Universidad
Rey Juan Carlos

DOCTORAL THESIS

Trophic cascades and plant-frugivore mutualisms: Ecological consequences of rewilding apex predators on Mediterranean ecosystems

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Table of contents

Resumen / Abstract

Resumen

Antecedentes

Las cascadas tróficas son mecanismos poderosos que estructuran los ecosistemas a través de las redes tróficas, afectando en última instancia a los productores primarios. Los depredadores apicales juegan papeles clave en los ecosistemas y desencadenan efectos en cascada en los niveles tróficos inferiores. Por tanto, dada la reciente recuperación de los grandes carnívoros en todo el mundo a través del *rewilding* activo y pasivo, evaluar los efectos ecológicos potenciales de la reintroducción de grandes depredadores en los ecosistemas es primordial para obtener información sobre el funcionamiento de las redes tróficas y la correcta aplicación de medidas de conservación. Las interacciones planta-animal pueden verse alteradas por la presencia novedosa de los depredadores apicales, afectando potencialmente a funciones relevantes que las especies subordinadas mantienen en los ecosistemas. Por ejemplo, interacciones mutualistas y antagonistas como la zoocoria o la herbivoría pueden resultar afectadas por las cascadas tróficas, con consecuencias contrastantes para el crecimiento, supervivencia y reproducción de muchas poblaciones de plantas. Esto es especialmente importante en los ecosistemas defaunados, donde algunos frugívoros oportunistas juegan el papel de dispersores de semillas legítimos para muchas plantas de fruto grande debido a las históricas y recientes extinciones de megafauna frugívora. Este es el caso de los carnívoros frugívoros, que son dispersores de semillas funcionalmente únicos para muchas plantas mediterráneas con frutos de gran tamaño, pero cuya abundancia y comportamiento es frecuentemente controlado por depredadores más grandes a través de la competencia intra-gremial. Los pequeños mamíferos también juegan un doble papel en las redes tróficas, actuando como presas para los carnívoros, pero también como depredadores de semillas para muchas plantas. Así, la cantidad (es decir, el número de semillas dispersadas) y la calidad (p. ej. supervivencia de semillas o de plántulas) del servicio de dispersión de semillas que brindan los mamíferos frugívoros a las plantas puede verse modificado bajo riesgo de depredación a través de las sucesivas fases post-dispersivas, afectando en última instancia a la demografía de las plantas, la composición de las comunidades y a procesos de regeneración vegetal. El actual escenario de cambio global requiere investigación sobre como las alteraciones ecológicas mediadas por el ser humano afectan al funcionamiento del ecosistema y a las dinámicas de las redes tróficas.

Objetivos

El objetivo principal de esta tesis es proporcionar una perspectiva novedosa de las consecuencias del *rewilding* en el funcionamiento de los ecosistemas, para comprender mejor los potenciales efectos en cascada de la recuperación de las poblaciones de depredadores apicales en las redes tróficas. Específicamente, evaluamos las cascadas tróficas en un ecosistema mediterráneo al comparar zonas con presencia de un depredador apical, el lince ibérico (*Lynx pardinus*), con áreas sin linces, simulando un escenario pre and post-*rewilding*. En el capítulo 1, evaluamos los efectos de arriba hacia abajo del lince ibérico (el mayor depredador terrestre en el sur de España) sobre la abundancia y patrones de actividad de depredadores subordinados, y como las fuerzas de abajo hacia arriba pueden modular la coexistencia entre carnívoros en un contexto de liberación de presas (pequeños mamíferos). En los capítulos 2 y 3 identificamos la existencia de cascadas tróficas mediadas por el comportamiento, desencadenadas por el riesgo de depredación, sobre el comportamiento de frugivoría y los patrones de dispersión de semillas de mesocarnívoros frugívoros. Esta cascada de efectos compromete el servicio clave de dispersión de semillas que proporcionan los mesocarnívoros a la comunidad de plantas mediterráneas de fruto carnoso. Específicamente, en el capítulo 2, evaluamos experimentalmente como las alteraciones comportamentales de los frugívoros determinaron las visitas y el consumo de frutos en el piruétano *(P. bourgaeana),* un árbol principalmente dispersado por carnívoros frugívoros. En el capítulo 3, nos centramos en la comunidad completa de plantas con frutos carnosos y evaluamos si las poblaciones reducidas y los cambios comportamentales de los carnívoros frugívoros afectan negativamente a varios patrones de dispersión de semillas a nivel de comunidad. Finalmente, el capítulo 4 evalúa los efectos en cascada desencadenados por el lince ibérico en el reclutamiento de plántulas, teniendo en cuenta varios procesos posteriores a la dispersión (depredación de semillas, emergencia y supervivencia de plántulas) del ciclo de vida del piruétano. En general, los hallazgos de esta tesis proporcionan un conocimiento valioso para entender el funcionamiento de las redes tróficas en un contexto de *rewilding*, considerando las alteraciones de la biodiversidad que están ocurriendo y que se avecinan (sobre-explotación, defaunación, invasiones, etc.) según el escenario actual de cambio global.

Metodología

A pesar de la recuperación de las poblaciones de lince ibérico en los últimos 20 años, la distribución del lince es aún muy parcheada a lo largo de la Península Ibérica.

Seleccionamos el Parque Natural Sierra de Andújar in el sur de España como área de estudio porque se trata de un bosque mediterráneo bien conservado donde la población de lince ibérico es espacialmente heterogénea, debido a la distribución variable de su presa principal, el conejo de monte (*Oryctolagus cuniculus*). Así, usamos un diseño experimental donde los sitios con presencia de lince emularon un escenario de *rewilding*, mientras que los sitios sin linces fueron controles (5 +5 sitios). Además, el gremio de carnívoros y la comunidad de plantas de fruto carnoso son muy diversas en esta reserva natural, proporcionando el escenario idóneo para evaluar como los efectos en cascada desencadenados por un depredador apical pueden alterar funciones clave del ecosistema, como la dispersión de semillas. Llevamos a cabo todos los experimentos de campo y observaciones comparando los sitios con presencia y ausencia de lince, pero la metodología específica varió con el objetivo específico de esta tesis.

En el capítulo 1, determinamos las abundancias absolutas y los mecanismos espacio temporales de evitación a pequeña escala de dos mesocarnívoros respecto del lince, la garduña (*Martes foina*) y la gineta (*Genetta genetta*), que tienen diferente plasticidad dietética. Colocamos 120 cámaras trampa durante ocho meses en dos años consecutivos e identificamos a los carnívoros individualmente (basándonos en sus patrones de pelaje) para estimar densidades de mesocarnívoros usando modelos bayesianos espacialmente explícitos de captura-recaptura. La disponibilidad de presas también se estimó mediante fototrampeo, para evaluar el solapamiento espacio-temporal entre depredadores y presas. Finalmente, usamos modelos lineales generalizados mixtos para evaluar el efecto del lince y de la disponibilidad de presas sobre (i) la densidad de mesocarnívoros, (ii) el tiempo entre encuentros y (iii) la probabilidad de encuentro entre los mesocarnívoros y el lince.

En el capítulo 2, llevamos a cabo un experimento de campo de 15 días de duración en el que colocamos frutos de piruétano bajo piruétanos silvestres y monitoreamos las visitas y el consumo de frutos por toda la comunidad de carnívoros frugívoros usando cámaras trampa (n = 30), en sitios con y sin linces. Seleccionamos los mejores modelos (según AICc) para evaluar el efecto de la presencia de lince, la disponibilidad de frutos y la cobertura forestal en la (i) tasa de visita, (ii) tasa de consumo, (iii) el consumo de frutos (0/1), (iv) los frutos consumidos por unidad de tiempo y (v) el tiempo empleado por visita.

En el capítulo 3, evaluamos la dispersión de semillas a nivel de comunidad (15 especies de plantas con fruto carnoso) durante dos estaciones de fructificación consecutivas, recogiendo y analizando excrementos de mesocarnívoros (n = 1575). Comprobamos nuestro grado de acierto en la identificación fecal a nivel de especie realizando análisis de ADN mediante *barcoding* genético. Específicamente, evaluamos el efecto de la presencia de lince en (i) la ocurrencia y la biomasa de frutos por excremento, (ii) el número de excrementos con semillas y (iii) la diversidad de semillas movilizada. Los efectos mediados por el tipo de hábitat pueden determinar el reclutamiento de plantas y su supervivencia, especialmente cuando las cascadas tróficas mediadas por el comportamiento llevan a un uso del hábitat diferencial por los dispersores de semillas. Así, consideramos el tipo de hábitat (bosque vs. abierto) y la disponibilidad de frutos en la selección de modelos (según AICc).

Finalmente, en el capítulo 4, usamos la cuantificación de la dispersión de semillas del piruétano incluida en el capítulo 3, y añadimos dos experimentos de campo adicionales para obtener datos de las etapas posteriores a la dispersión del ciclo de vida de este árbol. Llevamos a cabo un experimento de depredación de semillas en dos sitios con presencia y ausencia de lince, donde colocamos depósitos de semillas de piruétano durante 5 noches consecutivas y registramos las tasas de depredación de semillas por roedores. También sembramos semillas de piruétano en un sitio de estudio y monitoreamos la emergencia y supervivencia de plántulas durante un año completo. Consideramos el tipo de hábitat (bosque vs. abierto) y el micrositio de llegada de semillas (arbusto, roca, abierto) in todos los experimentos de campo. Finalmente, calculamos las probabilidades de transición entre las distintas fases vitales del piruétano y, usando modelos de ecuaciones estructurales, analizamos los efectos de las variables exógenas (presencia de lince, hábitat y micrositio) en (i) la dispersión de semillas, (ii) supervivencia de semillas, (iii) emergencia de plántulas y (iv) supervivencia de plántulas.

Resultados

Capítulo 1: El lince ibérico redujo la abundancia de mesocarnívoros hasta 10 veces, pero la fuerza de este efecto de supresión fue específica de la especie. La especie de carnívoro con un espectro alimenticio más amplio, la garduña, prácticamente despareció de los territorios del lince, mostrando una elevada segregación espacial y temporal con el depredador apical. Mientras tanto, los mecanismos de evitación a escala fina permitieron a la gineta persistir en baja densidad en los territorios de los linces, probablemente aprovechando la alta disponibilidad de su presa preferida (es decir, los roedores) en los sitios con linces. Así, las fuerzas de abajo hacia arriba parecieron ser también importantes en la red trófica al facilitar la coexistencia entre depredadores.

Capítulo 2: Los carnívoros frugívoros alteraron su actividad de búsqueda de alimento en presencia de lince, visitando menos a menudo los piruétanos y comiendo menos frutos. El riesgo de depredación no afectó a la tasa de visita y consumo del tejón (*Meles meles*), pero no se registró ninguna visita de garduña en sitios con linces. El zorro rojo fue el dispersor de semillas más frecuente, aunque los depredadores de semillas y consumidores de pulpa (p. ej. el ciervo) consumieron una gran cantidad de frutos. El comportamiento de forrajeo de los zorros se alteró en presencia de lince ya que consumieron menos frutos por unidad de tiempo y realizaron visitas más cortas. La abundancia local de frutos, la cobertura forestal y la variación intra-específica también fueron variables importantes para entender el comportamiento de forrajeo de los zorros en un paisaje del miedo.

Capítulo 3: La cantidad y la diversidad de semillas dispersadas por carnívoros frugívoros fue drásticamente reducida bajo riesgo de depredación, pero el tamaño del efecto varió entre las especies de mesocarnívoros. El servicio de dispersión de semillas brindado por las garduñas fue el más afectado por el lince debido a una marcada reducción de la cantidad de excrementos con semillas (93%) y diversidad de semillas dispersadas (46%). El número de excrementos de zorro con semillas también disminuyeron en presencia de lince, pero la diversidad de plantas movilizada fue más equilibrada entre escenarios de riesgo de depredación debido al reemplazo de especies. Las garduñas duplicaron la biomasa de frutos por excremento bajo riesgo de depredación y centraron su dieta frugívora en la especie de fruto preferida para los carnívoros, el madroño (*A. unedo*). Sin embargo, deberíamos tomar estos resultados con cautela debido al reducido tamaño de muestra para la garduña en escenarios con lince, atribuible a la drástica reducción en su abundancia. De manera remarcable, los zorros dispersaron un 68% menos semillas en hábitats abiertos cuando coexistieron con el lince, probablemente llevando a una contribución diferencial en la efectividad de la dispersión de semillas entre hábitats, basada en las condiciones ambientales más favorables para cada especie de planta.

Capítulo 4: Ya que la dispersión de semillas se redujo bajo riesgo de depredación (hasta un 80%) y se sesgó hacia hábitats forestales dado el patrón de dispersión de semillas del zorro, encontramos una extremadamente baja supervivencia de plántulas de piruétano en presencia de lince (1%) respecto a escenarios de ausencia de lince (8%). Este árbol mediterráneo es principalmente dispersado por mamíferos carnívoros y normalmente crece en paisajes abiertos, lo que favoreció la supervivencia de las plántulas de primer año en nuestro experimento. Como consecuencia de las elevadas condiciones de estrés hídrico e insolación de los hábitats abiertos en nuestra área de estudio, la supervivencia de plántulas de esta especie se vio favorecida bajo plantas nodrizas (es decir, arbustos). Sin embargo, este micrositio de llegada de las semillas mostró las tasas de depredación por roedores más altas. No encontramos un efecto directo de la presencia de lince sobre la depredación de semillas. No obstante, los efectos mediados por el hábitat y el microhábitat sobre los procesos posteriores a la dispersión combinados con el marcado patrón de dispersión de semillas de los mesocarnívoros, contribuyeron a llevar a las semillas de piruétano hacia sitios de baja calidad, especialmente en escenarios de presencia de lince.

Conclusiones

El escenario de *rewilding* de grandes depredadores en todo el mundo puede desencadenar cascadas tróficas sobre niveles tróficos inferiores, afectando en última instancia a la comunidad de plantas de fruto carnoso principalmente dispersada por carnívoros subordinados. La recuperación del lince ibérico a lo largo de la Península Ibérica puede revertir la potencial liberación de mesodepredadores en zonas donde este depredador apical fue previamente extinguido o controlado, sin embargo, la fuerza de este efecto supresor es probablemente específico de la especie. Los depredadores más flexibles en su dieta mostraron una alta segregación espacial y temporal con este superdepredador, incluso llevando a la exclusión de frugívoros importantes como las garduñas de los territorios de los linces. Sin embargo, los mesodepredadores con un nicho trófico más estrecho, coexistieron en abundancia reducida con el lince ibérico cuando la disponibilidad de las presas preferidas fue alta. La abundancia de pequeños mamíferos fue elevada dentro de los territorios de los linces, facilitando así la coexistencia entre depredadores bajo un patrón de regulación de abajo hacia arriba en la cadena trófica. No obstante, el escenario actual de restauración de las poblaciones de lince podría también alterar el comportamiento de alimentación de frugívoros clave. Específicamente, los zorros visitaron menos a menudo árboles frutales, consumieron menos frutos y emplearon menos tiempo buscando alimento bajo riesgo de depredación, resultando en una dispersión de semillas menos eficiente en escenarios de presencia de lince. Consecuentemente, este efecto supresor y la creación de un paisaje del miedo en el escenario de *rewilding* causó una reducción en la dispersión y la diversidad del ensamblaje completo de plantas de fruto carnoso dispersadas por la comunidad de carnívoros frugívoros. Sin embargo, los efectos compensatorios por otros frugívoros podrían equilibrar la falta del servicio de dispersión de semillas por frugívoros importantes cuantitativamente, pero no cualitativamente, debido a la diversidad funcional entre frugívoros. Este es el caso del piruétano, cuya dispersión de semillas por mamíferos carnívoros es drásticamente reducida y sesgada hacia hábitats no adecuados (es decir, bosques) para la supervivencia de plántulas en presencia de lince. Además, la depredación de semillas por roedores fue mediada por el micrositio de llegada de semillas (baja escala espacial) en lugar de por el hábitat de llegada (alta escala espacial), resultando en mayores tasas de depredación de semillas en el micrositio más adecuado para la supervivencia de semillas y revelando un conflicto semilla-plántula en el ciclo de vida de este árbol mediterráneo. Sin embargo, los escenarios de *rewilding* de grandes depredadores podrían estar favoreciendo a aquellas plantas más sensibles a la insolación o al estrés hídrico en ecosistemas mediterráneos, al desplazar a los frugívoros y la dispersión de semillas hacia hábitats forestales de bajo riesgo. Así, considerar el sitio de llegada de las semillas cuando se evalúa la efectividad de la dispersión de semillas es crucial para estudiar los efectos potenciales de reintroducir depredadores a lo largo de paisajes heterogéneos. Los especialistas de la conservación deberían considerar las interacciones complejas de las comunidades y promover una distribución heterogénea de los depredadores apicales, lo que probablemente lleve a efectos netos para los diferentes niveles tróficos en los ecosistemas.

Summary

Background

Trophic cascades are powerful mechanisms structuring ecosystems across food webs, ultimately affecting primary producers. Apex predators play key roles in ecosystems and can trigger cascading effects across lower trophic levels. Therefore, given the recent recovery of large carnivores worldwide through active and passive *rewilding*, assessing the potential ecological effects of reintroducing top-order predators in ecosystems is paramount to gain insights on the functioning of trophic webs and the correct enforcement of conservation measures. Plant-animal interactions can be altered by the novel presence of apex predators, potentially affecting relevant functions that subordinate species maintain in ecosystems. For instance, mutualistic and antagonistic interactions such as zoochory or herbivory can be affected by trophic cascades, with contrasting consequences for the growth, survival and reproduction of many plant populations. This is especially important in defaunated ecosystems, where some opportunistic frugivores play the role of legitimate seed dispersers for many large-fruited plants due to the historical or recent extinction of frugivore megafauna. Such is the case of frugivorous mesocarnivores, which are functionally unique seed dispersers for many Mediterranean large fleshy-fruited plants, but whose abundance and behaviour is often controlled by larger predators via intra-guild competition. Small mammals also play a double role in trophic webs, acting as prey for subordinate carnivores but also as seed predators for many plants. Thus, the quantity (i.e. number of dispersed seeds) and the quality (e.g., post-dispersal seed survival, seedling survival) of the seed dispersal service that mammal frugivores provide to plants may be modified under predation risk across the successive post-dispersal stages, ultimately affecting plant demography, community composition and forest regeneration processes. The current global-change scenario demands research on how human-mediated ecological changes affect ecosystem functioning and the entire dynamics of food webs.

Objectives

The main purpose of this thesis is to provide a novelty perspective of *rewilding* consequences on ecosystem functioning, to better understand the potential cascading effects of the recovery of apex predator’s populations on trophic webs. Specifically, we assess trophic cascades in a Mediterranean ecosystem by comparing areas with presence of a top predator (Iberian lynx, *Lynx pardinus*), to areas without lynxes, simulating a pre and post-*rewilding* scenario. In chapter 1, we evaluate the top-down effects of the Iberian lynx (the largest terrestrial predator in Southern Spain) on the

abundance and activity patterns of subordinate predators, and how bottom-up forces can modulate carnivore co-existence in a context of prey release (small mammals). Chapter 2 and 3 identify the existence of behaviorally-mediated trophic cascades triggered by predation risk on the foraging behaviour and seed dispersal patterns of frugivorous mesocarnivores. Such cascade of effects compromises the key seed dispersal service provided by mesocarnivores to the fleshy-fruited community of Mediterranean plants. Specifically, in chapter 2, we experimentally assess how behavioural shifts of frugivores determined the visitation and fruit consumption in fruiting *P. bourgaeana* trees, a fleshy-fruit tree mostly dispersed by frugivorous carnivores. In chapter 3, we focus on the entire community of fleshy-fruited plants and evaluate whether reduced populations and behavioural shifts of frugivorous carnivores negatively impact on several seed dispersal community patterns. Finally, chapter 4 evaluates the cascading effects triggered by the Iberian lynx on seedling recruitment, accounting for several post-dispersal process (seed predation, seedling emergence and seedling survival) of the life cycle of *P. bourgaeana*. Overall, the findings of this thesis provide valuable knowledge to understand the functioning of trophic webs in a context of apex predator *rewilding*, considering the ongoing and oncoming drivers of biodiversity alterations (overexploitation, defaunation, invasions, etc.) conforming the current global change scenario.

Methodology

Despite the recovery of Iberian lynx populations over the last 20 years, lynx range is still very patchy throughout the Iberian Peninsula. We selected Sierra de Andújar Natural Park in Southern Spain as study area because is a well-preserved Mediterranean forest where the lynx population is spatially heterogeneous, due to the variable distribution of its main prey, the wild rabbit (*Oryctolagus cuniculus*). Thus, we used an experimental sampling design where lynx presence localities emulated a *rewilding* scenario meanwhile absence localities were used as controls (5 + 5 localities). Furthermore, the carnivore guild and the fleshy-fruited plant community are highly diverse in this Natural Park, providing the suitable scenario to assess how cascading effects triggered by an apex predator can alter key ecosystem functions, such as seed dispersal. We carried out all the field experiments and observations comparing presence and absence localities of our study area, but the specific methodology varied with the specific objective of this thesis.

In chapter 1, we determined the absolute abundance and fine-scale spatiotemporal avoidance mechanisms respect to the Iberian lynx of two sympatric mesocarnivores, the stone marten (*Martes foina)* and the common genet (*Genetta genetta*), with different dietary plasticity. We placed 120 camera-traps for eight months in two consecutive years and identified carnivores individually (based on their coat patterns) to estimate mesocarnivore densities using spatially explicit capture-recapture Bayesian models. Prey availability was also estimated from camera-trapping data, in order to assess spatio-temporal overlapping among predators and prey. Finally, we used generalized linear mixed models to evaluate the top-down effect of the Iberian lynx presence and the bottom-up effect of prey abundance on (i) the density of mesocarnivores, (ii) time-to-encounter and (iii) encounter probability among mesopredators and lynx.

In chapter 2, we carried out a 15-days field experiment in which we placed *P. bourgaeana* fruits beneath fruiting trees and monitored visitation and fruit removal by the entire community of frugivorous carnivores using camera-traps (n = 30), in localities with presence and absence of lynx. We selected the best models (based on AICc) to assess the effect of the presence of lynx, the cropsize and the vegetation coverage on the (i) visitation rate, (ii) consumption rate, (iii) consumption/non consumption, (iv) fruits consumed per time and (v) time spent per visit.

In chapter 3, we assessed seed dispersal at the fleshy-fruited plant community level (15 plant species) during two consecutive fruiting seasons by collecting and analysing mesocarnivore scats (n = 1575). We checked our visual faecal species identification success by conducting barcoding DNA analysis. Specifically, we assessed the effect of lynx presence on (i) fleshy-fruit occurrence and biomass per scat, (ii) number of scats with seeds and (iii) diversity of seeds mobilised. Habitat-related effects can determine plant recruitment and survival, especially when behaviourally-mediated trophic cascades drive to differential habitat use by seed dispersers. Thus, we considered habitat (forest vs. open) and fruit availability in model selection (based on AICc).

Finally, in chapter 4, we used the seed dispersal quantification for *P. bourgaeana* included in Chapter 3, and we added up two additional field experiments to obtain data for the post-dispersal stages of the tree life-cycle. We conducted a seed predation experiment in two localities with lynx presence and lynx absence, where we placed *P. bourgaeana* seed depots during 5 consecutive nights and recorded the seed removal rates by rodents. We also sowed *P. bourgaeana* seeds in one study locality and monitored the seedling emergence and survival rates during a whole year around. We considered habitat (forest vs. open) and seed-arrival microsite (shrub, rock and open) in all field experiments. Lastly, we calculated transition probabilities among vital phases of *P. bourgaeana* and, using path analysis, analysed the effects of exogenous variables (lynx presence, habitat and microsite) on (i) seed dispersal, (ii) seed survival, (iii) seedling emergence and (iv) seedling survival.

Results

Chapter 1: The Iberian lynx reduced mesopredator abundance up to 10 times, but the strength of this top-down effect was highly species-specific. The mesopredator with a broader food spectrum, the stone marten, practically disappeared from lynx territories, showing a high spatial and temporal segregation with the apex predator. Meanwhile, fine-scale avoidance mechanisms allowed the genet to persist in low density inside lynx territories, probably taking advantage of the high availability of its preferred prey (i.e. rodents) inside lynx territories. Thus, bottom-up forces also seemed to be important in the trophic web by facilitating coexistence among predators.

Chapter 2: Frugivorous carnivores altered their foraging activity in presence of lynx, visiting less often *P. bourgaeana* fruiting trees and eating less fruits. Predation risk did not affect badger (*Meles meles*) fruit consumption and visitation, yet no visits of stone marten were recorded inside lynx territories. The red fox was the most frequent seed disperser, although seed predators and pulp-feeders (e.g. red deer) consumed a greater amount of fruits. The foraging behaviour of foxes was also altered by lynx presence whereby they consumed less fruit per unit of time in shorter visits. Local availability of fruit resources, forest coverage and intra-specific variation also were important variables to understand the foraging behaviour of foxes in a landscape of fear.

Chapter 3: The quantity and diversity of dispersed seeds by frugivorous carnivores were drastically decreased under predation risk, but the effect size differed among mesocarnivore species. The seed-dispersal service provided by martens was the most affected by lynx due to a markedly reduction of the quantity of scats with seeds (93%) and diversity of dispersed seeds (46%). The number of scats with seeds of foxes also decreased in lynx presence, but the diversity of plants mobilised was more balanced among predation risk scenarios due to species turnover. Stone martens duplicated the fruit biomass per scat under predation risk and focused its frugivory diet towards the preferred fruit resource for carnivores, the strawberry tree (*A. unedo*). However, we should take cautiously such findings due to the reduced sample size for the stone marten in lynx scenarios, attributable to a drastic decrease in abundance. Importantly, foxes dispersed a 68% fewer seeds in open habitats when co-existed with lynx, probably leading to a differential contribution to seed-dispersal effectiveness among habitats, based on the most favouring environmental conditions for each plant species.

Chapter 4: Since seed dispersal decreased under predation risk (up to 80%) and was biased towards forest habitats owing the red fox seed dispersal pattern, we found extremely low *P. bourgaeana* seedling survival in lynx presence (1%) respect to lynx absence scenarios (8%). This Mediterranean tree is mainly dispersed by mammal carnivores and grows often in open landscapes, which favoured first year seedling survival in our experiment. As consequence of the high insolation and water-stress conditions linked to open habitats in our study area, the survival of seedlings of this plant species was favoured under nurse plants (i.e. shrubs). However, this seed-arrival microsite showed the highest rate of rodent seed predation. No direct effect of lynx presence was found for post-dispersal seed predation. Nevertheless, microhabitat- and habitat-mediated effects on post-dispersal processes combined with the markedly seed dispersal pattern of carnivores, contributed to drive *P. bourgaeana* seeds towards low-quality seed-arrival sites, especially in lynx presence scenarios.

Conclusions

The *rewilding* scenario of large predators worldwide can trigger trophic cascades on lower trophic levels across food webs, ultimately affecting the community of large-fruited plants mainly dispersed by subordinate carnivores. Iberian lynx recovery across the Iberian Peninsula can reverse the potential mesopredator release scenario in areas where this apex predator was previously extinct or decimated, however the strength of the top-down suppression is likely species-specific. Dietary-flexible mesopredators, showed high spatial and temporal segregation with the apex predator, even leading to the exclusion of key frugivorous carnivores such as stone martens from lynx territories. However, mesopredators with a narrower trophic niche breadth, co-existed in reduced density with the Iberian lynx when the availability of the preferred prey was high. Small mammal abundance was high inside lynx territories, facilitating co-existence among predators under a bottom-up regulation pattern. Nevertheless, the current scenario of restoration of lynx populations could also alter the foraging behaviour of key mammal frugivores. Specifically, foxes visited fruiting trees less often, consumed less fruit and spent less time searching for fruits under predation risk, resulting in less efficient seed dispersal in lynx presence scenarios. Consequently, this top-down suppression and the creation of a landscape of fear in the *rewilding* scenario caused a reduction on the seed dispersal and plant diversity of the entire assemblage of large-fruited plants dispersed by the community of frugivorous carnivores. However, compensation effects by other frugivores could balance the lack of the seed dispersal service of key frugivores quantitatively but not qualitatively, due to functional diversity among frugivores. This is the case of *P. bourgaeana*, whose seed dispersal by mammal carnivores is drastically reduced and biased towards non-suitable habitats (i.e. forests) for seedling survival in presence of lynx. Furthermore, seed predation by rodents was microsite-mediated (low spatial scale) instead of habitat-mediated (high spatial scale), resulting in higher seed predation rates in the microsite most suitable for seedling survival and revealing a seed-seedling conflict in the life-cycle of this Mediterranean tree. Nevertheless, apex predator *rewilding* scenarios could be favouring those plant species more sensitive to insolation and water-stress in Mediterranean ecosystems, by displacing frugivores and dispersal towards low-risk forest habitats. Thus, considering seed-arrival site when assessing seed dispersal effectiveness has been demonstrated to be crucial to study the potential effects of reintroducing apex predators across heterogeneous landscapes. Conservation practitioners should consider complex community interactions and promote a heterogeneous distribution of apex predators, likely resulting in balanced net effects for the different trophic levels in ecosystems.

General introduction

Background

Current global change drivers for biodiversity

A primordial objective of conservation biology is to ensure the persistence of biodiversity and ecosystem functioning in the face of the current context of global change. During the 21st century, new predictions on the trajectory of biodiversity have become increasingly important due to new emerging threats (Pereira et al. 2010, O'Connor et al. 2020). Consequently, the analysis of present and future potential drivers of biodiversity alterations is paramount to conservation planning in a world of trade-offs (Mace 2010). Impactful factors on ecosystems are often a complex chain of synergistic components interacting with socio-economic scenarios, making difficult their interpretation by separate (Didham et al. 2007, Brook et al. 2008). However, several direct and indirect drivers have been described in previous research. The most important are relatively well-known and have been traditionally grouped into five major categories: land use change, overexploitation, impacts of invasive species, pollution and climate change (Purvis et al. 2000, Mace 2010, Jaureguiberry et al. 2022). Most of them are related in some way to globalization and human-footprint, impacting directly or indirectly on species and ecosystems (Di Marco et al. 2018, Williams et al. 2020). Effective adaptation to cope with the global change context that threaten the worldwide biodiversity is necessary to warrantee the persistence of species and ecosystem services (Armsworth et al. 2015). However, the global application of conservation actions requires clear knowledge of the drivers, their interactions, and how they impact the changing dynamics of ecosystems and ecological communities (Sage 2020).

Global change drivers can initiate secondary responses in ecosystems that cascade along the different ecological levels which compound food webs, magnifying the initial impact on biodiversity. This is particularly important in case that ecological disturbances affect apex consumers, triggering reverberating effects on the successive lower trophic levels, and ultimately reaching primary producers (Schmitz et al. 2004, Choquenot and Forsyth 2013). This top-down process across food webs is known as a trophic cascade, and is considered as a powerful mechanism structuring ecosystems in natural but also in altered landscapes (Terborgh et al. 2010, Estes et al. 2011, Ripple et al. 2016). Estes and Palmisano (1974) described for the first time trophic cascades in natural marine ecosystems, revealing that sea otters reduced urchin abundance and overconsumption of kelp forests. Hence, the current global change scenario can promote the arise of novel trophic cascades trough of a great variety of triggering drivers, such as overexploitation or the invasion of exotic species (Wallach et al. 2015). For instance, the unleashing of trophic cascades due to overfishing and its ecological impacts across food webs has been widely documented in oceanic ecosystems, with the potential to alter marine communities from large predators fish down to phytoplankton (Murdoch et al. 2007, Heithaus et al. 2008, Ferretti et al. 2010). Novel assemblages of native and introduced species have also promoted top-down effects in terrestrial and marine ecosystems, affecting multi-trophic interactions among predators, prey and primary producers (Wallach et al. 2015). In this line, Wallach et al. (2010) showed that a non-native predator promoted the extinction of several Australian mammals due to the human-mediated extirpation of the legitimate apex predator. Although studies on ecological alterations driving novel trophic cascades are increasing recently (Ostfeld and Holt 2004, Vavra et al. 2007, Anderson et al. 2009), research is still insufficient due to the wide range of ecological responses among species, ecosystems and contexts.

Ecological implications of rewilding apex predators

Apex predators are at global depletion state as a result of past massive extinctions such as the late Pleistocene extinction, that implied the disappearance of many megafauna species (Zeller and Göttert 2021). More recently, the exploitation of predator species has driven many of modern large carnivores at high risk of extinction worldwide, with important consequences for ecological functioning (Wolf and Ripple 2018). Habitat loss and fragmentation, coexistence with humans (often due to livestock-related conflict), prey depletion, trophy hunting or utilisation of body parts for traditional medicine are among the current most important threats for large carnivores across the globe (Ripple et al. 2014a, Wolf and Ripple 2018). In total, 64% of these species are highly endangered currently and many of their populations have suffered range contractions (Wolf and Ripple 2018). As a result, most of large carnivores have lost more than 50% of their historical ranges in the last 500 years (Wolf and Ripple 2017). Nevertheless, large carnivores are paramount for the maintenance of healthy ecosystems, delivering economic and ecosystem services via direct and indirect pathways. They contribute to maintain global biodiversity, affecting several ecosystem processes such as scavenger subsidies, disease dynamics, carbon storage, stream morphology, and crop production (Ripple et al. 2014a). Thus, the maintenance or recovery of ecologically effective densities of large carnivores is an important tool for maintaining the structure and function of diverse ecosystems. In this scenario of decreasing populations of large carnivores worldwide, active *rewilding* —which is based on reintroducing species into their former ranges through planned conservation programs— has become a necessary and successful conservation tool (Wolf and Ripple 2018). This strategy aims to save carnivore populations from extinction and to restore the top-down ecological regulation in ecosystems. However, the natural recovery of the former distribution range when the main threats have been mitigated is also contributing to enhance the conservation status of large carnivores worldwide throughout passive *rewilding*. In any case, several large carnivores such as the wolf (*Canis lupus*), the brown bear (*Ursus arctos*) or several species of lynx (Eurasian lynx *Lynx lynx* and Iberian lynx *Lynx pardinus*) have recovered in large major portions of their former ranges worldwide (Fig. 1) (Chapron et al. 2014, Ingeman et al. 2022). Nevertheless, the novel presence of top-order species in food webs has increased the attention of ecologists and conservation practitioners towards the potential cascading effects on ecosystems (Ritchie et al. 2012, Ripple et al. 2016). Reintroducing apex predators potentially would restore the ecosystem functions prior extinction, but a novel trophic cascade can trigger unexpected top-down effects across food webs in ecosystems where apex predators were lost for a long time (Schmitz 2006, Winnie and Creel 2017).

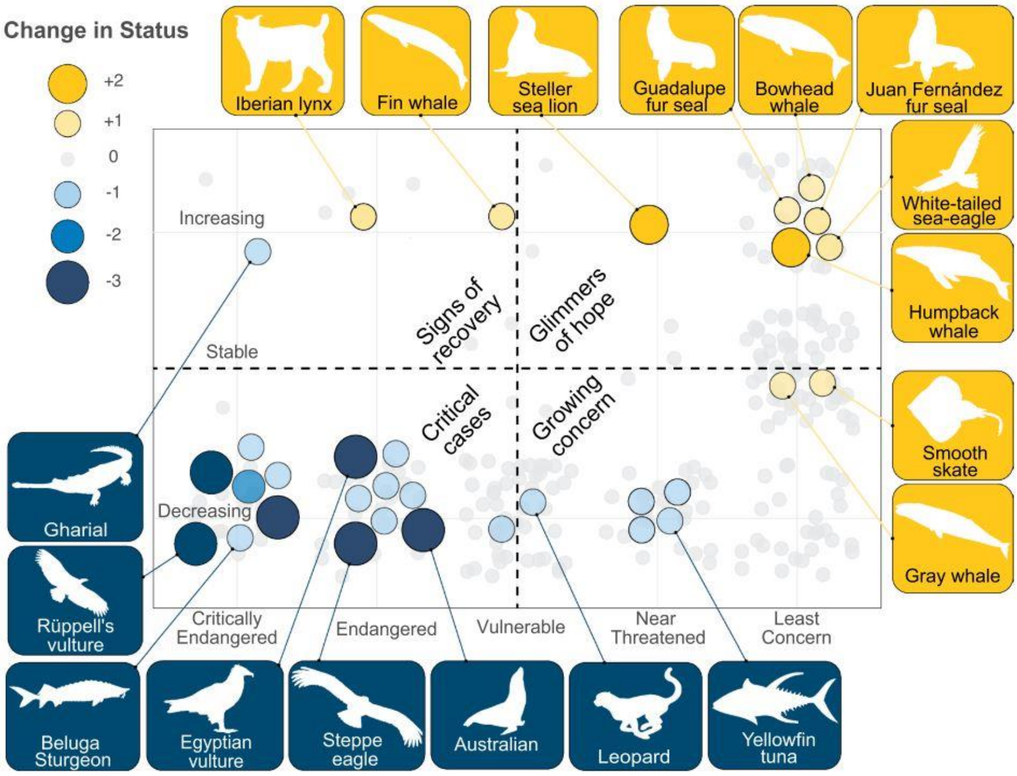

Figure 1. Distribution of large carnivore species across categories of current IUCN status (x-axis) and population trend (y-axis). Improvements in status are indicated by gold and declines by blue, with bubble size indicating the number of status category changes. The majority of species have not undergone any changes in status (shown in light gray). Note: No change in status may indicate lack of recent assessment, insufficient data, or, in the case of species designated Least Concern, effective conservation efforts. Figure extracted from Ingeman et al. (2022).

Pioneer research revealed direct killing by apex predators causes reductions in herbivore population sizes, which often translates into positive cascading effects for primary producers (Estes 1996, Terborgh et al. 1999). However, the indirect relationships between different trophic levels can be complex and context-dependent. For instance, increased killer whale predation in nearshore marine ecosystems was the likely cause of sea otter decline, with the subsequent deforestation of kelp forests due to an increase in sea urchin abundance (Estes et al. 1998). Regarding terrestrial ecosystems, it was not until 1995 the assisted reintroduction of the grey wolf in Yellowstone National Park (USA) provided the ideal scenario for experimentally demonstrating trophic cascades (Smith et al. 2003, Ripple and Beschta 2005, 2007). Ripple and Beschta (2012a) revealed a tri-trophic cascade showing that woody riparian plants increased recruitment after 15 years of wolf reintroduction, growing taller and increasing canopy cover due to a browsing decrease by elks over time. Thus, trophic cascades can also occur by non-lethal interactions through a behavioural change in response to a perceived predation risk, which is reported as behaviourally-mediated trophic cascades (Schmitz et al. 1997, 2004). Apex predators promote a landscape of fear— an animal's perception of spatial variation in predation risk (Gaynor et al., 2019)— that not only alters abundance, but also the foraging behaviour of subordinate species (Fig. 2) (Laundré et al. 2010). Nevertheless, the ecological consequences of these kind of anti-predatory behaviours are scarcely documented (but see Ripple and Beschta 2004) in spite of their potential impact on the ecosystem functioning in a context of apex predator *rewilding*.

Coexistence among predators in a landscape of fear

Although predator–prey interactions usually involve carnivore-herbivore relationships, subordinate carnivores (i.e. mesocarnivores or medium-sized carnivores) can also be susceptible to predation risk when coexist with larger predators (Holt and Polis 1997). Apex predators often control abundances of smaller predators by inter-specific killing, as a result of exploitative competition for resources or intra-guild predation (Polis et al. 1989, Linnell and Strand 2000, Caro and Stoner 2003). Oppositely, ecosystems where top-order predators have been extirpated or their abundance has been severely reduced are usually in a mesopredator release state (Crooks and Soulé 1999, Newsome et al. 2017). Nevertheless, coexistence among carnivores can be facilitated by several factors according to the coexistence theory (Schoener 1974). Sympatric carnivores can avoid intra-guild competition by performing behaviourally adaptations, mostly by spatial, temporal or dietary niche segregation (Karanth et al. 2017, Ferreiro-Arias et al. 2021). Subordinate species are often restricted to safer but less productive habitats as a result of asymmetric competition with larger predators (Sergio and Hiraldo 2008, Di Bitetti et al. 2010, Gallagher et al. 2017), especially when resources are limited (Newsome et al. 2017). Furthermore, mesopredator suppression may be amplified outside protected areas, because fear of large carnivores can drive mesopredators into even more risky areas due to human influence (Prugh et al. 2023). Consequently, this intensive foraging at the periphery of the dominant predator ranges, can lead to marked increases of the local abundance of small prey species in the core areas of apex predator territories (Letnic et al. 2012, Jiménez et al. 2019). In this line, Miller et al. (2012) showed that in a *rewilding* scenario, rodent numbers increased particularly in the surroundings of wolf dens, probably due to an overall decreased predation pressure resulting from mesopredator control. Moreover, previous studies have suggested that prey abundance and preferences can also drive coexistence patterns as a bottom-up force structuring carnivore communities (Santos et al. 2019, Ferreiro-Arias et al. 2021). Despite the most evident cascading effects across trophic webs are mediated by herbivores, little is known about the potential top-down effects of reintroducing apex predators on plants via intra-guild interactions of carnivores, which are also likely but much less known (but see Estes et al. 1998; Bartel and Orrock 2021; Roffler et al. 2023).

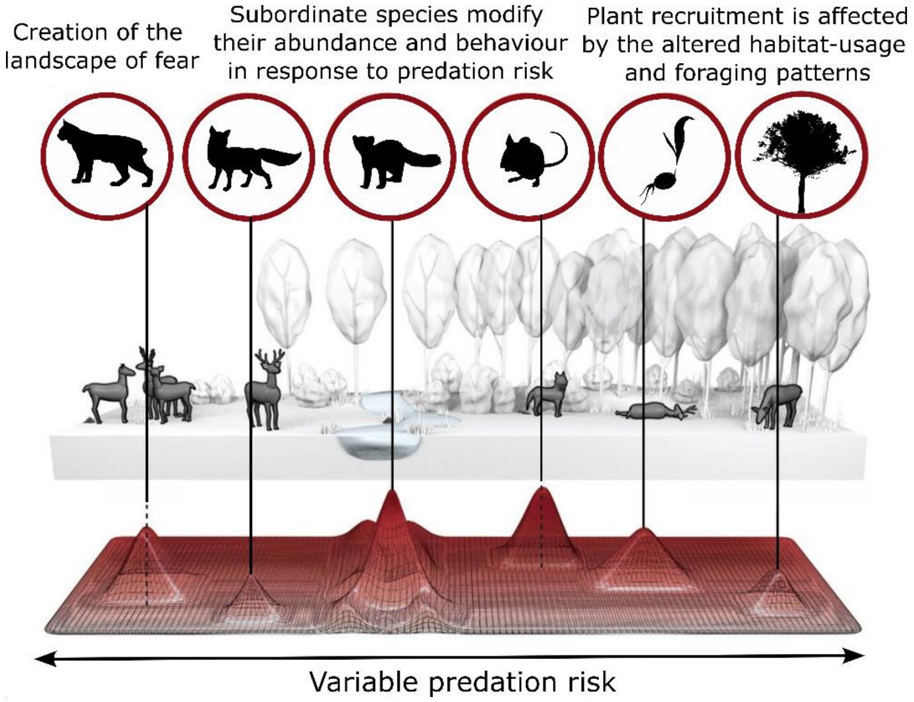

Figure 2. Illustration of the differential responses of subordinate species to the creation of the landscape of fear after apex predator reintroductions. Behavioural alterations respond to the variable predation risk across the landscape (predation risk peaks), modulated by the different perception of risk among habitats. Such shifts trigger alterations in the foraging and habitat-use patterns of herbivores but also of subordinate competitors, which affect plant recruitment and population dynamics. Figure adapted from Weinstein et al. (2018).

