0.047	0.094	0.059
Thymus zygis	Jn	0.138	0.094	0.133
Ulex parviflorus	Jn	0.158	0.375	0.178
Viburnum tinus	Jn	0.178	0.281	0.207
Acer granatensis	Sg	0.091	0.167	0.095
Amelanchier ovalis	Sg	0.120	0.167	0.181
Berberis hispanica	Sg	0.135	0.083	0.164
Cistus albidus	Sg	0.221	0.375	0.267
Cistus salvifolius	Sg	0.120	0.167	0.112
Crataegus laciniata	Sg	0.216	0.250	0.259
Crataegus monogyna	Sg	0.130	0.208	0.181
Cystus scoparius	Sg	0.067	0.083	0.086
Daphne laureola	Sg	0.005	*NA*	0.009
Juniperus communis	Sg	0.582	0.583	0.552
Pinus nigra	Sg	0.221	0.542	0.276

Prunus spinosa	Sg	0.048	0.042	0.138
Quercus faginea	Sg	0.063	0.083	0.164
Quercus ilex	Sg	0.159	0.292	0.207
Quercus pyrenaica	Sg	0.144	0.250	0.233
Rosa sp. Segura	Sg	0.019	0.083	0.095
Sorbus torminalis	Sg	0.149	0.167	0.164
Thymus mastichina	Sg	0.091	0.167	0.069
Thymus zygis	Sg	0.207	0.333	0.241

Table S1.2 Diversity (number of effective partners) of each fungal functional guild and site (Jn = Jaén, Sg = Segura) in each plant species.

Plant species	Site	Diversity saprotrophs	Diversity epiphytes	Diversity pathogens
Acer monspessulanum	Jn	5.088	1.000	4.593
Amelanchier ovalis	Jn	11.383	2.071	9.016
Cistus albidus	Jn	15.607	1.258	11.192
Cistus salvifolius	Jn	13.881	1.000	6.675
Crataegus monogyna	Jn	15.864	1.109	10.788
Daphne gnidium	Jn	5.695	1.000	3.884
Genista cinerea	Jn	7.413	5.143	5.402
Juniperus oxycedrus	Jn	21.152	2.543	22.273
Juniperus phoenicea	Jn	17.060	11.630	8.278
Lavandula latifolia	Jn	26.414	1.421	9.310
Phillyrea angustifolia	Jn	5.134	1.948	1.993
Phillyrea latifolia	Jn	9.384	5.492	5.120
Phlomis purpurea	Jn	19.821	1.480	9.465
Pinus halepensis	Jn	15.201	3.334	4.625
Pistacia lentiscus	Jn	11.747	3.864	3.531
Pistacia terebinthus	Jn	8.081	1.000	5.098
Quercus coccifera	Jn	13.230	2.010	7.108
Quercus faginea	Jn	12.155	1.126	8.415
Quercus ilex	Jn	22.057	5.058	11.382
Rhamnus lycioides	Jn	8.705	2.951	8.238
Rosa sp. Jaén	Jn	2.000	*NA*	*NA*

Rosmarinus officinalis	Jn	6.751	1.491	6.776
Ruscus aculeatus	Jn	21.412	5.497	6.942
Thymus mastichina	Jn	11.462	1.933	6.162
Thymus zygis	Jn	12.483	1.303	5.699
Ulex parviflorus	Jn	18.132	8.424	13.069
Viburnum tinus	Jn	14.406	3.538	12.430
Acer granatensis	Sg	4.080	1.055	7.823
Amelanchier ovalis	Sg	9.733	3.267	8.205
Berberis hispanica	Sg	8.961	1.624	14.734
Cistus albidus	Sg	14.291	1.685	17.014
Cistus salvifolius	Sg	13.771	2.347	8.690
Crataegus laciniata	Sg	13.106	3.301	15.269
Crataegus monogyna	Sg	9.090	3.770	8.417
Cystus scoparius	Sg	6.858	1.089	4.573
Daphne laureola	Sg	1.000	*NA*	1.000
Juniperus communis	Sg	31.979	6.832	13.990
Pinus nigra	Sg	16.515	8.491	11.940
Prunus spinosa	Sg	6.401	1.000	8.018
Quercus faginea	Sg	7.919	1.722	12.089
Quercus ilex	Sg	11.844	5.874	15.949
Quercus pyrenaica	Sg	11.609	2.151	9.596
Rosa sp. Segura	Sg	3.289	1.469	8.864
Sorbus torminalis	Sg	13.690	3.175	10.310
Thymus mastichina	Sg	15.850	3.821	6.182
Thymus zygis	Sg	12.084	3.795	16.103

Table S1.3 The cover of the canopy and the recruit species and their canopy service and recruit width for each plant species and site.

Plant species	Site	Contribution sapling bank	Structure sapling bank	Recruitment service	Recruitment width	Cover of recruits	Cover of canopy
Acer monspessulanum	Jn	17	36	5	7	579.8	668.8
Amelanchier ovalis	Jn	12	13	6	5	399	429.4
Cistus albidus	Jn	150	81	20	14	4595.5	4597.4
Cistus salvifolius	Jn	7	17	5	8	266.3	266.3
Crataegus monogyna	Jn	101	318	15	16	3267.1	3481.1
Daphne gnidium	Jn	23	23	10	8	932.1	939.7
Genista cinerea	Jn	0	0	0	0	83.6	83.6
Juniperus oxycedrus	Jn	92	64	22	13	3306.5	3637
Juniperus phoenicea	Jn	0	4	0	4	10.5	14.5
Lavandula latifolia	Jn	138	61	12	13	1419.2	1419.2
Phillyrea angustifolia	Jn	79	42	15	14	3529.2	3700.2
Phillyrea latifolia	Jn	108	408	20	22	3564.6	4202
Phlomis purpurea	Jn	68	48	11	12	1201.4	1201.4
Pinus halepensis	Jn	294	824	17	21	124323.5	4142.9

