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REDES DE REEMPLAZAMIENTO ENTRE PLANTAS: AVANCES TEÓRICOS, METODOLÓGICOS Y EMPÍRICOS

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REDES DE REEMPLAZAMIENTO ENTRE PLANTAS: AVANCES TEÓRICOS, METODOLÓGICOS Y EMPÍRICOS

Memoria presentada por

D. Manuel Pulgar Ramírez

para optar al Grado de Doctor por la Universidad de Jaén

Esta tesis ha sido realizada bajo la dirección de:

Dr. Pedro J. Rey y Dr. Julio M. Alcántara

UNIVERSIDAD DE JAÉN

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Certifican:

Que el trabajo recogido en la presente memoria, titulada: “Redes de reemplazamiento entre plantas: avances teóricos, metodológicos y empíricos”, presentada por D. Manuel Pulgar Ramírez, ha sido realizado bajo nuestra dirección y autorizamos su presentación y defensa para optar al grado de Doctor por la Universidad de Jaén.

Jaén, Febrero de 2017

Fdo. Dr. Pedro José Rey Zamora

Fdo. Dr. Julio Manuel Alcántara Gámez

A todas las personas queridas

a las que esta tesis les robó mi presencia,

ÍNDICE GENERAL

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SUMMARY

Over the last decades many studies have assessed the recruitment dynamics of woody species of Mediterranean plant communities. These studies provide valuable information, both in terms of basic science and aspects applied to the conservation of Mediterranean vegetation. However, because they are focused on the recruitment of specific species, these studies do not use a community approach that really allows understanding the origin and coexistence of a high diversity of species in these ecosystems. In this thesis we use the concept of replacement network as a conceptual framework for the study of plant-plant interactions and their role in the structure and dynamics of the community. These networks describe the recruitment of new individuals of each species of the community under the canopy of established adults of any other species, which they could eventually replace. The study of these networks, and the plant-plant interactions that configure them, can help us to improve our knowledge on the stability and dynamics of a community, as well as their assembly mechanisms, with the aim of understanding the coexistence of species and the maintenance of the biodiversity in Mediterranean forest ecosystems. Nowadays this is a critical objective because of the urgency of preserving these communities threatened by global change. The four chapters of the thesis describe different theoretical, methodological and empirical aspects related to replacement networks sampled in 12 mixed pine-oak forests in southern Spain. The aim is to gain a broad understanding of the dynamics of recruitment in these Mediterranean forest communities.

In the first chapter we analyze theoretically the role of transitivity and intransitivity of plant-plant interactions on the coexistence of species in replacement networks. Previous studies have shown that intransitively assembled interactions can support the coexistence of competing species, while transitively assembled (hierarchical) interactions are prone to species loss through competitive exclusion. However, as the number of species grows, the complexity of ecological interaction networks grows disproportionately, and species can get involved simultaneously in transitive and intransitive groups of interactions. In such complex networks, the effects of intransitivity on species persistence are not straightforward. Dissecting networks into intransitive/transitive components can help us to understand the complex role that intransitivity may play in supporting species diversity. We show through simulations that those species participating in the largest group of intransitive interactions

(the core of the network) have high probabilities of persisting in the long term. However, participation in a group of intransitive interactions other than the core does not always improve persistence. Likewise, participating in transitive interactions does not always decrease persistence because certain species (the satellites) transitively linked to the core have also a high persistence probability. Therefore, when networks contain transitive and intransitive structures, as it can be expected in real ecological networks, the existence of a large intransitive core of species can have a disproportionate positive effect on the maintenance of high species richness.

In the second chapter we analyze the effects of the sampling effort on the parameters that describe the structure of replacement networks. The qualitative and functional structure of these networks can provide information about community stability, as described in the first chapter. So, to reinforce the conclusions arising from any stability and dynamics analysis, it is critical to analyze how much sampling effort would be required to obtain complete and reliable networks. Using the replacement networks from the plots of each forest we constructed curves relating network descriptor estimates to sampling effort. Additionally we calculated the completeness of basic network elements (number of species and interactions) using rarefaction and extrapolation techniques. The results indicated that some network descriptors (such as the number of species, connectivity, persistence and attack and error sensitivity) reach stable values even with low sampling efforts. However, the number of interactions in the network and the mean number or interactions per species did not stabilize, although they reached relatively high values of completeness. In any case, most parameters describing the functional structure of the network and those related to community stability stabilized within our range of sampling effort (around 1 ha).

In chapters 3 and 4 we study the general factors that determine whether a given canopy-recruit interaction is realized or not in the different localities where both species co-occur. The aim of these studies is to back the conclusions on the structure and stability of these forest communities. Although both intra- and inter-specific interactions have fundamental roles in coexistence theory, studies of canopy-recruit interactions in plant communities focus largely on negatively density-dependent conspecific interactions. The realization or not of a given heterospecific interaction in a local community is thus

presumed to result from stochastic (neutral) processes (i.e., any species could recruit in principle under the canopy of any other species in a local community; whether it occurs or not would be just a matter of chance). However, many empirical studies suggest that although initial seed dispersal may have a strong neutral component, there are post-dispersive recruitment limitations (pair-wise specific) that may ultimately determine the pattern of interactions observed. In chapter 3 we explore the relative influence of neutral stochastic processes and species identity on the frequency of canopy-recruit interactions. Moreover, we estimate the effect (depressor, neutral or enhancer of recruitment) and spatial consistence (across study sites) of intra- and inter-specific canopy-recruit interactions. The results showed that, even though spatial stochasticity is intrinsic to the recruitment process, the assembly of canopy-recruit interactions is far from neutral. In fact, a given species can have depressor, neutral or enhancer effects on the recruitment of other species. In addition, adult-recruit interactions are found (or are absent) more consistently across different study sites than expected under a null model that takes into account only the abundance of species.

In order to reinforce the hypothesis that the assembly of canopy-recruit interactions is not an exclusively neutral process, in chapter 4 we explored whether the mechanisms shaping these interactions leave a detectable phylogenetic signal. Current theories on species coexistence disagree on the relative importance of interspecific niche differences and neutral outcomes of interactions. Studies addressing this issue use the phylogenetic relationships between species as a surrogate for niche differences, since it is assumed that more closely related species should have more similar niche requirements and hence would compete more strongly with each other. In this chapter we address whether the set of canopy-recruit interactions observed in a local community is a function of phylogenetic distances between species. Moreover we assess whether the degree of phylogenetic relatedness between two interacting species may influence the spatial consistency and outcome of their interaction. To do this we incorporate the phylogeny of the plant community into the replacement networks of each of the study forests. We found that canopy-recruit interactions exhibit significant phylogenetic structure in some communities, so that interactions tend to be established between more distantly related species (phylogenetic overdispersion pattern). Furthermore, key properties for the assembly of plant

communities, such as spatial consistency of the interactions and their effects on recruitment, are also related to phylogeny. Taken together, our results strongly suggest that phylogenetic distance, and therefore niche differences, play an important role in structuring canopy-recruit interactions. Once again, our results demonstrate that canopy-recruit interactions are not exclusively due to neutral mechanisms, reinforcing the view that ecologically driven canopy-recruit interactions determine the structure and stability of these forest communities.

RESUMEN

En las últimas décadas se han acumulado un buen número de estudios sobre la dinámica de reclutamiento de especies leñosas de bosques y matorrales mediterráneos. Estos estudios aportan valiosa información, tanto en términos de ciencia básica como en aspectos aplicados a la conservación de la vegetación mediterránea. Sin embargo, al estar enfocados en el reclutamiento de especies concretas, estos estudios no tienen una visión de comunidad que realmente permita comprender el origen y la coexistencia de una alta diversidad de especies en estos ecosistemas. En esta tesis utilizamos el concepto de red de reemplazamiento como marco conceptual para el estudio de las interacciones planta-planta y su papel en la estructura y dinámica de la comunidad. Estas redes describen el reclutamiento de nuevos ejemplares de cada especie de la comunidad en la zona de influencia de ejemplares ya establecidos de cualquier otra especie, a los que eventualmente podrían llegar a reemplazar. El estudio de estas redes, y de las interacciones planta-planta que las configuran, puede ayudarnos a conocer la estabilidad y dinámica de una comunidad así como los mecanismos de ensamble de esta, con el fin último de comprender la coexistencia de especies y el mantenimiento de la biodiversidad en ecosistemas forestales mediterráneos. En la actualidad este es un objetivo crítico debido a la urgencia de preservar este tipo de comunidades amenazadas por el cambio global. Los cuatro capítulos de esta tesis describen diferentes aspectos teóricos, metodológicos y empíricos sobre redes de reemplazamiento muestreadas en 12 bosques mixtos de pino y quejigo en el Sur de España. Con ello se pretende obtener un amplio conocimiento de la dinámica de reclutamiento en estas comunidades forestales mediterráneas.

En el primer capítulo analizamos el papel de la transitividad e intransitividad de las interacciones planta-planta sobre la coexistencia de especies en redes de reemplazamiento. Estudios previos mostraron que las redes de interacción de especies ensambladas intransitivamente pueden potenciar la coexistencia de especies, mientras que redes ensambladas transitivamente tienden a perder especies por exclusión competitiva. Sin embargo, cuando el número de especies crece también crece la complejidad de las redes ecológicas de interacciones, y las especies pueden participar simultáneamente en grupos de interacciones transitivos e intransitivos. En este tipo de redes complejas los efectos de la intransitividad sobre la coexistencia de especies no es tan directa. Diferenciar claramente

los componentes transitivos e intransitivos de la red nos ayudó a comprender el complejo papel que puede jugar la intransitividad manteniendo la diversidad de especies. Mediante simulaciones de un modelo no-lineal de dinámica de reemplazamiento mostramos que aquellas especies que participan en el mayor grupo de interacciones intransitivas (el núcleo de la red) tienen altas probabilidades de persistir en el tiempo. Sin embargo, la participación en un grupo de interacciones intransitivas distinto del núcleo no siempre asegura la persistencia de las especies. Por otra parte, participar en grupos de interacciones transitivas no siempre disminuye la persistencia porque ciertas especies unidas transitivamente al núcleo (especies "satélite") tienen también una alta probabilidad de no extinguirse. Por tanto, cuando las redes contienen estructuras transitivas e intransitivas (como sucede en las redes ecológicas reales) la existencia de un gran núcleo intransitivo de especies puede tener un enorme efecto potenciador de la riqueza de especies.

En el segundo capítulo analizamos los efectos del esfuerzo de muestreo sobre los parámetros que describen la estructura de las redes de reemplazamiento. La estructura cualitativa y funcional de estas redes puede dar información sobre la estabilidad de la comunidad, como se describe en el primer capítulo. Entonces, para reforzar las conclusiones derivadas de cualquier análisis de estabilidad y dinámica, es fundamental analizar cuánto esfuerzo de muestreo sería necesario para obtener redes completas y fiables. En cada bosque, usando la red de reemplazamiento de las diferentes parcelas de muestreo, se generaron curvas para relacionar los parámetros descriptores de la red con el esfuerzo de muestreo. Adicionalmente se calculó la proximidad ("*completeness*") de los descriptores básicos de la red (número de especies e interacciones) al valor esperable asintóticamente usando técnicas de rarefacción y extrapolación. Los resultados indicaron que algunos descriptores de la red (como la conectancia, persistencia de especies y sensibilidad a la extinción de especies) alcanzan valores estables incluso con bajos esfuerzos de muestreo. Por el contrario, los descriptores de número y densidad de conexiones no llegan a estabilizarse con el máximo esfuerzo de muestreo. En cualquier caso, la mayoría de los descriptores pueden ser calculados de manera fiable a partir de áreas de muestreo de alrededor de 1 ha.

En el tercer y cuarto capítulo hacemos una primera aproximación al estudio de los factores genéricos que hacen que una interacción adulto-juvenil se establezca o no en las

distintas comunidades en que están presentes ambas especies. Con ello se pretende una vez más reforzar las conclusiones sobre estructura y estabilidad de estas comunidades forestales. Aunque las interacciones intra- e inter-específicas tienen papeles fundamentales en las teorías de coexistencia, los estudios de interacciones adulto-recluta publicados hasta la fecha se centran especialmente en el análisis de interacciones intra-específicas en el contexto de procesos de reclutamiento dependientes negativamente de la densidad (efecto Janzen-Connell). Por su parte, las interacciones inter-específicas han sido consideradas por muchos ecólogos un resultado de procesos estocásticos o neutrales (es decir, cualquier especie podría reclutarse bajo la copa de cualquier otra especie en una comunidad local y si no lo hace es por causa del azar). Sin embargo, muchos estudios empíricos sugieren que, aunque la dispersión inicial de semillas puede tener un fuerte componente neutral, existen limitaciones post-dispersivas del reclutamiento (específicas entre pares de especies) que puede ser determinantes en última instancia del patrón de interacciones observado. En el tercer capítulo de la tesis exploramos la influencia relativa de los procesos neutrales (estocásticos) y la identidad de las especies sobre la frecuencia de las interacciones adulto-juvenil. Además, determinamos el efecto neutral, potenciador o depresor de las interacciones sobre el reclutamiento. También calculamos la consistencia espacial (a través de los diferentes sitios muestreados) de las interacciones adulto-recluta. Los resultados mostraron que, aunque la estocasticidad espacial (relacionada con la abundancia y distribución espacial de las especies) es intrínseca al proceso de reclutamiento, el ensamblaje de interacciones adulto-recluta está lejos de la neutralidad. De hecho, una especie dada puede tener efectos neutrales, potenciadores o depresores sobre el reclutamiento de otras especies. Además, las interacciones adulto-recluta son encontradas (o están ausentes) de manera más consistente a través de los diferentes bosques muestreados que lo esperado bajo un modelo nulo que tiene en cuenta únicamente la abundancia de las especies.

Por otra parte, para reforzar la hipótesis de que el ensamble de las interacciones adulto-juvenil que estructuran las redes de reemplazamiento no es un proceso exclusivamente neutral, en el cuarto capítulo se exploró si hay determinismo filogenético en la formación de estas interacciones. Ya que las teorías actuales discrepan en la influencia relativa de las diferencias de nicho y los mecanismos estocásticos sobre la coexistencia de

especies, algunos estudios abordaron esta cuestión usando las relaciones filogenéticas entre especies como una medida indirecta de las diferencias de nicho (especies más cercanas filogenéticamente deberían tener requerimientos ecológicos, es decir nichos, más similares). En este capítulo abordamos si el conjunto de interacciones adulto-recluta observadas en una comunidad es función de la distancia filogenética entre las especies (y por tanto de las diferencias de nicho). Para ello incorporamos la filogenia de la comunidad de plantas a las redes de reemplazamiento de cada uno de los bosques de estudio. Usando el índice NRI ("*Net Relatedness Index*") mostramos que las interacciones adulto-juvenil que estructuran las redes exhiben una significativa sobredispersión filogenética en algunos bosques de estudio. Esto se traduce en que las interacciones tienden a ser establecidas entre especies lejanas filogenéticamente. Por otra parte los resultados mostraron que propiedades clave para el ensamble de comunidades de plantas, como la consistencia espacial y el efecto de la interacción, están también relacionadas con la filogenia. En conjunto, los resultados sugieren que la distancia filogenética, y por tanto las diferencias de nicho, juegan un papel muy importante en la formación de interacciones adulto-juvenil que estructuran las redes de reemplazamiento. Una vez más se puso de manifiesto que las interacciones adulto-recluta no son debidas exclusivamente a mecanismos neutrales, lo que reforzó las conclusiones sobre estructura y estabilidad de estas comunidades forestales.

INTRODUCCIÓN

Las interacciones entre especies son uno de los principales mecanismos que gobiernan la dinámica de las comunidades, así que el estudio de las mismas es fundamental para comprender la coexistencia de especies, el mantenimiento de la biodiversidad y cómo las comunidades cambian con el tiempo (Tilman 1982; Silvertown 2004; Hubbel 2001). Responder a estas cuestiones ha sido uno de los objetivos de la ecología clásica, pero en la última década se ha convertido en un objetivo crítico debido a la necesidad urgente de preservar comunidades naturales amenazadas por el cambio global. Durante gran parte del siglo pasado los ecólogos abordaron diferentes aspectos de las interacciones entre especies desde una perspectiva poco holista, limitando sus estudios a la simple interacción entre pares de especies concretas coexistiendo en una comunidad. Sin embargo en las últimas décadas se han desarrollado diferentes teorías para el estudio de interacciones múltiples entre especies en el ámbito de las redes ecológicas complejas, lo que ha permitido grandes avances en el campo de las redes tróficas y redes mutualistas (Dunne 2002; Dunne 2006; Bascompte y Jordano 2014). El concepto de red proporciona una visión explícita de la complejidad de los sistemas ecológicos sólidamente basada en teoría matemática (teoría de grafos y teoría de sistemas complejos) por lo que tiene un gran potencial para transformar la manera en que se abordan los estudios de ecología de comunidades (Bascompte y Jordano 2014). Más aún, al ir más allá de las interacciones por pares, el axioma central de la ecología de redes es que este nivel de observación, aún cuando es menos familiar que las aproximaciones clásicas a la ecología de comunidades, es en última instancia un reflejo más realista del mundo natural (Poisot et al. 2016).

Sin embargo estos avances en el estudio de redes ecológicas no han sido empleados para abordar cuestiones sobre la estructura, estabilidad o dinámica de comunidades de plantas. Profundizar en el conocimiento de esta estructura de interacciones planta-planta y los mecanismos ecológicos que las configuran es fundamental ya que las comunidades de plantas constituyen el principal soporte vital para los ecosistemas terrestres. Una manera de alcanzar este nivel de conocimiento sobre las interacciones planta-planta desde una perspectiva de comunidad es integrar la ecología de comunidades vegetales y la teoría de redes ecológicas complejas a través del concepto de red de reemplazamiento (Alcántara y Rey 2012; Alcántara y Rey 2014). La dinámica de comunidades de plantas ha sido

interpretada frecuentemente como un proceso de reemplazamiento donde la muerte de una planta deja un espacio vacante en el que pueden reclutarse o crecer individuos de la misma u otra especie, reemplazando en última instancia al individuo muerto (Horn 1975; Grubb 1977; Myster 2012). Por tanto, las redes de reemplazamiento describen el conjunto de todos los posibles reemplazamientos entre individuos de todas las especies de la comunidad. En comunidades de plantas leñosas mediterráneas no es posible observar directamente estos reemplazamientos entre especies, ya que el proceso puede ser extremadamente lento (debido al largo ciclo de vida de estas plantas). Sin embargo, se puede asumir que la especie que reemplazará a la planta adulta será alguna de las que encuentren las condiciones micro-ambientales favorables para reclutarse bajo ella (George y Bazzaz 1999; Inderjit et al. 2011; Van der Voorde et al. 2011). Las interacciones en una red de reemplazamiento no representan otra cosa que el reclutamiento del juvenil de una planta bajo el dosel de otra planta adulta (Imagen 1). Como puede verse en la Figura 1 en una red de reemplazamiento cada especie es un nodo (representado por un círculo). Las flechas van desde las plantas adultas hacia los juveniles que se reclutan bajo su copa, lo que se interpreta como "parte del espacio dominado por los individuos adultos puede llegar a ser dominado en última instancia por los individuos de las especies que se reclutan bajo su copa". En la Figura 1 la región verde representa un conjunto de especies reemplazándose directa o indirectamente a través de ciclos de reemplazamiento (A→B→C→A). Este ciclo forma un componente fuertemente conectado (en inglés "*Strongly Connected Component"* ó simplemente "*SCC"*) que constituye el núcleo de la red. Por otra parte las especies fuera de este núcleo pueden ser especies satélite ("*Satellite species*") si se reclutan bajo alguna de las especies del núcleo o especies transitorias ("*Transient species*") si no lo hacen. Cada uno de estos componentes será descrito con un mayor nivel de detalle en los dos primeros capítulos de esta tesis.

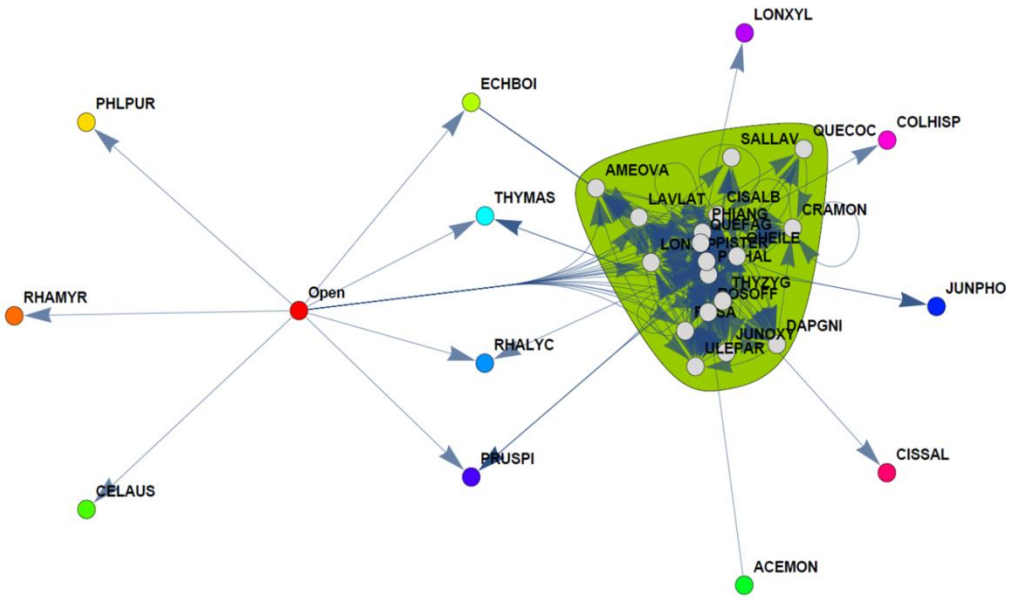

Figura 1: Ejemplo de red de reemplazamiento. Cada especie es representada por un círculo y está codificada con las tres primeras letras de su género y especie (ej. ACEMON es *Acer monspessulanum*).

Imagen 1. Ejemplos de reclutamiento de juveniles bajo plantas adultas a) *Crataegus monogyna* bajo *Genista cinerea* b*) Pistacia lentiscus* bajo *Phlomis purpurea* y c) *Quercus faginea* bajo *Lavandula latifolia.*

Una propiedad fundamental de las redes de reemplazamiento es que, al igual que otras redes de interacciones (e.g. Williams 2008; Stouffer y Bascompte 2011), pueden ser incorporadas de forma explícita en modelos dinámicos de comunidad (Alcántara et al. 2015). Esta característica ha convertido a estas redes en una herramienta útil para explorar de una manera directa las relaciones entre la estructura de la red y la estabilidad de la comunidad por medio del análisis cualitativo y cuantitativo del sistema. Alcántara y Rey (2012) mostraron que la estructura cualitativa de las redes de reemplazamiento (basada solamente en la presencia/ausencia de interacciones entre adultos y juveniles de plantas leñosas) permite inferir aspectos de la estabilidad de estas comunidades, como el número e identidad de especies que coexisten de forma estable en ausencia de perturbaciones (persistencia) o las que podrían hacerlo en caso de perturbaciones que impliquen la extinción de una especie (robustez). Por ejemplo, volviendo a la red de la Figura 1, si la dinámica de la comunidad es lineal e invariante en el tiempo (por ejemplo, si esta es modelada por modelos markovianos; Hill et al. 2002; Siles et al. 2008) solo las especies del núcleo y las satélites podrían persistir en el tiempo.

Sin embargo, la existencia de algunas propiedades estructurales de estas redes y su verdadero papel manteniendo la diversidad de especies no ha sido todavía abordado en profundidad. Un claro ejemplo sería la intransitividad en las interacciones planta-planta asociada a la presencia de ciclos de reemplazamiento (SCCs). Estudios teóricos del siglo pasado manifestaron que efectivamente la intransitividad en las interacciones puede favorecer la coexistencia de especies en una comunidad local (Gilpin 1975). Estos estudios se centraron en tríos de especies donde A vence a B, B vence a C, y C vence a A, como en el popular juego “piedra, papel o tijera”. Si lo representamos gráficamente las tres especies estarían conectadas por flechas formando un ciclo. La intransitividad promueve la coexistencia de especies porque en un sistema intransitivo no hay una especie competitivamente dominante sobre todas las demás. Por el contrario en un ensamblaje transitivo (o jerárquico) de interacciones A vence a B, B vence a C, y A vence a C. En la representación gráfica de las redes transitivas las especies no formarían ningún ciclo sino una estructura jerárquica. A diferencia de la in-transitividad, la transitividad en las interacciones de competencia conduce a la exclusión competitiva de todas las especies menos la dominante (Hardin 1960). Estudios más recientes extendieron estos conceptos

simples a redes de competencia más complejas (Laird y Schamp 2006; Allesina y Levine 2011; Rojas-Echenique y Allesina 2011). Sin embargo, cuando el número de especies crece, también crece la complejidad de las redes, donde las especies pueden estar involucradas simultáneamente en grupos de interacción transitivos e intransitivos. En este escenario de redes complejas los efectos de la intransitividad sobre la persistencia de especies dejan de ser tan obvios. En esta situación, diferenciar los componentes transitivos e intransitivos de las redes de reemplazamiento puede ser clave para comprender en qué medida la intransitividad puede estar actuando como motor de coexistencia y diversidad de especies en las comunidades de plantas mediterráneas.