Frugivory and seed dispersal by carnivores

Mutualistic interactions that plants maintain with animals are also susceptible to be altered by trophic cascades, such as plant-frugivore mutualisms (Cochrane 2003, Kurten 2013, Rogers et al. 2021). Therefore, *rewilding* apex predators could not only limit the abundance and alter the behaviour of mesopredators but also affect their relevant ecological role as seed dispersers. Mammal carnivores are often generalist and opportunistic predators able to feed on a wide variety of food resources, among which are fleshy-fruits (Herrera 1989, Rosalino et al. 2010). They release seeds on optimum conditions for germination and recruitment (Escribano-Ávila et al. 2013, Draper et al. 2022), contributing to long-distance dispersal events (González-Varo et al. 2013). Furthermore, carnivores have important functional differences respect to other important groups of frugivores. Firstly, they are non-migrant animals and potentially can feed on the whole available fruit diversity independently of ripe phenology, in contrast to migrant frugivore birds (López-Bao and González-Varo 2011). Secondly, although most fleshy-fruited plant species often interact with a diverse guild of frugivores (Jordano 2000), large-fruited plants imposes significant physical constraints for fruit consumption and seed dispersal for the remain frugivore species in altered ecosystems, drastically reducing the availability of effective dispersal agents (González-Varo et al. 2015, Escribano-Avila et al. 2018). Here, abundant generalist seed dispersers can dampen the loss of specialized frugivores, being essential for the persistence of the community dispersal service (Guimarães et al. 2008, Rumeu et al. 2017, Escribano-Avila 2019). In defaunated ecosystems lacking of frugivore megafauna, generalist and opportunistic frugivores such as mammalian carnivores can take over the role of primary seed dispersers for many large-fruited plants which have lost their legitimate seed dispersers (Guimarães et al. 2008, Draper et al. 2022). It is true that other groups of large mammals such as ungulates can also ingest the whole large fruits and continue to disperse certain megafaunal-dispersed plants, but their digestive systems are very restrictive and often cause damages to seeds (Spengler 2019), contrarily to the minimal processing caused by carnivore consumption (Fedriani and Delibes 2009a, Perea et al. 2013). Last but not least, carnivores are often highly mobile and show relatively long seed retention times, often leading to disperse many seeds to long distances into a single deposition (González-Varo et al. 2015). Thus, a growing number of studies are focusing on carnivoran frugivory and seed dispersal in temperate, Mediterranean and tropical forests worldwide and new insights are increasing in short time (Draper et al. 2022, Nakashima and Do Linh San 2022).

Cascading impacts of apex predators on seed dispersal effectiveness

Recent and past defaunation processes have been demonstrated to alter mutualistic interactions and ultimately impact plant recruitment (Cordeiro and Howe 2003, Wotton and Kelly 2011) and survival (Traveset et al. 2012, Fedriani et al. 2020a), compromising the persistence and expansion of many plant species. Therefore, altering the abundance and behaviour of frugivorous carnivores can cascade down to the key ecosystem service that they provide (Fig. 3) (Fedriani et al. 2020a, Draper et al. 2022). Large predators *rewilding* may indirectly reduce the seed rain (i.e. number of dispersed seeds) attributable to mesocarnivores, but also compromise the quality of this seed dispersal service (de Paula Mateus et al. 2018, Carreira et al. 2020). Seed dispersal effectiveness (i.e. SDE) is defined as the contribution of a seed disperser to the fitness of a plant and is calculated as the product of the quantity and the quality of seed dispersal. The quantity of seed dispersal is the number of dispersed seeds and the quality component describes the probability of a dispersed seed survive to become a new reproductive adult (Schupp 2007, Schupp et al. 2010). Thus, the local environmental factors which influence recruitment after seed deposition (i.e. seedscape; Beckman and Rogers 2013) depend on the faecal-marking behaviour of frugivores (Morales et al. 2013, Farris et al. 2017).

The creation of a landscape of fear in reintroduction scenarios of apex predators may drive frugivorous mesocarnivores to reduce their use of high-risk habitats (Fig. 2) (Fedriani et al. 2000, Miller et al. 2012, Haswell et al. 2018), contributing to alter the seed-arrival patterns and subsequent probabilities of plant recruitment (García-Cervigón et al. 2018). Mammalian carnivores are relevant seed-dispersal agents in forest expansion processes, especially in plant regeneration of abandoned lands (Escribano-Ávila et al. 2014, Fedriani et al. 2018), and therefore their spatial-usage patterns could also influence the structure and composition of plant communities under a predation risk scenario (Cancio et al. 2016). Although it is clear that plant functional connectivity —effective seed dispersal among habitat patches in a landscape— can be affected by several external factors such as behaviour of individuals and species interactions, such factors have only rarely been considered in previous research (Bélisle 2005, Carlo et al. 2013, Auffret et al. 2017). The reintroduction of large predators has been widely proved to limit mesopredator populations (Elmhagen and Rushton 2007, Jiménez et al. 2019), but the subsequent potential consequences on ecosystem functions involving subordinate carnivores are still uncertain. Therefore, understanding the ecological consequences of *rewilding* apex predators on frugivore behaviour is necessary to gain insights into the altered seed-arrival patterns and their effects on plant demography and landscape structure.

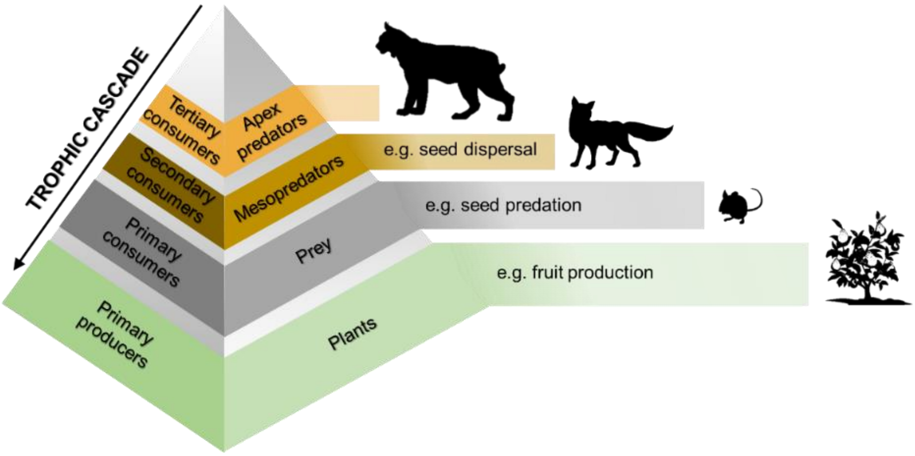

Figure 3. Trophic cascade scheme showing the multi-trophic interactions among tertiary consumers (i.e. apex predators), secondary consumers (i.e. mesopredators), primary consumers (i.e. prey) and primary producers (i.e. plants), and some examples of ecological functions performed by these trophic levels.

Since *rewilding* apex predators can also lead to small prey release due to mesopredator suppression (Letnic et al. 2012, Miller et al. 2012), an abundance increase of seed predators could cascade in contrasting ways until post-dispersal processes (Meserve et al. 2003, Garrote et al. 2019). Small mammals (e.g. rodents) are important seed predators in many ecosystems (Fedriani and Manzaneda 2005, Fedriani and Delibes 2013, Galetti et al. 2015) and their high abundance could suppose an extra limitation for SDE in mesopredator-free ecosystems (Fig. 3). Seed predators may significantly alter the initial pattern of seed rain marked by frugivores, modifying the availability and spatial distribution of seeds, and ultimately affecting plant recruitment (García et al. 2005a, Calviño-Cancela 2007). Thus, the potential impact of seed predation would also vary within the same population, being the highest where plant recruitment is seed limited (Calviño-Cancela 2007). Post-dispersal seed predation could compromise seed-dispersal and lead to seed survival trade-offs (Culot et al. 2017). For example, de Paula Mateus et al. (2018) found that the loss of large frugivores could reduce seed dispersal and increase seed predation at the same time, cascading across the different vital phases of plants and ultimately reducing future forest biomass and tree species-richness by impacting seed survival. Only a few studies have addressed how ecological alterations affecting predators and prey communities can cascade into post-dispersal process, and ultimately affect plant recruitment (Gutiérrez et al. 1997, Meserve et al. 2003, Maron and Pearson 2011, Pender et al. 2013, Bartel and Orrock 2021). Furthermore, integrative investigations across several life-cycle stages of plants are needed, because most of previous research about trophic cascades have focused on the different post-dispersal phases of the life cycle of plants separately (Cordeiro and Howe 2003, Traveset et al. 2012, Galetti et al. 2015).

The importance of environmental conditions for SDE

The concentration of many seeds in carnivore depositions (from tens to thousands) often drives density-dependent mortality episodes (e.g. pathogen infection or seedling competence; Howe 1990, Fricke et al. 2014), decreasing ultimate emergence and plant recruitment rates (Draper et al., 2021). Conversely, the fertilizing effect of faeces can improve emergence and recruitment conditions for seeds, overcoming the negative consequences of clumped seed dispersal (Sugiyama et al. 2018). In this line, Escribano-Ávila et al. (2013) showed that even considering the potential negative effects of aggregation, seeds of a Mediterranean shrub dispersed by carnivores led to more seedlings than those dispersed by birds. Janzen-Connell escape hypothesis predicts that the success of plant propagules increases with distance from the maternal surrounding (Janzen 1970, Connell 1971). Then, the density of seeds and seedlings decreases, and survival increases due to a reduced impact of distance- and density-responsive enemies. However, the accumulation of seeds in a single location can also attract seed predators (LoGiudice and Ostfeld 2002, Fragoso et al. 2003), limiting some of the many benefits that long distance seed dispersal by carnivores provides to plants (López-Bao and González-Varo 2011). Here, the seed-arrival site may play a key role dampening post-dispersal seed predation in scenarios where some seed predators can reach high abundances locally (Hulme 1994, Garrote et al. 2019, Bartel and Orrock 2021). For instance, García-Cervigón et al. (2018) showed that carnivores preferably dispersed seeds towards open interspaces, microsites which frequently keep low levels of seed predation by rodents (Suárez-Esteban et al. 2018).

Seed–seedling conflicts can be also frequent on plant establishment processes, since local environmental conditions may favour seed survival but disfavour at the same time seedling recruitment (Schupp 1995). For instance, to land beneath nurse plants often offers an advantage for plant recruitment (Gómez-Aparicio et al. 2004, Garrote et al. 2021), but vegetation cover decreases the perceived predation risk by small prey such as rodents (Rosalino et al. 2011, Loggins et al. 2019) and hence can favour seed predation (Rey et al. 2002, Sanguinetti and Kitzberger 2010). Thus, monitoring seed-arrival sites is paramount to know the local environmental conditions which could affect plant life stages after seed dispersal (Izhaki et al. 1991, Calviño-Cancela 2007, González-Varo et al. 2014, Donoso et al. 2016). Alternatively, scatter-hoarding rodents can also affect positively plant recruitment by frequently acting as rescuers of seeds confined within aborted fruits (Fedriani et al. 2020b) and contributing passively to secondary seed dispersal (Gómez et al. 2008, Cao et al. 2011), conferring the plants one more opportunity to reproduce successfully. Furthermore, since habitat selection cause non-random seed deposition and seed predation patterns, understanding how landscape structure affects post-dispersal patterns is paramount. This is especially relevant in *rewilding* scenarios, where the behavioural responses of animals to predation risk are key for determining the direction of natural plant colonization or planning well-informed conservation efforts (Fig. 4).

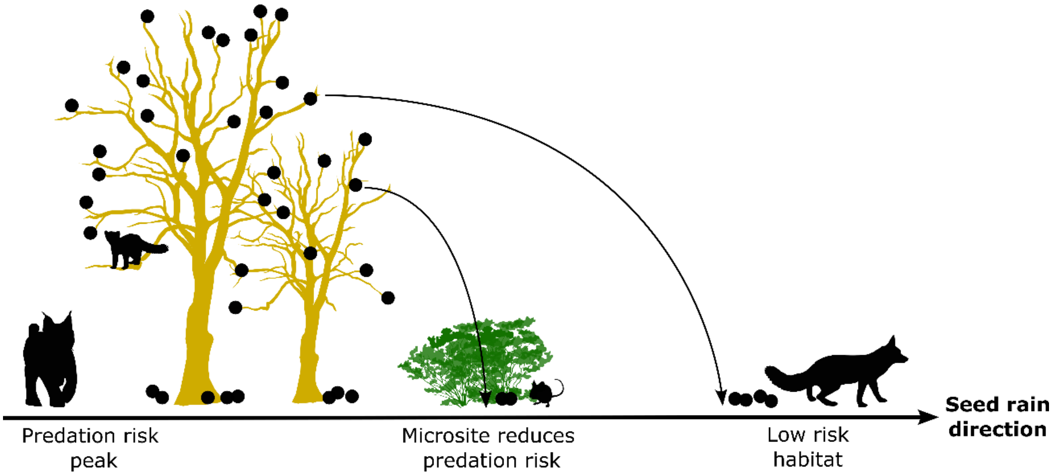

Figure 4. Scheme showing the potential directionality of seed dispersal in a landscape of fear. Apex predators increase the perceived predation risk and frugivores disperse seeds in low-risky areas outside top-order predator influence or in refuge habitats. Microsite can alter the perceived predation risk by small mammals, affecting post-dispersal seed predation rates.

Objectives

The general objective of the present thesis project was conceived under the ecological context introduced in the previous section. We argue that even though much progress has been made in the study of plant-frugivore mutualisms and predator-guild interactions separately, it is necessary to pool our knowledge of both interaction types for a better understanding of trophic webs functioning, especially under the oncoming ecosystem alterations in view of the current global change scenario. Thus, we assessed the potential cascading effects of the reintroduction of an apex predator, the Iberian lynx, on the seed dispersal service provided by frugivorous mesocarnivores to the fleshy-fruit plant community in Mediterranean ecosystems. This general objective comprises five specific aims that were organized into four chapters thesis:

- To assess the top-down effect of the Iberian lynx on the abundance and activity patterns of mesocarnivores and how bottom-up effects can modulate co-existence among carnivores (Chapter 1).
- To experimentally evaluate the effect of the landscape of fear created by the Iberian lynx on the foraging behaviour and fruit consumption of subordinate frugivorous carnivores (Chapter 2 and 3).
- To find out whether the abundance suppression and the behavioural shifts of frugivorous mesocarnivores under predation risk can trigger a trophic cascade up to plants, altering the seed dispersal patterns of a community of fleshy-fruited plants (Chapter 3).
- To assess the cascading effects of the Iberian lynx presence on the small mammal abundance, which play the joint role of prey species for mesocarnivores and post-dispersal seed predators for fleshy-fruited plants (Chapter 1 and 4).
- To determine the ecological consequences of apex predator rewilding scenarios on seedling recruitment and establishment, considering seed dispersal and also post-dispersal processes (seed survival, seedling emergence and seedling survival) in a fleshy-fruit plant widely consumed by frugivorous mesocarnivores (Chapter 4).

Main hypotheses

In the present section we propose and describe the main hypothesis which we pose in this thesis project. In a wide perspective, we predict that in a rewilding scenario, apex predators would reduce the establishment of fleshy-fruited plants dispersed by frugivorous mesocarnivores through a cascading effect. The predicted suppression effect on the abundance of medium-sized predators (Fig. 3) and their behavioural and foraging alterations (Fig. 2) would alter the entire food-web dynamics and the different trophic levels involved.

We detail different alternative hypotheses for each trophic level we studied throughout the food web (Fig. 5), which are inter-connected with each other, contributing to understand the whole food web functioning. Predicted direct top-down effects among different trophic levels are based on the current "state of the art" as detailed above. The specific top-down effects hypothesized here triggered by apex predators are:

- H1: The Iberian lynx would supress mesopredator abundance and alter their spatio-temporal activity patterns and foraging behaviour, leading to a less effective seed dispersal by mesocarnivores (Chapter 1 and 2).
- H2: The overall reduced predation pressure on small mammal communities inside lynx territories would lead to a small mammal release, leading to a higher abundance of prey for mesocarnivores. This high availability of small mammals within lynx range, would drive co-existence patterns among subordinate carnivores in spite of the perceived predation risk (Chapter 1).
- H3: The number of dispersed seeds by frugivorous mesocarnivores will be reduced, affecting the diversity of the seed rain and the composition of plant assemblages. The quality of the seed-arrival habitat and microsite would vary among predation risk scenarios due to the behavioural shifts of the habitat use and scent-marking patterns of subordinate carnivores (Chapter 3 and 4).
- H4: The positive cascading effect for small mammal (i.e. rodents) abundance in lynx presence scenarios would increase post-dispersal seed predation rates for fleshy-fruit plants (Chapter 4).
- H5: An ultimately cascading effect on plants would be derivate from the presence of the apex predator. In reintroduction scenarios, the reduced seed rain would lead to decrease the overall recruitment probability of fleshy-fruit plants. However, in heterogeneous landscapes, the alteration of the seed dispersal

spatial patterns could compensate this seed rain limitation at qualitative level. The habitat of arrival and microsite mediated effects on seed-dispersal effectiveness could vary among plant species, favouring or disfavouring plant establishment mediated by the experienced transition probabilities on post-dispersal stages (seed predation, seedling emergence and survival) (Chapter 4).

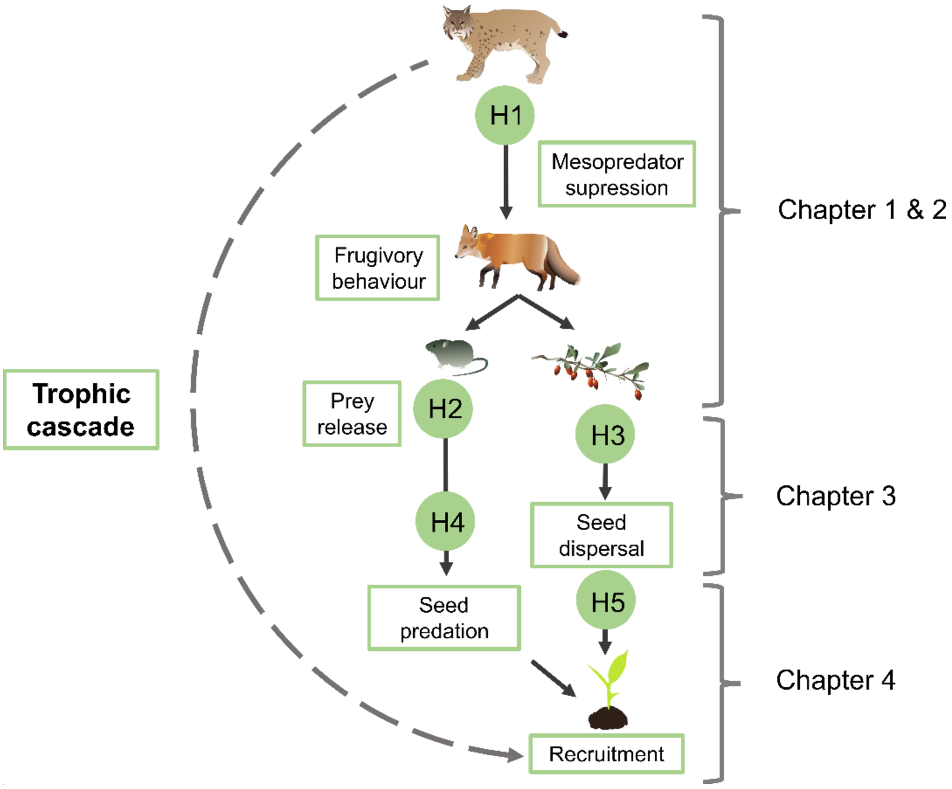

Figure 5. Hypotheses proposed across the trophic cascade of the study system. The Iberian lynx is the apex predator triggering cascading effects on the lower trophic levels, mesopredators, prey and plants. (H1) The apex predator controls the abundance of subordinate carnivores and alter their foraging behaviour and habitat-usage patterns. In consequence, (H2) small mammal prey species release their abundance due to a reduced overall predation pressure. (H3) Fearful frugivorous carnivores disperse less seeds under predation risk and alter the seed dispersal patterns, ultimately affecting seed dispersal effectiveness (SDE) through (H4) an increase on post-dispersal seed predation and a (H5) reduction on the plant recruitment.

Methods

The choice of the study system

The community of frugivorous carnivores and fleshy-fruit plants of Mediterranean ecosystems are highly diverse assemblages in template regions. Mediterranean mesocarnivores represent a group of generalist frugivores able to feed on a great variety of food resources but also disperse a large amount of seeds of diverse fleshy-fruits (Herrera 1987, Monterroso et al. 2015). They contribute to ensure the persistence of large-fruited plant species by dispersing their seeds (Fedriani and Delibes 2009a, González-Varo et al. 2015), for which extinct megafauna represented the legitimate frugivorous partners (Galetti et al. 2018). In tropical ecosystems, where the diversity of fruit-frugivore interactions is broadly studied (Kurten 2013, Vidal et al. 2013, Escribano-Avila et al. 2018), several large-body sized mammal frugivores (e.g. muriquis or tapirs; Bueno et al. 2013) are present in spite of the severe megafauna (>44 kg) extinctions occurred during the late Quaternary worldwide (Faurby and Svenning 2015, Galetti et al. 2017). Contrastingly in Europe, historical and recent extinctions of large vertebrates have supposed the loss of primary dispersers for many plants owing to other large and specialized frugivores are absent nowadays (Pérez-Méndez et al. 2016, Spengler 2019). In this defaunated scenario, generalist carnivores represent a key guild of seed dispersers, especially for large-fruited plants (Fedriani and Delibes 2009a, Draper et al. 2022).

Thus, a well-preserved Mediterranean forest was the ideal study system to test our hypotheses on a rich community of fleshy-fruit plant species. We chose Sierra de Andújar Natural Park, located in Central Sierra Morena Mountains of Southern Spain (province of Jaén), as the experimental scenario where to conduct this thesis. This Natural Park was one of the last refuge for the largest predator inhabiting Southern Spain, the Iberian lynx (6.1 – 15.9 kg). Thus, Sierra de Andújar NP sheltered the whole population of this endemic feline species together with Doñana (less than 100 individuals) when was close to extinction in the beginning of the XXI century (Simón et al. 2012). However, the plenty conservation efforts focused on this apex predator over the last 20 years (captive breeding, genetic management, reintroductions, prey supply, habitat suitability, etc.) have achieved its first assisted and then natural recolonization across the Iberian Peninsula via *rewilding* (Simón et al. 2012). Nowadays, the current wild population size of this feline reaches more than 1600 individuals although is still one of the most endangered feline species worldwide (Rodríguez and Calzada 2015, MITECO 2022).

This rewilding scenario brought us the opportunity to assess the potential cascading effects of a top-order predator across lower trophic levels in the food web. Several studies have documented that the restoration of the Iberian lynx have caused significant reductions in mesopredator abundances (Palomares et al. 1996, Jiménez et al. 2019) and behavioural alterations (Fedriani et al. 1999), leading to cause a small prey release (Palomares et al. 1995, Jiménez et al. 2019). Thus, to assess its impact on key ecosystem functions where subordinate carnivores and prey species are involved in arose as a paramount research question to contribute to the understanding of trophic cascades in altered ecosystems.

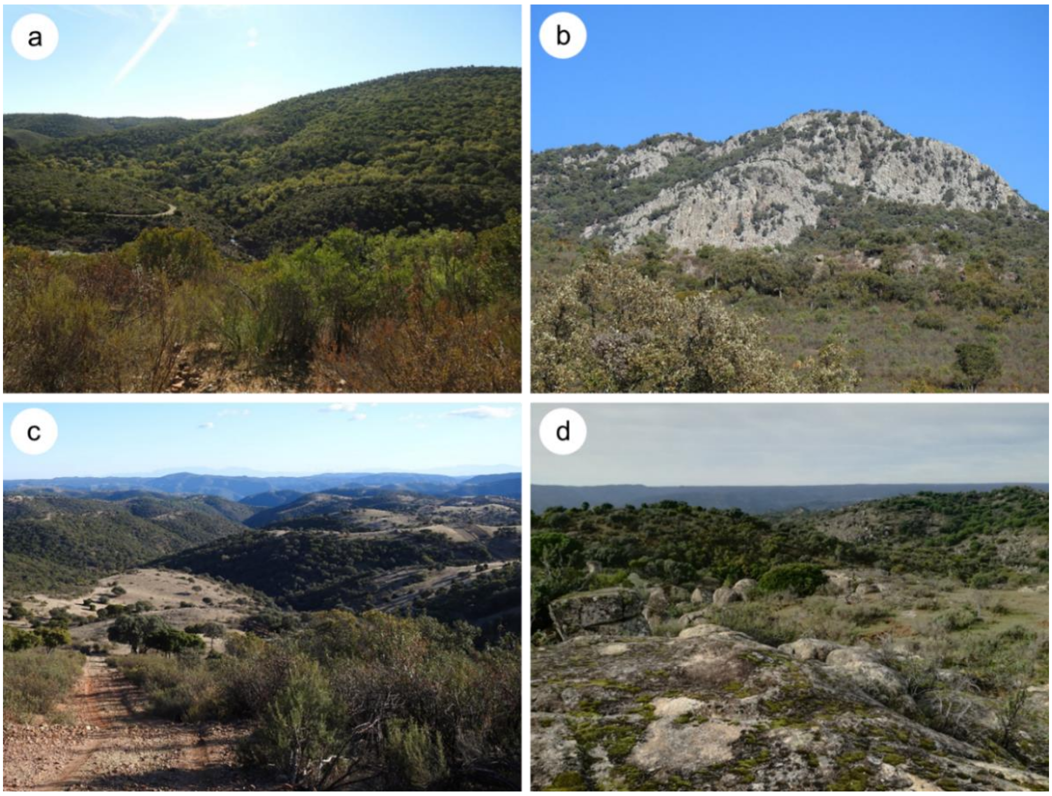

Figure 6. Pictures of the Mediterranean shrubland ecosystem selected for the study in Sierra de Andújar Natural Park. Mature forests (A) and open areas (B) shape the landscape, dotted with quartzite (C) and granitic (D) rocky outcrops (Photo credit: Tamara Burgos).

Study area

Sierra de Andújar NP (38° 14’ 27.71” N, 4° 4’ 45.03” W, near 740 km^2) is a typical Mediterranean shrubland ecosystem, with a landscape structured by mature forests, open areas and quartzite or granitic rocky outcrops (Fig. 6). The altitude ranges from 400 to 800 m and the Mediterranean climate is characterised by moderate-high annual average temperature (~18 ºC) and scarce precipitation (~700 mm) (data from Andújar meteorological station, AEMET Opendata, 2020). Extensive private and public game lands compound the Natural Park, where hunting of large wild game ungulates such as the red deer (*Cervus elaphus*) or the wild boar (*Sus scrofa*) is frequent. However, small game hunting and predator control is unusual or absent.

Mesocarnivore community

A rich community of frugivorous mesocarnivores inhabit the Natural Park such as the red fox (*Vulpes vulpes*), the stone marten (*Martes foina*), the common genet (*Genetta genetta*) and the Eurasian badger (*Meles meles*). Other wild medium-sized carnivores can co-exist in the study area, but they are strict carnivorous such as the European wildcat (*Felis silvestris subsp. silvestris*) and the Eurasian otter (*Lutra lutra*) or feed on fruit anecdotally such as the Egyptian mongoose (*Herpestes ichneumon*) (Fig. 7) (Monterroso et al. 2020). Frugivorous carnivores are sympatric medium-sized predators (1-7 kg body mass) of similar habitat and food requirements but markedly differ on their trophic niche breadth (Clavero et al. 2003, Monterroso et al. 2020). Small mammals (e.g. rodents) are the most common prey for mesocarnivores in Mediterranean ecosystems, but their opportunistic foraging behaviour allow them to feed on a wider spectrum of food resources such as invertebrates, carrion or fruits whenever is possible (Monterroso et al. 2020). The common genet and the stone marten have similar body weight (~1 kg) and select positively woody areas with forestry refuge (Santos-Reis et al. 2004, López-Martín 2006). However, the stone marten feeds predominantly on fruits (>90% occurrence in some study areas), in comparison to the more specialized diet of the common genet on micromammals (Virgós et al. 1999, López-Martín 2006). In overall, the red fox and the stone marten are the main frugivorous mesocarnivores in Southern Europe (>60% occurrence in diet), in spite of their generalist dietary nature (Monterroso et al. 2020). However, the red fox tends to specialize its diet on rabbits (*Oryctolagus cuniculus*) when this trophic resource is abundant (Delibes-Mateos et al. 2008). Fruits also represent a dominant food resource in the diet of badgers in Mediterranean ecosystems, but invertebrates can represent the bulk of the badger’s diet even when their availability is low (Virgós et al. 2004, Rosalino et al. 2005). Although the Eurasian badger and the common genet often keep moderate levels of frugivory (~30% occurrence) respect to foxes and martens (Monterroso et al. 2020), previous research has documented a key role of badgers for seed dispersal of several Mediterranean large-fruited plants (Fedriani and Delibes 2009a, Fedriani et al. 2020a, Garrote et al. 2022a).

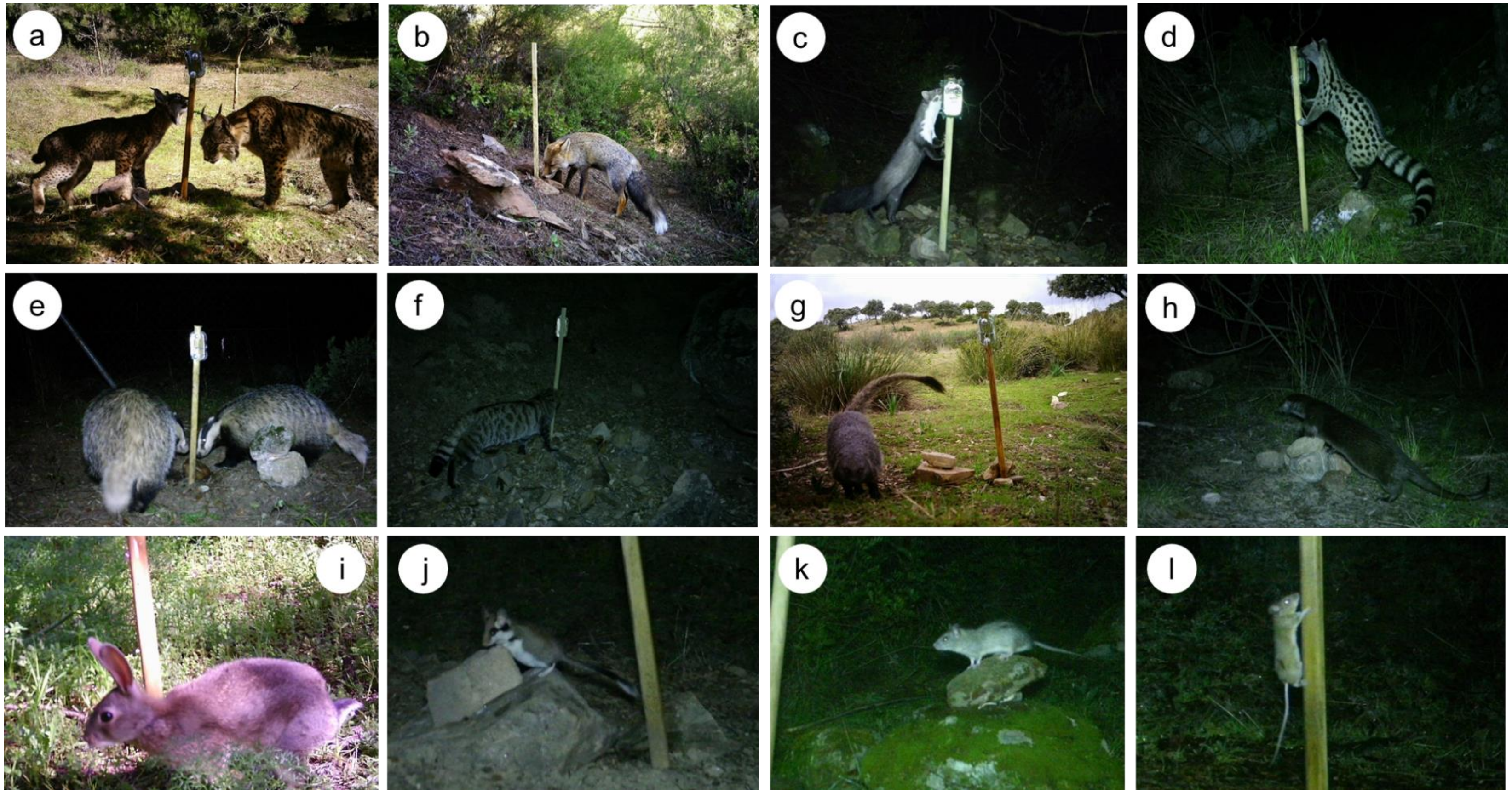

Figure 7. Medium-sized carnivores and its main prey in Sierra de Andújar Natural Park. (a) Iberian lynx (*Lynx pardinus*), (b) red fox (*Vulpes vulpes*), (c) stone marten (*Martes foina*), (d) common genet (*Genetta genetta*), (e) Eurasian badger (*Meles meles*), (f) European wildcat (*Felis silvestris subsp. silvestris*), (g) Egyptian mongoose (*Herpestes ichneumon*), (h) Eurasian otter (*Lutra lutra*), (i) European wild rabbit (*Oryctolagus cuniculus*), (j) garden dormouse (*Elyomis quercinus*), (k) Balck rat (*Rattus rattus*) and (l) wood mouse (*Apodemus sylvaticus*). (Photo credit: Tamara Burgos).

Small prey community

Small mammals are an important prey resource for mesocarnivores in Southern Europe (Monterroso et al. 2020). The most common small mammal species that inhabit our study area are the European rabbit and smaller species (<1 kg body mass) called 'micromammals' such as the garden dormouse (*Eliomys quercinus),* the wood mouse (*Apodemus sylvaticus*), the Algerian mouse (*Mus spretus*) and the black rat (*Rattus rattus*) (see Fig. 7). Rabbits represent more than 90% in occurrence of the Iberian lynx diet (Gil-Sánchez et al. 2006). This dietary specialization leads this feline to track rabbits at fine-scale across the landscape, leading to a high overlap in lynx and rabbit distribution in our study area. Besides to be prey of the mesocarnivore community, rodents play also the ecosystem role of seed predators and rabbits are often pulp-feeders for many fleshy-fruited plants in Mediterranean ecosystems (Rey et al. 2002, Fedriani and Delibes 2013). Thus, this antagonistic interaction with plants could suppose an extra limitation for plant production (Fedriani and Delibes 2009a). However, scatter-hoarding rodents are able to act as seed rescuers confined within aborted fruits and potentially contribute passively to secondary seed dispersal (Fedriani et al. 2020b).

Fleshy-fruited plant community

The rich fleshy-fruited plant community in Sierra de Andújar NP is compound by more than 15 fruiting species (see Fig. 8). Fruiting periods of the main fleshy-fruited plant species goes from late summer (end of August and beginning of September) until the beginning of spring (late March), although the ripening peaks are variable across the autumn and winter for each plant species (Herrera 1984). The dominant fleshy-fruited species are the strawberry tree (*Arbutus unedo*) in northward slopes and the mastic (*Pistacia lentiscus*) and the prickly juniper (*Juniperus oxycedrus*) in southern slopes. Other less abundant fruiting plants but also dispersed by carnivores are the oleaster (), the Iberian pear (*Pyrus bourgaeana*), the blackberry (*Rubus ulmifolius*), the myrtle (*Myrtus communis*), the common fig (*Ficus carica*), the terebinth (*Pistacia terebinthus*), the laurustinus (*Viburnum tinus*) and the Mediterranean buckthorn (*Rhamnus alaternus*). However, frugivorous mesocarnivores only select certain types of fruits in their diet, often large fruits with a pulp-rich content (Debussche and Isenmann 1989, Herrera 1989). In our study area, the common fig (30.6 mm average fruit diameter), the Iberian pear (23.3 mm), the strawberry tree (17.1 mm) and the blackberry (11.8 mm) are the fruiting species with largest fruit size (Jordano 2013a).

Many of these plant species have fruit diameters larger than the gape width of main avian seed dispersers (range 7.1-8.6 mm in *Sylvia melanocephala* and *Erithacus* *rubecula*, respectively). However, some species have specific fruit traits which make them easier to consume and handle by birds in spite of their large fruit size. For instance, *A. unedo* has a very soft ripe fruits and birds can consume some seeds by pecking at the pulp content and *R. ulmifolius* fruits are composed by multiple polydrupes (Herrera 1984). In our system, a few bird species (e.g. *Cyanopica cooki*) exceeding the gape width to consume large fruits such as *P. bourgaeana* or *A. unedo*. This evident physical limitation to swallow the whole fruit of large fruited species is likely due to they have not evolved as proper bird-dispersed plants (Herrera 1984). In a previous study conducted in a Mediterranean mountain of southern Spain, *A. unedo* fruits accounted for an average of 1% of estimated fruit mass ingested by small birds (e.g. *S. atricapilla*, *E. rubecula, Turdus sp.*) with respect to the total number of fruits ingested, meanwhile the coriaceous fruits of *J. oxycedrus* represented only 0.01% (Herrera 1994). The main seed dispersers for fruiting plant species of genus *Juniperus sp.* are often thrushes (*Turdus philomellos* and *T. viscivorus*) in Mediterranean ecosystems (Santos et al. 1999) due to its lower fruit size (9.7 mm average fruit diameter), but seed dispersal by carnivores have been proved to be a higher probability of recruitment (Escribano-Ávila et al. 2013).

We selected *P. bourgaeana* (Fig. 8b) as the plant study species in Chapter 2 and 4 because is mainly dispersed by frugivorous carnivores, which ingest whole ripe fruits (Fedriani & Delibes, 2013). This tree shows an aggregated spatial pattern derived from seedling clustering and induced by a mammal seed dispersal syndrome, mostly due to fox and badger seed dispersal (Fedriani and Delibes 2009b, Fedriani et al. 2010). The wild boar (*Sus scrofa*) occasionally disperses seeds, while the red deer (*Cervus elaphus*) and the fallow deer (*Dama dama*) act as seed predators. Furthermore, rodents, birds and rabbits function primarily as seed predators or pulp feeders (Fedriani & Delibes, 2009b; Fedriani & Delibes, 2013; Fedriani, Zywiec, & Delibes, 2012). Eurasian large-fruiting rosaceous species are largely reliant on rapid colonization of forest openings or open landscapes (Spengler 2019). This explains the relatively isolated and scarce spatial pattern of the Iberian pear in Sierra de Andújar NP (< 1 individual ha-1), growing most often in substrates lacking vegetation or coexisting with small-fruited shrub species such as *P. lentiscus*.

Figure 8. Several fleshy-fruited plants of Sierra de Andújar Natural Park. (a) Strawberry tree (*Arbutus unedo*), (b) Iberian pear (*Pyrus bourgaeana*), (c) prickly juniper (*Juniperus oxycedrus*), (d) blackberry (*Rubus ulmifolius),* (e) myrtle (*Myrtus communis*) and (f) oleaster (*Olea europaea* subsp*. europaea* var*. sylvestris*). (Photo credit: Tamara Burgos).

Broad experimental design

Despite the recovery of Iberian lynx populations over the last 20 years, lynx home ranges are heterogeneously distributed throughout the Sierra de Andújar NP (Fig. 9) and this feline is still absent in many lands as consequence of the lack of its main prey, the European rabbit. Thus, we used a common experimental design for all chapters of this thesis, comparing localities with territorial presence of Iberian lynx and localities out of lynx distribution range as controls (Fig. 9), based on our previous long-term knowledge of the study area and system. Habitat characteristics and fruit-producing plant communities were similar among localities. We selected five control localities (Selladores, Risquillo, Cereceda, Fontanarejo1, Lugar Nuevo) with confirmed absence of reproduction and territorial lynx pairs and five lynx presence localities (Puerto Bajo, Valquemado, Gorgogil, Chopos, Fontanarejo2) distributed along the lynx distribution area shown by the IUCN Red List of Threatened Species (Rodríguez and Calzada 2015) (Fig. 9). Furthermore, we previously verified reproduction of Iberian lynx during the previous five years before this study in presence localities. Study localities were considered spatially independent. Controls were situated far enough apart from the nearest Iberian lynx home range (Mean ± SD = 7.9 ± 3.0 km; Fig. 9) and presence localities included a territory of a different lynx couple (average lynx home range radius in our study area is 1.62 km; Gil-Sánchez et al. 2011)

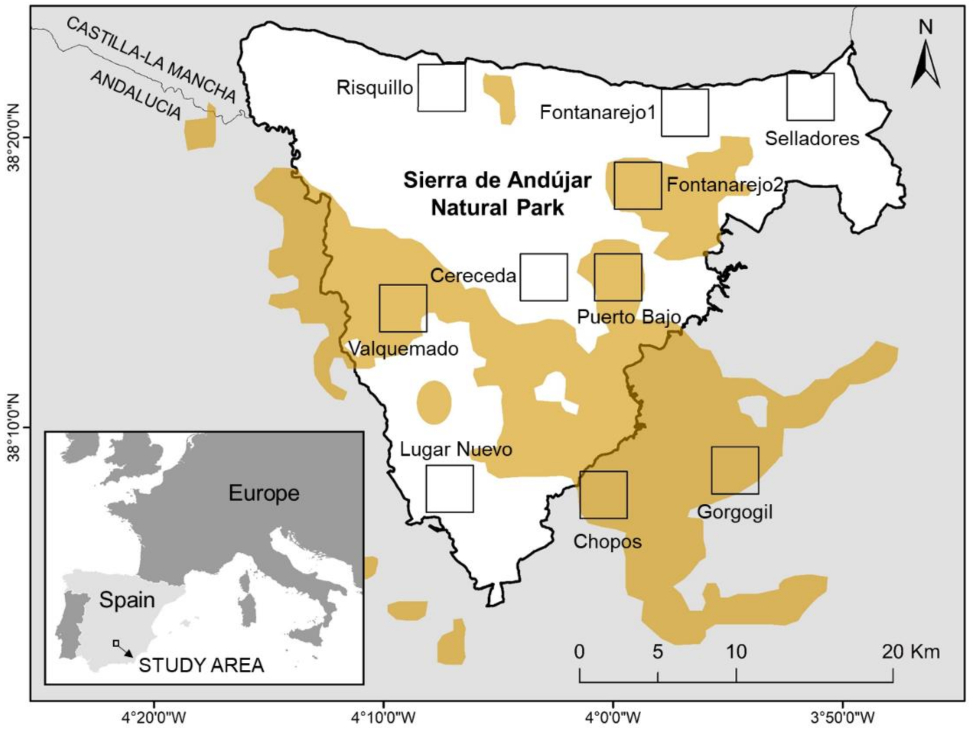

Figure 9. Map of study area showing the ten experimental localities along the Sierra de Andújar NP and the Iberian lynx range (orange shadowed) adapted from the distribution map available in the IUCN Red List of Threatened Species (Rodríguez and Calzada 2015). Map was generated in ArcMap 10.6 https://www.esri.com/.

Field sampling

We selected all the study localities described above or a subsample of them to carry out the specific objectives of each chapter of this thesis. The detailed methodologies are described in each chapter separately. However, we presented in this section a brief explanation of the specific field samplings conducted in order to achieve the main objectives of this thesis.

Chapter 1: In order to estimate the absolute abundance of two sympatric mesopredators and fine-scale spatiotemporal coexistence mechanisms among predators, we placed 120 camera-traps across the ten study localities during eight months in two consecutive years (2018-19 and 2019-20). Each camera worked an average of 44 days and reached an overall effort of 10644 trap-days. In particular, we identified stone martens (*Martes foina*) and common genets (*Genetta genetta*) individually, based on their coat patterns, to estimate population densities using spatially explicit capture-recapture Bayesian models. We also estimated small prey (rabbits and rodents) occurrence and relative abundances as the number of captures by 100 cam-days (Fig. 7).