Pistacia lentiscus	Jn	110	72	17	15	6203.8	6330.3
Pistacia terebinthus	Jn	90	127	15	17	5926.2	5637.9
Quercus coccifera	Jn	18	92	12	13	1768.3	1768.3
Quercus faginea	Jn	325	184	21	17	21007.5	10343.2
Quercus ilex	Jn	931	201	26	23	31998.4	18173.9
Rhamnus lycioides	Jn	19	40	8	13	1609	1686.4
Rosa sp. Jaén	Jn	33	38	10	12	747.5	762.4
Rosmarinus officinalis	Jn	161	20	23	9	7479.6	7479.6
Ruscus aculeatus	Jn	3	13	2	7	252.9	252.9
Thymus mastichina	Jn	6	10	4	6	259.2	259.2
Thymus zygis	Jn	7	15	4	6	100.1	100.1
Ulex parviflorus	Jn	32	15	12	6	1055.6	1055.6
Viburnum tinus	Jn	4	36	4	8	462	462
Acer granatensis	Sg	1	5	1	3	0	50.1
Amelanchier ovalis	Sg	0	1	0	1	0	7.5
Berberis hispanica	Sg	10	3	3	3	349.2	351.8
Cistus albidus	Sg	0	4	0	2	1	3
Cistus salvifolius	Sg	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
Crataegus laciniata	Sg	15	6	4	3	556.1	601.5
Crataegus monogyna	Sg	287	121	11	10	10264.9	10405.9
Cystus scoparius	Sg	75	39	11	7	2141.8	2168.4
Daphne laureola	Sg	9	22	5	8	617.6	635.8
Juniperus communis	Sg	13	4	3	4	814.2	822.6
Pinus nigra	Sg	485	415	13	11	70797	3509.8

Prunus spinosa	Sg	15	149	8	10	1326.2	1390.5
Quercus faginea	Sg	134	248	11	12	18480.5	2067.6
Quercus ilex	Sg	144	138	12	10	6572.3	5560.1
Quercus pyrenaica	Sg	25	38	8	7	3907.4	481.1
Rosa sp. Segura	Sg	51	65	13	10	2339.8	2370
Sorbus torminalis	Sg	3	10	3	6	254	147.7
Thymus mastichina	Sg	0	0	0	0	1.1	1.1
Thymus zygis	Sg	1	0	1	0	42.8	42.8

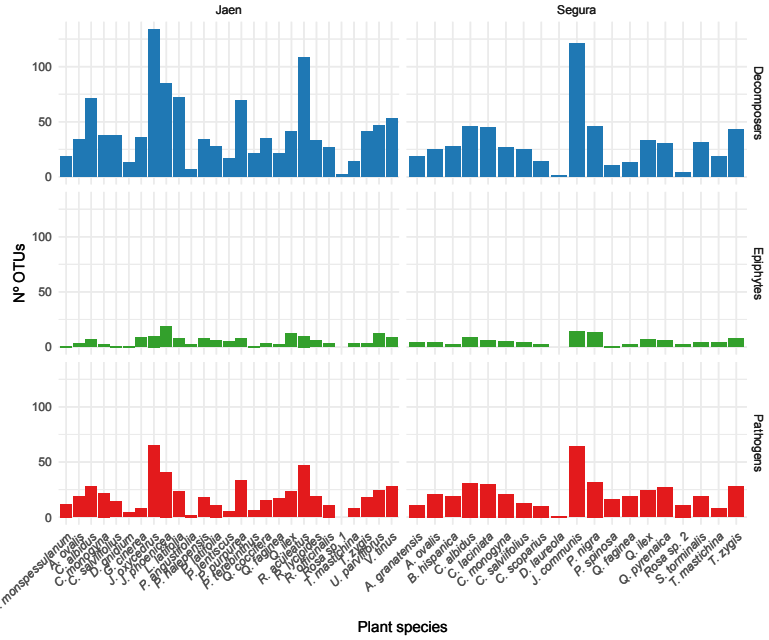

Figure S1.1 Density distribution of OTU abundances by guild and site.

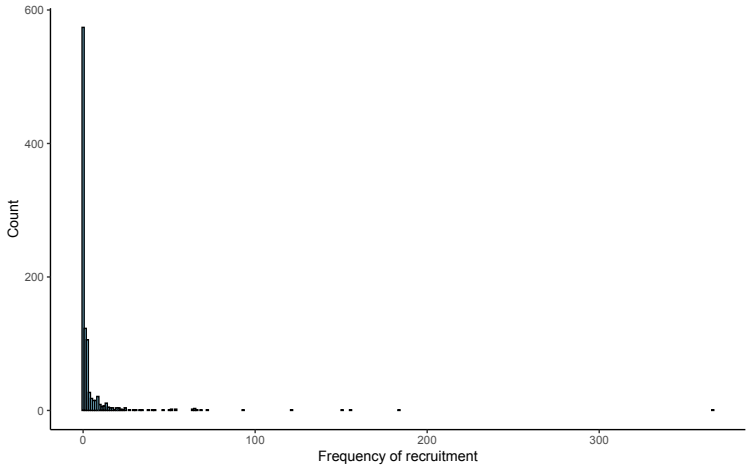

Figure S1.2 Histogram showing the data distribution of the frequency of recruitment.

