Ecological interactions mediated by the European mistletoe,

Viscum album subsp. *austriacum,* in Mediterranean forests—an integrated perspective

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Ecological interactions mediated by the European mistletoe, *Viscum album* subsp. *austriacum,* in Mediterranean forests—an integrated perspective

Memoria presentada por Ana Mellado García para optar al Grado de Doctor con Mención Internacional en Ciencias Biológicas por la Universidad de Granada. Esta memoria ha sido realizada bajo la dirección de Dr. Regino Zamora Rodríguez, Catedrático de la Universidad de Granada

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Certifica que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral: "Ecological interactions mediated by the European mistletoe, *Viscum album* subsp. *austriacum*, in Mediterranean forests—an integrated perspective", son aptos para ser presentados por Ana Mellado García ante el Tribunal que en su día se designe, para aspirar al Grado de Doctor con Mención Internacional en Ciencias Biológicas por la Universidad de Granada. Y para que así conste, en cumplimiento de las disposiciones vigentes, extiendo el presente certificado a 28 enero de 2016.

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La doctoranda Ana Mellado García y el director de la tesis Regino Zamora Rodríguez, garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por la doctoranda bajo la dirección del director de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

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Content

This Thesis is based on the following papers:

Chapter 1 Mellado A. & Zamora R. (2014) Linking safe sites for recruitment with host-canopy heterogeneity: The case of a parasitic plant, Viscum album subsp. austriacum (Viscaceae). American Journal of Botany, 101, 1-8.

Chapter 2 Mellado A. & Zamora R. (2014) Generalist birds govern the seed dispersal of a parasitic plant with strong recruitment constraints. Oecologia, 176, 139-147.

Chapter 3 Mellado A. & Zamora R. (2015) Spatial heterogeneity of a parasitic plant drives the seed-dispersal pattern of a zoochorous plant community in a generalist dispersal system. Functional Ecology doi: 10.1111/1365-2435.12524.

Chapter 4 Mellado A., Morillas L., Gallardo A. & Zamora R. Temporal dynamic of parasite-mediated linkages between the forest canopy and soil processes and microbial community. Submitted.

Chapter 5 Mellado A. & Zamora R. Parasites structuring ecological communities: the mistletoe footprint in Mediterranean pine forests. Manuscript.

Summary

In this thesis, we seek a deeper and broader understanding of the ecological interactions mediated by the mistletoe *Viscum album* subsp. *austriacum* in Mediterranean forests. Mistletoes constitute an interesting group of hemiparasitic epiphytes widely distributed around the world that have been thoroughly investigated with respect to their physiology, morphology, pharmacology and their role as forest pest. However, many aspects of their ecology remains a mystery. Better known as harmful parasites that decrease the vigor of their host trees, today we have little knowledge about the ecological interactions mediated by these parasites, as well as their direct and indirect effects on natural communities and ecosystems. In this thesis, we show that, although being a minor component of the forest community (in terms of abundance and biomass), mistletoe can play a relevant role in the forest. Besides its detrimental effect on the host, mistletoe parasitism promotes a series of direct and indirect effects able to affect the structure and dynamic of the forest community, as well as key ecosystem processes, such as nutrient cycling. Spread over five chapters we study the close relationship of mistletoe with its host trees and seed dispersers, as well as the effect of the parasite on different organisms of the natural community and ecosystem properties.

In *Chapter I*, we focus on the mistletoe-host interaction. We analyze different factors shaping the spatial distribution of *Viscum a. austriacum* in the forest canopy, including the host specificity and the variation of biotic and abiotic fac-

tors limiting safe sites for mistletoe recruitment. We found that mistletoe shows a strong host specificity for pine species, in particular for *Pinus nigra* (the most abundant species) at the local scale. We also found that biotic (seed predation) and abiotic (light and temperature) factors exert a non-random filter on mistletoe regeneration, resulting in recruitment hotspots at the periphery of tree branches, under pine-needle protection, and sites with high probability of recruitment failure at thicker and more exposed locations.

In *Chapter II*, we focus on the mistletoe-vector interaction. We study the seed dispersal effectiveness of an unspecialized group of frugivorous birds providing dispersal services to a parasitic plant with extremely narrow ranges of safe sites to recruit.

Our results indicate that a wide and heterogeneous assemblage of generalist birds successfully disperse mistletoe seeds by two different mechanisms: endozoochory and ectozoochory. Large generalist birds (Thrushes) constitute the most effective dispersal group; however, they provide low-efficiency services, consuming numerous fruits in order to successfully disperse a single seed. In contrast, small generalist birds provide better quality and more efficient dispersal, but of low-quantity. Finally, opportunistic birds are very efficient dispersers by moving seeds externally and directly to safe sites of the tree periphery. By having a wide and heterogeneous range of seed dispersers mistletoe ensures the maintenance of re-infection processes within the population and the colonization of new infection foci.

In *Chapter III*, we examine interactions between mistletoe and fleshy-fruited plants of the understory. We analyze the effect of *Viscum a. austriacum* spatial heterogeneity on the seed-deposition pattern of the zoochorous plant community. We observed that frugivorous birds respond to mistletoe patchiness by visiting parasitized trees preferentially to unparasitized ones, generating a differential deposition of mistletoe seeds on tree branches, increasing re-infection processes within the host, while dispersing seeds of co-fruiting species under the host canopy. Moreover, as mistletoe fruit-crops vary little from one year to the next, there is a temporal persistency of seed deposition sites, with the canopy of parasitized trees consistently receiving large mistletoe seed rain. Otherwise, understory species show strong fruit-crop fluctuations between different years, which is reflected in the abundance of seeds reaching microsites beneath the host each year.

In *Chapter IV*, we explore mistletoe-mediated aboveground-belowground interactions. We analyze the effect of *Viscum a. austriacum* and the parasitic and mutualistic interactions it mediates from the forest canopy on soil-chemical and biological properties. We found that mistletoe modifies the linkages between the forest canopy and the soil through time, increasing the amount, quality and diversity of organic matter inputs beneath the host canopy, directly through its nutrient-rich litter and indirectly through the decrease of host litterfall and the increase of bird-derived debris. This greater abundance and diversity of organic compounds gives rise to enriched hotspots able to support greater and more functionally diverse soil microbial communities beneath parasitized hosts, the effects of which are accentuated after host death. Thus, mistletoe, enhanced by the biotic interactions it mediates, intensifies soil-resource availability, regulating composition, abundance and spatial distribution of soil microbial communities.

In *Chapter V*, we study the effect of mistletoe on the structure and dynamics of the plant community. We seek to understand whether mistletoe-induced changes under host patches, coupled to the effect of post-dispersal processes, ultimately result in changes in the plant community assemblage. We found that *Viscum a. austriacum* plays an important role in structuring the plant community. Mistletoe induces significant changes on host growth through its parasitic interaction, leading to greater light infiltration to the forest floor. At the same time, mistletoe facilitates seed arrival of understory species on the fertilization islands generated beneath the host, where conditions for seedling establishment improve. Consequently, parasitized trees concentrate a more abundant and richer fleshy-fruited plant assemblage than non-parasitized trees, and, in turn, enhance plant growth. Moreover, by coupling detrimental effects on their hosts and facilitative effects on the woody-plant community over long time periods, mistletoe affects patch dynamics and community succession, promoting the replacement of a dominant host tree (*Pinus nigra*) by a diverse community of subdominant zoochorous plants.

In conclusion, by taking a broader view of mistletoe and considering a wider breadth of its biotic interactions, we found several direct and indirect facilitative effects of the parasite on different organisms in the community. On the one hand, mistletoe induces significant changes on the growth of the dominant tree through its parasitic interaction, while facilitating colonization and establishment of less represented species (zoochorous-shrub species), leading to changes in the configuration of the plant community. On the other hand, through modified organic matter input to the soil and affecting belowground processes, mistletoe exerts facilitative effects on soil microbial communities and also enhances the growth of understory shrubs. Thus, far from being a harmful organism, our findings show that mistletoe can play an important role in regulating the spatial-temporal dynamic of the forest ecosystem, enhancing the structural and biological complexity of the forest its inhabits.

Resumen general

En esta tesis doctoral exploramos diferentes interacciones ecológicas establecidas por el muérdago *Viscum album* subsp. *austriacum* en un pinar mediterráneo con el fin de ampliar el conocimiento del papel ecológico de este peculiar parásito en los ecosistemas forestales en los que habita. Se trata de una planta parásita ampliamente distribuida por los pinares del continente europeo que ha despertado el interés del ser humano desde tiempos remotos. Distintos aspectos biológicos del muérdago, tal como su anatomía, fisiología o su papel como plaga forestal, han sido ampliamente estudiados; sin embargo, su ecología sigue siendo, en muchos aspectos, un misterio. El muérdago forma parte de un grupo taxonómicamente diverso de angiospermas hemiparásitas epífitas que obtiene agua y nutrientes de sus plantas hospedadoras. Representa un organismo singular en la comunidad biológica en la medida que, siendo una planta, comparte el segundo nivel trófico con animales herbívoros. Más conocida como planta dañina capaz de disminuir el vigor vegetativo y reproductivo de su hospedador, a día de hoy disponemos de poca información sobre las interacciones ecológicas que establece el parásito, así como su efecto directo e indirecto sobre las comunidades y ecosistemas naturales. A lo largo de esta tesis mostramos cómo, a pesar de ser un componente minoritario de la comunidad (en términos de abundancia y biomasa), el muérdago puede ejercer un papel relevante en el ecosistema. Además de afectar al vigor del hospedador, el parasitismo provoca una cascada de efectos directos e indirectos capaces de afectar la composición y dinámica de la comunidad, así

como procesos ecosistémicos clave, como el reciclado de nutrientes. A lo largo de cinco capítulos estudiamos la relación de *Viscum a. austriacum* con sus plantas hospedadoras y sus vectores de transmisión (aves frugívoras), así como sus efectos directos e indirectos sobre diferentes organismos de la comunidad y propiedades del ecosistema.

En el *capítulo I* nos centramos en la interacción parásito-hospedador. Analizamos la capacidad de infección del muérdago, así como múltiples factores bióticos y abióticos que afectan la capacidad de reclutamiento del parásito sobre su hospedador. Los resultados que obtenemos confirman la fuerte especificidad de hospedador del muérdago, capaz de parasitar a distintas especies del género *Pinus,* especialmente a *Pinus nigra* (la especie dominante en el área de estudio) a escala local. Observamos que diferentes factores bióticos (como la depredación de semillas o la capacidad de adhesión de las semillas) y abióticos (como la cantidad de luz o la temperatura) afectan considerablemente la regeneración del parásito sobre el hospedador, limitando los sitios óptimos de reclutamiento a zonas periféricas de las ramas, bajo la protección de las acículas del pino.

En el *capítulo II* estudiamos la interacción del parásito y sus vectores de transmisión. La mayor parte de los trabajos que analizan la interacción del muérdago con sus dispersores de semillas se centran en el papel de aves especialistas capaces de dirigir las semillas del parásito a los escasos lugares donde el parásito puede reclutar. Sin embargo, numerosas especies de muérdago, incluido *Viscum a. austriacum*, dependen exclusivamente de aves no especializadas para llevar a cabo esta tarea. En este trabajo analizamos la eficacia de dispersión de este grupo de aves que dispersa las semillas del muérdago. Nuestros resultados indican que tanto la endozoocoria como la ectozoocoria son mecanismos válidos de dispersión, ya que las semillas no necesitan atravesar el tracto digestivo de las aves para germinar. Los zorzales son los dispersores más eficaces, pero los más ineficientes. Por el contrario, las aves de pequeño tamaño (frugívoras generalistas u oportunistas), aunque en poca cantidad, ofrecen una dispersión de alta calidad, dirigiendo las semillas a los puntos óptimos de reclutamiento. Al disponer de un amplio y diverso grupo de dispersores con diferente capacidad de movimiento, *Viscum a. austriacum* puede dispersar sus semillas dentro y fuera de sus núcleos de población, manteniendo los procesos de re-infección dentro de la población y generando nuevos puntos de colonización.

En el *capítulo III* analizamos el efecto del muérdago sobre el patrón de dispersión de semillas de la comunidad de especies productoras de fruto carnoso con las que coexiste, compartiendo el mismo grupo de dispersores de semillas. Observamos que las aves frugívoras responden a la heterogeneidad espacial del parásito, visitando árboles parasitados con más frecuencia que los no parasitados y, como consecuencia, reciben una mayor lluvia de semillas del parásito sobre sus ramas. Al ser frugívoros generalistas, estos pájaros dispersan simultáneamente semillas de otras especies incluidas en su dieta, provocando un proceso de re-infección sobre el hospedador al mismo tiempo que concentran abundantes cantidades de semillas del sotobosque bajo su copa. Además, como la cosecha de frutos del muérdago se mantiene relativamente constante año tras año, los árboles parasitados sirven como núcleos de concentración de semillas a lo largo del tiempo. Por el contrario, la producción de frutos de las especies del sotobosque fluctúa considerablemente entre años, viéndose reflejado en la cantidad de semillas que se encuentran bajo los árboles parasitados en diferentes años. En conclusión, el muérdago influye sobre el patrón de dispersión de semillas de las especies productoras de fruto carnoso con las que comparte dispersores de semillas, concentrando año tras año una elevada cantidad de semillas bajo árboles parasitados en comparación con los no parasitados.

En el *capítulo IV* nos enfocamos en las interacciones sobre y bajo el suelo mediadas por el muérdago. Analizamos el efecto directo del parásito a través de su hojarasca, así como el efecto indirecto a través de su interacción parásita y mutualista, sobre diferentes propiedades químicas y biológicas del suelo. Observamos que el muérdago incrementa la cantidad, calidad y diversidad de materia orgánica bajo el hospedador, directamente a través de su hojarasca rica en nutrientes e indirectamente disminuyendo el aporte de acículas recalcitrantes del hospedador y aumentando los restos orgánicos derivados de la actividad de las aves. Esta mayor diversidad y abundancia de compuestos orgánicos está asociada a un enriquecimiento local del suelo y a un cambio en la abundancia y diversidad funcional de la comunidad microbiana, un efecto que se prolonga y enfatiza incluso después de la muerte del hospedador. Por tanto, el muérdago, junto con la red de interacciones que establece, incrementa la disponibilidad de nutrientes en el suelo, regulando la composición, abundancia y distribución espacial de la comunidad microbiana.

En el *capítulo V* estudiamos el efecto del muérdago sobre la estructura de la comunidad vegetal leñosa. Analizamos cómo afectan los cambios inducidos por el muérdago bajo árboles hospedadores (incremento de luz, nutrientes y semillas), así como la depredación de semillas, a la comunidad de matorrales productores de fruto carnoso. Para ello cuantificamos la infiltración de luz a través del dosel arbóreo, así como el porcentaje de semillas depredadas, el éxito de establecimiento, la abundancia de reclutas y el crecimiento de especies de matorral productor de fruto carnoso. Observamos que los árboles parasitados funcionan como centros de colonización, establecimiento y crecimiento de estos arbustos, cuyo efecto se prolonga hasta después de la muerte del hospedador. Si sumamos el efecto negativo que ejerce el muérdago sobre el hospedador al efecto facilitador que ejerce sobre la comunidad de matorral, observamos cómo este parásito puede promover, a largo plazo, la sustitución de un árbol parasitado por una rica comunidad de arbustos productores de fruto carnoso.

Como conclusión final, si consideramos el efecto del muérdago desde una perspectiva más amplia, teniendo en cuenta una mayor cantidad de interacciones bióticas en las que éste organismo está involucrado, encontramos un fuerte efecto facilitador del parásito sobre diferentes organismos de la comunidad. Por un lado, a través de un selectivo patrón de especificidad, el muérdago disminuye el vigor (y a largo plazo la densidad) del árbol dominante en la comunidad al mismo tiempo que facilita la llegada y el establecimiento de especies arbustivas menos representadas. Por otro lado, el incremento de aportes orgánicos y su relación con los microorganismos del suelo, la fertilización local y el crecimiento del matorral, indican un efecto positivo sobre la comunidad microbiana y vegetal. Por tanto, lejos de ser un simple organismo dañino, podemos concluir que el muérdago juega un papel relevante en dinámica espacio-temporal del ecosistema forestal, incrementando la complejidad estructural y biológica del bosque que habita.

Introduction

Parasitic plants have always fascinated humans since ancient times when they had an important place in legends and folklore to modern ones which are the focus of naturalists and scientific research. These plants have adopted one of the most ubiquitous life forms on the planet (Poulin & Morand 2004), sharing this feature with organisms as different as viruses, bacteria, fungi, worms, arthropods and vertebrates. Over 4,500 species in 20 families of angiosperms have adopted a parasitic habit (approximately 1 % of all angiosperms), looking like common herbs, vines, shrubs or trees, ranging from annual to long-lived plants. They are widely distributed in natural and managed ecosystems of all vegetation types, from tropical rain forests, mangroves and savanna to deserts, salt marshes and tundra (Kuijt 1969; Press & Graves 1995). Despite their widespread prevalence, many fundamental aspects of parasitic plant ecology remain poorly studied and most research has traditionally been limited to their anatomy, physiology and systematic (Kuijt 1969; Press & Graves 1995; Pennings & Callaway 2002; Press & Phoenix 2005), and their role as agricultural pests (Knutson 1979; Parker and Riches 1993; Riches and Parker 1995). However, increasing evidence from other types of parasites suggests that these life forms are integral components in shaping community and ecosystem structure, as their impact on reducing host fitness and modifying competitive and trophic interactions amongst species has profound effects on species diversity and the interactions amongst them (e.g., Lafferty et al. 2008; Mitchel 2003). Although much of the parasite literature focuses on animals, plant parasites are ecologically and economically important and share many features in common with them. Under this new ecological approach, the role of parasitic plants on natural systems remains largely unexplored, especially in forest ecosystems where numerous species of parasitic plants, commonly known as mistletoes, inhabit.

From the wide diversity of parasitic plants, mistletoes constitute an interesting group of over 1,500 species very common in woodland and forest ecosystems worldwide. They form a functional group defined by their growth form, being shrubby hemiparasites that attach to their host above the ground. Mistletoes are often seen as harmful parasites that decrease the vigor of their host trees. Such negative effects are especially magnified in forestry literature, which commonly considers mistletoes as noxious pests for forest and agriculture stands based on their impact on the host's growth and survival (e.g., Sallé et al. 1983; Pérez-Laorga et al. 2001; Sallé & Frochot 2002). Contrary to this view, the few studies adopting a broader perspective have revealed that these plants can mediate important top-down (as host consumers) and bottom-up (as resources) forces able to affect the structure and function of natural systems. They can modify different ecosystem processes and exert positive and negative effects on neighboring vegetation (Pennings y Callaway 1996), having cascading effects over multiple trophic levels— including pollinators, seed dispersers, herbivores, predators and detritivores (Watson 2012,2015; Hartley 2015).

In particular, the European mistletoe *Viscum album* has been thoroughly investigated with respect to its physiology, morphology, pharmacology and its role as a forest pest, but little is known about its impact at a wider scale. In European forests, this mistletoe can negatively affect some pine plantations, increasing mortality of intensively parasitized hosts (Sallé & Frochot 2002; Pérez-Laorga et al. 2001). However, besides its detrimental effect on host trees, the negative or positive effect of mistletoe at the community and ecosystem scales is still to be determined. Thus, an up-to-date ecosystem-based vision must contemplate mistletoe as an integral part of the forest community and ecosystem, which makes it necessary to abandon the traditional view chiefly based on pest control and eradication. In this thesis, we seek a deeper knowledge of the ecological role that *Viscum album* subsp. *austriacum* plays in forest ecosystems, going beyond the simplified view of the host-parasite interaction to understand the direct and indirect effects this has on biological communities and ecosystems. This knowledge is necessary to achieve a holistic understanding of the functioning of ecosystems and to develop appropriate management strategies for these parasites that appear to threaten the vigor and stability of an ecosystem.

Mistletoes — an overview

Mistletoes constitute a polyphyletic group currently distributed in five families within the Santalales (Kuijt 1969, Nickrent et al. 1998). The little known Eremolepidaceae and Misodendronace, which are restricted to South America; the Santalaceae, present in south-east Asia and Central America; and the Loranthaceae and Viscaceae, which account for more than 98 % of all mistletoe species, with over 940 and 350 species respectively (Nickrent 2001). This last group contains the European mistletoe *Viscum album*. This is the focal species of this thesis, and is perhaps one of the most renowned mistletoes due to its close relationship with humans throughout history—first having a mystical status for Druids in Great Britain, being part of an English Christmas tradition from the seventeenth century on, while being used in cancer therapy today.

Mistletoes are found on most continents and oceanic islands where woody species grow—being absent from the highest mountains, polar regions and the driest deserts. Most species grow in woodlands, where they parasitize mainly trees and shrubs (Kuijt 1969). However, they are able to parasitize a wider range of hosts, including succulent euphorbs and cacti in African and Latin American deserts (Martínez et al. 1996), and orchids and ferns in Mesoamerican cloud forest (Kuijt & Mulder 1985).

Mistletoe parasitic and mutualistic interactions

A very interesting aspect of most mistletoe systems is the complex relationships they have between their hosts and dispersers. Mistletoes are simultaneously mutualists of their seed animal-dispersers and parasites of their host plants.

Mistletoes, instead of taking nutrients and water from the soil through roots, take them from other plants using a specialized structure known as haustoria. They tap into the host shoots above ground by either penetrating the host xylem or forming close connections with the phloem thus obtaining water and minerals from the host (Lamont 1983; Marshall & Ehleringer 1990). Unlike holoparasites that have no chlorophyll and rely on their hosts (plants or fungi) for all their needs (Musselman & Press 1995), mistletoes are hemiparasites able to manufacture their own carbohydrates using photosynthesis. By diverting important resources from their hosts, mistletoes are often detrimental to them, reducing their growth and fecundity and, in the case of heavy parasitic loads, causing the premature death of the host (Hawksworth 1983). However, this is not always the case and frequently parasites only affect the host's fitness (e.g., fecundity) but not its survival (Reid & Lange 1988; Sterba et al. 1993).

Across their wide distributional range, mistletoes show a wide variety of patterns of host specificity. Some species are generalist parasites able to grow on a wide range of host species, whilst others are specialized on just a few hosts. Relative host abundance is thought to be key in establishing the pattern of host specialization (Norton & Carpenter 1998). As species richness increases the relative abundance of any potential host species decreases, making it difficult for a parasite to specialize exclusively on it. Otherwise, in areas where few species dominate the community, greater relative abundances of the host make mistletoe specialization more viable. In the European mistletoe flora, for example, four subspecies of the

Viscum album species can be clearly distinguishable by their host specialization (Zuber & Widmer 2000). The subsp. *austriacum,* subsp *brutia* and subsp. *abietis* are all host specialists, respectively parasitizing conifers of the genus *Pinus* (the first two subspecies) and *Abies* (the third subspecies) as the primary host, while the subsp. *album* is a host generalist that has been recorded parasitizing a large number of woody angiosperm species (Zuber 2004). Although many aspects of the parasite-host interaction have been studied, little is known about the effect of the environmental heterogeneity of the forest canopy on mistletoe recruitment, being this the main question addressed in the first chapter of this thesis.

As mistletoes can recruit in just a narrowly limited range of safe sites within the host, they are especially dependent on animal dispersal services, the behaviour being critical for an effective dispersal. As fleshy-fruited parasitic plants, animals that consume mistletoe berries and disperse their seeds are both seed dispersers and parasite vectors (Aukema 2003). Thus, similar to other parasites, mistletoes have adopted a proper strategy to "manipulate" them. The fruit is generally brightly coloured (red, white or yellow), rich in sugars, fat and protein and highly attractive for frugivorous animals. In addition, the seeds are surrounded by a sticky substance (viscin) that makes them difficult for birds to expel, inducing dispersers to rub their bodies against a perch to remove seeds after regurgitating or defecating them, thus facilitating seed attachment to a host branch (Reid 1990 1991).

With few exceptions, such as the thermogenesis-triggered seed discharge of dwarf mistletoes (Rolena et al., 2015) or the wind-dispersed Misodendraceae, most mistletoes are dispersed by birds (Restrepo *et al.*2002), with few mammal species performing this task (Amico & Aizen 2000). The main mechanisms for seed dispersal are endozoochory (Reid 1991; Restrepo *et al.* 2002), performed by specialists and generalists frugivorous animals, and ectozoochory, which has been described for insectivorous, nectarivorous, granivores or omnivorous species that occasionally consume mistletoe fruit (e.g. Watson 2012). Mistletoe specialists have evolved independently in eight lineages around the world showing specific behavioral and anatomical adaptations to mistletoe fruit consumption (Reid 1991; Restrepo et al. 2002; Watson 2004). These are considered among the most specialized diets of all frugivorous birds (Restrepo *et al*. 2002) and typically have small bodies, bright colors, with relatively large gapes and modified guts for a rapid passage of fruits (Watson 2004). Although much of the attention has been paid to specialist seed dispersers, in several regions around the world, including Europe, many mistletoes rely on generalists that perform all seed dispersal duties. In these sites, how parasitic plants requiring precision in seed dispersal can exclusively rely on non-specialized dispersers is unknown, being this the main question addressed in the second chapter of this thesis.

Mistletoes in forest communities and ecosystems

Because of the epiphytic life form, mistletoes often are not considered in descriptions of vegetation types or in community theory and only the hosts are known as the typical accompanying species. The other plants of the understory are not taken into account, because of the idea that they have no interaction with the parasite (Zuber 2008 references therein). Only recently has attention been directed at understanding interactions of parasitic plants with other elements of the community and fundamental ecosystem processes (Pennings & Callaway; Press & Phoenix 2005).

On the one hand, the complex net of interactions around mistletoes has led to hypothesize their role as keystone species (sensu Paine 1969), as they can exert disproportional effects to their abundance and biomass on the community, which frequently favours local and regional wildlife diversity (Watson 2001; 2012; 2015). Mistletoes serve as important food and structural resources for many organisms of the community, offering nutritive nectar, flowers, fruit, seeds and leaves, as well as good structures to build a nest or to get shelter (Parks *et a*l. 1999). They concentrate large amounts of nutrients and water in their tissues

(Quested 2002, March & Watson 2007), which are consumed by a wide variety of folivorous, frugivorous, nectarivorous and detritivorous animals, including birds, mammals, fishes and arthropods (see Watson 2001 and references therein). In addition, because of their extended phenology, mistletoes often constitute the most reliable food resource in many regions, being the only source of fruit and nectar available for some periods and many low-productivity habitats. For example, many species, including *Viscum album*, fruit for more than half of the year, and initiate flower production while still bearing fruit.

On the other hand, mistletoes, as with other parasitic plants, have been considered ecosystem engineers (sensu Jones 1994) due to their capacity to modulate litter input on the soil and thus to affect nutrient cycling. As they can just suck out more resources from their hosts, parasitic plants usually have over 15 to 20 times more concentrated nutrients than their hosts do, at times 20 times more K (Lamont 1983). Moreover, they do not care about conserving their nutrients at senescence. Thus, contrarily to other plants which absorb nutrients before allowing them to fall, parasitic plants drop their leaves with all the nutrients included (Quested 2003), increasing nutrient-rich organic matter input in the soil beneath their hosts. In addition, litter from parasitic plants decomposes faster, releases nutrients more rapidly, and favours decomposition of recalcitrant litter of neighbouring plants, enhancing nutrient cycling and soil fertility around the host (Quested 2002).

To date, no study has determined the simultaneous effect of mistletoe and its network of interactions on soil nutrient cycling or soil biota, and little is known about the ecological consequences of this fertilization effect beneath host in comparison to non-host trees (but see March & Watson 2011), which may ultimately change the composition and structure of plant assemblages. These questions are addressed in the last three chapters of this thesis.

Study system and research objectives

In this thesis we seek a deeper and broader understanding of the ecological interactions mediated by *Viscum album* subsp. *austriacum* in Mediterranean forests. This mistletoe is a frequent, although minor, component of European pinelands. As most mistletoe species, *Viscum a. austriacum* shows a strong aggregated spatial distribution within the forest, with most hosts having no parasites, or just a few, whereas a small number of hosts have most of the population (Aukema 2001). Such spatial aggregation often depends on the probability of infection among hosts, where vector behavior and host occurrence ultimately limit mistletoe potential distribution. In Mediterranean pinelands, primarily dominated by pine trees, *Viscum a. austriacum* grows on pine species and relies on its seed dispersal exclusively by generalist birds which are responsible for the strong mistletoe patchiness, from the individual host to the landscape scale (Fig 1).

In these forests, *Viscum a. austriacum* coexists with a wide variety of fleshy-fruited plant species, and seed dispersers commonly create highly heterogeneous seed deposition patterns, with some microhabitats, such as isolated trees, shrubs or stones receiving large amounts of seeds, whilst others, such as open gaps bereft of vegetation receive less seeds (Jordano &Schupp 2000; García *et al*. 2000). Particularly interesting is the frequency with which zoochorous seeds concentrate close to trees and shrubs that birds use as perches (e.g., Verdú & García-Fayos 1998; Martínez *et al*. 2008; Carlo et al. 2013). Aware of the spatial distribution of *Viscum a. austriacum* in the forest canopy, we expect generalist birds to respond to mistletoe patchiness by preferentially visiting parasitized trees where they can feed on mistletoe fruit and probably disperse seeds of other species included in their diet, affecting this way the seed-deposition pattern of the fleshy-fruited plant community. Simultaneously, visiting birds can deposit significant amounts of organic debris (food leftovers, wings, excrements, etc.) localized in the same sites where the seeds arrive. We expect the nutrient-rich mistletoe litter and the additional input of animal-derived debris to gain especial importance in Mediterranean pinelands, where dominant plants are slow growing trees, with perennial, nutrient-poor and recalcitrant litter, frequently accompanied by sclerophillous woody plants, which often reabsorb nutrients from leaves before falling to achieve a higher nutrient use efficiency (Fioretto *et al*. 2003; Sardans *et al*. 2005). Therefore, mistletoe may concentrate seeds of zoochorous species under host trees at the same time that it enhances nutrient resources in the soil, which could ultimately affect soil biota, as well as the composition and structure of the plant community if a differential recruitment and growth occurs under host trees compared to non-host trees.

Fig.1

a) Mistletoe spatial patchiness at the landscape scale

b) Mistletoe spatial patchiness at the individual scale

Our *general hypothesis* is that *Viscum a.austriacum* promotes a series of ecological changes in the forest patches where it grows, inducing significant effects on the host tree through its parasitic interaction while mediating different facilitative mechanisms that may contribute to the spatial-temporal organization of the forest community and ecosystem. To address this, we first analyze the relationship between mistletoe and its host trees (Chapter I) and seed dispersal vectors (Chapter II), and then explore the direct and indirect effects of mistletoe-mediated interactions on the forest community and ecosystem properties (Chapters III, IV, and V). More specifically, the following objectives are addressed in this thesis:

In *Chapter I*, we focus on the mistletoe-host interaction. We seek to understand different factors shaping the spatial distribution of *Viscum a. austriacum* in the forest canopy, including the host specificity and the variation of biotic and abiotic factors limiting safe sites for mistletoe recruitment.

In *Chapter II*, we focus on the mistletoe-vector interaction. The main objective is to understand how unspecialized seed dispersers (generalist frugivorous birds) can provide dispersal services to a parasitic plant with extremely narrow ranges of safe sites to recruit.

In *Chapter III*, we examine interactions between mistletoe and fleshy-fruited plants of the understory. The main objective is to analyze the effect of *Viscum a. austriacum* spatial heterogeneity on the seed-deposition pattern of the zoochorous plant community.