La importancia de la estructura de las redes de interacciones para comprender cuestiones clave en la dinámica de las comunidades hace imprescindible el desarrollo de metodologías que permitan su determinación de la manera más adecuada. De hecho, limitaciones metodológicas en los primeros estudios sobre la estructura de redes tróficas obligaron a reconsiderar algunas de los primeros patrones generales que se describieron en este campo (Pascual y Dunne 2006). Para evitar los errores del pasado y avanzar con veracidad en el estudio de las redes de reemplazamiento (Alcántara y Rey 2012) se hace necesario obtener redes completas y fiables. Si esto es necesario en cualquier estudio de redes ecológicas (Martínez et al. 1999; Nielsen y Bascompte 2007), en el análisis cualitativo es crítico ya que la existencia o no de una interacción puede implicar conclusiones diametralmente opuestas acerca de la dinámica y estabilidad de la comunidad (Allesina y Bodini 2004; Fath y Halnes 2007). En un análisis cualitativo de la red, la fiabilidad la proporcionaría la certeza de que se han determinado todos los pares de interacciones adulto-juvenil existentes en la comunidad; es decir, la certeza de que las interacciones ausentes son interacciones prohibidas ("*forbidden links*" sensu Olesen et al. 2011) y no interacciones que han pasado desapercibidas ("*missing links*" sensu Olesen et al. 2011).

Por otra parte, pocos estudios han sido publicados hasta la fecha sobre mecanismos de ensamblaje en redes planta-planta (Valiente-Banuet y Verdú 2013). En el caso de las redes de reemplazamiento, el estudio de los factores que hacen que una interacción adulto-recluta esté ausente o presente beneficiaría al conocimiento sobre la estructura y estabilidad en dos direcciones: aportaría fiabilidad a las conclusiones y permitiría conocer los factores y mecanismos que participan en su proceso de ensamblado (Martínez et al.

1999). Verdú y Valiente-Banuet (2011) pusieron de manifiesto que la abundancia de cada especie y las distancias filogenéticas entre ellas (consideradas como una medida aproximada de las diferencias de nicho de regeneración entre especies; Grubb 1977; Brooks y McLennan 1991) pueden explicar, aunque solo en parte, la frecuencia de las interacciones y algunas características estructurales de las redes de facilitación (anidamiento y conectividad). La abundancia de cada especie se relaciona con la mera probabilidad de encuentro entre semillas dispersadas y plantas adultas. Ahora bien, una vez que las semillas caen bajo una planta, la probabilidad de reclutamiento puede estar influida por los procesos que tienen lugar a lo largo del ciclo de reclutamiento (escape a la depredación o infección postdispersiva de semillas, germinación, supervivencia de las plántulas y crecimiento), que podrían depender de características propias de cada especie de planta (Rey y Alcántara 2000; Traveset 2003). Sería lógico pensar que las interacciones de reclutamiento podrían ser en parte establecidas por procesos estocásticos relacionados con la limitación espacial en la dispersión de semillas (dependientes de la abundancia y distribución de las especies). Sin embargo la existencia de mecanismos de "filtrado" post-dispersivos implicaría que las interacciones de reclutamiento podrían ser en última instancia explicadas por procesos no neutrales relacionados con el nicho de regeneración de las especies (Grubb 1977; Stokes y Archer 2010). Basándose en la premisa de que los rasgos de nicho pueden ser evolutivamente conservados, diversos estudios sugirieron que la distancia filogenética entre un juvenil y una planta adulta puede aportar un papel explicativo en estos mecanismos post-dispersivos de ensamble, como por ejemplo los mecanismos de facilitación-competencia, infestación y herbivoría (Castillo et al. 2010; Ness et al. 2011; Valiente-Banuet y Verdú 2013). Por tanto, las relaciones evolutivas entre las especies que coexisten en una comunidad podrían ser determinantes del ensamblaje de las interacciones adulto-juvenil estructurando las redes de reemplazamiento en ecosistemas mediterráneos.

HIPÓTESIS Y OBJETIVOS

En las últimas décadas se han acumulado un buen número de estudios sobre la dinámica de reclutamiento de especies leñosas de bosques y matorrales mediterráneos. Estos estudios aportan valiosa información, tanto en términos de ciencia básica como en aspectos aplicados a la conservación de la vegetación mediterránea. Sin embargo, al estar enfocados en el reclutamiento de especies concretas, estos estudios no proporcionan una visión de comunidad que realmente permita comprender el origen y la coexistencia de una alta diversidad de especies en estos ecosistemas. En este contexto, la presente tesis aborda las siguientes **hipótesis de partida**:

1- La complejidad de las redes de reemplazamiento podría hacer que los efectos de la intransitividad de las interacciones sobre la persistencia de especies no sean tan directos como en redes más simples (*Capítulo 1*).
2- La determinación de las propiedades de las redes de reemplazamiento depende del esfuerzo de muestreo, pero es posible alcanzar estimas fiables de estas propiedades con esfuerzos de muestreo similares a los que se emplean en estudios de comunidades forestales (*Capítulo 2*).
3- La frecuencia de interacciones adulto-recluta que estructuran las redes de reemplazamiento debería ser explicada en gran medida por la identidad de las plantas y no solo por procesos neutrales de estocasticidad espacial (*Capítulo 3*).
4- Existe un fuerte determinismo filogenético en el ensamblaje de las interacciones adulto-juvenil que estructuran las redes de reemplazamiento. Esto apoyaría la importancia de procesos biológicos no neutrales en el reclutamiento (*Capítulo 4*).

Para comprobar estas hipótesis se han planteado los siguientes **objetivos generales**:

1- Evaluar la influencia de componentes transitivos e intransitivos presentes en las redes de interacciones planta-planta sobre la persistencia de especies a través de simulaciones ("Non-linear time-invariant model"). Diseccionar las redes de reemplazamiento en estos tipos de componentes puede ayudarnos a comprender el complejo papel que la in-transitividad puede jugar en el mantenimiento de la diversidad (*Capítulo 1*).

2- Analizar cómo el esfuerzo de muestreo afecta a la determinación empírica de diferentes descriptores de la estructura de las redes de reemplazamiento. Es decir, identificar si existen limitaciones metodológicas que puedan obstaculizar futuras investigaciones sobre estabilidad y dinámica de comunidad basadas en las redes de reemplazamiento (*Capítulo 2*).

3- Explorar la influencia de los procesos neutrales estocásticos (relacionados con la abundancia) y la identidad de las especies sobre la frecuencia de las interacciones adulto-recluta que estructuran las redes de reemplazamiento. Este objetivo nos permite además explorar la variación espacial y el efecto (depresor, neutral o potenciador) de las interacciones adulto-recluta sobre el reclutamiento (*Capítulo 3*).

4- Examinar si el ensamble de las redes de reemplazamiento, su consistencia espacial y su efecto sobre el reclutamiento dependen de las relaciones evolutivas de las especies que coexisten en una comunidad**.** (*Capítulo 4*).

ÁREA DE ESTUDIO

El sistema de estudio está formado por comunidades de plantas leñosas de los bosques mixtos de quercíneas (*Quercus faginea, Quercus ilex, Quercus pyrenaica*) y Pinos (*Pinus halepensis*, *P. nigra*, *P. pinaster*) en el sur de España. Además de estas especies arbóreas dominantes, la mayor parte de la riqueza específica de estos bosques es aportada por especies del sotobosque (ej. *Crataegus monogyna, Crataegus laciniata, Juniperus communis, Juniperus oxycedrus, Prunus mahaleb, Ilex aquifolium, Olea europaea* var. *sylvestris, Phillyrea angustifolia, Pistacia lentiscus, Pistacia terebinthus*, *Quercus coccifera*) y por especies de matorral bajo como las diversas especies de los géneros *Thymus, Cistus, Phlomis* o *Rosa*, entre otros. La distribución actual de este tipo de bosques en el Sur de España está restringida a áreas donde la precipitación anual es aproximadamente de unos 800 mm y la temperatura media está entre 10 y 14°C, con suelos profundos y donde la vegetación no ha sido afectada por incendios forestales recurrentes ni por sobrepastoreo u otra perturbación de origen antrópico en las últimas décadas. En la actualidad, este tipo de bosques mixtos puede ser frecuentemente resultado de la colonización de Quejigares por parte de pinos procedentes de repoblaciones forestales de principios del s. XX. Sin embargo, estudios palinológicos han mostrado que tales formaciones mixtas han sido comunes en el sur de la península Ibérica durante el Holoceno (Carrión et al. 2000, 2001). El trabajo de campo se desarrolló en el *Parque Natural de las Sierras de Cazorla, Segura y Las Villas* (38.29° N, -2.57° W) y el *Parque Periurbano Monte La Sierra de Jaén* (37.64° N, -3.73° W). Se seleccionaron 12 zonas de estudio entre los dos parques, donde serían establecidas las parcelas de muestreo. Todas las zonas de estudio presentaron masas boscosas de composición específica y estructura espacial homogénea. Estas zonas habían aumentado en densidad de cobertura durante los últimos 50 años, lo que evaluamos comparando ortofotografías recientes de la zona (vuelo de 2007) frente a las más antiguas (vuelo de 1956). Además, las zonas fueron seleccionadas cumpliendo los siguientes criterios: para las especies más frecuentes debían existir (i) ejemplares juveniles ("saplings"), (ii) ejemplares reproductores de distintos tamaños y (iii) ejemplares adultos senescentes o muertos por causas naturales. Estos criterios garantizaron la existencia en las parcelas de muestreo de una dinámica de reclutamiento activa (no impedida de forma significativa por sobrepastoreo u otras perturbaciones de origen antrópico durante más de 50 años).

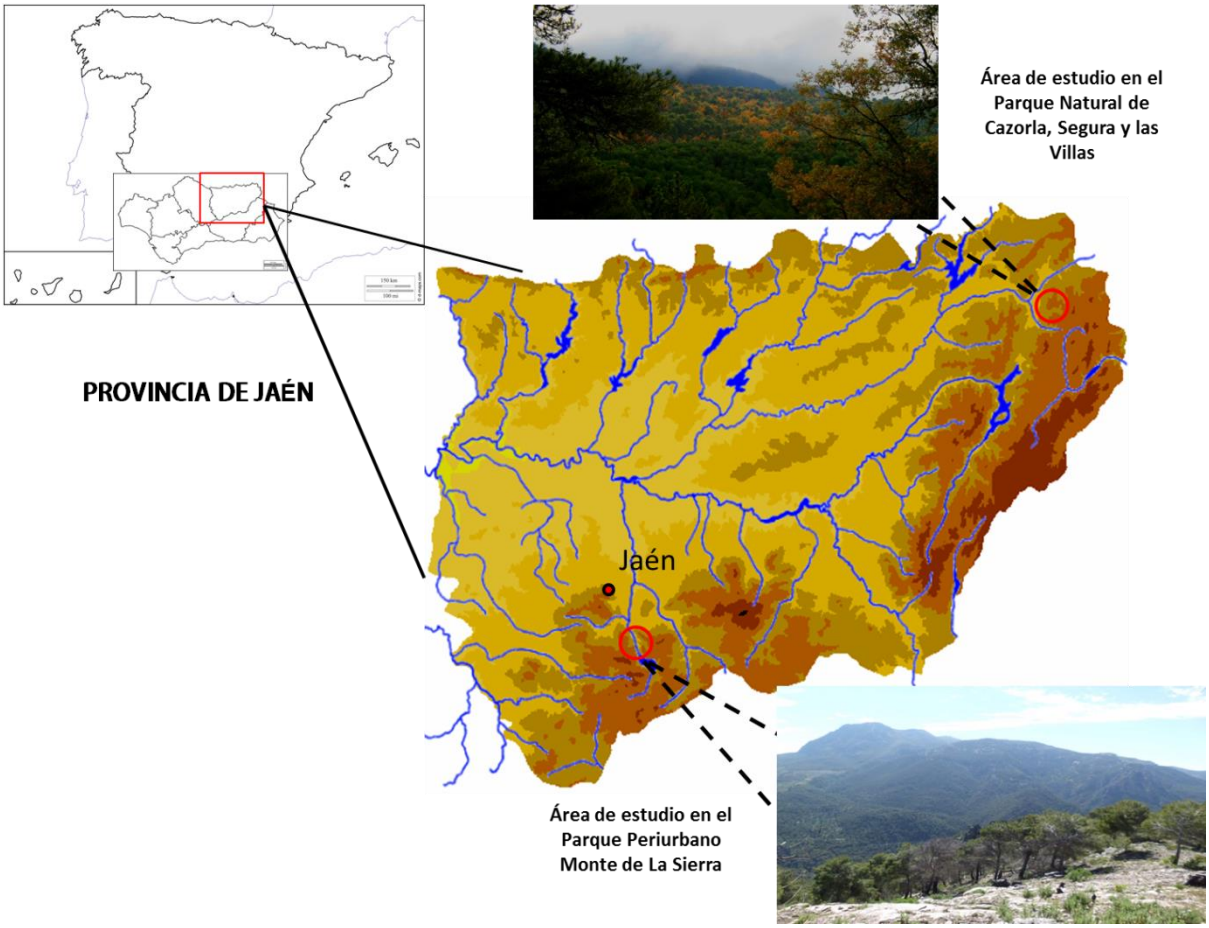

Figura 2. Situación geográfica del estudio.

Diseño espacial y metodología de muestreo

En 10 zonas de estudio se establecieron 5 parcelas de 50 x 50m (0.25ha), separadas menos de 300m entre sí y distribuidas de manera que sus condiciones ambientales fueran homogéneas. En cada zona de estudio una de estas cinco parcelas fue dividida en 25 sub-parcelas de 10 x 10m, donde además de las interacciones de reclutamiento muestreamos la cobertura de cada especie adulta. Para ello usamos una cinta métrica para las especies de matorral y realizamos una estimación visual para las especies del sotobosque y arbóreas. Las cuatro parcelas restantes fueron divididas en sub-parcelas de 25 x 25m donde sólo se muestreó el reclutamiento. En las 2 zonas de estudio restantes sólo se pudo establecer una

parcela de 50 x 50m ya que la topografía del terreno no permitió el establecimiento de 5 parcelas como en las restantes zonas de estudio. La única parcela establecida fue dividida en 25 sub-parcelas de 10 x 10m como en las otras zonas de estudio, donde también se muestreó la cobertura de las especies adultas presentes en la parcela. Para determinar las redes de reemplazamiento en todas y cada una de las anteriores parcelas recorrimos toda su área de manera intensiva buscando juveniles de especies leñosas, y anotamos si estos estaban reclutándose bajo el dosel de una planta adulta o al descubierto (cuantificando la frecuencia con la que ocurrían estas interacciones). Recorrimos un total de 13 hectáreas, donde identificamos 63 especies, un total de 26134 juveniles y 13144 interacciones de reclutamiento.

Imagen 2. Aspecto de 4 parcelas de estudio. a) Quiebrajano b) Cañada de las Azadillas c) Barranco de los Ladrones d) Nava del Espino.

Imagen 3. Método empleado para delimitar las parcelas. a) y b) Vértices de una parcela de 50 x 50 metros, c) y d) cuadrados de 25 x 25 metros delimitados con una cinta métrica.

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CHAPTER 1

Dissecting the role of transitivity and intransitivity on coexistence in competing species networks

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ABSTRACT

It is well established that intransitively assembled interaction networks can support the coexistence of competing species, while transitively assembled (hiearchical) networks are prone to species loss through competitive exclusion. However, as the number of species grows, the complexity of ecological interaction networks grows disproportionately, and species can get involved simultaneously in transitive and intransitive groups of interactions. In such complex networks the effects of intransitivity on species persistence are not straightforward. Dissecting networks into intransitive/transitive components can help us to understand the complex role that intransitivity may play in supporting species diversity. We show through simulations that those species participating in the largest group of intransitive interactions (the core of the network) have high probabilities of persisting in the long term. However, participation in a group of intransitive interactions other than the core does not always improve persistence. Likewise, participating in transitive interactions does not always decreases persistence because certain species (the satellites) transitively linked to the core have also a high persistence probability. Therefore, when networks contain transitive and intransitive structures, as it can be expected in real ecological networks, the existence of a large intransitive core of species can have a disproportionate positive effect on species richness.

Keywords: competition; ecological networks; intransitive interactions; plant community; replacement dynamics; replacement networks

INTRODUCTION

It is well established that intransitivity in competitive interactions can support the coexistence of species in a local community. Early theoretical studies on the intransitivity of interactions (Gilpin 1975; May and Leonard 1975) focused on triplets of species where species A defeats species B, B defeats C and C defeats A, like in the famous rock-scissors-paper game. Graphically pictured, the three species form the nodes of a network connected

by arrows forming a cycle. Intransitivity promotes coexistence because in a perfectly intransitive system there is not a competitive dominant. The counterpart to intransitivity is the transitive, or hierarchical, assembly of interactions, where for example species A defeats B, B defeats C and A defeats C. In terms of graphs, transitive networks lack any cycles. Transitivity in competitive interactions leads to competitive exclusion of all but the dominant competitor (Hardin 1960). Recent studies have moved forward these simple concepts from the original purely transitive or intransitive groups of competitors to complex competition networks called tournaments (Laird and Schamp 2006, 2008, 2009; Allesina and Levine 2011; Rojas-Echenique and Allesina 2011). These studies have largely confirmed the key role of intransitivity on coexistence at the community level. However, as network complexity increases, the relationship between intransitivity and species coexistence becomes less straightforward, among other things because some species may simultaneously participate in transitive and intransitive sets of interactions. For example, Laird and Schamp (2009) concluded that topological variation in competitive tournaments that is not captured by intransitivity indices can affect species coexistence. Along the same lines, Allesina and Levine (2011) found that those species that persisted in competitive tournaments were part of intransitive cycles, but membership in a cycle did not necessarily lead to persistence. Thus, when species can be involved in transitive and intransitive interactions simultaneously, which species will coexist? What will happen to species involved both in transitive and intransitive interactions? What should we expect if there is more than one set of intransitive interactions within the same network? Current studies have explored through simulations how many species may coexist in these complex networks but none has proposed explanations as to which ones will persist.

A detailed view of networks' structure can be obtained by dissecting them into transitive and intransitive components and considering how they are interconnected. Such dissection is achieved through the identification of the Strongly Connected Components (SCCs) of the network (e.g. Allesina et al. 2005; Alcántara and Rey 2012; Corominas-Murtra et al. 2013). SCCs of a directed network are the largest subsets of nodes such that there is at least one cycle connecting all members of the subset. The smallest SCC in a network can contain a single node (called a trivial SCC) and the largest nontrivial SCCs may contain from two to all the nodes of the network. By definition, nontrivial SCCs are intransitive structures. One can imagine an endless number of networks with complex arrangements of trivial and nontrivial

SCCs (e.g. Corominas-Murtra et al. 2013) but reality seems simpler. Random networks (Dorogovtsev et al. 2001), food webs (Allesina et al. 2005), networks of plant-plant interactions (Alcántara and Rey 2014), the WWW (Broder et al. 2000), metabolic networks (Zhao et al. 2006) and economic networks (Vitali et al. 2011) have the same macroscopic structure integrated basically by: (1) a core formed by the largest nontrivial SCC of the network, (2) in-component SCCs that have at least one path going to some member of the core, and (3) out-component SCCs that receive at least one path from some member of the core (see the random network example in Fig. 1). Thus, the nodes of most networks can be dissected into three basic components defined by their transitive-intransitive relations: the core (representing the largest intransitive structure) and the in- and out-components (representing network elements transitively connected to the core).

To assess the effect of network structure on species coexistence we need to combine the structure of interactions with a model of community dynamics. Most theoretical and modeling approaches to plant community dynamics conceptualize the dynamics in terms of a process of replacement of individuals by individuals: when a plant dies, another takes its place (e.g. Horn 1975; Grubb 1977; Tilman 1994; Hubbell 2001; Snyder and Chesson 2003; Allesina and Levine 2011; Myster 2012; Alcántara et al. 2015). Just like predator-prey interactions are rendered as food webs, such plant-plant replacements can be rendered in the form of replacement networks with arrows pointing from the replaced to the recruiting species, indicating that part of the space and resources that were dominated by individuals of the replaced species can eventually pass to individuals of the recruiting species. In the context of replacement dynamics (Alcántara and Rey 2012), species of the in-component are called *transient*, and the species of the out-component are called *satellites* (Fig. 1). Alcántara and Rey (2012) showed that when the replacement network represents the structure of the interactions matrix in a linear time-invariant (LTI) model, Gantmacher's theorems (1959) can be used to formulate qualitative predictions on the persistence of species as follows: each SCC has a corresponding set of eigenvalues in the interactions matrix of the LTI model; the SCC with the largest eigenvalue is the basic SCC (the core of the network is likely to be the basic SCC, especially when it is much larger than any other SCC); only the members of the basic SCC and those SCCs that receive arrows from them (i.e. the satellite species) will persist in the stable state of the model. Transient species will eventually become extinct. Therefore, the location of a species in the core, in- or out-components may determine its

possibilities of persisting in the long term, at least under LTI dynamics. However, qualitative conclusions based on LTI models cannot be directly extrapolated to non-linear models.

In this study we show that the qualitative implications of Gantmacher's theorems for LTI systems may help understanding the role of transitive and intransitive parts of a network in allowing the coexistence of competing species under nonlinear replacement dynamics. We first conduct simulations of a nonlinear model to show that it reproduces the classic results (1) that competitive exclusion occurs under purely hierarchical competition (i.e. in fully transitive networks), (2) that in the absence of dominant competitors (i.e. in fully intransitive networks), all species can coexist. Then, we apply the model to more complex networks combining transitive and intransitive structures and found that (3) species of the core and the out-component (one of the transitive components of the network) have much higher probability of persisting than species of the in-component, and (4) that networks containing just one intransitive structure allow the coexistence of more species than networks with multiple intransitive structures.

MATERIAL AND METHODS

To represent the structure of the networks in terms of its basic elements we use the proportion of transient, core and satellite species as the coordinates of each network in a ternary (mixture) plot we call the TCS triangle (for the Transient, Core and Satellite species; Fig. 1). For our analyses we built hierarchical, panmictic and random networks of S = 5, 10, 20, 50, and 100 species. In hierarchical networks (e.g. Tilman 1994) species are ordered so that individuals of the first species are able to replace individuals of any other species, those of the second species can replace individuals of all but the first species, and so on until the last species which is not able to replace any other (Fig. 1). Panmictic networks are those in which individuals of any species can replace individuals of any other species, so the network contains all the possible arrows between species (Fig. 1). Panmictic replacement is assumed for example in neutral models (Hubbel 2001). Random networks are much more complex than the hierarchical and panmictic ones because they do not have a fixed number of links per species (k) and the number of links varies between species. For each network size we developed series of networks as follows (Fig. 1): the first network in each series was a simple chain ($k = (S-1)/S$) with the direction of each arrow chosen at random; next we increased k randomly adding $0.2S$ links until reaching k = 10. As the number of links increased from the

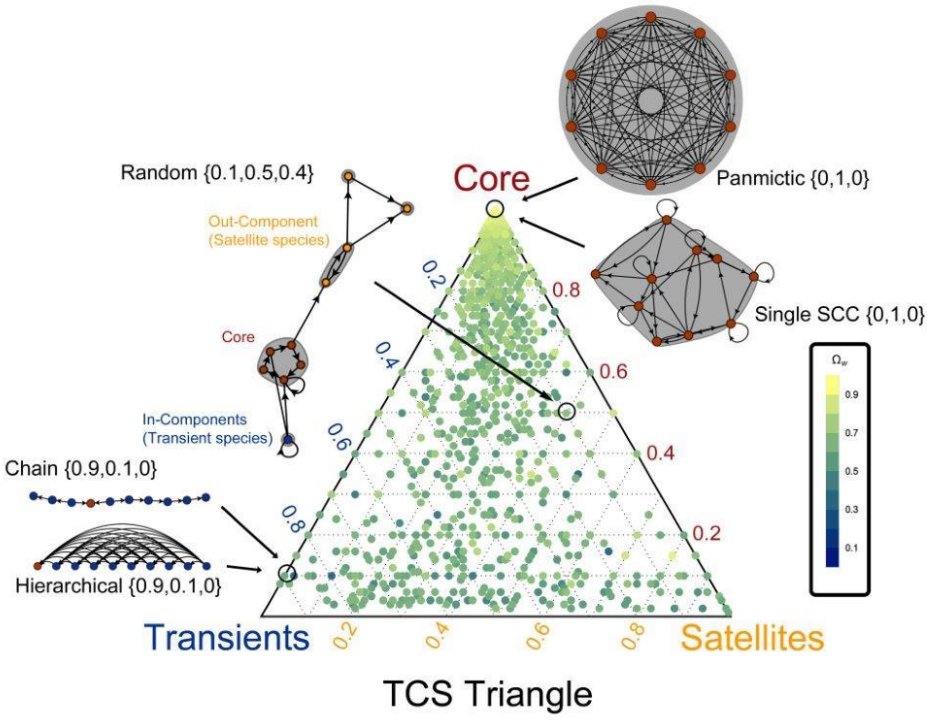

Fig. 1 Location of the studied networks in the TCS triangle. The axes of the triangle correspond to the proportion of Transient, Core and Satellite species in a network. Each point indicates the TCS composition of a network and its color indicates the mean persistence (according to Weibull replacement rates; see Methods) of species in random networks placed in the same coordinates (color scale is indicated by the bar on the right side). The figure also illustrates some examples of the studied networks. In each network, gray shaded groups of nodes indicate different SCCs. Node colors indicate that the species is transient (blue), core (red) or satellite (orange). The arrows point from the replaced to the recruiting plant, indicating that the space formerly occupied by the first is passed to the second. As the random network illustrates, interactions within non-trivial SCCs (those formed by more than one node) are intransitive by definition, but the interactions between SCCs are transitive. The higher the proportion of core species, the larger the intransitivity of the network. Panmictic networks and many random networks are formed by a single SCC which includes all the species, so they are maximally intransitive. Hierarchical and chain networks lack any cycles, so they are minimally intransitive (or maximally transitive). Most random networks have a mixture of transient, core and satellite species, so they mostly occupy inner positions of the triangle.

initial network of each series, a small number of non-trivial SCCs emerged but soon collapsed into a large core. The size of the core continued growing until it included all nodes. There is a close to 1 probability that a 100 species random network with $k > 10$ has all nodes in the core (Dorogovtsev et al. 2001), so increasing the length of the series beyond this value would not increase the range of random network structures explored.