Appendix S2 Pairwise analyses tables

Table S2. GLMM analyses performed to explore the effect of fungal richness (OTU proportion) and fungal diversity (number of effective partners), the site and the cover of canopy and recruit on the recruitment niche width (a, c) and the recruitment service (b, d) of plant species on the probability of canopy-recruit interactions. Significant effects are bold typed.

a) Recruitment niche width

Fixed effects	**Estimate**	**Std. error**	***Z* value**	***p***
Pathogens richness	-0.256	1.826	-0.140	0.889
Saprotrophs richness	0.218	1.833	0.119	0.905
Epiphytes richness	-0.367	0.563	-0.652	0.514
Site	-0.487	0.149	-3.268	**0.001**
Cover of recruit species	0.210	0.029	7.324	**<0.001**

b) Canopy service

Fixed effects	**Estimate**	**Std. error**	*Z value*	*p*
Pathogens richness	1.073	1.695	0.633	0.526
Saprotrophs richness	-0.370	1.738	-0.213	0.832
Saprotrophs richness	-0.549	0.527	-1.042	0.297
Site	-0.330	0.144	-2.290	**0.022**
Cover of canopy species	0.472	0.041	11.377	**<0.001**

c) Recruitment niche width

Fixed effects	**Estimate**	**Std. error**	*Z value*	*p*
Pathogens diversity	-0.004	0.016	-0.236	0.814
Saprotrophs diversity	-0.0002	0.012	-0.021	0.983
Epiphytes diversity	-0.016	0.027	-0.590	0.555
Site	-0.498	0.139	-3.575	**<0.001**
Cover of recruit species	0.207	0.026	8.042	**<0.001**

d) Canopy service

Fixed effects	Estimate	Std. error	*Z value*	*p*
Pathogens diversity	0.018	0.015	1.199	0.230
Saprotrophs diversity	-0.004	0.012	-0.330	0.741
Epiphytes diversity	-0.029	0.028	-1.012	0.311
Site	-0.334	0.140	-2.389	**0.017**
Cover of canopy species	0.468	0.039	11.917	**<0.001**

Appendix S3 DNA extraction and sequencing analyses

The dataset analysed phyllosphere fungal DNA from 276 leaf samples from 138 individuals belonging to 27 plant species in Jaén and 19 in Segura. Three adult individuals of each species were randomly selected from different zones. From each cardinal direction of the canopy, healthy and damaged leaves were taken to build respective composite samples of approximately 20 g per individual plant each, and immediately kept in silica-gel and kept at 4ºC until DNA extraction. The number of leaves variated depending on the plant species. In the laboratory, the 5 g of leaves per individual plant and type of sample (healthy or damaged leaves) were homogenized and DNA from 25 mg of dry leaf tissue extracted using the ISOLATE II Plant DNA kit (BioLine), strictly following the manufacturer's instructions. The DNA was finally eluted in a volume of 50 μL. Negative extraction controls were included in every DNA isolation round to check for cross-contamination.

DNA was sequenced with Illumina NovaSeq using specific fungal primers of ITS3 and ITS4 (ITS2 region, Tedersoo *et*

al. 2014). These primers included the Illumina sequencing primer sequences attached to their 5' ends. PCRs were carried out in a final volume of 25 µL, containing 2.5 µL of template DNA, 0.5 µM of the primers, 12.5 µL of Supreme NZYTaq 2x Green Master Mix (NZYTech), and ultrapure water up to 25 µL. The reaction mixture was incubated as follows: an initial denaturation step at 95 ºC for 5 min, followed by 35 cycles of 95 ºC for 30 s, 49 ºC for 45 s, 72 ºC for 30 s, and a final extension step at 72 ºC for 10 min. Negative controls that contained no DNA (BPCR) were included in every PCR round to check for contamination during library preparation. The oligonucleotide indices which are required for multiplexing different libraries in the same sequencing pool were attached in a second PCR round with identical conditions but only 5 cycles and 60 ºC as the annealing temperature. PCR products were pooled in equimolar amounts according to the quantification data provided by the Qubit dsDNA HS Assay (Thermo Fisher Scientific). This pool also contained a testimonial amount (1 µL) of each of the negative controls (both the extraction negative controls and the PCR negative controls). The pool was sequenced in a fraction of a NovaSeq PE250 run (Illumina) aiming for a total output of 20 gigabases. The indices and sequencing primers were trimmed during the demultiplexing step.

Forward and reverse primers were removed using cutadapt. Forward and reverse sequences were quality filtered to allow as maximum two expected errors per read. Sequences were dereplicated and the rate error model was inferred and used to implement the sample inference algorithm. The inferred error model was corrected in case that the error rate inferred in qualities below Q40 would be lesser than those of Q40. Forward and reverse reads were merged and chimeric sequences removed, resulting in 18668 ASVs from 32317404 reads. Afterwards,

LULU curation tool (Frøslev *et al.* 2017) was used to remove further sequencing errors. To correct possible mistagging, ASVs present in less than 0.01% of reads in a sequenced sample were discarded (Bokulich *et al.* 2013). Taxonomic assignment was determined for each ASV against the UNITE database v. 8.2. (Abarenkov *et al.* 2020), complemented with representative sequences of the plant genera involved in the study, using the RDP algorithm implemented in DADA2 pipeline. ASVs non assigned at a fungal phylum level were discarded. This resulted in a set of ASVs whose sequence varied from 50 to 460 bp, averaging 303.8 bp. ASVs were clustered into 97% sequence similarity using VSEARCH v. 2.8.1 (Rognes *et al.* 2016) to finally obtain 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.