In *Chapter IV*, we explore mistletoe-mediated aboveground-belowground interactions. The principal objective is to know whether *Viscum a. austriacum* and the parasitic and mutualistic interactions it mediates from the forest canopy affect soil-chemical and biological properties at different phases of parasitism.

In *Chapter V*, we analyze the effect of mistletoe on the structure and dynamics of the plant community. We seek to understand whether mistletoe-induced changes under host patches, coupled to the effect of post-dispersal processes, ultimately result in changes in the plant community assemblage.

Study species, site and general experimental design

Viscum album subsp. *austriacum*

Viscum album is a perennial hemiparasitic fleshy-fruited shrub native to most parts of Europe. *Viscum album* distribution ranges from southern Sweden, the Black and Caspian Seas, the Mediterranean Sea and the Atlantic Ocean, being reported in many islands, including Great Britain, Corsica, Sicily and Crete (Zuber 2004 and references therein). As previously mentioned, this species differentiates in four subspecies. In this study, we particularly focus on *Viscum album* subsp. *austriacum*. The distribution range of the parasite is directly related to the availability of suitable host trees. According to Zuber & Widmer (2009), Iberia (with *Pinus pinaster*, *P. sylvestris* and *P. nigra)*, as well as Corsica and the Balkans (with *P. nigra)*, were glacial refugia for *Viscum a. austriacum*.

This mistletoe species is dispersed by non-specialist birds, including generalist frugivores that regularly take mistletoe fruit as part of a broader diet dominated by fruit during the fruiting season and insects the rest of the year (hereafter, generalists), and insectivorous, granivorous or omnivorous species that occasionally consume fruit, including mistletoe fruit (hereafter, opportunists). Among these, some are migratory (*Turdus viscivorus*, *T. merula*, *T. philomelos*, *T. pilaris*, *T. iliacus*, *T. torquatus,* and *Sylvia atricapilla*), which migration routes,

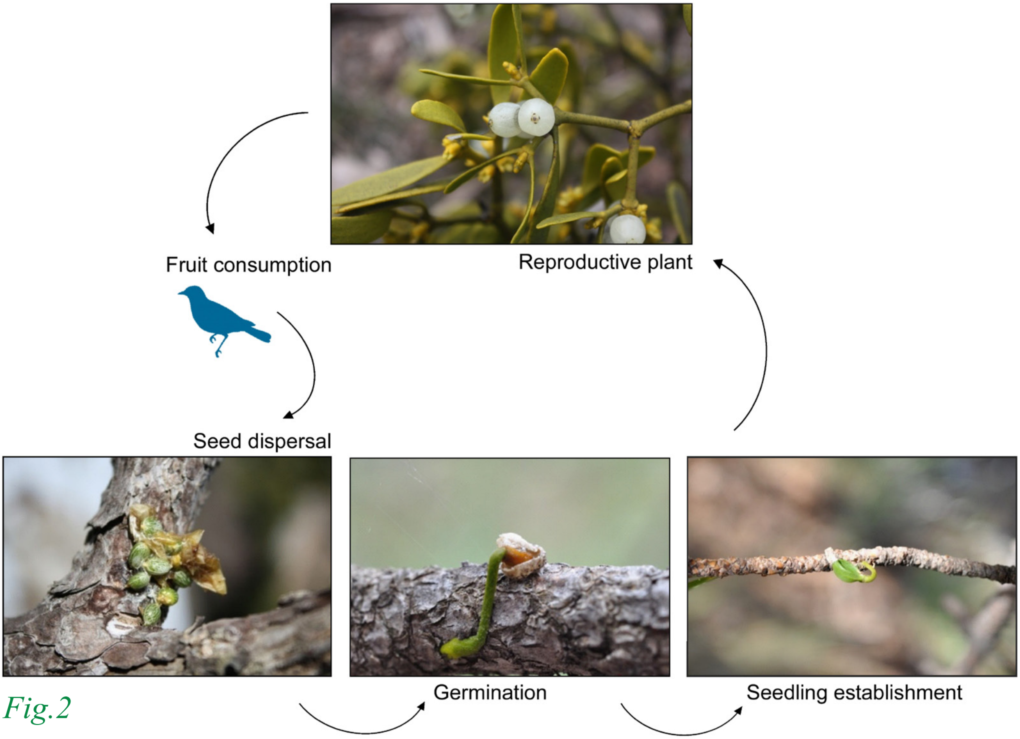

Fig.2

breeding and wintering areas greatly fits the distribution area of mistletoe. These birds, depending on their breeding site in Northern Europe, take a migration route to their wintering area in southeast direction either to Italy or Turkey or in a southwest direction to Spain (Huttunen 2004), where the study site is located.

Viscum album can live for over 30-35 years. It grows up to 1m high, with numerous, regularly forked branches. The plant first reproduces during the fifth year of life, flowering and fruiting once every year (Sallé 1983). The leaves are greenish-yellow, borne in pairs and have a distinctive leathery texture. The flowers are greenish-yellow, inconspicuous and insect (and perhaps wind) pollinated, appearing from February to April in clusters of 3 to 5. The parasite is dioecious, frequently growing three female plants per each male on the same host (authors' obs.). The berries are 1cm in diameter, white and sticky. They ripen in October in southern European regions (authors' obs.) and in November-December in northern regions (references in Zuber 2004). The berries are dispersed between October and February in the study site, being from February to May in northern regions (Stopp 1961)—when migratory birds (mainly thrushes) fly back. Seeds germinate everywhere during March-April, being independent of the host in their earliest stage of development. If deposited on a proper host, the haustorium penetrates the host bark until connecting with the host xylem, and then takes water and nutrients from the tree. In the second year of life, the cotyledons die and the first pair of leaves appear (Sallé 1983). See Figure 2.

Study site

The study site is located in the Sierra de Baza (over 550 Km^2 in area), a Mediterranean mountain in the southeast of the Iberian Peninsula. This mountain joins the Sierra de Los Filabres, forming the Baza-Filabres cordillera with prevailing west-east orientation. The cordillera constitutes a "bioclimatic island" situated between two extensive plains, Hoya de Baza in the north and Llanos del Marquesado in the south. The altitude ranges from 1,200m and 2,269 m at the mountaintop, where over two months of heavy snowfall are common. As in many eastern Iberian mountains, precipitation is distributed unevenly due to elevation gradients and localized rain shadow effects. Therefore, precipitation increases with altitude, exceeding 600mm above 1,400m and decreases below 350mm along the southern slopes (Gómez-Mercado & Valle 1998). Otherwise, mean annual temperatures decrease from 13ºC to 9º C with increasing altitude (unpublished data registered in 2010 and 2011).

The Sierra de Baza comprises a complex mosaic of plants, where tree vegetation is dominated by pine species. *Pinus nigra* is the dominant tree across the mountain, distributed from 1,200 m to the tree line reaching up to 1,900 m. *Pinus nigra* coexists with *Pinus halepensis* and *Pinus pinaster* at the lower altitude (1,200-1,400m) and with *Pinus sylvestris* at the higer altitude (1,750-1,900). Other arboreal species, such as *Quercus ilex* subsp. *ballota*, *Quercus coccifera* and *Acer* *granatensis* are also present. Many fleshy-fruited shrubs, lianas and trees form part of the plant community across the mountain, including *Crataegus monogyna*, *Berberis hispanica*, *Prunus ramburii*, *Juniperus oxycedrus*, *J. communis*, *J. sabina*, *Daphne gnidium, D. laureola, Hedera helix, Lonicera arborea, Phillyerea angustifolia*, *Sorbus aria* and *Rosa* sp. At lower altitudes non-fleshy-fruited shrubs such as *Cytisus reverchonii*, *Adenocarpus decorticans*, *Genista cinerea* subsp. *speciosa*, *G. scorpius*, and *G. umbellate* are common. Higher altitudes are characterized by open vegetation with a basal layer of hard-leaf grasses (*Festuca hystrix*, *Poa ligulata*, *Koeleria vallesiana*); cushion shrubs of junipers, Genisteae (*Erinacea anthyllis*, *Genista versicolor*, *Echinospartum boissieri*); and other thorns such as *Vella spinosa*, *Ptilotrichum spinosum*, *Bupleurum spinosum*, *Daphne oleoides* and *Dianthus subacaulis*, amongst others. Currently *Viscum album* concentrates from 1,300 to 1,900 m on the northern and western slopes of the Sierra de Baza, including the entire altitudinal range occupied by the different pine species. This mountain constitutes the mistletoe's southernmost distribution limit.

General experimental design

With some differences, chapters III, IV and V share the following general experimental design. On the northern slope of Sierra de Baza we selected 125 *Pinus nigra* focal trees, from which 55 were parasitized by mistletoe (hereafter 'parasitized tree'), 55 were not parasitized (hereafter 'unparasitized tree') and 15 were dead trees parasitized in the past (hereafter 'dead trees'). To cover a large range of the mountain's environmental heterogeneity we selected focal trees distributed at three altitudes (1,300, 1,650 and 1,850 m) within stands of different tree densities. At 1,300 and 1,650 m we differentiated two stand types of high (150.99 ± 9.43 trees / Ha) and low (58.88 ± 7.44 trees / Ha) tree densities, where 10 parasitized and 10 unparasitized trees were selected per altitude and stand type. At 1,650 m, there was only one stand type (54, 90 ± 9.06 trees / Ha), in which we selected 15 parasitized and 15 unparasitized trees. Trees were taken at random and spatially paired (one parasitized and one unparasitized) at distances of 40-80 m, being of similar architecture, size (trunk perimeter = 97.5 ± 4.01 cm) and height

(6.87 ± 0.23 m), and were located in similar environmental contexts. Parasitized trees presented moderate to intense parasitic loads (from 6 to 372 mistletoes per host], holding at least one mistletoe older than 30 years of age. Dead trees are less numerous than the other categories because they are scarce and difficult to find in the study area (although they are easy to identify because dead mistletoes remain on the host); thus, they were selected throughout the altitudinal gradient.

Chapters I and II follow different experimental designs from the rest of the chapters. The experiments in Chapter I were carried out at 1,300 m altitude in a forest plantation where all pine species (*Pinus nigra*, *P. pinaster*, *P. sylvestris* and *P. halepensis*), of similar age (40 yr) and architecture, were planted under similar ecological conditions. Experiments and observations in Chapter II were carried out on the same focal and additional pine trees at 1,300 and 1,650 m altitude. A detailed methodology is specified in each chapter.

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Chapter 1

Linking safe sites for recruitment with host-canopy heterogeneity:

The case of a parasitic plant, *Viscum album* subsp. *austriacum* (Viscaceae)

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(2014) Linking safe sites for recruitment with host-canopy heterogeneity: The case of a parasitic plant, *Viscum album subsp. austriacum* (Viscaceae). American Journal of Botany, 101, 1-8.

Abstract

Canopies are ecologically relevant compartments of forests. Multiple sources of heterogeneity interact within forest canopies due to their structural complexity, which exert major influences on the structure and composition of epiphyte communities. Here, we explore canopy environmental heterogeneity of a Mediterranean pine forest, identifying the key biotic and abiotic factors determining mistletoe (*Viscum album* subsp. *austriacum*) recruitment at coarse and fine spatial scales. Through field experiments, we assessed the range of suitable host species for *V. album* subsp. *austriacum* (hereafter, *V. a. austriacum*). We characterized the variation in abiotic factors at a fine spatial scale on the host species. Finally, we examined the effects of biotic (predation) and abiotic (light, temperature) factors on the fate of mistletoe seeds and seedlings along host branches. We confirmed the tight specificity of *V. a. austriacum* to pine species, in particular to *P. nigra* at the local scale. Biotic constraints increased toward the branch interior, with minor effects on apical locations due to the positive effect of pine-needle coverage. Contrarily, abiotic constraints increased toward branch extremities, harming mistletoe seeds by encouraging their desiccation. In conclusion, biotic and abiotic variables exert a strong, nonrandom filter on *V. album* regeneration, resulting in recruitment hotspots at the periphery of the branches and sites with a high probability of recruitment failure at thicker and more exposed locations. The narrow range of suitable host species and the scarcity and spatially restricted recruitment hotspots for *V. a. austriacum* leads to the clumping of mistletoe populations at the finer spatial scale.

Key words

canopy heterogeneity; host specificity; microhabitat; mistletoe; parasitic plant; recruitment; spatial pattern; seed survival; spatial heterogeneity; Viscaceae; Viscum album subsp. austriacum.

Introduction

The forest canopy, as a structurally diverse and ecologically relevant component of the forest, hosts numerous living organisms, including thousands of epiphytic species (Nadkarni et al., 2001; Nieder et al., 2001). Multiple sources of heterogeneity vary in space and time, giving rise to a rich mosaic of habitats for epiphytic communities (Parker, 1995; Shaw, 2004). Such environmental heterogeneity can be noted at diverse spatial scales (stands, crowns, branches, or microsites) directly affected by the structural complexity of the canopy, which filters incoming light (e.g., Gates, 1980; Canham et al., 1993), rain water (e.g., Doley, 1981; Parker, 1983) and organic matter (Ingram and Nadkarni, 1993) and also influences temperature, humidity (e.g., McNaughton, 1989; Raupach, 1989), and turbulence regimes (e.g., Raupach and Thom, 1981; Bohrer et al., 2008) within this forest compartment. Being aware of this, we might expect environmental heterogeneity to influence the dynamics of epiphyte populations, depending on host availability and on their capacity to reach suitable recruitment sites.

Mistletoes are a particular type of epiphyte that dwells in forest canopies around the world. They form a group of hemiparasitic plants capable of deriving water and nutrients from host trees by inserting a specialized structure, the haustorium, into the host vascular system. This host–parasite connection is very specific, occurring within a limited set of host species (Barlow and Wiens, 1977; Hawksworth and Wiens, 1996; Norton and de Lange, 1999) and at narrowly defined sites within such hosts (Reid, 1989; Sargent, 1995), as determined by biochemical and physiological constraints. Host availability (Norton and Carpenter, 1998; Kavanagh and Burns, 2012) and physiological condition (Norton and Stafford, 1999; Miller et al., 2003; Watson, 2009), together with the parasitic capacity of the mistletoe (Hoffman et al., 1986; Yan, 1993), determine to a large extent the spatial pattern of mistletoe populations on the basis of the mistletoe–host interaction. In this sense, the relative abundance of the host seems to drive the degree of mistletoe–host specialization because the species that are suggested to be major hosts at a given place are the dominant ones (Norton and Carpenter, 1998).

To move their propagules to safe sites, most mistletoes rely on animals, with birds being the most common dispersers (Restrepo et al., 2002). Thus, features of the canopy that influence the foraging and territorial behavior of seed dispersers—e.g., availability and distribution of food and nesting resources—shape the patterns of mistletoe seed deposition. Taller and isolated trees, for example, are more prone to be parasitized (Donohue, 1995; Aukema and Martínez del Río, 2002; Shaw et al., 2005; Roxburgh and Nicolson, 2008). After feeding on ripe berries, birds remove the fruit pericarp and deposit seeds on branches, either by defecation, regurgitation, or external transport on their bodies (e.g., Restrepo, 1987; Mathiasen, 1996; Mellado and Zamora, 2014). Seeds adhere to branches due to a sticky substance (viscin) that allows their fixation to the bark until germination begins. In a matter of days, seeds produce a haustorium that begins to penetrate into the bark layer to establish a functional connection with the host (Lamont, 1983; Yan, 1993; Fadini, 2011) and are able to survive for long periods (from 6 to 12 mo) before becoming successfully established (e.g., Zuber, 2004; Roxburgh and Nicolson, 2005; Fadini, 2011). Rather than producing real seeds, mistletoes produce photosynthetic embryos enclosed in an endocarp, lacking any protective layer (Kuijt, 1969). Therefore, these hemiparasites require light conditions from the very beginning (Tubeuf, 1923) and become highly susceptible to environmental adversities (e.g., dehydration, freezing, or seed predation) while striving to penetrate the host vascular system. While many aspects of mistletoe interactions with their hosts and seed dispersers have been explored, there is a lack of available information concerning this last matter of the effect of environmental heterogeneity of the canopy on the fate of mistletoe seeds and seedlings.

In this study, we explore how canopy environmental heterogeneity of a Mediterranean pine-forest influences the early stages of mistletoe recruitment (seeds and seedlings). For this, we examine patterns of mistletoe-host specificity, considering an ample range of possible coexisting host species, and evaluate the effects of multiple biotic and abiotic factors of the canopy with respect to the fate of mistletoe seed and seedlings. We used *Viscum album* subsp. *austriacum* (Wiesb.) Vollman (hereafter, *V. a. austriacum*) as a study case, which is widely distributed across Europe and parasitizes conifer species. This mistletoe produces abundant crops of a whitish fleshy fruit available for birds from early September to the end of March, when seed dispersal takes place. Once it adheres to a branch, a mistletoe seed remains in that position throughout the entire winter until germination begins (usually in April), taking about a year to tap into the xylem of the host. Using a field-experimental approach under natural conditions, we identified the key biotic and abiotic factors determining mistletoe recruitment at coarse and fine spatial scales, considering a wide gradient of ecological conditions affecting seeds during early establishment. Specifically, we explored (1) the range of tree species that are suitable hosts for *V. a. austriacum*; (2) within those suitable hosts, the variation of environmental factors that exists at the fine spatial scale (i.e., within host canopy); and (3) how environmental heterogeneity influences the fate of mistletoe seeds and seedlings, shaping safe sites for mistletoe regeneration at the fine spatial scale.

Material & Methods

Study site

The study site was located in a Mediterranean pine forest at the foothills of Sierra de Baza (1300 m a.s.l.; 2°51′48W″, 37°22′57″N), a mountainous natural reserve of southeastern Spain. Sierra de Baza presents a very heterogeneous landscape, including a sharp altitudinal gradient of 850 m to 2270 m, and a varied substrate composed of calcareous rocks, schists, and quartzites. This area presents hot and dry summers (June–September), cold winters (December–March), and rainy autumns and springs, following the typical continental Mediterranean climate. The principal forest vegetation is pine, mainly Austrian (*Pinus nigra* Arn.), Aleppo (*P. halepensis* Mill.), maritime (*P. pinaster* Ait.), and Scots (*P. sylvestris* L.); all naturally present at the Sierra de Baza, although under human management (Carrión et al., 2007). Additional vegetation includes Holm oaks (*Quercus ilex* L.), *Sorbus aria* L., and *Acer opalus* L. subsp. *granatensis* Boiss as well as native fleshy-fruited shrubs such as *Berberis hispanica* subsp. *hispanica* Boiss. & Reut., *Crataegus monogyna* Jacq, *Lonicera arborea* Boiss., *Juniperus oxycedrus* L., *J. communis* L., *Prunus ramburii* Boiss, and *Rosa* sp.

Range of suitable host species for mistletoe recruitment

Our first objective was to explore the range of tree species susceptible to parasitism by *V. a. austriacum*. For this, we used individuals of each pine species inhabiting Sierra de Baza (*Pinus nigra*, *P. sylvestris*, *P. sylvestris* subsp. *nevadensis, P. pinaster*, and *P. halepensis*) and three common nonconifer species occupying the same location as control hosts (*Crataegus monogyna*, *Sorbus aria*, and *Quercus ilex*). Ten young individuals of the same age (10 yr) and similar size (1.5 m tall for *Pinus* sp., 1 m for *C. monogyna*, *S. aria*, *and Q. ilex*) of each species were used as hosts, all coming from a local forest nursery. Trees were planted in pots and placed within the same location, exposed to natural environmental conditions. Mistletoe seeds were placed on thin branches of each host (twig diameter < 1.5 cm). Host saplings came from local sources, while planted seeds were collected from one mistletoe population of Sierra de Baza to avoid genetically based effects on local host specificity (Norton and Carpenter, 1998). Because seeds of *V. a. austriacum* do not require intestinal processing to adhere to branches or to germinate (Mellado and Zamora, unpublished manuscript), seeds were directly collected from 15 source plants growing on different host trees; these were pooled and mixed for randomization. To allow seed germination, we removed the fruit exocarps (Ladley and Kelly, 1996), then seeds were carefully placed on branches 1.5 to 2 cm apart. Three branches per tree were randomly selected and 10 seeds per branch were used for the experiment, which began in early March 2010. Seeds were monitored every 30 to 40 d for 18 mo after planting. Percentages of mistletoe seed survival (proportion of the number of surviving seeds from total of initial placed) and early seedling establishment (proportion of emerged seedlings from total of surviving seeds) were estimated for each individual tree.

Fine-scale variation of environmental factors

To explore environmental heterogeneity at the tree level, we analyzed the fine-scale variability of a set of abiotic factors that can affect mistletoe recruitment. We restricted our exploration to four pine species naturally occurring in Sierra de Baza: *P. nigra*, *P. sylvestris*, *P. halepensis* and *P. pinaster*. Ten trees per pine species and three branches per tree were randomly selected to characterize the variability of abiotic factors. Trees were alike in age (40 yr) and size (diameter at breast height [dbh]: 10.39 ± 1.49 cm), coexisting under similar ecological conditions. Age was estimated as the number of whorls (Edenius et al., 1995). As mistletoe seeds are generally dispersed on tree branches, rather than other structures (e.g., tree trunks), we limited our fine-scale exploration to these. At the branch scale, four locations were defined differing in diameter and environmental conditions: (1) "basal", as the nearest position to the trunk (<2.5 cm); (2) "middle", as the middle position of the branch (2.49–1 cm), and two apical positions (0.99–0.2 cm): (3) "apical uncovered", one uncovered (situated after the middle location) and (4) "apical covered",

covered by pine needles (the branch tip). Light incidence was quantified by hemispherical photography (Valladares and Guzman 2006) at each branch location and tree. Photographs were taken from a high tripod while pointing to the sky at the level of each branch position. A fish-eye lens with a 180° field of view (FCE8, Nikon, Tokyo, Japan) was used with a digital camera (Coolpix 995 digital camera, Nikon), horizontally levelled. Photographs were taken during completely cloud-covered days; later, images were analyzed using Hemiview canopy analysis software version 2.1 (1999, delta-T Devices Ltd, Cambridge, UK). The software estimates the global site factor (GSF), which is an estimator of the total amount of light, ranging from 0 (total darkness) to 1 (100% of light available). Temperature was estimated with HOBO data loggers (TidbiT v2 Water Temperature Data Logger, UTBI-001, Onset Computer Corp., Bourne, Massachusetts, USA) directly located on the selected branch positions; three data loggers per position and host species were used, for a total of 48. Average values of GSF, annual mean temperature, summer mean temperature (July, August, and September), and winter mean temperature (December, January, and February) were calculated for further statistical analyses.

Spatial pattern of safe sites for mistletoe regeneration

We performed a third experiment to identify safe sites for *V. a. austriacum* recruitment at the fine scale. We sought to determine where mistletoes recruit and how environmental variables affect early mistletoe recruitment. For this, we collected mistletoe seeds and immediately placed them on the same branch locations characterized in detail in the second experiment (basal, middle, apical uncovered, and apical covered). Seed collection and placement were performed in the same manner as mentioned for the first experiment. A total of 3600 seeds were used: 10 on basal branch locations, 10 on middle locations, and 5 on each of the two apical locations (i.e., apical uncovered and apical covered); the seed-reduction number in the latter locations was due to limited branch length. Seeds were placed during early March of 2010 and were monitored every 30 to 40 d for 15 mo. During seed monitoring, we registered whether a seed failed to adhere on the branch, and, if still attached, whether it was alive, depredated or desiccated. Adhesion failure (i.e., the number of seeds that were lost before their initial attachment to host bark) was quantified during the first monitoring (30 d after seed placement). Subsequently, we quantified (1) the number of surviving seeds (either established on the host or not); (2) the number of seedlings successfully established (seeds that had developed a functional haustorium and whose cotyledons had extended); (3) seed predation, which was easily quantified by recognizing traces of bird pecking on mistletoe seeds, and (4) seed desiccation, which was clearly distinguished by the yellowish and wrinkled appearance. Depredated and desiccated seeds were removed from branches after each monitoring event. One month after seed placement, the percentages of adhesion failure were calculated (proportion of seeds that disappeared with respect to the total initially placed); at the end of the experiment, seed survival (proportion of the number of surviving seeds and seedlings to the total attached on host branches), seed predation (proportion of depredated seeds with respect to the total attached on host branches) and desiccation (proportion of desiccated seeds to the total attached to host branches), and seedling establishment (proportion of emerged seedlings with respect to the total of surviving seeds) were estimated for each branch position and pine species.

Data analysis

To test whether seed survival and early establishment of mistletoe seeds differed between host species (first experiment), we used generalized linear models (GLM) assuming a binomial error distribution and logit-link function (Quinn and Keough, 2002). Comparisons between host species were made with a Tukey's pairwise comparison method. To explore the overall variation of abiotic factors along pine branches (second experiment), we tested whether abiotic microclimatic variables—i.e., light availability (GSF), annual mean temperature, summer and winter mean temperatures (°C)—differed between branch locations by using linear mixed models (LMM), including branch microsite as a fixed factor and tree and species as random factors. Tukey's pairwise comparison method was applied to compare branch microsites. For the third experiment, we first analyzed the effect of host species and branch microsite on mistletoe seed-adhesion failure, seed survival, seed mortality (caused either by predation or desiccation) and seedling establishment, applying a GLM with a binomial error distribution and logit-link function. Tukey's pairwise comparison method was used to compare microsites and species separately. Finally, we explored the effect of light, temperature (annual, summer, and winter means), branch diameter, and needle coverage (yes/no) of branch locations on mistletoe seed survival and quantified seed mortality and seedling establishment. For this, we used GLMM with binomial error distribution and logit-link function, including all the aforementioned abiotic variables as fixed factors and host species and tree as random factors. To identify the most parsimonious combination of explanatory variables, we followed a backward stepwise selection analysis based on Akaike's information criterion corrected for small sample sizes (AICc) (Burnham and Anderson, 2002) and likelihood ratio tests (Zuur et al., 2009), applied for each response variable. Each model was sorted on the basis of its difference with the lowest AICc (ΔAICc), then ΔAICc values were used to obtain the Akaike weights (wi) for each model. The pseudo-R^2 for GLMM was used as a measure of model goodness of fit (Nakagawa and Schielzeth, 2013 (Appendix S1). Statistical analyses were made in R 2.15.1, R Development Core Team, 2012. Linear mixed models and generalized linear mixed models were performed with the 'lme4' package (Bates et al., 2012). Tukey's pairwise comparisons with 95% confidence level were conducted with the "multcomp" package (Hothorn et al., 2008). The "MuMIn" package was used to obtain information from the model selection process (Bartón, 2013).

Results

Range of suitable host species for mistletoe recruitment

Seed survival and seedling establishment of *V. a. austriacum* differed between host species (seed survival: $\chi^2 = 35.17$, df = 4, $p < 0.0008$; seedling establishment: $\chi^2 = 12.53$, df = 4, $p < 0.0098$), being feasible only on pine species (Experiment 1) and excluding as possible hosts any of the angiosperms (*Crataegus monogyna*, *Sorbus aria*, or *Quercus ilex*) (Fig. 1). Among pines, *Pinus nigra* was the main host species, followed by *P. sylvestris* and *P. halepensis* (Fig. 1), while *P. pinaster* showed signs of being a possible host only in the Experiment 3 (see below for further explanation). Although *P. nigra* showed higher seed survival, only half of the seeds were successfully established (Fig. 1), whereas for the other pine species the seed survival and establishment matched (i.e., most surviving seeds were finally established).

Fine-scale variation of environmental factors within pine branches

Branch locations differed in their local abiotic environment (Fig. 2). Light was not uniformly distributed within pine branches ($F_{3,57} = 4.33$, $P = 0.008$); it was greater in the most apical locations, decreasing with proximity to the trunk. In the same manner, annual mean temperature species (seed survival: $\chi 2 = 35.17$, df =

4, $p < 0.0008$; seedling establishment: χ 2 = 12.53, df = 4, $p < 0.0098$), differed along pine branches. Again, most apical locations were exposed to the most extreme temperatures, the warmest during summer and the coldest during winter, showing the warmest values when mean annual temperatures were considered.

Influence of environmental heterogeneity on the fate of mistletoe seeds and seedlings

Host species and branch microsite had significant effects on seed adhesion failure, seed predation, and seed survival; on the other hand, branch microsite significantly influenced seedling establishment (Table 1).

Fifteen months after seed placement (June 2011), 6.47% of the 3600 seeds initially placed survived, while only 3.15% became successfully established on pine branches (i.e., cotyledons displayed). Seed detachment was considerably high; roughly half of the initial seeds disappeared from the pine branches (56.38%) due to adhesion failure, with seed loss decreasing toward the branch tips (Fig. 3A). Although seed loss was considerably high for all host species, *P. halepensis* and *P. sylvestris* showed

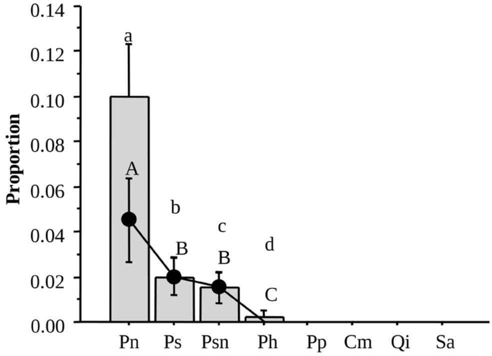

Fig. 1. Suitable host species as shown by proportion of seed survival (gray bars) and seedling establishment (black dots) of Viscum album subsp. austriacum on eight tree species coexisting at the Sierra de Baza (Pn: Pinus nigra; Ps: P. sylvestris; Psn: P. sylvestris subsp. nevadensis; Ph: P. halepensis; Pp: P. pinaster; Cm: Crataegus monogyna; QI: Quercus ilex; Sa: Sorbus aria). The experiment was performed in 2010 for 18 mo after seed placement. Results correspond to the first generalized linear modeescribed in methods (Experiment 1); seed survival: χ 2 = 35.17, df = 4, $p < 0.0008$ and seedling establishment: χ 2 = 12.53, df = 4, $p < 0.0098$. Comparisons between host species were performed with a Tukey's pairwise comparison method. Lowercase letters indicate differences in seed survival between host species, while uppercase letters indicate differences in seedling establishment between host species. Mean values and standard errors are represented; n = 10.