Chapter 2: In order to determine whether the presence of the Iberian lynx altered the foraging behaviour and fruit consumption of subordinate frugivorous carnivores, we carried out a 15-days field experiment in October-November of 2019 (Fig. 10). We placed *P. bourgaeana* fruits marked with a white natural thread beneath fruiting trees, and monitored visitation and fruit removal using camera-traps (n = 30) in a subsample of four localities with presence and absence of lynx. Overall, we reached a sampling effort of 437 trap-days and offered 2700 Iberian pear fruits. Specifically, we recorded visitation, fruit consumption, and time spent per visit by the entire community of frugivorous mesocarnivores. We also estimated fruit availability (individual cropsize) in the field and calculated the percentage of forest around a 100-m buffer from each individual tree or group of trees (MCP) by digitizing forest cover from a high-resolution (0.5 m) orthophotography.

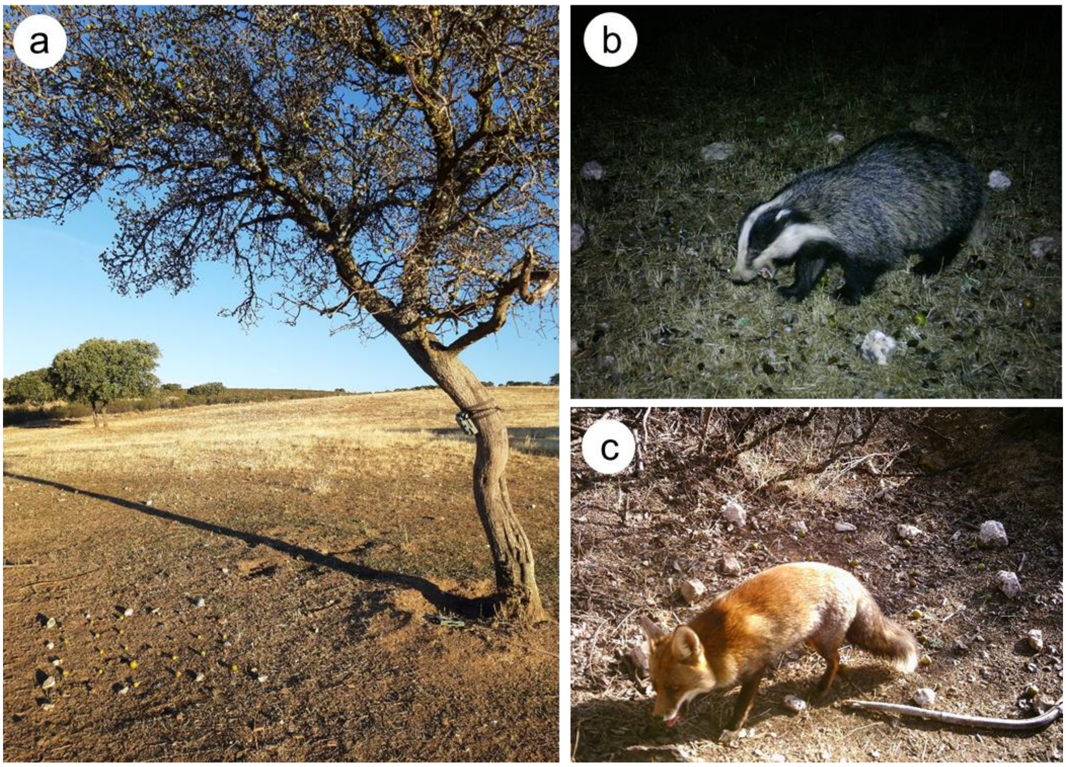

Figure 10. Photographs of the *Pyrus bourgaeana* fruit offering experiment. (a) Camera-trap installed on an isolated Iberian pear tree in front of the fruit offer. (b) European badger (*Meles meles*) and (c) red fox (*Vulpes vulpes*) eating pear fruits in our experiment.

Chapter 3: We compared fruit consumption and seed dispersal patterns of the main frugivorous mesocarnivores (the red fox *V. vulpes* and the stone marten *M. foina*) among lynx presence and absence localities (n = 10). We selected two habitat types (forest vs.

open) to sample mesocarnivore scats along 1.5 km transects during two consecutive fruiting seasons (2018-19 and 2019-20). We reached an overall sampling effort of 160 visits and collected 1575 scats. Then, we estimated seed content visually (plant species and number of seeds) for each dried scat (Fig. 11). We also performed DNA-barcoding analysis to verify our visual identification success of stone marten scats. Finally, we estimated fruit availability along the sampled transects by counting the number of fruits and the coverage percentage inside 15-m plots during the fruiting peak of each plant species.

Figure 11. Photographs illustrating the scat sampling conducted. (a-d) Examples of dispersed seeds by mesocarnivores found along sampled 1.5-km transects: (a) a stone marten (*Martes foina*) scat with strawberry tree (*Arbutus unedo*) seeds; (b) defecated Iberian pear seeds by the red fox (*Vulpes vulpes*); (c-d) a germinated strawberry tree and Iberian pear seed in a stone marten and red fox scat, respectively. (e-f) Seed manipulation in laboratory: (e) strawberry tree seeds and (f) prickly juniper (*Juniperus oxycedrus)* seeds extracted from one single mesocarnivore scat.

Chapter 4: We used the seed dispersal quantification for *P. bourgaeana* included in Chapter 3, and we added up two additional field experiments to obtain data for the post-dispersal stages of *P. bourgaeana* (Fig. 12). We conducted seed predation and recruitment field experiments in November of 2021 in two different habitat types (forest, open) and three microsites (shrub, rock, open). Pear seeds were manually taken from wild *P. bourgaeana* fruits. Seed predation was estimated in one locality with lynx presence and one control locality during 5 consecutive nights. We offered 1152 pear seeds in Petri dishes and monitored seed predation by rodents by using camera-traps (n = 36) in a subsample of the seed depots. Seedling emergence and survival was estimated in one study locality, due to the presence of lynx is not relevant for plant recruitment. In particular, we sowed 360 seeds and monitored the emerging and survival rates during a whole year around.

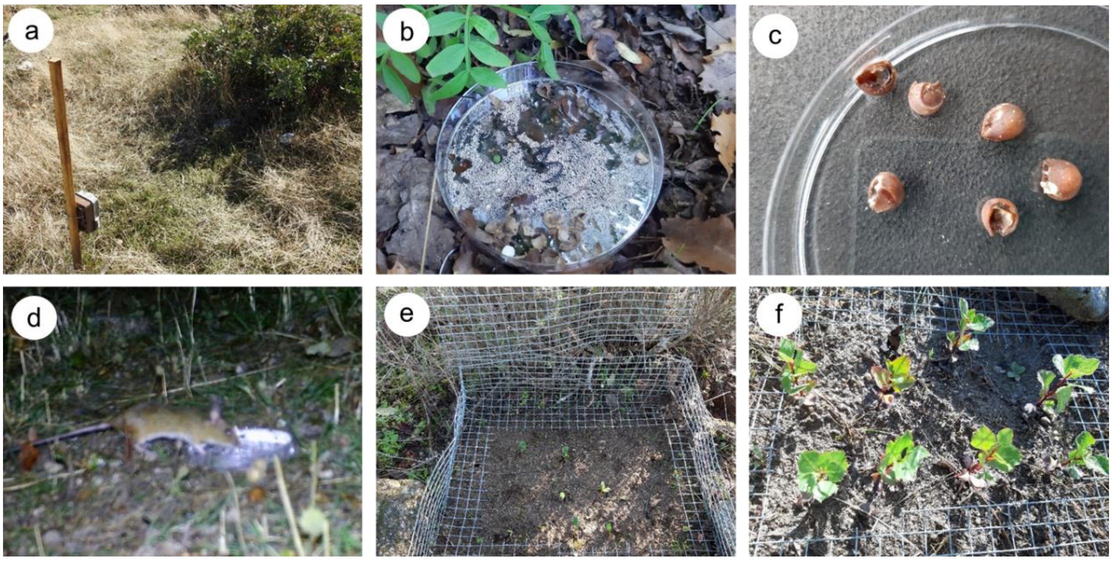

Figure 12. Photographs of the seed predation and seedling emergence and survival field experiments for *Pyrus bourgaeana*. (a) Installation of camera-traps (Scoutguard SG562-C; white led) in front of the Petri dishes with Iberian pear seeds dispensed during the experiment; (b-c) Predated Iberian pear seeds by rodents in our experiment; (d) wood mouse (*Apodemus sylvaticus*) predating pear seeds in our experiment (image taken by camera-trapping); (e-f) *P. bourgaeana* seedling emergence and survival experiment: (e) Iberian pear seedlings in the microsite 'shrub', under a rosemary (*Rosmarinus officinalis*), (f) 1st year Iberian pear seedlings that survived at the end of the experiment.

Chapters

Chapter 1

Top-down and bottom-up effects modulate species co-existence in a context of top predator restoration

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Abstract

Mesopredators abundance is often limited by top-order predators and also by key food resources. However, the contribution of these bidirectional forces to structure carnivore community is still unclear. Here, we studied how the presence and absence of an apex predator which is currently recovering its former distribution range, the Iberian lynx (*Lynx pardinus*), determined the absolute abundance and fine-scale spatiotemporal avoidance mechanisms of two sympatric mesocarnivores (stone marten *Martes foina* and common genet *Genetta genetta*) with different dietary plasticity. We hypothesized that the lynx causes a mesopredator suppression and subordinate predators develop segregation strategies in respect to their trophic niche breadth. We placed 120 camera-traps in Southern Spain for 8 months in two consecutive years to estimate mesocarnivore abundances by using SCR Bayesian models, prey availability and assess spatio-temporal patterns. We found that the lynx reduced mesocarnivore abundance up to 10 times. Stone marten, a mesopredator with a broader food resources spectrum, showed a total spatial exclusion with the apex predator. Meanwhile, fine-scale avoidance mechanisms allowed the genet to persist in low density inside lynx territories, probably taking advantage of high availability of its preferred prey. Thus, the strength of these top-down and bottom-up effects was rather species-specific. Given the recent recovery of large carnivore populations worldwide, variation in suppression levels on different mesopredator species could modify ecosystem functions provided by the carnivore community in contrasting ways.

Keywords: Capture-recapture, dietary plasticity, intra-guild interactions, mesopredator release, rewilding, spatiotemporal avoidance

Introduction

Apex predators play critical roles in ecosystem functioning, and their conservation is paramount for enhancing and maintaining global biodiversity (Alston et al. 2019). They are at the top of trophic webs and can exert a top-down regulation on prey populations by direct killing (Estes and Duggins 1995, Ripple and Beschta 2012b) and behaviourally-mediated trophic cascades due to behavioural responses to predation risk (Schmitz et al. 1997). However, food availability can also trigger bottom-up effects on predators limiting their numbers (Power 1992), especially for dietary or habitat specialists (Stoessel et al. 2019, Travers et al. 2021). Therefore, considering the recent recovery and rewilding processes involving large carnivores across the world (Wolf and Ripple 2018), a better understanding of how these bidirectional forces between predators and prey structure trophic webs would help to improve our knowledge on food web dynamics and to adopt well-informed conservation strategies at the whole community level (Krofel and Jerina 2016).

Top-down regulation from apex predators can also affect carnivores in lower trophic levels through complex intra-guild interactions (Prugh and Sivy 2020). Large carnivores can reduce abundances of medium-size predators (i.e. mesopredators) by exploitative competition or inter-specific killing (Linnell and Strand 2000, Caro and Stoner 2003). Therefore, extirpation or significant reduction of top predator numbers can lead to a mesopredator release (Crooks and Soulé 1999, Newsome et al. 2017). Nevertheless, according to the coexistence theory (Schoener 1974), the negative impacts of competitive interactions can be relaxed by several factors such as high availability of resources or adequate environmental conditions (Fedriani et al. 2000, Monterroso et al. 2020). Thus, sympatric species can avoid intra-guild competition by performing adaptive behavioural mechanisms, specifically by spatial, temporal and/or dietary niche segregation (Di Bitetti et al. 2010, Karanth et al. 2017, Ferreiro-Arias et al. 2021). These adaptations take special relevance in complex carnivore communities, where niche partitioning could involve positive cost-benefit balance to facilitate the coexistence among carnivores (Carvalho and Gomes 2004, Monterroso et al. 2014, Gil-Sánchez et al. 2021).

As a consequence of asymmetric competition with larger predators, subordinate species (i.e. smaller predators) are often restricted to safer but less productive habitats (Sergio and Hiraldo 2008, Di Bitetti et al. 2010, Gallagher et al. 2017), at the periphery of the dominant predator ranges, especially when resources are limited (Newsome et al. 2017). Local and intensive foraging of subordinate carnivores persisting in safe but restricted areas could markedly decrease the local abundance of susceptible prey species (Crooks and Soulé 1999). In contrast, some prey populations can spread as a result of a reduced overall predation pressure in areas frequented by a dominant predator (Palomares et al. 1996, Jiménez et al. 2019). Therefore, the presence of an apex predator could displace mesopredators to lower-quality habitats but also encourage certain specialized mesopredators to persist in areas with high risk of intra-guild predation due to a balanced effect of high prey abundance (Linnell and Strand 2000). Prey availability is especially relevant in a scenario of apex predator restoration where responses to competition by subordinate predators could be more complex than just fear or death (Gallagher et al. 2017, Wooster et al. 2021). Other studies have suggested that prey abundance and preferences can drive coexistence patterns as a bottom-up force structuring carnivore communities (Barrientos and Virgós 2006, Santos et al. 2019, Ferreiro-Arias et al. 2021). For instance, Monterroso et al. (2020) found that predator species with high overlapping dietary niches can coexist locally by prey switching when the subordinate species has a wide trophic niche breadth, accordingly with the optimal foraging theory (MacArthur and Pianka 1966).

In Mediterranean ecosystems of southern Spain, the recent recovery of the apex predator, the Iberian lynx (*Lynx pardinus*), allowed us to investigate how intra-guild competition and prey availability structures the predator community. Here, we studied the top-down suppression and coexistence mechanisms among two mesopredators and an apex predator, the Iberian lynx, through a natural experiment. We studied areas with and without predation risk and measured top-down effects on the abundance and spatio-temporal behaviour of mesocarnivores by using spatial capture-recapture (SCR) and time-to-encounter methods, which was rarely used in previous similar research on top-down suppression(Jiménez et al. 2019). We focused on two sympatric mesocarnivores with a high trophic niche overlap but different dietary plasticity: the common genet (*Genetta genetta*), specialized on small rodents and the stone marten (*Martes foina*), a more generalist and opportunistic carnivore (López-Martín 2006). Moreover, we assess how the availability of food resources modulates this trophic web through bottom-up effects. We hypothesize that the presence of the apex predator leads to a reduced mesopredator density in comparison with lynx absence scenarios. Furthermore, we expect mesopredators to show species-specific segregation or coexistence mechanisms with the apex predator and that prey availability and trophic plasticity to play an important role in facilitating coexistence. Specifically, we predict that the trophic-specialist, the common genet, should be able to develop spatio-temporal mechanisms to coexist with the apex predator due to the increase of preferred prey abundance (rodents) inside lynx home ranges. In contrast, we expect that for the mesopredator with a wider trophic-niche breadth, the stone marten, it should be easier to avoid lynx home ranges because is able to feed on a wider food spectrum.

Methods

Study area and target species

Our study was conducted in Sierra de Andújar Natural Park (Fig. 1). The annual average temperature (~18 ºC) and rainfall (~700 mm) are typical for Mediterranean climate areas (data from Andújar meteorological station, AEMET Opendata, 2020). The altitude ranges between 400 and 800 m. Vegetation is dominated by Mediterranean shrubland and an arboreal stratum of holm oak (*Quercus ilex*). Extensive private and public lands compound the Natural Park, where hunting of large wild game ungulates is frequent, but small game hunting and predator control is rare or absent.

The Iberian lynx is the largest carnivore inhabiting the study area (6.1 – 15.9 kg; Simón et al. 2012). Sierra de Andújar NP was one of the last refuge for this species in the 1990’s and 2000s when less than 100 individuals were left in the wild (Simón et al. 2012). Despite the recovery of Iberian lynx populations in the last 20 years, lynx home ranges are heterogeneously distributed throughout the Natural Park (Fig. 1) and this feline is still absent in many lands as consequence of the lack of its main prey, the European rabbit (*Oryctolagus cuniculus*) (Simón et al. 2012).

Nine wild mammal mesocarnivores inhabit the study area, including the red fox (*Vulpes vulpes)*, the stone marten, the common genet and the Eurasian badger (*Meles meles)*. Given that we aim to assess the suppression effect of top-order predator on mesopredator abundances by using SCR methods which require individual recognition and a high recapture rate (Royle et al. 2014), we focussed this study on the common genet and the stone marten because they are abundant mesocarnivores with easily identifiable coat patterns (see Appendix S1). They are sympatric carnivores of similar size (around 1 kg), habitat and food requirements but markedly differ in their trophic plasticity (Santos-Reis et al. 2004, López-Martín 2006). Small mammals are their main prey, followed by birds, fruits and invertebrates in decreasing order of importance (López-Martín 2006). Rabbits also represent a selected prey by these mesocarnivores when reach high abundances (Barrientos and Virgós 2006). However, the stone marten is more adaptable to food availability, feeding on fruits more often in comparison with the more specialized diet of the genet, which is mainly focused on micromammals (Virgós et al. 1999, López-Martín 2006). Thus, to assess the importance of prey availability on coexistence among predators, we focused on the most abundant small mammal species that inhabit our study area, the European rabbit, the garden dormouse (*Eliomys quercinus*), the wood mouse (*Apodemus sylvaticus*), the Algerian mouse (*Mus spretus*) and the black rat (*Rattus rattus*) (see Appendix S1: Fig. S3).

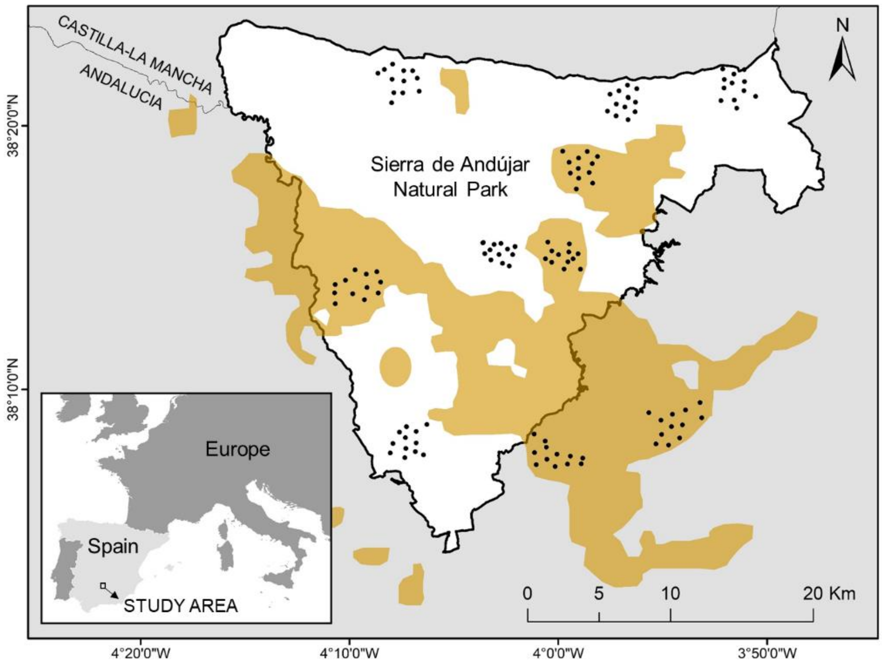

Figure 1. Map of study area showing the camera-trap sites (black dots) distributed in ten localities along the Sierra de Andújar NP and the Iberian lynx range (orange shadowed) adapted from the distribution map available in the IUCN Red List of Threatened Species (Rodríguez and Calzada 2015). Map was generated in ArcMap 10.6 https://www.esri.com/.

Experimental design and camera-trap data collection

We carried out a camera-trapping survey between October and May of 2018-19 and 2019-20 (Appendix S3: Table S4). We used an experimental design comparing five localities with territorial presence of Iberian lynx and five localities out of lynx distribution range as controls with the same habitat characteristics (Fig. 1). We selected these localities based on our previous long-term research in the study system and following the recommendations of the wildlife monitoring program of the Natural Park. Control localities confirmed absence of reproduction and territorial lynx pairs, mostly due to the absence or scarcity of its main prey, the wild rabbit. Localities with lynx presence were distributed along the lynx distribution area shown by the IUCN Red List of Threatened Species(Rodríguez and Calzada 2015) (Fig. 1) and had verified lynx reproduction in the previous five years. These localities were considered spatially independent because they were situated far enough apart (Mean ± SD = 7.9 ± 3.0 km; Fig. 2) in order to absence localities kept out of the nearest Iberian lynx home range and presence localities included a territory of a different lynx couple (average lynx home range radius in our study area is 1.62 km; Gil-Sánchez et al. 2011).

We placed 120 camera traps (12 in each locality; Fig. 2) the first year and we set them again at the same locations the second year (Scoutguard SG562-C; white led). Cameras were placed at the height of 40-60 cm above the ground, operated 24 h/day and were programmed with a trigger delay of 1 s, to take three consecutive images. Each camera worked an average of 44 days and reached an overall effort of 10,644 trap-days (see Appendix S3: Table S4 for more details). At each camera station, we placed under a small rock a hygienic tampon soaked with Iberian lynx urine (collected from several captive individuals in breeding centres, males and females mixture) and a sardine can on the top of a wood stick at a height of 60 cm to 1.5 m from the camera (see photographs in Appendix S1). The use of lures has been proved not to affect abundance estimates or temporal activity in camera-trapping studies and is a widely-used strategy to improve detectability in SCR models (Gerber et al. 2012, Jiménez et al. 2022). Lynx urine is one of the most effective attractants of mesocarnivores (Ferreras et al. 2018) and sardines are useful to keep the animals long enough in front of the camera-trap to take several images, thus improving the likelihood of obtaining individual identification. Two experts successfully individually identified predators based on unique coat patterns by consensus in 70% of lynx detections, 93% for stone marten and 87% for genets inside lynx territories and 82% for martens and 92% for genets outside lynx range (see Appendix S1 for more details). Detection of small-mammal prey species was also improved due to the use of lure, because most of them climbed up to the top of the stick where sardines were placed, making it easier to identify the species (see Appendix S1 Fig. S3). We classified small mammals into three categories: rabbit, garden dormouse and other rodents which grouped rodent species of similar morphology, such as the wood mouse, the Algerian mouse and the black rat. We considered an event as independent when we identified the individual as different from the previous recording or the time interval between successive images was at least 30 min for non-identified or the same individuals (Ridout and Linkie 2009). Within each locality, camera-traps were spaced an average distance of 683 ± 145.61 m (Mean ± SD) from the nearest camera. We set this distance because it was lower than 2σ (σ is the movement parameter (Jiménez et al. 2017) of the carnivore species with the smallest averaged home range size (381 ha for the stone marten (Genovesi et al. 1997) following Royle and Converse (2014) recommendations. We covered a surveyed surface of ca. 900 ha in each locality, enough for the mesopredator with the biggest home range size, in our case the genet (781 ha; Palomares and Delibes 1994).

Abundance estimates

We estimated the density (individuals/km^2) of genet and stone marten in each locality with and without Iberian lynx presence for each study year by using a capture-recapture database of identified records. Population was considered demographically closed because density estimates were carried out with data from October to May, out of the main juvenile dispersal period of these species which occurs during late summer and early autumn (Camps 2017). We used spatial capture-recapture (SCR) models (Efford 2004) to estimate capture probability as a decreasing function of the distance between activity centres and detectors (camera-traps) and calculated density estimates(Royle et al. 2014). In our study we assume the capture history y_{ij} are mutually independent outcomes of a binomial random variable, which we express as:

$$y_{ij} \sim Binomial\left(K, \lambda_{ij}\right) \, (1)$$

The link function between the location of detectors and the activity centres of the individuals follows a half-normal distribution:

$$\lambda_{ijk} = \lambda_0 exp\left(\frac{-d_{ij}^2}{2\sigma^2}\right)(2)$$

where d_{ij} is the Euclidean distance between the activity centre for each individual and camera-trap location, σ is the scale parameter from half normal distribution (that described the movement), and λ_0 is the baseline encounter rate. We implemented spatially-explicit models in a Bayesian framework using R (R Core Team, 2018) and Nimble package (de Valpine et al. 2017, NIMBLE Development Team 2017) similarly to Jiménez et al. 2019 (Jiménez et al. 2019). In order to get better parameter estimates for the stone marten, we used models with shared movement parameter sigma (σ) and baseline detection rate (λ_0) among localities with lynx presence and lynx absence because we recorded few spatial re-captures in localities with lynx (Royle and Converse 2014, Morin et al. 2018, Jiménez et al. 2019). For common genets, we estimated σ and λ_0 separately for localities with and without lynx. Posterior probabilities were calculated using 3 independent MCMC chains. For stone marten we used 55,000 iterations each, and a burn-in of 5000 iterations. For genet we used 160,000 iterations with an initial 10,000 iterations as a burn-in, and thinned the remainder by 5. We confirmed model convergence by examining trace plots (see Appendix S2; Fig. S2-S4) and ensuring that the potential scale reduction factor (R-hat) statistic for each parameter was < 1.1 (Gelman et al. 2013).

Temporal overlap

Temporal analysis was carried out by generating the kernel density estimation of the activity patterns of lynx, genet, stone marten and prey species (rabbits, garden dormice and other rodents) using the overlap package (Ridout and Linkie 2009). We carried out these analyses separately for the breeding season (i.e. denning period from March to June) and for the non-breeding season (excluding the detections from March to June; see Appendix S3: Table S5) of genets (Camps 2017), stone martens (Mangas 2007) and lynx (Simón et al. 2012). We took this decision due to previous research showing effects of breeding behaviour on circadian rhythms of these predators (Monterroso et al. 2014).

We calculated the overlap coefficients (Δ) of temporal activity among 1) lynx and genet and lynx and stone marten, 2) genet and marten inside and outside lynx range and 3) carnivores with prey species, considering only species with ≥10 detections (Appendix S3: Table S5). In line with Ridout and Linkie (2009), we used the estimator Δ_4, which is recommended for large sample sizes ($\bar{n} \pm SE = 1238.41 \pm 214.22$ detections; see Appendix S3: Table S5). The overlap coefficient varies from 0 (no overlap) and 1 (total overlap) (Weitzman 1970). We defined three overlap categories according the overlap values obtained from pairwise comparisons carried out with our data: Δ_4<50th percentile was considered as “low overlap”; 50th<Δ_4<75th as “moderate overlap”; and Δ_4>75th as “high overlap”; being the 50th and 75th percentiles for our overlapping coefficients 0.8 and 0.9, respectively (Monterroso et al. 2014). Confidence intervals for the overlap coefficients were obtained as percentile intervals from 1000 bootstrap samples. Then, we compared the activity patterns of each species pairs by using Watson’s two-sample test of homogeneity (U^2) for circular data (Jammalamadaka and Sengupta 2001).

Spatio-temporal segregation

We applied two methods to assess spatio-temporal segregation only among lynx and genets, because the stone marten was recorded almost exclusively in cameras where lynx did not occur (see Table S7). Firstly, we built a matrix of detections per hour of the daily cycle for each camera-trap, locality and year for both species. Then, we calculated the averaged proportion of cameras with co-occurrence and exclusive occurrence for each species along the diel cycle. Confidence intervals were calculated by empirical bootstrapping (Efron and Tibshirani 1994). Secondly, we estimated time-to-encounter between a lynx detection and the following genet detections in each locality and year using the multi-response permutation procedures showed by Mielke et al. (1976). We calculated the probability of time-to-encounter by comparing the median observed value with the simulated expected distribution (1000 simulations) and obtaining the proportion of randomly generated values that are greater than the observed time-to-encounter. A higher value of observed time-to-encounter in comparison to the expected value meant avoidance and a lower value indicated aggregation. We carried out these analyses by using the R codes provided by Karanth et al. (2017).

Modelling

We fitted Generalized Linear Mixed Models (GLMM) to evaluate 1) the top-down effect of Iberian lynx presence and the bottom-up effect of the prey relative abundance on the abundance of mesocarnivores, 2) whether the time-to-encounter between genets and lynx was related with prey and genet visitation rate and 3) whether the encounter probability between genet and lynx was affected by the availability of prey (see Table 1). The number of captures by 100 cam-days were used to calculate visitation rates at camera level and relative abundance indices (RAI) at locality level.

The presence/absence of Iberian lynx was included as a fixed factor for the response density of mesocarnivores and we added up second-order interactions among this variable and the fixed factors prey species (rabbits, garden dormice and other rodents) and carnivore species (stone marten and common genet) and the covariates prey relative abundance (RAI) and carnivore density. The locality was used as a random factor. We used gaussian errors and applied log transformation. Regarding time-to-encounter, the response variable was the median time to encounter in days from every visit of a lynx up to a genet visit in each camera and the fixed effects were the genet and prey visitation rate and its interaction with the micromammal species (garden dormice and other rodents) at camera level. Camera station was included as a random effect and Poisson errors were used. We excluded the rabbit of these analysis because it does not seem to be an important prey for genets based on our temporal overlap results. We upper-limited the garden dormouse visitation rate by camera to 200 because we detected five outliers which biased our results. Lastly, for the spatial encounter probability among genet and lynx, we used a binomial response of encounter/non-encounter in each camera and we included the presence/absence of the garden dormouse and other rodents as fixed effects and the camera site as random effect.

We calculated the marginal (contribution of fixed effects) and conditional (both fixed and random effects) coefficient of determination (pseudo-R^2) for all proposed mixed models (Nakagawa et al. 2017). We used R base functions (version 3.6.1; R Core Team

2019) and specialized packages (lme4 v.1.1-21 for GLMMs; Bates et al. 2020; MuMIn v.1.9.5 for pseudo-R^2; Barton 2013).

Results

Overall patterns

Camera-traps recorded 2.30 ± 0.56 (Mean ± SE) (n=156), 13.79 ± 3.77 (n=1546) and 21.59 ± 5.92 (n=1708) independent captures per 100 cam-days of adult Iberian lynx, common genets, and stone martens, respectively. Only 22% of independent genet detections and 0.9% of marten detections were recorded at localities inside lynx range (Appendix S3: Table S2). Genets and martens occupied, respectively, 41.6% and 5.8% of the camera-traps inside lynx territories and 80.8% and 87.5% outside (Appendix S3: Table S2). We identified 5.0 ± 0.76 genets and 1.4 ± 0.24 stone martens per locality inside lynx territories, and 9.3 ± 1.16 genets and 9.3 ± 1.61 martens outside (Appendix S3: Table S1). From the total of carnivore captures, 86.2%, 91.2% and 76.6% was recorded outside breeding season (March-June) for the lynx, genet and marten, respectively (Appendix S3: Table S5). We recorded 3.5 ± 0.54 lynx adults per locality (Appendix S3: Table S1) and we detected reproduction (kittens from the previous breeding season) in all 'lynx presence' localities . We found on average 55.8% ± 6.1% lynx occurrence and 2.9 ± 0.60 detections/100 cam-days (Appendix S3: Table S2). We registered only four lynx detections (2.5% of the overall lynx detections in two years) in two localities considered as controls (Appendix S3: Table S1-S2), probably connected with sub-adult dispersal or occasional exploratory incursions of territorial males from territories in the vicinity (see Fig. 1). We did not register any lynx territorial couple or reproductive event within control localities.

Regarding prey species, detections by 100 cam-days were 80.62 ± 30.40 (n=5445), 27.69 ± 6.29 (n=2913) and 5.94 ± 1.38 (n=416) for dormice, other rodents and rabbits respectively, of which the 98%, 73% and 99% were obtained inside lynx territories (see Appendix S3: Table S3). Occurrence was 44.2% for rabbits, 57.5% for dormice and 80.8% for other rodents inside lynx ranges. In localities without lynx, the highest percentage of occupation was for other rodents (62.5%) but the garden dormouse and rabbit occupancy was very low (only 4.2% and 2.5%, respectively) (Appendix S3: Table S3). We calculated that the 89.87% and 88.5% of the prey events occurred in lynx territories outside and inside breeding season, respectively (Appendix S3: Table S5). During breeding season and considering together both predation risk scenarios, we detected 2.6 higher and 0.73 lower relative abundance than out of breeding for the garden dormouse and other rodents, respectively (Appendix S3: Table S5).

Table 1. Coefficients with standard errors (SE), F or Likelihood ratio test statistics, significance values and marginal and conditional pseudo-R2 for the independent variables included in the generalized lineal mixed models (GLMM) for carnivore density, median time-to-encounter and encounter probability responses. The variance of the random effect ‘locality’ was 0.01 ± 0.08 (σ ± SE) for carnivore density response. The variance of the random effect ‘cam’ was 0.59 ± 0.77 for median time-to-encounter response and 2630 ± 51.28 for the encounter probability.

Response	Df	Fixed effects	Coeff. ± SE	F/LRT	P	R^2_m	R^2_c
Carnivore density	1	Intercept	-1.24 ± 0.22	-	-	0.75	0.79
	1	L	-0.76 ± 0.26	8.45	<0.01**		
	1	CS	0.19 ± 0.17	1.20	>0.05		
	1	PRAI	-0.02 ± 0.43	0.001	>0.05		
	1	L*CS	-1.54 ± 0.22	50.94	<0.0001***		
	1	L*PRAI	0.06 ± 0.46	0.02	>0.05		
	2	PS:PRAI	-	0.10	>0.05		
Median time-to-encounter	1	Intercept	2.32 ± 0.28	-	-	0.09	0.92
	1	GVR	0.25 ± 0.23	1.23	>0.05		
	1	PVR	0-0.53 ± 0.31	2.96	>0.05		
	1	PS	0.29 ± 0.19	2.37	>0.05		
	1	PS:PVR	-	2.17	>0.05		
Encounter probability	1	Intercept	-23.48 ± 6.50	-	-	0.10	0.99
	1	OR	12.80 ± 4.79	13.49	<0.001***		
	1	GD	33.16 ± 9.34	15.88	<0.001***		

Mesocarnivore density estimates

The abundance of mesopredators (individuals/km^2) was significantly higher outside lynx ranges (Table 1) and we observed stronger suppression on the stone marten density (Mean ± SE: 0.43 ± 0.07 vs. 0.04 ± 0.01; 10.75-fold decrease in lynx ranges) than on the common genet density (0.33 ± 0.05 vs. 0.15 ± 0.02; 2.20-fold decrease in lynx ranges) (Appendix S2: Table S1; Fig. 2). At level of locality, the lowest stone marten densities always occurred in localities with lynx (Appendix S2: Table S1). We did not find a significant effect from prey availability on carnivore density, even considering interactions among lynx presence/absence and prey species (Table 1).

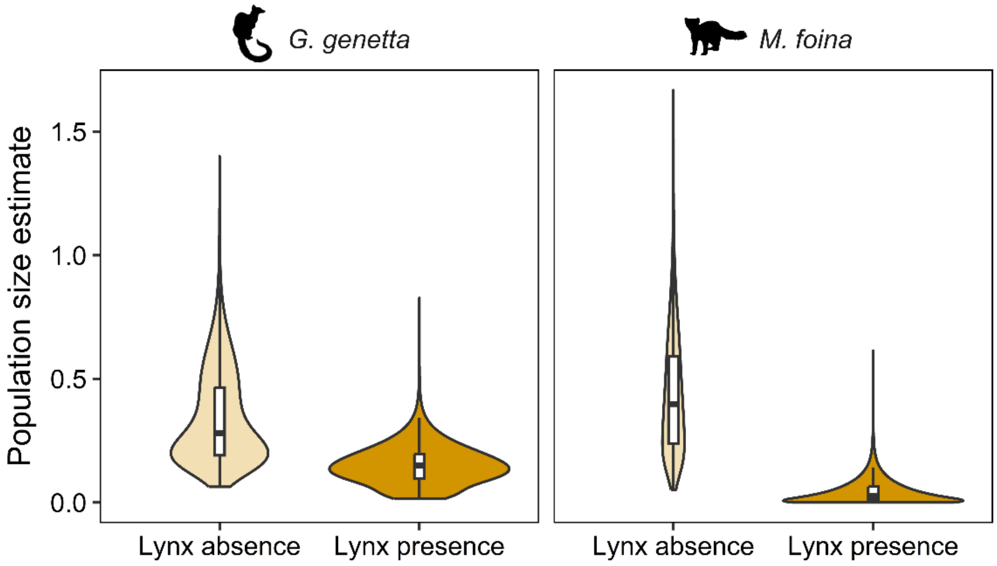

Figure 2. Estimated density (individuals per km^2) for the common genet (*G. genetta*) and the stone marten (*M. foina*). The width of each density curve corresponds with the frequency of data in each abundance interval and boxplots show the median and data quartiles with upper and lower whisker limits.

Temporal overlap among lynx, mesocarnivores and prey

Lynx activity was mostly crepuscular with some of diurnal activity, but turned into more nocturnal during the breeding period (Fig. 3). Genet and marten showed a strictly nocturnal behaviour in all periods and predation risk scenarios (Fig. 3). Genets showed a clear peak of activity after sunset inside lynx ranges outside breeding season but their activity pattern was more uniform in localities without lynx, showing a later peak around midnight during the breeding season (Fig. 3). Martens showed a single activity peak around midnight when inhabiting Iberian lynx ranges and two peaks of activity in localities without lynx (Fig. 3). We were not able to calculate the activity patterns of stone marten inside lynx territories during breeding season because we did not get enough records due to its very low abundance in this scenario (Appendix S3: Table S5). We found significant differences between lynx and genet and lynx and marten activity patterns outside breeding, with a low temporal overlap (Fig. 3). However, lynx-genet overlap during the breeding season was intermediate, showing non-significant differences in their activity patterns (Fig. 3). There was high overlap in genet and marten activity patterns comparing predation risk scenarios for both reproductive periods (Fig. 3).

Rabbits showed a high activity overlap with the Iberian lynx outside breeding period and low overlap inside breeding season (Fig. 4). We found significant differences between rabbits and mesocarnivore activity patterns inside lynx ranges during both reproductive periods (Fig. 4). The activity pattern of rodents (excluding dormice) overlapped highly with mesocarnivores in all scenarios, but the overlap was intermediate with the Iberian lynx (Fig. 4). We only found significant differences for the pair marten-rodents outside breeding and outside lynx territories (Fig. 4). However, significant differences were found among the circadian activity of carnivores (including lynx) and dormice outside breeding season in both predation risk scenarios, except for the pair marten-dormouse inside lynx territories (Fig. 4). Contrastingly, during the breeding season, we found no significant differences among the temporal patterns of mesocarnivores and dormice for any predation risk scenario and the same result was found for the pair lynx-dormouse during this period (Fig. 5).

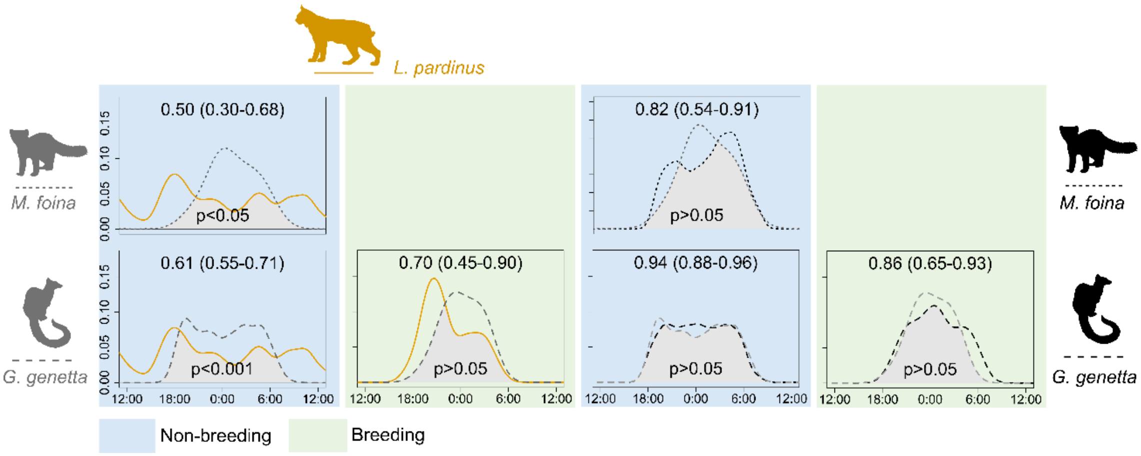

Figure 3. Temporal activity patterns of carnivores in localities with Iberian lynx presence (lynx silhouette) and localities without lynx (no lynx silhouette) outside the breeding season (blue boxes) and inside (green boxes). The Iberian lynx is represented by orange solid line, the stone marten by dotted line and the common genet by dashed line. Mesocarnivores co-existing with lynx are coloured in dark grey and mesocarnivores living outside lynx ranges in black. Empty plots refer to overlap unrealized combinations.

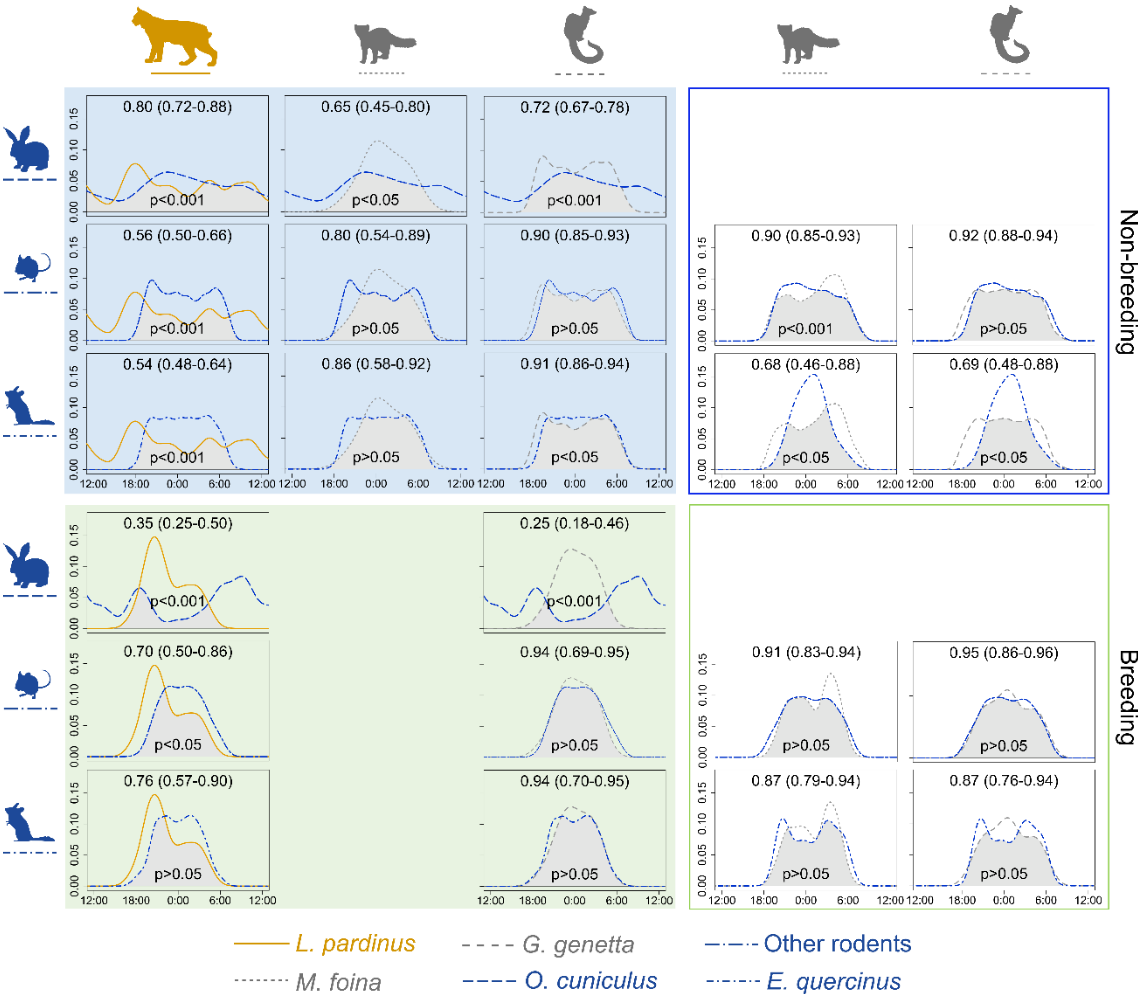

Figure 4. Temporal activity patterns of carnivores and prey species in localities with Iberian lynx presence (colour-filled boxes) and localities without lynx (non-colour filled boxes) outside breeding season (light blue) and inside (green). Apex predator is represented by orange, mesocarnivores by grey and prey by blue. Iberian lynx (solid line), common genet (dashed line), stone marten (dotted line), European rabbit (long dash line), other rodents (long dot–dash line) and garden dormouse (dot– dash line). Empty plots refer to overlap unrealized combinations.