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Appendix S4 Generalized linear mixed model formulas and figures

- Model structure of the generalized linear mixed models of the pairwise-level analyses:

Frequency of recruitment:
Model 2: frequency of recruitment ~ Bray Curtis dissimilarity of pathogens + Bray Curtis dissimilarity of saprotrophs + Bray Curtis dissimilarity of epiphytes + log (cover of recruits + 1) + log (cover of canopy + 1) + Site + sqrt (phylogenetical distance) + (1 | plant 1) + (1 | plant 2)

Probability of recruitment:
Model 1: probability of recruitment ~ Bray Curtis dissimilarity of pathogens + Bray Curtis dissimilarity of saprotrophs + Bray Curtis dissimilarity of epiphytes + log (cover of recruits + 1) + log (cover of canopy + 1) + Site + sqrt (phylogenetical distance) + (1 | plant 1) + (1 | plant 2)

- Model structure of the generalized linear mixed models of the species-level analyses:

Frequency of recruitment:
Model 1: Structure of the sapling bank ~ Pathogens richness + Saprotrophs richness + Epiphytes richness + Site + log (Cover of recruit + 1)
Model 2: canopy contribution to the sapling bank ~ Pathogens richness + Saprotrophs richness + Epiphytes richness + Site + log (Cover of canopy + 1)
Model 3: Structure of the sapling bank ~ Pathogens diversity + Saprotrophs diversity + Epiphytes diversity + Site + log (Cover of recruit + 1)
Model 4: canopy contribution to the sapling bank ~ Pathogens diversity + Saprotrophs diversity + Epiphytes diversity + Site + log (Cover of canopy + 1)

Probability of recruitment:
Model 1: Recruitment niche width ~ Pathogens richness + Saprotrophs richness + Epiphytes richness + Site + log (Cover of recruit + 1)
Model 2: Canopy service ~ Pathogens richness + Saprotrophs richness + Epiphytes richness + Site + log (Cover of recruit + 1)
Model 3: Recruitment niche width ~ Pathogens diversity + Saprotrophs diversity + Epiphytes diversity + Site + log (Cover of recruit + 1)
Model 4: Canopy service ~ Pathogens diversity + Saprotrophs diversity + Epiphytes diversity + Site + log (Cover of canopy + 1)

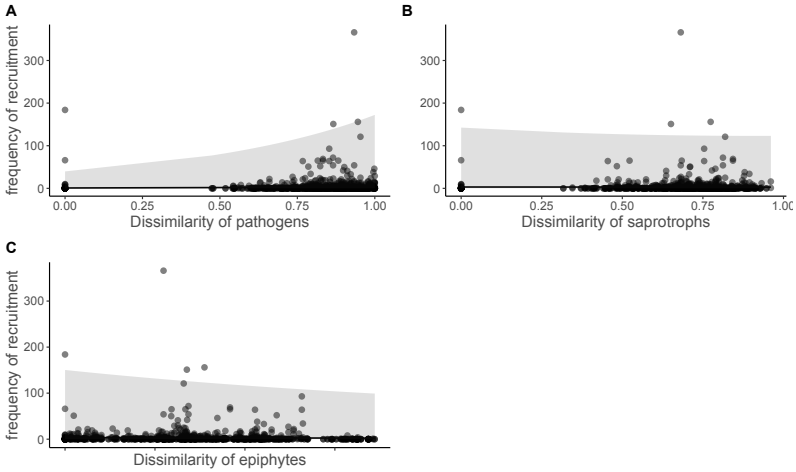

Figure S4.1 Predictions of the GLMM analysis performed to explore the effect of the dissimilarity of phyllosphere fungal communities of (**a**) pathogens, (**b**) saprotrophs and (**c**) epiphytes measured as Bray-Curtis distance on recruitment frequency between pairs of plant species.

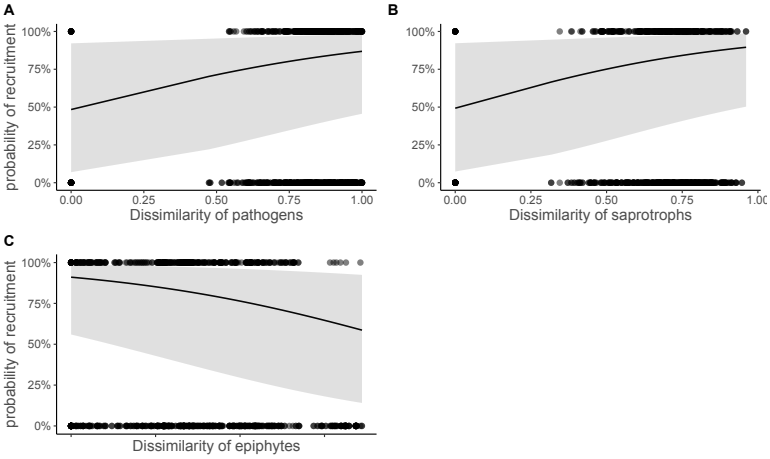

Figure S4.2 Predictions of the GLMM analysis performed to explore the effect of the dissimilarity of phyllosphere fungal communities of (**a**) pathogens, (**b**) saprotrophs and (**c**) epiphytes measured as Bray-Curtis distance on recruitment probability between pairs of plant species.

Información suplementaria Capítulo 3

Pajares-Murgó, M., Garrido, J. L., Perea, A. J., López-García, Á., Bastida, J. M., Prieto-Rubio, J., Lendínez, S., Azcón-Aguilar, C., & Alcántara, J. M. (2024). Intransitivity in plant–soil feedbacks is rare but is associated with multispecies coexistence. *Ecology Letters*, 27(3), e14408.