We used the Graph and Network Analysis functions of Mathematica (v. 10; Wolfram Research Inc. 2015) to find the largest SCC (core) of a directed graph, along with the corresponding in- and out-components (satellites and transients).

Replacement dynamics model.-

We conduct simulation analyses using a compartmental model of replacement dynamics formed by coupled populations of different species (a local assemblage) which compete for space so that when one individual dies, the space it occupied passes to a new individual of the same or different species. The identity of the replacing species is determined by the structure of the replacement network. Compartmental models are based on mass balance considerations so that the dynamics of a given species "*i*" (i.e. the temporal variations in its population size, x_i), are determined by its per-capita input and output rates. A simple general formulation for a continuous time, density dependent (non-linear) model can be:

$$\frac{dx_i}{dt} = x_i \sum_{j=1}^{S} \alpha_{i,j} x_j - x_i \sum_{\substack{j=1 \\ j \neq i}}^{S} \alpha_{j,i} x_j$$

In this formulation, the instantaneous inputs and outputs depend on the replacement rates between species (α_{ij}) and the abundance of the recruiting (x_i) and the replaced species (x_j). However, although the rates of interchange between species should increase as species density increases, the rates cannot grow indefinitely, so there must be some maximum replacement rate. We introduce a saturating non-linearity in the replacement rates using a Monod equation: $\alpha^*_{ij} = \alpha_{ij}\, x_i/(x_i+K_i)$; where K_i is the half-saturation constant (the value of x_i at which the replacement rate is half its maximum). Equations like Monod have been profusely used in the study of predator-prey interactions (the Type II functional response of the predator) and also in models of competing species (Gross 2008).

Persistence.-

The qualitative expectation of persistence under LTI dynamics is that all the core and satellite species would persist (Alcántara and Rey 2012). However, this qualitative expectation is likely to overestimate quantitative estimates because it will classify a species as persisting even if its abundance were to decline to unsustainably low values. Therefore, we can use the qualitative estimate as an expected maximum potential value of persistence.

Since the systems of equations of the replacement dynamics model cannot be solved analytically, we conducted a series of simulations to determine the dynamics of species in the assemblage. We set K_i = 10 for all species and the initial state $x_i[t_0]$ = 10000 individuals of each species. For each simulation we first constructed an S x S matrix of α_{ij} values, and multiplied it by the adjacency matrix of the corresponding replacement network. We used three parameterizations of α_{ij} in the model. First we assumed fixed replacement rates by setting all α_{ij} = 0.01. As a second option, we introduced heterogeneous replacement rates by assigning random values to the α_{ij} elements between 0.001 and 0.019 from a uniform distribution. In the third case the values of α_{ij} were chosen randomly from a Weibull distribution with mean approximately 0.01. We solved numerically the system of continuous time ordinary differential equations parameterized for each simulation using the function NDSolve of Mathematica (v.10; Wolfram Research Inc. 2015). In all simulations, we estimated quantitative persistence as the proportion of species with at least one individual after 5000 time units (results using 10000 time units were qualitatively the same). In this study the replacement rates lack an explicit time unit. The time unit in a real case implementations of the model (e.g. Alcántara et al. 2015) would depend on the units in which the replacement rates were measured (replacements per year, per season, per day, per decade…), which in turn depend on the system (e.g. woody species in a forest, annual plants in a pasture, bacterial colonies in a petri dish). We will use the symbol Ωf for persistence estimated in the model with fixed α_{ij}, Ωu for persistence in the model with replacement rates following a uniform distribution, and Ωw for persistence in the model with replacement rates taken from the Weibull distribution. Besides species persistence, we also obtained the mean time to extinction of transient, core and satellite species that did not persist at the end of each simulation.

For each network we conducted one simulation with the fixed replacement rates parameterization. For hierarchical and panmictic networks of each size, we conducted 200 simulations with the Uniform and Weibull parameterizations. For each of the 13500 random networks we conducted one simulation with the Uniform and one with the Weibull parameterization. In total, we conducted 44510 simulations, but the numerical solution of the model did not converge in 1611 of the random network parameterizations, so finally our results are based on 42899 simulations.

RESULTS

Panmictic, maximally intransitive, systems are among the simplest networks from the point of view of their TCS structure. They have all species forming a maximally dense core and so, regardless of their size, they are always at the tip of the TCS triangle (point {0,1,0}; Fig. 1). These networks always allowed the coexistence of all the species, independently of the size of the network or the distribution of replacement rates (Fig. 2). Similarly, those random networks with all species forming a single SCC (thus also located at point {0,1,0} in the TCS triangle; Fig. 1) had an average persistence of more than 97% of species regardless of the size of the network and the type of replacement rates distribution. At the other extreme, hierarchical, maximally transitive, systems have a slightly wider distribution in the TCS triangle, but they are still very narrowly restricted to the lower part of the left side (the positions with coordinates {(S-1)/S, 1/S, 0}). Hierarchical networks are not capable of sustaining a large assemblage of coexisting species (Fig. 2). Under fixed replacement rates all our simulations of hierarchical networks collapsed to a monospecific assemblage. With uniform distribution of replacement rates, most final assemblages were monospecific but there were some simulations that retained up to two species. With Weibull distribution of replacement rates, the average final assemblage had two species, and there were up to six species coexisting at the end of some simulations of the larger networks.

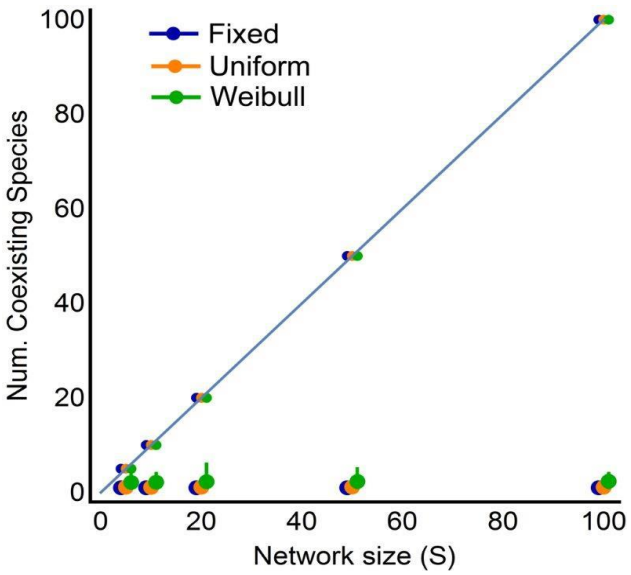

Fig. 2 Coexistence in panmictic (small points) and hierarchical networks (large points). Points and bars indicate the mean and range number of species persisting at the end of 200 simulations of each network run for 5000 time units and with three types of replacement rate distribution (Fixed, Uniform and Weibull). Some bars are shorter than the points so they do not show up in the figure. Points are slightly displaced in the x-axis for clarity. The 1:1 line indicates the maximum possible number of species that could coexist in a network of a given size

Random networks occupied most of the TCS triangle (Fig. 1), although they tend to concentrate towards the upper tip. This trend is caused by the fast growth of the core as *k* increases. The probability of persistence of a given species depended on its position within

the network: regardless of the type of replacement distribution and size of the network, core and satellite species had the largest probability of persistence always, while transient species had significantly lower probability of persistence, especially in larger networks (Fig. 3a, Table 1A). The shortest mean time to extinction corresponded to transient species, while satellite species had the longest time to extinction (Fig. 3b, Table 1B). Mean times to extinction of transient species were always longer in simulations conducted under Weibull than under fixed or uniform replacement rates and decreased with network size (Fig. 3c, Table 1C). Independently of the type of distribution of replacement rates, persistence varied non-linearly with the number of nontrivial SCCs, so that networks with just one nontrivial SCC had higher persistence than networks with none or more than one nontrivial SCC (Fig. 4, Table 1D).

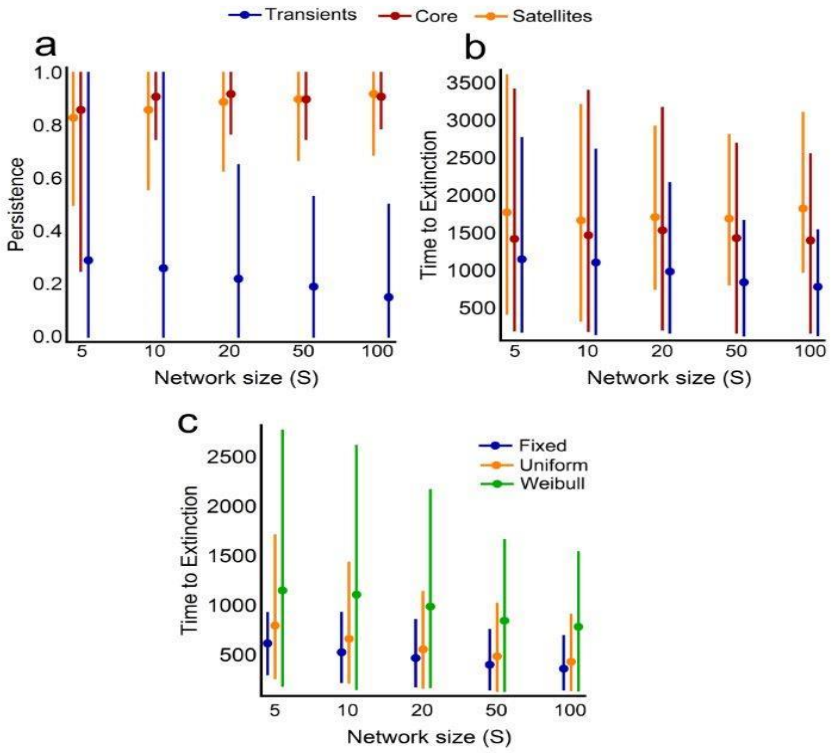

Fig. 3 Average persistence and time to extinction in random networks of 5, 10, 20, 50 and 100 species. Panels a) and b) show the persistence and mean time to extinction of transient, core and satellite species corresponding to simulations using Weibull replacement rates. Simulations using fixed or uniform replacement rates rendered very similar patterns but with stronger differences between groups. Panel c) shows the mean time to extinction of transient species under fixed, uniform and Weibull replacement rates (the same patterns were observed for core and satellite species). In all panels, bars spanning from percentile 10 to 90 are provided to show the variability among different runs of the simulations. Statistical analyses of these data are provided in Table 1A-C. Points are slightly displaced in the x-axis for clarity.

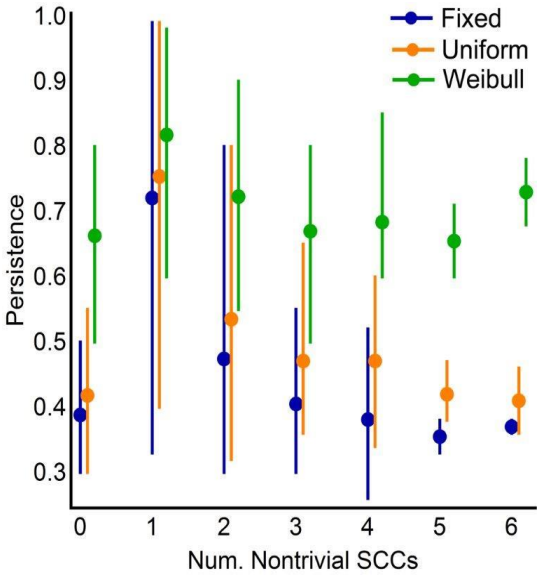

Fig. 4 Variation of mean persistence with the number of non-trivial SCCs in a network. Networks with zero nontrivial SCCs are fully transitive. Networks formed by a single SCC (i.e. fully intransitive networks located at the {0,1,0} coordinates of the TCS triangle) were excluded from the calculations, so that all the estimates correspond to networks with core, transient and/or satellite species. Including {0,1,0} networks would increase the mean persistence of networks with one nontrivial SCC to values around 0.92 in all cases. Bars span from percentile 10 to 90. Statistical analyses of these data are provided in Table 1D. Points are slightly displaced in the x-axis for clarity

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Dependent variable	**Multivariate factor**	**Multivariate Effect**[1]	**Network size**	**Effect x Network size**[1]
A) Persistence	Species type	F(2,2045) = 3288.23; P < 0.001	F(4,2046) = 1.72; P =0.14	F(8,4090) = 14.94; P < 0.001
B) Time to extinction	Species type	F(2,442) = 32.04; P < 0.001	F(4,443) = 0.41; P = 0.8	F(8,884) = 0.88; P = 0.53
C) Time to extinction of transient species	Parameterization	F(2,2867) = 789.84; P < 0.001	F(4,2868) = 47.81; P < 0.001	F(8,5734) = 8.83; P < 0.001

Dependent variable	**Multivariate factor**	**Multivariate Effect**[1]	**Number of non-trivial SCCs**	**Effect x Num. non-trivial SCCs** [1]
D) Persistence	Parameterization	F(2,4701) = 54.78; P < 0.001	F(6,4702) = 334.19; P < 0.001	F(12,9402) = 69.09; P < 0.001

[1]Wilks test. Results of Pillai's, Hotelling and Roy's multivariate tests were qualitatively the same.

Table 1. Results of MANOVA tests comparing persistence or time to extinction across types of species (transient, core and satellites) or among types of parameterization (fixed, uniform and Weibul replacement rates) obtained for random networks. A) Comparison of persistence (from simulations parameterized with Weibul replacement rates) of satellite, core and transient species across network sizes. B) Comparison of time to extinction (from simulations parameterized with Weibul replacement rates) of satellite, core and transient species across network sizes. C) Comparison of time to extinction of transient species calculated from different parameterizations (fixed, uniform and Weibul) across network sizes. D) Comparison of network persistence calculated from different parameterizations (fixed, uniform and Weibul) for networks with different number of non-trivial SCCs.

DISCUSSION

Fully transitive and fully intransitive networks as extreme cases

One of the major challenges in community ecology is understanding how such complex systems allow the coexistence of multitude of competing species. The early view of competition as a force driving plant community dynamics was that if all plant species rely on the same resources, even the slightest differences in resource use efficiency between species would lead to the competitive exclusion of all but the best competitor (Hardin 1960). Not surprisingly, our analyses of hierarchical replacement networks agree with this expectation. Similarly, spatially-explicit model analyses conducted by Laird and Schamp (2006) and Rojas-Echenique and Allesina (2011) also found that maximally transitive tournaments could sustain only one or two species regardless of the conditions of their simulations. Therefore, our findings reinforce the idea that strictly hierarchical competition systems are prone to lose most of their diversity. At the other extreme, panmictic networks, which are maximally intransitive, allow the coexistence of all the species. The assumption of panmictic replacement is present in lottery models (Chesson and Warner 1981) and neutral models (Hubbell 2001). Our results agree with these models in that panmictic replacement may allow coexistence in species rich communities. Interestingly, Gross (2008) found that a strictly hierarchical system can sustain most species if positive interactions are incorporated so that superior competitors confer benefits to inferior competitors. This addition introduces cycles, and thus intransitivity, transforming the initially hierarchical network in a panmictic one, what would explain the improved coexistence.

Although accumulated evidence suggests that these theoretical results for hierarchical and panmictic interactions between competitors are solid, we are far from knowing the structure of real competitive networks, so we do not know yet how commonly competitive interactions assemble intransitively or transitively in nature. It is not clear whether strict hierarchies of competitors exist in plant communities. Keddy and Shipley (1989) concluded that transitive hierarchies are common among herbs, however Soliveres et al. (2015) recently concluded that intransitivity is widespread in natural plant communities. A similar state of things occurs concerning competition in marine benthic communities (Quinn 1982, Chadwick and Morrow 2011) or in bacterial communities (Wright and Vetsigian 2016, Kirkup and Riley 2004). On the other hand, the existence of neutral (panmictic) competition has not been studied explicitly in real plant communities. However, there is circumstantial evidence

against it. For example, many studies have found that adult trees can filter the set of species that can grow beneath their canopy, thus limiting the set of species that could eventually replace them (Janzen 1970; Uriarte *et al*. 2010; Inderjit et al. 2011; Lebrija-Trejos et al. 2014). Indeed, the few replacement networks described to date (Alcántara and Rey 2014) contain transitive (transient and satellite) and intransitive (core) elements. Consequently, as noted by Laird and Schamp (2009), network complexity cannot be captured by a simple measure of intransitivity. All this evidence suggests that understanding the conditions allowing species coexistence requires understanding how transitive and intransitive structures combine within real networks. There is a universe of possibilities between the strictly hierarchical and the panmictic networks, so no wonder if real networks are somewhere in between.

Dissection of networks into transitive and intransitive components

Our results confirm that Gantmacher's theorems for LTI systems can provide guidance to understand the complex relationships between transitive/intransitive structures and species persistence also in nonlinear competition models. Accordingly, being part of the core or being a satellite species confers a high probability of persistence. Core species are involved in an intransitive cycle, so there is not a competitive dominant. In the case of replacement dynamics, this core implies that the space occupied by one species can be exploited in the future by individuals of any of the other species of the core. The consequence of this condition is that core dynamics approach those of panmictic networks even though interactions within the core can be far from panmictic. The core performs like a self-supporting system that could persist in the absence of transient or satellite species. It must be noted however that this conclusion assumes that the core plays the role of the basic SCC (we say *plays the role* rather than *is the* basic SCC because the concept of eigenvalue of a LTI system cannot be directly applied in nonlinear systems; Halás and Moog 2013). If a satellite species played the role of the basic SCC (for example by combining an outstanding longevity with a high rate of replacement of all other species, and also conspecifics), it would drive to extinction all core and transient species. However, if a transient species could play the role of the basic SCC, it would contribute to the persistence of the core and satellite species. In any case, it is highly likely that the core plays the role of the basic SCC as its size is much larger than the size of any other SCC (Alcántara and Rey 2012).

The persistence of satellite species emerges simply from their reliance on core species for recruitment. Although this result is not surprising, its implications in terms of the role of transitive interactions on coexistence have not been noted before. Many theoretical studies have demonstrated that species participating in intransitive interactions (i.e. the rock, the scissors and the paper of the famous game) can coexist. Our results show that intransitive interactions can accrue extra benefits for biodiversity maintenance beyond the coexistence of the species participating in the intransitive group, because satellite species, although transitively linked to the core, have also increased probabilities of persisting. Interestingly, this result also implies that participating in a transitive (hierarchical) set of interactions does not necessarily lead to competitive exclusion; it all depends on the position of this transitive set relative to the core. In the few replacement networks studied to date, including communities from Southern Spain, Northern Morocco and Mexico (Alcántara and Rey 2014), most species are core or satellites. If this finding were generalizable to other communities, our results would imply that the long term coexistence of most plant species in a local assemblage could be supported by the existence of a core of species interacting intransitively.

A corollary of these results is that species richness in stable communities can increase through the addition of core or satellite species. In the first case, there would be a positive relationship between richness and intransitivity, while in the second case the relationship would be negative. Therefore, again, a simple index of intransitivity may not capture the intricacies of how intransitivity affects coexistence. For example, in the study of Soliveres et al. (2015) the index of intransitivity explained less than 10% of variance in species richness. It would be interesting to assess whether a more detailed consideration of the structure of interactions as we propose could help explain a larger portion of the variability in their datasets.

Counterintuitively, even though intransitive interactions in the core can sustain a large part of the community, the presence of separate intransitive elements in the network can decrease general persistence. This result resembles the finding of Allesina and Levine (2011) that membership in a cycle did not necessarily lead to persistence in competitive tournaments. It also agrees with the decreased qualitative persistence with increased modularity found in replacement networks (Alcántara and Rey 2012). To understand this result we can resort to the qualitative estimates of persistence. Suppose that a network

contains a single nontrivial SCC with *n* species, all of which can potentially persist. Suppose now a compartmented network with the *n* species distributed in two equally sized SCCs. In this compartmented network the *n*/2 species of the core can persist but the *n*/2 species of the other SCC may or may not persist depending on whether it is located as satellite or as transient relative to the core. Thus, if both SCCs have the same probability of playing the role of the basic SCC, the maximum potential persistence in this compartmented network will be either 100% or 50%, so the average persistence across simulations will be lower than in the not compartmented network. According to this result, we would expect that stable systems have a single nontrivial SCC. The fact that food webs (Allesina et al. 2005) and replacement networks (Alcántara and Rey 2014) have a single core may be explained by the higher persistence conferred by this configuration to the members of the network. But the single core may emerge through processes of random assembly (Dorogovtsev et al. 2001). Therefore, the emergence of persistence in complex communities does not necessarily require the existence of finely tuned ecological assembly mechanisms because randomly assembled networks are likely to have high persistence. However, replacement networks seem to have lower persistence than randomly assembled networks with the same size and density of links (Alcántara and Rey 2012), what suggests that nonrandom (niche-dependent) assembly mechanisms operating in real communities may counter the stabilizing effects of stochastic assembly processes, decreasing the maximum potential persistence of species in the community.

CONCLUDING REMARKS

The complex nature of transitive and intransitive interactions in a network cannot be subsumed into a single index. We have shown that dissection of these networks into their transitive and intransitive components provides new interpretations of the role of intransitivity on the coexistence of competing species. Particularly, we show that intransitivity may sustain the persistence of more species than those participating in the intransitive set (core) of interactions, thus having a disproportionate impact on biodiversity by inflating the number of species that can coexist in a complex community.

Our results show that the analysis of ecological interaction networks based on SCCs opens new ways to improve our knowledge on the role of complex network structure on community dynamics. In Alcántara and Rey (2012) we showed how the structure of

interaction networks conditions the qualitative behavior of the community in terms of species persistence under LTI dynamics. In the present study we extend these findings to the qualitative dynamics of NLTI systems, and place the analysis of complex network structure in the context of studies on the intransitivity of interaction networks. So far we have addressed the relationships between network structure and species persistence using deterministic mean field models of community dynamics. Thus, there are many avenues for future developments. Particularly, it will be important to explore whether the persistence of transient, core and satellite species may vary under spatial constraints (e.g. local vs. global replacement, metacommunity structure) and stochastic interactions of the type implemented in individual based models of competitive communities.

ACKNOWLEDGEMENTS

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CHAPTER 2

Effects of sampling effort on estimates of the structure of replacement networks

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ABSTRACT

Aims: Important aspects of plant community dynamics depend on interactions between plant species which affect the processes of recruitment and the replacement of dead individuals by new ones. These interactions blend in the replacement network of the community. The qualitative and functional structure of replacement networks can provide insights on community stability properties. The goal of this study was analysing how sampling effort affects estimates of different descriptors of replacement networks.

Location: Mixed Pine-Oak forests in Southern Spain.

Methods: We sampled the replacement networks of nine forest patches. In each forest we surveyed 16 plots of 25 x 25m, locating all the saplings of woody species and noting whether they were recruiting under the canopy of some plant species or in open interspaces. Using the replacement networks from the plots of each forest we constructed curves relating network descriptor estimates to sampling effort. Additionally we calculated the completeness of basic network elements (number of species and interactions) using rarefaction and extrapolation techniques.

Results: Number of species (*S*) and connectance (*C*) stabilized within our range of sampling effort. The estimates of *S* reached completeness values above 97%. However, the number of interactions in the network (*L*) and the mean number or interactions per species (*k*) did not stabilise although the estimates of *L* reached completeness values above 86%. With few exceptions, the parameters describing the functional structure of the network and those related to community stability stabilized within our range of sampling effort.

Conclusions: Our results suggest that most replacement networks descriptors in the studied forests can be reliably estimated from samples of around 1 ha. Since plots used in forest ecology are commonly around that size, replacement network monitoring can be easily incorporated in forest ecology studies as a highly cost-effective tool to explore community dynamics. The reliability of replacement network estimates will allow both a solid foundation for further developments and also a straightforward comparison of results obtained from multiple communities.

Keywords: Replacement networks; sapling recruitment; plant community; forest dynamics; ecological networks; competitive interactions; facilitation; strongly connected components;

Nomenclature: Blanca et al. 2011

Abbreviations. RNs: Replacement Networks; SCCs: Strongly Connected Components

INTRODUCTION

The dynamics of plant communities are frequently interpreted as a series of replacement events: when any one plant individual dies, a gap is created and a new individual (of the same or another species) ultimately can take its place (Van Hulst 1992; Pacala et al. 1996; Hubbell 2001). More realistically, one or more individuals can grow in the space released by the death of a plant, or by the fall of a branch or a shoot, so replacement events do not involve only one-by-one individual replacements nor do they require the death of the whole plant (Grubb 1977; Myster 2012; Alcántara et al. 2015). Similarly, replacement events in theoretical models are most often thought as involving plants of similar size (e.g. trees replace trees and herbs replace herbs) but this is not necessarily the case. When a tree falls, the space it occupied can become dominated in the short term by shrubs or small trees, and when a tree recruits under a tussock grass, it will eventually replace the grass. Beyond these details, the crucial question is: which plant species will replace each dead individual (or branch or shoot) occupying the vacated space? The answer to this question will determine the dynamics of change in the community and the possibilities for the stable coexistence of a rich and diverse assemblage of species. In the case of long lived plants we cannot observe directly these replacement events because the process is extremely slow. Studies of replacement dynamics have assumed that the replacing species will be one of those able to recruit under individuals of other species (Horn 1975; McAuliffe 1988; Alcántara et al. 2015). Studying which plant species recruit under which others in a local assemblage can help to describe replacement networks (RNs hereafter; Alcántara & Rey 2012, 2014).