Spatio-temporal segregation among carnivores

Inside Iberian lynx territories, the proportion of visits of the common genet at camera sites without Iberian lynx ranged from 0% to 93% across localities in the first year (Mean ± SE: 47.58% ± 0.18) and from 40% to 93% in the second year (68.96% ± 0.09) (Appendix S3; Table S6). Lynx and genets were detected simultaneously in fewer than 8% of the camera-traps at each time interval whereas genet and lynx co-occurred exclusively at up to 36% and 14% of the cameras, respectively (Appendix S3: Table S7). The genet showed a notable avoidance behaviour mainly in the first year, reflected by an average time-to-encounter with lynx of 18.36 days (SE=1.80) but also in the second year with 14.43 days (SE=1.59) (Fig. 5). For the stone marten, we only detected one record spatially overlapping with lynx, while the 99.95% of the visits occurred in cameras where the lynx was not recorded, so that was impossible to calculate time-to-encounter.

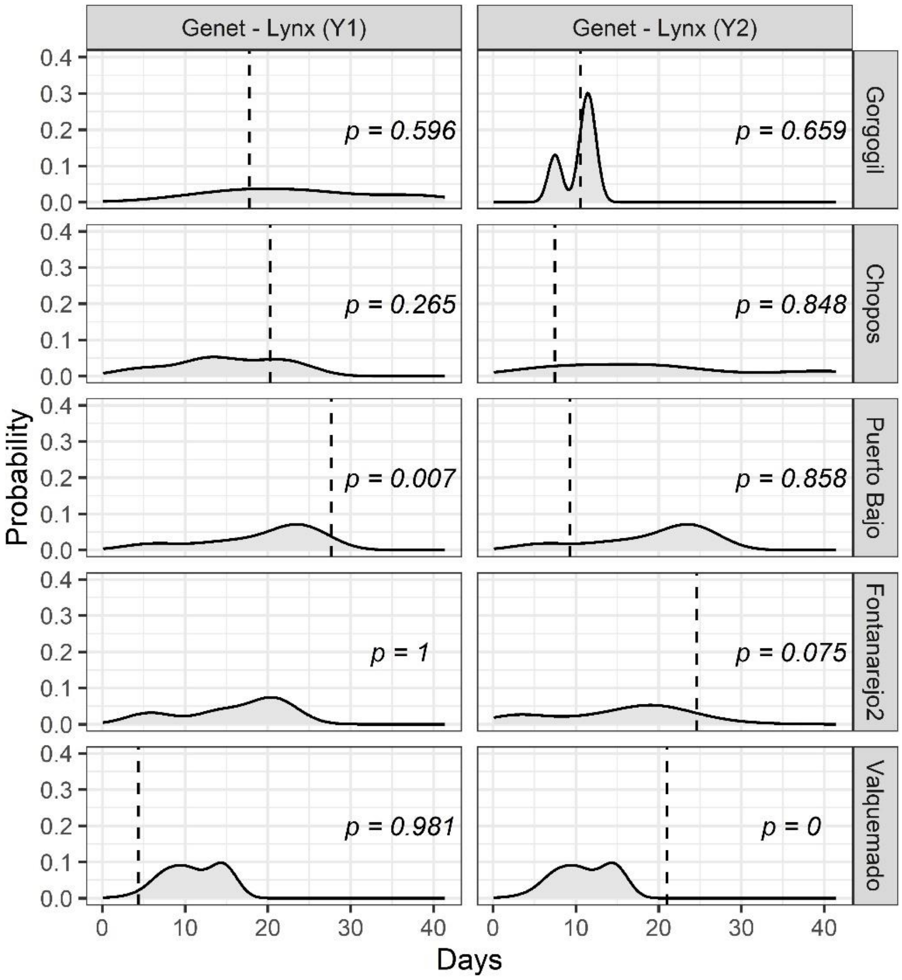

Figure 5. Spatio-temporal segregation patterns of common genets respect to the Iberian lynx in each locality and year. The vertical dashed lines represent the observed median minimum time-to-encounter between a lynx and a genet and the shaded grey area shows randomly simulated times-to-encounter. The p-values represents the proportion of randomly generated times-to-encounter values that are greater than the observed time-to-encounter.

Time-to-encounter and spatial encounter probability

We found that the median time interval between a visit of lynx and the following visit of a genet was not significantly affected by genet visitation rate and micromammal availability at camera level (Table 1). However, we found that the spatial encounter probability between lynx and genets was positive and significantly related to the presence of prey (both of garden dormouse and other rodents) (Table 1; Fig. 6).

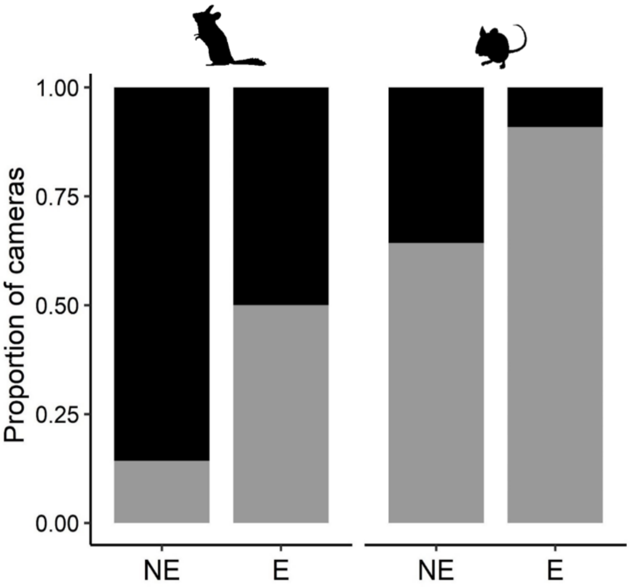

Figure 6. Proportion of camera-traps with presence of common genet inside lynx territories which in occurred an encounter with an Iberian lynx (E) or not (NE). Black colour shows the proportion of cameras with absence of micromammals and grey colour shows presence of micromammals. The first silhouette refers to the garden dormouse (*E. quercinus*) and the second silhouette to other rodents (*A. sylvaticus*, M. spretus and *R. rattus*).

Discussion

Our natural experiment in Southern Spain reveals that competitive intra-guild interactions among carnivores can be relaxed by bottom-up forces in a scenario of apex predator restoration in Mediterranean ecosystems (see Fig. 7). The largest predator in the area, the Iberian lynx, exerted a strong top-down control on absolute abundances of two sympatric mesopredators with a high dietary overlap but different trophic plasticity. However, the suppression strength and the spatio-temporal avoidance strategies developed by subordinate predators markedly varied among species, which could lead to contrasting ecological implications for the ecosystem functioning. Although previous studies have documented the negative impact of the Iberian lynx on relative mesopredator abundances (Palomares et al. 1996, Sarmento et al. 2021), our study is pioneer on estimating the impact of an apex predator on the absolute mesopredator densities (Forsyth et al. 2019, Jiménez et al. 2019). We documented for the first time the impact of Iberian lynx on common genet abundance and provide one of the most accurate density estimates for stone marten in Mediterranean ecosystems, which seems to be the species most sensitive to the Iberian lynx. Moreover, by using spatially explicit capture-recapture methods to estimate absolute abundance of mesocarnivores, we likely avoid bias which could lead us to underestimate movements and hence overestimate density.

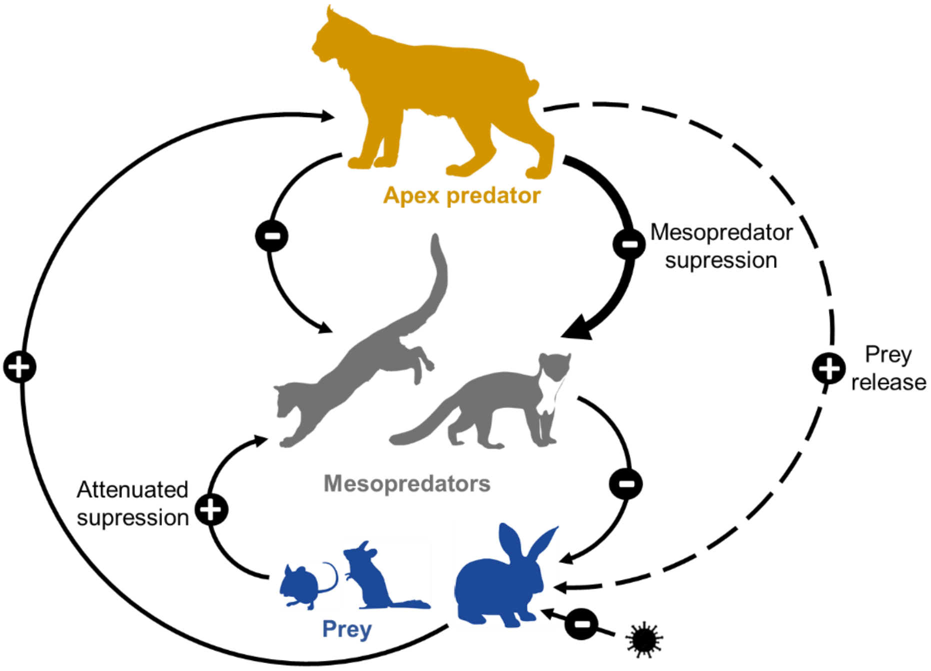

Figure 7. Direct (solid lines) and indirect trophic interactions (dashed lines) in the Mediterranean forest ecosystem. Trophic levels of the network are linked by top-down (descendent arrows) and bottom-up (ascendant arrows) forces which regulates the trophic web. Plus and minus symbols represent a positive or negative effect, respectively, and width of line indicates the relative strength of the interaction. Epizootic diseases (virus silhouette) determine the distribution of the European rabbit (*O. cuniculus*), the main prey of the Iberian lynx (*L. pardinus*) which is the apex predator in this ecosystem. Lynx controls mesopredator abundances and releases prey (rodents and rabbits) from their predation pressure. However, the suppression of the common genet (*G. genetta*) is lower compared to suppression of the stone marten (*M. foina*) possibly attenuated due to its more specialized diet in micromammals.

The recovery of the Iberian lynx in our study area is recent and still spatially heterogeneous, primarily due to the patchy distribution of its main prey, the wild rabbit, which is in turn connected with localized epizootic eruptions leading to frequent local extinctions of rabbits (Monterroso et al. 2016). Where the lynx was absent, the density of two sympatric mesopredators, the common genet and the stone marten, was up to 10-times larger than inside lynx territories. Similar mesopredator suppression has been shown by several apex predators across the world, such as the grey wolf, the dingo or the Eurasian lynx (Ritchie et al. 2012, Newsome et al. 2017). However, the strength of this top-down regulation differed considerably between the two mesopredator species, despite their similar size, diet and habitat use (Santos-Reis et al. 2004, Barrientos and

Virgós 2006). A narrower trophic niche of genets compared to martens could play a key role in dampening suppression and thus favour coexistence with the apex predator (Monterroso et al. 2020, Ferreiro-Arias et al. 2021). Another potential contributing factor to explain this species-specific suppression could be different behavioural mechanisms to avoid agonistic encounters or tolerance to predation risk. For example, both genets and martens are able to climb trees easily to escape from a lynx but the perception of predation risk may be variable among habitats or species (Santos-Reis et al. 2004). Stone martens use the three-dimensional space (i.e. movements above ground in tree crowns) less frequently than other sympatric species such as the pine marten (*Martes martes*) (Goszczyński et al. 2007), which could increase the likelihood of agonistic encounters with a lynx. This habitat-use segregation could also occur in respect to the genet, but fine-scale ecological studies on habitat use of these sympatric species are still insufficient for a comprehensive understanding of species-specific anti-predatory responses.

Although previous research has revealed that several species can modify their temporal activity patterns according to factors such as predation risk or human disturbance (Díaz-Ruiz et al. 2016, Gil-Sánchez et al. 2021), the circadian rhythms of genets and martens were predominately nocturnal and consistent among predation risk scenarios and seasons (Monterroso et al. 2014, Zanón Martínez et al. 2021). However, these mesocarnivores showed temporal segregation with the Iberian lynx, in contrast to Sarmento et al. (2021) findings for a more diurnal and adaptable mesopredator, the red fox. Thus, temporal segregation among subordinate carnivores and larger predators is often a result of an evolutionary adaptation to resource and habitat partitioning (Di Bitetti et al. 2010, Monterroso et al. 2014, Allen et al. 2020).

As we expected for a micromammal specialist consumer, genets synchronized their activity patterns with rodents in all scenarios but did not so with rabbits (Vilella et al. 2020). Small mammals offer energetically richer food intake than other resources, such as fruits (Santos et al. 2020), but due to scarce abundance of rabbits in our study area, this prey seems to be less preferred by generalists (Ferreras et al. 2011). In contrast, stone marten activity overlapped highly with small mammals only inside lynx territories and during the breeding season, when the availability of prey was maximum. Breeding coincides with the spring, when the garden dormouse comes out from its winter lethargy and increases activity (Moreno 1988). Bakaloudis et al. (2012) also found an animal-type prey specialization by stone martens in spring, contrasting with the general tendency to consume fruits during winter in Mediterranean ecosystems. Thus, several pieces of evidence indicate that martens are shifting their activity rhythms and likely their diet according to prey availability, as was already observed in several other facultative predators (Pereira et al. 2014). However, our results on behaviour of martens under predation risk must be taken with caution because it may be a consequence of the limited amount of records of stone marten inside lynx territories (11 detections).

The higher dormice availability during the breeding season could be increasing the tolerance of genets to predation risk (Santos et al. 2019, Vilella et al. 2020), as temporal partitioning with lynx did not occur. Despite the general synchronized activity patterns found among genets and other rodent species, they only overlapped highly with dormice during the breeding season, and the same pattern was found for the Iberian lynx. Although the Iberian lynx is a rabbit-specialist, their low temporal overlap with this prey species could be explained because lynx is complementing its diet with the abundant garden dormouse (Gil-Sánchez et al. 2006), especially when reproductive females are breeding and would benefit from additional, easy to catch prey, as was also observed in the Eurasian lynx (Krofel et al. 2011).

Genets and martens are behaviourally and ecologically similar and could be expected to develop analogous strategies to cope with larger predators. However, the stone marten almost disappeared from the Iberian lynx territories, which is in accordance with the strong avoidance patterns found by Monterroso et al. (2020) and Virgós et al. (2020). In contrast, the common genet co-existed spatially with the apex predator in low density by developing fine-scale spatio-temporal mechanisms (Karanth et al. 2017, Sarmento et al. 2021), similarly to observations of Jiménez et al. (2019) for other mesocarnivores following Iberian lynx reintroduction. Although previous studies described a high spatial segregation of genets in respect to lynx home ranges (Palomares et al. 1996, Jiménez et al. 2019, Monterroso et al. 2020), we found a surprising number of detections of genets in cameras where the lynx appeared (circa 50%), probably due to the higher abundance of genets and/or the greater forestry cover and refuge availability in our study area compared to other Mediterranean forests.

Bottom-up effects can also regulate the spatiotemporal coexistence patterns among carnivores (Karanth et al. 2017, Santos et al. 2019, Monterroso et al. 2020, Ferreiro-Arias et al. 2021). Similarly to previous research found for other prey (Gordon et al. 2015, Jiménez et al. 2019), a reduced overall predation pressure inside lynx territories seemed to benefit small mammal abundances in our study area. A more specialized diet on rodents probably led some genets to persist inside lynx ranges in spite of the risk of being killed. Martens however were able to feed on a wider spectrum of food resources available in non-risky nearby areas. Encounters among genets and lynx were more likely when rodent availability was higher locally, what may suggest that both predators are taking advantage of the abundance of prey or genets visiting these food hotspots are less cautious because of high motivation to forage there, as predicted by the optimal foraging theory (MacArthur and Pianka 1966).

Considering the strong lynx suppression of stone marten abundance, the impact on its spatial distribution patterns likely has indirect community level impacts. Carnivores are effective seed dispersers of many fleshy-fruited plants, dispersing large numbers of seeds to long distances but showing functional diversity among species (González-Varo et al. 2013, Draper et al. 2022). Fruit seeds are found in up to 94% scats of stone marten, contrasting with the more specialised genet diet on micromammals (Virgós et al. 1999, López-Martín 2006). Therefore, a suppressed abundance of key frugivorous carnivores as martens could trigger cascading effects for plants by limiting seed dispersal inside lynx territories (Escribano-Ávila et al. 2013, Fedriani et al. 2020a, Burgos et al. 2022). However, the exclusion of stone martens from rodent-rich patches inhabited by lynx, could impact body condition and fitness (Santos et al. 2020) and shift diet composition of marten population towards higher consumption of fruits (Gazzola and Balestrieri 2020), triggering a two-told effect on the ecosystem functioning by limiting seed dispersal inside lynx territories but boosting it outside. Thus, the patchy lynx distribution and the paired suppression of mesopredators as a consequence of spatially variable abundance of lynx main prey, the rabbit, could be contributing to build a mosaic ecosystem structure where apex predators, mesopredators and prey have variable local abundances and connected ecological roles (Fig. 7).

Our findings suggest that Iberian lynx reintroduction or recovery could reverse a potential mesopredator release scenario in areas where this apex predator was lost or severely reduced, but the strength of this top-down effect in the trophic web is species-specific. The most diet-flexible mesopredator, the stone marten, is drastically reduced by the apex predator and shows high spatial and temporal segregation with the apex predator. Nevertheless, the mesocarnivore with a narrow trophic niche breadth, the common genet, co-exists spatially in reduced density with the Iberian lynx and also overlaps temporally during the breeding season. Furthermore, bottom-up forces also seem to be important in the trophic web by dampening the mesopredator suppression and facilitating coexistence. The contrasting suppression found for two sympatric mesocarnivores may alter important ecosystem functions and processes involving them. Thus, conservation practitioners should consider to promote a locally-patchy distribution of the Iberian lynx through the landscape because it may result in balanced net effects for different trophic levels at a regional scale. Further future detailed studies are essential to assess how apex predator reintroductions are restoring the ecosystem functioning prior to their extinction. This knowledge will lead to more effective conservation programs of carnivores by taking evidence-based decisions and a better understanding on the working of the ecosystem at community level.

Supporting information – Chapter 1

Top-down and bottom-up effects modulate species co-existence in a context of top predator restoration

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Appendix S1. Individual identification

Figure S1. a-d show four different stone martens (*Martes foina*) individuals identified in the study based on distinctive fur marks (red boxes). The stone marten has a uniform coat pattern which makes this species particularly difficult to identify individually. Due to stone martens stood up to reach the bait at the top of the wooden sticks used in this study, we were able to identify successfully the most of the stone marten individuals because each individual presents several unique marks along the white throat.

Figure S2. Spot patterns (red boxes) used for individual identification of the common genet *Genetta genetta* (ab) and the Iberian lynx *Lynx pardinus (cd)*. We needed to have photographs from the two sides of these species because the spot patterns vary. Thank we used alternative baits (sardine and lynx urine), we usually obtained more than one photograph of each individual in different positions during the same event, which allowed us to identify correctly the individuals.

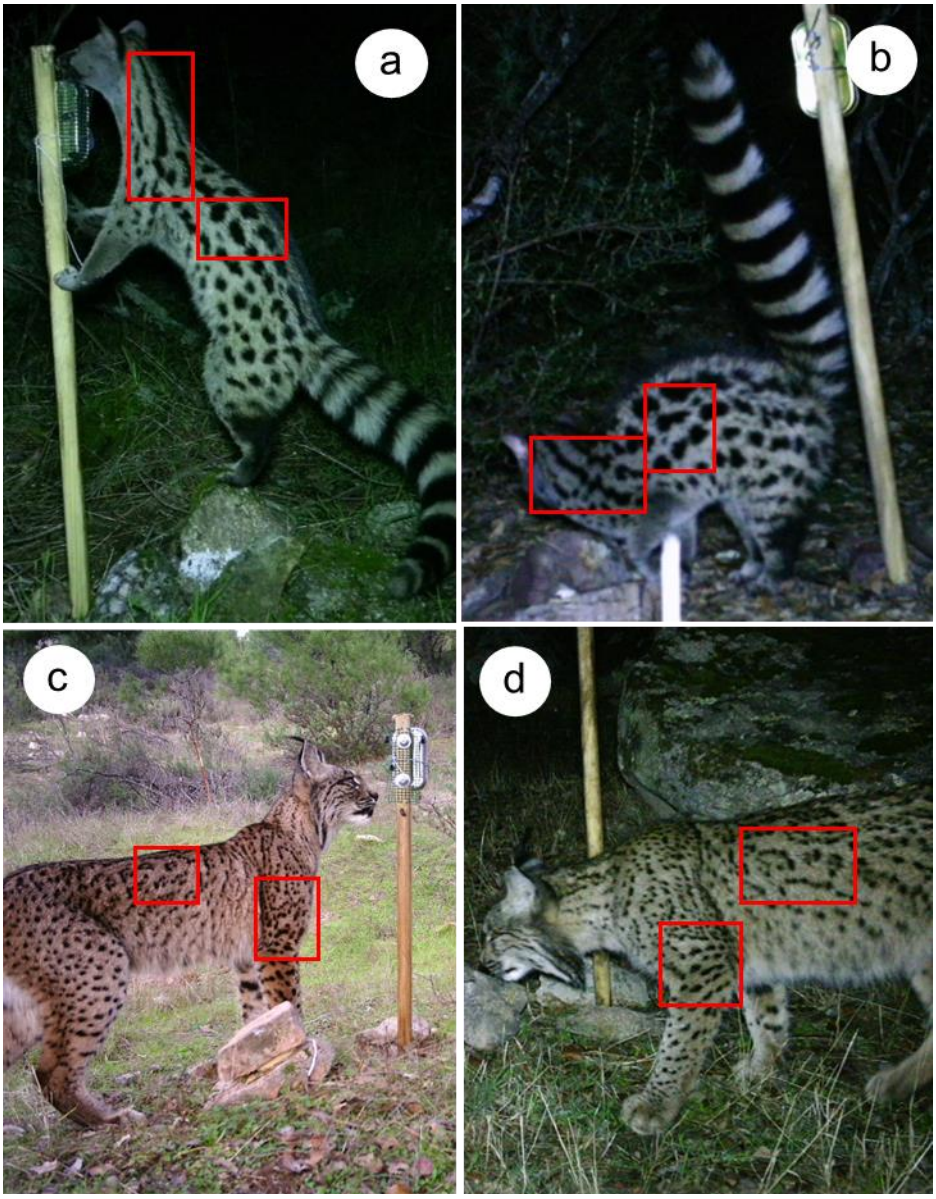
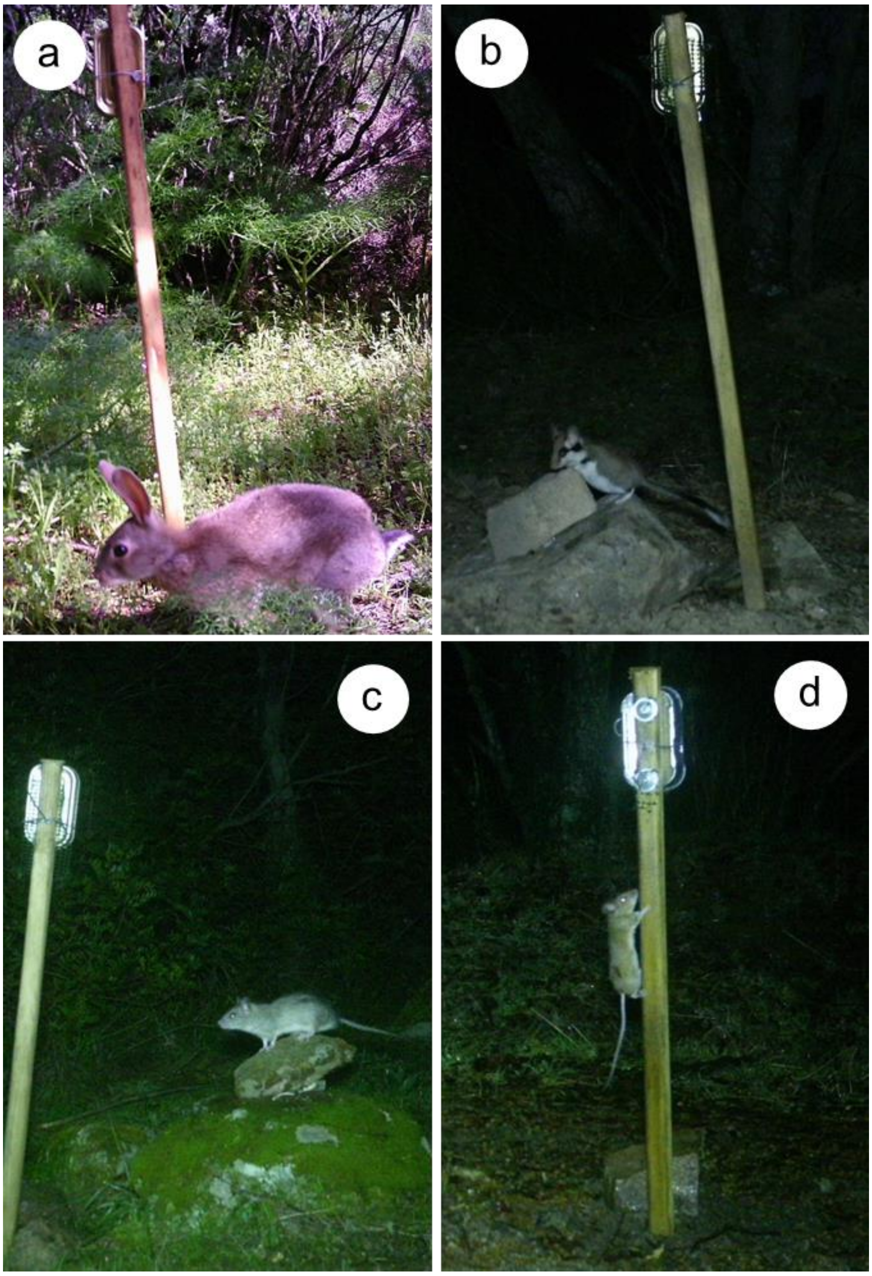


Figure S3. Photographs of the main small-mammal prey species of the studied carnivorous guild: a) European wild rabbit (*Oryctolagus cuniculus*), b) Garden dormouse (*Eliomys quercinus*) and other rodent species such as c) the black rat (*Rattus rattus*) or d) the wood mouse (*Apodemus sylvaticus*). Our camera-trapping scenario has been proved useful to get good-quality images of small mammals because in mostly occasions, they appeared on the top of the wood stick attracted by the lure.



Appendix S2. Density estimates

Table S1. Mean density (individuals/km^2) with 95% Bayesian credible intervals (BCI) estimated for mesocarnivores in each locality and year. The shared movement parameter (σ) estimated for stone marten was 0.56 (BCI: 0.51-0.61) in the first year and 0.65 (BCI: 0.60-0.70) in the second year. Genet sigma was 0.92 (BCI: 0.75-1.19) and 1.02 (BCI: 0.85-1.26) for the first year and 0.97 (BCI: 0.81-1.17) and 0.64 (BCI: 0.59-0.68) for the second year, in localities with lynx and without lynx respectively.

Year	**Lynx**	**Locality**	*G. genetta* **density**		*M. foina* **density**	
			Mean	BCI	Mean	BCI
1	Presence	Gorgogil	0.22	0.12-0.39	0.03	0.00-0.12
		Puerto Bajo	0.16	0.07-0.33	0.09	0.02-0.24
		Valquemado	0.08	0.03-0.19	0.03	0.00-0.13
		Fontanarejo2	0.16	0.07-0.32	0.03	0.00-0.14
		Chopos	0.17	0.08-0.32	0.03	0.00-0.13
	Absence	Lugar Nuevo	0.22	0.13-0.38	0.32	0.15-0.56
		Selladores	0.27	0.16-0.48	0.81	0.52-1.17
		Fontanarejo1	0.17	0.09-0.31	0.63	0.37-0.97
		Cereceda	0.19	0.10-0.35	0.38	0.18-0.68
		Risquillo	0.22	0.12-0.36	0.26	0.12-0.48
2	Presence	Gorgogil	0.18	0.09-0.31	0.02	0.00-0.09
		Puerto Bajo	0.04	0.01-0.12	0.07	0.01-0.18
		Valquemado	0.14	0.07-0.25	0.02	0.00-0.09
		Fontanarejo2	0.15	0.07-0.27	0.10	0.03-0.22
		Chopos	0.23	0.14-0.37	0.02	0.00-0.09
	Absence	Lugar Nuevo	0.64	0.42-0.92	0.20	0.10-0.36
		Selladores	0.51	0.33-0.76	0.66	0.45-0.95
		Fontanarejo1	0.54	0.33-0.81	0.47	0.28-0.72
		Cereceda	0.22	0.10-0.41	0.43	0.24-0.68
		Risquillo	0.40	0.23-0.63	0.17	0.07-0.32

Figure S1. Convergence of Markov chains for the density of common genet *G. genetta* for the first (left) and the second study year (right) in the ten localities.

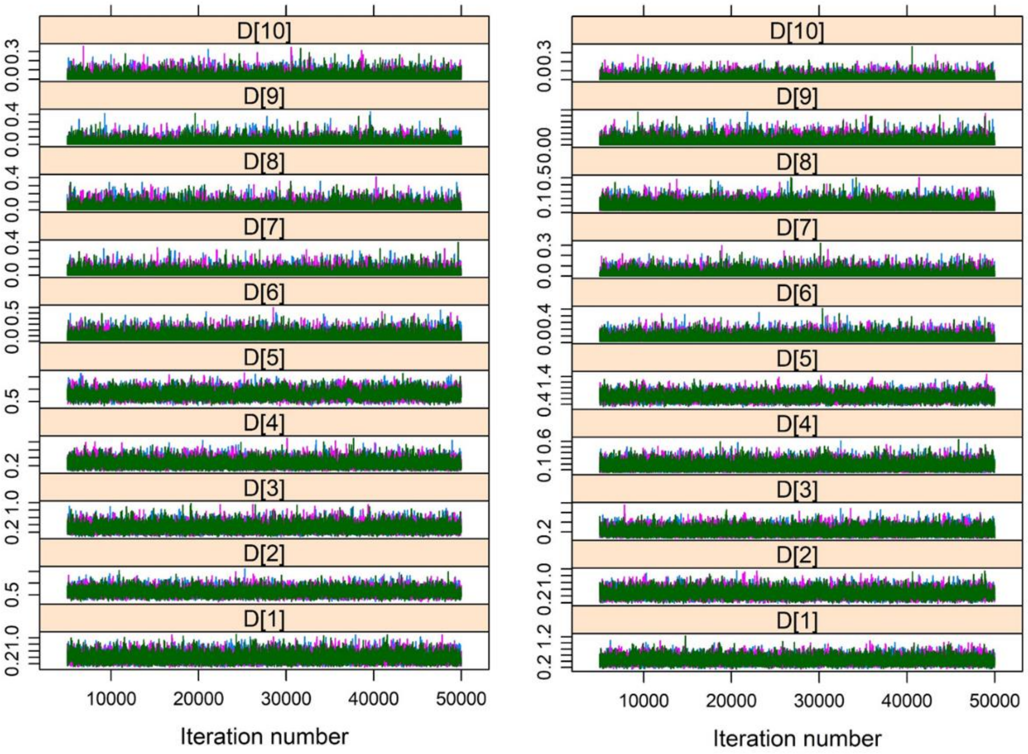


Figure S2. Convergence of Markov chains for the density of stone marten *M. foina* for the first (left) and the second study year (right) in the ten localities.

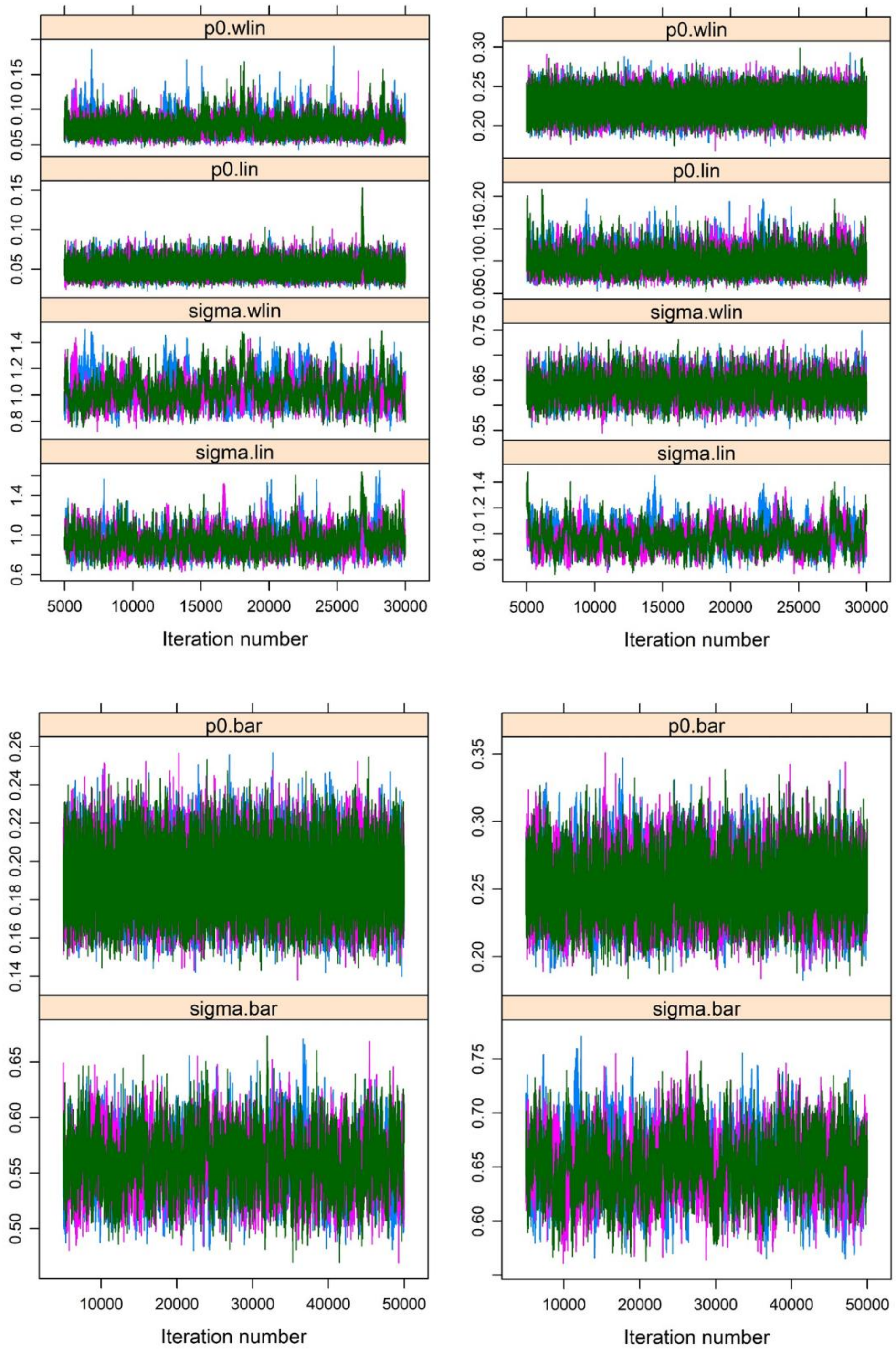
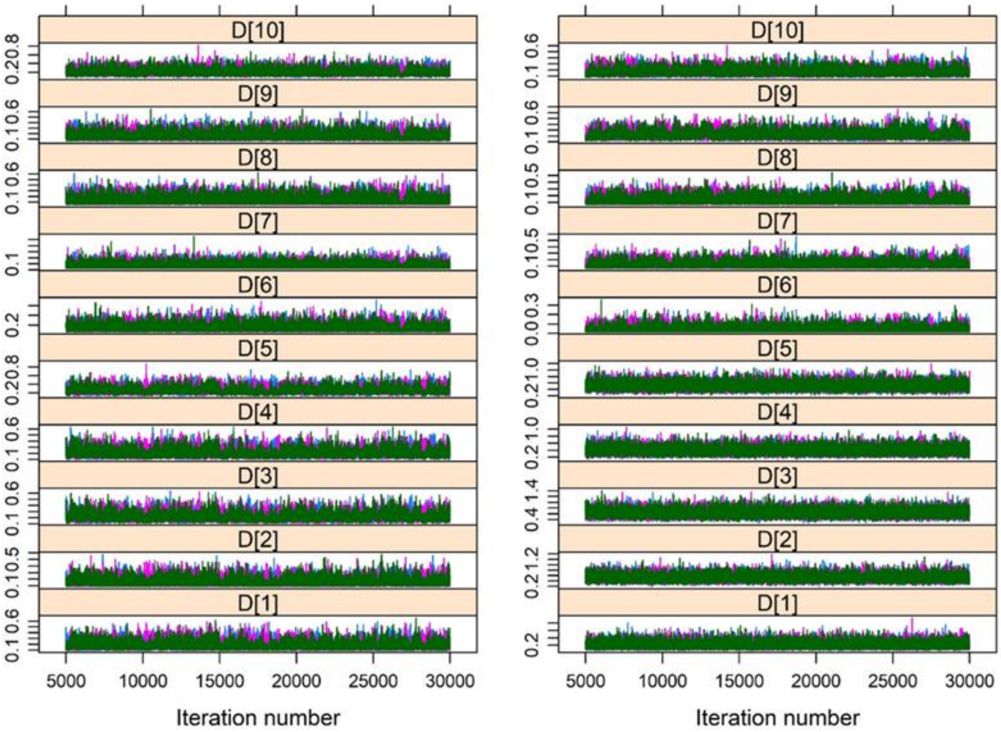


Figure S3. Convergence of Markov chains for the basal detection rate (λ_0) and movement parameters (σ) of common genet *G. genetta* (upper) and stone marten *M. foina* (lower) for the first (left) and the second study year (right) in localities with Iberian lynx presence (lin) and without lynx (wlin). For the stone marten we used a shared σ and λ_0 for all study localities.



Appendix S3. Overall results from camera-trapping

Table S1. Number of adult photo-identified individuals in each study locality and year.

Year	Lynx	Locality	Adult identified individuals		
			L. pardinus	*G. genetta*	*M. foina*
1	Presence	Gorgogil	3	7	0
		Puerto Bajo	3	4	1
		Valquemado	7	2	0
		Fontanarejo2	3	4	0
		Chopos	4	5	0
	Absence	Lugar Nuevo	0	8	6
		Selladores	0	10	17
		Fontanarejo1	0	6	12
		Cereceda	1	4	9
		Risquillo	0	8	5
2	Presence	Gorgogil	2	7	1
		Puerto Bajo	3	1	1
		Valquemado	6	5	0
		Fontanarejo2	2	6	2
		Chopos	2	9	2
	Absence	Lugar Nuevo	0	16	5
		Selladores	0	13	18
		Fontanarejo1	0	12	11
		Cereceda	1	4	9
		Risquillo	1	10	4

Table S2. Proportion of cameras with presence and number of independent captures by 100 cam-days of carnivores in each study locality and year.

Year	**Lynx**	**Locality**	**Occurrence**			**Captures/100 cam-days**		
			L. pardinus	*G. genetta*	*M. foina*	*L. pardinus*	*G. genetta*	*M. foina*
1	Presence	Gorgogil	0.50	0.42	0.00	1.82	4.19	0.00
		Puerto Bajo	0.83	0.25	0.08	3.36	1.31	0.19
		Valquemado	0.75	0.17	0.00	6.76	6.96	0.00
		Fontanarejo2	0.33	0.58	0.00	1.55	1.98	0.00
		Chopos	0.67	0.42	0.00	1.60	7.98	0.00
	Absence	Lugar Nuevo	0.00	0.92	0.67	0.00	32.24	15.61
		Selladores	0.00	1	1	0.00	10.72	50.65
		Fontanarejo1	0.00	0.83	1	0.00	12.96	54.44
		Cereceda	0.08	0.67	0.83	0.22	10.99	23.08
		Risquillo	0.00	0.58	0.67	0.00	8.43	10.73
2	Presence	Gorgogil	0.42	0.33	0.08	2.05	3.42	0.23
		Puerto Bajo	0.42	0.25	0.08	2.02	1.00	0.20
		Valquemado	0.83	0.5	0.00	5.93	9.41	0.00
		Fontanarejo2	0.42	0.50	0.08	2.13	5.23	1.16
		Chopos	0.42	0.75	0.25	1.82	21.69	0.99
	Absence	Lugar Nuevo	0.00	1	0.92	0.00	77.27	15.42
		Selladores	0.00	1	1	0.00	54.86	67.19
		Fontanarejo1	0.00	0.92	1	0.00	20.33	65.65
		Cereceda	0.08	0.42	0.83	0.23	4.28	22.52
		Risquillo	0.17	0.75	0.83	0.40	7.34	14.68

Table S3. Proportion of cameras with presence and captures by 100 cam-days of prey in each study locality and year.

Year	Lynx	Locality	Occurrence			Captures/100 cam-days		
			Rabbit	Dormouse	Other rodents	Rabbit	Dormouse	Other rodents
1	Presence	Gorgogil	0.33	0.67	0.83	8.20	19.85	39.98
		Puerto Bajo	0.58	0.83	1.00	7.46	287.13	71.46
		Valquemado	0.25	0.75	1.00	2.78	160.44	117.70
		Fontanarejo2	0.42	0.33	0.92	7.77	30.93	16.53
		Chopos	0.58	0.33	0.92	8.87	14.72	47.34
	Absence	Lugar Nuevo	0.00	0.00	0.50	0.00	0.00	4.09
		Selladores	0.00	0.00	0.42	0.00	0.00	6.28
		Fontanarejo1	0.00	0.00	0.42	0.00	0.00	4.63
		Cereceda	0.08	0.17	0.92	0.44	21.98	12.09
		Risquillo	0.08	0.17	0.92	0.19	0.96	56.71
2	Presence	Gorgogil	0.25	0.33	0.50	2.97	5.02	24.66
		Puerto Bajo	0.58	0.92	0.50	16.94	254.64	32.66
		Valquemado	0.58	0.50	0.75	7.57	20.04	22.70
		Fontanarejo2	0.33	0.75	0.92	11.24	230.62	15.50
		Chopos	0.50	0.33	0.75	2.65	1.16	17.22
	Absence	Lugar Nuevo	0.00	0.00	0.42	0.00	0.00	2.86
		Selladores	0.00	0.00	0.83	0.00	0.00	9.90
		Fontanarejo1	0.08	0.00	0.67	0.20	0.00	23.78
		Cereceda	0.00	0.00	0.50	0.00	0.00	4.50
		Risquillo	0.00	0.08	0.67	0.00	0.60	28.17

Table S4. Camera-traps working days in each study locality and year.