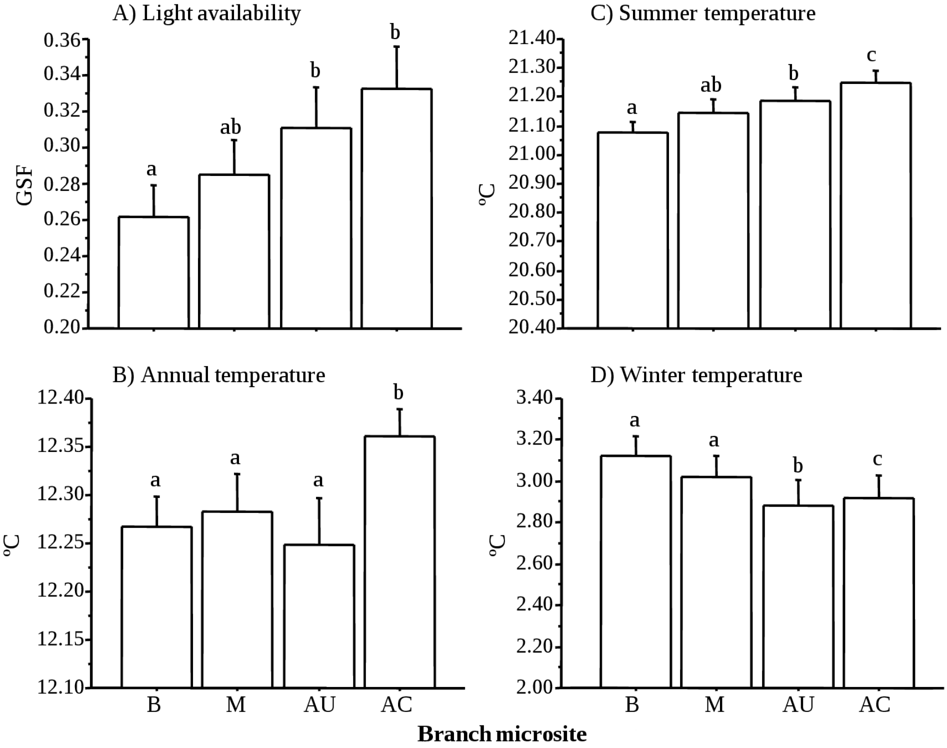

Fig. 2. Abiotic environment within host branches. Mean values of light (GSF) and temperature (°C) between different branch microsites of pine hosts. Basal, corresponds to the nearest branch location to the trunk of the tree (diameter: <2.5 cm); followed by middle location (diameter: 2.49–1 cm), apical uncovered by pine needles (diameter: 0.99–0.2 cm) and apical covered by pine needles (diameter: 0.99–0.2 cm). Results correspond to the LMM described in methods (Experiment 2); GSF: F3,57 = 4.33, P = 0.008; annual mean temperature: F3,82932 = 5.317, P = 0.0012; summer mean temperature: F3,185457 = 12.376, P < 0.0001 and winter mean temperature: F3,183441 = 20.432, P < 0.0001. Tukey's pairwise comparison method was applied to compare branch microsites. Differences between microsites are indicated with different lower case letters. Error bars represents standard errors.

the highest percentage of losses (Fig. 3A). From the seeds still remaining on pine branches, 51.38% desiccated on the bark, whereas 34.63% were depredated by birds. In general terms, seed predation decreased with distance from the trunk, while seed desiccation increased in this same direction (Fig. 3B, C). Seed mortality differed between pine species; seeds placed on *P. pinaster* and *P. sylvestris* suffered higher predation pressure, whereas desiccation was greater for *P. nigra and P. halepensis*. Despite the enormous seed losses, a few survivors were found, mainly under the coverage of pine needles (Fig. 3 D). This same result applied to seedling establishment, which was successful only in apical locations and, again, more frequently in locations covered by pine needles. Seedlings established preferentially on *P. nigra* followed by *P. halepensis* (Fig. 3E).

The results for the most parsimonious GLMMs (Table 2) showed that the coverage offered by pine needles to seeds significantly boosted seed survival, while mean winter temperature was associated with lower seed survival. On the other hand, needle coverage lowered seed predation, while increasing branch diameter encouraged seed predation. Seed desiccation significantly rose with increasing light incidence and mean annual temperature, whereas warmer winter temperature lessened seed desiccation. Seedling establishment was positively influenced by light incidence and, to a greater extent, by needle coverage. Overall, needle coverage was the relatively most important factor influencing seed survival (odds ratio [OR] = 8.531) and seedling establishment (OR = 7.803).

Dicussion

Safe sites for mistletoe recruitment are limited not simply by the availability of suitable hosts in a given place, but rather by a complex amalgamation of biotic and abiotic factors that interact with hosts, limiting locations where mistletoes can be recruited. In our experiments, by standardizing the seed amount provided in different tree microhabitats, we were able to disentangle microclimatic conditions from seed-rain effects, the latter being critical to recognize the role of key environmental variables as the basic template for natural regeneration of *V. a. austriacum*. Our results clearly indicate that host trees are not homogeneous niches for mistletoe regeneration, providing a mosaic of suitable and unsuitable locations for mistletoe recruitment. Additionally, we suggest that the negative consequences on host trees derived from the parasitic interaction can be seen as an opportunity for increasing forest heterogeneity, by modifying canopy structure and opening new gaps (Parks and Shaw, 1996). Thus, the results of this study should increase our understanding of the complexity of forest canopies.

Table 1. Results of generalized linear model analysis (with binomial error distribution and logit-link function) showing the effects of branch microsite and host species on mistletoe seed-adhesion failure, seed survival, seed predation, seed desiccation, and seedling establishment.

Variable	Model term	df	X^2	*p*
Adhesion failure	Species	3	7.84	**0.049**
	Microsite	3	28.27	**<0.0001**
	Interaction	9	3.28	0.952
Predation	Species	3	22.59	**<0.0001**
	Microsite	3	8.84	**0.0315**
	Interaction	9	8.75	0.461
Desiccation	Species	3	7.75	0.052
	Microsite	3	5.27	0.153
	Interaction	9	3.56	0.938
Survival	Species	3	15.46	**0.0015**
	Microsite	3	11.39	**0.0098**
	Interaction	9	4.43	0.880
Establishment	Species	3	5.03	0.051
	Microsite	3	41.04	**<0.0001**
	Interaction	9	2.73	0.974

Notes: In the model analyses, "species" and "microsite" are categorical explanatory variables used as factors. The first one corresponds to pine species used as hosts in the experiment (*Pinus nigra*, *P. pinaster*, *P. halepensis* and *P. sylvestris*) and the latter corresponds to each of the four branch locations in which mistletoe seeds were placed (basal; middle; apical uncovered and apical covered). Significant *p*-values are in boldface. Differences between species and microsites are shown in Fig. 3.

Range of suitable host species for mistletoe recruitment

Viscum album subsp. *austriacum* is able to parasitize a narrow range of closely related hosts, being limited to pine species at the study site (Experiments 1 and 3), as well as to conifers of the *Pinus* and *Larix* genera throughout its distribution range (Zuber, 2004). This tight specialization is strongly manifested at the local scale, where a single species—*Pinus nigra*—of all the ones available, acted as the primary host (Fig. 1). A similar pattern of local specialization is found for *V. a. austriacum* throughout the Iberian Peninsula, with *Pinus sylvestris* being the major host in northern regions, *P. halepensis* in the east, and *P. nigra* in the south (López-Sáez and Sanz de Bremon, 1992), as well as in mistletoes worldwide, e.g., *Arceuthobium* (Hawksworth and Wiens, 1996) and *Phoradendron*

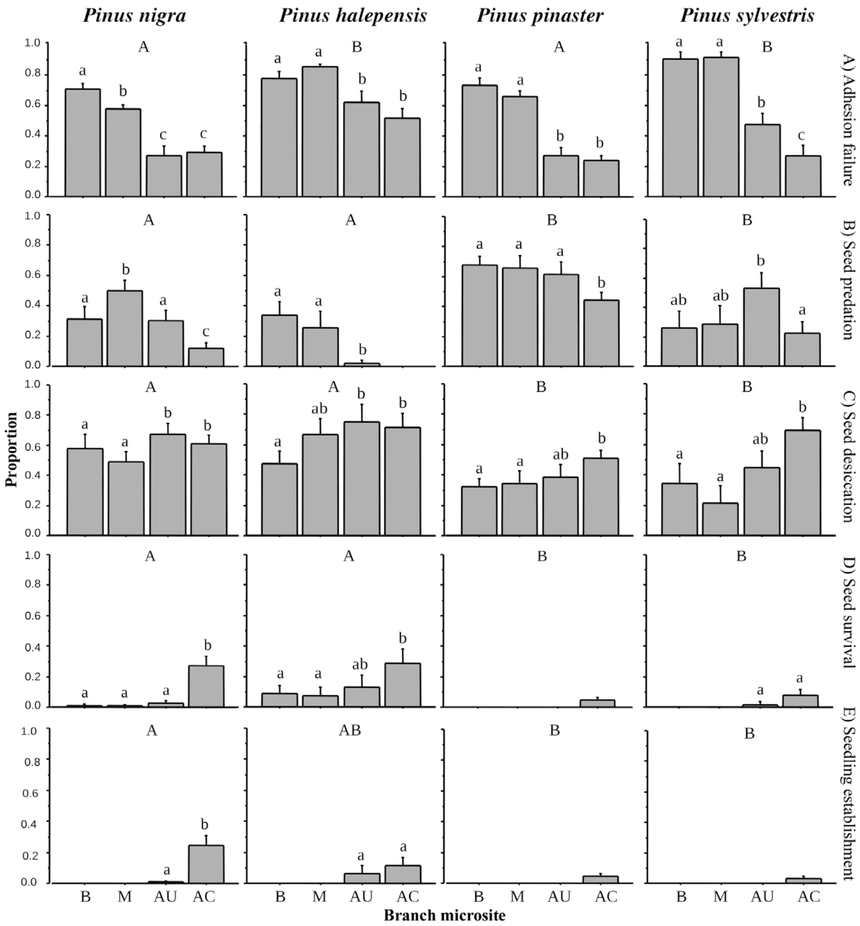

Fig. 3. Fate of seeds and seedlings as shown by proportions of (A) seed-adhesion failure, (B, C) seed mortality, (D) seed survival, and (E) seedling establishment in different branch microsites of four host species. Microsites: "Basal", nearest branch location to the trunk of the tree (diameter: <2.5 cm); followed by 'Mid.', middle branch location (diameter: 2.49–1 cm); "Ap. U.", apical location uncovered by pine needles (diameter: 0.99–0.2 cm); and "Ap. C.", apical location covered by pine needles (diameter: 0.99–0.2 cm). Mean values ± SEs are represented. Tukey's pairwise comparison method was used to compare microsites and species separately (see Table 1 for statistical results). Differences between microsites for each host species are indicated with different lowercase letters. Differences between host species are shown with different uppercase letters.

Table 2. Results of generalized linear mixed model analysis (with binomial error distribution and logit-link function) showing the effects of abiotic variables on mistletoe seed survival, seed mortality (either caused by predation or desiccation) and seedling establishment.

Variable	Model term	Estimate ± SE	Wald statistic	*p*	Odds ratio
Predation	Intercept	-1.370 ± 0.549	-2.492	**0.0126**	0.254
	Coverage	-1.059 ± 0.185	-5.704	**<0.0001**	0.346
	WT	0.066 ± 0.045	1.459	0.144	1.068
	Diameter	0.014 ± 0.005	2.798	**0.005**	1.014
Desiccation	Intercept	0.154 ± 0.439	0.351	0.726	1.166
	AT	0.116 ± 0.042	2.746	**0.006**	1.123
	WT	-0.121 ± 0.044	-2.771	**0.005**	0.886
	ST	-0.050 ± 0.028	-1.757	0.078	0.951
	GSF	0.062 ± 0.026	2.380	**0.017**	1.064
Survival	Intercept	-3.649 ± 0.803	-4.546	**<0.0001**	0.026
	Coverage	2.144 ± 0.292	0.292	**<0.0001**	8.531
	WT	-0.172 ± 0.062	-2.764	**0.0057**	0.842
	ST	0.062 ± 0.048	1.308	0.191	1.064
Establishment	Intercept	-5.343 ± 2.031	-2.631	**0.0085**	0.005
	Coverage	2.055 ± 0.746	2.753	**0.0059**	1.450
	GSF	0.372 ± 0.155	2.405	**0.0162**	7.803

Notes: In the model analyses, annual mean temperature (AT), winter mean temperature (WT), summer mean temperature (ST), light incidence (GSF), twig diameter (Diameter) and microsite coverage (yes/no) by pine needles (Coverage) are considered as fixed factors and species and tree as random factors. The models shown are the best models according to the AIC. Estimate ± SE (standard error); Wald statistic is from the coefficient evaluation test; odds ratio is used to compare the relative magnitude of various factors for a particular outcome: values = 1 mean that the exposure to the variable of interest does not affect odds of outcome; values < 1 mean that the exposure to the variable of interest is associated with lower odds of outcome; values >1 mean that the exposure to the variable of interest is associated with higher odds of outcome. (See Appendix S1 for information on model selection).

(Clay et al., 1985) in North America; *Psittacanthus schiedeanus* in Mexico (López de Buen and Ornelas, 2002) and *Amyema miquelii* in Australia (Norton et al., 1995). Notably, *P. nigra* constitutes the most abundant pine species of Sierra de Baza, making, as suggested by Norton and Carpenter (1998), host specialization more probable. As stated by these authors, specializing on a relatively abundant host species may increase a parasite's benefits of efficiently interacting with a frequently encountered host while decreasing the disadvantages of poorer interactions with a wider range of hosts. This latter aspect is advantageous in heterogeneous communities where finding proper hosts becomes unlikely.

Host trees as mosaics of safe sites for mistletoe recruitment

Aggregated spatial pattern of mistletoe populations is not determined only by the mistletoe-host specificity. In this study, we found that the high environmental heterogeneity of the pine-forest canopy strongly influences the fate of mistletoe seeds and seedlings, limiting safe sites for recruitment at an extremely narrow range within proper hosts. The first postdispersal filter against parasitism was seed adhesion failure, which eliminated more than half of the seeds initially placed on branches. Seeds on branches then began the long process of establishing on the host, coping with seed predators and the particularly high summer temperatures and strong light irradiance of the Mediterranean, as well as cold temperatures during the winter. Shortly after 1 yr, about 51.38% of the seeds dried, and 34.63% were depredated by granivore birds. Of the surviving 6.47%, 3.15% became successfully established.

Overall, *V. a. austriacum* show considerably high seed losses when compared with other mistletoe species, e.g., 44% for *Phoradendron robustissimum* (Sargent, 1995), 16% for *Alepis flavida* (Norton and Ladley, 1998), and 5–12% for *Psittacanthus schiedeanus* (Lopez de Buen and Ornelas, 2002). This apparent deficiency is probably counteracted by the abundant fruit production of *V. a. austriacum*, which can reach crops of approximately 2000 fruits/m^2 (Mellado and Zamora, personal observations), ensuring the recruitment of a small fraction of seeds. Despite the strong seed losses, few seeds were able to adhere to host branches, showing different adhesion capacity between host species and branch positions (Table 1, Fig. 3). Physical differences among hosts could determine seed adhesion. The flaky bark surface of *P. sylvestris* and the quite smooth bark of *P. halepensis* probably induce seeds to slough or slip and then fall soon after they have been deposited on the branch, whereas the rough bark of *P. nigra* and *P. pinaster* could facilitate seed adhesion. This physical barrier impeding the attachment of mistletoe seeds to host branches is the first postdispersal obstacle that seeds face, even before chemical host recognition promotes seed germination (e.g., Röld and Ward, 2002). Regardless of the host species, seed adhesion was greater toward the branch extremity, perhaps from the retention effect of the cohort of host needles growing at branch ends, which prevents seeds from falling off.

Additional postdispersal constraints to mistletoe recruitment were defined by multiple interacting biotic and abiotic factors. On the one hand, abiotic conditions (light and temperature) become harsher toward the tree periphery (Fig. 2), increasing seed mortality by desiccation (Table 2, Fig. 3C). Despite the fact that *V. a. austriacum* is a light-demanding, temperate species, excess light and heat harm seeds. On the other hand, seed mortality due to biotic factors (predation) followed an inverse pattern (Fig. 3B); s seed predators (birds) generally preferred to feed on branch interiors—where perching sites are sturdier and the accessibility to seeds is much easier—rather than the thin and unstable periphery twigs where seeds are difficult to reach (Mellado and Zamora, personal observations). Several titmouse species inhabiting forests of southern Spain could be potential mistletoe seed predators, with some of them, such as *Parus caeruleus* and *Parus ater*, being common feeders of mistletoe seeds at the study site (Mellado and Zamora, unpublished data) and throughout Europe (Zuber 2004). Although abiotic factors increased seed mortality toward the branch periphery, the lower seed predation counteracted seed mortality at these sites. In this sense, pine needles may provide a protective layer for seeds located under their coverage, just as several Mediterranean ground-dwelling plant species benefit from being shielded under nurse plants (Gómez-Aparicio et al., 2005).

The final outcome is that, overall, mistletoe seed survivors and established seedlings were found at the terminal points of tree branches under host-needle protection (Fig. 3D and 3E). These locations correspond not only to fine branches where mistletoes can more easily connect with the host (e.g., Sargent, 1995), but with those locations where seeds are protected against seed slippage during the initial attachment and safeguarded from seed predation. Therefore, the outermost locations of pine branches (tree periphery) provide suitable sites for earlier establishment as well as further growth and reproduction of mistletoes, given the light-dependence of this hemiparasitic plant for photosynthesis.

In conclusion, we experimentally confirmed the strong host specificity of *V. a. austriacum*, which is confined to pine species and in particular to *P. nigra* at the local scale. More broadly, we affirmed that canopy environmental heterogeneity of a Mediterranean pine forest influences the critical stages of early mistletoe recruitment, shaping safe sites for mistletoe regeneration at the fine spatial scale. Our approach demonstrated that biotic and abiotic variables exert a strong, nonrandom filter on *V. album* recruitment, resulting in recruitment hotspots (suitable microsites at the tips of the branches) and sites with a high probability of recruitment failure (unsuitable microsites in thicker and exposed branch locations). The narrow range of suitable host species and the scarce and spatially restricted recruitment hotspots for *V. a. austriacum* encourage the clumping of mistletoe populations at coarse and fine spatial scales. On the other hand, understanding how multiple environmental factors interact at the fine spatial scale to shape mistletoe distribution may give some clues about the distribution of the parasite at coarser scales. For instance, the finding that an excess of radiation and high temperature exert harmful effects on mistletoe seeds and limit recruitment at the fine scale can be seen, in larger terms, as a major agent impeding the expansion of *V. a. austriacum* to southern European regions, just as low temperatures limit its expansion into northern European regions (Zuber, 2004). These results expand our current knowledge of multiple factors driving the distribution of parasitic epiphytes in canopy ecosystems, including the degree of parasite–host specialization, as well as the physiological and environmental constraints defining the narrow range of safe sites for recruitment of these plants.

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Chapter 2

Generalist birds govern the seed dispersal of a parasitic plant with strong recruitment constraints

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Abstract

Mistletoes constitute instructive study cases to address the role of generalist consumers in the study of plant-animal interactions. They show among the most restricted range of safe sites for recruitment of any plant; therefore, frugivores specializing in mistletoe have been considered almost indispensable for the seed dispersal of these parasitic plants. However, the absence of such specialists in numerous regions inhabited by many mistletoe species raises a question whether unspecialized vectors may successfully disperse mistletoe seeds to narrowly defined safe sites. Using the European mistletoe *Viscum album* subsp. *austriacum* (hereafter, *V. a. austriacum*) as a study case, we recorded a broad range of 11 bird species that disperse mistletoe seeds. For these species, we studied the mistletoe-visitation rate and feeding behavior to estimate the quantity component of dispersal effectiveness, and the post-foraging microhabitat use, seed handling, and recruitment probabilities of different microhabitats as a measure of the quality component of effectiveness. Both endozoochory and ectozoochory are valid dispersal mechanisms, as seeds do not need to be ingested to germinate, increasing seed-dispersal versatility. Thrushes were the most effective dispersers, although rather inefficient, whereas small birds (both frugivores and non-frugivores) offered low-quantity but high-quality services for depositing seeds directly upon safe sites. As birds behave similarly on parasitized and non-parasitized hosts, and vectors have broad home ranges, reinfection within patches and the colonization of new patches would be ensured by an ample assemblage of generalist birds. In doing so, a parasitic plant requiring precision in seed dispersal can rely on unspecialized dispersers.

Key words

Ectozoochory; endozoochory; generalist system; mistletoe; Viscaceae.

Introduction

Most theoretical and empirical studies on plant-animal interactions have traditionally outlined the role of specialization as a prominent feature of interactive systems. However, an increasing amount of scientific evidence strongly supports the idea that generalization is more frequent than hitherto thought, with most plants interacting with multiple types of pollinators, seed dispersers, and herbivores (Herrera 1988; Waser et al. 1996; Gómez and Zamora 2000; Olesen and Jordano 2002; Bascompte and Jordano 2007). Mistletoes are widely distributed worldwide as common dwellers of tree canopies and constitute excellent models to delve into the issue of generalization versus specialization. Their parasitic nature limits them to a narrowly defined range of safe sites for seed recruitment, i.e. particular branch diameters of specific host species (Reid 1989, Van 1993, Sargent 1995; Norton and de Lange 1999; Aukema and Martínez de Río 2002; Arruda et al. 2006). Such strong specificity suggests that mistletoes must depend on highly precise dispersal mechanisms to guide their seeds to safe sites. In this regard, many frugivore species reportedly have close mutualist ties with mistletoes, exchanging effective seed-dispersal services for food rewards (Reid 1990; Wenny 2001; Amico and Aizen 2000; Carlo and Aukema 2005), reflecting a likely coevolution (Reid 1991; Aukema and Martínez del Río 2002). Notwithstanding, this tight dependence of mistletoes on specialized vectors has been increasingly questioned, since many dietary generalists have been described as frequent mistletoe seed dispersers (e.g. Restrepo 1987; Reid 1991; Hawksworth and Wiens

1996; Zuber 2004; Rawsthorne et al. 2011; Arruda et al. 2012). In fact, several mistletoe species inhabiting large territories where adept vectors are completely absent depend exclusively on such dietary generalists –e.g. most mistletoe species dwelling in the Northern Hemisphere, on many ocean islands, and in several regions of the Southern Hemisphere (Reid 1991; Watson and Rawsthorne 2013).

Mistletoes relying on specialist vector—all bird species (Restrepo et al. 2002) —may take advantage of the exclusive, direct seed dispersal of a few legitimate dispersers (Davidar 1983; Reid 1989; Martínez del Río et al. 1995; Sargent 1995; Larson 1996). These birds, with their extremely restricted diet, ensure that mistletoe fruits are consumed, subsisting almost completely upon mistletoe fruits (Reid 1991; Restrepo et al. 2002; Watson 2004); they also guarantee high-quality dispersal services due to special anatomical and behavioral adaptations (Walsberg 1975; Richardson and Wooller 1988) which enhance seed germination or seedling establishment after passing through the digestive tract (e.g. Martínez del Río and Restrepo 1993; Murphy et al. 1993) and encourage seedling recruitment by depositing seeds properly on safe sites (e.g. Reid 1989; Wenny 2001). On the other hand, mistletoes that depend on generalist vectors may risk uncertainties that fruits may not to be consumed or seeds may not arrive to suitable habitats (Reid 1989; Larson 1996; Montaño-Centellas 2012; Watson 2012). Despite of the frequency of these parasitic plants, the way in which mistletoes relying exclusively on generalists achieve their dissemination remains unknown.

In this study, we investigate dispersal by a group of birds that feed on the mistletoe *Viscum album* subsp. *austriacum* (hereafter *V. a. austriacum*). This parasitic plant is widely distributed across Europe, where no mistletoe specialists exist and, therefore, where seed dispersal depends exclusively on generalists (Snow and Snow 1984, 1988; Zuber 2004; Watson and Rawsthorne 2013). We explored how this bird assemblage affects the demography of mistletoe by analyzing relative dispersal effectiveness (i.e. the contribution of a seed disperser to mistletoe fitness, *sensu* Schupp 1993) and efficiency (i.e. the probability that a mistletoe seed will arrive to a safe site). For each mistletoe visitor, we studied the visitation rate and feeding behavior to estimate the "quantity" component of the effectiveness; also we examined post-foraging microhabitat use and the consequent seed shadows to assess the "quality" component of effectiveness. Concurrently, through an experimental approach, we quantified recruitment probabilities of different microhabitats within suitable hosts. We also noted whether these birds were capable of depositing seeds at appropriate safe sites beyond the mother plant, acting as colonizers of new infection foci. With this approach, we seek to understand how a parasitic plant requiring precision in seed dispersal can rely on an imprecise, unspecialized dispersal assemblage for reinfection, (establishing "infection centers"), as well as for transmission to suitable new hosts.

Material & Methods

Study system and site

Viscum a. austriacum (Wiesb.) Vollman, a mistletoe species native to most regions of Europe, specializes in parasitizing conifers, mainly *Pinus* species across its distribution range. The study site, Sierra de Baza (2º51' 48" W, 37º 22' 57" N), represents the southernmost limit of its geographical distribution. This is a mountainous natural reserve of southeastern Spain, which shows a sharp altitudinal gradient of 850m to 2270m. The climate is typically Mediterranean, with hot, dry summers (June-September), cold winters (December-March), and rainy autumns and springs. Pine is the dominant forest tree, with Austrian pine (*Pinus nigra* Arn.) and Scots pine (*P. sylvestris* L.) being major hosts for *V. a. austriacum* at this site. Other pines, Aleppo (*P. halepensis* Mill.) and Maritime (*P. pinaster* Ait.), as well as non-conifer tree species, Holm oaks (*Quercus ilex* L.) and *Acer opalus* L. subsp. *granatense* Boiss., are also abundant. As a bird-dispersed plant, *V. a. austriacum* produces copious crops of whitish fleshy fruits that ripen at the beginning of September and remain available for frugivores until early March. This mistletoe shares an assemblage of seed dispersers with a diverse community of fleshy-fruited shrubs formed chiefly by *Berberis hispanica* subsp. *hispanica* Boiss. & Reut., *Crataegus monogyna* Jacq, *Lonicera arborea* Boiss., *Juniperus oxycedrus* L., *J. communis* L., *Prunus ramburii* Boiss, and *Rosa* sp.

Mistletoe recruitment and seed-deposition sites

We studied the natural seed-deposition pattern of *V. a. austriacum* on pine branches after a seed-dispersal season (beginning of March of 2010). Mistletoe seed rain was measured on 41 branches of 41 parasitized *P. nigra* trees of similar parasitic loads (hosting from 5 to 10 large-sized fruiting mistletoes). One of the accessible branches per tree was randomly selected for study. Each branch was divided into 4 positions according to branch thickness: (1) 'basal position', the thickest (>2.5cm) and nearest position to the trunk; (2) 'middle position', the middle position of the branch (2.49-1cm), and two apical positions (0.99-0.2 cm): (3) one uncovered (pine twigs 2- 3 years old) and the other (4) covered by pine needles (the most recent pine growth)—hereafter, 'apical uncovered' and 'apical covered', respectively. Branch diameter was measured with a precision caliper. In each branch position we quantified the number of mistletoe seeds, considering losses due to seed predation as predators feed mostly on the embryo (Grazi and Urech 2000 in Zuber 2004), leaving easily detectable scrapes on the branch.

Also, we estimated the quality of each branch position for mistletoe recruitment by calculating recruitment probabilities at those sites. To do so, we performed a sowing experiment under field conditions. Ten *P. nigra* trees, alike in age (40 yr), size (diameter at breast height [dbh]: 10.27 ± 1.38 cm), architecture, and ecological environment, were chosen in Sierra de Baza. Three branches per tree were randomly assigned and divided into four positions, following the same criteria as for seed counts. A total of 900 seeds were placed onto the 30 branches: 10 seeds in basal positions (>2.5 cm), 10 in middle positions (2.49-1cm), 5 in uncovered apical positions (0.99-0.2 cm), and 5 in needle-covered apical positions. Fruits collected in Sierra de Baza during early March of 2010 from 15 source plants growing on different host trees were pooled and randomized. Fruit exocarps were removed to permit seed germination (Ladley and Kelly 1996) and seeds were rapidly and carefully placed on branches, leaving 1.5- to 2-cm spaces between seeds. After 15 months (considered sufficient time for seeds to germinate and to become established), we quantified the number of seedlings at each branch position. Then we estimated recruitment probabilities of each site as the number of seedlings counted at the end of the experiment to the initially placed seeds.

Identification of mistletoe seed dispersers and seed-deposition patterns

We investigated the behavioral patterns of all members of the frugivore bird assemblage inhabiting the study site, as well as their linkage to mistletoe dispersal. For this, we recorded bird visitations to parasitized *P. nigra* and described their feeding and post-foraging behavior on different host structures. Additional bird watching in non-parasitized trees served to compare avian behavioral patterns when mistletoes were present or absent. For two seed-dispersal seasons, from September to February of two consecutive years (2009-2010 and 2010-2011), birds were observed by direct observations as well as videotaping. Direct observations, from 07:00 to 12:00 h at four localities of the study site, were randomly conducted over the sampling period from hiding places at a minimum distance of 15 m from focal trees (using binoculars when needed for bird identification). For videotaping, high-resolution video cameras were placed directly in the field, from 07:00 to 12:00 h. Recordings were made on 48 parasitized *P. nigra* trees from distances of 5-15 m, enabling bird identifications and accurate descriptions of feeding behavior.

In the study of avian feeding behavior, a “visit” was considered each time a bird made contact with the mistletoe. During each visit, the visitor was identified to the species level, the activity (feeding or perching), fruit-removal rate (number of fruits swallowed per min) and visit duration were recorded. We disregarded incomplete observations to estimate visit durations, i.e. birds not observed from the moment they arrived into the mistletoe until the moment they left it. After identification, the birds were classified into four guilds (see Table 1) according to their body size (after Mullarney et al. (2000): large frugivores of 60-120 g, small frugivores of 12-20 g) and resource acquisition (generalist or opportunist frugivore, after Watson 2012). The first group, "large generalist frugivores" (LG) consisted of large birds which were dietary generalists, feeding on a broad range of fruits during autumn and winter. The second group, "small generalist frugivores" (SG), was composed of small birds that consumed a diverse range of fruits. The third group, "opportunist" (O), was formed by small birds, mainly granivores and insectivores that occasionally consumed fleshy fruits. Finally, the last group, "small mistletoe visitors" (SV), was composed of other small birds, either insectivores or granivores, that visit mistletoe but do not manipulate fruits. The variables "number of visits", "visit duration (min)" and "fruit-removal rate (fruits per min)" were used to estimate the quantity component of the dispersal effectiveness of each visitor species that could potentially act as a seed disperser.

Once the mistletoe visitors were identified, we analyzed their preferences for different host structures, on both parasitized and non-parasitized trees. First, we divided the trees into three portions: "upper-third" (the treetop), "middle-third" (the middle third of the tree) and "lower-third" (the bottom of the tree). Next, we distinguished between four structures visited by birds: (1) the crown of the pine, and 3 positions within branches: (2) basal, (3) middle and (4) apical. The branches were divided following the same criteria as for the exploration of seed-deposition and seedling-distribution patterns. The frequency of use of different host structures was used to assign locations with higher probabilities of receiving seeds. This, multiplied by the mistletoe recruitment probabilities previously calculated, was used to characterize the dispersal quality of each bird species. By multiplying the "quantity" and the “quality” components, we calculated the dispersal effectiveness of each bird species. Finally, disperser efficiency was estimated by dividing the “quality” term by the “quantity” term.

Seed condition

A sowing experiment was performed to evaluate the suitability of mistletoe seeds after they were internally or externally dispersed. For this, we collected bird-dispersed and non-dispersed mistletoe seeds that were placed on safe sites of *P. nigra* branches. For bird-dispersed seeds, we gathered seeds in the field with clear signs of previous consumption by frugivore birds, expelled either by regurgitation or defecation (simulating an endozoochorous dispersal event); they were mixed to randomization and immediately placed onto tree branches. For non-dispersed seeds, fruits were collected directly from mother plants, the exocarps manually removed (simulating an ectozoochorous dispersal event), and the seeds randomized and placed on host branches. During early March of 2010, a total of 900 dispersed seeds and another 900 non-dispersed seeds were inoculated onto twigs (0.99- 0.2 cm in diameter) of three limbs of seventy *P. nigra* trees (all alike in age, size, and architecture); then, seeds were monitored every 30-40 days for up to 15 months after planting. In the first monitoring the number of seeds that were lost before their initial attachment to the host bark was quantified. During seed monitoring, we noted whether a seed was absent or present, and if present, seed condition (alive or dead). We distinguished two stages within live seeds: germinated and established. Germinated included seeds that had started haustoria development, whereas established included seeds with a functional haustoria and emerged cotyledons. Proportions of seed germination (number of germinated seeds vs. the total remaining on tree branches after the first month) and seedling establishment (number of germinated seeds vs. the total remaining on tree branches) were calculated for further analyses.