The recruitment of a species is inextricably influenced by the presence of other plant species in the community. For example, there is ample evidence that the probability of recruitment of a given species depends on the species under which the

seeds are deposited (Rey & Alcántara 2000; Traveset et al. 2003; Gómez-Aparicio 2008). At the small spatial scale of individual plants, already established individuals withhold most of the resources (both above and below ground) and can modify the biotic and abiotic microenvironment at ground level where recruitment of new individuals should take place. Such dominance on resources and microenvironmental modifications can have positive, negative or neutral effects on the recruitment of different species (George & Bazzaz 1999; Inderjit et al. 2011; Van der Voorde et al. 2011). Once a plant has successfully recruited under the canopy of another plant, it can eventually replace the canopy species via a number of interactions (neighbour effects) that can be negative, neutral or positive, depending on the identities of the overstory species and particular disturbance regimes (e.g., Frelich and Reich 1999). Further, some neighbour effects can change over time from facilitative to competitive (Valiente-Banuet & Verdú 2008; Verdú et al. 2010), especially in drier environments or when some plants act as a seed attractors initially but as competitors later (e.g., Álvarez–Yépiz et al. 2014; Dovciak et al 2005). But RNs do not focus on the types of interactions or mechanisms that affect recruitment, such as competition, facilitation, seed predation, seed dispersal, pest attraction, shading or allelopathy. Rather, RNs place the focus on whether the combined outcome of these complex mechanisms is allowing or preventing the recruitment of a given species under another. By analogy, food webs focus on whether a species feeds on another, it does not matter which factors (or mechanisms or processes) may explain why a predator feeds on particular preys but not on others. Obviously, understanding the role of different mechanisms involved in recruitment is key to understand community dynamics (Grubb 1977), but important aspects of community dynamics can be ascertained simply through the study of RNs (Alcántara and Rey. 2012).

Replacement networks belong to the wide group of ecological networks, such as food webs (Dunne et al. 2002; Dunne 2009), plant-pollinator (Olesen et al. 2006, 2007), plant-seed disperser (Bascompte & Jordano 2014), host-parasitoid (Wilson et al. 1996; Muller et al. 1999), and plant facilitation networks (Valiente-Banuet & Verdú 2013). In the context of plant facilitation networks, the plants under which others recruit are called “nurse species”, denoting that the interaction is positive or necessary for the recruiting plant. However, in the context of RNs we will use the more neutral

term “canopy species” or “canopy plant”, which does not suggest a necessarily positive, negative or neutral interaction between plants. The canopy plant dominates the space (and the resources it contains) in the spot where another plant could recruit. In the same way as prey populations in food webs transfer biomass or energy to predator populations, in RNs the canopy plant population may transfer part of the space it dominates to the populations of the species recruiting beneath. This transference can occur when individuals of the canopy plant population die or lose part of their structures (e.g. branches or shoots lost after strong winds or through the action of pests or large herbivores).

The dynamics of a local plant assemblage are largely determined by the rates at which space is transferred between canopy and recruiting species (e.g. through their different death, growth and recruitment rates) and by the way canopy-recruit pairs are structured in the RN (Alcántara et al. 2015). Although the relative importance of interaction rates (or interaction strength) and network structure in determining community stability properties are still subject to debate (Williams 2008; Wootton & Stouffer 2015), the intuitive idea that there must be some connections between network structure and community dynamics underlies studies of ecological networks (Pascual & Dunne 2006; Thompson et al. 2012; Alcántara & Rey 2012). To firmly ground this connection, recent works with food webs and pollination networks have not only described the structure of the networks, but also have addressed how sensitive this structure is to sampling effort (Nielsen & Bascompte 2007; Chacof et al. 2012; Rivera-Hutinel et al. 2012). All of them found that sampling intensity does not affect all network metrics in the same way, and they encourage researchers to continue investigating to obtain information on the robustness of network metrics against sampling effort and to control such effects in the search for reliable patterns.

The study of community-level networks depicting interactions between plants during recruitment is slowly developing since the seminal studies by Verdú and Valiente-Banuet on facilitation networks (Verdú & Valiente-Banuet 2008; Verdú et al. 2010). Our recent studies have shown that the analysis of RNs can provide important insights into plant community dynamics and stability (Alcántara & Rey 2012, 2014). For example, we found that RNs of real plant communities are structured as a large group of interconnected species accompanied by much smaller groups that most often

consist of a single species. This structure of real RNs differed from what could be expected if RNs were constructed by randomly assembling interactions. Although the structure of real RNs could allow the persistence of most of the species in the community in the long term, the non-random structure of real RNs implies a potentially higher number of species extinctions than in randomly assembled networks. Learning the lessons from the historical development of food web studies, at this early stage in the study of RNs is fundamental addressing the possible sampling limitations that might handicap further developments. In this study we explore how sampling effort affects the estimates of 13 descriptors of RNs of mixed pine-oak forests from Southern Spain. We obtained RNs from local assemblages using 16 sampling plots of 625m^2 distributed through continuous and homogeneous areas of forest. Our results indicate that most networks descriptors were reliably estimated from plots covering around 1 ha, which is a size commonly used in forest ecology. Thus, replacement network monitoring could be easily incorporated in forest ecology studies as a highly cost-effective tool to explore important aspects of community structure and dynamics.

MATERIALS AND METHODS

Study area

The study focused on three types of mixed pine-oak forests from Southern Spain: mixed forests of *Pinus halepensis* and *Quercus ilex*, mixed forests of *P. halepensis* and *Q. faginea*, and mixed forests of *P. nigra* subsp. *salzmanii, Q. faginea* and *Q. pyrenaica*. The upper canopy of these forests is dominated by pines, with oaks representing the second most abundant trees, and other tree species much scarcer. The woody species richness of these forests is concentrated in the understory of subcanopy trees (e.g. *Crataegus monogyna* and *C. laciniata, Juniperus communis* and *J. oxycedrus, Sorbus aria* and *S. torminalis, Prunus mahaleb, Ilex aquifolium, Olea europaea* var. *sylvestris, Phillyrea latifolia* and *P. angustifolia*, *Pistacia lentiscus* and *P. terebinthus*), and the shrub layer that includes species of *Thymus*, *Cistus*, *Phlomis*, *Genista* and *Rosa*, among others (a complete list of species and the location of study sites is presented in Table S1). The current distribution of these forests in Southern Spain is restricted largely to areas where annual rainfall levels are above 800mm and mean temperature is

between 10-12°C, with deep soils in isolated areas with historically low human population density where vegetation has escaped severe fire and human management.

The study sites were located in the protected areas of Parque Periurbano Monte la Sierra (37.64°N, -3.73°W), for mixed forests dominated by *Pinus halepensis*, and Parque Natural de las Sierras de Cazorla, Segura y Las Villas (38.29°N, -2.57°W) for mixed forests dominated by *P. nigra* subsp. *salzmannii*. Within contiguous forest areas of these nature reserves we located forest patches belonging to their corresponding types of mixed forests. We used aerial photographs from 1956 onwards to select nine forest patches that have not been affected by fire for more than 60 years, and that were located more than 500m from each other. Within each forest patch we set 16 plots of 25 x 25m (totalling 1ha), distributed so that their physiographical conditions (slope, aspect, soil depth, elevation) were as homogeneous as possible. The plots within a forest patch were 300m from each other.

Sampling design

We obtained the RNs of 144 plots. Within each plot we systematically looked for saplings of every woody species and noted whether they were recruiting under the crown of a canopy plant species or in open interspaces. The minimum and maximum sizes of saplings depend on the species (note that each network can include a wide range of plant sizes, from small shrubs like *Thymus* to large canopy trees like pines). Thus, rather than setting strict size limits to classify a plant as a sapling, we defined a sapling as a plant older than a seedling, that did not bear signs of stunted growth, and that did not reach the minimum size of reproductive individuals of its species, which was determined from observation of small individuals that showed signs of having already reproduced, like hanging dried fruits, flowers, or their pedicels. Furthermore, since we were interested in recruitment processes, only free standing recruits were considered. Thus, in the case of species with sprouting ability we took care not to count vegetative sprouts as recruits. Likewise, we decided not to include in the study rhizomatous or stoloniferous species whose young plants cannot be nondestructively distinguished from vegetative sprouts; in the study area these species are mostly vines (like *Rubia*, *Clematis*, *Lonicera, Smilax* or *Hedera*) which covered less than 1% of the surface in the studied plots. Operationally, we considered that a sapling was recruiting

under a canopy plant when the recruit was growing less than 0.5m from the base of the trunk of a plant and/or the lowest branches of the canopy plant were less than 1.5m above the recruit. Otherwise, we considered that the plant was recruiting in open interspace. These criteria are based on observation of the patterns of aggregation of juveniles around the trunks of dead canopy plants (personal observation), and represent a compromise decision since every pair of canopy and recruiting species could require different limits. In any case, the logic behind these criteria is that the strength of direct interactions between plants (e.g. facilitation, competition) should decrease with increasing physical distance between them. All the interaction data were collected throughout years 2013 and 2014.

Replacement Network description

Replacement networks are directed graphs, so interactions are depicted by arrows directed from the node representing the canopy species to the node representing the recruiting species (Fig. 1). More precisely, nodes represent the populations of the different species occurring in an assemblage plus a node representing open spaces. The open spaces node accounts for the fact that some plant species recruit in spaces not dominated by other plants (e.g. shade intolerant species), but for simplicity we will refer to all the nodes as species. Open spaces can be colonized (replaced) by plants, so the node representing open spaces can send arrows to some species. However, this open node does not receive any arrows because replacement dynamics models assume that the space released by a dead plant does not remain vacant but is colonized by a new plant. Thus, plants are never replaced by open spaces in RNs. The arrows in ecological networks represent pairwise interactions between populations. In a RN such interactions can be interpreted from an operational perspective or from a community dynamics perspective. From an operational perspective, interactions in a RN indicate simply that individuals of a given species have been observed recruiting under the other. From a community dynamics perspective, the interactions in a RN can be interpreted in two main ways: as flows of resources or as replacement probabilities. Taken as flows (e.g. Alcántara et al. 2015), arrows can be interpreted as indicating that part of the resources dominated by individuals of the canopy species can eventually pass to individuals of the recruiting

species population when individuals of the canopy species die or lose part of their branches or shoots (e.g. during storms or by herbivores). Taken as replacement probabilities (e.g. Alcántara & Rey 2012), arrows can be interpreted as indicating that individuals of the recruiting species have a non-zero probability (i.e. they can have a chance, even if it is small) of replacing dead individuals of the canopy species or of growing in the space released by a fallen branch or stem of the canopy plant.

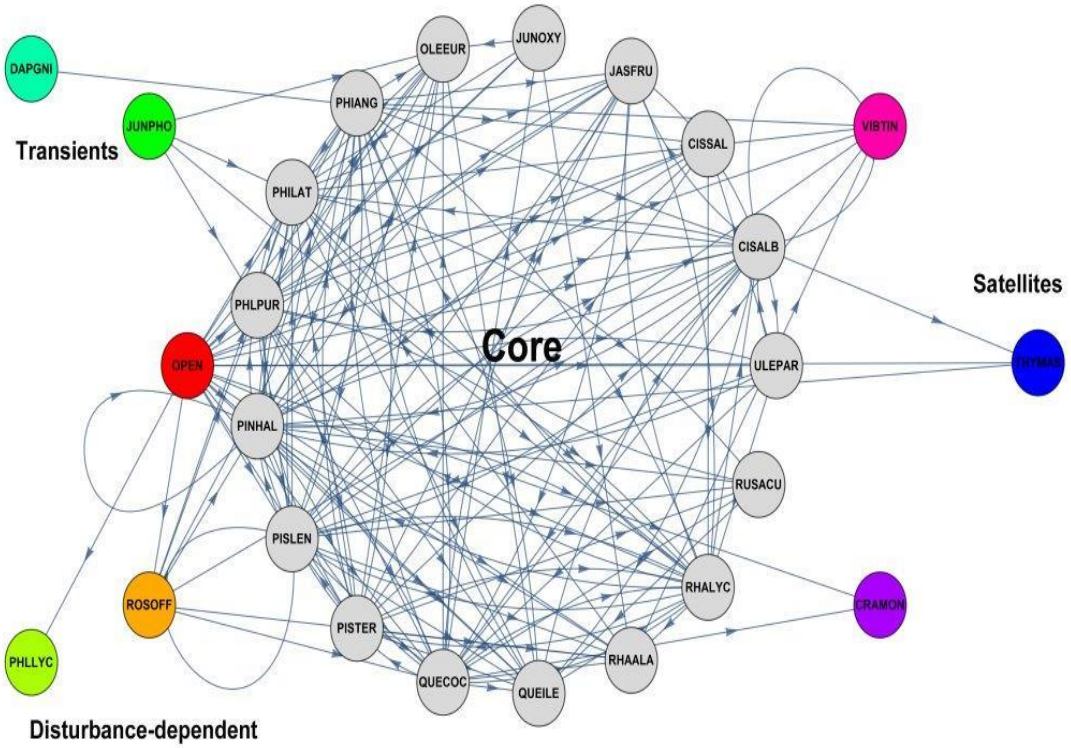

Fig.1. Replacement network of one of the studied forests (Cañada de las Azadillas Alto). Each node represents a species or 'open' space (species acronyms are the three-first letters of genus and species; full species names are given in Appendix S1). Nodes with the same colour belong to the same strongly connected component (see Methods). Arrows point from the canopy to the recruiting species. Self-loops represent species that can recruit under the canopy of conspecific plants. In the studied networks there is one large group of species (in grey) which forms the 'core' of the network. Within the core one can go back and forth from any node to all other nodes following the directions of the arrows. Species that send arrows to the core are transient species, which can be divided in: disturbance-dependent transients (in the lower left side of the graph), if they receive a path only from node open, or strictly transients if they do not receive a path from the node open or from the core (in the upper left side of the graph). Finally, nodes that receive a path from the core but do not send a path back to it, are classified as satellite species (placed to the right of the core).

For each network we obtained three sets of properties describing its basic elements, functional structure and stability. The basic elements of each network describe its size and density of interactions. The most general metrics used are network size (*S*), which is the number of species observed in the assemblage, total number of interactions observed in the assemblage (*L*), mean number of interactions per species (*k*) and connectance (*C*) which indicates the proportion of recruitment interactions relative to the maximum possible in the assemblage. The maximum number of interactions would be S^2 if each species recruited under all others. However, under the assumption that plants are never replaced by open spaces, the maximum possible number of interactions in a RN is $S^2 - S$. If the recruitment of a species were not affected by the presence of others, we would find all species recruiting under all others (what we call "panmictic replacement"), although probably some canopy-recruit pairs would not be formed just by chance or some would not be detected, so there would be a very high connectance. On the contrary, if species posed strong constraints on the recruitment of other plants under their canopy, then we would find small connectance.

Descriptors of the functional structure consider the qualitative role of each species in the dynamics of the assemblage under a replacement dynamics model. In turn, the stability properties describe qualitatively how the size of the assemblage would change under a replacement dynamics model subject to different disturbances. Full details on the definition and estimation of the functional structure and stability descriptors of replacement networks were provided in Alcántara & Rey (2012). To define the components of the functional structure we first find the Strongly Connected Components (SCCs) of the network. SCCs are groups of nodes such that there is a path from node *i* to node *j* and from *j* to *i* for every pair of nodes belonging to the same SCC (e.g. the core in Fig. 1). In terms of replacement dynamics, SCCs can be described as groups of species such that the space occupied by individuals of one of them can be occupied in the future by individuals of any of the species of the same group. Each species belongs only to one SCC. In a network with *S* species, the number of species within a SCC can range from 1 (in so-called trivial SCCs) to *S* (in non-trivial SCCs). The largest SCC forms the core of the network (the grey nodes in Fig. 1). The description of the functional structure is based on the position of species relative to the core and to

the open node, what determines their possibility of persistence in the long term. By definition, the node corresponding to open interspaces is a trivial SCC. All the species within the core can replace each other directly or indirectly through cycles of replacement. In models of replacement between competing species based on linear time invariant (LTI) dynamics (e.g. Horn 1975; Alcántara & Rey 2012; Ulrich et al. 2014), core species can be expected to coexist in the long term. By definition, the open node can only persist if new disturbances create open interspaces. The open node would disappear in the absence of disturbance, meaning that plants would cover all of the space available. We can classify the rest of the nodes as follows: strictly transients, disturbance-dependent transients and satellites (Fig. 1). We define a species as a satellite (Sat) when there is a path from the core to the species but not the other way around. Satellite species can persist in the long term because they will always have recruitment spaces available (i.e. spaces under the canopy of core species). Disturbance-dependent transient species (ddT) recruit only in open spaces, so they can only persist as long as there are recurrent disturbances creating new open interspaces. In this case, there is a path from open to these species but not from any core or satellite species. Lastly, strictly transient species (T) may not be able to recruit in the mature community (e.g. some species may require severe disturbances, like fire, to germinate and recruit). In this case, there may be a path from these species to the core, or the species may be totally disconnected from the rest of the network.

Finally, we obtained measures of network stability following Alcántara & Rey (2012) based on the structure of the networks: persistence and robustness. Persistence is the proportion of species that would be able to coexist in the long-term under LTI dynamics in the absence of disturbance (proportion of core plus satellite species). Robustness measures the number of species in the network that would become extinct if another species were removed from the community (because they would lose all the suitable canopy species to recruit under). The number of such secondary extinctions is the length of the secondary extinctions cascade. Error sensitivity (ES) is a measure of robustness that indicates the mean length of the cascade of secondary extinctions, and Attack sensitivity (AS) is the maximum length of the cascade of secondary extinctions.

Effect of sampling effort

Completeness of basic network elements.- The basic elements of a network are its nodes and links. There is a long tradition in estimating the true number of species (*S**) in a community from incomplete surveys through different rarefaction and extrapolation techniques (see Gotelli & Chao 2013 and references therein), but there are not specific procedures to estimate the true number of links (*L**). Following other authors (Olesen et al. 2011, Chacof et al. 2012), we will apply the same rarefaction and extrapolation techniques to both *S* and *L*.

In most empirical studies, *S* is an incomplete estimate of *S** because rare species can be easily missed. The fraction of *S** represented in *S* is called sample coverage, and is a measure of the completeness of a survey. Contrary to intuition, information theory demonstrated that coverage can be accurately estimated from a sample, even when *S** can be much more difficult to estimate (see Chao et al. 2014 and references therein). Recently, Chao et al. (2014) have developed procedures to estimate a sample coverage curve as a function of sample size for incidence data. For a reference sample of *n* plots, curves generated through this procedure include an intrapolation part (covering from 1 to *n*-1 samples) and an extrapolation part (from *n*+1 to 2*n* samples), which join smoothly at the point of the reference sample (*n*). With such a curve, ecologists can objectively quantify the sample completeness for any incomplete incidence (i.e. presence/absence) data sets. We use these curves to determine the values and completeness of *S* and *L* at different levels of sampling effort and to estimate the number of samples needed to reach 95% of the asymptotically estimated *S** and *L**. Finally, we derive curves for network *C* and *k* from the curves of *S* and *L*. In this way we can evaluate the effect of completeness of *L* and *S* on estimates of *C* and *k*.

Reliability of functional structure and stability descriptors.- There are not procedures to evaluate the completeness of functional and stability network descriptors or to extrapolate their values beyond the values observed in the reference sample. Alternatively, we follow the classic approach of building curves of cumulative sampling effort vs. estimated values, as it has been used in several studies of networks of food webs (e.g. Martínez et al. 1999) and pollinators (e.g. Nielsen & Bascompte 2007). The

procedure to obtain these curves was as follows. For a given forest, we chose a random permutation of its 16 plots. Then, we estimated network descriptors from the first plot in the sequence. Next, we combined the information from first and second plots into a common RN and obtained the new network descriptors. This process was repeated combining one more plot at each step until all 16 plots were combined forming the forest patch's RN. These sampling effort curves thus represent the estimates of RN descriptors obtained from plots covering from 625 to 10000m^2. Five hundred random permutations of plots were evaluated for each forest to obtain the mean curves of the descriptors.

All analyses necessary for RN description and sampling effort were conducted with the software *Mathematica version 10.0*.

RESULTS

Basic elements

The studied RNs had an observed mean of $\bar{S}$ = 25.33 nodes, with a range from 17 to 32 (Table 1). Most sampling effort curves for *S* tended to level off within the range of sampling effort in our study (Fig. 2a), reaching coverage values above 0.97 in the reference sample (Fig. 2b). In all cases, using just 7 plots sufficed to reach coverage values above 0.95. The observed mean number of links was $\bar{L}$ = 163 (range: 75 – 287, Table 1). The sampling effort curves for *L* did not show signs of stabilizing within the range of sampling effort in this study (Fig. 2c), but coverage of the reference sample was always above 0.86, reaching 0.94 in some cases. The sample size required to reach coverage values of *L* above 0.95 was always larger than our reference sample. In any case, the gain in coverage of *L* per unit increase in sampling effort beyond our reference sample would be very small (Fig. 2d).

Mean directed connectance was $\bar{C}$ = 0.257 (range: 0.208 - 0.298, Table 1). The sampling effort curves for connectance were very similar among forests, with quite constant values estimated along the range of sampling effort (Fig. 3a). The behaviour of *C* in relation to coverage of *L* and *S* (Fig. 3b and c) suggests that reaching a very high coverage of *S* is more critical to obtain accurate estimates of *C* than reaching very high coverage of *L*. Note that the values of *C* are relatively low until a very high coverage of

S is reached (Fig. 3c); however, the value of *C* increases smoothly with the coverage of *L* (Fig. 3b).

The mean observed value of *k* across forest patches was 6.47 (4.57 - 9.26, Table 1). The sampling effort curves did not stabilize within the range of sampling effort of the reference sample, but they showed a very small increase when extrapolated beyond the observed value (Fig. 3d). The behaviour of *k* in relation to coverage of *L* and *S* (Fig. 3e and f) suggests that reaching a very high coverage of *S* is more critical to obtain accurate estimates of *k* than reaching very high coverage of *L*.

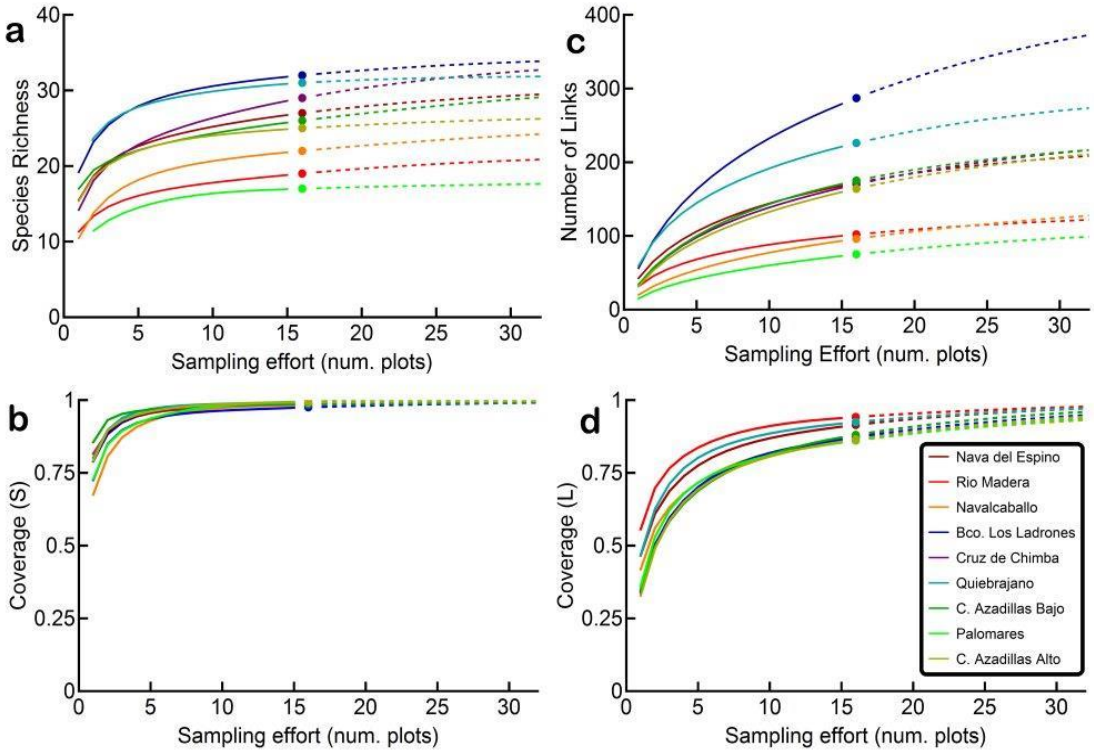

Fig. 2. Curves of sampling effort vs estimates and their coverage for the basic elements of the studied replacement networks: (a) species richness (*S*), (b) effect of sampling effort on sample coverage for *S* (fraction of the true number of species present in a sample), (c) number of links (*L*), and (d) effect of sampling effort on sample coverage for *L*. The curves were obtained following methods proposed in Chao et al. (2014). Each curve is divided in three parts. The solid line represents the results of intrapolation (or rarefaction) of the estimates for sampling efforts comprising *n* = 1–15 plots. The point indicates the estimate based on the whole reference sample (*n* = 16). The dashed line represents the estimate based on extrapolation from *n* + 1 to 2n plots. Each curve represents the results of a forest patch (see the image for colour codes).

Table 1. Properties of the studied replacement networks. Basic elements of the networks include the observed number of nodes (*S*; including species and node open), number of links (*L*), mean degree (*k*) and directed connectance (*C*). The values in parenthesis under *S* and *L* correspond to their estimated coverage (fraction of the true number of species or links present in a sample) in the reference sample (n = 16 plots). *n(S95)* and *n(L95)* indicate the number of samples needed to reach 95% coverage in *S* and *L*. The functional components are: the proportion of species forming the core (Core) and the proportion of species that are satellite (Sat), strict transients (T) or disturbance-dependent transients (ddT). Stability properties are: persistence (Pers.), error sensitivity (*ES*) and attack sensitivity (*AS*).