Appendix S1.1 Species-specific feedbacks

We conducted a fully crossed experiment to determine the recruitment success of 10 species of Mediterranean woody species cultivated in soil from each of them. The existence of species-specific PSFs can be determined by comparing the success of a plant (in our experiment, the ISEs of a given recruit species) grown in soil from conspecific individuals against its success in soil from a different species (Brinkman *et al.* 2010; Leckberg *et al.* 2018). Since each recruit species is represented by five ISEs, we averaged recruitment success of each ISE across the six ISOs. This resulted in five estimates of recruitment success probability (s_{ij}) for each species *i* in each soil-source species *j*. We tested for the existence of species-specific PSFs for each recruit species separately, using generalized linear mixed models (GLMMs) with mean ISE recruitment success probability as dependent variable and the soil-source species as predictor variable. All models were fitted using the beta family distribution. This distribution allows values of proportions excluding zero and one. When some ISEs had recruitment success in some soil species of one ($s_{ij} = 1$), we transformed the dependent variable to $1\text{-}s_{ij}$, what changes the sign of the estimates but does not affect the tests. Besides, since some ($s_{ij} = 0$), we included a zero-inflation intercept. We included the ISE in the models as a within-subject random effect so that the

success of each ISE in conspecific soil was paired with its success in each soil source. In each analysis, the soil from the recruit species was used as the aliasing level (intercept of the model) so that the GLMM directly tested the difference in recruitment success probability between conspecific and each heterospecific soil. Significant terms in the model (other than the intercept) indicate that the PSFs are species-specific. The results of analyses are shown in Table S1. Note that the pair *Juniperus oxycedrus* was not recruited in soil of *Colutea atlantica*, therefore, we obtained 89 pairs of species.

To assess whether the experimental results reflect the efficiency of canopy-recruit interactions occurring in the field, we used the data from Alcántara *et al.* (2018) obtained in the same study site as the experimental seeds and soils. This dataset contains the frequency of recruitment for the 10 studied species under the canopy of the soil-source species (*Fij*) estimated as the number of saplings of species *i* recruiting under species j. Briefly, the frequency of recruitment was sampled in 5 sites in Jaén (located between 0.78 and 3.69 km from each other) and 4 in Segura (1.10-3.58 km from each other). Each site consisted in a 50 x 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 m2) 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. Therefore, from

the frequency of recruitment (*Fij*) we estimated the efficiency of recruitment for the 10 studied species under the canopy of the soil-source species (*Eij*) as the number of saplings of species *i* recruiting under species *j* divided by the cover of the canopy and recruit species.

Table S1. Results of analyses testing for the existence of species-specific PSFs in each species. The dependent variable was the mean recruitment success (0/1) of each of the five individual seed sources (ISEs) in the six individual soil sources (ISOs) of each species. The dependent variable was the soil species, with the conspecific soil used as aliasing (intercept) level in the model. The ISEs was included as a random (within-subject) effect to control for possible interindividual differences in seed viability. The models were fitted using *glmmTMB* package in R (Brooks *et al.* 2023). The dependent variable was modelled using beta family distribution and logit link-function.

Recruit: *Acer monspessulanum*	**Estimate**	**Std. Error**	**z value**	***P***
(Intercept)	-0.80	0.39	-2.02	**0.043**
C. albidus	1.30	0.37	3.50	**<0.001**
C. atlantica	0.59	0.34	1.71	0.088
G. cinerea	0.25	0.34	0.73	0.467
J. oxycedrus	0.45	0.34	1.33	0.185
J. phoenicea	0.17	0.36	0.48	0.633
P. halepensis	0.85	0.34	2.51	**0.012**
P. terebinthus	0.24	0.36	0.67	0.506
Q. faginea	1.05	0.37	2.83	**0.005**
Q. ilex	0.77	0.35	2.23	**0.026**

Recruit: *Cistus albidus*	**Estimate**	**Std. Error**	**z value**	***P***
(Intercept)	-0.74	0.30	-2.48	**0.013**
A. monspessulanum	0.07	0.37	0.20	0.844
C. atlantica	-0.08	0.41	-0.18	0.855
G. cinerea	0.12	0.37	0.31	0.755
J. oxycedrus	0.25	0.37	0.69	0.491
J. phoenicea	-0.16	0.38	-0.41	0.680
P. halepensis	0.17	0.39	0.43	0.671
P. terebinthus	-0.06	0.41	-0.15	0.882
Q. faginea	-0.66	0.43	-1.53	0.127
Q. ilex	-0.15	0.38	-0.39	0.693

Recruit: *Colutea atlantica*	**Estimate**	**Std. Error**	**z value**	***P***
(Intercept)	0.02	0.29	0.06	0.952
A. monspessulanum	-0.07	0.37	-0.18	0.856
C. albidus	0.48	0.38	1.27	0.204
G. cinerea	0.40	0.38	1.06	0.291
J. oxycedrus	0.64	0.38	1.69	0.092
J. phoenicea	-0.59	0.38	-1.55	0.121
P. halepensis	-0.43	0.38	-1.14	0.254
P. terebinthus	0.45	0.38	1.19	0.233
Q. faginea	-0.07	0.38	-0.19	0.849
Q. ilex	-0.14	0.38	-0.37	0.714