Statistical analysis

To analyze the distribution of mistletoe seeds and seedlings

Table 1. List of bird species visiting *V. a. austriacum* grouped in different guilds depending on their body size and feeding behavior, followed by the sum of mistletoe visits for 306 observation hours, visit duration, mean number of fruits removed per minute, number of ectozoochory and endozoochory events and relative dispersal quantity.

Vernacular name (*scientific name*)	[1]Guild	[2]Σ Visits	[3]Σ Feedings	[4]Minutes/visit	[5]Fruits/min	[6]Σ Ectozoochory	[7]Σ Endozoochory	[8]Relative Quantity
Mistle thrush (*Turdus viscivorus*)	LG	605	177	2.58(0.30)	6.27(1.05)	-	27	1,00
Ring ouzel (*Turdus torquatus*)	LG	190	42	2.33(0.18)	8.17(0.98)	-	3	0,28
Song thrush (*Turdus philomelos*)	LG	82	20	2.31(0.17)	8.6(2.94)	-	1	0,14
Redwing (*Turdus iliacus*)	LG	15	6	2.45(0.28)	4.83(1.88)	-	3	0,025
Common blackbird (*Turdus merula*)	LG	26	6	1.94(0.32)	6.10(1.02)	-	3	0,025
Fieldfare (*Turdus pilaris*)	LG	12	3	0.99(0.24)	8.37(1.80)	-	1	0,009
Blackcap (*Sylvia atricapilla*)	SG	38	9	1.62(0.24)	6.31(1.15)	-	12	0,032
Robin (*Erithacus rubecula*)	SG	4	3	0.58(0.08)	4.25(0.25)	-	-	0,003
Coal tit (*Parus ater*)	O	37	4	0.50(0.12)	3(0.30)	4	-	0,002
Great tit (*Parus major*)	O	34	3	0.52(0.13)	3(0.30)	3	-	0,002
Blue tit (*Parus caeruleus*)	O	17	9	0.87(0.20)	1(0)	9	-	0,003
Long-tailed tit (*Aegithalos caudatus*)	SV	5	-	-	-	-	-	-
Hawfinch (*C. coccothraustes*)	SV	1	-	-	-	-	-	-
European goldfinch (*Carduelis carduelis*)	SV	1	-	-	-	-	-	-
Crested tit (*Lophophanes cristatus*)	SV	1	-	-	-	-	-	-

[1] LG, large generalist frugivore birds (60-120 g) ; SG, small generalist frugivore birds (12-20 g); O, opportunist birds (12-20 g); SV, other mistletoe visitors.

[2] Number of mistletoe visits over 306 observation hours, either by direct watching (208 hours) or video-taping (98 recording hours).

[3] Number of visits in which birds ingested or pecked on fruits.

[4] Visit duration, in minutes (mean ± S.E.), was estimated from complete observations of birds visiting mistletoes. When birds stayed more than 3 minutes, this was the maximum time recorded.

[5] Number of consumed or pecked fruits per minute (mean ± S.E.). Complete and partial observations were included to estimate rates of consumption.

[6] Number of times birds were observed carrying seeds externally and depositing them on pine branches.

[7] Number of times birds were observed feeding on mistletoe fruits and defecating or regurgitating seeds.

[8] Quantity values have been estimated as ΣFeedings x Fruits/minute x Minute/visit; then were divided by the highest value to calculate relative estimates.

in the different positions of pine branches, we used generalized linear models (GLMs), assuming a Poisson error distribution and log-function, followed by a Tukey-HSD pair-wise comparison method. The portion of host used (upper third, middle third or bottom third) and host structures used (crown and branch positions: basal, middle or apical) were compared between avian dispersing guilds (LG, SG and O) with a Pearson's Chi-squared test. Simulated p-values based on 2000 replicates were used when needed. Germination and establishment success of internally and externally dispersed seeds were compared using GLMs, assuming a binomial distribution of errors and logit-link functions, followed by a Tukey-HSD pair-wise comparison method. R software (version 2.10.0, R Development Core Team 2010) was used to perform the statistical analyses, while figures were made in StatView 5.0.1 (SAS Institute 1998). Tukey's pairwise comparisons with 95% confidence level were conducted with the "multcomp" package (Hothorn et al. 2008).

Results

Mistletoe recruitment and seed-deposition sites

Recruitment probabilities differed between branch positions (GLM: $\chi^2 = 66.30$, *d. f.* =3, $P < 0.0001$), being nil at the thickest sites (basal and middle positions of branches), 0.67% for apical uncovered twigs and 17.3 % for needle-covered twigs (Fig 1). Seed deposition also differed between branch positions (GLM: $\chi^2 = 649.11$, *d. f.* =3, $P < 0.0001$), although this followed a different pattern (Fig. 1): 393 seeds (66.16 % of total seeds) were on basal positions, 150 seeds (25.25 %) were on needle-covered apical twigs, only 50 seeds (12.67 %) reached the middle positions, while apical uncovered twigs hardly received 1 seed (0.002%).

Identification of mistletoe seed dispersers and seed-deposition patterns

During bird observations, we recorded 15 different bird species that visited fruiting *V. a. austriacum*. Eight species were classified as generalist frugivore seed dispersers, three as opportunists, and four as mistletoe visitors that never manipulated fruits. Of the seed dispersers, 6 were large and 2 small birds; while opportunists and other visitors were all small (Table 1). All seed dispersers swallowed the fruits and defecated or regurgitated the seeds, while opportunists only pecked at the fruit pericarp and externally

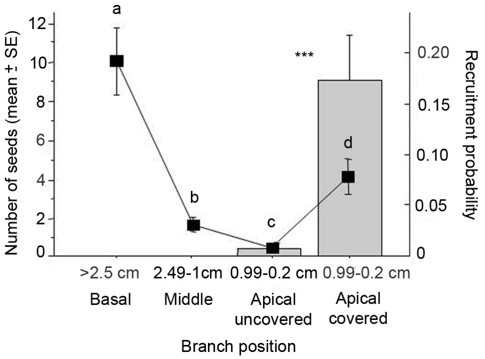

Fig.1. Pattern of mistletoe seed deposition (black squares) and recruitment probability (gray bars) according to branch diameter. Branches were divided into four categories: (1) basal position, >2.5 cm (closest to the tree trunk); (2) middle position, 2.49-1 cm and two apical positions: (3) uncovered apical position, 0.99-0.2 cm, and (4) needle-covered apical position, 0.99-0.2 cm. Number of seeds: mean ± S. E., N = 41 branches (Generalized linear model, $\chi^2 = 649.11$, d.f. = 3, $P < 0.0001$); differences among branch positions are indicated by different letters. Recruitment probabilities: mean ± S. E., N = 30 branches (Generalized linear model, $\chi^2 = 66.30$, d.f. = 3, $P < 0.0001$); *** P-value < 0.0001.

dispersed mistletoe seeds that adhered to their feathers or beaks. During 98 videotaping hours, for 48 parasitized *P. nigra* trees, we recorded 148 birds visiting mistletoe. For 208 h over 50 days of direct bird observations, we recorded another 361 visits to mistletoe on parasitized pines. An additional 564 bird observations were made on non-parasitized trees, which were used to compare the birds' behavioral patterns when mistletoe was absent. Large generalist frugivores accounted for 90% of the visits, small generalist frugivores for 4.26% and opportunists for 5.74%. From all the bird visitations, we clearly discerned 282 feeding events (in which birds were observed pecking or swallowing fruits) of which 50 were clear episodes of endozoochory (in which birds defecated seeds after feeding on whole mistletoe fruits), all involving *Turdus* spp. and *Sylvia atricapilla*. Also, we recorded scattered events of ectozoochory by *Parus caeruleus* (n = 9 events), *P. major* (n = 3 events) and *P. ater* (n = 4 events). In these cases, as birds pecked at fruits to feed on the pulp, sticky seeds occasionally adhered to their beaks, which were immediately rubbed against pine needles at the tip of the branches to remove the seeds. Quantitatively, thrushes were major contributors to mistletoe seed dispersal, mainly *Turdus viscivorus* (Table 1).

The three guilds of birds ("other visitors" were excluded as they showed no interaction with

Table 2 Seed-dispersal quality and frequencies of post-foraging host-use of mistletoe visitors, differentiating between three tree portions and four tree structures.

		[2]Tree portion			[3]Tree structure				
Vernacular name (*scientific name*)	[1]Σ Observations	Upper-third	Middle-thid	Lower-thid	Crown	Basal	Middle	Apical	[4]Relative Quality
Mistle thrush (*Turdus viscivorus*)	605	0,73	0,26	0,01	0,49	0,13	0,37	0,01	0,54
Ring ouzel (*Turdus torquatus*)	190	0,73	0,24	0,04	0,45	0,35	0,19	0,01	0,11
Song thrush (*Turdus philomelos*)	82	0,79	0,18	0,02	0,51	0,22	0,27	0,00	0,03
Common blackbird (*Turdus merula*)	26	0,50	0,38	0,12	0,23	0,31	0,46	0,00	0,02
Redwing (*Turdus iliacus*)	15	0,67	0,33	0,00	0,47	0,53	0,00	0,00	0,00
Fieldfare (*Turdus pilaris*)	12	0,67	0,33	0,00	0,33	0,67	0,00	0,00	0,00
Blackcap (*Sylvia atricapilla*)	38	0,13	0,79	0,08	0,00	0,26	0,45	0,29	0,39
Robin (*Erithacus rubecula*)	4	0,25	0,50	0,25	0,25	0,00	0,75	0,00	0,00
Coal tit (*Parus ater*)	37	0,03	0,95	0,03	0,00	0,00	0,19	0,81	1,00
Great tit (*Parus major*)	34	0,09	0,79	0,12	0,00	0,00	0,15	0,85	0.96
Blue tit (*Parus caeruleus*)	17	0,06	0,82	0,12	0,00	0,00	0,06	0,94	0,53

[1] Number of mistletoe visits over 306 observation hours, either by direct watching (208 hours) or video-taping (98 recording hours).

[2] Frequency of bird observations classified by the portion of the tree visited: "upper-third" (the treetop), "middle-third" (the middle third of the tree) and "lower-third" (the bottom third of the tree). Frequencies were calculated for each species as the number of visits at each tree portion by the total number of observations.

[3] Frequency of bird observations classified by the structure of the tree used: (1) the tree crown, and 3 different positions within branches: (2) basal (closer to the tree trunk), (3) middle and (4) apical (the branch periphery). Frequencies were calculated for each species as the number of visits at each tree structure divided by the total number of observations.

[4] The quality term was calculated as the number of bird observations on different branch positions—'basal, 'middle' or 'apical'—, multiplied by the recruitment probability of each position: 'basal', 0; 'middle', 0; 'apical' 0.173. Recruitment probabilities were experimentally calculated by estimating the proportion of mistletoe seedlings establishing on 4 different branch positions, where seeds are commonly deposited by birds: (1) basal branch position (diameter of <2.5 cm), (2) middle position (diameter of 2.49-1cm), (3) uncovered apical position (diameter of 0.99-0.2 cm) and (4) needle-covered apical position (diameter of 0.99-0.2 cm). As most birds visited needle-covered apical positions, we used the recruitment probability of this site for calculations. Quantity values were divided by the highest value to calculate relative estimates.

mistletoe fruits) differed in the use of host structures ($\chi^2 = 731.76$, *d. f.* = 6, *P* < 0.0001) and frequented different portions of the tree ($\chi^2 = 207.25$, *d. f.* = 4, *P* < 0.0001) (Table 2). Small birds (generalist frugivores and opportunists) differed in the use of the host's structures ($\chi^2 = 50.11$, *d. f.* = 3, *P* = 0.0005) but not in the portion of the tree ($\chi^2 = 2.92$, *d. f.* = 2, *P* =

0.272). Large generalist frugivores mostly frequented structures located at the treetop (72.5%), while the majority of small passerines visited medium heights of the pine (82.6%). About half of large birds (all *Turdus* species, except for *T. merula*) visited mistletoes located at the treetop, and fed on fruits while perched on the crown. After feeding, they kept on watch from the crown, moved to a nearby crown, or moved to basal (20% of the visits) and middle positions (30% of the visits) of pine branches. *Turdus merula*, however, frequently fed on fruits fallen on the ground. Otherwise, small birds, either generalist frugivores or opportunists, visited the middle portions of the tree. However, while small generalists commonly perched on the middle sites of branches (47%) and at times used apical twigs (26%), opportunists followed the opposite pattern with mostly perching on branch tips (85%) and rarely using thicker structures (15%). Small opportunists offered the highest quality seed dispersal services, followed by the large generalist frugivore *Turdus viscivorus* and the small generalist frugivore *Sylvia atricapilla* (Table 2). Overall, *Turdus viscivorus* was the most effective seed disperser, while opportunist species were the most efficient dispersers (Table 3).

Birds behaved similarly in parasitized and non-parasitized pines regarding the portion of the host used (high, middle, low) by each group of dispersers (LG: $\chi^2 = 0.37$, *d. f.* =2, $P = 0.83$; SG: $\chi^2 = 10.17$, *d. f.* = 2, $P = 0.062$; O: $\chi^2 = 2.28$, *d. f.* = 1, $P = 0.13$; the "low" position was eliminated from the latter analysis because of the lack of data). No differences were found, either, in the structures (crown or branch positions: basal, middle and apical) preferred by birds when the host was parasitized or not (LG: $\chi^2 = 16.73$, *d. f.* = 3, $P = 0.051$; SG: $\chi^2 = 2.54$, *d. f.* = 3, $P = 0.11$; O: $\chi^2 = 1.28$, *d. f.* = 3, $P = 0.26$) (Fig. 2). Overall, the preferred structure by LG was the tree crown, 41% of the visits in parasitized trees and 53% in non-parasitized.

Seed condition

With regard to seed handling, no differences were found between treatments in seed germination and seedling establishment. Of the seeds remaining on host branches (disregarding seeds that failed in their

Table 3 Seed dispersal effectiveness (quality x quantity) and efficiency (quality/ quantity) of mistletoe visitors.

Vernacular name (*scientific name*)	[1]Quantity	[2]Quality	[3]Relative Effectiveness	[4]Relative Efficiency
Mistle thrush (*Turdus viscivorus*)	2863,26	2,87	1,00	0,00
Ring ouzel (*Turdus torquatus*)	799,52	0,59	0,06	0,00
Song thrush (*Turdus philomelos*)	397,32	0,15	0,01	0,00
Common blackbird (*Turdus merula*)	71,00	0,08	0,00	0,00
Redwing (*Turdus iliacus*)	71,00	0,00	0,00	0,00
Fieldfare (*Turdus pilaris*)	24,86	0,00	0,00	0,00
Blackcap (*Sylvia atricapilla*)	92,00	2,02	0,02	0,02
Robin (*Erithacus rubecula*)	7,40	0,02	0,00	0,00
Coal tit (*Parus ater*)	6,00	5,24	0,00	0,81
Great tit (*Parus major*)	4,68	5,05	0,00	1,00
Blue tit (*Parus caeruleus*)	7,83	2,77	0,00	0,33

[1] The quantity term was estimated as the number of visits in which birds fed on mistletoe fruits, multiplied by the number of fruits consumed per minute and the duration of the visit (minutes).

[2] The quality term was calculated as the number of bird observations on different branch positions—'basal, 'middle' or 'apical'—, multiplied by the recruitment probability of each position: 'basal', 0; 'middle', 0; 'apical' 0.173. Recruitment probabilities were experimentally calculated by estimating the proportion of mistletoe seedlings establishing on 4 different branch positions, where seeds are commonly deposited by birds: (1) basal branch position (diameter of <2.5 cm), (2) middle position (diameter of 2.49-1cm), (3) uncovered apical position (diameter of 0.99-0.2 cm) and (4) needle-covered apical position (diameter of 0.99-0.2 cm). As most birds visited needle-covered apical positions, we used the recruitment probability of this site for calculations.

[3] Seed dispersal effectiveness is defined as the probability of a seed being handled by a particular bird that reach a 'safe site'. It was calculated as the product of the 'quantity' and the 'quality' terms, then divided by the highest value to calculate relative estimates.

[4] Disperser's efficiency is a measure of the number of dispersed seeds relative to the number of handled seeds. It was as calculated as the ratio of 'quality' and 'quantity' terms, and then divided by the highest value to calculate relative estimates.

initial attachment to host branches), 69% of internally dispersed seeds and 71.4% of those externally dispersed germinated (GLM: $\chi^2 = 0.17$, *d. f.* =1, $P = 0.68$), while 8.2 % of externally dispersed seeds and 5.6% of internally dispersed seeds got successfully established (GLM: $\chi^2 = 2.24$, *d. f.* =1, $P = 0.14$).

Discussion

This study explicitly examines the poorly explored topic of how generalist birds, in the absence of specialized dispersers, provide seed dispersal for a parasitic plant with strong recruitment constraints. We first identified the potential group of seed dispersers of *V. a. austriacum* in a Mediterranean mountain of southeastern Spain. Then, by linking species-specific behavior to seed-dispersal patterns and to demographic consequences for the parasite in different microhabitats, we discovered that this mistletoe can rely on unspecialized vectors to disperse seeds to narrowly defined safe sites.

A broad and diverse group of 11 bird species (except for strict granivores or insectivores) disperse seeds of *V. a. austriacum* in Sierra de Baza. Any bird can be a potential disperser, as seeds do not need to be ingested to germinate. This flexibility guarantees that *V. a. austriacum*, irrespective of the composition and abundance of the avian community in a pine forest, will satisfy its seed dispersal needs. Regardless of the dispersal mode, all vectors have to deposit seeds on proper safe sites—narrowly limited to thin branches of the tree periphery— in order to exert a real impact on mistletoe demography. Small epizoochorous and endozoochorous birds disperse, respectively, by rubbing their beaks against pine needles or directly defecating on twigs. Meanwhile, large endozoochorous birds disperse by haphazardly and abundantly defecating or regurgitating from the treetop. In short, any bird able to deposit a seed on the proper site qualifies as a seed disperser.

Among endozoochorus vectors, large generalist frugivores (LG) constitute the most effective dispersal guild; however, they provide low-efficiency services, consuming numerous fruits in order to suitably disperse a single seed. Their preferences for firm and robust structures as perching sites finally result in abundant seed rain with most seeds reaching basal, thick positions of branches. At these sites the seeds have no chance of survival, the haustorium being unable to penetrate the xylem of the host, and furthermore

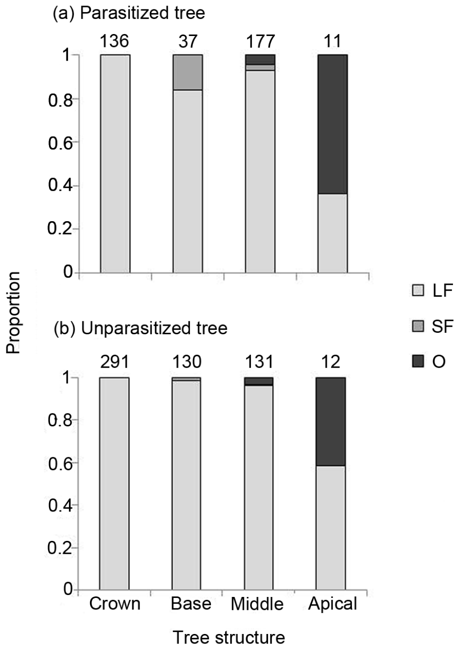

Fig.2 Post-foraging microhabitat use of mistletoe seed dispersers on parasitized and non-parasitized trees. Shown is the relative use frequency of different host structures by major groups of mistletoe seed dispersers. Tree structures are indicated as: (1) crown, the tree-top, and 3 different positions within branches: (2) basal, as the closest position to the tree trunk; (3) middle, as the middle position and (4) apical, as the branch periphery. Groups of seed dispersers are shown as: LG, large generalist frugivores, including *Turdus viscivorus; T. torquatus; T. philomelos; T. merula; T. iliacus* and *T. pilaris;* SG, small generalist frugivores, including *Sylvia atricapilla* and *Erithacus rubecula;* O, opportunists, including *Parus ater, Parus major* and *Parus caeruleus*. Pearson´s Chi squared test: LG, $\chi^2 = 16.73$, d.f. = 3, $P = 0.051$; SG, $\chi^2 = 2.54$, d.f. = 3, $P = 0.11$; O, $\chi^2 = 1.28$, d.f. = 3, $P = 0.26$. Sample sizes are shown above the bars.

are likely to become desiccated by extreme summer temperatures or to be depredated (Mellado and Zamora 2014). On the other hand, as thrushes provide high-quantity dispersal services, the few times (1.1%) they used apical sites of branches led to a considerable number of favorable dispersal events, which notably contributed to the final recruitment of the parasite. In the light of these results, large generalist frugivores might act as effective seed dispersers whenever mistletoe fruit availability is enough to offset their high inefficiency. *Viscum a. austriacum* can afford this wasteful dispersal mechanism by producing abundant and constant crops of small fruits over most of the fruiting season (Zuber 2004). In contrast, although small generalist frugivores (SG) provide better-quality and more efficient dispersal, they were so scarce that their contribution to the parasite was almost negligible during our study.

On the other hand, opportunist tits (O) can be considered very efficient dispersers by moving seeds externally and directly to safe sites of the tree periphery. This external seed transport by dietary generalists and opportunist frugivores has been frequently reported among *Viscaceae* mistletoes (e.g. Punter and Gilbert 1989; Restrepo et al. 2002), which may confer several advantages to the parasite. First, single deposition onto safe sites may help seeds to escape high post-dispersal seed predation—both because of lower seed densities (Davidar 1983) and, particularly in this system, because of the higher protection of seeds under needle coverage, where they have optimum recruitment conditions (Mellado and Zamora 2014). Secondly, once seedlings become established, sibling competition may be diminished (Davidar 1983). In this sense, the demographic consequences of tits would be akin to those of mistletoe specialists, if not for the scarcity of the dispersal events. Notwithstanding, this indicates that morphological adaptations do not necessarily confer advantages for mistletoe dispersal over less specialized vectors, and further highlights the great importance that unexpected dispersal agents can have on the demography of a plant (Calviño-Cancela 2002; Heleno et al. 2011; Frick et al. 2013).

An interesting finding is that birds visit parasitized as well as non-parasitized pines, behaving similarly on both. This, coupled with the large home ranges of dispersal vectors (especially thrushes) and the wide and heterogeneous range of seed dispersers, may increase the overall dispersal effectiveness of the mistletoe population, with several species moving seeds at different distances; this ensures local population functions, including reinfection processes, and the establishment of new populations or expansion of the plant's range, which might relieve host populations from intense local reinfection processes (Watson and Rawsthorne 2013). *Viscum a. austriacum*, being able to cope with massive seed losses (by producing large fruit crops) and to accept any dispersal mechanism (either endozoochorous or ectozoochorous), can achieve an effective dispersal function by an unspecialized dispersal group. In conclusion, the mutualistic interaction between the European mistletoe and zoochorous birds is a generalized system; almost all birds feeding on fruits can act as effective seed dispersers. Plant dispersal success relies on the correspondence between a large fruit crop and a diverse guild of endozoochorous and/or ectozoochorous dispersal vectors. These generalistic features enable the spatio-temporal replacement of dispersers, allowing the presence of *Viscaceae* species in geographical ranges lacking specialist dispersers, such as occur in Europe.

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Chapter 3

Spatial heterogeneity of a parasitic plant drives the seed-dispersal pattern of a zoochorous plant community in a generalist dispersal system

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Abstract

Biota plays a central role as sources of spatial heterogeneity, having great potential to define ecological processes and patterns in the landscape. Mistletoes are fleshy-fruited parasitic plants that dwell in forest canopies showing a strong aggregated spatial distribution. Parasitized trees potentially concentrate frugivore activity on their canopy, where birds find food, places to perch, and protection against predators. Thus, seed-deposition patterns generated from the canopy are expected to reflect the heterogeneity associated with the parasite. This becomes especially important in generalist dispersal systems; however, so far, we do not know the implications of mistletoe spatial heterogeneity on the seed-dispersal pattern of other plants with which they simultaneously fruit. In a Mediterranean pineland, we analyze the impact of *Viscum album* subsp. *austriacum* on the seed-deposition pattern of a zoochorous plant community, taking into consideration the spatial and temporal variability of environmental factors influencing the frugivore's habitat use, such as fruit availability and forest tree density. For four consecutive years, we studied 55 pairs of trees parasitized and unparasitized by mistletoe, analyzing zoochorous fruit availability, frugivore visits, and the zoochorous seed rain in selected trees. As expected, frugivorous birds responded to mistletoe heterogeneity by visiting parasitized trees preferentially to unparasitized ones, generating a differential deposition of mistletoe seeds on tree branches while dispersing seeds of co-fruiting species under the host canopy. Availability of understory fruits remained similar in patches of parasitized and unparasitized trees, but showed strong temporal fluctuations reflected in the seed rain.

On the other hand, mistletoe proved more copious in patches of parasitized trees and their fruit crops varied little between years, making mistletoes reliable food resources likely to lead to consistency in fruit-deposition patterns. In conclusion, mistletoes, by patchily growing on the canopy layer and concentrating zoochorous seeds underneath, can shape the spatial seed-deposition pattern of fleshy-fruited plants in the forest. Moreover, as seeds constantly reach the same deposition sites over long periods, the soil beneath the host canopy could become hotspots for community regeneration. In degraded areas, such mistletoe effects might be critical, possibly promoting recolonization and vegetation recovery through the frugivore's activity.

Key words

frugivory, mistletoe, plant-animal interactions, plant-plant interactions, spatial-temporal variability, *Viscum album.*

Introduction

Biota constitutes an important agent of spatial heterogeneity, defining ecological processes and patterns in the landscape by interacting with other organisms and by causing physical changes in the environment. Understanding its role as sources of heterogeneity and the subsequent ecological implications is very useful to gain comprehension of the spatial configuration and functioning of ecosystems (Lovett et al. 2005). In this study, we show how the spatial heterogeneity of mistletoes (i.e. hemiparasitic epiphytes) can define the seed-dispersal pattern of a zoochorous plant community by influencing seed-disperser behavior. Mistletoes are common dwellers of forest canopies that offer abundant and nutritious fleshy-fruited resources, serving as keystone species in ecosystems worldwide (Watson 2001; Watson, McGregor & Spooner 2009; Burns, Cunningham & Watson 2011; Watson & Herring 2012; Ndagurwa et al. 2014). They show an extremely patchy distribution, aggregated within very specific hosts and neighborhoods (Aukema 2004), that results in highly heterogeneous forests with clumps of abundant fruit supplies available on top of the canopy layer, having a great potential to entice frugivores and therefore to concentrate zoochorous seeds.

The patchy distribution of mistletoes arises from the disproportional seed rain that frugivores generate upon already parasitized trees, creating a positive re-infection feedback that enforces mistletoe clumping (Aukema & Martínez del Río 2002; Medel et al. 2004; Carlo & Aukema 2005). As mistletoe recruitment is limited to an extremely narrow range of safe sites—requiring seeds to attach on particular branch diameters of specific host species (Reid 1989; Norton & Ladley 1998) — their seed dispersal is frequently carried out by the activity of specialized animals that guarantee an effective dispersal service to the parasite (e.g., Reid 1989; Sargent 1995; Larson 1996; Restrepo et al. 2002). Nevertheless, many mistletoe species live where specialists are absent, their seed dispersal depending on generalist birds (e.g., Zuber 2009; Mellado & Zamora 2014a). In these systems, instead of providing directed seed dispersal, frugivores need many attempts to successfully disperse a single mistletoe seed (Mellado & Zamora 2014a). In addition, generalist frugivores feed on multiple fleshy-fruited resources and disperse seeds of different species all together, commonly under trees that serve as perches or sites to feed (Herrera 1984; Clark et al. 2004; Kwit, Levey & Greenberg 2004). In such generalist seed-dispersal systems, mistletoe spatial patchiness may have important implications on the seed-dispersal pattern of other plants with which they simultaneously fruit, as parasitized trees have the potential to concentrate frugivore activity on their canopy, which simultaneously provide food, resting sites, and protection against predators. Therefore, seed-deposition patterns generated from the canopy are expected to reflect the heterogeneity associated with the parasite. However, so far, we have no information about the implications of mistletoe patchiness on the seed-dispersal patterns of co-fruiting plants.

To address this question, we need to consider that the attractiveness of parasitized trees (and thus the placement of seed-deposition sites) may depend on many environmental factors influencing frugivore habitat use, such as the availability and distribution of alternative food resources and forest tree density (Saracco et al. 2005; Carlo 2005; Blendinger, Blake & Loiselle 2010; Herrera, Morales & García 2011). These factors, in turn, might be subject to temporal changes, being very frequent in certain ecosystems (e.g., Herrera 1998; Prasad & Sukumar 2010). On the other hand, mistletoes produce regular fruit crops through time (Larson 1996; van Ommeren & Whitham 2002), which might permit the maintenance of repeating seed-dispersal patterns over long periods, with zoochorous seeds reaching the same deposition sites time and again.

In this study, we analyze the impact of *Viscum album* subsp. *austriacum* (Wiesb.) Vollmann (*V. a. austriacum*, hereafter) patchiness on the seed-deposition pattern of a zoochorous plant community, taking into consideration the spatial and temporal variability of forest tree density and fruit availability of mistletoe and co-fruiting plants. The study was conducted in a Mediterranean pineland of southeastern Spain, where *V. a. austriacum* coexists with a diverse community of zoochorous understory plants, sharing a broad assemblage of generalist seed dispersers. In this pineland, parasitized trees, constituting the only (or most abundant) nutritive resource offered on the canopy layer, might be particularly noticeable for frugivorous birds. Under this scenario, we expect frugivorous birds to respond to mistletoe patchiness by visiting parasitized trees preferentially to unparasitized ones, driving a differential deposition of mistletoe seeds towards parasitized trees (Figure 1). At the same time, while staying on the host canopy, frugivores concurrently disperse seeds of other zoochorous plants, giving rise to multispecies seed-deposition sites underneath parasitized trees (Fig. 1b). Finally, we expect the seed rain to reflect the temporal variability of the environment.

We studied 55 pairs of mistletoe parasitized and unparasitized trees for four consecutive years (2009-2012), analyzing fruit availability of mistletoe and co-fruiting species in focal trees and nearby neighborhoods, frugivore visits and the zoochorous seed rain generated in selected trees, in an effort to answer three main questions: (1) Does mistletoe presence on top of pine canopies, through its influence on the activity of frugivores, shape the spatial pattern of zoochorous seeds in the forest? (2) What environmental factors are behind the patterning of seed-deposition? (3) What is the temporal consistency of mistletoe and understory fruit crops and seed rain at deposition sites?