Forest patch	S	n(S95)	L	n(L95)	k	C	Core	Sat	T	ddT	Pers.	ES	AS
Bco. Los Ladrones	32 (0.99)	5	287 (0.87)	>32	8.97	0.29	0.871	0.032	0.032	0.065	0.90	0.002	0.03
Cruz de Chimba	29 (0.98)	7	170 (0.87)	>32	5.86	0.21	0.607	0.214	0.036	0.143	0.82	0.004	0.04
Quiebrajano	31 (0.99)	4	226 (0.92)	23	7.29	0.24	0.700	0.200	0.033	0.067	0.90	0.004	0.07
C. Azadillas Bajo	26 (0.99)	3	175 (0.88)	29	6.73	0.27	0.680	0.240	0.040	0.040	0.92	0.006	0.04
C. Azadillas Alto	25 (0.99)	5	164 (0.87)	>32	6.56	0.27	0.708	0.125	0.083	0.083	0.83	0.003	0.08
Palomares	17 (0.99)	7	75 (0.86)	>32	4.41	0.28	0.750	0.63	0.063	0.125	0.81	0.007	0.13
Rio Madera	19 (0.99)	5	102 (0.94)	19	5.37	0.30	0.667	0.333	0.000	0.000	1.00	0.012	0.11
Nava del Espino	27 (0.99)	5	172 (0.92)	24	6.37	0.25	0.692	0.115	0.000	0.192	0.81	0.004	0.04
Navalcaballo	22 (0.98)	7	96 (0.87)	>32	4.36	0.21	0.571	0.333	0.048	0.048	0.91	0.004	0.10

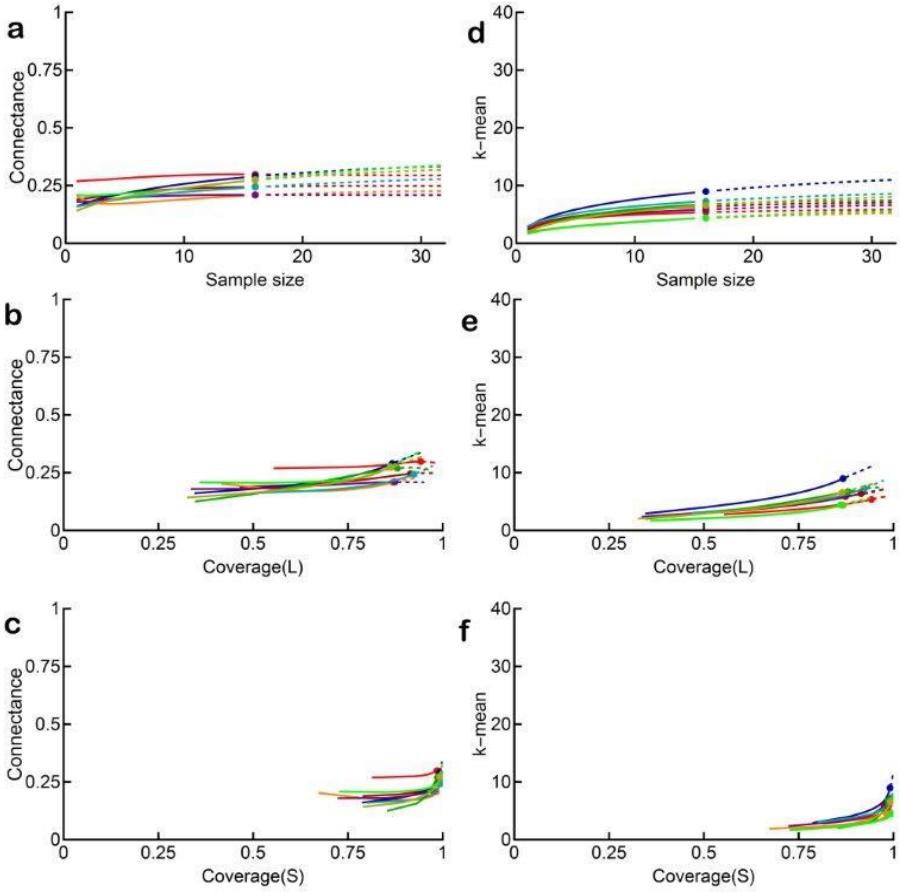

Fig. 3. Effect of sampling effort and coverage of the number of links (*L*) and species (*S*) on estimates of connectance and mean node degree. Left panels show how the estimates of connectance vary with sample size (a) and with the coverage of *L* (b) and *S* (c). Right panels show how the estimates of mean node degree vary with sample size (d) and with the coverage of *L* (e) and *S* (f). Panels (a) and (d) show that estimates of connectance and mean degree are scarcely affected by sampling effort. Panels (b, c, e) and (f) show that increasing sample coverage of *L* or *S* slightly increases the estimates of connectance and mean degree, but the estimates obtained from the reference sample (the point placed along each curve) are close to the estimates that would be obtained with full coverage (coverage = 1). The curves are formatted as in Fig. 2.

Functional structure and stability

Table 1 summarizes the estimates of functional structure and stability parameters of the forests obtained by combining the 16 plots in each forest patch. All the studied networks exhibited a single non-trivial SCC (the core) comprising on average 69.41% of the species, with a range between 57.14% and 87.10%. Between 3.23% and 33.33% of

the species (mean = 18.40%) were satellite species, 2.74% were transient (range: 0% - 8.33%) and 8.47% (range: 0% - 19.23%) were disturbance-dependent species. The studied networks had a mean of 87.81% of persistent species, with a range from 80.76% to 100%. Furthermore networks had a mean error sensitivity of 0.51% (0.2 – 1.2%).The average attack sensitivity was 7.11% (3 – 13%). The accumulation curves for these parameters (Fig. 4) suggest that their estimated values stabilize, or get close to a stable value, within the range of sampling effort of our reference sample in most forests.

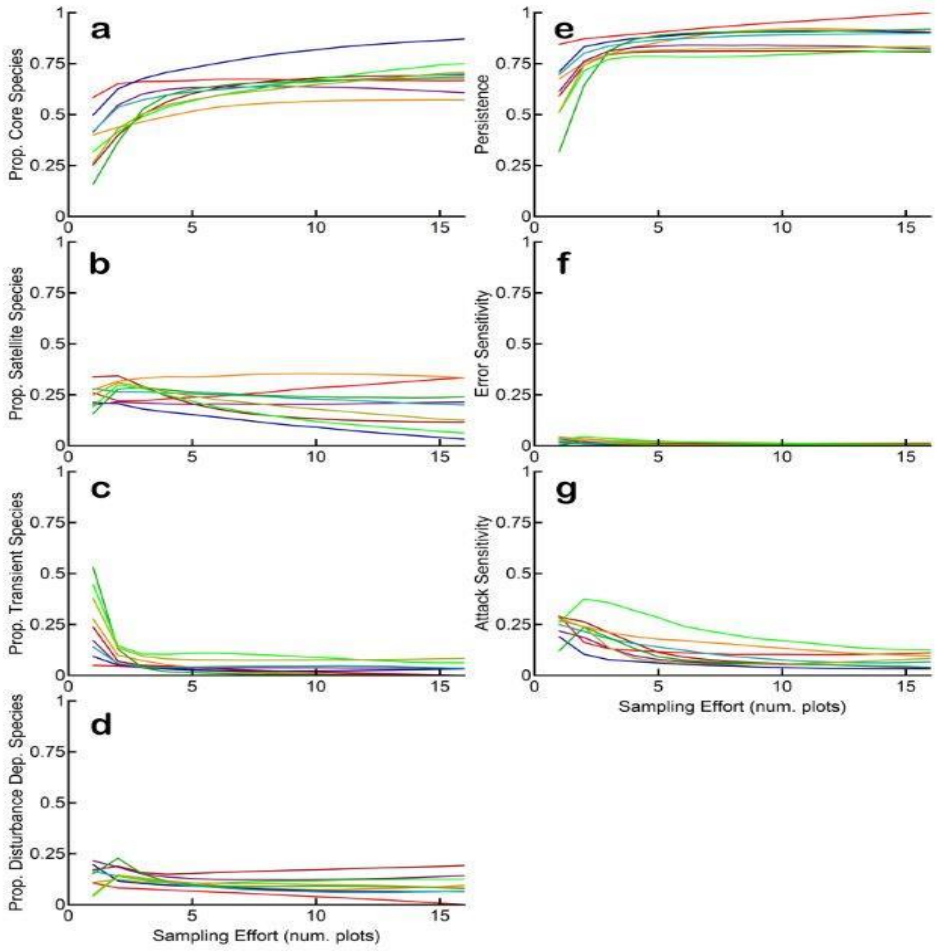

Fig. 4. Sampling effort curves for the functional structure (panels a–d) and stability properties (panels e–g) of the studied replacement networks. Each curve represents the results of a different forest patch (colour codes as in Fig. 2). For each patch, the curves represents the means of cumulative values obtained from 500 random permutations of 16 replacement networks for plots of 25 m x 25 m. Thus, sampling effort ranges from 1 plot (covering 625 m^2) to 16 plots (covering 10 000 m^2). The panels represent: (a) proportion of core species, (b) proportion of satellite species, (c) proportion of transient species, (d) proportion of disturbance-dependent species, (e) proportion of species persisting in the long term, (f) error sensitivity and (g) attack sensitivity.

DISCUSSION

The complex nature of species interactions at the community level is explicitly addressed through the study of ecological networks which are fuelling important advances in our understanding of community and ecosystem structure and dynamics (Emmerson et al. 2005; Bascompte & Jordano 2014). Such advances must be based on a solid knowledge of network structure, otherwise we may reach misleading conclusions (Cohen et al. 1993). We propose that the study of replacement networks can provide important insights about plant community structure and dynamics (Alcántara & Rey 2012; Alcántara et al. 2015), so it is critical at this stage to address how reliable our estimates of the structure of replacement networks can be. Determining which characteristics of networks can be more reliably estimated will allow both a solid foundation for further developments and a straightforward comparison of results obtained from multiple communities.

Basic network elements

The studied RNs are small compared to the size of other ecological, technological or economical networks (Dunne et al. 2002). This small size is clearly related to the relatively low diversity of woody species present in Mediterranean plant communities, so it is not surprising that species richness stabilized quickly with the amount of sampling effort, and that the completeness values obtained were above 0.97. In contrast the number of links never seemed to stabilize in any of the studied communities within our level of sampling effort. Still, its coverage values were above 0.86 in all patches, reaching 0.94 in some cases. This underestimation of the number of links has been also detected by Nielsen & Bascompte (2007), Chacof et al. (2012) and Rivera-Hutinel et al. (2012) for plant-pollinator networks, and by Goldwasser & Roughgarden (1997) and Bersier et al. (1999) for food webs. Thus, it seems a general issue for different types of ecological networks that a proper determination of the number of links might require larger sampling efforts. Two properties of the studied communities may contribute to this problem. On the one hand, it is possible that the studied assemblages include a large proportion of low-frequency (rare) canopy-recruit pairs. In the case of species richness, Coddington et al. (2009) suggest that in communities with many rare species, sample richness might never stabilize as sample

size increases, under any realistic sampling scheme. If this were the case, it might be worth extending the sampling effort beyond the reference sample but focusing this extra effort exclusively on rare species. For example, one could try to locate individuals of rare species outside the sampled plots and survey the area beneath and around them looking for interactions in which those species participate. On the other hand, it is also possible that the number of links inevitably increases continuously as new samples are added due to the intrinsic heterogeneity of the environment. Indeed, each new species added in a RN would contribute between 1 and 2*S* new links. This limitation could be tackled only in cases where the whole area occupied by the community can be exhaustively sampled (e.g. well defined habitat patches, islands). In any other cases, as new samples are added, spatial heterogeneity across samples will inevitably lead to the inclusion of new links between species already observed or to the inclusion of new species with the concomitant addition of their particular set of links.

The underestimation of the number of links seems to pose a dilemma because if we cannot observe all the links in a network, we cannot be sure that we can determine its structure properly. However, in the case of RNs this is not a serious limitation. As our results indicate, there are many structural, functional and dynamic properties of networks that stabilize within our sampling effort even though the number of links did not. On the other hand, the links missing as sampling effort increases must be the most infrequent ones, and so are those with a less likely effect on replacement dynamics at the community level. Moreover, a model of plant community dynamics based on RNs suggests that the addition of single links does not have a significant effect on the community dynamics (Alcántara et al. 2015), so adding a few more links to a network that has already been intensively sampled is not likely to cause a relevant deviation in its dynamic properties.

The structural property that more clearly reached stability was connectance. Studies conducted on plant-pollinator networks (Nielsen & Bascompte 2007) also found that estimates of connectance become remarkably constant with relatively small sampling effort. The values estimated for our RNs were relatively high (mean C = 0.26) compared to food-webs (mean C = 0.11, range 0.026 – 0.315; Dunne 2002) and non-ecological types of networks (mean C = 0.01 in a set of metabolic, neural, social

information and technological networks; Dunne 2006). It is remarkable that even this relatively high connectance implies that most potential interactions (around 3/4) do not take place in these RNs, and suggests that plant species have some limitations regarding the set of species under which they can recruit, as well as on the set of species that can recruit under their canopy. This result is robust against sampling effort limitations because we found that the estimates of *C* are remarkably stable even with sampling sizes much lower than those used in this study. In other words, our results strongly suggest that recruitment interactions would not lead to "panmictic" processes where all species can replace all species. Since a panmictic RN should have $C = 1$, our conclusion rejecting panmictic recruitment would be wrong only if our estimates of *S* were strongly biased upwards and/or the estimates of *L* were tremendously biased downwards. However, given the high values of completeness of *S* and *L* we do not expect such strong biases. This result may have important implications for models of plant community dynamics, and especially models of forest dynamics, which usually assume that replacement follows a lottery process (e.g. Pacala & Rees 1998; Hubbell 2001). Replacement events in lottery models do not consider the identity of the dead plant, so an individual of a given species can be replaced by an individual of any of the species in the community (i.e. panmictic replacement is possible). The identity of the replacer in lottery models can be determined by relative species abundance, seed production or spatial constraints, but it is never determined by the identity of the dead plant. If the values of *C* we found in our small sample of 9 networks could be generalized to a wider set of communities, then new models that do consider the identity of the dead plant would make an improvement to classical lottery models. A theoretical study comparing lottery models with RN models is necessary to understand the importance of those simple assumptions of lottery models (i.e. identity of dead plant).

Functional structure and stability

Our sampling effort curves clearly indicate that stable estimates of functional and stability descriptors can be obtained in most forest patches with the sampling effort of our reference sample, which covered 1 ha. The size of the core stabilized with a relatively small sampling effort, although the effort needed to approach stability

varied slightly among forests. In the case of the proportion of satellite, transient and disturbance-dependent species, the estimates with very small sampling efforts were unstable but in most cases the estimates stabilized with relatively low sampling efforts. Robustness of functional structural parameters against sampling effort is important since stability properties depend on them (Alcántara & Rey 2012). Accordingly, the stability parameters were also robust against sampling effort. Stable estimates of persistence and error sensitivity were obtained with relatively low sampling effort while a stable estimate of attack sensitivity required a slightly higher effort. In general these results suggest that the functional structure and stability properties of the RNs can be properly determined even with a relatively low sampling effort.

All of the studied networks contain a single non-trivial SCC that forms the core of the network. Most species (around 2/3) in the studied forests belong to the core, the second most abundant functional type was that formed by satellite species (with nearly 20% of the species in the community), disturbance-dependent species scarcely represented 10% and finally strictly transient species were usually absent or represented the smallest part of the community. A non-trivial SCC (the core) is the expression of an intransitive set of interactions which is a well-known mechanism of coexistence between competing species (Soliveres et al. 2015; Laird & Schamp 2006). Assuming that replacement dynamics represented by the RNs can be described through a LTI model, the core and satellite species will persist in the long term (Alcántara & Rey 2012). Accordingly, our results suggest that around 90% of the diversity of woody species in the studied communities is supported directly (core species) or indirectly (satellite species) by a system of intransitive interactions. In addition, there is a small set of species that depend ultimately on the continuous creation of open spaces for recruitment (disturbance-dependent species). Therefore the occurrence of disturbance (such as fire or falling trees) would allow those species to persist over time, what may contribute to increased diversity of those communities depending on the strength and recurrence of the disturbance regime (Alcántara et al. 2015).

CONCLUSIONS

Overall the results from our study indicate that reliable estimates of RN properties can be obtained with sampling areas of around 1ha (equivalent to the sum of our 16, 25 x 25 m plots). Plots of this size are frequently used in studies of forest plant communities (Mendoza et al. 2009; Liu et al. 2012; Piao et al. 2013; Jansen et al. 2014). Thus, the sampling effort required to obtain reliable data in the context of RNs falls within the magnitude of sampling efforts that are commonly conducted in studies of forest ecology, so RN analyses can be easily incorporated. Obviously, this sampling effort cannot be extrapolated to other types of plant communities (e.g. annual plants) or to other types of forests (e.g. tropical rain forests), where nevertheless studies like this one can be easily performed to determine the appropriate sampling effort.

Our study suggests that direct comparisons of RNs from different communities must not be based on estimates of link density or link number, because it is not possible to obtain stable estimates in most communities. However, there are many properties of these networks that could be safely compared across communities provided a minimum sampling effort has been achieved. This makes replacement networks a solid approach for the study of plant community structure and dynamics.

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Table S1. Species composition of the Replacement Network of each forest patch. The values indicate the number of 25x25m plots in which each species occurred, out of 16 plots sampled in each forest.

Forest type:	**Mixed *P. halepensis* and *Q. ilex***		
UTM Coordinates:	30S 4168934N 436119E	30S 4169328N 435590E	30S 4167257N 437904E
Altitude (m):	857	804	1118
SPECIES	**C. Azadillas Alto**	**C. Azadillas Bajo**	**Palomares**
Celtis australis		2	
Cistus albidus	15	15	15
Cistus salvifolius	10	16	
Colutea hispanica		2	
Crataegus monogyna	2	1	11
Daphne gnidium	1	6	
Genista scorpius		1	
Jasminum fruticans		10	11
Juniperus oxycedrus	4	12	2
Juniperus phoenicea	3	3	2
Olea europaea var. sylvestris	13	13	
Phillyrea angustifolia	14	16	
Phillyrea latifolia	15	16	
Phlomys lychnitis	1		4
Phlomys purpurea	14	13	2
Pinus halepensis	16	16	14
Pistacia lentiscus	15	16	
Pistacia terebinthus	15	16	11
Prunus spinosa			1
Quercus coccifera	13	14	3
Quercus faginea			2
Quercus ilex	13	11	16
Rhamnus alaternus	8	1	
Rhamnus lycioides	14	16	4
Rosa sp.	2		9
Rosmarinus officinalis	4	13	
Ruscus aculeatus	4	1	
Thymus mastichina	3		13
Ulex parviflorus	5	13	
Viburnum tinus	4	5	

Forest type:	**Mixed *P. nigra subsp. salzmannii, Q. faginea* and *Q. pyrenaica***		
UTM Coordinates:	30S 4238960N 538619E	30S 4238431N 535111E	30S 4237375N 535473E
Altitude (m):	1409	1366	1254
SPECIES	**Nava del Espino**	**Navalcaballo**	**Rio Madera**
Acer opalus subsp. granatens	16		
Amelanchier ovalis	9		
Astragalus granatensis		1	
Berberis hispanica	6	3	4
Cistus albidus	2	11	
Cistus crispus	5		3
Cistus populifolius	1		
Cistus salvifolius	4		3
Crataegus laciniata	15	7	
Crataegus monogyna	16	10	16
Cytisus scoparius	3	5	11
Daphne laureola	7		10
Fraxinus angustifolia	1		
Genista pseudopilosa		1	
Ilex aquifolium	1		1
Jasminum fruticans			
Juniperus communis	8		4
Lavandula latifolia	3		
Malus sylvestris	10		
Phlomys lychnitis		4	1
Pinus nigra subsp. salzmannii	16	16	16
Pinus pinaster	7	5	
Prunus mahaleb	1		
Prunus spinosa	14	10	16
Quercus coccifera		5	1
Quercus faginea	16	16	16
Quercus ilex	16	15	16
Quercus pyrenaica	12	2	14
Rhamnus saxatilis		2	
Rosa sp.	16	9	16
Sorbus aria	2	1	
Sorbus torminalis		3	7
Thymus mastichina	6		
Thymu zygis		8	
Viburnum lantana			6

Forest type:	**Mixed *P. halepensis* and *Q. faginea***		
UTM Coordinates:	30S 4166886N 437339E	30S 4165630N 434416E	30S 4163073N 437049E
Altitude (m):	1053	903	1002
SPECIES	**Cruz de Chimba**	**Bco. los Ladrones**	**Quiebrajano**
Acer monspessulanum	1	4	11
Amelanchier ovalis	7	1	5
Berberis hispanica			11
Celtis australis	1		
Cistus albidus	14	16	8
Cistus salvifolius	1	1	
Colutea hispanica	1		2
Crataegus monogyna	11	12	16
Daphne gnidium	13	6	14
Echinospartum boissieri	2		
Fraxinus angustifolia		5	1
Genista cynerea		3	11
Hormatophylla spinosa		1	
Jasminum fruticans		2	
Juniperus oxycedrus	7	12	15
Juniperus phoenicea	2		1
Lavandula latifolia	8	8	13
Olea europaea var. sylvestris		15	5
Osyris alba			1
Phillyrea angustifolia	11	14	
Phillyrea latifolia		15	16
Phlomys purpurea	1	16	
Pinus halepensis	16	16	16
Pistacia lentiscus		13	
Pistacia terebinthus	12	16	15
Prunus mahaleb		4	5
Prunus spinosa	2		7
Quercus coccifera	3	7	
Quercus faginea	16	16	16
Quercus ilex	15	15	16
Rhamnus alaternus		3	16
Rhamnus lycioides	4	15	
Rhamnus myrtifolius	1	3	
Rosa sp.	11	5	16
Rosmarinus officinalis	12	2	
Ruscus aculeatus			7
Salvia lavandulifolia	5		
Sorbus aria			7
Stahelina dubia			1
Teucrium fruticans		6	
Thymus mastichina	5	10	8
Thymu zygis	4		5
Ulex parviflorus	12	5	12
Viburnum tinus			10

CHAPTER 3

Dissection of community-level intra- and inter-specific plant-plant interactions in forest communities

Unpublished manuscript

SUMMARY

1 Although both intra- and inter-specific interactions have fundamental roles in coexistence theory, studies of canopy-recruit interactions in plant communities focus largely on negatively density-dependent conspecific interactions. Heterospecific interactions are thus presumed to result from stochastic or neutral processes (i.e., any species could be expected to recruit under the canopy of any other species in a local community).

2 In this study we explore the relative influence of neutral stochastic processes and species identity on the frequency of canopy-recruit interactions. Moreover, we estimate the effect (depressor, neutral or enhancer of recruitment) and spatial consistence (across sites) of intra- and inter-specific canopy-recruit interactions.

3 To this end we collected information on sapling recruitment in 12 mixed pine-oak forest communities of Southern Spain. We monitored recruitment under 56 shrub and tree species, participating in 4161 interactions.

4 We show that neutral stochastic processes affect the frequency of canopy-recruit interactions at the community level, although with a very small effect size. Species-specific effects on recruitment are strong enough to be detectable above this stochasticity. Canopy-recruit interactions are found (or absent) more consistently across sites than expected from a null model. Around 40% of the potentially occurring interactions were not observed, and only 25% of them could be explained by the scarcity of the participants. Most interactions (57%) were neutral, 28% had enhancing effect on recruitment and 15% had depressing effects. Canopy species tend to depress the recruitment of conspecific saplings and facilitate the recruitment of heterospecifics.

Synthesis. Even though spatial stochasticity is intrinsic to the recruitment process, the assembly of canopy-recruit interactions is far from neutral. A given species can have depressor, neutral or enhancer effects on the recruitment of other species. The simplifying dichotomy of conspecific vs. heterospecific effects on recruitment must be avoided since it can blur the rich diversity of interactions that form fundamental part of plant community dynamics.

Keywords: complex networks, conspecifics, ecological interactions, facilitation, forbidden links, heterospecifics, interaction consistence, missing links, plant recruitment.

INTRODUCTION

The recruitment phase of a plant population involves the part of the life cycle from seed deposition on the ground to the establishment of a juvenile individual. Recruitment is frequently a bottleneck for plant demography and by extension it is a limiting process of plant community dynamics (Zeiter *et al.* 2006; Sankaran *et al.* 2013). Recruitment is a multistage process driven by biotic and abiotic factors acting simultaneously or sequentially (Herrera *et al.* 1994; Wang & Smith 2002). These multifarious factors impose an inherent stochasticity on recruitment. For example, chance can affect where seeds are deposited, whether they escape from predators or whether they meet suitable microenvironmental conditions for germination and seedling survival. However, the pooled outcomes of direct and indirect effects of established plants (canopy plants hereafter) on individual plants recruiting beneath their canopy can overpower those stochastic drivers. For example, many studies have found that the prospects for recruiting individuals of a given species vary depending on the canopy species under which they were dispersed (Rey & Alcántara 2000; Castillo-Landero & Valiente-Banuet 2010; Rother *et al.* 2013). Therefore, community species composition can feed back on community dynamics through the rates of recruitment of species under each other (Alcántara *et al.* 2015).