Lynx	Locality	Year	Date set	Working days
Presence	Gorgogil	1	19/12/2018	549
		2	14/11/2019	438
	Puerto Bajo	1	04/02/2019	536
		2	12/05/2020*	496
	Valquemado	1	18/12/2018	503
		2	10/01/2020	489
	Fontanarejo2	1	13/10/2018	708
		2	12/02/2020	516
	Chopos	1	05/12/2018	564
		2	13/11/2019	604
Absence	Lugar Nuevo	1	19/10/2018	538
		2	15/11/2019	629
	Selladores	1	17/10/2018	541
		2	20/11/2019	576
	Fontanarejo1	1	12/10/2018	540
		2	13/02/2020	492
	Cereceda	1	05/03/2019	455
		2	11/05/2020*	444
	Risquillo	1	05/02/2019	522
		2	11/02/2020	504

Table S5. Number of detections per 100 cam-days of carnivores and prey species used for temporal analysis outside and inside breeding season for carnivores. Camera-traps reached an overall effort of 4543 trap-days in sites with lynx and 3883 trap-days in sites without lynx excluding breeding season and 1024 trap-and 1415 trap-days in sites with lynx and without lynx, respectively during the breeding season.

Species	Lynx	Detections/100 cam-days (n)	
Carnivores		Non-breeding	Breeding
Iberian lynx *L. pardinus*	Absence	-	-
	Presence	2.88 (131)	2.05 (21)
Common genet *G. genetta*	Absence	31.73 (1233)	8.41 (119)
	Presence	7.26 (330)	1.76 (18)
Stone marten *M. foina*	Absence	37.24 (1446)	23.75 (336)
	Presence	0.24 (11)	0.49 (5)
Prey			
Rabbit *O. cuniculus*	Absence	0.05 (2)	0.14 (2)
	Presence	6.80 (309)	9.96 (102)
Garden dormouse *E. quercinus*	Absence	0.28 (11)	6.86 (97)
	Presence	73.43 (3336)	187.99 (1925)
Other rodents	Absence	14.04 (545)	15.97 (226)
	Presence	40.97 (1861)	24.22 (248)

Table S6. Proportion of visits of the common genet and the stone marten in cameras where the Iberian lynx did not appear in localities with presence of lynx.

Locality	***G. genetta* (Y1)**	***G. genetta* (Y2)**	***M. foina* (Y1)**	***M. foina* (Y2)**
Gorgogil	0.7391304	0.9333333	-	1
Puerto Bajo	0	0.4	0	1
Valquemado	0.6	0.6086957	-	-
Fontanarejo2	0.9285714	0.7037037	-	1
Chopos	0.1111111	0.8015267	-	1

Table S7. Average proportion of cameras where each species was exclusively active and where lynx and genet overlapped (G x L) per hour of the daily cycle. Empirical bootstrapped CI are shown in brackets.

Species	**Year**	**Locality**				
		Gorgogil	Puerto Bajo	Valquemado	Fontanarejo2	Chopos
G. genetta	1	0.15 (0.11-0.19)	0.08	0.14 (0.12-0.17)	0.11 (0.09-0.14)	0.14 (0.11-0.18)
G. genetta	2	0.10 (0.08-0.12)	0.10 (0.07-0.14)	0.14 (0.11-0.17)	0.14 (0.11-0.18)	0.36 (0.30-0.42)
L. pardinus	1	0.11 (0.08-0.14)	0.11 (0.09-0.14)	0.14 (0.10-0.18)	0.11 (0.08-0.14)	0.13 (0.09-0.16)
L. pardinus	2	0.08	0.11 (0.06-0.16)	0.14 (0.10-0.17)	0.11 (0.08-0.12)	0.08
G x L	1	0.08	0	0.08	0.08	0
G x L	2	0	0	0.08	0	0.08

Chapter 2

Predation risk can modify the foraging behaviour of frugivorous carnivores: implications of rewilding apex predators in plant-animal mutualisms

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Abstract

Apex predators play key roles in food webs and their recovery can trigger trophic cascades in some ecosystems. Intra-guild competition can reduce the abundances of smaller predators and perceived predation risk can alter their foraging behaviour thereby limiting seed dispersal by frugivorous carnivores. However, little is known about how plant-frugivore mutualism could be disturbed in the presence of larger predators. We evaluated the top-down effect of the regional superpredator, the Iberian lynx (*Lynx pardinus*), on the number of visits and fruits consumed by medium-sized frugivorous carnivores, as well as the foraging behaviour of identified individuals, by examining the consumption likelihood and the foraging time. We carried out a field experiment in which we placed Iberian pear (*Pyrus bourgaeana*) fruits beneath fruiting trees and monitored pear removal by frugivorous carnivores, both inside and outside of lynx ranges. Using camera traps, we recorded the presence of the red fox (*Vulpes vulpes*), the Eurasian badger (*Meles meles*) and the stone marten (*Martes foina*), as well as the number of fruits they consumed and their time spent foraging. Red fox was the most frequent fruit consumer carnivore. We found there were fewer visits and less fruit consumed by foxes inside of lynx ranges, but lynx presence did not seem to affect badgers. We did not observe any stone marten visits inside of lynx territories. The foraging behaviour of red foxes was also altered when inside of lynx ranges whereby foxes were less efficient, consuming less fruit per unit of time and having shorter visits. Local availability of fruit resources, forest coverage and individual personality also were important variables to understand visitation and foraging in a landscape of fear. Our results show a potential trophic cascade from apex predators to primary producers. The presence of lynx can reduce frugivorous carnivore numbers and induce shifts in their feeding behaviour that may modify the seed dispersal patterns with likely consequences for the demography of many fleshy-fruited plant species. We conclude that knowledge of the ecological interactions making up trophic webs is an asset to design effective conservation strategies, particularly in rewilding programs.

Key words: fleshy-fruit, Iberian lynx, Iberian pear, trophic cascade, landscape of fear, mesopredator release, seed dispersal, individual variability

Introduction

A trophic cascade occurs when predators trigger a reverberating effect across lower trophic levels in food webs (Estes et al. 2011, Ripple et al. 2016). Apex predators are at the top of trophic webs and can lead to top-down effects on the abundance and behaviour of prey populations, critically influencing ecosystem structure and functions (Schmitz 2006, Winnie and Creel 2017). Recent recovery and rewilding processes involving large carnivores worldwide (Chapron et al. 2014) have increased the attention of ecologists and conservation practitioners towards those cascading effects (Ritchie et al. 2012). Some studies have revealed direct killing by carnivores causes reductions in herbivore population sizes, which translates into positive effects for plants (Ripple and Beschta 2007, 2012a). Nonetheless, trophic cascades can also occur by non-lethal interactions through a behavioural change in response to a perceived predation risk (Schmitz et al. 2004) and the subsequent anti-predatory behaviour modifies the ecosystem functioning (Ripple and Beschta 2004). The most evident cascading effects on plants are mediated by herbivores (Ripple & Beschta, 2012ab), but little is known about the potential top-down effects of apex predators on plant-carnivore mutualisms.

Predator-prey interactions usually involve large carnivores and herbivores, however medium-sized carnivores (i.e. mesocarnivores) can also play the role of prey when they coexist with larger predators, known as superpredators (Holt and Polis 1997). Superpredators often control population abundances of smaller predators by intraguild predation or other types of antagonistic interactions, resulting in competition for shared resources (Polis et al. 1989). The global extirpation of apex predators (Wolf and Ripple 2018) has shifted many ecosystems to a state of mesopredator release (Crooks & Soulé, 1999). Apex predators promote a landscape of fear—an animal's perception of spatial variation in predation risk (Gaynor et al. 2019)—that not only alters abundance, but also the foraging behaviour of mesopredators (Laundré et al. 2010). For instance, Haswell et al. (2018) found that foxes responded to perceived predation risk by being less efficient foragers and spending less time visiting food patches. Similarly, Leo et al. (2015) identified non-foraging behaviours, such as resting, were less frequent under predation risk by foxes. Food patches which offer fewer resources but a low exposure to predation risk may be the optimal foraging sites even if the available resources are potentially more challenging to obtain (Brown et al. 1999, Laundré et al. 2010). According to the optimal foraging theory (Charnov 1976), predation risk can select more efficient or cautious foragers that modify their foraging behaviour due to the fear of larger carnivores (Toscano et al. 2016, Eccard et al. 2020) resulting in a trophic cascade on the resources exploited (Suraci et al. 2016).

Trophic cascades can also shape ecosystem functions that involve plant–animal interactions other than herbivory, such as seed dispersal (Kurten 2013). Ripple et al. (2014) were pioneers in this regard after wolves were reintroduced in Yellowstone. They found seed dispersal by grizzly bears increased due to a less abundant population of elks competing with bears for fruit resources. This finding indicates the value of opportunistic foraging by carnivores (Fedriani, Palomares, & Delibes, 1999) and highlights their role in ecosystem functioning as agents of seed dispersal (Schupp et al. 2010). Frugivorous carnivores have functional differences with other main groups of seed dispersers, that make them especially effective in this key mutualism (Escribano-Ávila et al., 2014; González-Varo, Fedriani Laffite, & Suárez-Esteban, 2015). Carnivores can move many seeds over long distances and increase the probability of seed germination (Traveset and Verdu 2002, Escribano-Ávila et al. 2013). Despite literature studying the implications for seed dispersal of the lack of key frugivore-plant interactions is growing recently, the loss of big-sized frugivores has been usually linked to defaunation (de Paula et al., 2018; Fedriani, Ayllón, Wiegand, & Grimm, 2020) rather than to reintroductions of superpredators.

In the Mediterranean ecosystems of Southern Spain, the Iberian lynx *Lynx pardinus* is the largest regional predator and plays the role of superpredator (Fedriani, Palomares, & Delibes, 1999). This feline was near extinction in the 90's as a consequence of direct exploitation, habitat fragmentation and human-assisted spread of diseases affecting its main prey (Rodríguez and Calzada 2015). The Iberian lynx is still one of the most endangered feline species in the world (Rodríguez and Calzada 2015). However, populations have recently increased and are currently estimated at 855 individuals (MITECO 2019). The recovery of this superpredator is due to several conservation efforts mainly focused on prey and habitat management, captivity breeding, reintroductions and enhancement of the genetic diversity of extant populations (Simón et al. 2012). Lynx cause direct decreases in mesopredator abundance and alter their habitat use (Sarmento et al. 2021), and the suppression of mesopredator release has positive effects on small prey species (Jiménez et al. 2019). Nevertheless, mesocarnivores are important seed dispersers in Mediterranean ecosystems (Herrera, 1989), and a decrease in their abundance or alterations to their foraging behaviour could negatively influence their mutualistic interactions with plants (Pegman et al. 2017, Carreira et al. 2020). Thus, to shed light on whether the Iberian lynx may induce a trophic cascade on Mediterranean fleshy fruited plants, we performed a field experiment to study the foraging behaviour of the carnivore community in areas where the Iberian lynx is present, compared to areas where this apex predator is extinct. We hypothesized that the presence of lynx would affect: 1) the number of visits to fruit trees and the number of fruits consumed by mesocarnivores at constructed fruit depots and 2) the foraging behaviour of the main frugivorous mesocarnivore. In particular, we expected that the presence of lynx will decrease the number of visits of mesocarnivores and, consequently, the amount of fruit consumed. In addition, we expected frugivorous mesocarnivores to be less efficient under predation risk and to make shorter visits.

Materials and Methods

Study area

Our study was conducted during the autumn (October-November) of 2019 in Sierra de Andújar Natural Park (38°14’27.71”N, 4° 4’45.03”W, near 740 km^2). The annual average temperature (~18 ºC) and rainfall (~700 mm) are typical for Mediterranean climate areas (data from Andújar meteorological station, AEMET Opendata, 2020). The altitude ranges between 400 and 800 m. The study area is located within extensive private lands where large wild ungulates are frequently hunted, but small game hunting and predator control is unusual or absent. Vegetation is dominated by Mediterranean shrubland and holm oaks *Quercus ilex*. Some of the fleshy-fruited species present include: the strawberry tree (*Arbutus unedo)*, the mastic (*Pistacia lentiscus)* and the Iberian pear tree (*Pyrus bourgaeana)*.

This Natural Park is home to one of the largest Iberian lynx population and was the last redoubt together with Doñana for this species in the 90’s decade (Simón et al. 2012). The area does not count with lynx reintroduced populations, however lynx has expanded its distribution range over the last 20 years, due to several conservation efforts (Simón et al. 2012). Despite its recovery, Iberian lynx home ranges are heterogeneously distributed throughout the area which offered the opportunity to select otherwise similar areas that differ in the presence/absence of lynx. We studied distribution and abundance of lynx from camera-trapping surveys before designing this experiment (see Appendix S1: Table S2; Burgos et al., unpubl.) in order to select these locations. Lynx coexist with a rich community of mesopredator species in the study area, including frugivorous carnivores such as the red fox (*Vulpes vulpes)*, the stone marten (*Martes foina)*, the common genet (*Genetta genetta)* or the Eurasian badger (*Meles meles).* Frugivorous carnivores abundances are lower inside lynx ranges (with the exception of the Eurasian badger, see Appendix S1: Table S2), due to predation of the mesocarnivores populations by the Iberian lynx (Jiménez et al. 2019).

Study plant species

We selected the Iberian pear as the fruit study species because this species is mainly dispersed by frugivorous carnivores (Fedriani & Delibes, 2013), which ingest whole ripe fruits. This causes only minor mechanical damage to seeds (Fedriani & Delibes, 2009b). Foxes and badgers are long-distance legitimate dispersers of Iberian pear despite being less abundant than other frugivores such as ungulates (Fedriani & Delibes, 2009b; Fedriani, Wiegand, & Delibes, 2010). The wild boar (*Sus scrofa)* only occasionally disperses seeds, while the red deer (*Cervus elaphus)* and the fallow deer (*Dama dama)* act as seed predators. Rodents, birds and rabbits function primarily as seed predators or pulp feeders (Fedriani & Delibes, 2009b; Fedriani & Delibes, 2013; Fedriani, Zywiec, & Delibes, 2012).

This small tree produces fleshy-pomes (2-3 cm diameter; ~9.5 g wet weight) (Fedriani & Delibes, 2009b) with a high sugary and water-rich pulp content (Herrera, 1987). Fruits fall ripe during September-November and contain 1-5 viable seeds (Fedriani & Delibes, 2009b). The Iberian pear is a predictable food resource in the area, producing a large number of fruits (from 100 to >1000 fruits per tree). It has an aggregated distribution, linked to a pattern seed dispersal induced by mammals, with seed clustering and some seedling establishment beneath mother trees (Fedriani & Delibes, 2009b; Fedriani, Wiegand, & Delibes, 2010). In Sierra de Andújar, Iberian tree clusters occurred in very low densities in the study area (even lower than in other Mediterranean areas such as Doñana National Park; < 1 individual ha-1; Fedriani et al., 2010) and were composed of three to nineteen trees. Pear trees are relatively isolated from other fruiting species, growing most often in substrates lacking vegetation or coexisting with small-fruited shrub species such as *P. lentiscus* whose fruits are seldom consumed by mesocarnivores (Herrera, 1989).

Sampling design

Our experiment compared frugivorous carnivores foraging on pear fruits in areas where the Iberian lynx was present or absent. We selected the area with lynx based on camera-trapping data taken in a previous study (Appendix S1: Table S2; Burgos et al., unpubl.), where we recorded images of at least two territorial lynx couples. We placed fruit depots beneath 30 adult pear trees. Fifteen depots were located inside lynx distribution range, and the remaining 15 were used as controls outside lynx territories. Due to the patchy and scarce distribution of the Iberian pear tree, we distributed the fruit depots based on the availability of trees. The distance from a pear tree in lynx range to a control tree ranged from 4.6 to 19.3 km. We selected isolated trees and clustered trees (both within and outside lynx territories) to have a higher and representative sample from the wild distribution of the Iberian pear. We placed eight fruit depots beneath isolated trees (four of them within and four outside lynx territories) and the rest of the depots (n=22) were placed in clusters (n=11). The number of depots per cluster varied depending on its size and the number of trees that comprised the patch, ranging from one to three. We considered isolated trees and independent clusters as those located over a hundred metres away from the nearest pear tree (see Fig. 1).

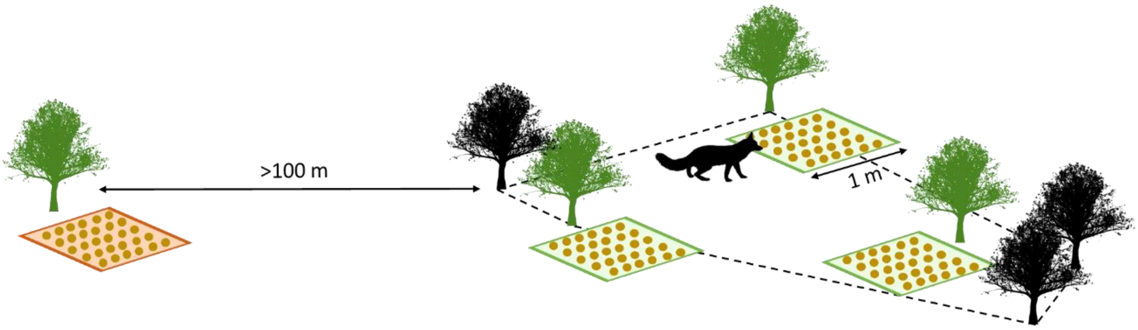

Figure 1. Field sampling design showing the depots of fruits beneath isolated Iberian pear trees (orange squares) and beneath clustered trees (green squares). Green trees show Iberian pear trees selected to locate the offers and camera traps. The dashed line shows the polygon-shaped linking trees in the clusters according to the method of Minimum Convex Polygon.

Each fruit depot was comprised of 30 ripe fruits within a 1 m side squared plot. Fruits were set regularly in 6 lines (5 fruits per line) about 10 cm apart (Fig. 1). We placed a camera trap (Scoutguard SG562-C; white led) on the pear tree trunk at the height of 60 cm with a slope of 45 degrees. We programmed the cameras to record three images per second when movement was detected, with a minimum time delay (0 s) between consecutive records to maximize the number of images taken per visit. The experiment lasted 15 consecutive days. We visited each camera trap site every 5-days to refill the fruit depots. We reached an overall effort of 437 trap-days in which cameras were working and offered 2700 Iberian pear fruits during the experiment.

Data collection

We processed a total of 130269 images taken by camera traps and recorded the species, the date, and the hour of each visit. The number of fruits consumed in each visit was estimated by comparing every image with the previous one to count the number of fruits left. We distinguished the fruits offered from the fruits fallen by marking all offered fruits with a white natural thread. The time spent per visit was calculated as the difference between the time of the first and the last image from each visit. Visits were considered independent events when separated by a period greater than 30 minutes (Linkie &

Ridout, 2011). When possible, different red foxes within the same image were identified and considered separately. Foxes have particular fur marks that often make them easily recognizable, mainly in the legs, face and tail (Sarmento, Cruz, Eira, & Fonseca, 2009; Appendix 2: Fig. S1). We identified individual foxes in 80% of all fox events in cameras placed in lynx territories and 74% outside of these. Two experts identified individually ten foxes outside of lynx ranges and four inside by consensus.

We accounted for habitat and fruit availability to assess the possible confounding effects of landscape structure and food resources. The cropsize of focal Iberian pear trees was estimated (both isolated and within cluster). We counted the number of individual fruiting trees in the clusters and visually estimated the individual cropsize. We transformed our data on cropsize into a logarithmic fruit abundance index (FAI): 0 = no fruits; 1 = 1–10 fruits; 2 = 11–100; 3 = 101–1,000; 4 = 1001–10,000; 5 > 10,000. (see Saracco, Collazo, & Groom, 2004). The cropsize of isolated trees was calculated by adding the number of fruits on the canopy to the number of ripe fruits fallen on the ground every time we replenish the fruits in the depots (three times in total). Similarly, we calculated the cropsize of Iberian pear clusters by adding up the average number of fruits per individual tree to the number of ripe fruits on the ground inside the cluster. We also recorded the forest cover inside a 100 m circular buffer around each isolated tree where we located the fruit depots. For tree clusters, we used a hundred-meter buffer around a minimum convex polygon (MCP) including all pear trees within the cluster (Fig. 1). We digitized the surface covered by forest from a high-resolution (0.5 m) orthophotography and calculated the percentage of forest cover within buffers (IGN, 2016).

All camera-trapping surveys were permitted by the land owner and manager and the Territorial Delegation of Environment of Jaén with expedient number 201899900431971. Ethical approval was not required for this study.

Statistical analysis

We built Generalized Linear Mixed Models (GLMM) to evaluate the effect of Iberian lynx presence on the number of visits, fruit consumption and foraging behaviour of frugivorous carnivores in the fruit depots. We built a GLMM for the following response variables: 1) number of visits, 2) proportion of fruits consumed in the fruit depots, 3) consumption/non-consumption of fruits in each visit, 4) fruits consumed per second in each visit and 5) time spent per visit in seconds. We used lynx presence/absence, cropsize and forest cover as fixed effects plus the interaction terms cropsize x lynx and forest cover x lynx. Because the number of stone marten visits and amount of fruits consumed by badgers were both low (n=15; n=31, respectively), we only used the red fox and badger data to analyse the number of visits (first response variable) and only the red fox data for the rest of response variables. We also added the species of carnivore for the number of visits as a fixed factor and as an interaction term with lynx presence, cropsize and forest cover. The models for the third, fourth and fifth response variables did not include interaction terms to avoid overfitting due to our low sample size (following Matuschek et al. 2017). For these response variables, we used a reduced dataset that included only the fox visits that were successfully identified (n=310). We only included the visits in which the identified foxes consumed fruits for the fruits consumed per second (n=106), and the visits that lasted between 1 sec and 15 min for the time per visit (n=161). We limited the time interval of the visits because we recorded many visits with very low (1 sec) and very high values (>15 min) that hampered model convergence and fit. Cluster (n=19) was added as random factor in all models including isolated trees and the identity of red foxes (n=14) was added as (partially) crossed random effect for the third, fourth and fifth response variables. The number of days that the camera traps were active was added as an offset in all models because some cameras failed in certain days. We used Poisson errors for the first response variable, binomial for the second and third and Gamma for the fourth and fifth. We inspected diagnostic graphs to assess normality and homoscedasticity of residuals. We checked the lack of spatial autocorrelation among clusters with Moran's Index for all the response variables (Appendix S1: Table S3).

Model selection on GLMMs was performed comparing models which contained all the possible combinations of fixed effects and interaction terms given above with models without our variable of interest, lynx presence/absence. We selected the best-fitting model that minimized the second-order Akaike information criterion (AICc). If only one model had an AICc > 2 with respect to the rest of the models, it was considered the best model. When differences between several models had an AICc < 2.0, we considered these models as set of confidence models with similar statistical support and we calculated the Akaike weights (Burnham and Anderson 2002). We carried out post-hoc simple-slope pairwise comparisons to estimate the coefficients of the interaction terms. We calculated the marginal and conditional coefficient of determination (pseudo-R^2) for the selected models (Nakagawa et al. 2017).

All the statistical analyses were conducted in R (version 3.6.1; R Core Team 2019). We used R base functions and specialized packages (lme4 v.1.1-27.1 for GLMMs: Bates, Mächler, Bolker, & Walker, 2020; MuMIn v.1.43.17 for pseudo-R^2 and model selection: Barton, 2013; ape v.5.5 for Moran's Index: Paradis et al., 2020; emmeans v.1.6.3 for post-hoc comparisons: Russell V., Buerkner, Herve, Love, & Singmann, 2021).

Results

We recorded 2370 independent visits to the fruit depots in which the frugivores consumed 69% of the Iberian pear fruits offered. Fruit depots were visited by 14 mammal species and 10 bird species, but only 11 species consumed fruits (Appendix S1: Table S1). Seed predators (ungulates) were the group of frugivores which consumed the most fruit, followed by the legitimate seed dispersers, the frugivorous carnivores (Appendix S1: Table S1). We did not find differences between predation risk scenarios (i.e. lynx presence vs. lynx absence) both for visitation (Chi^2=2.33; df=1; p=0.13) and consumption (Chi^2=0.60; df=1; p=0.44) considering the whole frugivores assemblage (Appendix S2: Fig. S2).

Visits and quantity of consumed fruits by carnivores

We recorded 458 visits of three species of frugivorous carnivores in the fruit depots (Appendix S1: Table S1). The red fox showed the highest percentage of visits (87%), while the badger (10%) and the stone marten (3%) were much less frequent. Each carnivore species showed a different pattern of visitation to fruit depots. Fox visited fruit depots much more frequently in areas outside of lynx ranges than inside (Mean ± SE: 2.18 ± 0.46, 6.96 ± 1.22) with about 70% of fox visits occurring outside of lynx territories. On the contrary badgers visited fruit depots located within lynx territories more frequently (0.73 ± 0.20 in lynx presence and 0.31 ± 0.11 in lynx absence scenario). No visits were recorded for the stone marten when lynx was present (0.33 ± 0.13 in lynx absence) (Fig. 2A).

Carnivores consumed 20.4% (n=380) of fruits removed from the depots (Appendix S1: Table S1). Red fox consumed by the far the highest percentage of fruits (90.79%) whereas badgers and martens consumed minor quantities (8.16% and 1.05%, respectively). The proportion of Iberian pear fruits consumed by foxes per depot was 9.7 ± 0.03% (n=213) and 15.7 ± 0.03% (n=132) in presence and absence of lynx, respectively. Thus, overall 38% less fruit was consumed within lynx territories. Badgers consumed fruits where the Iberian lynx was present in a greater proportion (1.4 ± 0.01%) than where the lynx was absent (0.8 ± 0.01%) (Fig. 2B). The proportion of fruits consumed per depot by stone marten was 0.3 ± 0.002%, and all the consumption events took place outside of lynx ranges (Fig. 2B).

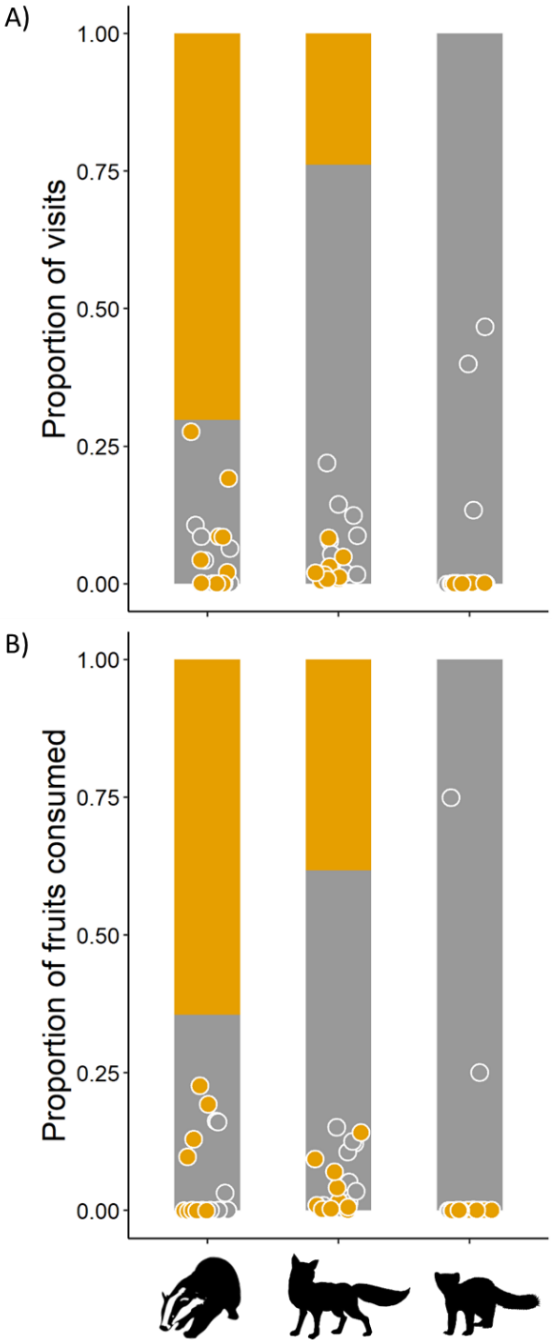

Figure 2. Proportion of the number of visits to fruit depots (A) and proportion of fruits consumed (B) by the three species of carnivore mammals that consumed pear fruits. First silhouette refers to the Eurasian badger *M. meles*. second refers to the red fox *V. vulpes* and the third to the stone marten *M. foina*. Orange refers to depots inside Iberian lynx ranges and grey represents depots outside. Circles denote tree cluster level observations.

Table 1. Generalized Linear Mixed Models for the number of mesocarnivore visits to experimental depots and for the proportion of pear fruits consumed. The list of candidate models within 2 ΔAICc is shown. K: number of parameters in the model. Loglik: log-likelihood. W: Akaike's weight. R^2_c and R^2_m give conditional and marginal proportion of variance explained.

Response	L	Sp	C	FC	SpxC	SpxFC	SpxL	LxFC	LxC	LxSpxC	LxSpxFC	K	Loglik	AICc	ΔAICc	W	R^2_c	R^2_m
(1) Nº visits	X	X	X	X		X	X	X	X		X	11	-296.23	616.03	0	0.76	0.90	0.63
(2) Prop. fruits	X	-			-	-	-			-	-	3	-281.40	569.09	0	0.26	0.27	0.08
		-	X		-	-	-			-	-	3	-281.88	570.05	0.95	0.16	0.27	0.01
		-		X	-	-	-			-	-	3	-282.05	570.38	1.28	0.14	0.27	0.01
	X	-	X		-	-	-			-	-	4	-281.06	570.60	1.51	0.12	0.27	0.08

L: lynx presence or absence, Sp: mesocarnivore species (fox and badger), C: cropsize (fruits/tree or cluster), FC: forest cover (%). The multiplicative symbol (x) indicates an interaction term.

Visitation modelling of foxes and badgers

We obtained one best model with ΔAICc<2 which included lynx presence/absence, species of carnivore, cropsize and forest cover and the interaction terms lynx x species x forest cover and lynx x cropsize (Table 1; Appendix S1: Table S4). Post-hoc comparisons showed that the carnivores most often visited pear trees with larger cropsize where the lynx was absent (CS: 560.48 ± 54.55; coeff. = 0.73, SE = 0.25). However, we found the opposite relationship when they coexisted with lynx (CS: 927.57 ± 307.03; coeff. = -0.16, SE = 0.07). We also found that foxes visited more often pear trees with higher forest cover when lynx was present, but we did not find this relationship for badgers (Appendix S2: Fig. S3).

Fruit consumption models by foxes

We obtained four models similarly plausible according to AICc and none of them included interaction terms (Table 1). The variables lynx presence/absence and cropsize were included in two models each and the variable forest cover was included only in one model (Table 1). Cropsize and forest cover had a positive effect on the proportion of fruit consumed by foxes (Appendix S1: Table S4).

Foraging behaviour modelling of identified foxes

We obtained six models with ΔAICc<2 for the fruit consumption/non-consumption response (Table 2). The variables lynx presence/absence, cropsize and forest cover were included in three models each (Table 2). The number of successful consumption events were similar to non-consumption events in lynx presence but events without consumption were higher in the absence of lynx (Fig. 3A). The probability of consuming fruit was positively related to cropsize and negatively related to forest cover (Appendix S1: Table S4). The proportion of random effects variance in the consumption probability attributable to differences among fox individuals was higher than that attributable to differences among clusters (Appendix S1: Table S4).

For the fruits consumed per second in each visit we obtained one best model with ΔAICc<2 which contained only the variable lynx presence/absence (Table 2). We found that foxes consumed less fruit s^{-1} when they coexisted with lynx (Fig. 3B). Fox identity was the random effect which explained almost the entire proportion of residual variance in the model (Appendix S1: Table S4).

For the time spent per visit we obtained four models with similar plausible AICc (Table 2). The variables lynx presence/absence and cropsize were included in three models each and the forest cover was included only in one model (Table 2). Foxes spent less time in visits to pear trees inside lynx territories than outside of lynx ranges (Fig. 3C). We found that the cropsize had a positive effect on the time per visit while the forest cover had a negative effect (Appendix S1: Table S4). The proportion of the explained residual variance by random effects was bigger for the tree cluster than for the fox individual (Appendix S1: Table S4).

Table 2. Generalized Linear Mixed Models within 2 ΔAICc built to explain the foraging behaviour of the main seed disperser, the red fox. K: number of parameters in the model. Loglik: log-likelihood. W: Akaike's weight. R^2_c and R^2_m give conditional and marginal proportion of variance explained.

Response	L	C	FC	K	Loglik	AICc	ΔAICc	W	R^2_c	R^2_m
(3) C/NC	X			4	-187.79	383.71	0	0.26	0.15	0.03
			X	4	-188.14	384.42	0.70	0.18	0.17	0.01
		X		4	-188.29	384.72	1.01	0.16	0.16	~0
	X	X		5	-187.56	385.32	1.61	0.11	0.15	0.03
	X		X	5	-187.65	385.501	1.78	0.10	0.15	0.03
		X	X	5	-187.71	385.61	1.90	0.10	0.17	0.01
(4) Fruits/s	X			5	159.49	-308.38	0	0.94	0.63	0.02
(5) Time/visit	X	X		6	-1053.04	2118.64	0	0.22	0.22	0.08
	X	X	X	7	-1052.01	2118.74	0.10	0.21	0.21	0.12
		X		5	-1054.32	2119.03	0.38	0.18	0.22	0.02
	X			5	-1054.55	2119.50	0.85	0.14	0.18	0.04

L: lynx presence or absence, C: cropsize (fruits/tree or cluster), FC: forest cover (%)

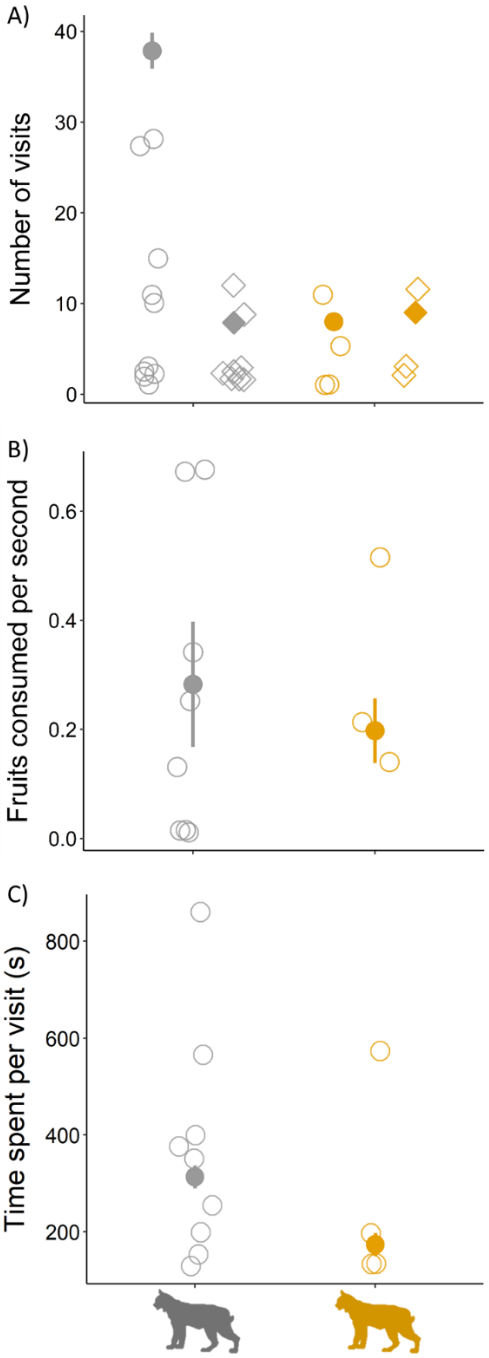

Figure 3. Filled circles show the average number of visits without fruit consumption and filled rombos show the average number of visits with consumption (A) by foxes. Filled circles show the number of fruits consumed per second (B) and time spent per visit in seconds (C) by foxes. Bars represent standard errors. Hollow circles refer to the average value found in each fox individual. Orange represents Iberian pear fruit depots inside of Iberian lynx ranges and grey represents depots outside of lynx ranges.

Discussion

The ecological interactions among the apex predator, frugivorous carnivores and a fleshy-fruited tree in Southern Spain suggest a trophic cascade induced by a predation risk scenario. Red fox—a legitimate seed disperser for the Iberian pear (Fedriani & Delibes, 2009b)—was the main fruit consumer among carnivores, but in territories with the presence of an apex predator, a smaller number of foxes conducted fewer visits to pear trees, consumed less fruit and spent less time searching for fruit. Although foxes had less opportunity to feed when under predation risk, they consumed fruits less efficiently. These results suggest the presence of lynx reduces the abundance of foxes and alters their foraging behaviour, which could inhibit the efficacy of foxes as seed dispersers. Overall, the results support our hypothesis that the Iberian lynx can affect a key plant–animal mutualism, that could result in a negative cascading effect on seed rain of plant species whose seed dispersal depends on mammal carnivores.

Lynx differentially affect mesocarnivore visitation

Frugivorous mesocarnivores actively search for Iberian pear fruits because these are a predictable and valuable resource in Mediterranean ecosystems (Fedriani & Delibes, 2009a). Therefore, less frequent visits to pear trees under predation risk could be due to lower population abundances, a behavioural avoidance of agonistic encounters, or a combination of both (Ripple and Beschta 2004, Carreira et al. 2020, Selwyn et al. 2020). We identified far fewer fox individuals and recorded fewer visits in a predation risk scenario, which supports previous work by Garrote et al. (2018), who proposed that in the Doñana National Park a low frequency of fox visitation to pear fruits was likely related to a high lynx density. Although our best model revealed that lynx presence was an important factor to understand the lower visitation of foxes, we also found that lynx can have a differential effect among mesocarnivores (Fedriani et al., 1999; Jiménez et al., 2019). We found a low number of visits of stone marten and all were recorded in pear trees outside of lynx ranges (Jiménez et al., 2019), indicating that the presence of lynx may lead to the virtual extinction of the seed dispersal service provided by stone martens. Conversely, we found more visits of badgers inside lynx territories. Badgers did not seem to show sensitivity to lynx presence, probably due to their larger size and corpulence (Fedriani et al., 1999). The smaller size of martens and foxes relative to the badger could lead them to be easily predated by a bigger competitor (Virgós et al. 2020) which may explain their low abundance in areas with lynx (see Appendix S1: Table S2; Burgos et al., unpubl.).

Habitat variables modulate the effect of predation risk on visitation

Foxes and badgers avoided high-production trees inside of lynx ranges despite making bigger cropsize on average. This behavioural change probably is linked to cost-benefit balance as stated by the optimal foraging theory (Charnov 1976). Low-production patches could be safer for foraging even if the available resources are potentially more challenging to obtain (Brown et al. 1999). However, we found the opposite relationship when carnivores did not coexist with lynx. In this scenario, carnivores invested foraging efforts to areas where food is easier to find, in accordance with other similar studies (Virgós et al. 2010, García et al. 2011, Selwyn et al. 2020). We found that while badger visits were positively related with forest cover around pear trees in both the presence and absence of lynx, foxes only demonstrated this pattern in lynx scenario. As Iberian pear trees were located in low-vegetated areas, foraging in fruit patches with higher forest refuge could be less risky for foxes which live in a landscape of fear (Laundré et al. 2010). In a trophic cascade context, these kind of behavioural adaptations could determine the spatial patterns, dispersal kernels and effectiveness of seed dispersal for fleshy-fruit plants in the long-term (Fedriani et al., 2010; Pegman et al., 2017; Rey & Alcántara, 2014).

Lynx presence limits the quantity of fruits consumed by foxes

The main seed disperser of Iberian pear was the red fox. We found that foxes consumed 38% less fruits under a perceived predation risk by lynx. Despite this reduction being balanced by the consumption of the rest of the frugivore assemblage (see Appendix S2: Fig. S2), their qualitative seed dispersal effectiveness (*sensu* Schupp et al. 2010) is very low. Carnivore mammals are the legitimate seed dispersers of the Iberian pear (Fedriani & Delibes, 2009b; Fedriani & Delibes, 2013) while the rest of the frugivores, such as ungulates or lagomorphs, that consumed a relevant proportion of fruits are largely seed predators or pulp feeders (Fedriani & Delibes, 2009b; Fedriani & Delibes, 2013). Although we did not record many visits of badgers in our study area, this species could potentially balance the loss of seed dispersal by foxes within lynx ranges in areas where they reach greater densities (Fedriani et al., 2020). For example, in Doñana National Park, Iberian pear seeds are mostly dispersed by badgers (Fedriani & Delibes, 2009b), and they are a key species to create new clusters of pear trees away from the tree neighbourhoods (Fedriani et al., 2010).

Implications of trophic cascades on plant ecology

The foraging behaviour of foxes was also disturbed by predation risk. We found that when under predation risk, foxes utilized the visits to pear trees to feed on fruits, in contrast to the high proportion of visits without consumption in areas outside of lynx distribution range, according to Leo et al. 2015 found. In lynx presence scenario foxes consumed less fruit per time (i.e. lower efficiency) and their visits to pear trees were shorter, behaviour likely linked to an anti-predatory response (Haswell et al. 2018, Carreira et al. 2020, Selwyn et al. 2020). The combination of a lower fruit consumption and the alteration of the feeding behaviour can cause a cascading effect that disrupts a plant-disperser mutualism (Kurten 2013, de Paula Mateus et al. 2018). The quantity of dispersed seeds of the Iberian pear could be limited by the presence of lynx because its main disperser (red fox) was less abundant, consumed less fruit and therefore dispersed fewer seeds. Cascading effects on the main disperser could also alter the qualitative seed dispersal (e.g. habitat and microhabitat of seed deposition), affecting plant demography and distribution (de Paula et al., 2018; Fedriani et al., 2020). As seed dispersal patterns are different among Iberian pear seed dispersers (Fedriani et al., 2010), fewer pear-fox interactions could modify the spatial distribution of pear trees, for instance limiting seed arrival to vacant habitats (e.g. oldfields; Fedriani et al., 2020). Moreover, disturbances on the spatial patterns of scat deposition triggered by predation risk could also occur on an intra-specific level as a consequence of behavioural changes (Virgós et al. 2020) and affect seed dispersal delivery. For instance, although foxes dispersed fewer seeds in a predation risk scenario, the possibility cannot be ruled out that they move seeds towards safe forest refuges, which could potentially be suitable habitats for seedling establishment of Mediterranean plants (Bustamante et al. 1992).

Effects of habitat and individual variation of fox foraging behaviour on fruit consumption

Fruit availability, forest cover and intra-specific variability also explained variation in the foraging behaviour of foxes, in addition to predation risk. Fruit consumption, both the amount of fruits consumed and the probability of consumption, increased with cropsize and the visits lasted for a longer time in patches with higher fruit availability, which supports several previous studies about usage intensity of fruit patches (Moegenburg and Levey 2003, García et al. 2011, Selwyn et al. 2020). Forest cover had a positive effect on the proportion of fruits consumed by foxes, but its effect on the probability of fruit consumption and activity time in the pear trees was negative. This opposite relationship could be due to the different sampling levels used for fruit consumption models (fruit depot) and foraging behaviour models (visit). Mediterranean forest patches can hold a high diversity of food resources for generalist carnivores such as foxes (Cavallini and Volpi 1996), and they probably also foraged on food items other than fruits, explaining the lower probability of fruit consumption per visit and shorter visits. However, a high forest cover could provide foxes a greater perception of safety (Laundré et al.

2010) allowing them to consume a larger amount of fruits in overall. Moreover, intra-specific variability seems to also be relevant to explain the variation found in the foraging behaviour of foxes such as the likelihood of consuming fruits. As individual variation can also modulate fruit resource exploitation (Araújo et al. 2011, Toscano et al. 2016), more research would be needed to understand how different predation-risk scenarios could select certain phenotypes (Eccard et al. 2020, Steinhoff et al. 2020) and how this could in turn alter ecological functions such as seed dispersal.