Material & methods

Study site and experimental design

The study was conducted in a Mediterranean pine forest located in the Natural Park of Sierra de Baza (southeastern Spain; 2º 51' W, 37º 22' N). This is a jagged mountain range, mainly calcareous, with minimum altitudes of 1200 m and maximum of 2269 m. The site shows the typical Mediterranean climate, characterized by cold winters and hot summers with pronounced summer drought (June-August), while precipitation concentrates in spring and autumn. Sierra de Baza contains a complex mosaic of plant formations. Dominant tree vegetation is pine forest, mainly Austrian (*Pinus nigra* Arn.) and Scots (*Pinus sylvestris* L.), but also Aleppo (*Pinus halepensis* Mill.) and Maritime (*Pinus pinaster* Ait.), coexisting with oaks (*Quercus ilex* L.) and maples (*Acer opalus* L. ssp. *granatense* Boiss). The landscape reflects the impact of past human activities, having stands of native and naturalized pinelands intermingled throughout the mountain range. A diverse and abundant

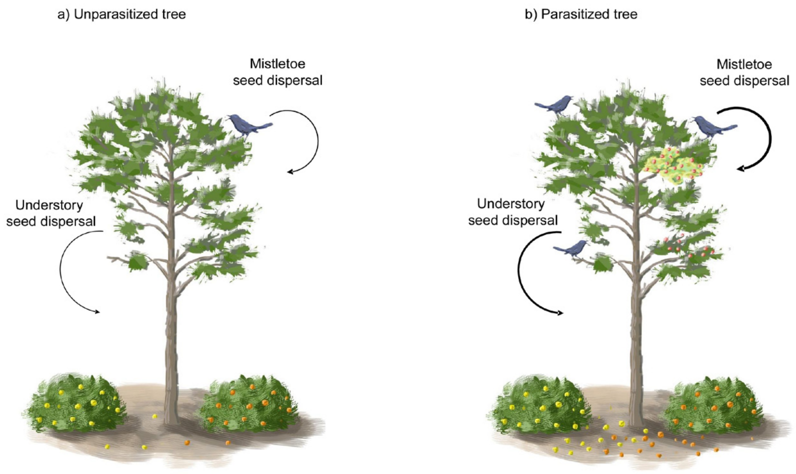

Figure 1. Hypothesis: In a pine forest, frugivores commonly perch on pine's canopy (a) looking for sites to watch or protect against predators. During this time, they can disperse sporadic quantities of zoochorous seeds. Otherwise, when mistletoes are concentrated in the canopy (b), parasitized pines become highly attractive because they simultaneously offer food, sites to perch, and protection. As a result, frugivores visit parasitized trees preferentially to unparasitized ones, driving a differential deposition of mistletoe seeds towards the canopy of parasitized trees, while carrying along seeds of co-fruiting species, giving rise to multispecies contagious seed-dispersal patterns underneath the canopy of parasitized trees.

ensemble of zoochorous fleshy-fruited shrubs accompanies pines, including *Berberis hispanica subsp. Hispanica* Boiss. & Reut., *Crataegus monogyna* Jacq, *Lonicera arborea* Boiss., *Juniperus oxycedrus* L., *J. communis* L., *Prunus ramburii* Boiss and *Rosa* spp. Part of the zoochorous plant community is the mistletoe *Viscum album austriacum*, a hemiparasitic, dioecious epiphyte that absorbs water and mineral salts from the xylem of its host trees. This species is widely distributed across Europe parasitizing conifers, *Pinus nigra* and *P. sylvestris* being the most common host species in southern Spain and at the study site (Mellado & Zamora 2014b).

The most usual species of avian seed dispersers in Sierra de Baza include non-migrants and seasonal migrants such as *Sylvia* spp., *Turdus* spp., and *Erithacus rubecula,* which feed on various fruit species during autumn-winter. Thrushes are the main seed dispersers of *V. a. austriacum* (Zuber 2009; Mellado & Zamora 2014a), as well as legitimate dispersers of other zoochorous species of the plant community (Herrera 1984); they swallow entire fruits and defecate or regurgitate intact seeds. Small passerines, such as Robin *Erithacus rubecula* and Blackcap *Sylvia atricapilla* also contribute to the dispersal of both ground and canopy-dwelling fleshy-fruited plants.

We selected 110 *Pinus nigra* (hereafter focal trees), half parasitized by mistletoe and half unparasitized, except for the first study year (2009) in which 80 *P. nigra* were used. A parasitized tree was chosen if it hosted at least one fruit-producing mistletoe. The experiment covered a large range of heterogeneity of terrain by choosing focal trees at 3 altitudes (1300, 1650 and 1850 m) within stands of different tree densities. Trees were randomly selected and spatially paired (one parasitized and one unparasitized). Paired trees were of similar architecture, size (trunk perimeter = 97.5 ± 4.01 cm) and height (6.87 ± 0.23 m), and were located in similar environmental contexts (staying 40-80 m apart). Sites were visited for four years from 2009-2012.

Mistletoe and understory species fruit availability

In all focal pines, we established circular plots of 20 m radius. In these plots, we measured fruit abundance (berries, drupes, and functionally analogous structures such as juniper berries) of every fleshy-fruited species, fruit-species richness and tree density (trees / Ha). Abundance of mistletoe fruit was quantified for all parasitized trees included within the plot, considering all mature female mistletoes as a whole. We visually estimated ripe fruit crops, using binoculars when required. To estimate fruit abundance of understory species, each circular plot was divided into four equal subplots (90º each), covering the whole sampling area. At the beginning of the dispersal season (October), in each subplot we visually counted the quantity of ripen fruits of all fruiting plants that had, at least, 30% of their canopy surface within the subplot. In the study locality, fruiting is quite synchronous among individuals and species, where early- or late-ripening species are rare and most of the ripening period is delimited within 1-2 months (although fruits remain attached to trees for 1-3 additional months). We thus considered that a single sampling to quantify fruit abundance at the beginning of the season would provide an appropriate estimate of the spatial template of fruit resources available for frugivores. For further analyses we differentiated (i) mistletoe fruits available within plots (sum of ripe mistletoe fruits produced in the focal pine and all parasitized pines included in the plot, hereafter 'mistletoe fruits') and (ii) fruits of other zoochorous species (sum of ripe fruits produced by all understory fleshy-fruited species in the plot, hereafter 'understory fruits'). Fruit richness was estimated as the sum of the available fruit species counted within the plot.

Frugivorous birds

We made direct observations of frugivore birds visiting parasitized and unparasitized focal pines. Each census was performed between 7:00 to 12:00 a.m., consisting in 5 min of observation per focal pine during different days throughout the dispersal season (from the end of September to the end February for the four study years). During each observation, a trained ornithologist (R. Zamora) covered the area in which focal trees were located, making stops at 20-40 m to get a full image of the whole canopy. Birds were identified to the species level. When the observation period ended, the process was repeated for all other focal trees. At the end of each season, we amassed 12 to 14 observations per focal pine, for a total of 70 minutes of observation time per tree per year, resulting in 478 h of observation time for the whole study (80 focal trees during 2009 and 110 trees for the following three years). We calculated frugivore-bird abundance per focal pine as the cumulative number of birds watched through the season divided by the total observation time.

Mistletoe and understory species zoochorous seed rain

We quantified the seed rain of all zoochorous plant species generated from the canopy of all focal pines. Seed traps were used during the complete dispersal season, from October to February, during the four study years. Seed traps consisted of flowerpots (0.125 m^2) covered with an aluminum mesh hanging on the lower branches of the tree canopy, to prevent seed predation by rodents and granivorous birds, while avoiding the intensive wild-boar uprooting that frequently occurs under these trees. Three seed traps hung on three randomly assigned branches of the lower third of the tree height (c. 2 m above the ground level). At the end of February, samples were transferred to the laboratory where seeds of all fleshy-fruited plant species were identified and counted, and seed-species richness calculated. Quantifying the seed rain by this approach directly estimates the seed dispersal of understory species, but not that of mistletoes, as seeds need to stick on tree branches. For this reason, we estimated the relationship between mistletoe seed rain and mistletoe seeds stuck on branches of 20 parasitized focal trees. For statistical analyses, abundance of mistletoe seeds were counted separately (hereafter, ‘mistletoe seeds’), whereas seed abundance of ground-dwelling plant species were all pooled (hereafter, ‘understory seeds’).

Statistical analyses

We performed separate statistical analyses for each year, since the sample size differed; for 2009, 40 pairs of *P. nigra* were used, while for the following three years it was increased to 55 pairs. Moreover, during the study period, three parasitized trees died and had to be replaced by others of similar characteristics. Therefore, for 4 years, we compared between parasitized and unparasitized focal pines: (i) total abundance of mistletoe and understory fruits available within the plot (ii) fruit richness, (iii) frugivore visitations to focal pines, and (iv) the mistletoe and understory seed rain generated on focal pines. We used GLMM (generalized linear mixed models) with Poisson error distribution and log-link function for fruit and frugivore abundance, negative binomial for seed abundance and LMM (linear mixed models) for fruit and seed richness, including tree condition (parasitized or not) as a fixed factor and paired trees as a random factor.

We analyzed the effect of focal-tree condition (parasitized or not) and neighborhood traits (i.e. mistletoe and understory fruit abundance, fruit richness, and tree density) on frugivore visitations, and on the abundance of mistletoe and understory seed rain on focal trees. Models were simplified using a backward stepwise selection analysis, starting with a maximal model that included all predictors and interactions, from which the most parsimonious combination of explanatory variables was identified following a hypothesis-testing approach with the 'drop1' command, which drops one explanatory variable in turn and each time applies an analysis of deviance test (Zuur et al. 2009). Following an inspection of model residuals and considering dispersion, we chose the most appropriate family of GLMM for each case. For frugivore counts we used Poisson error distribution and the log-link, while for mistletoe and understory species seed abundance, we applied negative binomial distribution and the log-link because the equidispersion assumption of the Poisson model was not fulfilled (Zuur et al. 2009). Explanatory variables were standardized (by subtracting the mean and dividing by one standard deviation) for better comparisons.

To analyze temporal variability of mistletoe and understory fruit production and seed rain at deposition sites, we restricted the data to the 40 parasitized *Pinus nigra* trees selected from the beginning of the study (i.e. excluding unparasitized trees and those parasitized that were latter added). Analyses were made with GLMMs using a log-link function and Poisson error distribution for fruits and a negative binomial distribution for seeds, followed by Tukey's pairwise comparisons with 95% confidence level. Replicates were included as random factors in the model to account for temporal pseudoreplication while the year was included as a single fixed term.

Analyses were carried out using the open source software Statistical R 2.15.1 (R Development Core Team 2012). GLMMs were run using lmer and glmer functions of the package lme4 (Bates, Maechler & Dai 2008) whereas Negative binomial GLMMs with the glmmadmb function of the "glmmADMD" package (Skaug et al. 2008). Tukey's pairwise comparisons with 95% confidence level were conducted with the "multcomp" package (Hothorn, Bretz & Westfall 2008). Results are presented as mean ± one standard error, unless otherwise specified.

Results

We recorded 10 plant species with fleshy fruits ripening simultaneously to *V. album*, which made up the diet of frugivorous birds at the study site. These were: *Juniperus communis*, *J. oxycedrus*, *J. sabina*, *Lonicera arborea*, *Crataegus monogyna*, *Berberis vulgaris*, *Sorbus aria, Prunus ramburii*, *Hedera helix*, and *Rosa* spp. Non-fleshy-fruited trees, which served as perches for frugivores, were also abundant in the vicinity; mainly *Pinus nigra*, *P. sylvestris*, *P. halepensis*, *P. pinaster*, *Quercus ilex,* and *Acer granatensis.* The density of trees surrounding focal parasitized and unparasitized trees were quite similar (*d.f.* = 1, $F_{1, 53} = 10.3$; *p-value* = 0.38), with ranges of 7.95 to 310.35 trees / ha (mean ± S.D., 102.29 ± 9.47 trees / ha) for unparasitized trees and ranges of 9.014 to 262.60 trees / ha (mean ± S.D., 80.30 ± 9.01 trees / ha) for those parasitized.

In 478 h of observation, we recorded 285 individuals of 8 frugivorous bird species visiting focal trees and consuming fruits of *V. album* and fleshy-fruited species of the understory. Thrushes were the most abundant frugivores, chiefly *Turdus viscivorus* (75.80% of the visits), followed by *T. torquatus* (10.50%), *T. philomelos* (7.70%), *T. iliacus* (1.80%), *T. merula* (1.40%) and *T. pilaris* (0.40%). Other species, *Sylvia atricapilla* (0.18% of the visits) and *Erithacus rubecula* (0.07%), were less frequently recorded.

Fruit availability, frugivore visits, and seed-dispersal spatial patterns

The fruit of understory species were similarly abundant around parasitized and unparasitized trees for all the study years (Fig. 2a) (GLMMs *d.f.* = 1, 2009: χ^2, 0.02; *p-value* = 0.91, 2010: χ^2, 0.14; *p-value* = 0.708, 2011: χ^2, 0.22; *p-value* =0.641, 2012: χ^2, 0.07; *p-value* = 0.792). On the contrary, mistletoe fruit abundance proved more copious in patches of parasitized trees, with significant statistical differences when compared to unparasitized ones (Fig. 2b) (GLMMs *d.f.*=1, 2009: χ^2, 2052; *p-value* <0.0001, 2010: χ^2, 4182; *p-value* <0.0001, 2011: χ^2,6447; *p-value* <0.0001, 2012: χ^2, 3991 ; *p-value* <0.0001). Fruit richness was greater in plots of parasitized trees (Fig. 2c) (LMMs *d.f.*=1, 2009: χ^2, 8.56; *p-value* = 0.003, 2010: χ^2, 8.39; *p-value* =0.004, 2011: χ^2,27.27; *p-value* <0.0001, 2012: χ^2, 0.52 ; *p-value* = 0.47). This was later reflected in a richer seed rain (Fig. 2c) (LMMs *d.f.* = 1, 2009: χ^2, 101.29; *p-value* <0.0001, 2010: χ^2, 95.14; *p-value* <0.0001, 2011: χ^2, 78.04; *p-value* <0.0001, 2012: χ^2, 74.63; *p-value* <0.0001). Frugivore visits were more abundant during the first two study years, and pines holding mistletoes were preferably visited (Fig. 2d) (GLMMs *d.f.* =1, 2009: χ^2, 31.64; *p-value* <0.0001, 2010: χ^2, 35.88; *p-value* <0.0001, 2011: χ^2, 21.87; *p-value* <0.0001, 2012: χ^2, 12.16; *p-value* =0.0005). Similarly, the zoochorous seed rain was substantially more abundant upon the canopy of parasitized pines, both mistletoe (Fig. 2b) (negative binomial GLMMs *d.f.*=1, 2009: χ^2, 77.24; *p-value* <0.0001, 2010: χ^2, 71.509; *p-value* <0.0001, 2011: χ^2,89.26; *p-value* <0.0001, 2012: χ^2, 99.68 ; *p-value* <0.0001), and understory seeds (Fig. 2a) (negative binomial GLMMs *d.f.*=1, 2009: χ^2, 0.42; *p-value = 0.53*, 2010: χ^2, 24.09; *p-value* <0.0001, 2011: χ^2,12.25; *p-value* = 0.0004, 2012: χ^2, 22.25 ; *p-value* <0.0001). Finally, mistletoe seed rain and seeds attached to pine branches showed a significantly positive relationship (R^2 = 0.32, *p-value* = 0.009, n=20), indicating that mistletoe seeds gathered with seed collectors can be a good estimate for mistletoe seed dispersal.

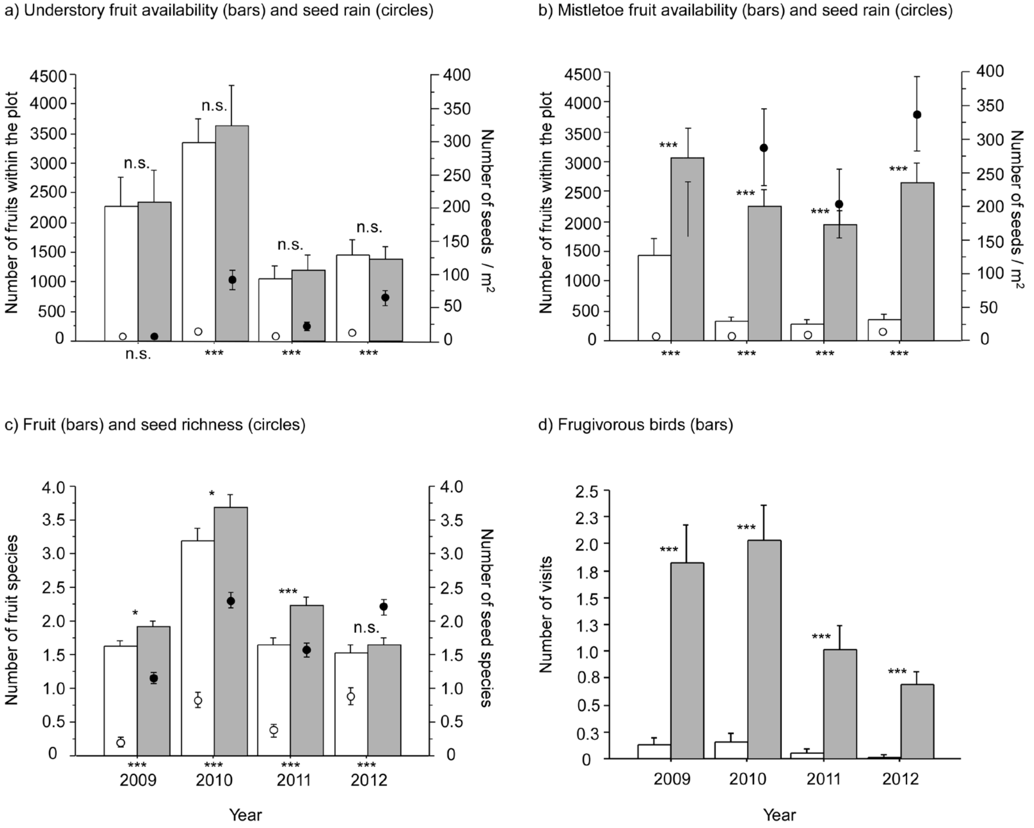

Figure 2. Spatial pattern of fruit availability, seed rain, and frugivore visits. Comparison between unparasitized (white) and parasitized (gray) trees in (a) understory fruit availability within the plot (r = 20 m) and the seed rain generated on focal trees; (b) mistletoe fruit availability within the plot and the seed rain generated on focal trees; (c) fruit richness within the plot and seed richness reaching focal trees, and (d) number of frugivore visits in focal trees. Generalized linear mixed models with Poisson error distribution were used for frugivore and fruit counts, negative binomial error distribution for seed counts, and linear mixed models for fruit and seed richness, with tree condition (parasitized or not) as the fixed factor and paired trees as the random one. Statistical differences between parasitized and unparasitized trees are indicated as non-significant (n.s.), p < 0.01 (*), p < 0.001 (**), p < 0.0001 (***). Bars represent fruits or frugivores (statistical significance indicated above bars), while circles represent seeds (statistical significance indicated below bars). Results correspond to mean ± 1 S.E, N=40 tree pairs for the year 2009 and N=55 tree pairs for the following years.

Environmental factors influencing seed-dispersal patterns

The number of frugivore visits strongly responded to the condition (parasitized or not) of individual hosts, showing weaker relationships with traits of the neighborhood (i.e. mistletoe and understory fruit abundance, fruit richness, and tree density) (Table 1a). Nevertheless, fruit abundance of mistletoe and understory species was in all cases positively correlated with frugivore visits (Table 1a). Otherwise, high tree densities, when significant, showed negative relationships with frugivores (Table 1a) and dispersed seeds (Table 1a and b). With regard to seeds, for both mistletoe and understory seed rain (Table 1b and c), the chief influence was the focal pine to be parasitized, while fruiting neighborhoods exerted different effects on the seed rain in different years. Overall, during years of high mistletoe and understory fruiting peaks (i.e. 2009 and 2010), fruiting neighborhoods positively influenced mistletoe and understory-species seed rain. An opposite effect was found for years of lower fruit abundances (i.e. 2011 and 2012).

Temporal variability of fruit availability and seed-dispersal patterns

Understory species showed great fruit-crop fluctuations over the study years (Figure 3b). The mean number of understory fruits produced in 2009 and 2010 was substantially more numerous than those produced in 2011 and 2012, varying significantly between years (Fig. 3b; GLMM, *d.f.*, 3; χ^2, 31.01; *p - value* < 0.0001). By contrast, milder temporal fluctuations were found in average mistletoe fruit production (Fig. 3a; GLMM, *d.f.*, 3; χ^2, 18.76; *p - value*, 0.002). Seed rain generated on focal pines varied accordingly to their respective fruit availability (Fig. 3; negative binomial GLMM: understory, *d.f.*, 3; χ^2, 97.19; *p – value* < 0.0001 and mistletoe, *d.f.*, 3; χ^2, 19.04, 7.68; *p - value*, 0.004).

Discussion

Our study highlights the important role mistletoe plays as sources of spatial heterogeneity in the forest. In this pineland, where generalist birds disperse seeds of mistletoe and co-occurring fleshy-fruited plant species, zoochorous seed-deposition patterns clearly reflect the spatial heterogeneity of the parasite.

Fruit availability, frugivore visits, and seed-dispersal spatial patterns

In the study site, parasitized pines constitute outstanding sites for frugivorous birds when compared to unparasitized ones. First, this is because mistletoes transform a conifer into a fleshy-fruited tree, which directly offers abundant fruit

Table 1. Results of generalized linear mixed-model effects for frugivore visitations (with Poisson error, Z-test) and mistletoe and understory seed rain (with negative binomial error, t-test) generated on focal trees as a function of focal-tree condition (parasitized or not) and features the context (mistletoe and understory fruit abundance, fruit richness, and forest tree density).

	Year	Variable	Estimate	SE	Z-t value	P value
a) Number of Frugivore visits	2009	Constant	-2.395	0.483	-4.962	**<0.0001**
		Parasitized-tree	2.603	0.481	5.417	**<0.0001**
		Tree density	-0.4947	0.168	-2.946	**0.00322**
	2010	Constant	-2.349	0.456	-5.152	**<0.0001**
		Parasitized-tree	2.493	0.448	5.564	**<0.0001**
		Mistletoe neighb.	0.253	0.113	0.133	**0.057**
		Understory neighb.	0.424	0.424	0.135	**0.002**
	2011	Constant	-3.289	0.631	-5.217	**<0.0001**
		Parasitized-tree	2.740	0.637	4.306	**<0.0001**
	2012	Constant	-4.074	1.035	-3.934	**<0.0001**
		Parasitized-tree	3.585	1.048	3.421	**<0.0001**
b) Anundance of mistletoe seeds	2009	Constant	0.411	1.098	0.370	0.709
		Parasitized-tree	6.915	0.817	8.460	**<0.0001**
		Understory neighb.	0.821	0.273	3.010	**0.003**
		Tree density	-0.642	0.232	-2.760	**0.006**
		Fruit richness	-1.110	0.518	-2.140	**0.032**
	2010	Constant	0.695	0.437	1.590	0.111
		Parasitized-tree	4.715	0.492	9.580	**<0.0001**
		Mistletoe neighb.	0.679	0.239	2.850	**0.004**
	2011	Constant	-1.031	0.548	-1.880	0.060
		Parasitized-tree	5.814	0.557	10.440	**<0.0001**
		Mistletoe neighb.	0.521	0.237	2.200	**0.028**
		Understory neighb.	-0.601	0.290	-2.080	**0.038**
		Tree density	-0.639	0.283	-2.250	**0.024**
	2012	Constant	0.174	0.515	0.340	0.740
		Parasitized-tree	5.708	0.569	10.040	**<0.0001**
c) Abundance of understory seeds	2009	Constant	-1.800	1.920	-0.940	0.350
		Parasitized-tree	-0.740	1.310	-0.570	0.570
		Fruit richness	1.880	1.200	1.570	0.120
	2010	Constant	1.997	0.333	6.000	**<0.0001**
		Parasitized-tree	2.274	0.408	5.570	**<0.0001**
		Understory neighb.	0.659	0.239	2.760	**0.006**
		Fruit richness	0.502	0.244	2.050	**0.040**
	2011	Constant	1.186	0.411	2.890	**0.004**
		Parasitized-tree	1.362	0.631	2.160	**0.031**
		Mistletoe neighb.	-0.275	0.282	-0.980	0.329
		Fruit richness	0.694	0.332	2.090	**0.037**
	2012	Constant	2.315	0.231	10.020	**<0.0001**
		Parasitized-tree	1.854	0.332	5.580	**<0.0001**
		Mistletoe neighb.	-0.452	0.178	-2.540	**0.011**
		Fruit richness	0.381	0.164	2.32	**0.02**

Note: In the model analyses, tree condition (parasitized or not), neighboring mistletoe fruit abundance (Mistletoe neighb.), neighboring understory fruit abundance (Understory neighb.), fruit richness, and tree density are considered as fixed factors, while tree pairs (see methods) are considered as the random factor. Models shown are the optimal models found following a hypothesis-testing approach (Zuur et al. 2009). N=40 tree pairs for the year 2009 and N=55 tree pairs for the following years.

rewards on top of the canopy layer where frugivores can feed at the time they perch. Second, because their nearest environment provides greater fruit supplies, as mistletoe fruit abundance proved more copious in patches of parasitized pines (Fig 2b). This reflects the typical aggregated disposition of the parasite at the scale of neighborhoods (Aukema 2004) and increases overall fruit availability within the patch. On the contrary, understory fruit availability and forest tree density remain spatially constant for all study years, being quite similar among patches of parasitized and unparasitized focal pines (Fig 2a). Abundant mistletoe fruit coupled with greater fruit richness (Fig. 2c), enhances the attractiveness of the surrounding environment of parasitized pines, where frugivores are likely to optimize their foraging (Carlo & Morales 2008; Morales et al. 2012).

Thrushes were major fruit consumers for all study years, the Mistle Thrush (*Turdus viscivorus*) being the most frequent species. These territorial birds frequently use parasitized trees as watchtowers to defend patches of abundant fruit densities from conspecifics or other fruit consumers (Snow & Snow 1984; Skórka & Wójcik 2005). By visiting parasitized trees more preferably (Fig. 2d), frugivores convert these trees into important sites for seed deposition in the forest landscape. Here great amounts of mistletoe seeds are deposited on host branches (starting new re-infection loops), while abundant and rich amounts of seeds of understory species reach the soil underneath the canopy (Figs 2a, b and c).

Environmental factors influencing seed-dispersal patterns

As expected, forest structure and the availability of other fruit resources influenced frugivore behavior and the seed rain generated on parasitized trees. In some habitats,

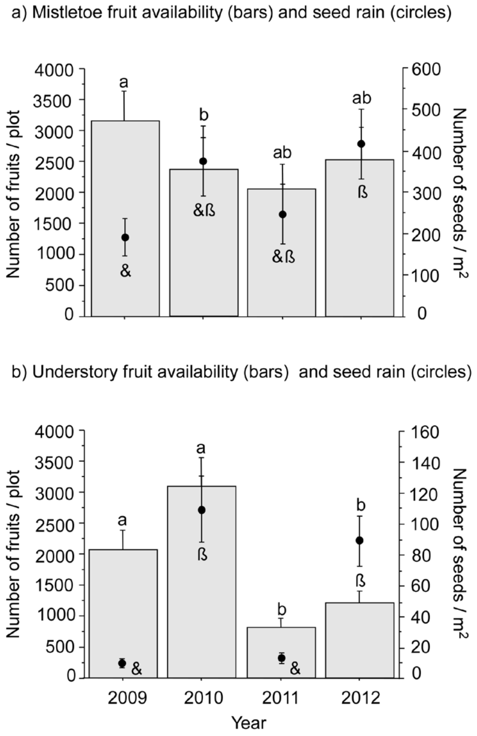

Figure 3. Temporal variation of mistletoe and understory fruit production and seed rain. Mistletoe fruit production and mistletoe seeds reaching parasitized focal pines remain relatively constant from one year to the next (Fruits: *d.f.*, 3; χ^2, 18.76; *p - value*, 0.002, Seeds: *d.f.*, 3; χ^2, 19.04; *p - value*, 0.004). On the other hand, understory fruit production sharply fluctuates between years (GLMM, *d.f.*, 3; χ^2, 31.01; *p - value* < 0.0001), as reflected in the seed rain (*d.f.*, 3; χ^2, 97.19; *p – value* < 0.0001). GLMMs with log-link function and Poisson error distribution were used for fruits and negative binomial distribution for seeds, followed by Tukey's pairwise comparisons with 95% confidence level. Replicates were included as random factors in the model to account for temporal pseudorreplication while the year was included as single fixed term. Results correspond to mean ± 1 S.E, N=40 for the subset of parasitized trees monitored from the beginning of the study. Different Latin letters (a, b) denote significant differences ($p < 0.05$) among fruit abundance of different years, while different symbols (&, β) refer to such differences for seed abundance of different years.

especially in those with spatially segregated food resources, frugivorous birds need to optimize their habitat use by compromising the search for fruits or the protection under forest coverage (Howe 1979; Fedriani & Boulay 2006). In our study, however, for the four study years and regardless of other environmental factors, mistletoe presence was the most influential variable over the number of frugivores visiting focal pines (Table 1), perhaps because frugivores find safe sites to feed in parasitized trees as fruits are concentrated in the canopy. With less intensity and only for some years, mistletoe and understory fruit abundance of the neighborhood boosted frugivore activity, whereas forest tree density exerted a negative influence (Table 1); this reflects Thrushes preferences for freestanding trees (Snow & Snow 1988) and patches of abundant fruit resources (Skórka &

Wójcik 2005). Yearly differences in the effects of these environmental factors could be related to the strong temporal fluctuations in fruit availability (Fig. 3b), which changes the environmental context each year.

Due to the wide generalism of frugivorous birds and zoochorous plants (with many plant species sharing seed dispersers), plant-plant interactions mediated by frugivores are likely to emerge (Bascompte et al. 2003). In previous studies, facilitative interactions between mistletoes and their fleshy-fruited hosts have been shown, as additional fruit resources of the parasite improve rates of host's seed dispersal (Van Ommeren & Whitham 2002; Carlo & Aukema 2005; Candia, Medel & Fontúrbel 2014). Here, we show that mistletoe-plant interactions, when considering the entire community of zoochorous plants, strongly vary from one year to the next, giving rise to a variety of scenarios in which fruiting plants facilitate or hinder the seed dispersal of other zoochorous species. In years of bountiful understory fruit supplies (2009 and 2010), more frugivores visited parasitized pines (Fig. 2) and facilitative interactions (i.e. increased seed dispersal) emerged between mistletoes and understory species (Table 1b and c). Otherwise, when food resources were more scarce (2011 and 2012), zoochorous plants competed for the limited availability of seed dispersers that visited a patch at a given time, with fruiting plants negatively influencing the seed dispersal of other species (Table 1b and c). Nevertheless, regardless of the year, the most influential variable over the seed rain generated on a tree was the fact of being parasitized by mistletoe (Table 1b and c).

Temporal consistency of fruit availability and seed-dispersal patterns

The attractiveness of parasitized trees remains constant through time as mistletoe's fruit-crop vary little from one year to the next, making them reliable food resources able to promote consistency in seed-deposition patterns. Such small variation in yearly fruit production is common among mistletoe species and has been recognized as an important phenomenon for the maintenance of frugivore populations at certain sites when other fleshy-fruited species reduce their crops (Larson 1996; van Ommeren & Whitham 2002; Watson 2001). On the contrary, understory species show strong fruit-crop fluctuations between different years (Fig. 3), a common phenomenon for many of the fleshy-fruited species involved in this study, which produce large fruit-crops synchronously at particular time intervals (Herrera 1998).

Besides yearly fluctuations of understory fruits and differences in plant-animal and plant-plant interactions, our four-year dataset shows the temporal persistency of seed-deposition sites, with the canopy of parasitized trees consistently receiving large mistletoe seed rain while microsites beneath these trees receive high seed inputs of understory species. In generalist systems, mistletoes, because of their extremely narrow range of safe sites for recruitment (Reid 1989; Sargent 1995; Norton & Ladley 1998; Mellado & Zamora 2014b), and understory species because of their high seed- and seedling-mortality rates (Zamora et al. 2010; Matías, Zamora & Castro 2012), have to withstand heavy seed wastage in order to find a single successful recruitment event. Thus, abundant and repeated seed-dispersal events are necessary to ensure a small fraction of recruits on the host canopy (Mellado & Zamora 2014a) and understory recruits in soil microsites (Howe & Mariti 2004; Hampe et al. 2008). Moreover, as seeds constantly reach the same deposition site over long time periods, these sites could become hotspots for community regeneration within the landscape (Hampe et al. 2008).