Unfortunately, most studies on the interactions between canopy and recruiting plants lack a community-level perspective. Community-level studies are accumulating on the prevalence, sign and strength of interactions between conspecific adult and recruiting plants (Webb & Peart 1999; Harms *et al.* 2000; Comita *et al.* 2010; Raventós *et al.* 2010; Swamy *et al.* 2011; Zhu *et al.* 2015; Liang *et al.* 2016), largely disregarding pairwise interspecific interactions. For example, Comita *et al.* (2010), Raventós *et al.* (2010) or Liang *et al.* (2016) found that the density of conspecific neighbors around seedlings had overwhelmingly negative effects on recruitment, while the density of heterospecific adults had negligible or weakly positive effects. However, by pooling

many species under the common label of heterospecifics, their results cannot rule out the possibility that recruitment may be enhanced, depressed or unaffected under different species, explaining the weak net general effect of heterospecifics. Another set of studies have demonstrated the prevalence of facilitation of recruitment in many plant communities (Valiente-Banuet & Verdú 2007; Verdú & Valiente-Banuet 2008; Cavieres *et al.* 2014; Soliveres & Maestre 2014; Rey *et al.* 2016). But again, these studies pooled the effect of all species (both con- and hetero-specific) under the common label of nurse plants, so a net positive effect on recruitment from the pool of nurse species could mask the existence of some recruitment depressing or neutral effects of different canopy species. Thus, the prevalent magnitude and effect (enhancer, neutral or depressing) of different species on recruitment remains largely unexplored. To evidence the magnitude of this gap in knowledge, think that all species (*S*) in the community can potentially interact with each other, so there are potentially up to S^2 interactions in the community. Studies on conspecific density-dependence of recruitment analyze *S* interactions (one per species). Therefore, for example, in a community of 100 species there could be up to 10000 pairwise interactions, but these studies would explore only 100 of them, pooling the effects of the remaining S^2-S = 9900 interactions as if they were equivalent. Coexistence theory (Chesson 2000) posits that strong intra-specific competition (e.g. a strong reduction of recruitment under conspecific plants) is an important mechanism stabilizing the communities, but that coexistence requires also the existence of fitness equalizing mechanisms between competing species. It is very likely that these S^2-S overlooked interspecific interactions have something to say on these last mechanisms (Alcántara & Rey 2012; Alcántara *et al.* 2016).

To start filling this gap, we explore the role of stochasticity and species-specific effects on the realization (frequency, effect and consistence) of potential canopy-recruit interactions between woody species in mixed pine-oak forest communities of Southern Spain. We characterize these interactions in terms of their effect (i.e. whether a canopy species depresses, enhances or has no significant effect on the density of recruitment of another), spatial consistence (i.e. whether these interactions can be found in different sites more often than expected by chance) and effect consistence (i.e. whether their effect changes across sites). Differently from other

studies of recruitment interactions (e.g. Verdú & Valiente-Banuet 2008), we explore the whole set of potential interactions that could be established in a local community. This implies that we not only study realized interactions (i.e. those actually observed in some local community) but also explore absent interactions. Following Olesen et al. (2011), absent interactions can be classified as missing interactions (those that are feasible and could be established in a local community because both partners co-occur, but that were not observed for some reason) or forbidden interactions (those that cannot occur due to some biological constraint). Discerning between missing and forbidden interactions is one of the current challenges in the study of ecological interaction networks (Jordano 2016).

If stochastic processes dominate the assembly of canopy-recruit interactions in a community, we would expect that the occurrence and effect of any given recruitment interaction would depend only on the abundance of canopy and recruiting species in the community. In this case, absent interactions could be considered as missing interactions caused by the low probability of encounter of seeds with rare canopy species (what has been also labeled as Neutral Forbidden interactions by Canard *et al.* 2012). Alternatively, the assembly of recruitment interactions may be strongly influenced by non-random factors determined by some properties of the canopy and recruit species. Studies of ecological interaction networks have found that the set of interactions observed in a community, although largely resulting from the distribution of abundance among species, is influenced significantly also by some properties of the participating species (Vázquez *et al.* 2005; Stang *et al.* 2007; Vázquez *et al.* 2007; Verdú & Valiente-Banuet 2011). We hypothesize that species-specific effects on recruitment are strong enough to be detectable above the random noise imposed by stochastic spatio-temporal processes that affect recruitment in plant communities. Rejection of this hypothesis would imply that the effects of heterospecific individuals on recruitment could be considered equivalent. Moreover, if our hypothesis is correct, then we would also expect that the frequency of interspecific interactions with enhancer, neutral and depressing effects on recruitment should be balanced, explaining why some studies found a negligible or weak pooled effect of heterospecific species on recruitment. Finally, since the predominant effect of canopy species on recruitment is known to vary depending on environmental conditions (Pugnaire &

Luque 2001; Maestre *et al.* 2005), we could expect finding some variation in the effects of pairwise interactions among sites. However, if our hypothesis is correct, we should find that canopy-recruit interactions are more consistent across sites than expected by chance, and that recruitment enhancing interactions should be found more consistently in different sites than recruitment depressing interactions (because the later should be more prone be lost in some sites). Here, we explored this hypothesis and its predictions by exploring the relative influence of neutral stochastic processes and species identity on the frequency of canopy-recruit interactions in mixed pine-oak forests of Southern Spain.

METHODS

Study sites and design

The observational study was conducted on two types of mixed pine-oak forests from Southern Spain: mixed forests of *Pinus halepensis*, *Quercus ilex* and *Quercus faginea*, and mixed forests of *P. nigra* subsp. *salzmanii*, *Q. faginea* and *Q. pyrenaica*. The upper canopy of these forests is dominated by pines, with oaks representing the second most abundant trees. However, the species richness of these forests is fed mostly by the understory of subcanopy trees and tall shrubs (e.g. species of *Acer*, *Crataegus*, *Juniperus*, *Sorbus*, *Prunus*, *Phillyrea* and *Pistacia*), and by a small shrub layer that includes species of *Thymus*, *Cistus*, *Phlomis*, *Genista, Rosmarinus* and *Rosa*, among others (a complete list of species is presented in table S1). In these forests the climate is Mediterranean, with rainfall concentrated in late autumn and winter and hot dry summers. Annual rainfall levels are above 800mm and mean temperature is between 10-12°C.

Altogether we located 12 study sites, spread between the two types of forest as follows: 8 study sites in the protected area of Parque Periurbano Monte la Sierra for mixed forests dominated by *Pinus halepensis*, and 4 study sites in the protected area of Parque Natural de las Sierras de Cazorla, Segura y Las Villas for mixed forests dominated by *P. nigra* subsp. *salzmannii*. Within each site we set a central plot of 50 x 50 m. Additionally, in 10 of the sites we established 16 accessory plots of 25 x 25 m, all of them distributed so that their physiographical conditions (slope, aspect, soil depth, elevation) were as homogeneous as possible. Within each site all plots were spaced

less than 300m from each other. In all central and accessory plots we systematically looked for saplings of every woody species and noted whether they were recruiting under the crown of some canopy species or in open interspaces (see Pulgar *et al.* 2017, for further methodological details of sampling). Each central plot was divided into 25 subplots of 10 x 10 m where we recorded the crown projection area of each canopy species.

Data analysis

Stochasticity and species-specific effects on interspecific canopy-recruit interactions.

Our central hypothesis states that species-specific canopy or recruit effects are strong enough to be detected above the stochasticity that affects the processes involved in recruitment. We tested this hypothesis by fitting a generalized linear mixed model (glmm) to the frequency of each potential interaction. Since our dependent variable consists in counts of the number of saplings with a mean of 1.17, and we wish to model three random effects (see below) we used Markov Chain Monte Carlo procedure (implemented in package MCMCglmm of R; Hadfield 2010) as suggested by Bolker *et al.* (2008). To model the influence of species abundance on the frequency of interactions we incorporated three fixed effect covariates consisting of the cover (in m^2) of the recruiting and canopy species and their crossproduct. Incorporating the crossproduct is necessary because the chances of interacting depend simultaneously (not independently) on the abundance of both species. Besides these effects of species abundance, a given interaction can be more or less frequent in some sites due to variation in environmental conditions. We model these spatial differences through a random effect for the study site as a blocking factor that also controls for autocorrelation in the frequency of interactions sampled in the same site. Finally, we incorporated two random effects that account for the effects of the recruiting and canopy species that are not explained by their cover. We excluded from the analysis the saplings recruiting in open spaces and those recruiting under conspecific individuals because we were interested only in interspecific interactions in this analysis. Note also that in the case of intraspecific interactions, the effect of cover on recruitment is not expected to follow a pattern similar to that of interspecific interactions because the probability of a seed to end up under a conspecific individual

(particularly under the mother plant) is far greater than that of falling anywhere else, irrespective of the abundance of conspecifics. Our dataset contained the frequency of 4161 local interactions found in 12 sites and involving 49 recruiting species and 49 canopy species (the lists of recruiting and canopy species do not fully overlap, Table S1). Inspection of the dataset suggested that there might be zero-inflation because it contains a proportion of 0.77 zeroes while the expected proportion under Poisson distribution is 0.31. However, a model using zero-inflated Poisson distribution showed a poor fit to the data. Thus, we used standard Poisson distribution with log link function. Procedure MCMCglmm automatically corrects for overdispersion. To improve the convergence of the fitted parameters we used 65000 iterations of the MCMC algorithm with a burn-in period of 15000 iterations and thin interval of 50 iterations. Inspection of the trace of the sampled posterior suggests that convergence of parameter estimates was achieved. The autocorrelation of parameter estimates for the random factors was: -0.05 for Site effect, 0.06 for recruit species, 0.05 for canopy species and 0.21 for the residuals.

Spatial Consistency of Interactions

If recruitment interactions are governed more by deterministic filters associated to particular canopy and recruit species, than by stochastic processes, we would expect that a particular canopy-recruit interaction would be realized whenever adult plants of the two participant species co-occur in a local community. In other words, canopy-recruit interactions should show spatial consistency. Spatial consistency of the interaction between recruiting species *i* and shelter species *j* (IC_{ij}), was calculated as the number of sites (*n*) where we detected their recruitment interaction relative to the number (*m*) of sites where (1) there were adults of both species and (2) there were saplings of the recruiting species in the site. For analyses of IC we only included interactions between species that fulfilled these conditions at least in three sites. To cover the largest possible number of interactions, in this analysis we compiled an interactions matrix for each site using data from its 16 accessory plots. These empirical interaction matrices contain only information on the presence/absence of each potential interaction between species present in the site. Taking into account all these restrictions, we finally obtained IC values for 505 interactions, including 204 absent

interactions in which two species co-occurred at least in three sites and even so their interaction was never observed ($IC_{ij} = 0$).

Since the realization of an interaction at a given site can be influenced by stochastic processes, we developed a null model to test whether the observed ICs differ from what could be expected simply from the abundance of the interacting species. From each site's empirical interactions matrix, we generated 1000 null matrices by distributing the interactions of each recruiting species (rows in the matrix) among the canopy species present in the site (columns in the matrix) with probability according to their relative frequency in the 16 plots of the site. In each iteration of the null model we obtained 10 matrices (one per site) from which we obtained the IC_{ij}* values. The average IC_{ij}* obtained for each interaction is the expected probability of finding its realization in a site if interactions were realized according to the null model. For each interaction we calculated the binomial probability of observing its realization in *n* out of *m* sites (i.e. the observed IC_{ij}) with expectation according to the null model (IC_{ij}*). To control for Type I errors caused by multiple testing, we adjusted the p-values to declare an IC as significant using False Discovery Rate (FDR: expected proportion of false rejections of the null hypothesis; Verhoeven *et al.* 2005). To visualize species co-occurrence and preparing data for our own downstream computations of IC, we used package cooccur (version 1.3) for R (R version 3.2.3; R Development Core Team 2015).

Interaction Effect

If the observed frequency of recruitment of species *i* under *j* (r_{ij}) in a given site were simply a matter of chance (what would imply a neutral effect of the canopy species on the recruiting species), we would expect r_{ij} to be proportional to the cover of *j* (c_j), so the null expectation for r_{ij} would be $r_{ij}^* = r_i c_j$, where $r_i = \sum_j r_{ij}$ However, if the frequency of recruitment were driven mainly by positive interactions (e.g. seed attraction, facilitation) we would find recruitment more frequently than expected from the cover of the canopy plant ($r_{ij} > r_{ij}^*$), concluding that species *j* has an enhancing effect on the recruitment of *i*. Alternatively, if the frequency of recruitment were driven mainly by negative interactions (e.g. increased pest pressure, presence of allelochemicals, competition), we would find recruitment less frequently than expected ($r_{ij} < r_{ij}^*$), concluding that species *j* has a depressing effect on the recruitment of *i*. We tested the

significance of these effects using a null model analysis as follows. If all the saplings of a recruiting species in a site could have occurred under any of the canopy species with probability given by c_j, then the distribution of the r_i samplings among the *j* species would follow a multinomial probability distribution. From this distribution we can calculate for each recruiting species in a given site the probability that exactly r_{ij} saplings (with r_{ij} = 0, 1, 2 … r_i) would recruit under a given canopy species. If the probability of obtaining the observed r_{ij} is lower than 0.05, we can conclude that species j has a significant (enhancing or depressing) effect on the recruitment of *i*.

Before running these tests we used two criteria to choose those interactions for which our data would allow sufficient power to reject the null hypothesis. We first restricted our analyses to those recruiting species with at least 10 saplings in the site. This resulted in 2797 interactions. To further refine this set of interactions, we calculated the minimum r_i necessary to reject the null hypothesis in a shelter plant with the observed c_j. This restricted our analyses to 585 interactions. All these analyses were conducted with the software *Mathematica* version 10.0. (Wolfram Research Inc.)

RESULTS

Effects of species abundance and identity on the frequency of interactions.- The mean density (± SD) of the observed interactions (i.e. disregarding absent interactions) was 0.11 ± 0.44 recruits/m^2 of the canopy species (range: 0.0014 – 10 recruits/m^2). Results of glmm analysis indicate that the simple effect of canopy species cover was highly significant and positive, but the effect of recruiting species abundance and the interaction term were not significant (Table 1). The site effect accounted for 8.25% of the variance in the frequency of interaction (credible interval CI = 2.65% - 15.33%), while the identity of the recruiting and canopy species accounted, respectively, for 22.93% (CI = 14.09% - 32.55%) and 30.29% (CI = 19.87% - 41.06%).

Table 1. Results of the fit of the MCMCglmm model to the frequency of interspecific interactions. Estimates of the fixed effects are the slopes of each covariate. Estimates of the random factors are their contributions to the variance of the frequency of interactions (numbers in parenthesis are the proportions of variance accounted for by each factor). N/A= not applicable.

Effect	Type	Effective size	Estimate	Lower 95% CI	Upper 95% CI	P(MCMC)
Intercept	Fixed	1000	-3.847	-4.591	-2.931	< 0.001
Recruit cover	Fixed	1000	0.00139	-0.00171	0.00516	0.442
Canopy cover	Fixed	1000	0.00592	0.00249	0.00951	< 0.001
Recruit x Canopy cover	Fixed	1000	0.0000141	-0.0000115	0.0000405	0.300
Site	Random	1112.91	0.5104 (0.0825)	0.1179 (0.0265)	1.075 (0.1533)	N/A
Recruit species	Random	788.68	1.419 (0.2293)	0.7601 (0.1409)	2.035 (0.3255)	N/A
Canopy species	Random	908.85	1.875 (0.3029)	1.144 (0.1987)	2.767 (0.4106)	N/A
Error	Random	578.26	2.385 (0.3853)	2.1 (0.3093)	2.669 (0.4642)	N/A

Interaction Consistency.- Considering all the potential interactions that could occur in at least 3 sites (N = 505), we found that 40% of them were consistently absent. However, only 11% should be absent under the null model (Fig. 1). Thus, absent potential interactions are more common than expected (Two proportions test $P < 0.0001$). Considering only realized interactions (N = 301), the mean observed IC was significantly higher than expected from the null model (mean $IC_{ij} = 0.4962$; mean $IC_{ij}^* = 0.1869$; Wilconxon's matched pairs test: $Z = 13.98$, $P < 0.0001$). Under the null model, the vast majority of interactions (76%) should have occurred with very low consistency ($IC_{ij}^* < 0.33$), and only 14% should have intermediate or high IC. However, 44% of the observed interactions had $IC_{ij} > 0.33$. Thus, observed recruitment interactions are significantly more consistently realized than expected under random assembly.

Interaction Effect frequency.- For all testable interactions pooled (N = 585) we found similar frequency of neutral (49.57%) and non-neutral (50.42%) interactions, and within the later, similar frequency of recruitment depressing and enhancing interactions (25.98% and 24.44% respectively). Figure 2A dissects these interactions. For interspecific interactions (N = 409) we found significantly larger ($P < 0.001$) percentage of neutral than non-neutral interactions. Among the non-neutral interspecific interactions, we found a lower percentage ($P < 0.0001$) of recruitment

depressing than enhancing interactions. In the case of intraspecific plant-plant interactions (N = 43) we found a similar percentage of neutral and non-neutral interactions (P = 0.83), and within the latter, a higher frequency of depressing than enhancing interactions (P < 0.016). . Finally, among the interactions between plants and open (N = 133) we obtained that neutral interactions were significantly less common than non-neutral ones (P < 0.0001), and within the latter there were many more depressing than enhancing interactions (P < 0.0001).

Interaction Effect Consistency.- For interactions that could be analyzed in more than one site (N = 125; Fig. 2B) we can explore whether they changed in intensity (varying between neutral and enhancer or between neutral and depressing) or in effect (being enhancer in some sites and depressing in others). Only 11 out of 125 studied interactions changed in effect between sites, and this change was significantly more frequent (P < 0.04) in plant-open interactions than in plant-plant interactions. Consistently depressing interactions and interactions that shifted in intensity between neutral and depressing were more frequent in open than under canopy plants (P < 0.01 in both cases). On the contrary, consistently enhancing or neutral interactions and interactions that varied in intensity between neutral and enhancing occurred more often under canopy plants than in open (P < 0.03 in all cases). Considering only plant-plant interactions, effect constancy is less frequent in recruitment depressing than in enhancing interactions (P < 0.007).

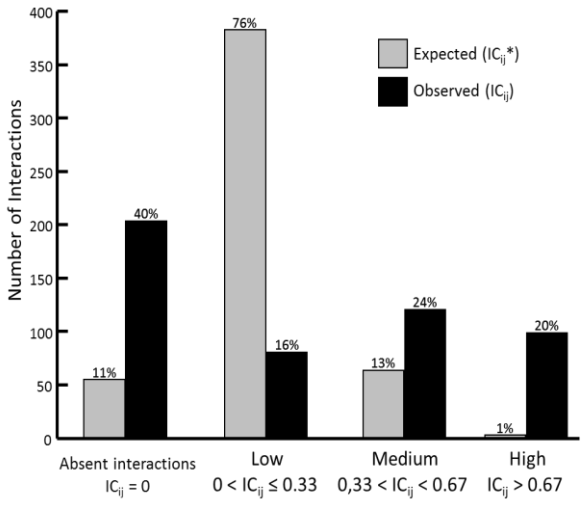

Figure 1. Histogram showing the distribution of IC values observed and expected under the null model.

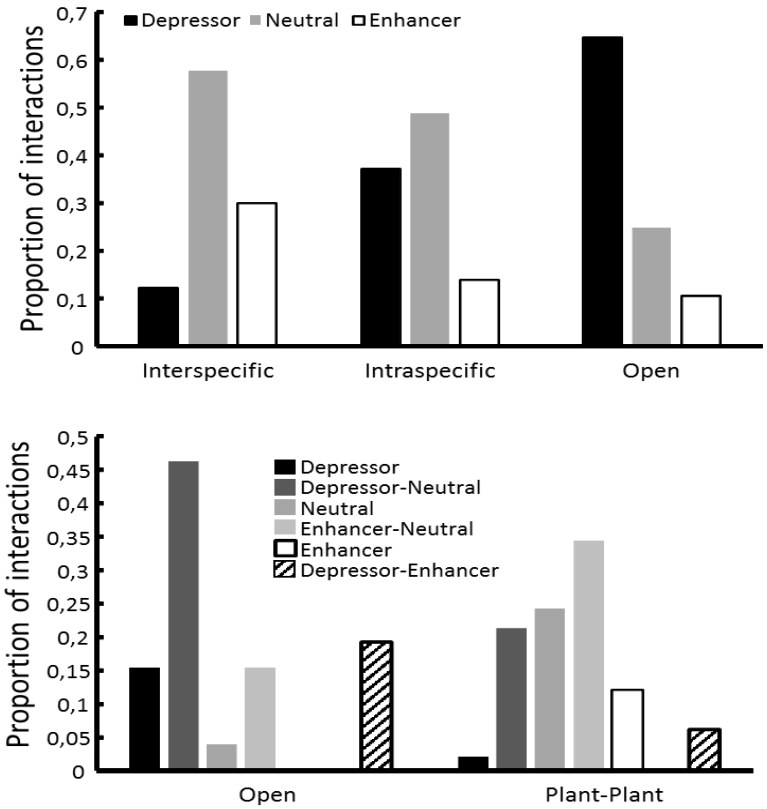

Figure 2. Frequency of interaction effects. A) Proportion of interactions between conspecific plants (intraspecific), between heterospecific plants (interspecific) and between plants and open. Interactions with depressor, neutral or enhancer effect occur when recruitment under the canopy species is respectively lower, equal or larger than expected from chance. B) Proportion of plant-plant and plant-open interactions that maintain or shift their effect between sites.

DISCUSSION

Stochastic and species-specific effects on recruitment.

The frequency of recruitment of a given species under another depends on many processes, most of them with a strong stochastic component (Chambers & MacMahon 1994; Wang & Smith 2002). It is unpredictable whether a seed will become a sapling and where. This unpredictability makes it easy to presume that the frequency of recruitment of a species under another should be largely a matter of chance, thus dependent only on the relative abundance of the participant species. We detected the signal of stochastic processes acting at the community level: the more abundant a canopy species is the larger the number of saplings recruiting underneath. Although this effect was significant, the magnitude of increase in recruitment frequency per unit increase in canopy cover is really small. Taking the average cover of recruiting species

(44.52 m^2), our fitted model predicts that an increase of 100 m^2 in the cover of a canopy species (from an average 44.4 m^2 to 144.4 m^2) would increase the average recruitment frequency under its canopy in barely 0.028 recruits (from 0.030 to 0.058). However, the frequencies observed for interactions with recruiting species cover between 40 and 50 m^2 and canopy species cover between 40 and 150 m^2 (N = 31) is 100 times larger than expected from species cover, averaging 3 recruits per interaction with a range between 0 and 25. Therefore, there is a very large amount of variability in interaction frequency that is not accounted for by species abundance.

Trees and shrubs produce large amounts of seeds every year, and reproduce many times during their lives. If each seed is a trial of recruitment, the sapling bank in a forest can be seen as the successes accrued after thousands or millions of trials, so if a given species expresses or confers even a very small recruitment advantage, then its success or impact on the frequency of recruitment can be large. There is accumulated evidence that such species-specific effects occur in some of the species included in our study communities (Rey & Alcántara 2000; Traveset *et al.* 2003; Gómez-Aparicio 2008). Our results demonstrate that these effects pervade to the whole community. Most of the variability in the frequency of interspecific interactions (around 53%) is explained by differential effects on recruitment associated to the canopy and recruiting species, what gives account of the strength of canopy-recruit interactions: not all species have the same capacity to recruit and, more importantly, not all canopy species have the same effect on recruitment.

Interaction consistency and unobserved interactions

Another indication of the strength of interactions is their propensity to be consistently realized or not wherever the species co-occur. For example, species in plant-pollinator networks tend to interact with each other more consistently than expected by chance (Carstensen *et al.* 2014; Trøjelsgaard *et al.* 2015), although recent results by McLeod *et al.* (2016) suggest that most pollinators adjust their preferences according to plant abundance. An indication of the strength of canopy-recruit interactions is provided by our results on interaction consistency. We found that canopy-recruit interactions are realized more consistently than expected from species abundance. It is worth noting that unlike ecological interactions involving animals,

canopy-recruit interactions are not actively established by the participants, so there is always some probability that a feasible interaction is not realized in a local community even though both participants co-occur. This probability increases as the probability of interspecific encounters decreases, what depends largely on the abundance of the interactors. Since species abundances typically follow a log-normal distribution, the expected probability of encounter of most pairs of species is so small that the interaction is never realized. This is what Canard et al. (2012) called neutral forbidden links in trophic networks. Our null model suggests that 11% of potential canopy-recruit interactions (those with IC* = 0) could be considered as neutral forbidden links explained by the low abundance of the interacting species. However, we found that 40% of the potential interactions were consistently absent, so we can estimate that 72.5% of unobserved interactions ((40-11)/40) are likely forbidden interactions because they were consistently absent even though the participant species were abundant enough to interact. Similarly high frequencies of forbidden interactions have been found in plant-seed disperser and plant-pollinator networks (Jordano 2016).

Effects of interactions

As the analysis of the frequency of interactions showed, canopy species differ in their effects on recruitment. In 57% of the studied interspecific plant-plant interactions, the frequency of recruitment did not differ from what could be expected from the cover of the canopy species. This result seemingly contrasts with our previous conclusion of a very small effect of canopy cover on interaction frequency. It is likely that the dispersion around the estimated regression line coefficients of canopy cover on interaction frequency results in a wide prediction band that includes most of the observed interactions, which would then be classified as neutral. In any case, the frequency of non-neutral interspecific interactions was very high (43%), clearly indicating that the dynamics of the studied communities cannot be approached through neutral theories (Hubbell 2001).

The analysis of interactions whose effect could be tested in more than one site allows assessing the consistency of such effects. Just like the realization of a canopy-recruit interaction does not necessarily occur whenever it is feasible, the strength or direction of its effect can also vary. Studies on plant-plant facilitation have shown that

a given interaction can change from facilitative to competitive as environmental conditions change (Maestre *et al.* 2005; Sthultz *et al.* 2007). We found that changes from depressing to enhancing plant-plant interactions are rare in the studied communities (6.1%) and more common in the case of plant-open interactions (19.2%). This suggests that plant-plant interactions are more buffered against environmental variation than plant-open interactions.