Recruit: *Genista cinerea*	**Estimate**	**Std. Error**	**z value**	***P***
(Intercept)	0.67	0.54	1.23	0.218
A. monspessulanum	-0.84	0.52	-1.60	0.109
C. albidus	0.06	0.48	0.13	0.899
C. atlantica	-1.36	0.54	-2.52	**0.012**
J. oxycedrus	-0.99	0.53	-1.87	0.061
J. phoenicea	-0.76	0.51	-1.48	0.138
P. halepensis	-1.22	0.57	-2.14	**0.032**
P. terebinthus	-0.56	0.53	-1.07	0.284
Q. faginea	0.05	0.49	0.11	0.913
Q. ilex	-0.82	0.50	-1.62	0.105

Recruit: *Juniperus oxycedrus*	**Estimate**	**Std. Error**	**z value**	***P***
(Intercept)	-1.33	0.42	-3.16	**0.002**
A. monspessulanum	0.37	0.48	0.77	0.440
C. albidus	0.45	0.57	0.79	0.431
G. cinerea	0.66	0.56	1.18	0.237
*J. oxycedrus*1*				
J. phoenicea	-0.14	0.49	-0.28	0.777
P. halepensis	0.94	0.55	1.71	0.088
P. terebinthus	-0.29	0.53	-0.55	0.581
Q. faginea	0.22	0.51	0.44	0.661
Q. ilex	0.14	0.51	0.27	0.790

Recruit: *Juniperus phoenicea*	**Estimate**	**Std. Error**	**z value**	***P***
(Intercept)	-1.03	0.26	-3.91	**<0.001**
A. monspessulanum	0.39	0.31	1.25	0.211
C. albidus	0.01	0.34	0.02	0.987
C. atlantica	0.74	0.32	2.30	**0.021**
G. cinerea	-0.42	0.39	-1.09	0.276
J. oxycedrus	-0.31	0.33	-0.92	0.356
P. halepensis	-0.09	0.34	-0.26	0.792
P. terebinthus	-0.19	0.36	-0.54	0.587
Q. faginea	-0.25	0.35	-0.73	0.468
Q. ilex	-0.16	0.33	-0.50	0.614

Recruit: *Pinus halepensis*	**Estimate*²**	**Std. Error**	**z value**	***P***
(Intercept)	-1.26	0.22	-5.80	**<0.001**
A. monspessulanum	-0.35	0.34	-1.04	0.298
C. albidus	-0.28	0.36	-0.76	0.446
C. atlantica	-0.31	0.33	-0.92	0.357
G. cinerea	0.00	0.35	0.00	0.999
J. oxycedrus	0.11	0.34	0.33	0.743
J. phoenicea	1.12	0.35	3.17	**0.002**
P. terebinthus	0.05	0.34	0.16	0.874
Q. faginea	0.61	0.28	2.19	**0.028**
Q. ilex	0.04	0.34	0.13	0.897

Recruit: *Pistacia terebinthus*	**Estimate*²**	**Std. Error**	**z value**	***P***
(Intercept)	-1.17	0.29	-4.03	**<0.001**
A. monspessulanum	0.68	0.35	1.93	0.054
C. albidus	0.56	0.35	1.59	0.111
C. atlantica	0.80	0.35	2.31	**0.021**
G. cinerea	0.61	0.35	1.76	0.078
J. oxycedrus	0.51	0.37	1.37	0.171
J. phoenicea	0.31	0.36	0.88	0.380
P. halepensis	0.19	0.36	0.52	0.601
Q. faginea	0.80	0.35	2.29	**0.022**
Q. ilex	0.84	0.35	2.42	**0.016**

Recruit: *Quercus faginea*	**Estimate*²**	**Std. Error**	**z value**	***P***
(Intercept)	-0.29	0.32	-0.93	0.355
A. monspessulanum	0.33	0.44	0.75	0.451
C. albidus	-0.15	0.44	-0.33	0.741
C. atlantica	-0.21	0.47	-0.44	0.662
G. cinerea	0.00	0.44	0.00	0.999
J. oxycedrus	-0.14	0.44	-0.31	0.754
J. phoenicea	0.27	0.44	0.62	0.536
P. halepensis	0.81	0.44	1.81	0.070
P. terebinthus	0.29	0.44	0.67	0.506
Q. ilex	0.48	0.44	1.10	0.273

Recruit: *Quercus ilex*	**Estimate*[2]**	**Std. Error**	**z value**	***P***
(Intercept)	-1.11	0.28	-3.99	**<0.001**
A. monspessulanum	0.89	0.37	2.39	**0.017**
C. albidus	0.19	0.39	0.50	0.619
C. atlantica	0.28	0.38	0.72	0.473
G. cinerea	0.73	0.38	1.95	0.051
J. oxycedrus	0.48	0.40	1.20	0.231
J. phoenicea	0.03	0.39	0.08	0.936
P. halepensis	0.62	0.38	1.64	0.101
P. terebinthus	0.77	0.40	1.95	0.051
Q. faginea	1.23	0.39	3.13	**0.002**

*[1] Not tested due to lack of variance between ISEs (all $s_{ij} = 0$)

*[2] The response variable was coded as 1-probability due to the presence of 1s.