Conclusions

In our study area, lynx recovery could reverse the release of the main seed disperser of the Iberian pear, the red fox, by decreasing its abundance and modifying its foraging behaviour. Specifically, foxes visited pear trees less often, consumed less fruit and spent less time searching for fruits under predation risk, resulting in less efficient frugivores. Different elements also affected the foraging behaviour and the decision-making of foxes, such as the availability of food resources, forest refuge and intra-specific variation. Cascading effects from lynx on this key mutualism could alter the demography and spatial structure of the Iberian pear, although a greater abundance of badgers could balance the lack of seeds dispersed by foxes. Thus, fleshy-fruited plants dispersed by medium- and large-sized seed dispersers could experience limitations of their quantitative seed dispersal due to trophic cascades, especially for scarce or endangered plant species. Understanding the ecological interactions among the different levels of food webs is essential to design suitable conservation strategies and predict potential cascading effects in altered ecosystems. Consequently, reintroduction programs of apex predators should consider trophic cascades as a powerful mechanism, which can alter key ecosystem functions in contrasting ways.

Supporting information – Chapter 2

Predation risk can modify the foraging behaviour of frugivorous carnivores: implications of rewilding apex predators in plant-animal mutualisms

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Appendix S1: Tables

Table S1. Visit rate and proportion of fruits rate for the assemblage of frugivorous species of the Iberian pear inside and outside lynx range. Visit and fruit consumption rates represent the quotient between the number of visits and fruit consumed by each specie and the sum of visits and consumed fruits of all species in the depots, respectively. Category refers to the role of these species on pear seed dispersal according to Fedriani & Delibes, 2009: LSD: legitimate seed disperser; SD: seed predator; PF; pulp feeder.

Species	Category	Visit rate		Fruit consumption rate	
		Lynx	No lynx	Lynx	No lynx
Carnivore mammals		0.056	0.147	0.082	0.122
Red fox *V. vulpes*	LSD	0.042	0.135	0.071	0.114
Badger *M. meles*	LSD	0.014	0.006	0.011	0.006
Stone marten *M. foina*	LSD	-	0.006	-	0.002
Ungulates		0.379	0.224	0.448	0.294
Red deer *C. elaphus*	SP	0.359	0.217	0.439	0.291
Fallow deer *D. dama*	SP	0.006	0.001	0.007	0.001
Wild boar *S. scrofa*	SP/LSD	0.014	0.006	0.002	0.002
Lagomorphs		0.006	0.047	0.001	0.028
Iberian hare *L. granatensis*	PF	-	0.044	-	0.028
E. rabbit *O. cuniculus*	PF	0.006	0.003	0.001	0.000
Birds		0.100	0.069	0.019	0.008
Iberian magpie *C. cooki*	PF	0.063	0.036	0.018	0.006
Common blackbird *T. merula*	PF	0.000	0.028	0.000	0.002
Mistle thrush *T. viscivorus*	PF	0.009	0.005	0.001	0.000

Table S2. Occurrence (percentage of camera-traps with presence) and relative abundance index (RAI) of the Iberian lynx and frugivorous mesocarnivores community in our study area. Occurrence and abundance were extracted from an unpublished study (Burgos et al.) in the same area during the previous year following the methodology used by Sarmento et al., 2009. Twenty-four camera-traps were placed in each lynx presence and lynx absence scenario with a total effort of 516 trap-days and an average effort of 43 days/trap.

Species	Lynx scenario	Occurrence	RAI ± SE
Iberian lynx *L. pardinus*	Presence	0.75	5.01 ± 0.90
	Absence	0	0
Red fox *V. vulpes*	Presence	0.42	5.57 ± 3.62
	Absence	0.96	35.27 ± 6.37
Badger *M. meles*	Presence	0.46	7.45 ± 5.35
	Absence	0.25	1.16 ± 0.67
Stone marten *M. foina*	Presence	0.04	0.08 ± 0
	Absence	0.75	15.77 ± 3.98

Table S3. Moran's Index (I) and significance (P) values from the autocorrelation analysis to test spatial autocorrelation among patches of Iberian pear trees for all the response variables included in GLMMs.

Response variable	Moran's Index (I)	*P* (0.05)
(1) Nº visits	-0.05	0.13
(2) Prop. Fruits consumed	-0.05	0.62
(3) C/NC	-0.05	0.33
(4) Fruits/s	-0.05	0.39
(5) Time spent per visit	-0.05	0.09

Table S4. Coefficients and standard errors for the fixed effects and variances for the random effects included in the selected models (ΔAICc<2) shown in Tables 1 and 2. Hyphen symbol indicates that variable was not included in the proposed models.

Response	Fixed effects (Estimate ± SE)										Random effects (σ)	
	Intercept	L	Sp	C	FC	SpxFC	SpxL	LxC	LxFC	LxSpxFC	Cluster	Fox id
(1) Nº visits	-3.29 ± 0.52	1.20 ± 0.65	3.56 ± 0.38	0.74 ± 0.25	0.70 ± 0.51	-0.81 ± 0.33	-2.39 ±0.44	-0.90 ± 0.26	0.47 ± 0.65	-0.08 ± 0.41	0.90	-
(2) Prop. fruits	-3.62 ± 0.56	-0.98 ± 0.79	-			-	-			-	2.71	-
	-4.15 ± 0.41		-	0.11 ± 0.15		-	-			-	2.97	-
	-4.14 ± 0.41		-		0.20 ± 0.43	-	-			-	2.92	-
	-3.61 ± 0.57	-1.04 ± 0.80	-	0.12 ± 0.15		-	-			-	2.75	-
(3) C/NC	-3.45 ± 0.30	0.75 ± 0.54	-			-	-	-	-	-	0.18	0.28
	-3.23 ± 0.28		-		-0.22 ± 0.22	-	-	-	-	-	0.17	0.46
	-3.25 ± 0.28		-	0.12 ± 0.15		-	-	-	-	-	0.20	0.42
	-3.45 ± 0.30		-	0.10 ± 0.14		-	-	-	-	-	0.18	0.28
	-3.41 ± 0.31	0.64 ± 0.60	-		-0.12 ± 0.23	-	-	-	-	-	0.17	0.32
	-3.24 ± 0.28		-	0.13 ± 0.15	-0.24 ± 0.22	-	-	-	-	-	0.17	0.44
(4) Fruits/s	-4.94 ± 0.50	0.80 ± 0.94	-			-	-	-	-	-	~0	6.15
(5) Time/visit	2.86 ± 0.19	-0.54 ± 0.31	-	0.15 ± 0.09		-	-	-	-	-	0.08	0.03
	2.95 ± 0.17	-0.70 ± 0.28	-	0.17 ± 0.09	-0.19 ± 0.12	-	-	-	-	-	0.06	0.02
	2.67 ± 0.20		-	0.13 ± 0.10		-	-	-	-	-	0.10	0.05
	2.86 ± 0.19	-0.41 ± 0.30	-			-	-	-	-	-	0.08	0.03

L: lynx presence or absence, Sp: mesocarnivore species (fox and badger), C: cropsize (fruits/tree or cluster), FC: forest cover (%). The multiplicative symbol (x) indicates an interaction term.

Appendix S2: Figures

Figure S1. Images of two of photo-identified foxes that visited the Iberian pear fruits depots during the field experiment. Red circles show the fur marks that allowed the individual identification. The couple of images AB and CD shows the same individual.

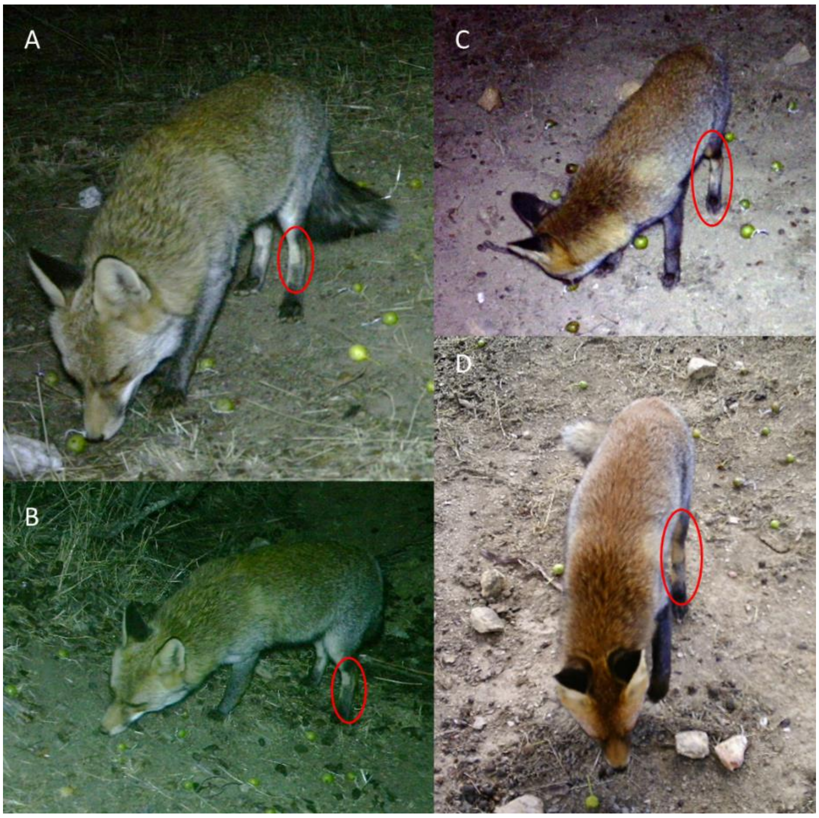


Figure S2. Proportion of visits and fruit consumption by the whole assemblage of frugivores which consumed Iberian pear fruits from the depots (Table S1) for the lynx presence (orange) and lynx absence scenario (grey). Circles denote tree patch level observations.

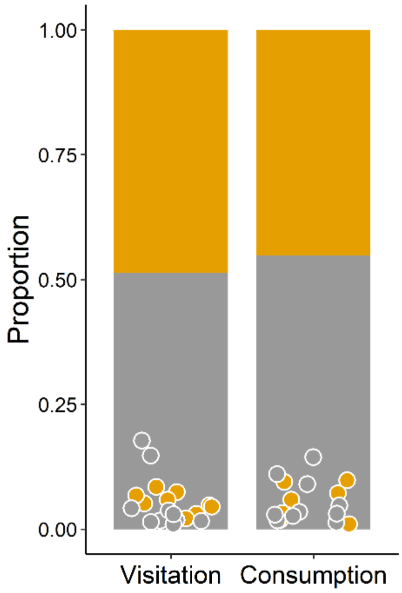
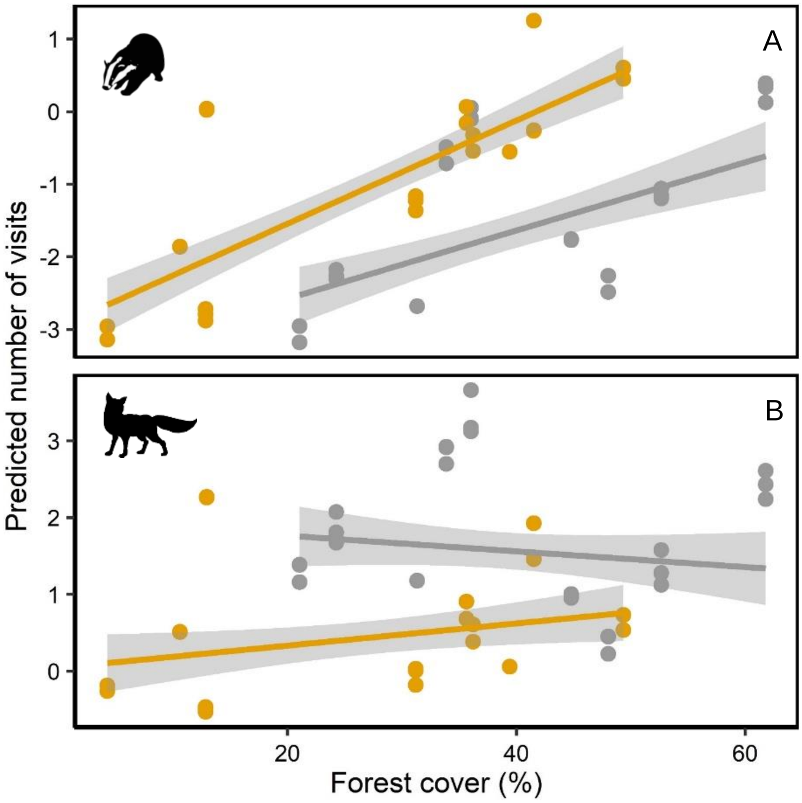


Figure S3. Predicted number of visits obtained from the generalized linear mixed model (GLMM) for the number of visits of badgers (A) and foxes (B) to fruit depots. The x axis shows the percentage of forest cover around the pear tree patches. Confidence intervals are represented by gray shading. Orange refers to depots inside of Iberian lynx ranges and gray represents depots outside of them.



Chapter 3

Apex predators can structure ecosystems through trophic cascades: linking frugivore behaviour and seed-dispersal patterns

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Abstract

1. The current global-change scenario demands research on how human-mediated ecological changes affect ecosystem functioning and the entire dynamics of food webs. Frugivorous mesocarnivores are a key link in trophic cascades, because they provide relevant services to plant communities acting as seed dispersers and at the same time, their abundance and behaviour are usually controlled by top-order predators. Thus, the recent rewilding scenario of large carnivores worldwide could trigger cascading effects to plants.
2. We investigated the top-down effects of a top-predator (Iberian lynx *Lynx pardinus*), on the seed dispersal services mediated by mesocarnivores (red fox *Vulpes vulpes* and stone marten *Martes foina*) at the plant community level, by comparing areas with lynx presence and absence in a Mediterranean mountain range of Southern Spain.
3. We collected mesocarnivore scats (n = 1575) to assess seed dispersal of 15 plant species in two consecutive fruiting seasons and two habitat types. Specifically, we assessed the effect of lynx presence on (i) fleshy-fruit occurrence and biomass per scat, (ii) number of scats containing seeds and (iii) diversity of seeds mobilised.
4. In the presence of lynx, the stone marten increased the fruit biomass per scat 2.4 times on average. However, the quantity and diversity of dispersed seeds were drastically decreased under predation risk for both mesocarnivore species. The seed-dispersal service provided by martens was the most affected by the lynx presence due to a markedly reduction of the quantity of scats with seeds (93%) and diversity of seeds mobilised (46%). Nevertheless, foxes dispersed a 68% fewer seeds in open habitats when co-existed with lynx, probably leading to a differential contribution to seed-dispersal effectiveness among habitats.
5. Our study reveals a novel trophic cascade from apex predators to plant community throughout behavioural responses of frugivorous mesocarnivores to predation risk and a reduction in their faecal deposition pattern, probably related to their lower abundance. Rewilding top-predators has been proved a successful conservation tool, yet attention should be paid to cascading effects across food webs in particular when frugivore megafauna is missing and mesocarnivores could provide unique services to plant communities.

Keywords: Iberian lynx, endozoochory, frugivory, plant-animal mutualism, Order Carnivora, mesopredator release, seed-dispersal services, DNA-barcoding

Introduction

Interactions between fruits and frugivores are of paramount relevance to guarantee the functioning of most terrestrial ecosystems (Jordano 2013b). Plants and fruit consumers have developed efficient mutualisms which do not necessarily require of mutual evolutionary adaptations, which is known as diffuse co-evolution (Herrera 1995, Valenta and Nevo 2020). For instance, alterations in habitat structure (e.g. habitat fragmentation; McConkey et al. 2012), in the frugivore community composition (e.g. defaunation; Donoso et al. 2020) or in frugivore behaviour (e.g. dietary shifts; Morales et al. 2013) can alter plant-frugivore mutualisms. As consequence, novel seed-disperser interactions can arise or even lead to the loss of key links and the consequent functional decay in plant-frugivore networks (Valiente-Banuet et al. 2015). Although studies on ecological factors shaping plant-frugivore mutualisms are increasing (Rey and Alcántara 2014, Corlett 2021, Rogers et al. 2021), the current context of global change is demanding more research regarding the oncoming ecosystem alterations that affect key ecological functions, such as seed dispersal by animals.

Since opportunistic and adaptable large frugivores represent a key ally for many native plant communities, mammalian carnivores play an important role in seed dispersal of fleshy-fruited plants (Herrera 1989, Escribano-Avila 2019). They are able to transport a great amount of seeds to long-distances (González-Varo et al. 2013) and release them on optimum conditions for germination and seedling establishment (Escribano-Ávila et al. 2013, Draper et al. 2022). Most fleshy-fruited plant species often interact with a diverse assemblage of frugivores, but seed dispersal of large-fruited plants imposes significant physical constraints, reducing the availability of dispersal partners to a few number of frugivores among which mammalian mesocarnivores play a relevant role (González-Varo et al. 2015, Escribano-Avila et al. 2018). Moreover, large and medium sized seed dispersers are the most vulnerable species in defaunation scenarios (Galetti and Dirzo 2013) and show low functional redundancy with other groups of frugivores (Pérez-Méndez et al. 2016, Naniwadekar et al. 2019). Mesocarnivores show functionally unique characteristics in respect to other specialized frugivores such as birds, usually derived from dietary preferences (Rumeu et al. 2020) and differential movement ability and habitat use (Jordano et al. 2007, González-Varo et al. 2013). However, only a few studies have addressed how ecological alterations affecting carnivore communities ultimately cascade into seed dispersal process (Ripple et al. 2014b, Rogers et al. 2021, Burgos et al. 2022).

Given the global worldwide extirpation of large predators, mesocarnivores (i.e. subordinate predators) are an abundant guild in many ecosystems following the mesopredator release hypothesis (Crooks and Soulé 1999, Ripple et al. 2014a). This means that abundance of medium-sized carnivores is higher than expected in comparison to complete communities in which top-order predators remain. Yet, not only apex predators can control mesocarnivore abundance, but also promote a landscape of fear. This is an animal change of behaviour that varies spatially according to the perceived predation risk (Gaynor et al. 2019), resulting in differential foraging strategies, for instance by reducing the use of risky habitats. These changes in subordinate predators behaviour may lead to further unforeseen consequences across the food web (de Paula Mateus et al. 2018, Carreira et al. 2020, Burgos et al. 2022). Since scientific literature has been biased towards the negative ecological effects of mesopredator release, such as an increased predation pressure on lower trophic levels (Brashares et al. 2010), the restoration of threatened apex predators to regulate the population of subordinate predators has become in a promising but still challenging conservation strategy (Wolf and Ripple 2018, Prugh and Sivy 2020). However, rewilding apex predators in places where they have been lost for a long time can trigger trophic cascades (i.e. top-down effects across lower trophic levels), altering the entire food web dynamics (Estes et al. 2011, Ripple et al. 2016) and affecting further ecological functions and services provided by mesocarnivores, such as seed dispersal (Tambling et al. 2018, Draper et al. 2022).

Altering the abundance and behaviour of key frugivores could affect both the quantity (i.e. number of seeds) and the quality (i.e. probability of a dispersed seed survive to become a new reproductive adult) of seed-dispersal effectiveness (Schupp 2007, Schupp et al. 2010) modifying the ecosystem service that frugivores provide to plants (Draper et al. 2022). For instance, Burgos et al. (2022) found that the presence of an apex predator not only reduced fruit consumption of a Mediterranean tree by mesocarnivores but also modified their foraging behaviour, turning them into less efficient frugivores and potentially reducing the number of seeds dispersed. In this line, Cancio et al. (2016) showed how a reduction in the intensity of use of degraded habitats of a keystone mammalian seed disperser led to the seed-dispersal collapse of a threatened shrub. Thus, a behavioural change in frugivores could alter the territorial-marking patterns and hence, the quality of deposition site (i.e. local environmental conditions a seed experiences after dispersal), affecting plant recruitment (Beckman and Rogers 2013). Since carnivores are relevant seed-dispersal agents in forest expansion processes (Escribano-Ávila et al. 2014, Fedriani et al. 2018), the structure and composition of plant communities may be also affected in a landscape of fear. Similar to what has been found in tropical defaunated ecosystems, predation risk could determine not only how diverse is the array of frugivores, but also the diversity of the plant assemblage dispersed by them (Kurten 2013). Moreover, dietary shifts or specialisation towards a low-risk fruit resource may also control how diverse is the assemblage of plants dispersed and eventually established (Gazzola and Balestrieri 2020).

The recent recovery of the threatened Iberian lynx (*Lynx pardinus*) along the Iberian Peninsula offers a unique opportunity to better understand trophic cascades on a very complex and scarcely explored system (Estes et al. 2011). There is little evidence of the potential effects of apex predators on the structure and functioning of Mediterranean ecosystems beyond mesopredator suppression (Palomares et al. 1995, Jiménez et al. 2019). However, most of the subordinate predators which co-exist with lynx are opportunistic frugivores and key seed dispersers for several fleshy-fruited plants (Herrera 1989, Fedriani et al. 2020a, Burgos et al. 2022). Thus, the main aim of this study is to assess the top-down effects of the Iberian lynx on the seed-dispersal patterns of mesocarnivores under a quasi-experimental scenario, by comparing areas with lynx presence and lynx-free areas in a Mediterranean forest of southern Spain. Since apex predators can negatively affect mesopredators' abundance and foraging behaviour, we hypothesized the lynx presence would alter the seed-dispersal service provided by mesocarnivores at a community level both in quantity and quality, although the strength and direction of such top-down effect could change across plant species. Firstly, we expected to find a reduced fruit consumption and faecal-marking behaviour of mesocarnivores in the presence of lynx. Secondly, we predicted that the type of seed-arrival habitat would vary among predation risk scenarios. In particular, we hypothesized a lower mobilisation of seeds to open-vegetation habitats in lynx presence scenarios because such a habitat type is more prone to predation risk owing to more scarce shelter for mesocarnivores. Lastly, we expected a reduced provision of seed-dispersal services in terms of quantity and diversity of dispersed seeds. Thus, the composition of the plant assemblage dispersed by mesocarnivores would vary among predation risk scenarios, resulting in potentially dissimilar plant communities.

Materials and Methods

Study area

This study was carried out in Sierra de Andújar Natural Park, in Southern Spain (Fig. 1). The annual average temperature (~18 ºC) and rainfall (~700 mm) are typical for Mediterranean climate areas (data from Andújar meteorological station, AEMET

Opendata, 2020), and altitude ranges between 400 and 800 m. The Natural Park is composed by extensive game lands, where hunting of large wild game ungulates is frequent, but small game hunting and predator control is deprecated. Vegetation is dominated by Mediterranean shrubland and an arboreal stratum of holm oak (*Quercus ilex*) mixed with a rich community of wild fleshy-fruited species. The mastic (*Pistacia lentiscus*), terebinth (*Pistacia terebinthus*), strawberry tree (*Arbutus unedo*), prickly juniper (*Juniperus oxycedrus*), wild olive (*Olea europaea* subsp*. europaea* var*. sylvestris*), blackberry (*Rubus ulmifolius*), myrtle (*Myrtus communis*), narrow-leaved mock privet (*Phyilyrea angustifolia*) and green olive tree (*Phillyrea latifolia*) are the most common fruiting plants in close-vegetation habitats meanwhile in open landscapes, the community of fleshy-fruited plants is basically composed by the Iberian pear (*Pyrus bourgaeana*), common hawthorn (*Crataegus monogyna*) and mastic.

This Natural Park provides shelter to one of the largest remnant populations of Iberian lynx. This threatened apex predator has expanded its distribution range over the last 20 years, due to several conservation efforts such as captive breeding and reintroductions (Simón et al., 2012). Despite the recovery of the Iberian lynx in the study area, its home ranges are heterogeneously distributed throughout the Natural Park and the population trend is stable (Fig. 1). Iberian lynx co-occurs with a rich community of mesopredator species in the study area, including frugivorous carnivores such as the red fox (*Vulpes vulpes*), the stone marten (*Martes foina*), the common genet (*Genetta genetta)* or the Eurasian badger (*Meles meles*). Mesocarnivore's abundance is lower inside Iberian lynx ranges (except for the Eurasian badger, Appendix S2: Table S4), due to top-down suppression by this apex predator (Jiménez et al., 2019).

Experimental design and scat sampling

We used an experimental design comparing five localities with year-round territorial presence of reproductive individuals of Iberian lynx and five localities without lynx as controls, with similar habitat characteristics and distributed along the Natural Park (Fig. 1). Thus, we were able to compare the fruit consumption and seed dispersal patterns of mesocarnivores among predation-risk and predation-free scenarios. We studied the lynx distribution and abundance of mesocarnivores from camera-trapping surveys before carrying out this study (Appendix S2: Table S4; Burgos et al. 2023). Since mesocarnivores can alter their habitat-usage patterns under predation risk, we selected two different types of habitat in each study locality which could affect the environmental conditions of the seed deposition sites and determine the quality of the seed-dispersal effectiveness (Schupp 2007, Schupp et al. 2010): a) mature forest with a dense shrub stratum; and b) open vegetation landscapes with scarce shrub and isolated trees (see Fig. 1 and Appendix S3: Fig. S1). Along transects of 1.5 km in each type of habitat, we conducted mesocarnivore scat sampling between October and March of 2018-19 and 2019-20. This methodology has been proved to be an efficient effort for sampling most mesocarnivore scats (Martin-Garcia et al. 2022). Sampling was conducted periodically at 1.5-months intervals to be able to replicate each transect four times per fruiting season, reaching a total effort of 160 visits (4 replicates per transect x 2 habitats x 10 sites x 2 seasons). Two mammal scat experts sampled the transects (~3 m in width) twice the same day and an extra 1-meter buffer from both sides of the transect. Mammal carnivores usually deposit their faeces in the border of the main trail, beneath vegetation or on the top of conspicuous shrubs or stones, thus we ensured to find all the scats by this sampling protocol. We did not find any scat of common genet because the faecal-marking behaviour of this species is often linked to the use of latrines on conspicuous and elevated spots inside forests with high understory cover, which would require a different type of sampling design (Espírito-Santo et al. 2007). Following Putman (1984), the experts identified visually the scats based on size, shape and odour characteristics specific to each carnivore species. In addition, we performed DNA-barcoding analysis for a subsample of 70 stone marten scats to confirm our visual identifications due to this species is susceptible of misidentification (see molecular procedures in Appendix S1). We reached an identification success of 95%, supporting our identification expertise. The scats were stored in paper bags and dehydrated at 30ºC in a drying oven during 3-4 days to avoid the appearance of fungi.

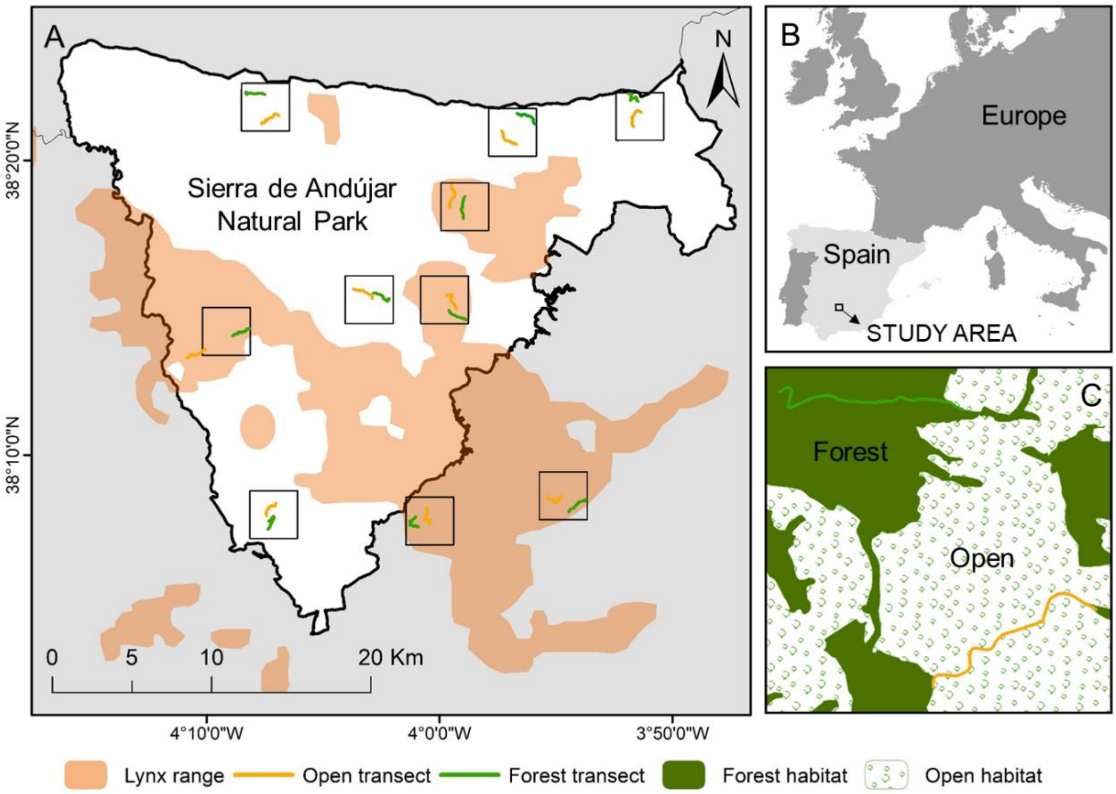

Figure 1. (A) Location of the ten study localities where we conducted the scat sampling. Orange shadow shows the distribution range of the Iberian lynx (*Lynx pardinus*) along Sierra de Andújar Natural Park adapted from the data available on the IUCN Red List of Threatened Species (Rodríguez and Calzada 2015). Orange and green routes represent the 1.5-km transects (n = 20) carried out in open and forest habitats in each locality and fruiting season (n = 160), respectively. (B) Location of the study area in Southern Spain. (C) One of the study locality illustrating the sampling design for recording mesocarnivore seed-dispersal along trails located inside the forest or crossed open landscapes; landscape map produced in ArcGIS v.10.6 by using the National Forest Map 1: 50,000 (MITECO. 2013).

Seed content, fruit biomass and seed diversity estimates

We estimated seed content from the collected faeces in order to quantify the potential effect of predation risk on the quantitative seed dispersal. The dried scats were broken up carefully and the seed content was extracted by using decreasing sieve-size (see photographs in Appendix S3: Fig. S1). Two experts visually identified the seeds of fleshy-fruited plants to species, according to morphology concordance with a reference collection (see González-Varo et al. 2021, for a similar approach). We quantified the number of seeds in each scat by counting them under lens in a Petri dish. When the seed number exceeded 100 (n = 24) within one scat, we divided the seed content up into four homogeneous quadrants and extrapolated the number of seeds in one quadrant to the rest by multiplying. Fleshy-fruit biomass per scat was estimated to quantify the effect of lynx on the fruit consumption behaviour of mesocarnivores. To do this, we used the number of quantified seeds per scat and the relationship between the average number of seeds per fruit and the average fleshy-fruit biomass obtained from the *frubase* database (Jordano 2013a) for each consumed plant species. Then, we calculated two metrics of alpha seed diversity for each locality and fruiting season in order to determine the differences on the effective number of equally common and also the dominant species among predation risk scenarios, as Hill-Shannon (1) and inverse Hill-Simpson (2) respectively (Chao et al. 2014, Roswell et al. 2021); where S was plant species richness and pi the quantitative contribution to seed rain (i.e. number of seeds mobilised) of each dispersed plant species in the community.

$$\left(e^{-\sum_{i=1}^{S} p_i \log(p_i)}\right) \quad (1)$$

$$\left(\frac{1}{\sum_{i=1}^{S} (p_i)^2}\right) \quad (2)$$

We used our scat level dataset to estimate sample coverage and sampling completeness in the lynx presence and absence scenario in order to prove that our results on alpha diversity were not driven by sampling effort (see details in Appendix S2: Table S5 and Appendix S3: Fig. S6 and S7). We followed the rarefaction and extrapolation method proposed by (Chao et al. 2014) using the R package `iNEXT` (Hsieh et al. 2016). We found no significant differences between predation risk scenarios both in sample coverage ($P = 0.11$) and sampling completeness (all $P \geq 0.12$). Lastly, we also obtained beta diversity between lynx presence and absence scenarios in order to determine whether the potential dissimilarity in seed dispersal diversity was due to species replacement (turnover) or to the loss of certain species (nestedness). We calculated the proportional contribution of these mechanisms to total dissimilarity using the R package `betapart` v.1.5.6. (Jaccard's index; Baselga 2010).

Fruit density and diversity estimates

We estimated the available fruit density during the fruiting peak in each study locality and dispersal season in order to account for potential effects of fruit availability on the fruit consumption and seed dispersal by mesocarnivores. At the same time that we carried out the scat sampling, we visually estimated the coverage percentage and the individual crop size inside plots of 15x15 m every 200 m for both sides of the transect for the strawberry tree, Iberian pear, prickly juniper, blackberry, myrtle and wild olive, which are frequently consumed by carnivores (Herrera 1989, Rosalino and Santos-Reis 2009). We estimated the crop size using a semi-logarithmic fruit abundance index: 0 = no fruits; 1 = 1–10 fruits; 2 = 11–100; 3 = 101–1000; 4 = 1001–10000; 5 > 10000. This fruit estimation was performed only along forest habitat type transects because in open habitats these plants were rare or absent except for the Iberian pear. The Iberian pear is a Mediterranean tree which is relatively isolated and clustered, growing at low densities (<1 ind / ha; Fedriani et al. 2010). In our study area, this small tree most often occurs in substrates lacking vegetation (Fedriani et al. 2010). For these reasons, the crop size of this species was estimated by searching adult individuals in a 100 m-buffer across the open habitat transects, and counting the observed number of fruits per tree. Seeds of common fig (*Ficus carica*) were found in mesocarnivore scats but no wild individual was found along the sampled transects or in their surroundings, probably because this plant is frequently associated to human shelters. Peak fruit density (fruits/m^2) within each locality and fruiting season was calculated from the averaged FAI of all sampled plots along each transect for each species. Fruit diversity was calculated as the Hill-Shannon/Simpson diversity index of available fruits similarly to describe for seed diversity.

Statistical analyses

We fit Generalized and Linear Mixed Models (GLMM and LMM) using the R package `lme4` v.1.1-27.1 (Bates et al. 2020) to evaluate the effect of Iberian lynx presence, the habitat type and fruit availability on fruit consumption and seed-dispersal patterns of mesocarnivores. We also performed a Pearson correlation test between the number of scats and the number of seeds dispersed by carnivores, in order to determine the relationship between the scent-marking and seed-dispersal patterns among predation risk scenarios.

Response variables

We modelled separately data the red fox and the stone marten on fruit consumption and seed dispersal and analysed the following response variables: 1) fruit occurrence per scat (1/0), 2) fleshy-fruit biomass per scat (zero non-included), 3) number of scats with seeds (zero included) and 4) seed diversity. We only found badger scats in sites without lynx and their low number (n = 26) did not allow us to consider this species in our analysis. We chose the fruit biomass and the number of scats with seeds as response variables as an unbiased measure of fruit consumption of the whole plant community given that the number of fruits and seeds consumed are influenced by fruit size and seed content of each plant species, respectively. We repeated the analysis for the first, second and third response variables a) by using a dataset with all dispersed plants and b) only with the most dispersed plant in our study area, the strawberry tree. Seeds of common fig and rare plant species (grouped in 'Others' category) were not included in modelling because of their low representativeness in carnivore diet (see Fig. 2), except for diversity analyses. We used Binomial errors for fruit occurrence, Gaussian errors with identity link function on log-transformed data for fruit biomass, Poisson with log link function for the number of scats with seeds and Gamma with log link function for seed diversity.

Fixed and random effects

We used lynx presence/absence (L) and fruit density (FD) as fixed effects in all models. Fruit density (fruits/m^2) was included for the fruit occurrence, fruit biomass and scats with seeds models. Fruit diversity was previously discarded because resulted in non-significant Pearson correlations with seed diversity (Appendix S3: Fig. S8), in order to avoid overfitting due to our reduced sample size (n = 20). We also added as fixed term the type of habitat (H) for the number of scats with seeds. Interactions FD x L and H x L were also included in the respective models to account for a differential effect of those variables among predation risk scenarios. Locality (n = 10) and fruiting season (n = 2) were added as crossed random effects in all mixed models except for seed diversity models where only fruiting season was included as random effect.

Model selection

Model selection for the response variables 1) fruit occurrence, 2) fleshy-fruit biomass and 3) number of scats with seeds was performed using models which contained all the possible combinations with biological sense of fixed effects and interaction terms explained above compared to models without our variable of interest, lynx presence/absence (L) and a null model (intercept), using the `MuMIn` package v.1.43.17 (Barton 2013). Due to the reduced sample size for 4) alpha seed diversity, we performed model selection by comparing models including the unique fixed term lynx (L) with null models (intercept) trough Likelihood-ratio tests in `lmtest` package v. 0.9-40 (Hothorn et al. 2022). Regarding model selection for fruit occurrence, fleshy-fruit biomass and scats with seeds, we selected the best-fitting model that minimized the second-order Akaike information criterion (AICc). If only one model differed more than 2 with respect to the AICc of the rest of the models, it was considered the best model. When differences between several models had an AICc < 2.0, we considered these models as set of confidence models with similar statistical support and we calculated the Akaike weights (Burnham and Anderson 2002). We calculated the marginal and conditional coefficient of determination (pseudo-R^2) for the selected models (Nakagawa et al. 2017). We carried out post-hoc simple-slope pairwise comparisons to estimate the model coefficients of the interaction terms using the R package `emmeans` v.1.6.3 (Russell V. et al. 2021). Residuals were inspected by using `DHARMa` package v. 0.4.6 (Hartig and Lohse 2022). All analyses were conducted in R (version 3.6.1; R Core Team 2019).

Results

General overview

We collected 1575 scats from two mesocarnivore species, the red fox (n = 1400) and the stone marten (n = 175). The 64% and 91% of red fox and stone marten scats were collected outside lynx territories, respectively. The 49% and 74% of scats contained seeds from fleshy-fruited plants for the red fox and the stone marten, respectively. The former dispersed a total amount of 30070 seeds and the later 3972, with the 63% and 88% respectively being dispersed outside lynx territories. We found a positive and significant correlation between the number of scats and the number of seeds dispersed by carnivores, except for the stone marten in lynx presence scenarios, probably due to the scarce sample size (Appendix S3: Fig. S3).

Mesocarnivores dispersed up to 15 fleshy-fruited plant species, but the most consumed fruit was the strawberry tree, appearing in a 67% of scats with seeds. The Iberian pear, the prickly juniper, the blackberry, the myrtle, the oleaster and the common fig were also widely dispersed in decreasing occurrence order (Fig. 2). Other seed species occurred in scats in very low percentages (less than 1%). These corresponded to small-size-fruited plant species rarely consumed by carnivores in Mediterranean ecosystems, such as the laurustinus (*Viburnum tinus*), the Mediterranean buckthorn (*Rhamnus alaternus*) or the mastic (Fig. 2). The fleshy-fruit most abundant along transects were the wild olive fruit (Mean ± SE: 9.83 ±4.23 fruits/m^2), followed by the prickly juniper fruit (7.84 ± 4.48 fruits/m^2) and the strawberry tree fruit (7.14 ± 1.84

fruits/m^2) whereas the Iberian pear fruit was the scarcest one (0.0008 ± 0.0002 fruits/m^2) (Appendix S3: Fig. S2). Fruit density, Hill-Shannon and inverse Hill-Simpson diversity of the wide-community of fleshy-fruited plants did not significantly differ between predation risk scenarios (F = 2.18, P = 0.17; F = 1.28, P = 0.27 and F = 1.08, P = 0.31, respectively). However, the average values of crop size, Hill-Shannon and Hill-Simpson diversity of available fruits were 2.0, 1.2 and 1.1 times higher inside lynx territories, respectively (Appendix S3: Fig S1).

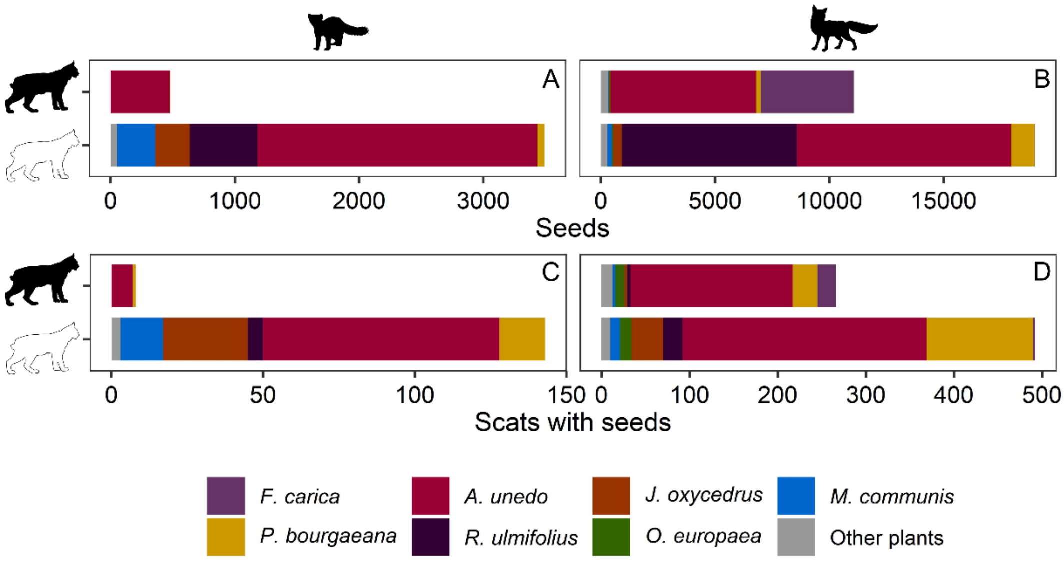

Figure 2. Additive number of seeds dispersed by the stone marten (A) and the red fox (B) and scats of the stone marten (C) and the red fox (D) with seeds in sites with Iberian lynx presence (coloured lynx silhouette) and absence (non-coloured lynx silhouette). Since a scat can contain multiple seed species, the total number of scats with seeds (C, D) shows the addition of scats with different plants species. Thus, the total number of scats with seeds was 239 and 8 in lynx presence scenarios and 452 and 121 in lynx absence, for the red fox and the stone marten respectively. Seed species are represented by different colours: *Ficus carica, Pyrus bourgaeana, Arbutus unedo, Rubus ulmifolius, Juniperus oxycedrus, Olea europaea, Myrtus communis* and Other plants.

Fleshy-fruit occurrence and biomass

Outside lynx territories, the frequency of appearance of fruits in scats (i.e. seeds) was 1.2 and 1.4 times greater than inside, for the red fox and the stone marten respectively (Appendix S3: Figure S4). Although lynx presence was selected in the best model of fruit occurrence for the red fox, this factor did not significantly affect the fleshy-fruit occurrence of all plants (Table 1) and either of *A. unedo* (Appendix S2: Table S1). Fruit density significantly interacted with lynx (Table 1), only in fruit occurrence of all plants for the red fox (Coeff. ± SE) (-0.10 ± 0.11 in lynx presence vs. 0.27 ± 0.10 in lynx absence). The overall effect of fruit density was significantly positive for the fox (0.27 ± 0.10) and negative for the marten (-0.59 ± 0.20), regarding all plants (Appendix S2: Table S2 and S3). Respect to *A. unedo*, we only found a significant and positive effect of fruit density (0.37 ± 0.10) for the red fox (Appendix S2: Table S2).

We did not find any effect of the lynx presence on the fleshy-fruit biomass per scat of all plants and either of *A. unedo* for the red fox (Table 1; Appendix S2: Table S1), but the best models included lynx presence/absence for the stone marten both for all plants and *A. unedo* (Table 1; Appendix S2: Table S1). Inside lynx territories, the fruit biomass per scat of marten was on average 2.4-fold significantly higher than outside (Table 1), especially for *A. unedo* fruits (Fig. 3). Fruit density was included in the best models of all plants only for the stone marten, with a significant and negative effect (Coeff. ± SE: -0.31 ± 0.10) on fruit biomass per scat (Table 1; Appendix S2: Table S3).