Many studies have shown that recruitment in open spaces under Mediterranean climate is severely limited in many species (Rey & Alcántara 2000; Traveset *et al.* 2003; Gómez-Aparicio 2008) what agrees with our finding that recruitment is depressed in open in the vast majority of cases. Only some pioneer species showed enhanced recruitment in open (*Cistus salviifolius*, *Cytissus scoparius*, *Rosmarinus officinalis* and *Ulex parviflorus*), and even in these cases the strength of the effect varied between positive and neutral. On the other hand, most interspecific interactions had neutral or enhancing effects on recruitment (respectively, 236 and 123 out of 409 interactions), and most tested species (21 out of 26) experienced depressed recruitment in open at least in one site. All these results clearly indicate that facilitation between plants prevails in the studied communities (Rey *et al.* 2016). Still, there is a not negligible group of recruitment depressing interactions (50 out of 409 = 12%). Thus, taken together the effects of all interactions the community-level effect of heterospecifics is slightly positive, what agrees with findings in other communities (Comita *et al.* 2010; Raventós *et al.* 2010). However, our results suggest that these studies could be giving the wrong impression that interspecific interactions during recruitment have neutral effects.

Conspecific adult-recruit interactions were not always negative as expected under Janzen-Connell model of negative conspecific density dependence (Janzen 1970; Connell 1971). Out of 16 species which we could test, we found that recruitment under conspecifics tended to be depressed in 8 species, while it was neutrally affected in 5 and enhanced in 3. Recent studies on plant-soil feedbacks (Bennett *et al.* 2017; Teste *et al.* 2017) suggest that these variable effects could be related with the type of mycorrhizal fungi associated with each species. Bennet et al. (2017) found that trees associated with ectomycorrhizal fungi (EM) actually facilitate conspecific seedlings whereas trees associated with arbuscular mycorrhizal fungi (AM) inhibit their

conspecific seedlings. Our dataset partly supports their findings: (1) all tested AM species (Table S1) had neutral or depressing effects on conspecific recruitment (*Crataegus monogyna*, *Genista cinerea*, *Juniperus oxycedrus*, *Lavandula latifolia*, *Phillyrea angustifolia*, *Phillyrea latifolia*, *Phlomys purpurea*, *Pistacia lentiscus*, *Pistacia terebinthus*, *Prunus spinosa* and *Rosmarinus officinalis*); (2) all the species significantly enhancing conspecific recruitment were EM species (*Pinus halepensis*, *P. nigra* and *Cistus albidus*; this last with ectendomicorrhizal association); (3) however 3 EM species (*Quercus coccifera, Q. faginea* and *Q. rotundifolia*) had depressing effects on conspecific recruitment. It is possible that the recruitment of *Quercus* under conspecifics becomes limited during pre-germination stages. For example, it is well known that intense acorn consumption by animals occurs under oaks (Gómez 2004), so the benefits of EM fungi would be enjoyed too late to actually enhance recruitment.

Discerning the mechanisms that determine canopy-recruit interactions awaits future experimental and observational approaches. The nature of the constraints behind forbidden interactions and the types of forces explaining the consistency observed interactions can be rather complex. Note that the studied communities are formed by a diverse array of species with very different traits: different seed dispersal adaptations (none, wings, feathery pappus, fleshy fruits, acorns), seeds differently protected against seed predators (from hard husks like in *Crataegus*, to soft covers in the acorns of *Quercus*), different nutrient-acquisition associations (ectomycorrhizal or arbuscular fungi, nitrogen-fixing bacteria), different degrees of shade tolerance (from pioneers like *Cistus* to forest floor specialists like *Ruscus*) and different types of structural defenses (like hard spines in *Berberis hispanica* or *Prunus spinosa*). It is unlikely that the consistency and effect of canopy-recruit interactions in diverse assemblages of species has a single cause. The approach based on functional traits of the interacting species can be promising as it will allow testing different causes simultaneously for large number of species and a wide set of traits (Adler *et al.* 2013). Unfortunately, this approach has been barely used in the study of recruitment interactions (Beltrán *et al.* 2012; Valiente-Banuet & Verdú 2013; Soliveres *et al.* 2014). The only consistent pattern described to date is that the balance between competition and facilitation in recruitment seems to relate to plant size so that taller species tend to be more dependent on facilitation from others for their recruitment. Plant-soil

feedbacks mediated by mutualistic and antagonistic fungi are beginning to be incorporated as very relevant for community dynamics (Van der Voorde *et al.* 2011; Liu *et al.* 2016; Bennett *et al.* 2017; Teste *et al.* 2017). The role of many other traits potentially functional in relation to recruitment interactions remains unexplored. The detailed analysis of traits potentially functional in relation to canopy-recruit interactions is an important avenue for further research to improve our knowledge on the organization and dynamics of ling-lived plant communities.

Author's Contributions.

JMA and PJR conceived the ideas and designed methodology; JMA, MP and PJR collected the data; JMA and MP analysed the data; JMA and MP led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Table S1. List of species acting as recruit and/or canopy and their associated mycorrhizal fungi.

	Recruit	Canopy	Mycorrhizal Association
Open		X	
Acer granatensis	X	X	Arbuscular
Acer monspessulanum	X	X	Arbuscular
Amenlachier ovalis	X	X	Arbuscular
Berberis hispanica	X	X	Arbuscular
Cistus albidus	X	X	Ectendomycorrhizal
Cistus clusii		X	Ectendomycorrhizal
Cistus salviifolius	X	X	Ectendomycorrhizal
Crataegus laciniata	X	X	Arbuscular
Crataegus monogyna	X	X	Arbuscular
Cytisus scoparius	X		Arbuscular
Daphne gnidium	X	X	Arbuscular
Daphne laureola	X	X	Arbuscular
Echinospartum boissieri	X	X	Arbuscular
Genista cinerea		X	Arbuscular
Hormatophylla spinosa	X	X	Arbuscular
Ilex aquifolium	X	X	Arbuscular
Juniperus communis	X	X	Arbuscular
Juniperus oxycedrus	X	X	Arbuscular
Juniperus phoenicea	X	X	Arbuscular
Ligustrum vulgare	X		Arbuscular
Olea europaea	X	X	Arbuscular
Phillyrea angustifolia	X	X	Arbuscular
Phillyrea latifolia	X	X	Arbuscular
Phlomis purpurea	X	X	Arbuscular
Pinus halepensis	X	X	Ectomycorrhizal
Pinus nigra	X	X	Ectomycorrhizal
Pistacia lentiscus	X	X	Arbuscular
Pistacia terebinthus	X	X	Arbuscular
Prunus spinosa	X	X	Arbuscular
Quercus coccifera	X	X	Ectomycorrhizal
Quercus faginea	X	X	Ectomycorrhizal
Quercus ilex	X	X	Ectomycorrhizal
Quercus pyrenaica	X	X	Ectomycorrhizal
Rhamnus alaternus	X	X	Arbuscular
Rhamnus lycioides	X	X	Arbuscular
Rhamnus myrtifolius	X	X	Arbuscular
Rhamnus saxatilis	X		Arbuscular
Rosa canina	X	X	Arbuscular
Rosmarinus officinalis	X	X	Arbuscular
Ruscus aculeatus	X	X	Arbuscular
Salvia lavandulifolia	X		Arbuscular
Sorbus torminalis	X	X	Arbuscular
Taxus baccata		X	Arbuscular
Thymus mastichina	X	X	Arbuscular
Thymus zygis	X	X	Arbuscular
Ulex parviflorus	X	X	Arbuscular
Viburnum lantana	X	X	Arbuscular
Viburnum tinus	X	X	Arbuscular

CHAPTER 4

Phylogenetic effects on plant-plant interactions in mixed Pine-oak Mediterranean forests

Unpublished manuscript

Abstract: Current theories on species coexistence disagree on the relative importance of interspecific niche differences and neutral outcomes of interactions. Studies addressing this issue use the phylogenetic relationships between species as a surrogate for niche differences, since it is assumed that more closely related species should have more similar niche requirements and hence would compete more strongly with each other. Many studies explored the phylogenetic structure of the plant community focusing on the patterns resulting from the assembly processes (i.e. on which species co-occur in local assemblages) but do not consider explicitly any assembly process or mechanism. It is already well known that evolutionary relationship among plants may affect the outcome of their interactions. One important assembly mechanism is the recruitment of juveniles of one species under the canopy of established plants (what we call canopy-recruit interactions), because established plants can exert a strong filter on the recruitment of different species. In this study we addressed whether the set of canopy-recruit interactions observed in a local community is a function of phylogenetic distances between species. Moreover we assess whether the degree of phylogenetic relatedness between two interacting species may influence the spatial consistency and outcome of their interaction. For this purpose we collected information on sapling recruitment and constructed phylogenetically informed interaction networks for 10 mixed pine-oak forest communities of Southern Spain. Using Net Relatedness Index (NRI) we show that canopy-recruit interactions are more often established between distantly related species (phylogenetic overdispersion pattern). Further, our results demonstrated that key properties for the assembly of plant communities, such as spatial consistency of the interaction and its effect on recruitment, are also related to phylogeny. Taken together, our results strongly suggest that phylogeny, and therefore niche differences, play an important role in structuring canopy-recruit interactions.

Keywords: Replacement networks, phylogenetic overdispersion pattern, pairwise-specific interaction, regeneration niche, phylogeny, spatial consistency, facilitation, competition, enhancer recruitment effect, depressor recruitment effect.

INTRODUCTION

Ecologists have long debated on whether patterns in species assemblages originate from niche differences (Tilman 1982; Silvertown 2004) or by stochastic processes (Hubbell 2001). Although many authors have claimed for the need to move beyond this dichotomy to a broader theory of community structure that can simultaneously accommodate both approaches (Adler et al. 2007; Stokes and Archer 2010), still today some ecologists aim to demonstrate the high importance of non-neutral biological processes structuring communities. To address these ecological questions, over the last decade network ecologists have combined information about the evolutionary history of species (phylogenetic) with species interaction networks (Mouquet et al 2012; Peralta 2016). They used phylogeny as a surrogate for niche differences assuming that more closely related species should have more similar niche requirements and hence would compete more strongly with each other. If phylogenetic relationships between species played an important role shaping interaction patterns, then the dominance of non-neutral biological mechanisms would be evidenced (Peralta 2016). To perform these eco-phylogenetic studies ecologists benefited from a simultaneous growth of approaches based on complex network theory. In mutualistic networks and food webs several studies revealed that phylogeny may be key in shaping species interaction patterns (Cattin et al. 2004; Bersier and Kherli 2008; Rezende et al. 2009; Cagnolo et al. 2011), highlighting the importance of interspecific niche differences versus neutral outcomes of interactions.

Specifically in plant community ecology, many studies conducted in diverse types of communities explored the phylogenetic structure of the pool of species. These studies demonstrate that the phylogenetic relatedness of co-occurring plants can be a useful tool to investigate ecological patterns (Webb 2000; Webb et al. 2002; Cavender-Bares et al. 2004; Kembel & Hubbel 2006; Emerson & Gillespie 2008; Cavander-Bares et al. 2009; Silva & Batalha 2009; Chen 2013). There are two central lines of argument in these studies. (1) If the main process involved in the assembly of the community were environmental filtering of species based on their tolerance to abiotic conditions, then closely related species would be more likely to co-occur, leading to a high relatedness between co-occurring species. In the literature this pattern is called

"phylogenetic clustering" (Webb 2000; Tofts & Silvertown 2000). (2) On the contrary, if competitive interactions between species govern the organization of the community and the coexistence of species, then phylogenetically distant species would be more likely to co-occur, leading to low relatedness between co-occurring species. This pattern is called "phylogenetic overdispersion" (Cavander-Bares et al. 2004; Kembel & Hubell 2006). Fewer studies have used phylogenetic information to understand plant-plant interactions and the processes of assembly of plant communities. The studies conducted by Valiente-Banuet, Verdú and co-workers (see Valiente-Banuet & Verdú 2013, and references therein) have demonstrated that the probability that a species facilitates the recruitment of another under its canopy increases with the phylogenetic distance between the species, what ultimately leads to a pattern of phylogenetic overdispersion in the community (Valiente-Banuet et al. 2007; Castillo et al. 2010; Verdú et al. 2012). The classical explanation for facilitation involved the amelioration of the abiotic stress under the canopy of the established plants (Callaway 2007), but this does not agree with the existence of a phylogenetic overdispersion in the recruitment niches. Accordingly, recent studies explored the importance of biotic interactions in shaping plant recruitment niches. For example, Montesinos-Navarro et al. (2012) and Teste et al. (2017) have found that recruitment is enhanced when the recruiting species and the canopy plant have different arbuscular mycorrhizal associates or different nutrient acquisition strategies (arbuscular mycorrhizal, ectomycorrhizal, ericoid mycorrhizal, N-fixing and nonmycorrhizal cluster-rooted), what is likely to differ more between distantly related species. Other mechanisms involved in plant-plant recruitment interactions are related to plant pathogens (or antagonists in general). Liu et al. (2012) searched experimental evidence for a phylogenetic Janzen-Connell effect (Janzen 1970) between one focal species and several canopy species in a subtropical forest, concluding that the probability that a herbivore or pathogen may infect two plant species decreases with phylogenetic distance between the plants (see also Becerra 2007; Gilbert & Webb 2007; Ness et al. 2011). It is now clear from these studies that the interactions between recruiting and established plants should not be expected to be based in neutral (e.g. stochastic) mechanisms, but the number of communities where phylogenetic (niche-dependent) effects on such interactions have been explored is very small: Mexican semi-arid

communities (Valiente-Banuet and Verdú 2007), subtropical forests from China (Liu et al. 2012), tropical forests from South and Central America (Paine et al. 2012, Zhu et al. 2015) and intermediate successional stages in post-fire communities of Mediterranean mixed Pine-Oak forests of Southern Spain (Verdú et al. 2009). Therefore, more studies are needed before any generalizations can be made on this topic.

In the present study we analyze the phylogenetic pattern of canopy-recruit interactions (sensu Pulgar et al. 2017, Chapter 2) in late successional or mature stages of mixed Pine-oak Mediterranean forests of similar type to those studied in Verdú et al. (2009). Valiente-Banuet and Verdú (2007) and Verdú et al. (2009) studied phylogenetic structure of bipartite plant-plant facilitation networks. The present study makes a first attempt to show the association of community phylogeny and the pattern of canopy-recruit interactions. Canopy-recruit interactions are the basic elements that integrate replacement networks (Alcántara & Rey 2012). Differently from facilitation networks, canopy-recruit interactions form unipartite replacement networks where all species can play simultaneously the roles of canopy and recruit (Alcántara and Rey 2012; Pulgar et al. 2017, Chapter 2). This may seem a subtle difference buy it has some relevant implications for the analysis of interactions in a phylogenetic context. For example, interaction matrices of bipartite facilitation networks contain a smaller number of potential interactions (S_1 x S_2) than unipartite replacement networks of the same community ([S_1 U S_2] x [S_1 U S_2]), so the null model testing phylogenetic distances scans a wider and/or denser set of possible values in replacement networks. This makes the test of phylogenetic distances more conservative in canopy-recruit than in facilitation interactions. This assembly mechanism increased the phylogenetic diversity of the community during intermediate successional stages of recovery after fire. However, in mature stages of the community they found phylogenetic randomness in species composition but they could not assess the phylogenetic structure of canopy-recruit interactions in the mature stages. Thus, one objective of the present study is to test the hypothesis that canopy-recruit pairs in the mature stage of these forests show random phylogenetic distances.

Interaction networks studies have often assumed that if two species are known to interact at one location, they will interact wherever they co-occur (Havens 1992).

However some studies in pollination networks and food webs called into question this assumption, arguing that it neglects the fact that local community composition and foraging preferences may intervene in the realization of interactions (Poisot et al. 2015). In this way, the same species that interact in one location may not interact in another when for example their local abundances vary. Consequently, networks can vary through space in their interactions pattern (Petanidou et al. 2008; Novotny et al. 2009; Poisot et al. 2012; Poisot et al. 2015). Like other ecological networks, canopy-recruit interactions in the studied communities are not always realized due to stochasticity associated with changing abundances (Chapter 3). However, we have found in Chapter 3 that canopy-recruit interaction consistence (IC; a measure of how often an interaction is realized) in the studied communities is higher than expected from the abundance the species. If this propensity of canopy-recruit pairs to be realized whenever species co-occur is the result of niche differences rather than the outcome of stochastic fluctuation in the abundance or spatial distribution of species in local communities, then we would expect that IC should be related to phylogenetic distance between canopy and recruiting species. Few studies to date have addressed the spatial variation of interactions (see Chapter 3 and references therein) and none has assessed how phylogenetic distance between interacting species may influence the spatial consistence of the interactions.

Besides their spatial consistence, another fundamental property of canopy-recruit interactions is their effect on recruitment. The complex nature of the recruitment process makes it unlikely that the assembly of all canopy-recruit interactions could be driven by the same mechanism (Baumeister & Callaway 2006; Kawai & Tokeshi 2007; Smit et al. 2009; Soliveres et al. 2011). For example, some will be more influenced by seed dispersal patterns, others, by post-dispersal seed predators, and others by the presence of pathogenic or mutualistic fungi on the soil. The different influence of canopy species on these drivers makes them more or less suitable for the recruitment of different species (Rey & Alcántara 2000; Traveset et al. 2003; Gómez-Aparicio 2008). We can classify the global effect of a canopy species (*j*) on the recruitment of another species (*i*) as: enhancing (if recruitment of *i* under *j* is higher than expected from the local abundance of *j*), depressing (if recruitment of *i*

under *j* is lower than expected from the local abundance of *j*) or neutral (if recruitment of *i* under *j* is not different from what could be expected from the local abundance of *j*). We avoid calling these effects as positive, negative or neutral (as most studies do) because even a recruitment depressing interaction can contribute positively to the population dynamics of the recruiting species (since recruitment under the canopy species is not zero, in which case the interaction would not exist). Few studies have assessed the role of phylogenetic distance in the outcomes of pairwise interactions (Castillo et al. 2010; Soliveres et al 2012). Soliveres et al. (2012) evaluated the phylogenetic signal on the outcome of pairwise interactions established with two canopy species (*Quercus coccifera* and *Stipa tenacissima*). They found that phylogenetic distance between interacting species can be even more important than abiotic conditions to determine the outcome of plant-plant interactions. In this study we address this topic from a community-wide perspective. We relate the effect for each pairwise interaction to their phylogenetic distances. Following the results of Castillo et al. (2010) and Soliveres et al. (2012), we hypothesized that distantly related species pairs should form recruitment enhancing interactions, while closely related species should form recruitment depressing interactions.

The combined results of our analyses suggest that the processes that assembly canopy-recruit interactions in the studied communities are strongly influenced at some stage by the phylogenetic history of the plants. We found that distantly related species are more likely to recruit under each other, are more likely to do so in spite of spatial variation in their abundance and local community context, and are more likely to have enhancing effects on recruitment.

METHODS

Study site

The study was conducted in mixed forests from Southern Spain dominated by pines (*Pinus halepensis*, *P. nigra* subsp*. salzmanii* and *P. pinaster*) and oaks (*Quercus ilex, Q. faginea*, *Q. pyrenaica* and *Q. coccifera*); see Pulgar et al. (2017, in Chapter 2) for details. We located mature forest stands in 10 study sites in two protected areas: 6 study sites in *Parque Periurbano Monte de la Sierra* (Sierra de Jaén hereafter) with

mixed forests dominated by *P. halepensis*, *Q. ilex* and *Q. faginea*, and 4 study sites in *Parque Natural de las Sierras de Cazorla, Segura y Las Villas* (Sierra de Segura hereafter) with mixed forests dominated by *P. nigra* subsp. *salzmannii*, *Q. faginea* and *Q. pyrenaica*. In both areas the climate is Mediterranean, with rainfall concentrated in late autumn and winter and hot dry summers. Annual rainfall levels are around 710 mm in Sierra de Jaén and 890 mm in Sierra de Segura, and mean temperatures are around 14.6 and 11.6°C respectively. All sites were located more than 500 m from each other. We selected patches of forest as less perturbed as possible by human activities such as livestock and forestry during the last 50 years. Within each site we set 5 plots of 50 x 50 m (0.25 ha) all of them distributed so that their physiographical conditions (slope, aspect, soil depth, elevation) were as homogeneous as possible. Within each site all plots were spaced less than 300m from each other. At each plot we systematically looked for saplings of every woody species and noted whether they were recruiting under the crown of some canopy plant species or in open interspaces (see Pulgar et al. 2017, in Chapter 2, for more methodological details of sampling). With these observations of recruitment we constructed an adjacency matrix per site, consisting of recruit species in rows and canopy species in columns. Additionally in each site one plot of 50 x 50 m was divided into 25 sub-plots of 10 x 10 m where we estimated the cover of the horizontal projection of the crown for each species using a tape-measure for shrub species and a visual estimate for trees (further details can be found in Chapter 3).

Data analysis

Phylogenetic pattern of the canopy-recruit interactions

We assembled a phylogenetic supertree (Figure 1) matching all sampled species in our study (63 species) with those contained in the megatree obtained of *TimeTree*, a public knowledge-base where thousands of published studies are assembled into a searchable tree of life scaled to time (Hedges & Kumar 2009). However 24 species of our study are not included yet in this database. To solve this, we searched the literature to find their closest congeneric species present in the megatree of *TimeTree* and considered them as if they were sister taxa. Even so, we removed 8 species of the study since they had not congeneric species in the *TimeTree* database (the list of

replaced and removed species is shown in Table S1). The pairwise phylogenetic distances were computed from the phylogenetic tree using the *cophenetic.phylo* function implemented in the package *ape* (version 4.0) in R (R Development Core Team 2010). In this way we obtained a phylogenetic distance matrix between all pairs of species in the study.

Sampled species	Surrrogate species
Berberis hispanica	Berberis bealei
Celtis australis	
Colutea hispanica	Colutea arborescens
Crataegus laciniata	Crataegus pubescens
Daphne laureola	Daphne oleoides
Echinospartum boissieri	
Fraxinus angustifolia	Fraxinus excelsior
Genista cinerea	Genista tinctoria
Jasminum fruticans	
Lavandula latifolia	
Osyris alba	
Prunus mahaleb	Prunus avium
Quercus pyrenaica	Quercus robur
Quercus faginea	Quercus alba
Quercus coccifera	Quercus variabilis
Quercus ilex	Quercus fusiformis
Retama sphaerocarpa	
Rhamnus myrtifolius	
Salvia lavandulifolia	Salvia officinalis
Sorbus torminalis	Sorbus tianschanica
Teucrium fruticans	Teucrium scorodonia
Teucrium polium	
Thymus zygis	Thymus vulgaris
Thymus mastichina	Thymus pulegioides

Table S1. List of replaced and removed (bold text) species in our study community are presented in left column. Phylogenetically close species replacing them are presented in right column. Note that the surrogate species were always congeneric of the sampled species.

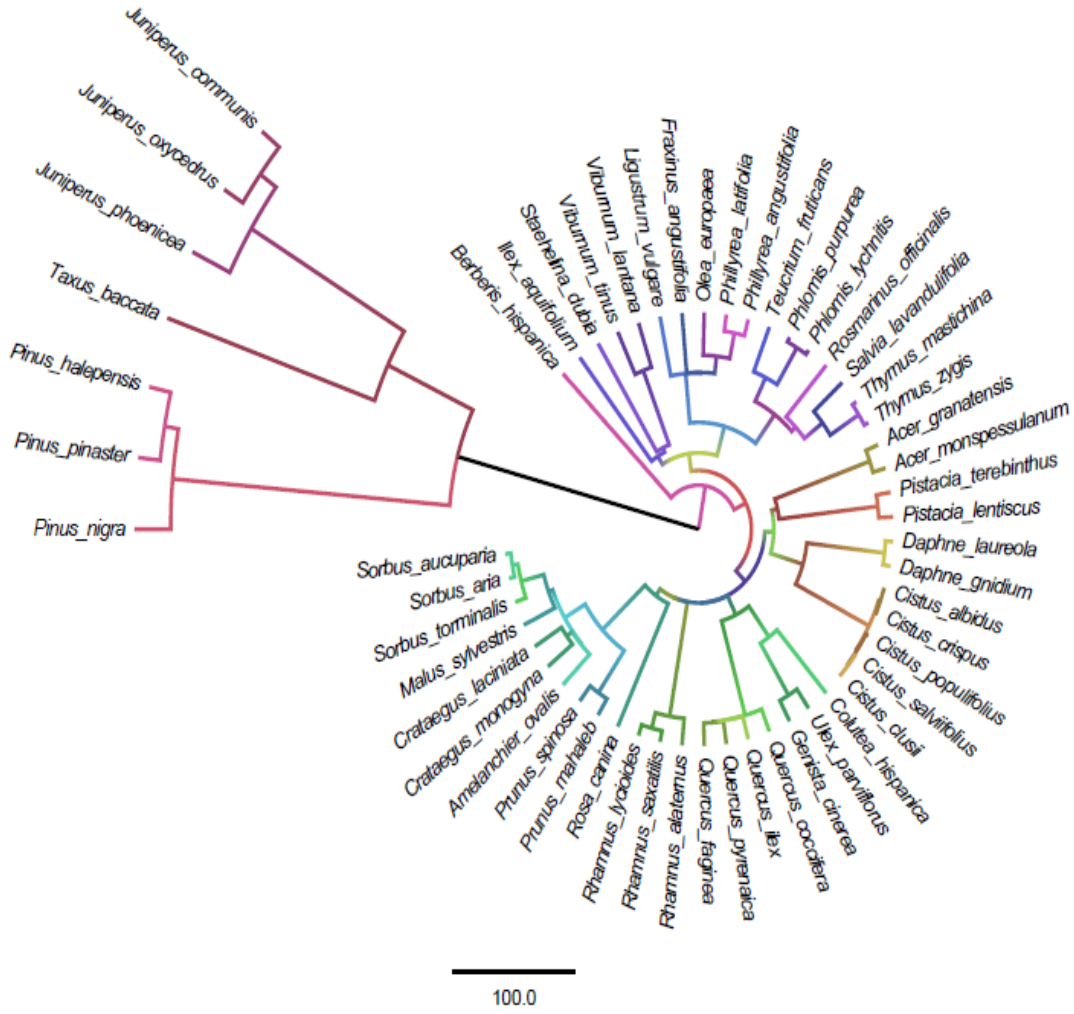

Figure 1. Phylogenetic tree of the species present in the study forests. The scale bar indicates million years.