The 100 estimates sij are shown in matrix A:

RECRUIT SPECIES \ SOIL SOURCE SPECIES	ACEMON	CISALB	COLATL	GENCIN	JUNOXY	JUNPHO	PINHAL	PISTER	QUEFAG	QUEILE
ACEMON	0.28	0.54	0.46	0.38	0.42	0.33	0.52	0.34	0.48	0.50
CISALB	0.34	0.33	0.25	0.35	0.40	0.29	0.31	0.25	0.16	0.28
COLATL	0.48	0.61	0.52	0.61	0.67	0.35	0.41	0.59	0.48	0.48
GENCIN	0.39	0.59	0.35	0.48	0.37	0.47	0.29	0.43	0.50	0.48
JUNOXY	0.18	0.06	0.00	0.07	0.03	0.11	0.08	0.06	0.10	0.10
JUNPHO	0.34	0.22	0.33	0.13	0.20	0.27	0.21	0.17	0.19	0.23
PINHAL	0.87	0.90	0.87	0.86	0.87	0.82	0.77	0.87	0.66	0.86
PISTER	0.63	0.66	0.59	0.64	0.73	0.70	0.72	0.80	0.59	0.58
QUEFAG	0.48	0.61	0.69	0.59	0.61	0.50	0.36	0.52	0.58	0.45
QUEILE	0.55	0.72	0.69	0.59	0.71	0.76	0.62	0.66	0.57	0.77

Species acronyms are: ACEMON (*Acer monspessulanum*), CISALB (*Cistus albidus*), COLATL (*Colutea atlantica*), GENCIN (*Genista cinerea*), JUNOXY (*Juniperus oxycedrus*), JUNPHO (*Juniperus phoenicea*), PINHAL (*Pinus halepensis*), PISTER (*Pistacia terebinthus*), QUEFAG (*Quercus faginea*) and QUEILE (*Quercus ilex*).

Appendix S1.2. Pairwise-level feedback

Table S2. Predicted outcome of competition driven by PSFs for each species pair.

Recruit	Canopy	SD	FD	Outcome
C. albidus	*A. monspessulanum*	0.357	0.199	Coexistence
C. atlantica	*A. monspessulanum*	0.204	0.090	Coexistence
G. cinerea	*C. albidus*	0.191	0.088	Coexistence
J. oxycedrus	*A. monspessulanum*	1.294	0.704	Coexistence
J. phoenicea	*J. oxycedrus*	0.343	0.128	Coexistence
P. halepensis	*A. monspessulanum*	0.375	0.048	Coexistence
P. halepensis	*J. oxycedrus*	0.348	0.298	Coexistence
Q. faginea	*A. monspessulanum*	0.166	0.097	Coexistence
Q. faginea	*J. oxycedrus*	0.507	0.240	Coexistence
Q. ilex	*A. monspessulanum*	0.105	0.080	Coexistence
Q. ilex	*J. oxycedrus*	0.404	0.277	Coexistence
C. atlantica	*C. albidus*	-0.057	0.104	Exclusion
G. cinerea	*A. monspessulanum*	0.008	0.258	Exclusion
G. cinerea	*C. atlantica*	-0.063	-0.129	Exclusion
J. oxycedrus	*C. albidus*	0.307	-0.701	Exclusion
J. oxycedrus	*C. atlantica*	-0.032	-0.957	Exclusion
J. oxycedrus	*G. cinerea*	0.069	-0.361	Exclusion
J. phoenicea	*A. monspessulanum*	0.277	0.706	Exclusion
J. phoenicea	*C. atlantica*	0.009	0.546	Exclusion
P. halepensis	*C. albidus*	0.040	-0.093	Exclusion
P. halepensis	*C. atlantica*	-0.042	0.101	Exclusion

RECRUIT SPECIES

P. terebinthus	*A. monspessulanum*	-0.064	0.375	Exclusion
P. terebinthus	*J. oxycedrus*	0.144	0.579	Exclusion
P. terebinthus	*P. halepensis*	-0.002	0.185	Exclusion
Q. faginea	*C. albidus*	-0.286	0.300	Exclusion
Q. faginea	*C. atlantica*	0.083	0.248	Exclusion
Q. faginea	*G. cinerea*	0.028	-0.051	Exclusion
Q. ilex	*C. atlantica*	-0.100	0.154	Exclusion
Q. ilex	*P. halepensis*	-0.068	0.077	Exclusion
J. phoenicea	*C. albidus*	-0.192	0.026	Priority
J. phoenicea	*G. cinerea*	-0.357	-0.241	Priority
P. halepensis	*G. cinerea*	-0.181	0.097	Priority
P. halepensis	*J. phoenicea*	-0.119	-0.094	Priority
P. terebinthus	*C. albidus*	-0.279	0.127	Priority
P. terebinthus	*C. atlantica*	-0.097	-0.020	Priority
P. terebinthus	*G. cinerea*	-0.204	0.049	Priority
P. terebinthus	*J. phoenicea*	-0.328	0.195	Priority
Q. faginea	*J. phoenicea*	-0.282	0.008	Priority
Q. faginea	*P. halepensis*	-0.294	0.025	Priority
Q. faginea	*P. terebinthus*	-0.246	-0.032	Priority
Q. ilex	*C. albidus*	-0.145	0.107	Priority
Q. ilex	*G. cinerea*	-0.144	-0.093	Priority
Q. ilex	*J. phoenicea*	-0.109	0.075	Priority
Q. ilex	*P. terebinthus*	-0.279	0.032	Priority
Q. ilex	*Q. faginea*	-0.299	0.060	Priority

To assess the robustness of these comparisons, we obtained 1000 matrices of simulated s_{ij} values obtained from a binomial

distribution with probability equal to the observed s_{ij}. We obtained simulated values of the number of recruits of species *i* in soil from species *j* (n_{ij}) by generating random numbers from a binomial distribution where N_{ij} was the number of trials conducted with each pair of species (i.e. number of pots seeded with species *i* in soil from species *j*) and $s_{ij} = n_{ij} / N_{ij}$ was the observed recruitment success in the simulation for this pair of species. From these matrices we obtained the SD and FD values and the frequency of each outcome (coexistence, competitive exclusion and priority effects) for each randomization.