Concluding remarks

In conclusion, by patchily growing on the canopy layer and concentrating zoochorous seed rain in parasitized trees, mistletoes can shape the spatial deposition pattern of zoochorous seeds in the forest, which may be reflected in the future configuration of the plant community. In degraded areas, this ability of mistletoes to concentrate zoochorous seeds might be of prime importance, as it could promote recolonization and revegetation recovery through frugivore activity (Watson 2009). In the case of pine plantations, mistletoes could allow their conversion into mixed forests, as many Mediterranean woody species have a comparatively higher probability to persist in the understory of plantations, benefitting from the mild microclimate generated by the canopy (Mendoza, Zamora & Castro 2009; Gómez-Aparicio 2009, Zamora et al. 2010). This has far-reaching ecological implications when we consider the large area covered by pine plantations in the European continent, characterized by a homogeneous spatial structure and monospecific composition. In these scenarios, mistletoes introduce diversity of zoochorous species with the seed rain and heterogeneity in their spatial distribution, breaking the prevailing species monotony of most pinelands while introducing new assembly rules within the plant community. As a final remark, it bears mentioning the geographic breadth of the system under consideration, with *Viscum album* and Mistle thrushes expanding from Northern to Southern Europe (Zuber 2009; Snow & Snow 1984), frequently accompanied by other fleshy-fruited plant species. Thus, it would not be fanciful to speculate that mistletoe-mediated seed-dispersal patterns of fleshy-fruited plant communities may be occurring at large geographical scales, driving, with each set of regional peculiarities, many aspects of the community and ecosystem dynamics. For future advancements, it would be of key interest to delve into the influence that mistletoe spatial heterogeneity exerts over more complex plant-frugivore systems (such as tropical forests), using long-term and multispecies approaches.

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Chapter 4

Temporal dynamic of parasite-mediated linkages between the forest canopy and soil processes and microbial community

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Temporal dynamic of parasite-mediated linkages between the forest canopy and soil processes and microbial community. *Submitted*.

Abstract

Parasitic plants are important drivers of community and ecosystem properties. In this study, we identify different mechanisms by which mistletoe and the parasitic and mutualistic interactions it mediates from the forest canopy can affect soil-chemical and biological properties at different temporal stages: before, during, and after parasitism. We quantified the effect of parasitism on host growth and the number of frugivorous mutualists visiting the host canopy. Then we collected, identified, and weighed the organic matter inputs underneath tree canopies and analyzed their nutrient content. Simultaneously, we analyzed soil samples under tree canopies and examined chemical properties, microbial abundance, and functional diversity of heterotrophic microbial communities. Mistletoe increased the amount, quality, and diversity of organic matter inputs beneath the host canopy, directly through its nutrient-rich litter and indirectly through the decrease of host litterfall and the increase of bird-derived debris. All these gave rise to enriched hotspots able to support greater and more functionally diverse soil microbial communities beneath parasitized hosts, the effects of which accentuated after host death. We conclude that mistletoe, enhanced by the biotic interactions it mediates, plays an important role in intensifying soil- resource availability, regulating composition, abundance, and spatial distribution of soil microbial communities.

Key words

ecosystem engineer, functional diversity, host-parasite interaction, microbial community, MicroResp, mutualistic interaction, parasitic plant, plant-soil interactions.

Introduction

There is an increasing awareness that plants and aboveground-belowground subsystems are tightly connected, and thus the need for integrated approaches to understand more clearly how terrestrial ecosystems function is being recognized (Wardle 2002). Recent studies with parasitic plants have shown their important role in regulating belowground processes by enhancing soil-nutrient availability and increasing its spatial heterogeneity (Press, 1998; Quested *et al.,* 2002, 2008; Bardgett *et al.,.,* 2006; March & Watson 2007, 2010; Ameloot *et al*., 2008; Spasojevic & Suding, 2011; Ndagurwa *et al*., 2013, 2014a, 2014b; Fisher *et al*., 2013, Muvengwi *et al*., 2015), which may in turn affect the growth of neighboring plants (Quested, 2003). Otherwise, many parasitic plants are known to be highly connected with organisms of different trophic levels, being considered keystone resources within communities (Watson, 2001; Press & Phoenix, 2005). However, we still have a limited understanding about the way in which complex interactions involving parasitic plants might change linkages between the forest canopy and the soil. Moreover, it remains unknown how such linkages between the host-parasite system and soils change through time and how they contribute to temporal ecosystem dynamics. This is especially important for long-lived plants, which substantially change their physical and biotic environment at different stages of their life and may leave behind a spatial footprint able to persist long after death (Rodríguez *et al*., 2011) due to the continuous enhancement of nutrient inputs, bearing important implications for local biogeochemical cycling (e.g., Dean *et al*., 1999; Bardgett *et al*., 2005; Stahlheber *et al*., 2015).

Mistletoes constitute good study systems to explore this question, as they are long-lived organisms that maintain multiple long-term biotic interactions. Mistletoes comprise a diverse group of aerial hemiparasitic plants that obtain water and mineral resources from host plants, considered keystone species in forest ecosystems around the world (Watson, 2001). In addition to parasitism, mistletoes establish mutualistic interactions with animal pollinators and seed dispersers, which move pollen and seeds in exchange for nutritive nectar and fleshy-fruit rewards (e.g. Aizen, 2003). Herbivorous insects and mammals consume mistletoe foliage (e.g. Umucalilar *et al*., 2007; Burns, 2009) and many other animals use the bulky structure of this plant as a refuge, sites to rest or install a nest (e.g., Cooney *et al*., 2006). Due to this variety and bounty of interactions, mistletoes can, both directly and indirectly, modulate soil organic matter and nutrient inputs underneath the canopy of parasitized hosts. Direct effects can be highly noticeable, as mistletoes often produce large biomass amounts, rich in nutrients and of fast decomposition rate, which enhances soil-nutrient cycling (March & Watson, 2010; Ndagurwa *et al*., 2014a, b; Muvengwi *et al*., 2015). Indirect effects through mistletoe parasitic and mutualistic interactions could be equally remarkable, but, although sometimes mentioned, these relationships still remain unexplored. On the one hand, the acquisition of host resources reduces host productivity and reproductive fitness (Reid *et al*., 1994; Silva & Martinez del Rio, 1996; Howell & Mathiasen, 2004), which might be reflected in lower inputs of host biomass into the soil. On the other hand, animal visitors may provide substantial nutrient sources, dropping excrements, feathers, wings, hair, or food leftovers underneath the host (Dean *et al*., 1999, Van der

Wal *et al*., 2004, Watson, 2009). After enough time, strong parasitic loads may culminate in host death, and thus in the death of the parasite. Once this occurs, the host-parasite system may expand its effects on the soil through the deposition of dead organic matter (e.g. Facelli & Facelli, 1993), through the persistence of past changes in nutrient cycling (e.g. Maron & Jefferies, 1999) or through the establishment of new interactions with animals that make new use of them, for instance, as perches for birds (e.g. McClanahan & Wolfe, 1993).

In this study, we seek to identify different mechanisms by which mistletoe, coupled to the parasitic and mutualistic interactions it mediates, jointly affect soil chemical and biological properties at different developmental stages of the host-parasite system: before, during, and after parasitism. We focus on the mistletoe *Viscum album* subsp. *austriacum* (hereafter *V. a. austriacum*), a long-living (over 35 years) and common parasite of European forests that specialized in hosts of *Pinus* spp. (Zuber, 2004; Mellado & Zamora, 2014a) and that maintains a mutualism with generalist frugivorous birds (Mellado & Zamora, 2014b). In a field study, we analyze (a) an early stage before parasitism (hereafter 'unparasitized trees'), (b) an intermediate stage during parasitism (hereafter 'parasitized trees') and (c) a late stage, after death of parasitized trees (hereafter 'dead trees'). Our principal hypothesis is that unparasitized, parasitized, and dead trees exert different influences in soil properties due to changes in biological interactions prevailing in the host canopy at each temporal stage. In the absence of the parasite, pine litter is expected to be the dominant resource reaching the soil. When the pine becomes parasitized, mistletoe may either directly or indirectly alter the overall nutrient inputs underneath the host. After host death, the remaining dead structures from the host and the parasite are expected to maintain litter accumulation. We expect differences in identity, quantity, and quality of organic compounds reaching the soil at different stages to be reflected in the soil-nutrient status and the microbial community. Particularly, we identify the main direct and indirect links between the host-parasite system and soil at different temporal stages in order to evaluate the effect of such linkages on the soil-nutrient status, as well as on the abundance and functional diversity of soil microbial communities.

Materials & Methods

Study site

The study was performed from 2010 to 2013 in a Mediterranean pine forest located in the Natural Park of Sierra de Baza (southeastern Spain; 2º 51’ W, 37º 22’ N). This jagged mountain range, mainly calcareous, has minimum altitudes of 1200 m and maximums of 2269 m. The site shows the typical Mediterranean climate, characterized by cold winters and hot summers with pronounced summer drought (June-August), while precipitation concentrates in spring and autumn. Sierra de Baza contains a complex mosaic of plant formations. The dominant tree vegetation is pine forest, mainly Austrian (*Pinus nigra* Arn.) and Scots (*Pinus sylvestris* L.), but also Aleppo (*Pinus halepensis* Mill.) and Maritime (*Pinus pinaster* Ait.), coexisting with oaks (*Quercus ilex* L.) and maples (*Acer opalus* L. ssp. *granatense* Boiss). A diverse and abundant ensemble of zoochorous fleshy-fruited shrubs accompanies pines, including *Berberis hispanica subsp. hispanica* Boiss. & Reut., *Crataegus monogyna* Jacq, *Lonicera arborea* Boiss., *Juniperus oxycedrus* L., *J.communis* L., *Prunus ramburii* Boiss, and *Rosa* spp. Part of the zoochorous plant community is the mistletoe *Viscum album austriacum* (Wiesb.) Vollmann (Viscaceae), a hemiparasitic, dioecious epiphyte widely distributed across European coniferous forests. *Pinus nigra* and *P. sylvestris* constitute the most common host species in southern Spain and at the study site (Mellado & Zamora, 2014a), where *V. a. austriacum* can live for more than 35 years (Mellado and Zamora, *pers. obs*). Most usual species of avian seed dispersers in Sierra de Baza include non-migrants and seasonal migrants such as *Sylvia*, *Turdus,* and *Erithacus,* which feed on various fruit species during autumn-winter (Herrera, 1995). Thrushes are the main seed dispersers of *V. a. austriacum* (Zuber, 2009; Mellado & Zamora, 2014b), as well as legitimate dispersers of other zoochorous species of the plant community. Small passerines, such as Robin *Erithacus rubecula* and Blackcap *Sylvia atricapilla* also contribute to the dispersal of both ground and canopy-dwelling fleshy-fruited plants.

To perform the study, we selected 125 *Pinus nigra* trees (hereafter focal trees), from which 55 were parasitized by mistletoe, 55 were unparasitized and 15 were recently dead parasitized trees—as dead individuals still retained needles and twigs, as well as thin branches of dead mistletoes that often disappear with the passage of time, tree death was estimated in about 4-6 years ago. Parasitized trees presented moderate to intense parasitic loads (mean ± SE: 68.5 ± 9.6 mistletoes/host), holding at least 1 mistletoe older than 30 years. Mistletoe age can be easily estimated based on the dichotomous growth pattern of *Viscum album* by counting the number of shoot segments of the plant. Dead trees are less numerous than the other categories because they are scarce and difficult to find at the study site. However, they are easy to identify because dead mistletoes remain on trees. The experimental site covers a large range of the mountain's heterogeneity, as focal trees were equitably distributed at 3 altitudes (1300, 1650, and 1850 m) within stands of different tree densities. Parasitized trees taken at random were spatially paired with unparasitized ones, except for dead trees that were scattered throughout the mountain due to their rarity. Paired trees were of similar architecture, size (trunk perimeter = 97.5 ± 4.01 cm) and height (6.87 ± 0.23 m), and were located in similar environmental contexts. All focal trees were 40 m-80 m apart. To address the above-mentioned questions, in all focal pines we quantified the effect of parasitism on host growth and the capacity of mistletoes to attract frugivorous mutualists to the host canopy. Then we collected, identified, and weighed the organic matter inputs underneath tree canopies and analyzed their nutrient content. Simultaneously, we analyzed soil samples under focal pines and examined chemical properties, nitrogen content in microbial biomass as a proxy of microbial abundance, and functional diversity of heterotrophic microbial communities.

Mistletoe-host interaction

We measured the effect of parasitism on host's growth by estimating annual internode growth in unparasitized and parasitized focal pines. Estimates were performed in three randomly selected branches per tree by measuring (with a tape measure) bud elongation (cm) of three consecutive years (from 2010 to 2012), this being an easy and precise procedure due to the presence of yearly bud scars (see Herrero & Zamora, 2014). We used average growth values within focal pines for statistical analyses.

Mistletoe-frugivorous bird interaction

We measured mistletoe effects on animal mutualists by making direct observations of frugivorous birds visiting parasitized and unparasitized focal pines for three consecutive years (from 2010 to 2012, data published in Mellado & Zamora, 2015). Each census consisted of 5-min observations per focal pine on different days throughout the dispersal season, from the end of September to the end February. A trained ornithologist (R.Z.) performed observations between 7:00 to 12:00, covering the area around each focal tree and identifying birds to the species level. At the end of each season, we amassed 12 to 14 observations per focal pine, for a total of 70 observation min per tree per year. We calculated frugivorous bird abundance per focal pine as the cumulative number of birds watched through the dispersal season.

Deposition of organic compounds

We collected, identified, and weighed all of the kinds of organic compounds reaching the ground beneath the canopy of each unparasitized, parasitized and dead focal pine. Litter collectors were used throughout the year for two consecutive years (2012-2013). These were flowerpots (0.125 m^2) covered with an aluminum mesh hanging on the lower branches of the tree canopy. These collectors avoided seed predation by rodents and granivorous birds, as well as the intensive wild-boar uprooting that frequently occurs under these trees. Three collectors were hung from three randomly assigned branches of the lower third of the tree height (c. 2 m above the ground level). Every 6 months, the collectors were emptied and the samples were transferred to the laboratory, where the litter was identified, separated, dried (70 ° C, for 72 h), and weighed (g) on a precision balance. We differentiated three main litter sources: (1) host-delivered compounds, i.e. needles, cones, flowers, nuts, and bark; (2) mistletoe-delivered compounds, i.e. leaves, stems, fruits, flowers, and seeds; and (3) debris of frugivorous birds, i.e. food remains, excrement, and seeds from zoochorous species (excluding mistletoe seeds, which are counted as a mistletoe-derived compound). We used average biomass per square meter values (g/m^2) within focal pines for statistical analyses.

Chemical composition of the collected organic compounds

Senescing pine needles and senescing mistletoe leaves, stems, flowers, fruits and seeds, were gathered from litter collectors of 5 parasitized focal pines at the beginning of spring 2012. The material was dried (50ºC, for 96 h), well cleaned, ground, and stored in plastic vials until analysis. Each material was analyzed for total carbon (TC) and nitrogen (TN) by combustion at 850ºC (Leco TruSpec autoanalyzer). Potassium (K^+) in the acid extract of organic compounds was determined by atomic-absorption spectrophotometry (David, 1960), while phosphorous (P) was determined by the molybdovanadate method (AOAC, 1975).

Soil chemical properties

Beneath the canopy of focal pines we analyzed the fraction of soil nutrients available for microbes and root plants in spring 2012 (beginning of April), coinciding with the moment of maximum soil biological activity. We estimated soil nutrients (nitrate [NO_3^--N], ammonium [NH_4^+-N] and phosphate [PO_4^{3-}-P]) and K^+ availability using ion-exchange membranes (IEMs; Subler *et al*., 1995; Durán *et al*., 2013) on a subset of 76 focal trees: 38 parasitized, 38 unparasitized, and 12 dead trees.

This technique takes into account the soil ion-diffusion rates, enabling the detection of nutrient accessibility to root plants and microbes over a certain time period. Resins were first expanded by submerging them in distilled water at 82–90°C for 48 h, and then they were cut into 2.5 × 2.5 cm squares and attached to a plastic rod with acrylic glue. Beneath the canopy of the selected focal trees, 3 cation and 3 anion IEMs were randomly buried in the soil at a 0.5–3 cm depth and were incubated in the field for 30 days (from April to May 2012). After removal, the IEMs were taken to the laboratory and dried at ambient temperature. They were carefully separated from the plastic rod, brushed to remove soil particles, and placed into 125-ml flasks for extraction with 25 ml of distilled water by orbital spinning (1 h at 200 rpm). The NH_4^+-N concentration was directly estimated with the indophenol blue method using a microplate reader (Sims *et al*., 1995). The NO_3^--N was first reduced to NH_4^+ with Devarda alloy, and its concentration was determined as described above. The NO_3^--N concentration in the extracts was calculated as the difference between the Devarda-incubated and unincubated samples. The potassium concentration in the extract was determined by atomic-absorption spectrophotometry (David, 1960), while PO_4^{3-}-P content was determined by the Olsen method (Watanabe & Olsen, 1965).

At the same time as the IEMs were installed, we randomly collected 3 soil cores underneath the canopy of each selected focal tree using a circular soil corer (5 cm in diameter x 10 cm in height). Samples were taken from the top 10 cm of the soil profile because most of soil nutrients in a Mediterranean ecosystem accumulate in the first few cm of the soil profile (Lugo *et al*., 1990). Soil samples were transported in polyethylene bags to the laboratory and sieved at 2 mm to remove stones, roots, and visible plant debris. Soil samples from each pine were merged in a composite sample for posterior analyses. A fraction of each composite soil sample was air-dried at ambient temperature for 7 days and stored until subsequent analyses, and another soil fraction was kept at 3°C for three days and then processed to determine N in microbial biomass (MB-N; Brookes et al.,. [1985])

as a proxy of microbial abundance and dissolved organic N (DON). To measure MB-N, 20 g of fresh soil were fumigated with chloroform for 5 days. The non-fumigated replicates were used to measure DON. The fumigated and non-fumigated samples were extracted with 100 ml of K_2SO_4 0.5 *M* by shaking for 1 h at 200 rpm at 20 ºC and filtered through a 0.45-µm Millipore filter (Jones and Willett, 2006). The extracts were first oxidized to NO_3^--N with potassium persulfate ($K_2S_2O_8$) in an autoclave at 121°C for 55 min and then reduced to NH_4^+-N with Devarda alloy (Sollins *et al*., 1999). The DON content was calculated as total dissolved N minus inorganic N in the digested extracts (Morillas *et al*., 2013) and determined by colorimetry (indophenol blue method) with a microplate reader (Sims et al.,. 1995). The MB-N concentration was estimated as the difference between the total N in fumigated and unfumigated digested extracts divided by a Kn (fraction of MB-N extracted after $CHCl_3$ treatment) of 0.54 (Brookes *et al*., 1985).

From dried soils, we determined the content of soil organic-matter (SOM) by incineration at 550ºC with a thermo-balance (Leco TGA 710, St. Joseph, MI, USA) to constant weight (Sparks, 1996). Total C (TC) and N (TN) were determined by combustion at 850ºC (Leco TruSpec autoanalyzer), while total inorganic C (IC) was measured by the acidification method with $HClO_4$ in a TIC analyzer (UIC CM-5014). Total organic C (TOC) was estimated as the difference between TC and IC. The gravimetric soil-water content was calculated in fresh 5 g subsamples after drying in an 80 ºC oven for 48 h, and the soil pH was measured in 1:5 soil-water solutions.

Functional diversity of soil microbial communities

We analyzed soil heterotrophic microbial communities with the MicroResp system (Campbell *et al*., 2003). This method is based on community-level physiological profiles (CLPP) obtained by testing of 15 carbon sources that vary in structural complexity (Oren &

Steinberger, 2008). Carbon sources were selected depending on their ecological importance to soil and their solubility in water. We used amino acids (L-alanine, L-lysine, arginine, L-cysteine HCl, and N-acetyl-glucosamine [NAGA]), carbohydrates (D-fructose, D-galactose, D-glucose, L-arabinose, and D-trehalose) and carboxylic acids (citric acid, L-malic acid, oxalic acid, oxoglutaric acid, and amino butyric acid [GABA]). In functional terms, the substrate utilization rates of the carbon sources correspond to the catabolic attributes of different soil microbial functional groups (Zak *et al.*, 1994). Even if we cannot evaluate microbial communities in relation to taxonomic or phylogenetic diversity (Øvreås, 2000), we can still use MicroResp results to explain functional diversity shifts. Before performing the MicroResp method, air-dried soils were placed in flasks and pre-incubated for five days at 25ºC. The moisture within the flasks was corrected to 40% water-holding capacity in order to condition the soils and reestablish active microbial populations. To avoid changes in soil-moisture content during incubation, we covered the flasks with parafilm. Each carbon source was dissolved in deionized water and added to soils to deliver 30 mg C g soil water^{-1}. Approximately 0.4 g of soil was placed volumetrically in the 96 deep-well plates. To estimate the evolved CO_2, a colorimetric method was used relying on the change in the pH of a gel-based solution of bicarbonate. Afterwards, the plates were incubated for 6 h and read at 570 nm. The results were calculated on the basis of water, which represents the basal respiration.

Statistical analyses

The effect of parasitism (parasitized or not) on tree growth was analyzed with linear mixed models (LMM), while the effect on frugivorous counts was analyzed using generalized linear mixed models (GLMMs) with Poisson error distribution and the log-link. To compare biomass inputs, we applied negative binomial distribution and the log-link because the equidispersion assumption of the Poisson model was not achieved (Zuur *et al.*, 2009). The nutrient content of pine and mistletoe litter were compared with analysis of variance models (ANOVA). Soil-chemical properties (total organic C [TOC], inorganic C [IC], total N [TN], DON, pH, moisture, IEMs-NH_4^+-N, IEMs-NO_3^--N, IEMs-PO_4^{3-}-P, and IEMs-K^+), as well as N in microbial biomass (MBN) and microbial functional diversity, were analyzed using LMMs, with variables square-root or log-transformed when required in order to meet assumptions of normality and homoscedasticity. For all analyses, we included the focal-tree condition (unparasitized/parasitized/dead) as a fixed factor and, for LMM and GLMM, paired trees as a random factor. We calculated the Shannon-Weaver Diversity Index (H') to assess microbial functional diversity using the soil-respiration response to the different C sources as (Shannon & Weaver, 1963):

$$H' = -\sum_{i=1}^{s} pi.\ln pi$$

where pi is the ratio of the activity of a particular C substrate to the sum of activities of all C substrates (Zak *et al*., 1994).We performed non-metric multidimensional scaling (nMDS) to explore differences in tree condition among hosts using soil microbial functional diversity. Significant differences were evaluated by using permutational multivariate analysis of variance with the PERMANOVA t-statistic. PERMANOVA analyses were performed using 9999 permutations and the Euclidean distance with Primer 6 and Permanova+ (PRIMER-E Ltd, Plymouth, UK). The rest of the analyses were carried out using the open source software Statistical R 2.15.1 (R Development Core Team 2012). GLMMs were run using lmer and glmer functions of the package lme4 (Bates *et al*., 2008), whereas negative binomial GLMMs with the glmmadmb function of the "glmmADMD" package (Skaug *et al*., 2008). Results are presented as the mean ± one standard error, unless otherwise specified.

Results

For all years, unparasitized trees grew more than did parasitized ones (LMM, 2010: d.f. = 1; $\chi^2 = 30.54$; $P < 0.0001$; 2011: d.f.= 1; $\chi^2 = 17.80$; $P < 0.000$; 2012: d.f. = 1; $\chi^2 = 19.23$; $P < 0.0001$), while parasitized trees received significantly more visits of frugivorous birds (GLMM, 2010: χ^2

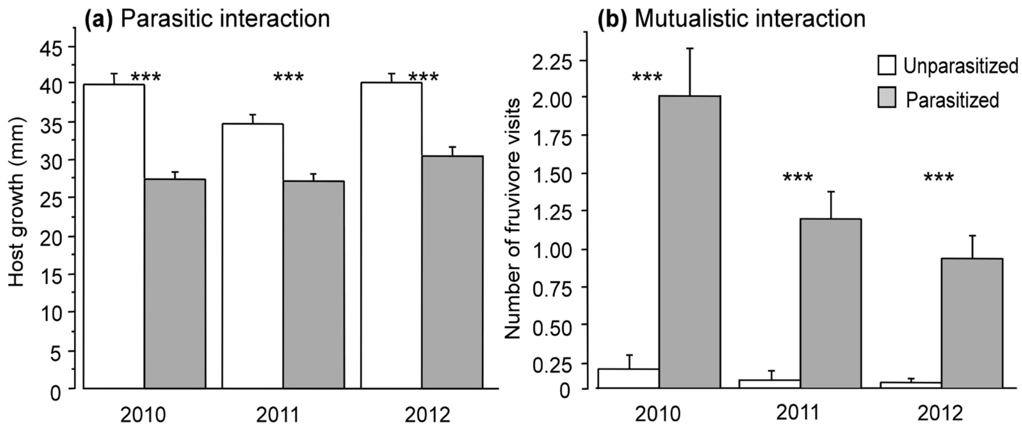

Figure 1. Tree growth (a) and number of frugivore visits (b) in parasitized and unparasitized *Pinus nigra* trees. Annual internode growth of focal trees was estimated by measuring bud elongations (cm) for three consecutive years (2010-2012) in three randomly selected branches per tree. The number of frugivore visits was estimated by direct observations in all focal trees during the dispersal season for three consecutive years (2010- 2012). We used average growth values within focal pines and the cumulative number of birds watched through the season for statistical analyses. Linear mixed models were used to compare tree growth, while generalized linear mixed models with Poisson error distribution and log-link were used to compare frugivorous bird counts. Statistical differences are indicated as $p < 0.0001$ (***). Results correspond to mean ± 1 S.E, n=75 unparasitized trees and n=75 parasitized trees.

= 35.88; d.f.= 1; $P < 0.0001$; 2011: $\chi^2 = 21.87$, d.f. = 1; $P < 0.0001$; 2012: $\chi^2 = 12.29$, d.f. = 1; $P = 0.0005$) (Fig. 1). In general, the largest biomass accumulated for the two sampling years was quantified beneath parasitized trees (309.85 g/m^2), followed by unparasitized (269.07 g/m^2) and dead ones (53.71 g/ m^2). The abundance of different organic matter sources (from the host, the parasite, and the activity of frugivorous birds) differed between unparasitized, parasitized, and dead trees: (i) host needles (χ^2= 302.32; *d.f.*= 2; $P < 0.0001$) and other structures—including cones, bark, and pine flowers ($\chi^2 = 14.91$; *d.f.* = 2; $P = 0.0006$); (ii) mistletoe leaves and stems ($\chi^2 = 220.68$; *d.f.* = 2; $P < 0.0001$) , seeds ($\chi^2 = 117.24$; *d.f.* = 2; $P < 0.0001$), fruits, and flowers (only present under parasitized trees); (iii) seeds of co-fruiting zoochorous plant species ($\chi^2 = 57.72$; *d.f.* = 2; *P*

< 0.0001) and bird excrement (χ^2 = 85.172; *d.f.* = 2; $P < 0.0001$) (Fig. 2). As expected, the soil beneath the canopy of parasitized trees received greater quantities of mistletoe, leaves, flowers, and fruits than beneath trees without mistletoe (unparasitized: 0.19 g/ m^2; parasitized: 103.21 g/ m^2; dead: 10.49 g/ m^2; χ^2 =519.07; *d.f.* = 2; $P < 0.0001$). On the contrary, litter from the host was more abundant under unparasitized trees (unparasitized: 268.68 g/ m^2; parasitized: 203.66 g/ m^2; dead: 39.98 g/ m^2; χ^2 =113.68; *d.f.* = 2; $P < 0.0001$). The biomass delivered by seed dispersers was greater under the canopy of live and dead parasitized trees (unparasitized: 0.20 g/ m^2; parasitized: 2.98 g/ m^2; dead: 3.23 g/ m^2; χ^2 =211.28; *d.f.* = 2; $P < 0.0001$), especially in the form of seeds of zoochorous plant species under parasitized trees (Fig. 2), and excrement under dead trees (Fig. 2). Overall, in unparasitized trees, pine needles constituted 99 % of total biomass input, while the remaining 1% was from frugivore activity. In parasitized pines, mistletoe litter comprised 33.5 % of overall biomass, host litter 65% and frugivore litter 1.5 %. In dead parasitized trees, 74% of biomass input pertained to the host, 19% to mistletoe, and 6% to frugivores.

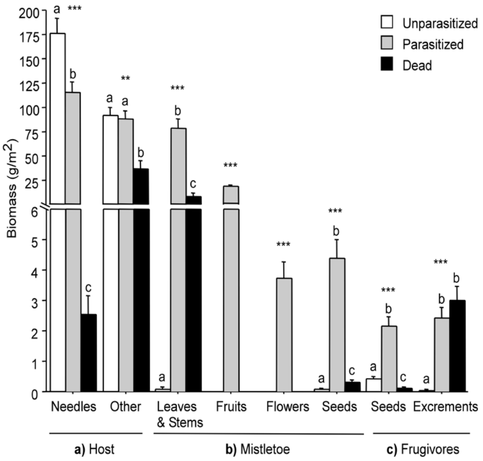

Figure 2. Comparisons of litter biomass (g/ m^2) reaching the soil beneath the canopy of unparasitized, parasitized and dead *Pinus nigra* trees. Different organic matter sources come from (a) the host, (b) the parasite and (c) the activity of frugivorous birds. Analyses were performed using negative binomial GLMMs. Statistical differences are indicated as $p < 0.001$ (**), $p < 0.0001$ (***). Results correspond to mean (± S.E.) values of two sampling years (2012 and 2013), n=75 unparasitized trees, n=75 parasitized trees and n=15 dead parasitized trees.

Nitrogen, P, and K concentrations in mistletoe tissues were greater than those of the host (Table 1). Except for seeds, all other mistletoe tissues (leaves, stems,

	Chemical composition				
Organic matter (N=5)	N %	C %	C/N	P (mg/g)	K (mg/g)
Pine senescent needle	0.71 (0.07)	49.30 (0.42)	71.83 (9.22)	0.65 (0.15)	2.99 (0.95)
Mistletoe senescent leave	1.47 (0.17)	44.61 (0.56)	31.92 (3.44)	2.55 (0.29)	29.25 (1.66)
Mistletoe stem	1.76 (0.27)	47.19 (1.13)	29.33 (4.24)	2.02 (0.32)	14.60 (1.18)
Mistletoe flower	1.39 (0.06)	52.08 (0.34)	37.65 (1.64)	2.97 (0.15)	13.70 (0.33)
Mistletoe fruit	1.07 (0.15)	44.45 (0.99)	45.13 (6.65)	2.88 (0.29)	16.98 (2.09)
Mistletoe seed	0.71 (0.06)	40.86 (0.99)	59.00 (6.65)	0.90 (0.29)	12.58 (2.09)
	$F_{5,24}$= 7.94; **P < 0.0001**	$F_{5,24}$= 30.06; **P < 0.0001**	$F_{5,24}$= 8.38; **P < 0.0001**	$F_{5,24}$= 13.22; **P < 0.0001**	$F_{5,24}$= 38.98; **P < 0.0001**

Table 1. The table summarizes N, C, C:N, P and K content of host senescent needles and mistletoe senescent leaves, stems, flowers, fruits, and seeds. Results of linear models (F-values, degrees of freedom and *P*-value). Statistical differences are indicated in bold, mean (±SE).

flowers, and fruits) contained 1.5 to 2.5 times more N, about 1.4- to 4.5-fold more P and 4.6- to 9.8-fold more K than in host needles, and showed lower C: N ratios ($P < 0.001$).