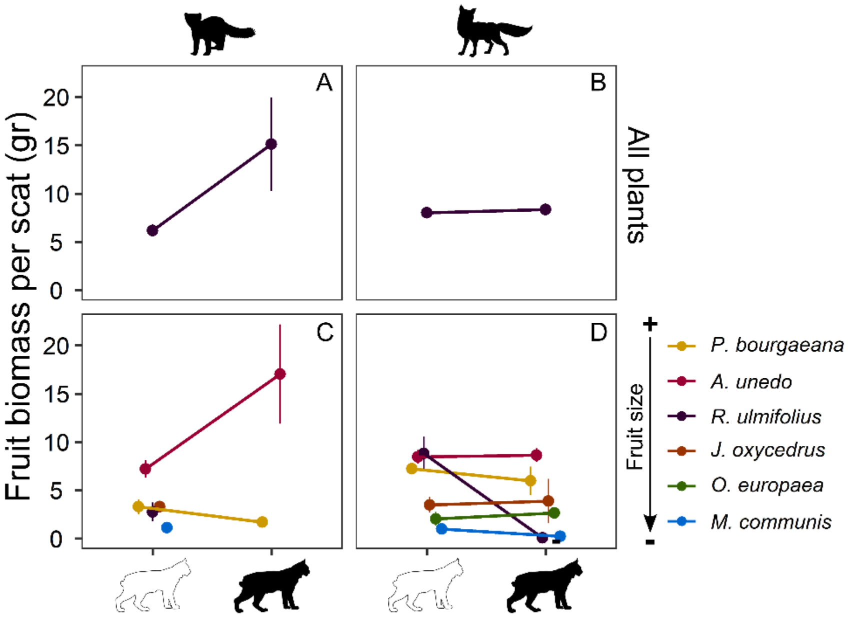

Figure 3. Average estimated fleshy-fruit biomass (gr) of all plants consumed per scat of (A) stone marten (n = 129) and (B) red fox (n = 691) and each plant species separately (CD) in sites with presence of Iberian lynx (coloured lynx silhouette) and absence of lynx (non-coloured lynx silhouette). Bars represent standard errors and lines show the trend between predation risk scenarios. Fruit biomass was calculated by using the number of quantified seeds per scat and the relationship between the average number of seeds per fruit and the average fleshy-fruit biomass obtained from the *frubase* database (Jordano 2013b). Plant species are represented by different colours. Arrow shows the decreasing variation in fruit size. All plants refer to the additive fruit biomass per scat of all plant species.

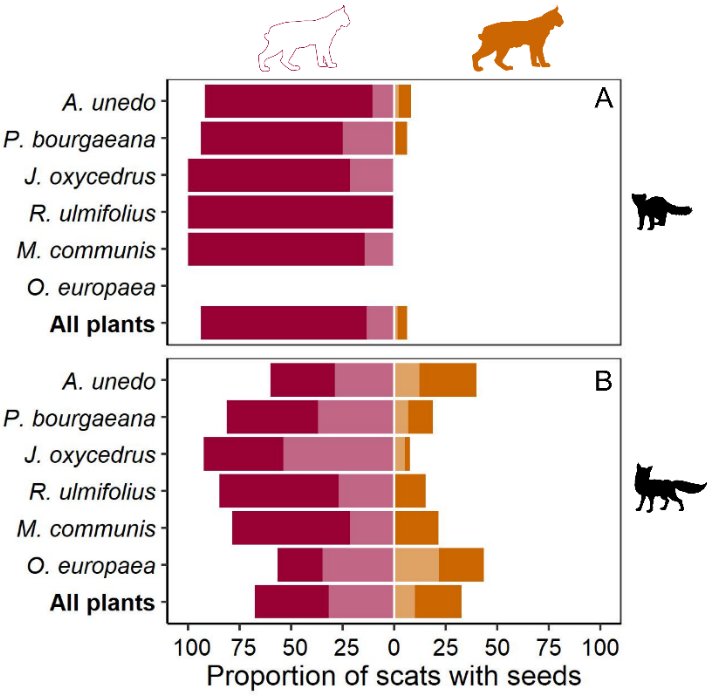

Figure 4. Proportion of scats with seeds of (A) stone marten and (B) red fox deposited in sites with presence of Iberian lynx (orange colour) and sites without lynx (magenta colour) for each plant species. Intensity of each colour represents the type of land habitat for seeds, meaning dark colour to forestry habitats and light colour open habitats. Values increase to the left on the plot for areas without lynx, and the opposite for the plot with lynx presence. All plants refer to the proportion of scats with seeds of any plant species.

Seed dispersal and seed-arrival habitat

All the best models included lynx presence/absence for both mesocarnivores regarding all plants and *A. unedo* (Table 1; Appendix S2: Table S1). We found a significant lower number of scats with seeds along transects inside lynx territories (Table 1), both for the red fox (52% lower) and the stone marten (93% lower) (Fig. 2). Habitat was also included in all best models for both mesocarnivores, but the effect on scats with seeds (all plants and *A. unedo*) was only significant for the stone marten (Table 1; Appendix S2: Table S1). Stone martens dispersed a significantly higher proportion of seeds to forest habitats (up to 6 times) regardless the predation risk scenario (Table 1; Fig. 4A). The interaction between habitat x lynx was significant in the case of the red fox (all plants and *A. unedo*) contrary to the stone marten (Table 1; Appendix S2: Table S1). Foxes deposited 0.5 times fewer scats with seeds in open than in forest habitats inside lynx territories (Coeff. ± SE) (2.69 ± 0.17 vs. 1.90 ± 0.19; Fig. 4B), but this proportion was similar between habitats in lynx absence scenario (53% vs. 47.0%) (Fig. 4B). Fruit density affected positive and significantly the number of scats with seeds of the red fox for all plants and *A. unedo* (0.20 ± 0.08; 0.43 ± 0.12), but not for the stone marten (Table 1; Appendix S2:

Table S2). We found a contrasting remarkable pattern between predation risk scenarios (Appendix S3: Fig. S5AC), a positive and significant effect of fruit density of all plants in absence of lynx, and negative in presence, only for the red fox (0.20 ± 0.08 vs. -0.06 ± 0.09).

Alpha and Beta seed-dispersal diversity

The effective number of equally common (Hill-Shannon) dispersed species was significant lower when the lynx was present for both the red fox ($Chi^2 = 4.35$, $p < 0.05$; $R_m^2 = 0.20$, $R_c^2 = 0.20$) and the stone marten ($Chi^2 = 6.13$, $p < 0.01$; $R_m^2 = 0.38$, $R_c^2 = 0.43$) (Fig. 5A). Respect to the hill number of dominant seed species, we found that the inverse Hill-Simpson seed diversity was significantly lower for the stone marten in lynx presence ($Chi^2 = 5.84$, $p < 0.01$; $R_m^2 = 0.16$, $R_c^2 = 0.16$), but not for the red fox ($Chi^2 = 3.32$, $p = 0.06$; $R_m^2 = 0.35$, $R_c^2 = 0.41$) (Fig. 5B). The red fox and the stone marten mobilised a similar average diversity of seeds outside lynx territories, but in lynx presence scenarios foxes dispersed a higher seed diversity than martens (Fig. 5AB), both considering common and dominant species (1.5 and 1.4 times higher, respectively). Lastly, we found high beta dissimilarity of seed species dispersed by the red fox and the stone marten (mean Jaccard's = 0.29 and 0.73, respectively) between predation risk scenarios (Fig 5E). Beta diversity was totally driven by the loss of species (nestedness) for the stone marten, but in the case of the red fox, the contribution varied between species replacement (turnover) and loss (i.e. nestedness) for each fruiting season (Fig. 5CD).

Table 1. List of candidate models (ΔAICc<2) for the response variables fruit occurrence, fruit biomass and scats with seeds of the red fox (*Vulpes vulpes*) and the stone marten (*Martes foina*) for all plants. K: number of parameters in the model. Loglik: log-likelihood. W: Akaike's weight. R^2_c and R^2_m give conditional and marginal proportion of variance explained. Hyphen symbol refers to non-selected variables. Bold x refers to significant *p* values; $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

	Response	L	FD	H	FDxL	LxH	K	Loglik	AICc	ΔAICc	W	R^2_c	R^2_m
V. vulpes	Fruit occurrence per scat	x	**x****		**x****		6	-952.03	1916.13	0	0.67	0.05	0.02
	Fruit biomass per scat	-	-		-		4	-1098.03	2204.11	0	0.80	0.03	-
	Scats with seeds	x	**x***	x	**x***	**x*****	8	-178.67	377.99	0	0.43	0.85	0.59
		x*	-	x	-	**x*****	6	-181.78	378.11	0.12	0.40	0.86	0.56
		x*	x	x	-	**x*****	7	-181.15	379.80	1.81	0.17	0.85	0.59
M. foina	Fruit occurrence per scat	-	**x*****		-		4	-97.74	203.71	0	0.42	0.21	0.08
		x	**x****		-		5	-97.29	204.94	1.23	0.23	0.19	0.08
		-	-		-		3	-99.74	205.63	1.92	0.16	0.11	-
	Fruit biomass per scat	**x****	**x****		x		7	-186.03	386.99	0	0.53	0.28	0.12
		x**	**x****				6	-187.56	387.82	0.82	0.35	0.27	0.12
	Scats with seeds	**x*****	-	**x*****	-	-	5	-68.21	150.18	0	0.42	0.92	0.80
		x***	x	**x*****	x	-	7	-66.89	151.27	1.09	0.24	0.94	0.86

L: lynx presence or absence, FD: fruit density, H: Type of habitat. The multiplicative symbol (x) indicates an interaction term.

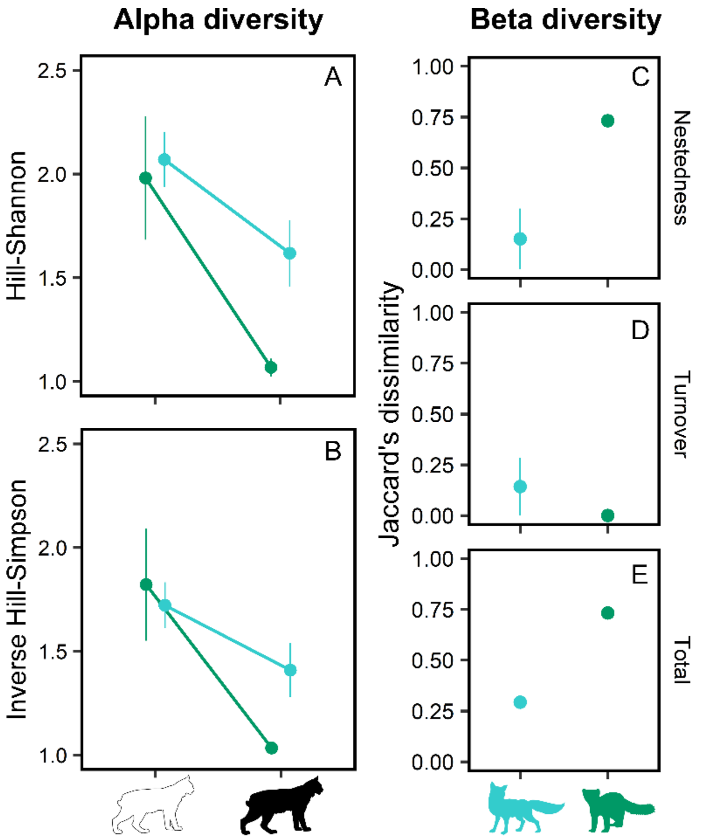

Figure 5. Alpha and Beta diversity of dispersed seed species for the red fox (blue) and the stone marten (green). Alpha diversity is shown in terms of Hill-Shannon (A) and inverse Hill-Simpson (B) indices and was estimated at level of locality and fruiting season. Beta diversity between predation risk scenarios of each fruiting season is expressed in terms of Jaccard's (E) and its respective components: nestedness (C) and turnover (D). Circles represent observed average values with their correspondent standard errors (bars). Coloured lynx silhouette denotes presence of Iberian lynx and non-coloured silhouette denotes sites without lynx.

Discussion

Our results show how the seed-dispersal patterns by mesocarnivores of a Mediterranean fleshy-fruited plant community were altered by the presence of an apex predator, supporting our initial prediction. The Iberian lynx modified the frugivory and faecal-marking behaviour of mesocarnivores, triggering a trophic cascade down to plants. Since apex predators can reduce mesocarnivore abundance (Newsome et al. 2017) and alter their behaviour (Ritchie and Johnson 2009), the resultant combined effect led frugivorous mesocarnivores to reduce seed dispersal (up to 0.9 times fewer seeds) and to mobilise a dissimilar plant-assemblage between predation risk scenarios, with unforeseen consequences for plant establishment. Despite the mutualistic interaction between frugivorous carnivores and fleshy-fruited plants can be altered, apex predator rewilding scenarios return seed dispersal to a previous and more natural state before lynx eradication.

Frugivory behaviour and seed dispersal in a landscape of fear

Frugivorous mesocarnivores are frequently suppressed by larger predators which search for subordinate competitors actively and kill them, as is the case of the Iberian lynx (Palomares and Delibes 1994, Jiménez et al. 2019). This top-down effect constrained mesocarnivore abundance and leaded to a reduced seed dispersal in areas inhabited by the lynx. Predation risk can also alter the foraging behaviour of frugivores or even reduce the visitation rate to fruiting trees in risky areas, both via top-down suppression and behavioural alterations (Carreira et al. 2020, Selwyn et al. 2020, Burgos et al. 2022). Although the fleshy-fruit biomass of certain preferred and abundant fruits was greater under predation risk for the stone marten (see *A. unedo* in Fig. 3), the lower abundance of frugivorous carnivores led to a reduction in the quantitative component of seed mobilisation for all plant species. Faecal scent-marking is a territorial behaviour evolved to inter-individual communication (Gorman and Trowbridge 1989) and therefore, fewer animals lead to fewer faecal-marks and seed-dispersal events. Moreover, the subtle negative effect of lynx presence found on fruit occurrence in mesocarnivore scats also contributes to decrease seed rain under predation risk. Consistent with our findings, previous studies have found the simplification of the frugivore community can lead to a decay in the dispersal function, either by causing a redistribution of interactions or altering interaction strength (Rogers et al. 2021).

Added to the quantitative effect on seed rain, the presence of an apex predator could also promote that seed-dispersal quality was modified. The red fox reduced the faecal-marking in open habitats, probably as consequence of a behavioural adaptation in the use of space to avoid intra-guild competition in risk-exposed areas (Schmitz et al. 1997, Ferreiro-Arias et al. 2021). The habitat-use adaptability shown by the red fox, which focused its marking behaviour in forest habitats in presence of lynx, is translated into shifts on the seed-arrival patterns (García-Cervigón et al. 2018), with fewer seeds being deposited in open environments. Habitat suitability represents a qualitative component of the seed-dispersal effectiveness (Schupp 2007) that condition the probability of recruitment of seeds and thereby influence the quality of the ecosystem service that frugivores provide to plants (Beckman and Rogers 2013, González-Castro et al. 2015). In Mediterranean ecosystems, where the shortage of water supply and high levels of herbivory severely limit plant recruitment (Mendoza et al. 2009, Perea et al. 2020), landing at covered habitats could affect positively seedling emergence and survival and ultimately plant spatial distribution (Fedriani and Delibes 2009a, Fedriani et al. 2018). Although lynx may be limiting the colonization of certain plant species towards open habitats affecting plant-community structure (such as the Iberian pear; see Fig. 4) and their colonization potential in response to global change (Schleuning et al. 2020), apex predators could contribute to maintain the valuable ‘mosaic landscapes’ (Curveira-Santos et al. 2017, Brotons et al. 2018).

The loss of the seed-dispersal service provided by martens

Contrary to the distinctive spatial pattern showed by the red fox, the stone marten did not modify the seed-arrival habitat in presence of the apex predator, probably because it is mainly a forest-dwelling species avoiding high-risky exposed habitats (Virgós et al. 2020). Stone marten diet can be composed by up to 90% of fruit and its arboreal habits allows martens to exploit fruit resources non-accessible for other carnivores (Padial et al. 2002, López-Martín 2006). However, martens barely dispersed seeds in presence of the Iberian lynx owing to its low abundance, which limited the quantification of its frugivory behaviour and seed-dispersal patterns. This result is in accordance with the strong avoidance patterns showed by the stone marten to large predators (Monterroso et al. 2020, Virgós et al. 2020) and the severe abundance decrease found in previous studies (Jiménez et al. 2019; see Appendix S2: Table S7). In addition, martens almost exclusively fed on strawberry-tree fruits under predation risk despite other fruiting plants were also abundant, probably because this is a rich-pulp fruit (Herrera 1987), highly abundant in this landscape of fear (Pyke et al. 1997). Accordingly, availability of this plant affected positively quantitative seed rain as previous studies have stated with other key fruits (Virgós et al. 2010, García et al. 2011, López-Bao and González-Varo 2011). However, available fruits of less-preferred species seemed to affect negatively the probability of seed appearance and fleshy-fruit biomass in stone marten scats. These findings suggest a possible dietary specialization towards the preferred food resource as a result a cost-benefit balance (Brown and Morgan 1995), similarly to the findings of Gazzola and Balestrieri (2020) for this species in a scenario of intra-guild competition. Thus, specific threats affecting keystone seed dispersers as the stone marten could lead to the rapid collapse of the community-level dispersal services in case of lacking of complementary frugivores (Rumeu et al. 2017).

Trophic cascades affect the diversity of dispersed seeds

A larger array of frugivores can promote the consumption and mobilisation of a more diverse ensemble of fruiting plants including non-preferred fruits, probably driven by functional complementarity (García et al. 2018, García-Rodríguez et al. 2022), as we found in scenarios of lynx absence. However, seed diversity decreased due to a trophic cascade triggered by an apex predator, as previously shown in defaunated ecosystems (Kurten 2013). The diversity reduction was controlled by two different mechanisms structuring communities, nestedness and turnover. The former was explained by the loss of species dispersed by the stone marten (*M. communis* or *J. oxycedrus*) and the later responded to different preference of fruit resources by foxes in the presence of the top-predator. Accordingly, some fruits dispersed by foxes outside lynx territories were replaced by other species with a similar number of seeds mobilised in sites with lynx, leading to a more balanced variation on beta diversity among predation risk scenarios (e.g. *F. carica* and *R. ulmifolius*; see Fig. 2B). Moreover, the effective number of dominant species (Inverse Hill-Simpson) dispersed by the red fox was not affected by lynx presence, compensating the markedly reduction on seed dispersal diversity found for the stone marten for both equally common and dominant species. Nevertheless, the largest and favourite fruit species (*A. unedo and P. bourgaeana*; see Fig. 3) may be the most affected by the trophic cascade triggered by lynx, because they usually depend on the seed-dispersal service provided by large frugivores, mainly carnivores (Fedriani and Delibes 2009a, Escribano-Ávila et al. 2013). This type of large fruits, such as the Iberian pear, have often high pulp to seed ratios (Fedriani and Delibes 2009a), what leads to a low quantitative seed-dispersal even in absence of predation risk, but that in the presence of the top-predator may lead to more limiting availability of seeds for recruitment constraining regeneration dynamics. Thus, the reduced diversity and quantity of seeds dispersed in presence of an apex predator could cascade up to forest regeneration (Rogers et al. 2021) and structure of plant communities in old-field colonization processes where mesocarnivores play key roles (Escribano-Ávila et al. 2014, Fedriani et al. 2018). This may be particularly relevant in ecosystems in which larger frugivores (i.e. megafauna) are extinct. That is the case of many Mediterranean ecosystems where the brown bear (*Ursus arctos*) likely played a key role for many plants species and in particular for those bearing larger fruits such as the Iberian pear.

Functional redundancy among frugivores

Alternatively, other large and medium-sized frugivores whose abundance and behaviour are not affected by the presence of lynx (e.g. badger) could compensate the lost seed-dispersal functions of foxes and martens (Fedriani et al. 2020a) inside lynx territories. This may be the case of top-order frugivorous carnivores which could also compensate the decreased seed-dispersal service produced after lynx recovery (Vidal et al. 2013, García-Rodríguez et al. 2022), but the current rewilding context for the Iberian lynx in Mediterranean ecosystems represents still a defaunation scenario, lacking in megafauna of seed dispersers such as the extinct brown bear. Therefore, understanding the cascading effects of rewilding apex predators could be particularly relevant in defaunated ecosystems, where mesocarnivores maintain a key mutualism with fleshy-fruited plants. The markedly functional diversity of carnivores and their low functional redundancy with other groups of frugivores such as birds (Rumeu et al. 2020, Nakashima and Do Linh San 2022) makes each species unique in terms of seed dispersal, especially when the availability of top-order carnivores with frugivore habits is scarce. For instance, foxes have been proved to be essential seed dispersers in arid ecosystems (Cancio et al. 2016, Escribano-Avila 2019), ultimately affecting the regeneration of keystone scrubland habitats over its range (Rey et al. 2018). Furthermore, although migratory birds can be important drivers of seed dispersal towards cooler latitudes under climate change (González-Varo et al. 2021), seed rain is frequently restricted to the parental plant surrounding. In this line, Escribano-Ávila et al. (2014) found that foxes and martens played a key role in land abandonment colonization processes for *Juniperus thurifera*, due to carnivores contributed largely to disperse seeds away from the adult plants, in contrast with the local movements of thrushes. Thus, this contribution to seed flow among contrasting type of habitats is highly valuable in disturbed landscapes, meanwhile frugivore birds frequently avoid open habitats lacking of tree vegetation cover (Garcia et al. 2010).

Concluding remarks

The present study supports our hypothesis that in a top-predator rewilding scenario, the fleshy-fruited plant community experienced a drastic decrease on the diversity of species and the quantity of seeds dispersed, with potential consequences for the recruitment patterns and the structure of the whole community. Trophic cascades are powerful mechanisms which can affect patterns and processes that drive seed dispersal. These complex community effects should be accounted for when planning future large predator reintroductions, mainly in defaunated ecosystems lacking of frugivore megafauna.

Supporting information – Chapter 3

Apex predators can structure ecosystems through trophic cascades: linking frugivore behaviour and seed-dispersal patterns

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Appendix S1. Molecular procedures

A sub-sample of 70 stone marten scats were stored frozen at -21ºC to DNA extraction before to analyse their seed content. We used DNA barcoding analysis (i.e., we amplified a fragment of the mitochondrial; cytochrome c oxidase subunit I -COI-) to test our success in the stone marten scat visual identification. For DNA extraction, we rub a sterilized cotton swab on all the surface of each scat and we introduced it in a 2-ml tube with extraction buffer. Briefly, on each tube we added the following reagents: 475 µL of extraction buffer4 (0.1 M Tris-HCl pH 8.0, 0.1 M EDTA pH 8.0, 0.01 M NaCl, 0.5% SDS and 0.25 mg/mL Proteinase K), 6 µL of proteinase K 20 mg/ml, 2 µL of β-mercaptoethanol and 22 µL of 10% PVP-360 (polyvinylpyrrolidone) as adjuvants or supplements against PCR inhibitors. The tubes were incubated in rotation at 50 ºC for 1 h 40 minutes, and then centrifuged 2 min at 10000 rpm. Supernatant (~475 µL) was transferred to a new 1.5 mL tub. Next, we added 0.5 volume (~ 235-255 µL) of binding buffer (5 M GuSCN, 0.1 M Tris-HCl pH 6.4, 0.02 M EDTA pH 8.0 and 1.3% Triton X-100) and 80 µL of silica suspension and the mix was incubated in rotation at room temperature for 1 h 40 minutes in the dark. This step allows the binding of DNA to silica particles in the presence of high salt concentration. After centrifugation (2 min at 4500 rpm), the supernatant was discarded, and the silica pellet was re-suspended in 300 µL of binding buffer and transferred to columns (MoBiTec, Germany, product # M1002S) with a glass microfiber filter (Whatman Grade GF/B 1.0 µm) on the top of the 10 µm column filter. After centrifugation (1 min at 13000 rpm), silica particles retained in the column were washed at least twice using 450 µL of washing buffer (50% Ethanol, 10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0 and 125 mM NaCl). Columns were placed in new 1.5-µL tubes and DNA was eluted twice, first with 60 µL of ultrapure water at 50ºC and then with 50 µL of diluted TE buffer at 50ºC (5 mM Tris-HCl pH 8 and 0.1 mM EDTA pH 8.0).

For PCR amplification, we used the primers In 1F (5-’GGNGAYGAYCARATNTACAATGT-3’) and In 1R (5’-GGNGGNAGNAGTCARAARC-3’) to amplify a 134-bp fragment of the mitochondrial COI gene (Schäffer et al. 2017). PCR amplifications were set up in a final volume of 10 µL including 5.0 µL 2 x QIAGEN Myltiplex PCR Master Mix, 0.5 µL (10 µM) of primers In 1F and In 1R, 2.5 µL ultrapure water and 1.5 µL of the DNA extract. Reactions were performed on a G-Storm GS2 thermal cycler (Somerton Biotechnology Centre, Somerset, UK) using the following conditions: initial 15 min of denaturation at 95 °C; 8 cycles at 94 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 1 min; 27 cycles at 94 ºC for 30 s, annealing at 50 ºC for 30 s and extension at 72 ºC for 1 min and final extension of 30 min at 60 °C. After verifying successful amplification by 1.5% agarose gel electrophoresis, samples were purified using the E.Z.N.A. Gel Extraction Kit OMEGA. Sequencing reactions were performed from only one end (forward primer) using the BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). Labelled fragments were cleaned on SephadexTM G-50 (GE Healthcare, UK) plates before electrophoresis in an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Amplified DNA fragments were aligned and edited using BioEdit v. 7.0.9 (Hall 1999) and Chromas v. 2.5.1 (http://technelysium.com.au/wp/chromas/).

Polymerase Chain Reaction (PCR) amplifications failed in 28.57% of samples, reaching higher values than we expected based on previous research (González-Varo et al. 2014, 2022). In total we retrieved 16 sequences (31.37%), which were identified at species level from the best sequence matches (>90% similarity) in BLAST (Altschul et al. 1997). The remaining 34 sequences either did not match in BLAST or we obtained a low percentage of similarity due to low sequence quality. In order to get more robust results, we aligned these sequences with our 'type' sequence from the stone marten and the red fox and we calculated the percentage of sequence identity by using Clustal Omega (Sievers and Higgins 2014). Pairwise comparison matrix revealed that 24 sequences matched in a poor percentage of identity (<70%) and we discarded them as non-reliable. Finally, we successfully identified the species of 28.57% (n=20) analysed scats due to PCR failure, low sequence quality or diet identification. Only one scat was wrongly visually identified because corresponded to red fox. This means that our visual identification in the field had a success rate of 95%. DNA-barcoding analysis was conducted at the molecular ecology laboratory of the Biodiversity Research Institute (IMIB).

Appendix S2. Tables

Table S1. List of candidate models (ΔAICc<2) for the response variables fruit occurrence, fruit biomass and scats with seeds of the red fox (*Vulpes vulpes*) and the stone marten (*Martes foina*) only for *A. unedo*. K: number of parameters in the model. Loglik: log-likelihood. W: Akaike's weight. R^2_c and R^2_m give conditional and marginal proportion of variance explained. Hyphen symbol refers to non-selected variables.

	Response	L	FD	H	FDxL	LxH	K	Loglik	AICc	ΔAICc	W	R^2_c	R^2_m
V. vulpes	Fruit occurrence per scat	-	**x*****		-		4	-874.28	1756.58	0	0.44	0.11	0.04
		x	**x*****		x		6	-872.39	1756.83	0.25	0.38	0.12	0.05
		x	**x*****		-		5	-874.17	1758.38	1.80	0.18	0.11	0.04
	Fruit biomass per scat	-	-		-		4	-760.50	1529.08	0	0.82	~0	-
	Scats with seeds	x	**x*****	x	x	**x*****	8	-141.06	302.76	0	0.63	0.82	0.44
		x	**x*****	x	-	**x*****	7	-143.21	303.91	1.15	0.36	0.79	0.45
M. foina	Fruit occurrence per scat	-	-		-		3	-108.94	224.02	0	0.42	0.26	-
		x	-		-		4	-108.33	224.89	0.88	0.27	0.28	0.01
	Fruit biomass per scat	**x***	-		-		5	-136.35	283.46	0	0.59	0.25	0.08
		x*	x		-		6	-136.17	285.41	1.95	0.22	0.35	0.13
	Scats with seeds	**x*****	x	**x*****	-	-	6	-59.50	133.55	0	0.31	0.89	0.73
		x***	-	**x*****	-	-	5	-60.99	133.74	0.19	0.28	0.89	0.68
		x***	x	**x*****	-	x	7	-58.84	135.18	1.62	0.14	0.86	0.67
		x***	x	**x*****	-	x	6	-60.32	135.19	1.64	0.14	0.87	0.60

L: lynx presence or absence, FD: fruit density, H: Type of habitat. The multiplicative symbol (x) indicates an interaction term.

Table S2. Coefficients and standard errors for the fixed effects and variances for the random effects included in the selected models shown in Table S1 for the red fox (*Vulpes vulpes*). Binomial, Gaussian (identity with $\log_{10}$ transformation) and Poisson errors (log link) were used to fit fruit occurrence, fruit biomass per scat and number of scats with seeds GLMMs, respectively. Hyphen symbol refers to non-selected variables.

Response	Fixed effects (Estimate ± SE)						Random effects (σ)	
	Intercept	L	FD	H	FDxL	LxH	Locality	Year
Fruit occurrence per scat (all plants)	0.09 ± 0.18	-0.28 ± 0.27	0.27 ± 0.10		-0.37 ± 0.14		0.13	~0
Fruit occurrence per scat (*A. unedo*)	-0.67 ± 0.21	-	0.37 ± 0.10		-		0.24	0.03
	-0.71 ± 0.28	0.14 ± 0.33	0.55 ± 0.14		-0.32 ± 0.17		0.22	0.05
	-0.75 ± 0.26	0.16 ± 0.33	0.36 ± 0.10		-		0.23	0.03
Fruit biomass per scat (all plants)	1.42 ± 0.09	-	-		-		0.05	~0
Fruit biomass per scat (*A. unedo*)	1.47 ± 0.06	-	-		-		~0	~0
Scats with seeds (all plants)	3.15 ± 0.17	-0.46 ± 0.25	0.20 ± 0.08	-0.12 ± 0.10	-0.26 ± 0.12	-0.68 ± 0.18	0.11	~0
	3.10 ± 0.15	-0.43 ± 0.21	-	-0.12 ± 0.10	-	-0.68 ± 0.18	0.07	~0
	3.13 ± 0.15	-0.48 ± 0.22	0.08 ± 0.06	-0.12 ± 0.10	-	-0.68 ± 0.18	0.08	~0
Scats with seeds (*A. unedo*)	2.66 ± 0.24	-0.19 ± 0.28	0.43 ± 0.12	-0.08 ± 0.12	-0.28 ± 0.14	-0.72 ± 0.21	0.14	0.38
	2.63 ± 0.21	-0.20 ± 0.27	0.25 ± 0.08	-0.08 ± 0.12	-	-0.72 ± 0.21	0.13	0.01

L: lynx presence or absence, FD: fruit density, H: Type of habitat. The multiplicative symbol (x) indicates an interaction term.

Table S3. Coefficients and standard errors for the fixed effects and variances for the random effects included in the selected models shown in Table S2 for the stone marten (*Martes foina*). Binomial, Gaussian (identity with $\log_{10}$ transformation) and Poisson errors (log link) were used to fit fruit occurrence, fruit biomass per scat and number of scats with seeds GLMMs, respectively. Hyphen symbol refers to non-selected variables.

Response	Fixed effects (Estimate ± SE)						Random effects (σ)	
	Intercept	L	FD	H	FDxL	LxH	Locality	Year
Fruit occurrence per scat (all plants)	0.97 ± 0.54	-	-0.59 ± 0.20		-		~0	0.50
	1.02 ± 0.47	-0.57 ± 0.60	-0.57 ± 0.21		-		0	0.41
	0.97 ± 0.54	-	-		-		~0	0.50
Fruit occurrence per scat (*A. unedo*)	-0.13 ± 0.73	-	-		-		0.31	0.87
	-0.31 ± 0.76	0.81 ± 0.74	-		-		0.26	0.96
Fruit biomass per scat (all plants)	1.19 ± 0.36	1.34 ± 0.43	-0.31 ± 0.10		1.12 ± 1.21		~0	0.30
	1.19 ± 0.35	1.17 ± 0.39	-0.30 ± 0.10		-		~0	0.22
Fruit biomass per scat (*A. unedo*)	1.24 ± 0.38	1.37 ± 0.55	-		-		0.13	0.17
	1.06 ± 0.51	1.78 ± 0.52	-0.29 ± 0.15		-		0.02	0.44
Scats with seeds (all plants)	2.11 ± 0.35	-2.66 ± 0.53	-	-1.74 ± 0.26	-	-	0.30	0.09
	2.13 ± 0.35	-2.66 ± 0.56	0.10 ± 0.17	-1.74 ± 0.26	-1.21 ± 0.76	-	0.28	0.08
Scats with seeds (*A. unedo*)	1.64 ± 0.46	-2.55 ± 0.58	0.36 ± 0.22	-1.91 ± 0.34	-	-	0.31	0.56
	1.57 ± 0.58	-2.39 ± 0.56	-	-1.91 ± 0.34	-	-	0.32	0.56
	1.65 ± 0.46	-2.76 ± 0.63	0.36 ± 0.22	-2.04 ± 0.37	-	1.12 ± 0.94	0.31	0.56
	1.58 ± 0.58	-2.61 ± 0.62	-	-2.04 ± 0.38	-	1.12 ± 0.94	0.32	0.44

L: lynx presence or absence, FD: fruit density, H: Type of habitat. The multiplicative symbol (x) indicates an interaction term.

Table S4. Occurrence (percentage of camera-traps with presence) and average relative abundance index (RAI) of the Iberian lynx and frugivorous mesocarnivores community in our study area. We registered only four lynx detections (2.5% of the overall lynx detections in two years) in two localities considered as controls, probably connected with sub-adult dispersal or occasional exploratory incursions of territorial males from territories in the vicinity (see Fig. 1), but we did not register territorial couples or reproduction. Occurrence and relative abundance were extracted from an unpublished study (Burgos et al.) in the same localities and period that this study following the methodology used by Sarmento et al., 2009. One hundred and twenty camera-traps were placed during two consecutive years in ten localities with a total effort of 10644 trap-days and an average effort of 44 days/trap.

Species	Lynx scenario	Occurrence	RAI ± SE
Iberian lynx *L. pardinus*	Presence	0.56	2.85 ± 0.57
	Absence	0.03	0.54 ± 0.06
Red fox *V. vulpes*	Presence	0.63	10.22 ± 3.53
	Absence	0.97	50.80 ± 7.09
Badger *M. meles*	Presence	0.45	7.43 ± 3.28
	Absence	0.25	1.25 ± 0.25
Stone marten *M. foina*	Presence	0.06	0.63 ± 0.19
	Absence	0.88	33.98 ± 7.17
Common genet *G. genetta*	Presence	0.42	6.36 ± 1.91
	Absence	0.81	24.07 ± 7.73

Table S5. Sample coverage (the proportion of the total number of plants in the entire assemblage that belongs to detected species in scats) and sampling completeness (the proportion of the assemblage's plants belonging to detected species, with each plant being proportionally weighted by the $(q - 1)^{th}$ power of its abundance) estimates for the red fox (*V. vulpes*) and the stone marten (*M. foina*) in sites with lynx presence and lynx absence with standard errors (SE). The absence of standard errors for the stone marten is because we performed this analysis by adding up the samples from all localities and fruiting seasons for each predation risk scenario, due to the scarce sample size separately, mostly inside lynx territories (n=14). Sample coverage values were high and almost identical in lynx presence and absence sites, with non-significant differences between predation risk scenarios for the red fox (F = 2.69 and *P* = 0.12). Sampling completeness values were also almost identical in lynx presence and absence sites, with non-significant differences between predation risk scenarios for the red fox (F = 2.55 and *P* = 0.13 and F = 0.67 and *P* = 0.43 for Hill-Shannon and Hill-Simpson diversity index, respectively). GLMs were fitted with Log-Normal distribution.

Species	Lynx scenario	Sample coverage	Diversity index	Sampling completeness
Vulpes vulpes	Absence	0.97 ± 0.01	Hill-Shannon	0.93 ± 0.01
			Hill-Simpson	0.96 ± 0.01
	Presence	0.92 ± 0.03	Hill-Shannon	0.87 ± 0.03
			Hill-Simpson	0.94 ± 0.01
Martes foina	Absence	0.99	Hill-Shannon	0.98
			Hill-Simpson	0.99
	Presence	1	Hill-Shannon	0.90
			Hill-Simpson	0.90

Appendix S3. Figures

Figure S1. (a-f) Photographs illustrating the landscape structure of forest and open types of habitats selected for this study. (a-b) Illustrate panoramic landscape perspective of a forest transect, (c-d) show the landscape transition between forest and open habitats and (e-f) illustrate transects along open landscapes lack of arboreal vegetation except isolated individuals or clusters of Iberian pear (*Pyrus bourgaeana*) or oaks (*Quercus sp.*). (g-l) Examples of dispersed seeds by mesocarnivores found along sampled 1.5-km transects: (g) a stone marten (*Martes foina*) scat with strawberry tree (*Arbutus unedo*) seeds; (h) defecated Iberian pear seeds by the red fox (*Vulpes vulpes*); (i-j) a germinated strawberry tree and Iberian pear seed in a stone marten and red fox scat, respectively. (k-l) Seed manipulation in laboratory: (k) many strawberry tree seeds and (l) prickly juniper (*Juniperus oxycedrus*) seeds extracted from one single mesocarnivore scat.

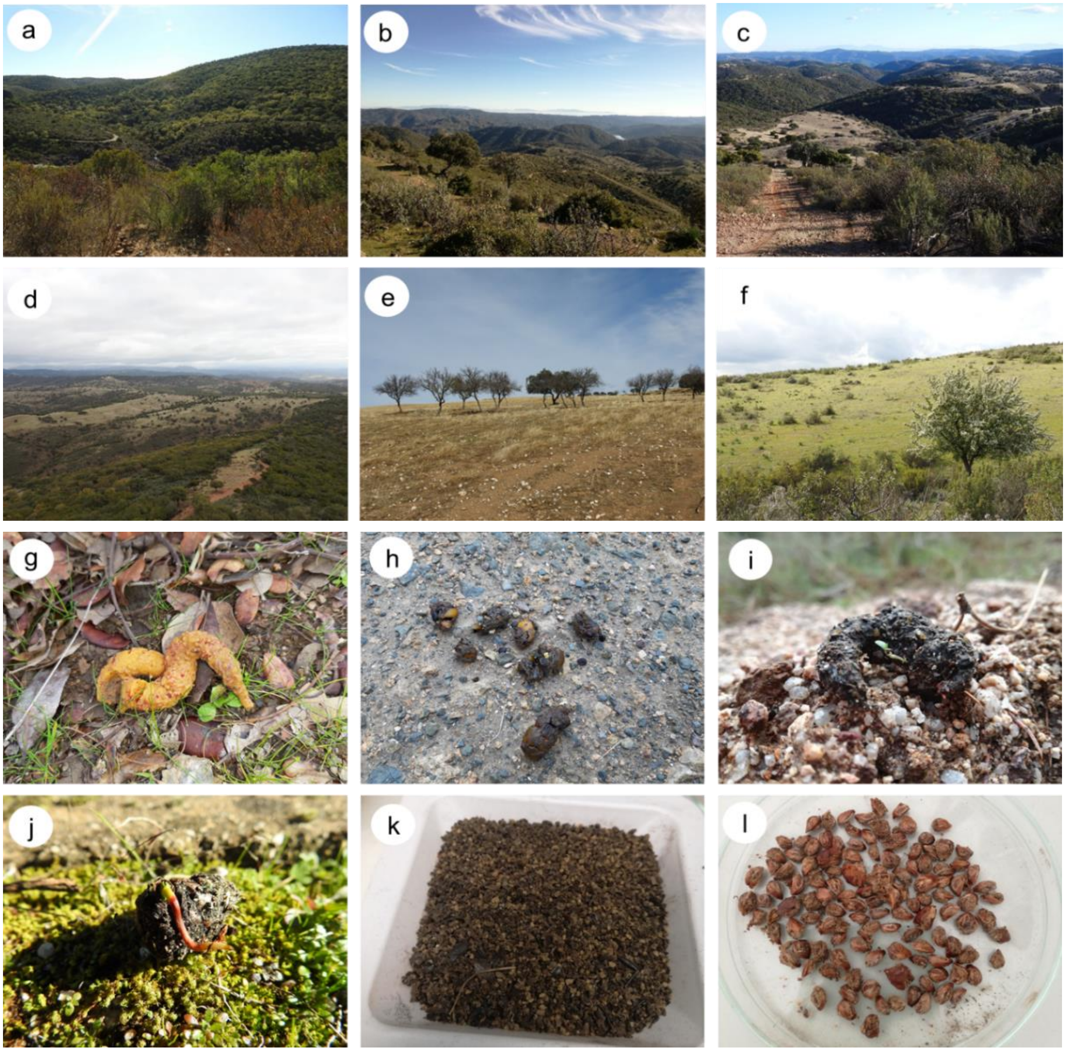


Fig. S2. (A) Observed average fruit density (fruits/m^2) and (B) fruit alpha diversity (Hill-Shannon and Inverse Hill-Simpson) in lynx presence scenario (circles) and lynx absence (rhombus). Bars represent standard errors.

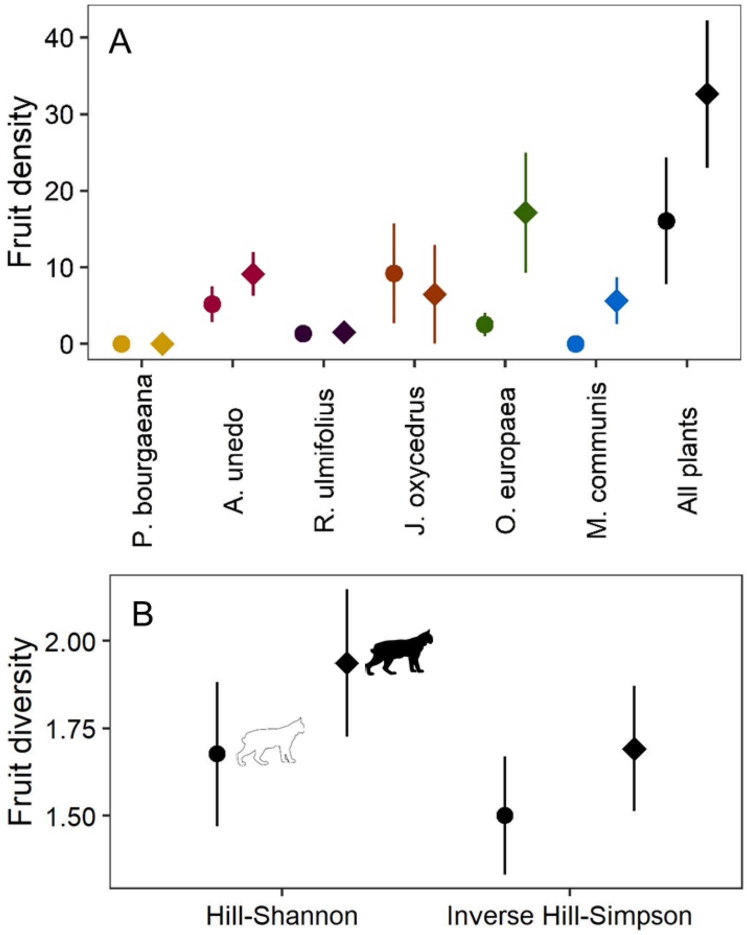


Fig. S3. Correlations between the number of seeds dispersed and the number of scats of mesocarnivores. Dashed lines represent correlations for the stone marten (*M. foina*) and solid lines for the red fox (*V. vulpes*). Orange colour refers to lynx presence scenarios and magenta to lynx absence scenarios. *R* represents the Pearson correlation value and *p* the significance (95% CI). Shadow area shows 95% confidence intervals.