Appendix S1.3. Community-level effects of plant-soil feedbacks

Table S3. Stability analysis of the 1013 subcommunities classified by the E-model in terms of feasibility and stability, and by the SCC analysis in terms of their transitivity or intransitivity.

	STABLE		NO STABLE		
	FULLY INTRANSITIVE	TRANSITIVE	FULLY INTRANSITIVE	TRANSITIVE	
FEASIBLE	32	22	9	793	856
NOT FEASIBLE	0	8	0	149	157
TOTAL	62		950		1013

Table S4. Confusion matrix showing the number of assemblages classified as E-persistent (i.e. all species can coexist stably according to the model of Eppinga *et al.* 2018) and as SCC-persistent by the topological analysis of the **A** matrix of the model (i.e. the network contains a single intransitive group integrating all species). Values in parenthesis indicate the residuals of the test. Table a) show the results for the full set of 1013 communities and

Table b) excluding communities with only two species.

a)		SCC-persistent		
		No	Yes	
E-persistent	No	950 (0.98)	9 (-4.14)	959
	Yes	22 (-4.78)	32 (20.17)	54
		972	41	1013

b)		SCC-persistent		
		No	Yes	
E-persistent	No	925 (0.37)	9 (-1.95)	934
	Yes	22 (-2.50)	12 (13.11)	34
		947	21	968

Table S5. Results of the agreement between E-persistence and SCC-persistence after applying different thresholds to binarize the A matrix. Max: transforming the maximum within each column into 1 and the rest to 0 (see Eppinga *et al.* 2018, Supplementary Information section S4). "<1" transforming all values above 1 into 1 and the rest into 0. Mean: transforming the values above the column mean into 1 and the rest into 0. The last rows present the results of Chi square test on the association between E-persistence and SCC-persistence and the Cohens' kappa statistic assessing the level of agreement between observed values (E-persistence) and predicted values (SCC-persistence). Columns marked with (+2) give the results using only subcommunities with more than 2 species.

	MAX	>1	MEAN	MAX (+2)	>1(+2)	MEAN (+2)
True negatives	950	122	162	925	97	137
False negatives	9	837	794	9	837	797
False positives	22	15	0	22	4	0
True positives	32	39	54	12	30	34
Sensitivity (%)	59.25	72.22	100	35.29	88.23	35.29
Specificity (%)	99.06	12.72	16.89	99.03	10.39	99.04
CCR (%)	96.94	15.18	21.32	96.80	13.12	96.80
TPR (%)	78.05	4.45	6.35	57.14	3.46	57.14
TNR (%)	97.74	89.05	100	97.68	96.04	97.98
Prop. Com. Core (%)	68.11	91.12	93.49			
Prop. Full Core (%)	4.05	67.92	79.27			
Chi square	447.73	9.91	10.859	182.18	0.07	182.18
	$p < 0.0005$	$p < 0.006$	$p < 0.002$	$p < 0.0005$	$p = 1$	$p < 0.0005$
Kappa	0.658	-0.018	0.0212	0.421	-0.0011	0.019

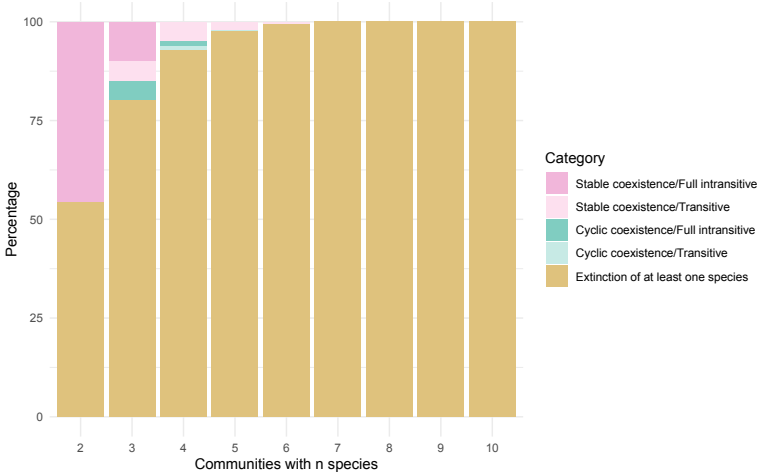

Figure S1. Stability analysis of the 1013 subcommunities classified by the E-model in terms of feasibility and stability, and by the SCC analysis in terms of their transitivity or intransitivity. Note that persistence of all species in the subcommunities can be achieved either through a feasible and stable equilibrium value, or through heteroclinic cycles which emerge in subcommunities that are not E-persistent but have full intransitivity. Interestingly, three out of 793 subcommunities that were not E-persistent and did not show full intransitivity supported persistence through heteroclinic cycles; however, their level of intransitivity was at least 0.75 and had negative *Ic*. Also, there are no subcommunities with the extinction of at least one species that are full intransitive.

Appendix S1.4 List of software used in the study

All model simulations and statistical analyses were performed in the R-environment version 4.1.1 (R Development Core Team, 2020) by means of RStudio IDE (Rstudio Team, 2020). GLMMs were fitted using *glmmTMB* R package (Brooks *et al.* 2023) and residual distributions were checked with *DHARMa* R package (Hartig & Lohse 2022). Simulations of the E-model were conducted with Runge-Kutta 4th and 5th order integration implemented in R-package *deSolve* (Soetaert *et al.* 2010). Figures were created with R-package *ggplot2* (Wickham 2016). Network visualization was conducted with R-package *igraph* (Csardi & Nepusz, 2006). Numerical simulations were performed with R-package *numDeriv* (Gilbert, 2019). For data manipulation we used R-package *dplyr* (Wickham *et al.* 2023).

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