According to differences in litter quantity and quality, soils at different stages of the host-parasite system exhibited different chemical and biological properties (Table 2; Fig. 3). In this regard, total organic carbon (TOC) was more abundant beneath dead trees, followed by parasitized and unparasitized trees (Table 2), while inorganic carbon (IC) followed the opposite pattern (Table 2). Soil moisture and pH were quite similar ($P = 0.920$ and $P = 0.872$, respectively), with parasitized and dead trees showing slightly more acidic soils. Although with no statistical significance, total nitrogen (TN) was higher in soils beneath dead trees, followed by parasitized and unparasitized ones (Table 2). The dominant N-form was different in each case. Beneath parasitized and unparasitized trees the soils contained a greater abundance of DON and NH_4^+-N, though not statistically significant, whereas beneath dead pines N prevailed in the form of NH_3^- (Table 2). While IEMs-PO_4^{3-}-P availability was more abundant beneath parasitized and dead trees (Table 2), IEMs-K^+ availability predominated under parasitized trees (Table 2).

Differences in soil chemical properties were similarly reflected in soil microbial features, with those beneath dead trees hosting more abundant and functionally diverse

Variable	Unparasitized (N=38)	Parasitized (N=38)	Dead (N= 12)	Model ($\chi 2$, *P-value*)
TOC (mg/kg)	291.93 ± 40.71^a	403.03 ± 40.54^a	536.06 ± 120.41^b	7.017 (**0.029**)
IC (mg/kg)	25.04 ± 6.01^a	16.19± 1.80^a	15.03 ± 3.47^a	3.886 (0.143)
TN (mg/kg)	52.58 ± 8.65^a	73.02 ± 11.76^a	117.20 ± 23.42^a	2.116 (0.347)
DON (mg/Kg)	53.38 ± 5.91^a	54.34 ± 4.62^a	45.37 ± 4.33^a	1.518 (0.468)
pH	7.29 ± 0.06^a	7.21 ± 0.06^a	7.22 ±0.15^a	0.273 (0.872)
Moisture (%)	4.50 ± 0.36^a	4.52 ± 0.28^a	4.39 ± 0.71^a	0.165 (0.920)
NO_3-IEM (ug/(cm^2*day)	0.249 ± 0.020^a	0.269 ± 0.027^{ab}	0.277 ± 0.048^b	6.132 (**0.046**)
NH_4^+-IEM (ug/(cm^2*day)	0.279 ±0.013^a	0.298 ± 0.016^a	0.247 ± 0.009^a	3.847 (0.172)
PO_4^2-IEM (ug/(cm^2*day)	0.043 ± 0.002^a	0.055 ±0.004^b	0.061 ± 0.007^b	14.932 (**0.0005**)
K^+-IEM (ug/(cm^2*day)	0.340 ± 0.026^a	0.465 ± 0.037^b	0.348 ± 0.022^a	28.811 (**0.0001**)

Table 2. Nutrient concentrations and availabilities in top 10 cm of the soil profile beneath the canopy of unparasitized and alive and dead parasitized trees. Total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), dissolved organic nitrogen (DON), pH, and moisture were analyzed in composite soil samples collected at 0-10 cm deep, whereas NO_3^-, NH_4^+, PO_4^{2-}, and K^+ availabilities were estimated using ion-exchange membranes (IEMs) incubated for 1 month in soils. Results (Chi-squared and *P*-values) of a linear mixed model including tree condition (unparasitized /parasitized/dead tree) as a fixed factor and paired trees as the random one). Statistical differences are indicated in bold, mean ± SE.

microbial communities, followed by parasitized trees and then by unparasitized ones (MB-N: LMM, χ^2 = 7.36; *d.f.* = 2; *P* = 0.0025; H' based on microbial functional diversity: LMM, χ^2 = 4.09; *d.f.* = 2; *P* = 0.129). The nMDS based on microbial functional diversity showed no clear separation among the three types of focal pines (PerMANOVA: *pseudo-F* = 1.361; *d.f.* = 2; *P* = 0.206; Fig.3c). However, we found significant differences between unparasitized and dead trees (*t* = 1.52; *P* = 0.04), and pseudo-significant differences between parasitized and dead trees (*t* = 1. 24; *P* = 0.164).

Discussion

According to our hypothesis, different biotic interactions prevailing before, during, and after mistletoe parasitism result in different linkages between the forest canopy and the soil, influencing soil-chemical and biological properties in different ways.

We identified three main organic sources reaching the soil beneath tree canopies: the host, the parasite, and frugivorous birds, differing in their relative abundance at different stages of parasitism. At

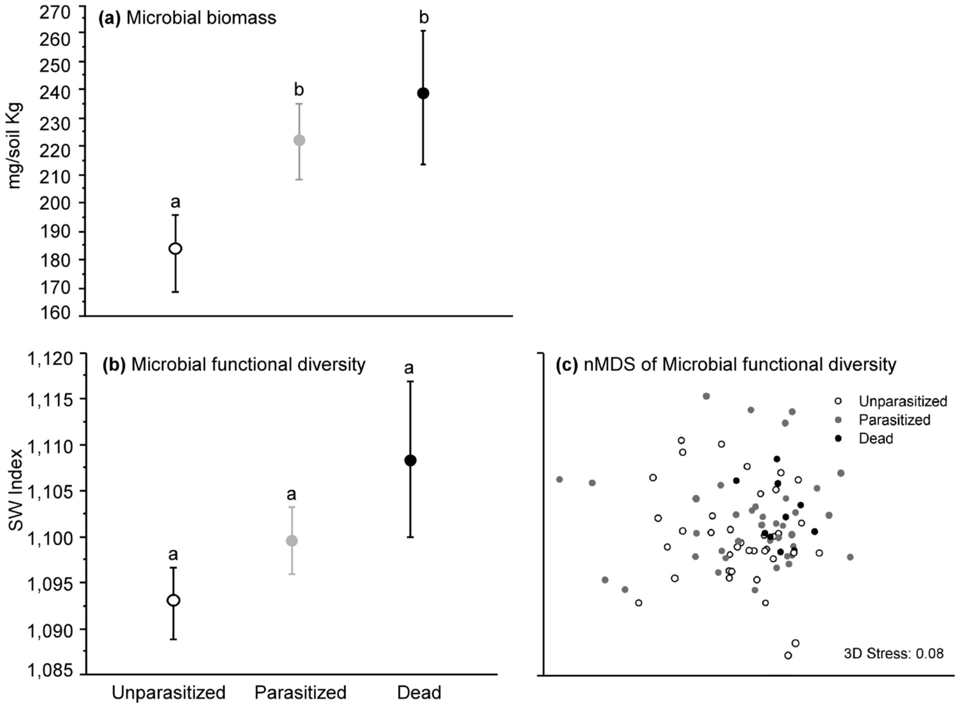

Figure 3. (a) Nitrogen in microbial biomass and (b) H' based on functional diversity of soil microbial communities and (c) non-metric multidimensional scaling (nMDS) ordination of soil microbial communities beneath unparasitized, parasitized, and dead *Pinus nigra* trees. Nitrogen in microbial biomass was estimated from composite soil samples collected beneath the canopy of focal trees. Shannon-Weaver Diversity Index and nMDS are based on functional diversity of microbial communities analyzed with the MicroResp system. The three-dimensional nMDS shown as a 2D plot, was based on Euclidean distance. The ordination 3D stress value was 0.08 (PerMANOVA: *d.f.*, 2; *pseudo-F*, 1.361; *P*=0.206). Results correspond to average values (± S.E.) of one sampling year (2012), n=38 unparasitized trees, n=38 parasitized trees and n=12 dead trees.

the earliest stage, before parasitism, pine litter proved to be the most important resource reaching the soil (99.9% of total organic compounds) together with small amounts of excrement that birds deposited while perching in the tree canopy (Fig. 2a). Parasitism changed the previous linkages between the host and the soil: the parasite increased overall litterfall quantity, including a wide variety of tissue types (i.e., leaves, flowers, trunks, fruits, and seeds) (Fig. 2b) of different chemical composition (Table 1), while, as expected, triggering the occurrence of additional indirect factors. First, the stressful effect of parasitism on the host was reflected by diminished host growth (Fig. 1a), which caused a 1.33-fold reduction in the amount of host litter reaching the soil (Fig. 2a). Second, the increase in frugivorous bird visits (Fig. 1b) raised the input in allochthonous organic compounds (feces and zoochorous plant seeds) by about 15-fold with respect to unparasitized trees (Fig. 2c). At the final stage, once the host died, senescing litter of both the parasite and the host continued to enrich the soil. Moreover, frugivorous birds used dead standing structures to perch, from where they dropped excrement (Fig. 2c). Overall, parasitized trees, either alive or dead, received organic matter inputs from more diverse sources than did unparasitized ones. Thus, parasitized trees accumulated under their canopy 1.16-fold more litter biomass than did unparasitized trees and 5.80-fold more than did dead unparasitized trees. These sources, in addition to their abundance, differed in quality (Table 1). In this sense, litter from *Viscum a. austriacum* presented about 2-fold more N, 4-fold more P, and 5.8-fold more K than did that from the host, these nutrients being especially concentrated in leaves, but also in stems, flowers, and fruits (Table 1). Moreover, litter of *V. a. austriacum* contained lower C: N ratios (Table 1), pointing to a faster decomposition rate and quicker nutrient release than more recalcitrant litter of the host (Quested, 2002, 2005; Ndagurwa *et al*., 2014b). Furthermore, considerable inputs of bird debris into the soil may provide an important supply of nutrients, mainly in form of N, P, and Na (Dean *et al*., 1999; Van der Wal *et al*., 2004).

Mistletoe, by increasing the amount, quality, and diversity of organic matter inputs, returned a more heterogeneous mixture of resources to the soil that enhanced C accumulation and increased the variety of soil resources that the biota could utilize, giving rise to local ‘fertilization islands’ under parasitized trees (Table 2). These soil spots were especially enriched in K^+ and PO_4^{3-}-P, reflecting the main elements provided by litter of *V. a. austriacum* (Table 1). This result has been noted in other mistletoe species (March & Watson, 2010; Ndagurwa *et al*., 2013; Muvengwi *et al*., 2015). However, soil N content was lower than expected, a fact also reported for mistletoes of the African savanna (Ndagurwa *et al.,* 2013; Muvengwi *et al*., 2015). This could be due to a greater mineralization and nitrification of the organic N, which could be stimulated by the lower C/N relationship of the mistletoe or by changes in the abiotic environment produced in the tree canopy as a consequence of parasitism (e.g., greater light infiltration and temperature). Otherwise, lower NH_4^+ availability could result from an antagonistic effect of NH_4^+ and K^+ at the soil-exchange surface (Ndagurwa *et al.,* 2013; Muvengwi *et al*., 2015). One main outcome to note is that the host-parasite system maintained its effects on soil after death, despite that organic matter inputs decreased at late stages. These soils showed greater amounts of organic C and PO_4^{3-}-P than those under parasitized trees, and the dominant N form switched from NH_4^+-N to $NO3^-$-N, perhaps because, once the tree dies, lower crown density permits greater light infiltration to the forest floor, which may increase soil temperature and stimulate nitrification processes. Our findings show that parasitized tree effects after death could be maintained by the additional organic matter inputs that feed soil imprints generated during a long past of parasitism, similarly occurring after past agricultural land use (Dupouey *et al*., 2002; Mattingly & Orrock, 2013) or after the effect of past tree crowns in savannas (Dean *et al*., 1999; Stahlheber *et al*., 2015), which could last for years in the soil (Rodríguez *et al*., 2011).

According to the diversity and quantity of resource availability, soil heterotrophic microbial communities became increasingly abundant (Fig. 3a) and functionally diverse (Fig. 3b) as stage development matured, reaching their maximum under dead parasitized trees. Composition of the microbial community under dead trees differed with respect to those inhabiting soils at earlier stages—differing significantly from unparasitized trees, Fig. 3c— perhaps because in these sites, which have been receiving mistletoe-derived organic matter for long time, the variety of organic compounds under different decomposition degree increases. In addition, different microbial composition could be one plausible explanation for the different N forms prevailing under their canopy (NO^{3-}-N) in contrast to that predominating under unparasitized and parasitized trees (NH_4^+-N). These results support the idea of aboveground diversity boosting functional diversity of belowground communities (Bardgett & Shine, 1999; Stephan *et al*., 2000; Zak *et al*., 2003) at different ecosystem developmental stages (Ohtonen *et al*., 1990; Schipper *et al.,* 2000), which may potentially affect fundamental ecosystem processes driven by soil microbial organisms (Zak *et al*., 2003).

In conclusion, mistletoe, enhanced by the biotic interactions that prevail in the forest canopy at different stages of parasitism, can play an important role in intensifying soil-resource availability and its spatial variability, regulating in turn the composition, abundance, and spatial distribution of heterotrophic microbial communities inhabiting the soil. This situation strengthens the idea of mistletoes as keystone species, as well as ecosystem engineers (*sensu* Jones *et al*., 1994) modifying their nearest abiotic environment and opening new habitats for other organisms. As these parasitic plants are so frequent in forest canopies around the world, there still remains much to be explored about their impact on aboveground and belowground communities, as well as general ecosystem functioning.

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Chapter 5

Parasites structuring ecological communities: the mistletoe footprint in Mediterranean pine forests

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Parasites structuring ecological communities: the mistletoe footprint in Mediterranean pine forests. *Manuscript*.

Abstract

The capacity of parasitic plants in structuring natural communities is increasingly recognize. They can affect the structure and productivity of plant communities by modifying the competitive balance between hosts and non-host species and through altering the quantity and quality of resources entering the soil. However, most studies available today limit to a small subset of herbaceous parasites and their effects on prairies and salt-marsh ecosystems. We still know little about the structuring role of parasitic plants on plant communities of long lifespan. In this study we evaluate the long-term impact of *Viscum album* subsp. *austriacum* on the woody-plant community of a Mediterranean pineland. This mistletoe maintains over several years on the same host, exerting long-lasting, spatially concentrated effects on community and ecosystem properties. Among these, mistletoe concentrates seeds of zoochorous plants and enhances soil nutrient availability beneath the host year after year. Here we analyze whether the close spatial association between seed-deposition sites and soil nutrient "hotspots" could result in nucleus of zoochorous woody-plants nourished by the abundant organic detritus accumulated under the host. We also analyze whether mistletoe effects can expand after host death. To address these issues, we selected unparasitized, parasitized and dead parasitized *Pinus nigra* trees in a Mediterranean pineland, in which we study the joined effect of mistletoe-mediated abiotic changes, with the seed-rain, seed predation, seedling establishment, plant recruitment and plant growth. We conclude that mistletoes can exert strong and lasting impact on the structure and dynamics of forest communities, with parasitized trees acting as centers for the establishment and growth of colonizing fleshy-fruited woody-species, which, over the long term, promotes vegetation changes by limiting dominant tree species and facilitating less represented fleshy-fruited shrubs.

Key words

forest heterogeneity, plant-animal interactions, community assemblage, parasitic plant, plant-soil feedback, nucleation process, *Viscum album*.

Introduction

Understanding the mechanisms that maintain biodiversity and structure natural communities is a central theme in ecology. Competition and predation are among biotic processes especially recognized as important, but increasingly also parasitism (Hatcher et al. 2012). Parasites can influence communities through their direct effects on host survival or reproductive output, or through their indirect effects on different habitat features that matter to other organisms (e.g., modulating availability or quality of resources). From the countless number of parasite species, over 4500 are plants. This group includes herbs, shrubs, vines, and trees that take water and nutrients from a wide variety of host plants in ecosystems worldwide (Musselma & Press 1995). An increasing body of evidence reveals their capacity to modify competitive relationships between host and non-host plants (e.g. Pennings & Callaway 1996; Gibson & Watkinson 1991) and to alter the soil-nutrient cycle (e.g. Quested *et al.* 2002; March & Watson 2007, Ameloot *et al.* 2008), showing a great potential to play a structuring role in natural communities. The few studies specifically addressing the role of parasitic plants at the community level limit to a small subset of herbaceous parasites and their effects on prairies and salt-marsh ecosystems (Gibson & Watkinson 1992; Pennings & Callaway 1996; Joshi et al. 2000; Quested et al. 2003; Bardgett et al. 2006; Fisher et al. 2013, but see March & Watson 2007). However, there is a lack of information about the impact of shrubby parasitic plants (mistletoes) on woody plant communities of long lifespan.

Although mistletoe shares many features with other parasitic plants, these parasites have a long generation time with individual plants living for several decades on the same host. Moreover, these parasites maintain on the same host over several generations because seed dispersers continuously deposit mistletoe seeds onto already parasitized trees, increasing re-infection probabilities (Aukema 2004; Mellado & Zamora 2014b). For this reason, mistletoe effects on host performance and the ecosystem are long-lasting and may take several decades until the host-parasite system die. Under this situation, intense and continued effects of the host-parasite system on the ecosystem may lead to community changes over long timescales of decades or centuries. Yet, to date, the long-term effect of mistletoes on the forest community remains unknown.

The ecological consequences of parasitism strongly depend on the ecological role that the host plays in the ecosystem. As the parasitic plant acts on the individual host, changes at the community scale might be accentuated in plant communities where keystone organisms or major, dominant species serve as primary hosts. The fleshy-fruited mistletoe *Viscum album* subsp. *austriacum* (hereafter *Viscum a. austriacum*) is a specialist parasite of dominant pine trees in Mediterranean pinelands (Mellado & Zamora 2014a), where it is dispersed by dietary-generalist frugivorous birds that serve as transmission vectors (Mellado & Zamora 2014b). In previous studies, we have identified two key mechanisms by which *V. a. austriacum* could affect the plant community. First, avian seed dispersers (mainly *Turdus* spp.) prefer to perch on parasitized trees where they drop disproportional amounts of seeds (Mellado & Zamora 2015). As dietary generalists, these birds not only disperse mistletoe seeds but also seeds of co-fruiting understory species that concentrate beneath the host canopy (Mellado & Zamora 2015). Second, mistletoe enhances soil nutrient availability beneath these perches (Mellado et al. submitted), which could favor the recruitment of colonizing species. The close spatial association between seed-deposition sites and soil nutrient "hotspots" could result in nucleus of zoochorous plants nourished by the abundant organic detritus accumulated under the host. This might occur by a differential establishment and subsequent growth of recruits in these sites, where factors such as seed-predation (Matías et al. 2009) or abiotic factors [such as drought (Mendoza et al. 2009)] do not constrain the survival and recruitment of colonizing species. If so, parasitized trees could drive a nucleation process within the forest landscape similar to that previously described for isolated trees within non-forest matrices (Verdú & García-Fayos 1996; Dean 1999), in which solitary trees facilitate later colonists by attracting seed-dispersing birds and by ameliorating environmental conditions.

Mistletoe effects on colonizing species might be accentuated by the parasite's typically constant and uniform fruit production (Larson 1996; Van Ommeren & Whitham 2002), which makes parasitized trees constant food resources for frugivorous birds, thereby providing consistent sites for seed- and organic matter-deposition in space and time (Mellado & Zamora 2015; Mellado et al. 20016). The persistent re-infection process that characterizes mistletoe populations only strengthens these tendencies of seed accumulation and soil fertilization at the same local site, which, combined with a steady decline in host's growth (Mellado et al. submitted) might permit greater amounts of light to penetrate through the forest canopy. Due to the long lifespan of the host-mistletoe system, mistletoe-driven changes beneath the host form slowly (Mellado et al. submitted) and their effect on the plant community may last after the host dies (Facelli & Facelli 1993, Stahlheber et al. 2015). Aware of this, in the present study we hypothesize that, by concentrating zoochorous seeds and inducing changes in the environment beneath the canopy of parasitized trees (soil and light resources) over several

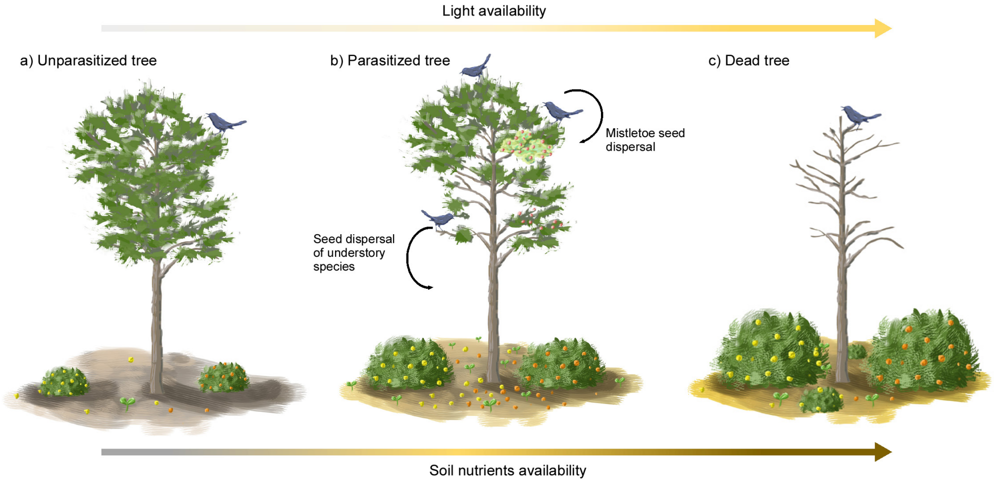


Fig 1. Schematic illustration of mistletoe effects driving long-term vegetation change in an individual host tree. In a pineland landscape, a tree lacking mistletoe (a) receives sporadic visitations of frugivorous birds. Once the tree is parasitized by mistletoe (b), it has greater chances to be re-infected as the parasites act as attractive milestones in the forest canopy, highly preferred by avian seed-dispersers. As parasitic loads increase due to the positive re-infection feedback driven by these birds, the host begins to suffer the effect of parasitism, which may finally hasten its death. Simultaneously, parallel processes are occurring in the understory plant community. Frugivorous birds, in addition to mistletoe seeds disperse seeds of co-fruiting plants under the host canopy. With time, intense parasitic loads coupled to greater frugivores activity not only enhance seed deposition, but also organic matter inputs beneath the host canopy, giving rise to local soil enrichment that favors seedling establishment and subsequent plant growth. Moreover, because of the gradual negative impact on the host, heavily parasitized trees produce less biomass, reflected in sparser leaf coverage. This permits greater amounts of light to penetrate the forest canopy, having a positive effect on growth of understory woody plants already established beneath the host. This positive loop (mistletoe re-infection - understory seed accumulation – increasing soil and light resources- understory plant establishment and growth) would intensify as the system matures, ending with the death of the host (c), which would finally be replaced by understory vegetation, essentially zoochorous species.

decades, *Viscum a. austriacum* has the potential to facilitate zoochorous-plant colonization, recruitment, and growth at the same time as it weakens the host. Such mistletoe-driven long-lasting effects may have far-reaching implications for vegetation change, which, by facilitating the establishment of zoochorous species, may, over the long-term, cause a tree-dominated patch to be replaced by a diverse patch of zoochorous species (Figure 1). By doing so, mistletoe may play a keystone role in the organization of pine forest communities where it inhabits, inducing disproportional changes to their abundance and biomass.

To understand the long-term effect of *Viscum a. austriacum* on the woody plant community of a Mediterranean pineland, we selected unparasitized pine trees (*Pinus nigra*), mistletoe parasitized and dead parasitized pines, in order to establish a broad temporal framework representing phases before, during, and after mistletoe parasitism. In particular, we ask whether changes in light availability coupled to soil enrichment and constant zoochorous-seed inputs, all spatially localized under the canopy of parasitized trees, promote changes in the plant community assemblage. To address this question, we analyze the effects of seed-deposition patterns and the abiotic environment of subcanopy patches where seeds arrive, coupled to the effect of post-dispersal processes, such as seed predation, seedling establishment, plant recruitment and plant growth before, during, and after mistletoe parasitism.

Materials & Methods

Study site and Experimental design

The study was performed from 2011 to 2013 in a Mediterranean pine forest located in the Natural Park of Sierra de Baza (southeastern Spain; 2º 51' W, 37º 22' N). This jagged mountain range is mainly calcareous, with minimum altitudes of 1200 m and maximum of 2269 m. The site has a typical Mediterranean climate, characterized by cold winters and hot summers with pronounced summer drought (June-August), while precipitation concentrates in spring and autumn. The dominant tree vegetation consists of pine forests, mainly Austrian (*Pinus nigra* Arn.) and Scots (*Pinus sylvestris* L.), but also Aleppo (*Pinus halepensis* Mill.) and Maritime (*Pinus pinaster* Ait.) pines, coexisting with oaks (*Quercus ilex* L.) and maples (*Acer opalus* L.

ssp. *granatense* Boiss). A diverse and abundant ensemble of zoochorous fleshy-fruited shrubs accompanies pines, including *Berberis hispanica subsp. hispanica* Boiss. & Reut., *Crataegus monogyna* Jacq, *Lonicera arborea* Boiss., *Juniperus oxycedrus* L., *J.communis* L., *Prunnus ramburii* Boiss and *Rosa* spp.. Part of the zoochorous-plant community is the mistletoe *Viscum album austriacum* (Wiesb.) Vollmann (Viscaceae), a hemiparasitic, dioecious epiphyte widely distributed throughout European coniferous forests. *Pinus nigra* and *P. sylvestris* constitute the most common host species in southern Spain and at the study site (Mellado & Zamora 2014a); where individual *V. a. austriacum* can live for more than 35 years (Zuber 2009; *pers. obs.*). The most common species of avian seed dispersers in Sierra de Baza include non-migrants and seasonal migrants such as Robin *Erithacus rubecula,* Blackcap *Sylvia atricapilla*, and *Turdus* spp.*,* which feed on various fruit species during autumn-winter (Herrera 1995), including mistletoe (Zuber 2004; Mellado & Zamora 2014b).

To perform the study, we selected 125 *Pinus nigra* focal trees, from which 55 were parasitized by mistletoe (hereafter 'parasitized tree'), 55 were not parasitized (hereafter 'unparasitized tree') and 15 were dead trees parasitized in the past (hereafter 'dead trees'). Parasitized trees presented moderate to intense parasitic loads [68.5 ± 9.6 (mean ± SE) number of mistletoes per tree], holding at least one mistletoe older than 30 years of age. Dead trees are less numerous than the other categories because they are scarce and difficult to find in the study area. However, they are easy to identify because dead mistletoes remain on the host. The experimental site covers a large range of the mountain's heterogeneity as focal trees were equitably distributed at three altitudes (1300, 1650 and 1850 m) within stands of different tree densities. Trees were taken at random and spatially paired (one parasitized and one unparasitized), excepting for dead trees that were scattered over the mountain. Paired trees were of similar architecture, size (trunk perimeter = 97.5 ± 4.01 cm) and height (6.87 ± 0.23 m), and were located in similar environmental contexts. All focal trees were at distances of 40-80 m. In previous studies, we measured zoochorous-seed density under the canopy of unparasitized and parasitized trees (Mellado & Zamora 2015) and soil-nutrient availability beneath the canopy of these same trees, including the latest stage after host death (Mellado et al. in prep). In this study, we add new information to fully understand the consequences of mistletoe parasitism in the dynamic of the forest community, including the seed density found under dead trees, and quantify light incidence, seed predation, seedling establishment, plant recruitment and growth of zoochorous plant species at the three different stages of the host-parasite system.

Light incidence

Light incidence was quantified by hemispherical photography (Valladares & Guzman 2006) under the canopy of 40 parasitized, 40 unparasitized and 15 dead focal trees. We took two photographs per tree, considering its northern and southern side, the average value of which was used for subsequent analyses. Photographs were taken from a high tripod while pointing to the sky at 1.70 m from the ground. A fish-eye lens with a 180° field of view (FCE8, Nikon, Tokyo, Japan) was used with a digital camera (Coolpix 995 digital camera, Nikon), horizontally leveled. Photographs were taken during completely cloud-covered days; later, images were analyzed using Hemiview canopy analysis software version 2.1 (1999, delta-T Devices Ltd, Cambridge, UK). The software estimates the global site factor (GSF), which is an estimator of the total amount of light, ranging from 0 (total darkness) to 1 (100% of light available). Average values of GSF were calculated and statistical analyses performed using linear mixed models (LMMs).

Seed rain

We quantified the seed rain of all zoochorous-plant species generated from the canopy of unparasitized, parasitized, and dead parasitized focal pines. Seed traps were used during the entire dispersal season, from October to February, for two study years (2011 and 2012). Seed traps consisted of flowerpots (0.125 m^2) covered with an aluminum mesh hanging on the lower branches of the tree canopy (c. 2 m above the ground level) or attached to the soil in the case of dead trees, where branches were more susceptible to breakage. Three seed traps were located on randomly assigned sites. At the end of February, samples were transferred to the laboratory where seeds of all fleshy-fruited plant species were identified and counted, and seed-species richness calculated. Seed density of zoochorous understory species were all pooled, and were analyzed using negative binomial generalized linear mixed models (GLMMs). Seed richness was analyzed with LMMs.

Seed predation

We quantified seed predation by recording seed removal of the dominant plant species, *Berberis hispanica* and *Viscum album,* quantified in the seed-rain. Seeds were offered to predators in the field under the canopy of unparasitized and parasitized focal trees. The experiment was run at the end of winter (March) of 2011 (n=40 replicates) and 2012 (n=55 replicates) and monitored after 30 days, coinciding with the end of the dispersal season. Each experimental unit consisted of a square seed depot of 6 x 6 cm of plastic mesh (1.5-mm pore) attached to the soil. In each seed depot, four seeds of each species were glued with a low-odor thermoplastic adhesive, resistant to wind and rain (see Matias et al. 2009 for similar methods). Three seed depots were randomly placed on the ground under the canopy of each tree, separated by a minimum distance of 50 cm. To camouflage seed depots, they were mixed with soil litter including seeds naturally dispersed by birds. Zoochorous seed density naturally reaching the soil was left intact. We considered a seed to have been consumed when missing from the plastic mesh or when present but with visible snag marks or empty. For the statistical analyses, we used the proportion of depredated seeds to the total seeds exposed per focal tree (composed of three seed depots), using GLMMs with binomial error distribution and a logit link function.

Seedling establishment

We performed a sowing experiment to quantify seedling establishment under the canopy of focal trees. In a subset of 38 unparasitized, 38 parasitized and 10 dead parasitized *P. nigra*, we sow seeds of the host (*Pinus nigra*) and the dominant understory zoochorous species (*Berberis hispanica*) counted in the seed rain. From each species, 15 seeds were sown inside individualized 15×25 cm side wire-mesh cages (1-cm mesh) to avoid seed predation by small mammals as well as herbivory losses. We placed two wire-mesh cages per tree. Before the sowings, seeds were visually inspected to reject non-viable seeds (predated or aborted); also, seeds of *B. hispanica* were kept refrigerated at 4º C throughout the winter, to simulate a cold-stratification period to boost the germination rates (see Matías et al. 2012 for similar methods). Seeds were sown 2 cm deep and 1.5 cm apart. A total of 2,580 seeds per species were sown in February 2011 and monitored for one year. For the statistical analyses (GLMMs), we used the proportion of emerged and surviving seedlings to the total seeds sown, using a binomial error distribution and a logit link function.