In each site in order to test whether interactions occur between phylogenetically distant species we compared the mean phylogenetic distance (MPD hereafter) between interacting canopy and recruiting species against the expected MPD of a null model (rndMPD). MPD for each site was calculated crossing the interactions and phylogenetic distance matrices of the regional species pool. In each site the null model was constructed by reshuffling 999 times the species labels across the phylogenetic tree of the regional species pool and in each iteration we estimated a new MPD value where interacting species had changed their phylogenetic relationship (the same procedure used in Verdú et al. 2009). We standardized the measures of phylogenetic relatedness between interacting species pairs for each site by calculating the Net Relatedness Index (NRI) as follows (Webb et al. 2002):

$$NRI = -1 \times (MPD - rndMPD/sdrndMPD)$$

where rndMPD is the mean of MPD values from 999 randomizations and sdrndMPD is the standard deviation of these values. NRI < 0 would indicate higher MPD than expected from the null model, indicating that interacting species pairs tend to be distantly related (phylogenetic overdispersion). By the contrary NRI > 0 would indicate lower MPD than expected from the null model, indicating that interacting species pairs tend to be closely related (phylogenetic clustering). Since this standardized index should follow the unit normal distribution with mean = 0 and variance = 1, if the index is less than -1.96 (or larger than 1.96) the overdispersion (or clustering) pattern identified may be regarded as statistically significant (Chen 2013). The analyses of phylogenetic structure were run with *R software* (*R Development Core Team* 2010).

According to Kraft et al. (2007), the size of each local community in relation to that of the regional pool influences the power of the phylogenetic analysis of plant community structure. In general, the greatest power is obtained for communities of intermediate size, ranging from approximately 30 to 60% of the pool (Swenson et al. 2006; Silva & Batalha 2009). Therefore, caution should be taken in rejecting the null hypothesis in cases where the community is very species poor or rich in relation to the regional pool (Kraft et al. 2007). In our study the regional pool was composed of 55 species while the study plots averaged 25.5 species (range 17-32; Table 1). Consequently, the percentages of the local to the regional species pool were intermediate (mean 46.36%, range 30.91-58.18%), representing the situation with the greatest statistical power to detect community structure.

Phylogenetic distance and the properties of canopy-recruit interactions

Spatial consistency of an interaction between recruiting and canopy species (IC) was calculated as the number of sites where we detected the interaction relative to the number of sites where (1) there were adults of both species and (2) there were

saplings of the recruiting species in the site. In addition, for our analyses we only included interactions between species that co-occurred at least in three sites. Taking into account all these restrictions, we finally obtained IC values for 526 interactions, including 204 unobserved interactions in which two species co-occurred at least in three sites but they never interacted (IC = 0). If the phylogenetic distance between two species increases their probability of interaction, then we would expect that distantly related species would interact more frequently than closely related ones. To address this prediction we used a generalized linear model (GLM) in which we entered pairwise phylogenetic distances and IC (log transformed) as independent and dependent variable respectively, assuming Gaussian error and log link function.

Additionally we assigned types of seed dispersal to each species in the community (Table 2) to determine if dispersal syndromes may constitute and early filter of the specifity and spatial consistency of the canopy-recruit interactions eventually found. We used a two-way analysis of variance (ANOVA) to examine whether seed dispersal of recruiting or canopy species (or one combination of both) could influence IC values. The dispersal mechanisms of shelter and recruiting plants were separated into three categories: 'zoochory' (those seeds with fleshy fruits and acorn dispersed mainly by birds or small mammals), 'anemochory' (seed dispersal by wind) and 'autochory' (self-dispersal of seeds either by gravity or by explosive discharge of seeds). Information on the dispersal mechanism was obtained from the literature (Herrera 1995, Blanca et al. 2009). All analyses were run using *R software* (R Development Core Team 2010).

ZOOCHORY	ANEMOCHORY	AUTOCHORY
Amelanchier ovalis	Acer granatensis	Cistus albidus
Berberis hispanica	Acer monspessulanum	Cistus clusii
Crataegus laciniata	Fraxinus angustifolia	Cistus crispus
Crataegus monogyna	Pinus halepensis	Cistus populifolius
Daphne gnidium	Pinus nigra	Cistus salviifolius
Daphne laureola	Pinus pinaster	Colutea hispanica
Ilex aquifolium		Genista cinerea
Juniperus communis		Phlomis lychnitis
Juniperus oxycedrus		Phlomis purpurea
Juniperus phoenicea		Rosmarinus officinalis
Ligustrum vulgaris		Salvia lavandulifolia
Malus sylvestris		Teucrium fruticans
Olea europaea		Thymus mastichina
Phillyrea angustifolia		Thymus zygis
Phillyrea latifolia		Ulex parviflorus
Pistacia lentiscus		
Pistacia terebinthus		
Prunus mahaleb		
Prunus spinosa		
Quercus coccifera		
Quercus faginea		
Quercus ilex		
Quercus pyrenaica		
Rhamnus alaternus		
Rhamnus lycioides		
Rhamnus saxatilis		
Rosa canina		
Sorbus aria		
Sorbus aucuparia		
Sorbus tominalis		
Taxus baccata		
Viburnum lantana		
Viburnum tinus		

Table 2. Dispersal mechanism assigned to each plant species in our study.

Using only interaction matrices arising from 50 x 50 m plots where cover of canopy species was sampled (see above), we performed a multinomial null model analysis testing whether interaction frequencies at each site are determined by canopy species abundances (i.e. neutral interactions) or they may be driven by recruitment enhancing interactions (such as seed attraction or facilitation) or by recruitment depressing interactions (such as presence of allelochemicals, pathogens or competition) (see chapter 3 for more methodological details). Thus, we obtained estimates of interactions effect (classified as recruitment enhancing, depressing or neutral) in each site. For this analysis we chose those canopy-recruit interactions for which we had enough power to test the interaction effect in at least two sites. We classified an interaction as recruitment enhancing if it did not show recruitment depressing effect in any of the sites where it could be tested (and vice versa). We excluded 6 interactions whose effect varied between enhancing and depressing among sites. In all, we could accurately classify 45 interactions as recruitment enhancing, 24 as neutral and 22 as recruitment depressing. If phylogenetic distance is related to the probability of interaction, we would expect that recruitment enhancing interactions would be formed between more distantly related species than recruitment depressing interactions. To test this prediction, we conducted a one-way ANOVA comparing the mean phylogenetic distance between interactions with different effects on recruitment. We performed a Tukey´s test as *post hoc* analysis for pairwise comparisons. All analyses were run using R software (R Development Core Team 2010).

RESULTS

Phylogenetic overdispersion was the prevalent pattern across forest patches (Table 1). The MPD in the set of studied communities was significantly larger than expected from the null model (Wilcoxon's matched pairs test: $Z = 2.60$, $N = 10$, $P < 0.01$). Accordingly, all but one of the forests had negative NRI, and 4 of them had significantly negative NRI. All the significantly negative NRI values belonged to Sierra de Jaén.

Generalized linear model showed that interaction consistence was significantly related to phylogenetic distance between species ($E = 0.000961 \pm 0.00038$, $P = 0.013$),

although phylogenetic distance explained very little of the total variance of IC (R^2 = 0.028). On the other hand, the two-ways analysis of variance showed that dispersal syndrome of recruiting species (F = 0.30, DF = 2, P = 0.74), dispersal syndrome of shelter species (F = 0.98, DF = 2, P = 0.38) and the interaction of both (F = 1.47, DF = 4, P = 0.21) were not significantly related to interaction consistence. Hence, dispersal syndrome did not apparently represent an early filter determining the specificity and spatial consistency of the canopy-recruit interactions eventually found.

The effect of the canopy-recruit interaction on recruitment as an assembly mechanism was significantly dependent on phylogenetic distances between interacting species (F = 4.36, DF = 2, P = 0.016). As shown in figure 2, post-hoc Tukey tests indicate that MPD did not differ between interactions with enhancing and neutral effects (P = 0.66), but these differed significantly from the MPD of recruitment depressing interactions (P = 0.05 and 0.02, respectively).

FOREST PATCH	PROTECTED AREA	*S*	MPD (Myr)	rndMPD (Myr)	sdMPD (Myr)	NRI
Barranco de Los Ladrones	*Monte de la Sierra*	32	381.76	261.24	59.13	**-2.04***
Cañada de las Azadillas alto	*Monte de la Sierra*	25	304.16	292.50	64.83	-0.18
Cañada de las Azadillas bajo	*Monte de la Sierra*	26	387.47	265.05	51.87	**-2.36***
Cruz de Chimba	*Monte de la Sierra*	29	393.96	272.12	60.40	**-2.02***
Nava del Espino	*Cazorla, Segura y Las Villas*	27	352.17	298.81	67.50	-0.79
Navalcaballo	*Cazorla, Segura y Las Villas*	22	341.63	295.78	84.58	-0.54
Palomares	*Monte de la Sierra*	17	321.84	289.95	93.98	-0.34
Quiebrajano	*Monte de la Sierra*	31	375.12	260.94	57.66	**-1.98***
Reserva	*Cazorla, Segura y Las Villas*	27	277.80	299.59	58.88	0.37
Rio Madera	*Cazorla, Segura y Las Villas*	19	387.88	283.39	88.11	-1.19

Table 1. Phylogenetic distance of canopy-recruit interactions in the studied forest communities. Table consists of the species richness (S), the observed (MPD) and randomized (rndMPD) mean phylogenetic distance of interacting species, its standard deviation (sdMPD) and the Net Relatedness Index (NRI). Significant values are marked by asterisks at the probability level (P<0.05).

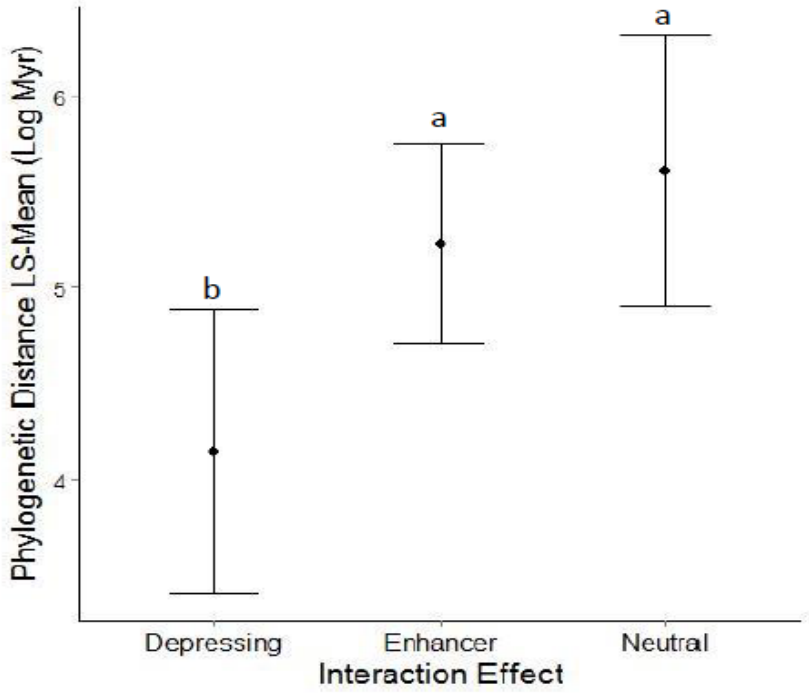

Figure 2. Comparisons of LS-Means Plot. Dots depict the mean of pairwise phylogenetic distances for each group of interactions according to their effect. Bars depict 95% Confidence intervals. The same letter above bars indicates that phylogenetic distance is not significantly different from each other.

DISCUSSION

Studies on ecological networks show that phylogenetic signals can be tracked in the processes structuring different types of interactions (Cattin et al. 2004; Bersier & Kherli 2008; Rezende et al. 2009; Cagnolo et al. 2011). However few studies addressed this issue at the level of plant communities. The most complete community-level studies published to date (Valiente-Banuet & Verdú 2007; Verdú et al. 2009) found that nurse-facilitated plant interactions occur between distantly related species. Overall our results suggest that the evolutionary relationships of species in the studied forest communities play a significant role in the assembly of canopy-recruit interactions. Interacting species pairs tend to be phylogenetically distant in all but one of the study sites, especially in 4 of the sites where the NRI was statistically significant, indicating phylogenetic overdispersion in the canopy-recruit pairs. Another five sites showed also negative NRI values but they were not different from expected under the null model, what would qualify them as phylogenetically random. According to

previous studies of nurse-beneficiary interactions, phylogenetic overdispersion of the interactions could be due to evolutionarily conserved regeneration niches so that closely related species display larger niche overlap and then are more likely to compete for resources (Valiente-Banuet & Verdú 2007; Castillo et al. 2010), or they can be more likely to share soil pathogens (Becerra 2007; Gilbert & Webb 2007; Ness et al. 2011; Liu et al. 2012). Discerning the relative roles of these and other possible drivers of canopy-recruit interactions is beyond the scope of the present study, but the existence of a significant phylogenetic signal clearly suggest that the assembly of these interactions is influenced by non-neutral processes.

It is noteworthy that all the forests studied in Sierra de Segura showed phylogenetic randomness in canopy-recruit interactions, while 4 out of 6 forests studied in Sierra de Jaén showed significant overdispersion. There is not a simple explanation for these differences. On the one hand, observational and experimental studies on facilitation suggest that nurse species tend to facilitate distantly related recruits (Valiente-Banuet & Verdú 2007; Castillo et al. 2010). On the other hand, the importance of facilitation for recruitment at the community level increases as abiotic stress increases (Rey et al. 2016). These two patterns could help explain our results. The abiotic conditions are more suitable for recruitment in Sierra de Segura (with milder summer drought), so niche differences would not impose a strong filter on which canopy-recruit interactions are realized. Consequently, the phylogenetic signal is not strongly expressed in these conditions. However, conditions for recruitment in Sierra de Jaén are more stressful (with stronger summer drought), so facilitation would be stronger and the phylogenetic distance between canopy and recruit would be more important for the realization of the interaction. However, this explanation is not complete since there are two forest areas in Sierra de Jaén where canopy-recruit interactions are phylogenetically random. These two forest areas may have experienced historically higher levels of disturbance by cattle, since they are close to a traditional cattle way, what may have altered the recruitment of those species preferred by browsing sheep and goats.

Part of the differences of the phylogenetic pattern of canopy-recruit interactions could be caused by spatial variation in the environmental scenario where

they must take place, so some interactions may not be realized in some sites just by chance. Nevertheless, in Chapter 3 we found that canopy-recruit interactions tend to be realized more often than expected from the abundance of species and that the frequency of non-neutral interactions is too high to be the result of neutral stochastic processes. These results led us to conclude that the assembly of canopy-recruit interactions is far from neutral. In the present study we have found further evidence on this direction since both IC and the effects of interactions are related to the phylogenetic distance between the interacting species. Interaction consistency across forest patches was positively related to phylogenetic distance between species. The propensity of two species to establish a canopy-recruit interaction under different community settings (e.g. different composition and abundance of species, different spatial distributions of individuals and even different degrees of climatic stress) is partly related to their phylogenetic distance. However, phylogenetic distance explained only around 2% of the variance of IC, so many other factors must influence interaction consistency. On the other hand, the effect of phylogenetic distance on IC may not by exactly linear and monotonic, since events of niche convergence can add noise on the studied relationship. More detailed analyses of the evolution of recruitment niches along phylogenetic trees are required to gain a clearer picture on this relationship.

Some of the non-explained variance of the specificity and spatial consistency of the interaction canopy-recruit could potentially be due to dispersal mechanism of canopy or recruiting species or even to the interaction of both as early filter of the recruitment (e.g. refuge effect could promote the specificity of interaction between species by means of nucleation processes). In fact, a large number of studies suggested that dispersal may be a crucial factor determining the interaction among species (Verdú & García-Fayos 1996; Dean et al. 1999; Pausas et al. 2006; Gómez-Aparicio 2008; Soliveres et al. 2012). However, according our results dispersal mechanism has no direct influence on specificity to shape canopy-recruit interactions, suggesting that relationship found between specificity and phylogenetic distances may be due exclusively to post-dispersal facilitative effects (Callaway 1995).

Interaction effects on recruitment also depend on the phylogenetic relatedness between species. Our results suggest that when the phylogenetic distance is under 135My it is very likely that the interaction has a recruitment depressing effect; above this distance, the interactions can have neutral or enhancing effects. Thus, taxa from lineages that diverged before the Cenozoic (more than c.a. 66Mya) are more likely to have neutral or enhancing effects on each other's recruitment. This result roughly agrees with the meta-analysis of nurse-based restoration experiments worldwide conducted by Verdú et al. (2012) who found that the minimum phylogenetic distance between nurse and facilitated species to enhance early survival of the latter was around 100My, what approximately corresponds to a divergence time of 50My. It seems possible that the evolution of regeneration niche divergence is an extremely slow process that takes tens of millions of years to be completed after a speciation event. For example, Valiente-Banuet & Verdú (2006) found that Mediterranean plant lineages that diversified during the Tertiary may have persisted to the present by conserving their regeneration niche thanks to facilitation by more drought tolerant lineages evolved in the Quaternary.

CONCLUDING REMARKS

Altogether our study suggests that evolutionary relationships among plant species may play an important role shaping the pattern of canopy-recruit interactions. Since we used phylogeny as a surrogate for regeneration niche, the phylogenetic structure found in our study makes it evident that the role of niche differences structuring plant communities could be at least as important as neutral processes. Specifically, phylogenetic over-dispersion indicates that established canopy plants would exert a stronger filter on the recruitment of ecologically similar species (niche overlapping) than on the recruitment of species with different ecological requirements (niche differentiation). Complementarily to this study, an interesting avenue for further research would be unraveling the tangible assembly mechanisms underlying phylogeny which could be structuring the canopy-recruit interaction patterns (e.g. functional traits, plant-mycorrhizal fungi mutualism or plant-antagonist interactions).

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SÍNTESIS DE LOS RESULTADOS

En todas las redes de reemplazamiento determinadas en este estudio existe un elevado número de especies formando parte de un gran ciclo de interacciones intransitivas (núcleo de la red). Según las simulaciones realizadas en el primer capítulo de la tesis, estas especies tienen una alta probabilidad de persistir en el tiempo. Sin embargo, la participación de una especie en un grupo de interacciones intransitivas diferente del núcleo no siempre aumenta su probabilidad de persistencia. Por otra parte nuestros resultados mostraron que la existencia de este gran grupo intransitivo de interacciones de reclutamiento puede contribuir a la persistencia de más especies de aquellas que participan directamente en él. Por ejemplo, las simulaciones mostraron que las especies satélite, unidas transitivamente al núcleo, también tienen una alta probabilidad de persistencia. La existencia de este núcleo intransitivo tiene un fuerte impacto sobre la biodiversidad, aumentando notablemente el número de especies que pueden coexistir en estas comunidades, incluso tratándose de especies relacionadas de forma transitiva con especies del núcleo. De hecho, en las redes de reemplazamiento muestreadas las especies del núcleo y las especies "satélite" aportan el 90% de la diversidad total en las comunidades de estudio. También encontramos un pequeño grupo de especies cuya persistencia dependía en última instancia de la creación de espacios abiertos para el reclutamiento (especies dependientes de perturbaciones). La existencia de perturbaciones (como fuego o caída de árboles) podría permitir a estas especies persistir, lo que puede contribuir también a un incremento de la diversidad. Sin embargo, esto puede depender en gran medida de la fuerza y recurrencia con la que ocurran estas perturbaciones.

Por otra parte, según los resultados obtenidos en el segundo capítulo, el esfuerzo de muestreo empleado para la determinación de las redes de reemplazamiento (aproximadamente 1 hectárea muestreada para cada red) es suficiente para obtener estimas fiables de la mayoría de las propiedades estructurales, y de los descriptores funcionales y de estabilidad. Estos resultados validan a nuestras redes para el análisis de dinámica y estabilidad. Los resultados mostraron que el número de especies y la conectividad se estabilizaron dentro de nuestro rango de muestreo, así como los descriptores funcionales y de estabilidad. Sin embargo, el

número de interacciones y el número medio de interacciones por especie no se estabilizaron con este esfuerzo de muestreo en la mayoría de los sitios de estudio. Las comparaciones entre comunidades no deberían basarse en ninguno de estos dos parámetros a menos que se tengan en cuenta explícitamente las posibles diferencias en esfuerzo de muestreo entre redes. Pero, en general, la baja superficie de muestreo requerida para el cálculo fiable de la mayoría de los descriptores de la red de reemplazamiento, convierte a estas redes en una herramienta altamente eficiente para analizar la dinámica y estabilidad de la comunidad. Además, dado que tamaños de parcelas de muestreo como el de las utilizadas en este estudio son usados frecuentemente en estudios de ecosistemas forestales mediterráneos, los análisis de dinámica basados en el concepto de red de reemplazamiento podrían ser fácilmente incorporados a los estudios de ecología forestal.

En el tercer y cuarto capítulo de la tesis estudiamos los factores que influyen en que las interacciones adulto-recluta que integran las redes reemplazamiento estén presentes o ausentes en las distintas localidades en que ambas especies aparecen. Los resultados del capítulo 3 mostraron que los efectos de la identidad de las plantas sobre la frecuencia de las interacciones adulto-recluta son lo suficientemente fuertes para ser detectados a pesar de la estocasticidad espacial intrínseca al proceso de reclutamiento. De hecho, nuestros resultados demuestran que los individuos adultos de la comunidad pueden tener un efecto neutral sobre el reclutamiento de bastantes especies, pero también un efecto potenciador o depresor del reclutamiento de otras muchas. Esto descarta la posibilidad de que, como plantearon algunas teorías, las interacciones inter-específicas sean exclusivamente debidas a procesos estocásticos. Además, la especificidad en el reclutamiento se pone de manifiesto en la consistencia geográfica de las interacciones adulto-recluta (a pesar de la estocasticidad espacial relacionada con la abundancia y distribución espacial de las especies). Por otro lado, los resultados del cuarto capítulo mostraron que las relaciones evolutivas entre las especies puede tener un papel importante formando el patrón de interacciones adulto-juvenil que estructuran las redes de reemplazamiento. Además de la influencia de las relaciones filogenéticas en el ensamblaje local, nosotros encontramos que la filogenia de la comunidad influye también en la consistencia espacial de las

interacciones y su efecto sobre el reclutamiento. Si consideramos la filogenia de la comunidad como un sucedáneo del nicho de regeneración, la estructura filogenética encontrada en las interacciones adulto-juvenil evidencia de nuevo que el papel de la diferenciación de nicho estructurando las comunidades de plantas puede ser más importante que los procesos neutrales. El patrón de sobredispersión filogenética apoya una vez más que las plantas adultas establecidas en la comunidad ejercerían un filtro más fuerte sobre el reclutamiento de especies ecológicamente similares que de especies con diferentes requerimientos de nicho. Por otra parte nuestros resultados mostraron que la sobredispersión filogenética solo parece ser significativa cuando el estrés abiótico es relativamente alto, sin embargo desaparece en las zonas de estudio donde el estrés hídrico disminuye ligeramente. Esto plantea la posibilidad de estudiar si la influencia de la filogenia en la estructuración de las redes planta-planta podría cambiar a lo largo de un gradiente ambiental, donde el ambiente podría tener una mayor (o menor) influencia sobre el patrón de interacciones observado.

En conjunto, los resultados de los capítulos 3 y 4 revelaron que el ensamblaje de las interacciones adulto-recluta que estructuran las redes de reemplazamiento no es un proceso exclusivamente neutral, lo que reforzó las conclusiones sobre estructura y estabilidad de estas comunidades forestales. Aunque puede ser una tarea bastante compleja, estos resultados animan a comprobar de manera observacional y/o experimental el tipo de mecanismos (subyacentes a la filogenia) que limitan el reclutamiento entre pares de especies, así como los tipos de fuerzas que pueden explicar la especificidad de las interacciones observadas.

GENERAL CONCLUSIONS

1. In plant-plant replacement networks species participating in the largest group of intransitive interactions (the core of the network) have high probabilities of persisting in the long term. However, participation in a group of intransitive interactions other than the core does not always guarantee persistence.

2. Participating in transitive interactions does not always decreases persistence because certain species (the satellites) transitively linked to the core have also a high persistence probability. The existence of large intransitive set (core) of interactions in replacement network can have a disproportionate positive effect on biodiversity, since it may sustain the persistence of more species than those participating in the core.

3. Reliable estimates of replacement network properties such as the number of species, connectivity, persistence, error and attack sensitivity, can be obtained with sampling areas of even less than 1 ha. The small sample area required makes this framework highly cost-effective to analyze forest community dynamics, structure and stability.

4. Estimates of the number of links and link density per species do not reach stable values within our sampling effort, but reached relatively high values of completeness, what suggests that the proportion of undetected links must be small (< 14% in all the studied communities).

5. Even though spatial stochasticity is intrinsic to the recruitment process, the assembly of canopy-recruit interactions structuring replacement networks is far from neutral.

6. A given species can have neutral but also enhancer or depressor effects on the recruitment of other species. Furthermore canopy-recruit interactions are found (or absent) more consistently across sites than expected from a neutral model that accounts for the abundances and spatial distribution of the species.

7. Evolutionary relationships among plant species may play an important role shaping the local pattern of canopy-recruit interactions. Further, key properties for the assembly of plant communities, such as spatial consistency of the interaction and its effect on recruitment, are also related to phylogeny.

8. The phylogenetic effect found in our study makes it evident that niche differences play a mensurable role in structuring plant communities, although they do not blur the influence of neutral processes.