Saplings and adult understory woody plants

At the end of summer of 2012, we estimated density and richness of woody species (zoochorous plants and pine trees) naturally growing under the canopy of all focal pines. We differentiated between the estimate of adult plants and that of saplings (immature individuals) and juveniles (young reproductive individuals), because adult individuals are difficult to differentiate in many of the species involved due to their resprouting capacity. For adult plants, we estimated the overall surface area (m^2) that they covered under focal trees. Otherwise, for saplings and juveniles, we counted individuals and estimated their density and richness under the coverage of the pine canopy. To estimate densities, we took into consideration only the surface area where plants could become established, excluding any rocky surface from the total area. Saplings and juveniles were identified to the species level and sorted into three categories: (1) young (2-5 years); (2) middle-aged (6-14 years) and (3) old (>15 years old), the age of which was estimated by counting growth scars (in species where this was possible) or by using plant size as a proxy (see Zamora & Matías 2014). Sapling density and richness, as well as coverage of adult plants, were analyzed using LMMs, with variables log-transformed when necessary to meet assumptions of normality and homoscedasticity.

Growth of understory plants

To estimate plant growth, we measured yearly bud elongations of adult zoochorous plant species growing under the canopy of unparasitized, parasitized, and dead *P. nigra*, as well as in open sites. We selected 24 replicates per treatment and paired unparasitized-parasitized trees on the one hand and dead trees-open sites on the other to compare plant growth. Comparisons between all treatments were not possible because plants meeting the criteria set were difficult to find. First, paired treatments must have understory plants of the same species and of comparable size. Second, these understory plants must have at least five non-grazed buds available for measurement. The measured understory species were *Berberis hispanica*, *Crataegus monogyna*, *Rosa* spp. and *Quercus ilex*. At the end of the growing season (July 2012) from 5 to10 buds per plant species were measured in each treatment (see Herrero & Zamora 2014). Linear mixed models were used including paired treatments as a fixed factor and pairs as the random one, with variables log-transformed when required to meet assumptions of normality and homoscedasticity.

Statistical analyses were carried out using the open source software Statistical R 2.15.1 (R Development Core Team 2012). GLMMs were run using lmer and glmer functions of the package lme4 (Bates et al. 2008), whereas negative binomial GLMMs with the glmmadmb function of the "glmmADMD" package (Skaug et al. 2012). For all analyses, we included tree condition (unparasitized, parasitized and dead tree) as a fixed factor and paired trees as a random factor. The results are presented as mean ± one standard error, unless otherwise specified.

Results

Light incidence

Light availability (GSF) increased with parasitism development, being lower under the canopy of unparasitized trees than under parasitized trees and maximum under dead parasitized ones (χ^2 = 67.32; d.f. = 2; P <0.0001).

Seed rain and seed predation

The seed rain of zoochorous species was significantly more abundant and richer under parasitized trees than under unparasitized and dead parasitized ones, following a similar pattern in the two study years (Seed density: 2011: $\chi^2 = 74.51$; d.f. = 2; $P < 0.0001$; 2012: $\chi^2 = 91.98$; d.f. = 2; $P < 0.0001$; Seed richness: $F_{2,122} = 38.28$; $P < 0.0001$). Seeds of *V. a. austriacum* were preferably consumed compared to seeds of *Berberis hispanica* (Table 1). Despite Nevertheless, none of the species had similar probabilities to be depredated under the canopy of unparasitized and parasitized trees (*Berberis hispanica*, year 2011: χ^2 = 0.708, d.f.= 1; P = 0.399; year 2012: χ^2 = 0.061; d.f.= 1; P = 0.805; *Viscum a. austriacum*, year 2011: χ^2 = 2.282, d.f.= 1; P = 0.131; year 2012: χ^2 = 0.774; d.f.= 1; P = 0.379).

		Unparasitized tree	Parasitized tree	Dead tree
Seed-rain	*Density*			
	2011	4.81 ± 1.47 ^{a}	218.48 ± 51.52 ^{b}	2.78 ± 0.74 ^{a}
	2012	21.45 ± 5.46 ^{a}	397.99 ± 61.22 ^{b}	39.06 ± 8.35 ^{a}
	Richness			
	2011	a	b	a
	2012	a	b	b
Seed predation	*Berberis hispanica*			
	2011	41 94 ± 3.64	37.39 ± 3.51	-
	2012	32. 20 ± 2.54	34.50 ± 3.28	-
	Viscum album			
	2011	69.91 ± 3.77	80.67 ± 2.97	-
	2012	77.72 ± 2.81	83.59 ± 2.15	-

*Table 1.*Density of zoochorous seeds, seed richness and seed predation percentage quantified under the canopy of unparasitized, parasitized and dead parasitized *Pinus nigra* trees for two consecutive years (2011 and 2012). Generalized linear mixed models with negative binomial error distribution were used for seed counts, binomial error distribution for seed predation percentage, and linear mixed models for seed richness, with treatment (unparasitized, parasitized or dead) used as the fixed factor and paired trees as the random one, followed by Tukey's pairwise comparisons with 95% confidence level. Differences between treatments are indicated with different lowercase letters. Results correspond to mean ± standard error.

Seedlings, saplings, juveniles and adult understory plants

Early establishment of *Pinus nigra* was extremely low, with no single seedling surviving the first year of life, whereas *Berberis hispanica*'s success was greater under parasitized trees ($\chi^2 = 11.203$, d.f. = 2; $P = 0.0036$). We counted a total of 3 pine saplings and 968 saplings and juveniles belonging to 10 zoochorous species (*Berberis hispanica, Crataegus monogyna, Prunus* spp., *Juniperus communis, J. oxycedrus, J. Sabina, Rosa* spp., *Lonicera arborea, Sorbus aria, Quercus ilex*). The three pine saplings were found under parasitized trees. Otherwise, increasing amounts and richness of zoochorous saplings and juveniles were found as the parasite-host system matured, reaching their maximum values under dead parasitized trees (sapling density: young, $\chi^2 = 33.47$, d.f.= 2; $P < 0.0001$; middle, $\chi^2 = 62.10$, d.f.<0.00012; P =; old, $\chi^2 = 56.55$, d.f.= 2; $P < 0.0001$; sapling richness: young, $F_{2,122} = 7.15$, $P < 0.0011$; middle, $F_{2,122} = 4.91$, $P < 0.0089$; old, $F_{2,122} = 19.02$; $P < 0.0001$; total sapling richness, $F_{2,122} = 14.7$; $P < 0.0001$). Adult plants showed greater coverage in dead trees, although without statistical differences when compared with unparasitized and parasitized trees ($\chi^2 = 1.18$, d.f. = 2; $P = 0.55$).

Growth of understory plants

Plant growth was enhanced by the influence of the parasite, being greater in parasitized pines than in unparasitized ones ($\chi^2 = 4.027$, d.f. = 1; $P = 0.044$), as well as in dead parasitized trees compared to open sites ($\chi^2 = 11.14$, d.f. = 1; $P = 0.0008$).

Discussion

Our study provides evidence of the strong influence of mistletoe in structuring the forest community, being able to change the composition and structure of woody plant assemblages and affect local species richness. By having a long generation time and maintaining persistent re-infections on the same hosts, mistletoe exerts long-lasting, spatially

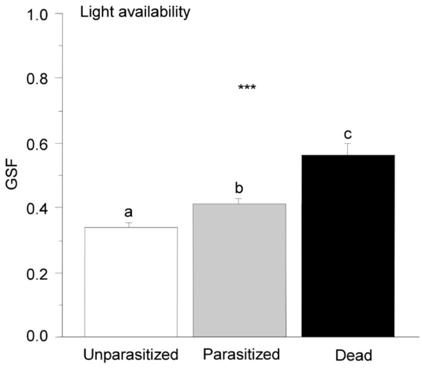


Fig 2. Mean values of light (GSF) penetrating the canopy of unparasitized, parasitized and dead *Pinus nigra* trees. Linear mixed models were applied, with treatment (unparasitized, parasitized or dead) used as the fixed factor and paired trees as the random one ($\chi^2 = 67.32$; d.f. = 2; $P < 0.0001$), followed by Tukey's pairwise comparisons with 95% confidence level. Statistical differences are indicated as $P < 0.0001$ (***), with differences between tree condition shown by different lowercase letters. Results correspond to mean ± standard error, N=40 unparasitized, N=40 parasitized and N=15 dead trees.

concentrated effects on community and ecosystem properties. Consequently, environmental conditions and plant assemblages in host patches vary with the development of parasitism, leaving a strong legacy that continues to affect the plant community and ecosystem processes long after the host dies.

Viscum a. austriacum, by modifying light and soil resources under the host canopy coupled to its capacity to concentrate zoochorous seeds in these same microsites, combines two facilitative mechanisms that result in the spatial aggregation of zoochorous species close to the parasite. Firstly, mistletoe fruits offered from the host canopy lead to predictable amounts of seed rain that concentrate beneath the host tree year after year (Mellado and Zamora 2015), with few seeds reaching the ground under unparasitized and dead pines lacking fruits (Table 1a). In such seed foci, which offer abundant and diverse food supplies to seed consumers, we expected greater seed-predation rates. However, contrarily, seeds showed similar depredation probabilities at all sites, irrespective of the seed density (Table 1b). Thus, the seed spatial pattern after seed-predation remained similar to that initially created by frugivorous birds, with most seeds concentrated preferentially under parasitized trees and few presented under non-parasitized or dead pines.

Secondly, by gradually reducing host growth and depositing nutrient-rich organic compounds beneath the host, *Viscum a. austriacum*

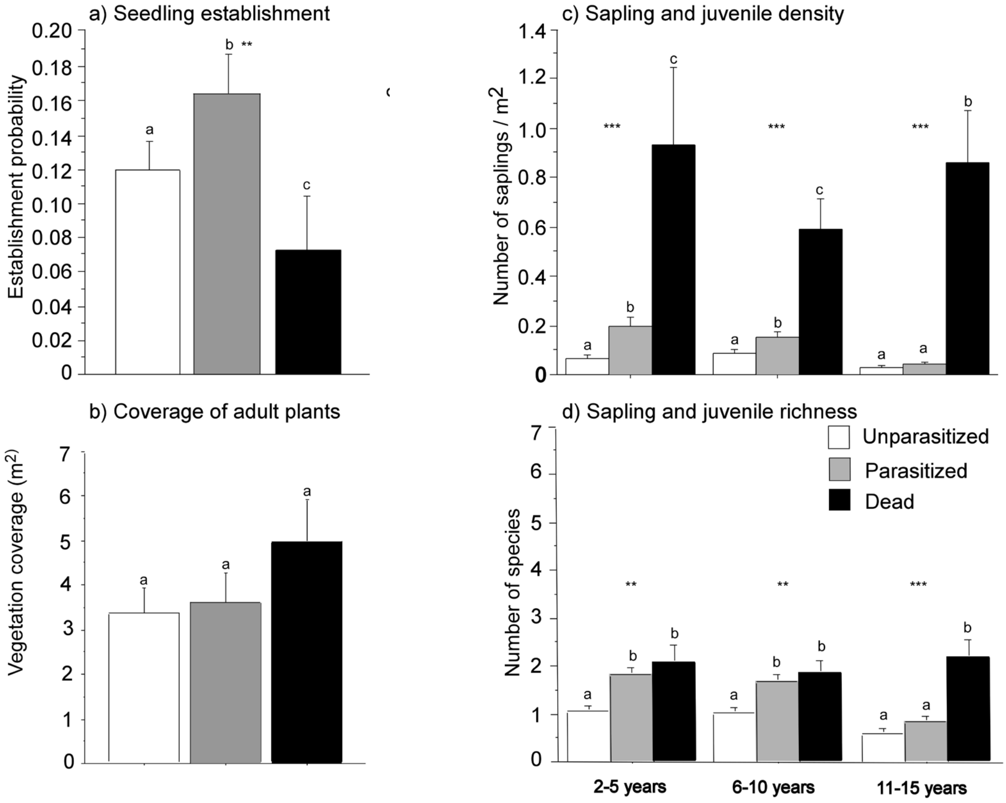


Fig 3. Establishment success and demographic structure of the zoochorous plant community. The figure shows the (a) probability of seedling establishment, (b) coverage of adult understory shrubs, (c) sapling and juvenile density, (d) and sapling and juvenile richness under the canopy of unparasitized, parasitized and dead *Pinus nigra* trees. Probability of seedling establishment was experimentally estimated by showing seeds of the zoochorous species *Berberis hispanica* and the host *Pinus nigra*. Only seedling establishment of the former species is shown because pine seedlings failed to survive [GLMMs, binomial error distribution and the logit-link]. Coverage of adult shrubs (m^2). As well as sapling and juvenile density and richness were calculated under the coverage of the focal pine canopy. Saplings and juveniles were identified to the species level and sorted into three categories: (1) young (2-5 years); (2) middle-aged (6-14 years) and (3) old (>15 years old) [LMM, with variables log-transformed when necessary]. Statistical differences are indicated as P < 0.001 (**), $P < 0.0001$ (***), with differences between treatments shown by different lowercase letters. Results correspond to mean ± standard error, N=38 unparasitized, N=38 parasitized and N=15 dead trees for plant establishment estimates, and N=55 unparasitized, N=55 parasitized and N=15 dead trees for all other variables.

creates a gradient of light (Fig 2) and soil-nutrient resources (Mellado et al. submitted) that increases as the host-parasite system matures. Before parasitism, pines grow a dense crown that shades the understory (Fig 2) and show poor soils due to the dominant deposition of recalcitrant pine litter (Mellado et al. submitted). Once parasitism develops, the density of the host crown diminishes (Mellado et al. submitted), allowing more light to penetrate to the understory (Fig 2). At this stage, more light availability coincides with greater soil resources directly provided by the parasite and indirectly by visiting birds (Mellado et al. submitted). Finally, when the host and the parasite die, their tissues detach from the canopy and accumulate on the ground; then, light becomes more intense (Fig 2) and soil nutrients more abundant (Mellado et al. submitted).

Thirdly, seedling establishment and survival of zoochorous species (*Berberis hispanica*) proved more likely in soils under parasitized trees. At these sites, intermediate light availability, coupled to intermediate values of soil nutrients, could be the best scenario for plant recruitment, especially in Mediterranean ecosystems where plants are subject to extreme drought and heat during summer. Otherwise, poor soils and shady conditions under unparasitized pines, and the strong light conditions under dead pines, could be detrimental for these plants at early recruitment phases (Gómez-Aparicio et al. 2008; Matías et al. 2012). On the contrary, seedling establishment of the host pine was extremely low, with no seedling able to survive the first year of life, probably due to summer drought. These results are consistent with those found in similar Mediterranean ecosystems, where seedlings of mesic tree species, such as *Pinus sylvestris*, showed higher rainfall requirements for survival, while shrub species were less affected by summer drought, being able to expand their habitat and colonize open areas and forest gaps (Matías et al. 2012).

Matching the greater and constant seed input and better probabilities for plant establishment, parasitized trees had higher abundance and diversity of zoochorous saplings than did unparasitized ones (Fig. 3). Notwithstanding, saplings reached

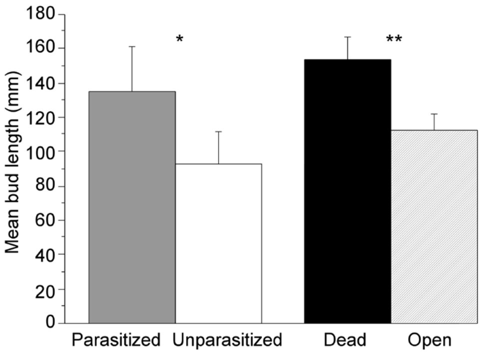


Fig 4. Yearly growth of zoochorous plants growing under the canopy of unparasitized, parasitized and dead trees, as well as in open sites. Understory plants were selected following strict criteria (see Methods) to analyze their growth in different treatments under natural conditions, comparing parasitized with unparasitized trees and dead trees with open sites. At the end of the growing season from 5 to 10 buds per plant species were measured in each treatment, then the average value was calculated and analyzed with linear mixed models, including treatments as a fixed factor and paired trees as the random one, with variables log-transformed when required to meet assumptions of normality and homoscedasticity. Statistical differences are indicated as $P < 0.01$ (*), $P < 0.001$ (**). Results correspond to mean ± standard error, N=24.

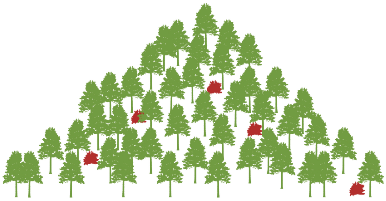
their maximum densities (especially the elder ones) and richness under dead trees (Fig. 3b). Higher abundance of older juveniles could be reflecting the greater plant recruitment occurring when the host was still alive, which growth is promoted after the host death, creating a halo of vegetation around the tree. Older, larger juveniles might then facilitate the establishment and survival of new colonizing plants by sheltering them against adverse abiotic conditions and herbivores (Gómez-Aparicio et al. 2008), a situation that could explain the greater abundance of young saplings under trees at this late stage.

Our results also indicate greater growth of understory plants subject to the influence of the parasite (either dead or alive) than in places lacking it (Fig. 4). An important fact is that plants grew more under dead trees than in open sites, despite that light conditions were similar in both places. In this case, plant growth could be attributed mainly to the effect of soil resources, resulting in plant-soil feedback acting at the community scale, with mistletoe changing soil composition over time in a way that these changes in turn enhance the growth of understory woody plants (Bever et al. 1997; Callaway et al. 2004). In addition, at this late stage, the death of the tree may increase soil-water availability, facilitating the growth of those saplings established underneath, which could now grow better than saplings growing in open sites. Such benefits for productivity resulting from nutrient-rich parasite litter have been previously described for the mistletoe *Amyema miquelii* (March & Watson 2007) and herbaceous parasites such as *Bartsia alpine* and *Rhinanthus minor* (Quested et al 2003; Fisher et al. 2013). However, our study is the first to show that the impact of a parasitic plant can affect biomass of long-lived woody plants, even long after host death, fostering growth of a diverse coterie of zoochorous species replacing a dead pine tree.

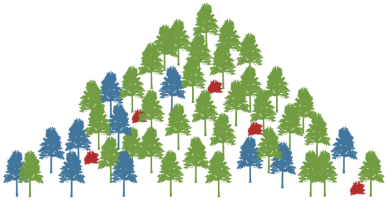
Conclusion

Our findings demonstrate that mistletoes can exert strong and lasting impact on the structure and com-

a) Forest landscape with no mistletoe



b) Initial phase of parasitism



c) Mature phase of parasitism

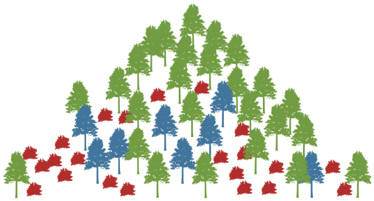


Fig 5. Temporal scheme of mistletoe-driven effects on community assemblage at the landscape scale. *Pinus nigra* are dominant trees in the pine forest studied (a), which coexists with other less represented species such as zoochorous shrubs. When *Viscum album* colonizes a given pine host (b), avian seed-dispersers begin a positive re-infection process that results in the spatial aggregation of the parasite within individual hosts, expanding to close neighborhoods and at larger scales on the landscape. As a consequence, mistletoe effects on the plant community at the individual host might translate to the landscape matching the patchy distribution of the parasite, resulting in a heterogeneous and dynamic forest where patches of unparasitized trees or trees with low parasitic loads are intermingled with patches of intense parasitism that are being replaced by a diverse community of zoochorous species as the host dies (c). Green represents unparasitized trees; red represents zoochorous shrubs, and blue mistletoe parasitized trees.

position of woody plant communities, with parasitized trees acting as centers for establishment and growth of colonizing fleshy-fruited woody-species. Moreover, by coupling detrimental effects on their hosts and facilitative effects on the woody-plant community over long time periods, *Viscum a. austriacum* affects patch dynamics and community succession, promoting the replacement of a host tree by a diverse community of zoochorous plants (Fig. 1). Further, as mistletoe's spatial aggregation occurs at the level of the host, neighborhood, and landscape (Aukema 2004), their local effects may translate at a hierarchy of spatial scales. At the landscape scale, patches of unparasitized trees or trees with low parasitic loads are intermingled with patches of intense parasitism where mistletoe accelerates the replacement of dominant trees by subordinate zoochorous woody-species (Fig. 5). Therefore, mistletoe plays a key role in the organization of forest communities, promoting disproportional changes to their abundance and biomass, leaving an ecological footprint that might be apparently subtle, but very patent in the spatial and temporal organization of the community. Over the long term, after the host and the parasite become locally extinct, different scenarios could be plausible. First, if the host species cannot return to the patch, the system could evolve towards a zoochorous shrub-dominated landscape. Second, if the host is able to return—for instance, facilitated by understory vegetation— then the parasite could re-establish on the new tree, initiating a new vegetation cycle. Thus, mistletoes in the forest could show a similar effect to that of parasitic grasses on prairies (Gibson & Watkinson 1992; Pennings & Callaway 1996), which seem to be "moving through" vegetation across the landscape, shifting patch configuration and dynamics along their route. In doing so, mistletoes constitute a disrupting force of the frequently assumed equilibrium dominating late stages of ecological succession, where the parasite follows a different successional trajectory from that of the non-parasitized matrix, increasing landscape heterogeneity in space and time.

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General discussion

Throughout this thesis we have explored different ecological interactions mediated by the mistletoe *Viscum album* subsp. *austriacum* in Mediterranean pinelands with the aim of achieving a greater understanding of its role in forest ecosystems. First, we analyzed the interaction between mistletoe and its hosts and seed dispersers. We found that *Viscum a. austriacum* shows strong host preferences for the dominant tree (*Pinus nigra*) of the forest community (Chapter I) and that a wide and heterogeneous group of non-specialized birds successfully disperses mistletoe seeds (Chapter II). Second, we analyzed the effect of the parasite at a broader scale. We found that mistletoe, coupled to the parasitic and mutualistic interactions it mediates, promotes important ecological changes in the forest patches where it grows, having important direct and indirect effects on the structure of forest communities and ecosystem processes (Chapters III, IV and V). Mistletoe influences seed dispersal, soil nutrient cycling and productivity, as well as affects the structure and composition of soil microbial and plant communities. Overall, this thesis provides evidence that mistletoe acts as a major driver of both aboveground and belowground properties of forest ecosystems.

Mistletoe tends to enhance heterogeneity in forests, with aggregated patches of infection (and concomitant habitat changes) interspersed between relatively uninfected patches. Such non-uniform spatial distribution is determined by host availability (Hawksworth & Wiens 1996; Shaw et al. 2004; Mathiansen et al. 2008) and the activity of seed dispersers (Aukema & Martínez del Río 2002; Restrepo 1987), and, as described in this thesis, by the environmental variability of the forest canopy (Chapter I). As an important source of spatial heterogeneity, mistletoe, by interacting with other organisms and by causing changes in the environment, defines ecological processes and patterns in the landscape. First, mistletoe affects aboveground properties, such as seed dispersal processes and plant productivity. As frugivorous birds respond to mistletoe patchiness, by visiting parasitized trees preferentially to unparasitized ones, they generate mistletoe reinfection feedbacks within the host, while dispersing seeds of co-fruiting species under the host canopy, shaping the spatial seed-deposition pattern of fleshy-fruited plants in the forest (Chapter III). Otherwise, mistletoe affects overall plant productivity by decreasing host growth through its parasitic habit (Chapter IV) and increasing growth of understory shrubs through multiple environmental changes beneath the host (i.e., light availability, soil fertilization) (Chapter V).

Second, mistletoe has strong direct and indirect effects on belowground properties. Through the massive production of fruit and flowers at the expense of its host (of which a large proportion reach the soil every year) mistletoe does not only ensure its dispersal, but indirectly puts at the disposal of other members of the community nutrients that would otherwise be retained in the pine host for long time. These nutrients return to the soil in form of litter that can be more easily utilized by decomposers, in comparison with the more recalcitrant litter of the host, which is more resistant to degradation (Chapter IV). In addition, organic matter input beneath parasitized trees does not only come from mistletoe and host litter, but from the wide assemblage of birds that feed on mistletoe fruit, which enhances overall nutrient availability inside the host-parasite system with the introduction of allochthonous compounds (Watson 2009; Chapter IV). The increasing diversity of organic compounds supplied to the forest floor is reflected in a greater abundance and functional diversity of the microbial community and higher nutrient availability in the soil. Thus, mistletoe, enhanced by the biotic interactions it mediates, plays an important role in intensifying soil-resource availability, regulating composition, abundance and spatial distribution of soil microorganisms (Chapter IV).

Finally, joining the effects of above- and belowground changes, mistletoe has an important impact on the woody-plant community. Mistletoe leads to greater light infiltration to the forest floor by decreasing host growth, creates local fertilization islands and facilitates seed arrival of understory fleshy-fruited species beneath the host. Consequently, parasitized trees concentrate a richer and more abundant fleshy-fruited plant assemblage than non-parasitized trees, and, in turn, enhance plant growth. Moreover, by coupling detrimental effects on their hosts and facilitative effects on the woody-plant community over long time periods, mistletoe promotes the replacement of a host tree by a diverse community of zoochorous plants.

At larger temporal and spatial scales, a major consequence of mistletoe parasitism is an increase of the structural complexity of the forest. *Viscum a. austriacum* influences the horizontal distribution of forest trees and other vegetation types by affecting the vigor of dominant trees (often reducing their density) and promoting tree replacement by subdominant shrub species (Chapter V). In this sense, mistletoe [as with other parasite systems (Pennings & Callaway 1992; Davies et al. 1997; Pywell et al. 2004)], could positively contribute to forest biodiversity by allowing subdominant species to coexist with a dominant one. In addition, due to the spatial patchiness of the parasite, such mistletoe-induced changes are also heterogeneously distributed, enhancing within-stand heterogeneity.

Another major consequence we could draw of mistletoe parasitism is a redistribution of biomass and energy flow among the multiple components of the forest ecosystem. Instead of being concentrated in the dominant canopy stratum, biomass and productivity is distributed somewhat more evenly among lower vegetation strata. In doing so, mistletoe indirectly enhances resource diversity and creates habitats for other organisms, which might expand the complex web of life that already exists around these parasites (Watson 2001). These changes are likely to exert knock-on effects for other organisms of the community. For example, the coexistence of different vegetation life forms (i.e., trees and shrubs) and different tree conditions (i.e., unparasitized, parasitized and dead pines) within the stand offers a wider variety of resources and the generation of different litter, snags and logs that provide structural complexity to the forest floor and habitat for many soil organisms. Even the acceleration of the host's death might benefit other species, as dead trees constitute a crucial resource directly used by many fungi, insects and vertebrates that depend upon dead or decaying wood (Schiegg 2000; Grove 2002), and indirectly by a countless number of species included in the saproxylic food web.

Thus, mistletoe parasitism may ultimately increase the structural and biological complexity of the forest, modifying the horizontal and vertical distribution of forest vegetation, and probably, the abundance of organisms directly or indirectly associated with these changes. In this sense, *Viscum a. austriacum* could be playing a keystone role in the organization of the forest community, and likely, in maintaining forest diversity. However, further studies should be performed to demonstrate cascading effects of mistletoe-induced changes across trophic levels.

Variation in mistletoe-mediated interactions and ecological consequences

The impact of mistletoe on community and ecosystem properties may vary from that discussed throughout this thesis due to multiple factors. The mistletoe's virulence and the host specificity may differ across environments. As mentioned in Chapter 1, *Viscum a. austriacum* shows different host preferences across its latitudinal distributional range and seems to adapt locally to the dominant host species in a given site. Changes in host preferences also occur along altitudinal gradients. At higher altitudes of the Sierra de Baza, populations of the dominant host *Pinus nigra* coexist with a remnant and abundant population of *P. sylvestris*, where the parasite uses both species as major hosts (Zamora & Mellado *in prep*). Both host species, however, greatly differ in their response to parasitism (Mellado & Zamora *in prep* a). *Pinus sylvestris* has lower tolerance to mistletoe parasitism, dying before reaching intense parasitic loads. Moreover, its reproductive fitness is severely affected, decreasing cone production by about 85 % when parasitized. Otherwise, parasitism does not considerably affect *P. syslvestris* growth. On the contrary, *Pinus nigra* tolerates greater parasitic loads, its reproduction is not as severely affected, but it clearly diminishes its growth as parasitism intensifies. Such different host responses to a shared parasite may change the web of biotic interactions mediated by different mistletoe-host systems (Mellado & Zamora *in prep* b), as well as the final impact on the community and ecosystem (Mellado *et al*. *in prep* a, b). For example, *Pinus sylvestris*, being able to resist lower parasitic loads offer less mistletoe-derived food resources to animal consumers and thus receives less animal visitations, which in turn contribute with lower organic matter input to the soil. In addition, as host growth is not as intensively affected by parasitism, the host contributes with greater proportions of recalcitrant litter to the soil, which may finally result in more subtle fertilization islands than those created beneath *P. nigra*. Furthermore, due to the strong reduction in seed production, *Pinus sylvestris* may have lower chances to regenerate in parasitized stands compared to *P. nigra* (Mellado & Zamora *in prep* a).

Even within the same host species, the virulence of the parasite and community interactions may change along altitudinal gradients or among stands with different characteristics (e.g., stands with greater or lower tree density), combining to produce different parasite effects at different elevations or stand types (Mellado & Zamora *in prep* c). In the Sierra de Baza, where moisture and temperature conditions considerably differ between altitudes, environmental variability may affect host performance and its capacity to resist mistletoe parasitism, as well as determining decomposition rates and thus nutrients return to the soil, which finally influences plant productivity. This variation in impact of mistletoe contributes to enhancing forest heterogeneity as well as its structural and biological complexity.

Conclusion

Beyond the detrimental effect that mistletoe has on the host, here we confirm that there is another side to this story. By taking a broader view of mistletoe and considering a wider breadth of its biotic interactions, we found several direct and indirect facilitative effects of the parasite on different organisms in the community. On the one hand, mistletoe induces significant changes on the growth of the dominant tree (*Pinus nigra*) through its parasitic interaction, while facilitating colonization and establishment of less represented species (zoochorous-shrub species), leading to changes in the configuration of the plant community. On the other hand, through modified organic matter input to the soil and affecting belowground processes, mistletoe exerts facilitative effects on soil microbial communities and also enhances the growth of understory shrubs. Thus, far from being a harmful organism, our findings show that mistletoe can play an important role in regulating the spatial-temporal dynamic of the forest ecosystem. Indeed, it can be positively associated with processes or characteristics that are often regarded as “positive” attributes for ecosystems, such as heterogeneity, diversity and productivity.

Overall, our study constitutes one of the many recently documented systems in which parasites appear to play an important structuring role in natural ecosystems (Hudson et al. 2006; Lafferty et al. 2008; Hatcher & Dunn 2011), contributing to this developing field which is challenging the conventional wisdom that parasites only have a negative impact on ecological communities. Our results are likely to have important implications for the understanding of ecosystems and to the rethinking of the role of mistletoe in the forest.

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Articles in prep

Zamora R. & Mellado A. ‘Range shifts of mistletoe along an altitudinal gradient: dissecting the abiotic and biotic drivers behind the spatial pattern of a parasitic plant’.

Mellado A. & Zamora R. a. Different host responses to a shared parasite: consequences for plant community structure.

Mellado A. & Zamora R. b. Effect of parasitic plants on trophic webs by trait-mediated indirect effects.

Mellado A. & Zamora R. c. Ecological consequences of parasitism are context-dependent.

Mellado A., L. Morillas, A. Gallardo & R. Zamora. a. ‘Complex biotic interactions regulate aboveground-belowground linkages in forest ecosystems’.

Mellado A., L. Morillas, A. Gallardo & R. Zamora. b. ‘Effect of multiple interactions mediated by a parasitic plant on litter decomposition

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