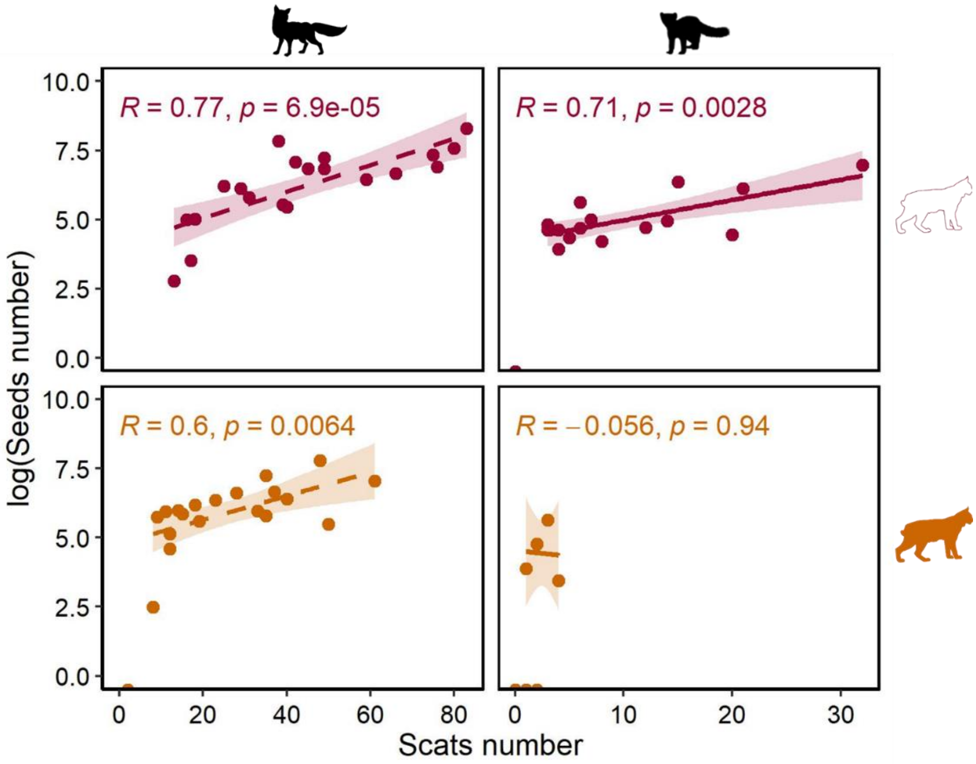


Figure S4. Frequency of appearance (%) of fleshy-fruits in (A) stone marten (n = 175) and (B) red fox (n = 1400) scats deposited in sites with presence of Iberian lynx (right side) and absence of lynx (left side) for each plant species. Values increase to the left on the plot for areas without lynx, and the opposite for the plot with lynx presence. Dark colours refer to scats with presence of fruits (1) and transparent colours refer to scats without fruits (0). Plant species are represented by different colours: *Arbutus unedo, Pyrus bourgaeana, Juniperus oxycedrus, Rubus ulmifolius, Myrtus communis, Olea europaea*. All plants refer to the proportion of scats with seeds of any plant species.

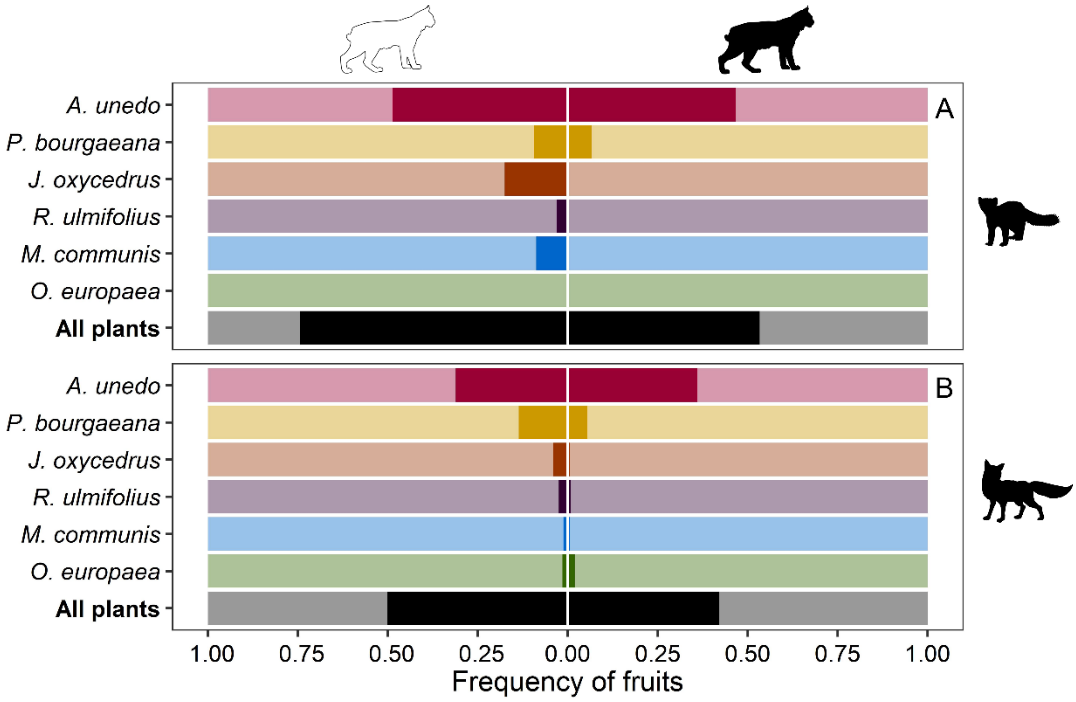


Figure S5. Relationship between the fruit density (fruits/m^2) and the predicted values by GLMMs of back-transformed number of scats with seeds for all plant species (left panel) and only for the strawberry tree (*A. unedo*) (right panel) in the lynx presence scenario (orange colour) and in absence of lynx (magenta colour). Shadow areas show the log-transformed confidence intervals derived from modelling. Asterisks denote interactions selected in the best models with AICc<2. First black silhouette refers to the red fox (*Vulpes vulpes*) and the second one to the stone marten (*Martes foina*).

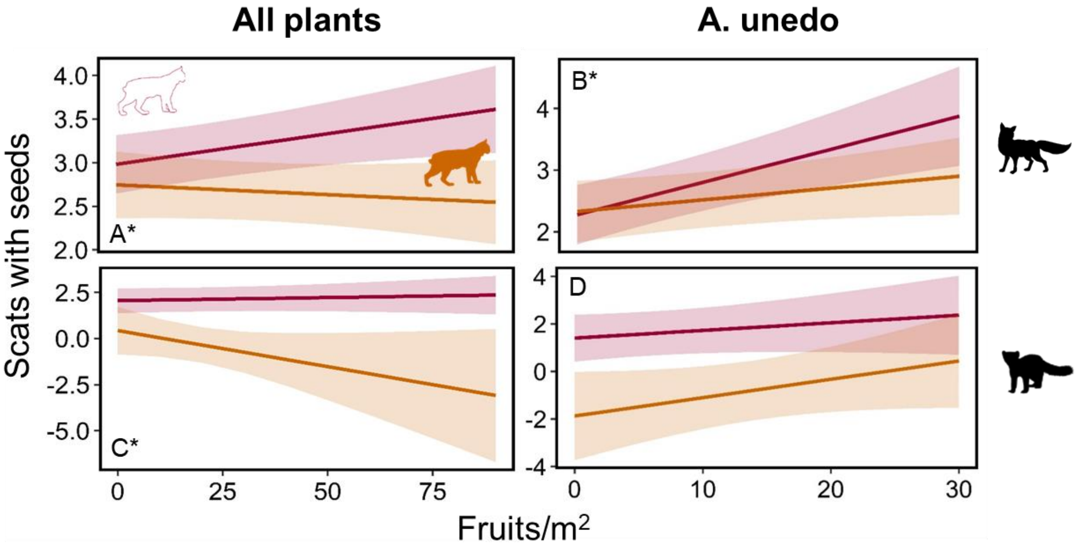


Figure S6. Sample-size-based rarefaction (solid) and extrapolation (dashed) curves for the Hill-Shannon (light purple) and Hill-Simpson (dark purple) diversity of seeds dispersed by the red fox (*V. vulpes*) in each study locality, fruiting season and predation risk scenario. Empty lynx silhouette represents the scenario without Iberian lynx and the black one with lynx presence.

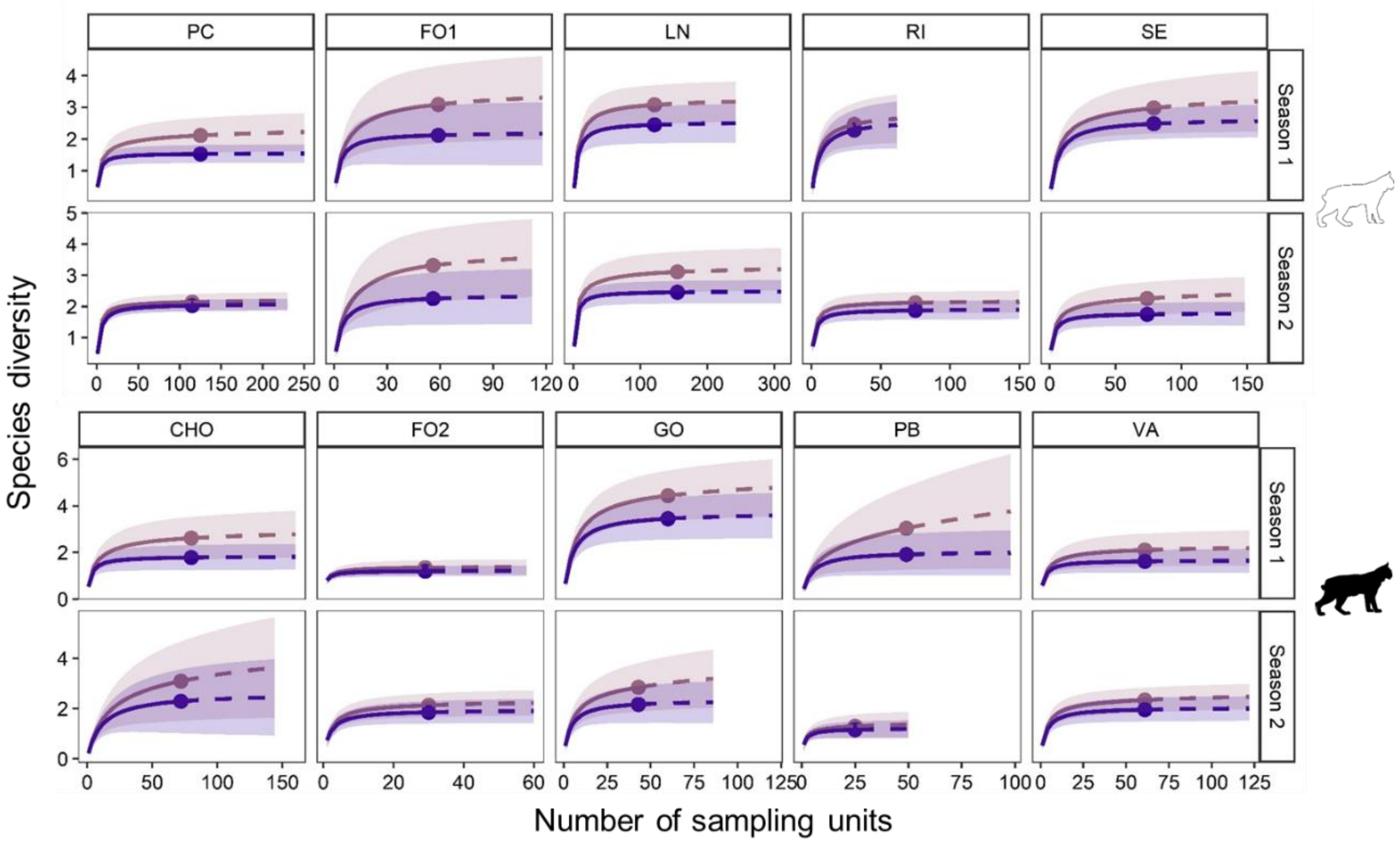


Figure S7. Sample-size-based rarefaction (solid) and extrapolation (dashed) curves for the Hill-Shannon (light purple) and Hill-Simpson (dark purple) diversity of seeds dispersed by stone marten (*M. foina*) in each predation risk scenario. Empty lynx silhouette represents the scenario without Iberian lynx and the black one with lynx presence. We performed this analysis by adding up the samples from all localities and fruiting seasons for each predation risk scenario due to the scarce sample size separately, mostly inside lynx territories (n=14).

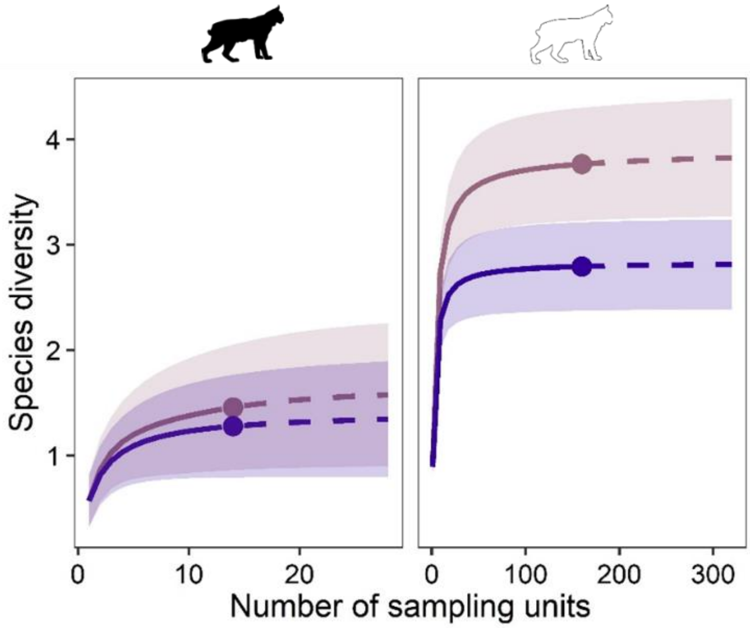
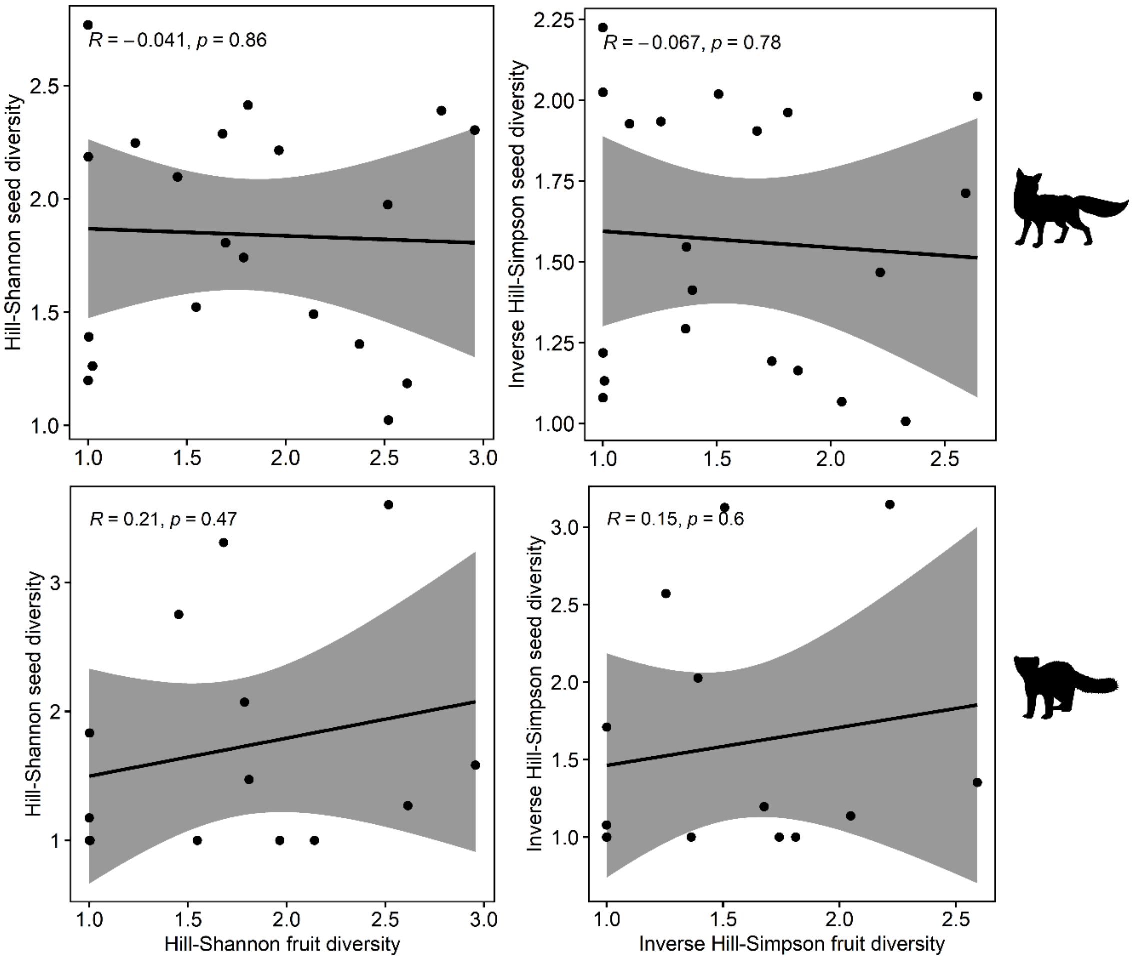


Figure S8. Correlations between the alpha diversity of dispersed seeds by mesocarnivores and the alpha diversity of available fruits, considering Hill-Shannon and Inverse Hill-Simpson index. The first row represents correlations for the red fox (*V. vulpes*), and the second one for the stone marten (*M. foina*). *R* represents the Pearson correlation value and *p* the significance (95% CI). Shadow area shows 95% confidence intervals.



Chapter 4

Cascading effects triggered by apex predators can ultimately affect plant recruitment: a study case on a Mediterranean fruit tree

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Unpublished

Abstract

Rewilding scenarios of large carnivores worldwide suppose a unique opportunity to study their cascading impacts on dynamics and demography of plant populations. Since subordinate carnivores are controlled by larger predators, the abundance of small prey can increase ant thus many plant-animal interactions may be altered. Thus, we investigated how the presence of an apex predator, the Iberian lynx (*Lynx pardinus*), led to direct effects on seed dispersal by mesocarnivores, triggering cascading effects on 1) post-dispersal seed predation by rodents, 2) seedling emergence and 3) seedling early survival of a Mediterranean tree, the Iberian pear (*Pyrus bourgaeana*). Habitat and microhabitat affect plant recruitment and survival. Thus, we conducted multiple field experiments to assess the effect of habitat and seed-arrival microsite on *P. bourgaeana* performance under lynx presence and lynx absence scenarios. Our path analysis revealed that seed dispersal was extremely decreased in lynx presence (up to 80%) and was biased towards forest habitats, what indirectly led to extremely low seedling survival in lynx presence (1%) respect to lynx absence scenarios (8%). No direct effect of lynx presence was found for post-dispersal seed predation. However, we found a higher seed predation by rodents beneath shrubs, negatively affecting plant recruitment. Since this microsite was the most suitable for seedling survival, especially in open habitats, our results identified a seed-seedling conflict that may compromise the potential of *P. bourgaeana,* and likely also for other plant species*,* to expand beyond their range in the current global change scenario. Reintroduction programs of threatened carnivores in defaunated ecosystems should take into account trophic cascades as mechanisms able to structure ecosystems and alter in variable ways mutualistic and antagonistic interactions across food webs.

Keywords: seed dispersal, post-dispersal seed predation, small mammals, mesocarnivores, predation risk, defaunation, Iberian lynx, seed-dispersal effectiveness

Introduction

Trophic cascades are powerful mechanisms structuring ecosystems across food webs (Terborgh et al. 2010, Estes et al. 2011, Ripple et al. 2016). Top-down effects can trigger reverberating effects on successive trophic levels, ultimately affecting the dynamics of plant populations and whole communities (Ripple and Beschta 2005, Choquenot and Forsyth 2013). Therefore, plant-animal mutualistic interactions (e.g. pollination or endozoochory) are susceptible to be altered by trophic cascades, triggered by a wide range of human impacts. For example, frugivore loss in defaunated ecosystems has been proved to ultimately affect plant demography components such as seedling recruitment (Cordeiro and Howe 2003, Wotton and Kelly 2011) and survival (Traveset et al. 2012, Fedriani et al. 2020a), compromising the persistence and spreading of many plant species. Thus, the loss of key links in highly specialized mutualistic networks could cascade across plant life stages (Wang and Smith 2002, Cochrane 2003, Rogers et al. 2021). Human-caused ecosystem alterations have been recently documented as important drivers of trophic cascades but we still lack research to cope properly with the current global change scenario.

Rewilding is a recent process referring to the active or passive restoration of animal species (Perino et al. 2019), whose populations are threatened or locally extinct, with promising results for the conservation of large carnivores worldwide (Wolf and Ripple 2018). However, the sudden presence of top-order predators in ecosystems can trigger trophic cascades across lower trophic links (Ripple and Beschta 2012a, Colman et al. 2015). The restoration of trophic interactions existing prior apex predator extirpation re-establishes the landscape of fear (Laundré et al. 2010), where smaller predators (i.e. mesocarnivores) are often displaced to risk-free but less productive areas as a result of asymmetric competition (Brown et al. 1999, Gallagher et al. 2017). Thus, predation risk can trigger behaviorally-mediated trophic cascades, by altering the behaviour of subordinate predators (Schmitz et al. 1997, Pasanen-Mortensen et al. 2013). For instance, Haswell et al. (2018) and Burgos et al. (2022) found that foxes were less efficient foragers under predation risk in order to avoid costly agonistic interactions. Apex predators can also limit the abundance of smaller predators by direct intra-guild predation (Linnell and Strand 2000, Newsome et al. 2017), resulting in a mesopredator-free state which could cascade across the ecosystem functions and ecological processes where mesocarnivores are involved in (Brashares et al. 2010, Tambling et al. 2018).

Mammalian carnivores play a key role as seed dispersers for many fleshy-fruit plants (Herrera 1989, Rosalino et al. 2010), especially for large-fruited plants for which extinct megafauna represented the legitimate frugivorous partners (Galetti et al. 2018). Thus, shifts in abundance and behaviour of frugivorous mesocarnivores triggered by restored top-predators could affect both the quantity (i.e. number of dispersed seeds) and the quality (i.e. probability of a seed results on a new adult plant) due to the seed-dispersal service provided by mesocarnivores (Schupp et al. 2010, Draper et al. 2022). Habitat and microsite selection patterns by mesocarnivores (Fedriani et al. 2000, Gosling and Roberts 2001, Miller et al. 2012) could change in response to predation risk by top-predators, modifying thus the quality of seed deposition (Schupp 2007, Beckman and Rogers 2013). Seed dispersal biased towards safer and high coverage areas, as result of the creation of a landscape of fear (Carreira et al. 2020), could cascade in multiple ways affecting plant establishment (García et al. 2005b, García-Cervigón et al. 2018). Whether the alteration on seed-arrival patterns drives plants to highly suitable habitats, then higher recruitment rates could partly or entirely compensate for the lower number of seeds dispersed under predation risk due to mesopredator suppression. Nevertheless, behavioural shifts can be also detrimental to plant establishment whether seeds are transported to unsuitable habitats, exacerbating the seed-limitation effect. Several studies have found that the seed-arrival microsite affected seed emergence and recruitment in Mediterranean ecosystems (Escribano-Ávila et al. 2013, 2014, Garrote et al. 2022b), where seed dispersal is only the first step in an obstacle career against herbivory and desiccation (Mendoza et al. 2009, Perea et al. 2020). However, few research has assessed how ecological alterations affecting seed dispersal by carnivores ultimately cascade into plant demography and population dynamics (Maron and Pearson 2011, Cancio et al. 2016, Fedriani et al. 2020a).

Apex predators can also exert a top-down regulation on prey populations in ecosystems where the landscape of fear and the mesopredator suppression has been re-established via *rewilding*. Even though prey suppression is one of the best documented cascading effect of reintroducing apex predators (Letnic et al. 2012, Miller et al. 2012), the size and sign of this top-down effect is species and context dependent. For example, Ripple and Beschta (2012) showed that the wolf reintroduction limited elk populations in Yellowstone due to an increase in the direct predation pressure. However, mesopredator-free scenarios can also cause a small prey release (Meserve et al. 2003, Miller et al. 2012). For instance, the Iberian lynx (*Lynx pardinus*) plays the role of apex predator in Mediterranean ecosystems where has been restored, limiting mesopredator abundance and hence, indirectly increasing the abundance of small-sized prey such as rodents (Jiménez et al. 2019, Burgos et al. 2023), which are important seed predators in many ecosystems (Fedriani and Delibes 2013, Galetti et al. 2015). Then, increased population of seed predators may significantly alter the initial pattern of seed rain, modifying the availability and spatial distribution of seeds, and ultimately affecting plant recruitment and establishment (García et al. 2005a, Calviño-Cancela 2007). Although several previous research have studied the ecological consequences of population regulation of large body-sized prey species (e.g. herbivory decrease and landscape restructuring; Beschta and Ripple 2009), very little is known about whether and how small prey release after top-predator restorations can affect key ecosystem functions.

Here, we studied the potential cascading effects triggered by apex predators across trophic levels and the ultimate ecological consequences of rewilding scenarios on seedling establishment of a mesocarnivore-dispersed Mediterranean tree, the Iberian pear (*Pyrus bourgaeana*). Since effective seed dispersal of pear fruits depends mostly on frugivorous carnivores (Fedriani and Delibes 2009a), presence of an intra-guild predator could suppose a serious limitation for the distribution and survival of this scarce tree throughout a trophic cascade. We combined field experiments and path analyses to assess the top-down effects driven by the Iberian lynx on mesocarnivore seed dispersal and the sequential plant ontogenetic stages (seed survival and seedling emergence and survival). We hypothesize that lynx presence would reduce 1) the number of dispersed seeds, 2) post-dispersal seed survival and ultimately 3) seedling emergence and recruitment, through suppressing the abundance of frugivorous carnivores and releasing seed predator's abundance (i.e. rodents). However, the environmental conditions of seed arrival habitats and microsites could condition the effect of lynx on seedling recruitment. Specifically, we predict that frugivorous mesocarnivores under lynx presence will modify their seed-dispersal patterns according to predation risk, leading to a lower seed dispersal in open highly-risky landscapes. We also expect an increase of seed predation rates and that the extreme environmental conditions of open Mediterranean habitats will dramatically limit seedling emergence and survival and thus, plant establishment. Consequently, whether seed-dispersers respond to predation risk by dispersing more seeds into more suitable habitats, then such shift in their spatial behaviour could compensate any reduction in the number of dispersed seeds in a top-predator *rewilding* context.

Methods

Study area

This study was performed in Sierra de Andújar Natural Park (SANP), in Southern Spain (38°14'27.71" N, 4° 4'45.03" W, near 740 km^2; see Chapter 3: Fig. 1). A soft annual average temperature (~18 ºC) and scarce rainfall (~700 mm) are the typical for

Mediterranean climate areas (data from Andújar meteorological station, AEMET Opendata, 2020). The altitudinal range is between 400 and 800 m. SANP is composed by extensive game lands, where hunting of large ungulates is frequent, but predator control is scarce. Mediterranean shrubland is the predominant vegetation, but pine plantations are also frequent. The most important habitat type is high-coverage mature forest with an arboreal stratum of holm oak (*Quercus ilex*) mixed with a rich community of wild fleshy-fruited species such as the strawberry tree (*Arbutus unedo*) or the mastic (*Pistacia lentistus*) and evergreen fragrant shrubs (e.g. *Rosmarinus officinalis and Thymus sp.*). Forests are frequently intercalated with open landscapes with scarce scrubland and isolated of small clumps of trees such as Iberian pears (*Pyrus bourgaeana*).

This Natural Park shelters one of the largest non-reintroduced wild populations of Iberian lynx, an endemic and threatened feline species endemic of the Iberian Peninsula. Lynx was close to extinction in 90's decade but recent successful conservation efforts (i.e. genetic management, captive breeding, reintroductions, habitat adequacy; see Simón et al. 2012) have driven the current wild population to count with more than 1500 individuals (MITECO 2023). Despite its recovery, the Iberian lynx range is still heterogeneous throughout the SANP (see Chapter 3: Fig. 1) due to the patchy distribution of its main prey, the European rabbit (*Oryctolagus cuniculus*). Lynx co-exists with a rich community of medium-sized mesocarnivores in the study area, including frugivorous species such as the red fox (*Vulpes vulpes*), the stone marten (*Martes foina*), the common genet (*Genetta genetta)* or the Eurasian badger (*Meles meles*), and exerts a top-down suppression by controlling their abundance (except for the Eurasian badger) (see Burgos et al. 2023). Fruits represent a key food resource for frugivorous mesocarnivores (Rosalino and Santos-Reis 2009), mainly when other more energetic food intakes are scarcely available, such as small mammals (Padial et al. 2002, Bakaloudis et al. 2012). The most common small mammal prey species for mesocarnivores in our study area are the wild rabbit (*Oryctolagus cuniculus*) and rodents such as the garden dormouse (*Eliomys quercinus*), the wood mouse (*Apodemus sylvaticus*) and the Algerian mouse (*Mus spretus*), which are also seed predators and pulp-feeders of several fleshy-fruited plant species (Rey et al. 2002, Fedriani and Delibes 2013).

Study tree species

The Iberian pear is a Mediterranean tree which produces high-sugar and water-rich fleshy-pomes of 2-3 cm diameter and ~9.5 g wet weight (Fedriani and Delibes 2009b).

Iberian pear is a predictable food resource from September to January (peaking in November; Fedriani and Delibes, 2009b), producing a large number of fruits (from 100 to >1000 fruits per tree) which contain 1-5 viable seeds with thin and easily breakable coats. Pre-dispersal seed losses by invertebrates are generally low (Fedriani and Delibes 2009b). In our study area, the Iberian pear occurs in very low densities (even lower than in other Mediterranean areas such as Doñana (< 1 individual ha-1; Fedriani et al., 2010), and is relatively isolated from other fruiting species, growing in open-vegetation landscapes. This tree shows an aggregated spatial pattern derived from seedling clustering induced by aggregated seed dispersal by mammals, mostly the red fox and Eurasian badger (Fedriani and Delibes 2009b, Fedriani et al. 2010). Thus, *Pyrus bourgaeana* was selected as the target plant species in this study. Mammal carnivores ingest the whole fruits, barely causing mechanical damage to seeds and dispersing them far from the maternal surrounding (Fedriani and Delibes 2009a). Fruit processing by frugivorous carnivores is an important service for this tree because seed germination requires removal of the attached fruit pulp (Fedriani and Delibes 2009b). Other abundant frugivores disperse Iberian pear seeds occasionally (e.g. wild boar or birds*)* but they act largely as seed predators or pulp feeders (Fedriani et al. 2012, Fedriani and Delibes 2013). Rodents are important seed predators for this plant and their specialized feeding behaviour could suppose an extra limitation for plant production (Fedriani and Delibes 2009a). However, scatter-hoarding rodents are able to act as seed rescuers confined within aborted fruits and potentially contribute passively to secondary seed dispersal (Fedriani et al. 2020b). Most *Pyrus bourgaeana* seedlings emerge from late February to late April, being the first summer the period of highest seedling mortality due to the severe droughts in Mediterranean areas (Fedriani & Delibes, 2009b).

Seed dispersal quantification

We quantified seed dispersal by frugivorous carnivores in five localities with permanent territorial presence of reproductive individuals of Iberian lynx and five localities without lynx (i.e. controls), following an experimental design throughout the Natural Park (Chapter 3: Fig. 1). Lynx presence and absence localities were selected based on a previous monitoring study of the community of carnivores in SANP (Burgos et al. 2023). These localities had similar habitat characteristics and fruit-producing Iberian pear trees were found in all of them (0.86 and 1.73 trees per km^2 on average in lynx absence and presence scenarios, respectively). Seed dispersal was quantified in two different habitat types per locality: a) mature forest with a dense shrub stratum; and b) open vegetation landscapes with scarce shrub and only isolated or small clumps of trees (see Fig. S1). Two mammal scat experts conducted mesocarnivores scat sampling along transects of

1.5 km x 3 m in each habitat type during two consecutive fruiting seasons, between October and March of 2018-19 and 2019-20. Sampling was conducted periodically (1.5-months intervals), reaching a total effort of 160 visits (4 temporal replicates per transect x 2 habitats x 10 localities x 2 seasons). We also recorded the microsite of scat deposition because is an important factor determining plant recruitment (Schupp 1993, Escribano-Ávila et al. 2014); a) beneath or over a small rock (hereafter rock), b) beneath or over a shrub (hereafter shrub) and c) on open terrain (hereafter open), corresponding to frequent microhabitats for scat deposition of mammal carnivores (García-Cervigón et al. 2018, Cortázar-Chinarro et al. 2019). The carnivore species was identified visually according to scat size, shape and odour characteristics (Putman 1984). We recorded scats of red fox, stone marten and badger. The few (n = 26) badger scats found were all located in two localities without lynx and strongly aggregated in a few latrines. Such an aggregated distribution of badger scats made very unlikely to finding them during the transects; thus, badger faecal samples were excluded from the analyses. DNA-barcoding analysis was performed for a subsample of stone marten scats (n = 70) to confirm our visual identification success, which reached 95% (see molecular procedures in Chapter 3). The scats were stored in paper bags and dehydrated at 30ºC in a drying oven during 3-4 days to avoid fungi appearance. Iberian pear seeds were identified visually according to their morphology, extracted manually from the dried scats (see photographs in Fig. S1).

Post-dispersal seed predation

We compared the post-dispersal predation of pear seeds by rodents between lynx presence scenarios in November of 2020, coinciding with the seed-dispersal peak for *P. bourgaeana* (Fedriani and Delibes 2009a) and the autumnal activity peak of rodents (Wilson et al. 1993). Seed predation rates were estimated in a field experiment performed in two localities, one with presence of Iberian lynx and the other one without lynx (control locality). We placed pear seed depots in Petri dishes (previously taken from wild Iberian pear fruits) in each type of seed-dispersal habitat (forest and open) and seed-arrival microsite (rock, shrub and open) mentioned above, simulating dispersed seeds (e.g. Garrote et al. 2019; see Fig. S1). The intensity of seed predation by rodents is known to markedly vary among habitats and microsites (Hulme 1994). We selected similar sized shrubs (~1-m height) and rocks (~0.5 m height) to place the seed depots. In each type of habitat, we placed seed depots in six spatially independent plots (15 x 15 m) spaced 200 m, far enough considering the reduced rodents home ranges (Rosalino et al. 2011). Microsite was replicated twice inside each spatial plot. We placed 8 seeds per Petri dish the first day of the experiment and we monitored it during 5 consecutive days to record the total number of eaten seeds. Non full-moon nights took place during the experiment in order to avoid a limited activity of rodents in moonlight (Navarro-Castilla and Barja 2014). We offered a total of 1152 seeds (8 seeds x 2 seed depots x 3 microsites x 6 plots x 2 habitats x 2 lynx scenarios). We assumed that seed removal is equivalent to seed predation because we frequently observed seed shells from predated seeds by rodents (Garrote et al. 2019; Fig. S2). To evaluate potential seed predation by other seed-consumers (e.g. ants, birds; Warzecha and Thomas Parker 2014, Suárez-Esteban et al. 2018), we installed 36 camera-traps in a subset of seed depots (25%; n = 144). Camera-traps confirmed that rodents were the only seed predators in our field experiment (see Fig. S2). Seed removal rate per depot was calculated as the number of seeds predated divided by the number of dispensed seeds.

Seedling emergence and survival

Because we discarded the possibility that the mere presence of lynx could have any effect on seedling emergence and survival, and due to logistical limitations, we estimated emergence and survival rates in just one locality within our study area and assumed the same rates for the remaining localities. We sowed pear seeds (previously taken from local trees) in the same seed-dispersal habitats (forest and open) and seed-arrival microsites (rock, shrub and open) mentioned previously (see Fig. S1). The experiment began in November 2020 (coinciding with the natural seed-dispersal peak of *P. bourgaeana*) and was monitored up to February 2022, with periodical visits (~2-months intervals) to check seedling emergence and survival. We replicated microsite across five spatially independent plots (200 m apart) in each habitat type. Inside each plot (15x15 m) we selected a rock, a shrub and an open interspace to sew 12 pear seeds per microsite. Shrub size and type was standardized to minimise potential bias due to plant-plant interactions (Gómez-Aparicio et al. 2004, Peláez et al. 2019). Thus, we selected ~1-m height evergreen fragrant plants (e.g. *Rosmarinus officinalis* or *Lavandula stoechas*) because were shared species between both type of habitats and were frequently selected by carnivores to faecal delivering in our study area (Authors *unpublished* data). Seeds were sowed north-facing in a grid of 4 x 3 rows (5 cm apart) and, to prevent vertebrate herbivory and trampling, were covered by bottom-opened metallic grid cages (40 x 30 x 20 cm; 15 mm light) (Fig. S1). We sowed a total of 360 seeds (12 seeds x 3 microsites x 5 plots x 2 habitats). We estimated emergence and survival rates per microsite and habitat as the number of seedlings emerged per the total sown seeds and the number of seedlings survived per the total emerged seedlings up to the end of the experiment, respectively.

Quantitative variation of seed dispersal and vital rates among scenarios

We fitted Generalized Linear Mixed Models (GLMMs) to study the effects of lynx presence and seed-arrival habitat and microsite on the i) number of dispersed seeds by carnivores and ii) seed predation rate by rodents. Also, we used GLMMs to estimate the effects of habitat and microsite of seed arrival on iii) seedling emergence and iv) seedling survival rates. We included the interactions 'lynx x habitat', 'lynx x microsite' and 'habitat x microsite' for seed dispersal and seed predation and 'habitat x microsite' for seedling emergence and survival. Random effects were 'Locality' and 'Fruiting season' for seed dispersal experiment, and 'Plot' for seed predation, emergence and survival experiments. Binomial errors were used for all models except for the number of dispersed seeds, where we used Poisson errors. We calculated marginal and conditional pseudo-R^2 for each single model. We carried out post-hoc simple-slope pairwise comparisons to estimate the model coefficients of the interaction terms using the R package `emmeans` v.1.6.3 (Russell V. et al. 2021). Residuals were inspected by using `DHARMa` package v. 0.4.6 (Hartig and Lohse 2022).

Transition probabilities estimates

Transition probabilities (TPs; seed survival, seedling emergence and seedling survival) were calculated as the ratio of the number of individuals completing a stage over the number of individuals entering that same stage (e.g. Rey and Alcántara 2000, Balcomb and Chapman 2003, Garrote et al. 2022b). Nonetheless, since sample sizes used to mean observed transition probabilities were rather limited, we fitted Bernoulli distributions (n = 100) to the observed values of TPs for each lynx scenario, habitat and microsite. Then, we resampled randomly without replacement each distribution truncated by the standard deviation of the observed TP (Manly 2006), in order to include a moderate stochasticity in path analysis. Resulting seed survival rates were multiplied by the number of seeds dispersed by mesocarnivores in each transect, attaining thus the number of surviving seeds. Then, we multiplied seed emergence rates by the number of surviving seeds, leading to the number of emerged seedlings per transect. Finally, seedling survival rates were multiplied by the number of emerged seedling, attaining thus the number of surviving seedlings per transect. By last, we rounded up to an entire value the number of surviving seeds, emerged seedlings and surviving seedlings due to the true count nature of these variables. These rounded numbers were used as input data in subsequent path analysis (below).

Path analysis

We performed structural equation models (SEMs) to assess the direct and indirect effects of lynx presence and the habitat and microsite of arrival on different *P. bourgaeana* life-cycle stages, from seed deposition by mesocarnivores until is established as a new plant, based on our previous knowledge of the study system (confirmatory path analysis; Shipley 2000). Path analysis is based on a structured system of nested relationships among responses and predictors that is composed by individual linear regression equations (Grace 2006). We used the `piecewiseSEM` R package (Lefcheck 2016) because it allows to run generalized lineal mixed models (GLMM) with non-gaussian errors from the `lme4` R package (Bates et al. 2020).

In these models, we specified the lynx presence/absence, type of habitat (forest and open) and type of microsite (rock, shrub and open) as exogenous categorical variables. The following response variables were specified as endogenous: number of i) mesocarnivore scats, ii) dispersed seeds, iii) post-dispersal surviving seeds, iv) emerged seedlings and v) 1st year surviving seedlings. To obtain path coefficients for categorical predictors, we also run the models by codifying them as exogenous ordinal variables: lynx (0 = absence, 1 = presence), habitat (0 = open, 1 = forest), microsite (0 = open, 1 = rock, 2 = shrub). Poisson errors and the random effects 'Locality' and 'Fruiting season' were used for all GLMMs. Since relationships can vary among mesocarnivore species, we proposed separately SEMs for the red fox and the stone marten and then also for both species jointly. Since stone marten barely dispersed pear seeds in lynx presence scenarios ($n = 2$), we did not include seed predation, germination and survival for the single-species modelling. Interaction between lynx and habitat type was statistically significant ($P < 0.05$) for the seed dispersal model with both mesocarnivores jointly and only with the red fox (see Table S1), thus we split up the full datasets into two subsets by lynx presence and absence, in order to determine if the path coefficients varied among predation risk scenarios.

SEMs were evaluated using tests of directed separation (d-sep; Shipley 2009). A path model was considered consistent when the C statistic is not significantly different from a $\chi 2$ distribution ($P > 0.05$). We used standardised path coefficients to compare direct effects of different scale relationships. We also calculated indirect and total effects to assess the total relative importance of each exogenous variable on seedling survival (Shipley 2000). Indirect effects were calculated as the product of all direct effects (coefficients directly linking predictor and response) along the paths linking predictors and seedling survival through at least one intermediate variable. Total effects were the sum of direct and indirect effects. We calculated marginal and conditional pseudo-R^2 to estimate the explained variance of each individual model.

Results

Overall patterns

We found 1400 and 175 scats of red fox and stone marten, respectively. The 64% and 91% of fox and marten scats were collected outside lynx territories, respectively. The 11% and 9% of scats contained Iberian pear seeds for the red fox and the stone marten, respectively. Foxes and martens dispersed a total amount of 1219 and 60 pear seeds, of which 84% and 97% occurred in lynx absence scenarios respectively. The red fox dispersed significantly less seeds inside than outside lynx territories, particularly in open habitats (Coeff. ± SE) (0.10 ± 1.15 vs. -0.32 ± 1.16; $P < 0.05$; Table S1 and Fig. 1). The stone marten mobilised significantly less seeds also in open habitats (-2.98 ± 1.48 vs. -5.00 ± 1.52; $P < 0.05$), but independently of the predation risk scenario (Table S1). Considering both mesocarnivores jointly, we found the same results that only for the red fox (Table S1). The most common seed-arrival microsite was the ‘open’ type ($P < 0.05$, Table S1), but in lynx absence scenarios foxes also selected positively the microsite ‘shrub’ ($P < 0.05$, Table S1 and Fig. 1). Rodents predated on 30% of dispensed pear seeds in the post-dispersal seed predation experiment. The seed predation rate was 0.35 ± 0.44 and 0.26 ± 0.40 (Mean ± SD) for lynx presence and lynx absence scenarios, respectively. Rodents predated seeds mainly in the ‘shrub’ microsite type (shrub: -0.42 ± 0.54, rock: -1.64 ± 0.55, open: -2.63 ± 0.56; $P < 0.05$), independently of the type of habitat and lynx scenario (see Table S1 and Fig. 1). The 85% of sow seeds emerged seedlings. Emergence rates were consistent across habitats and microsites (Table S1; $P > 0.05$). A considerable fraction (21%) of emerged seedlings survived the whole 1st year around. Seedling survival was significantly higher in open habitats (Fig. 1), especially in the microsite ‘shrub’ (shrub: -0.40 ± 0.39, rock: -2.14 ± 0.52, open: -1.48 ± 0.45; $P < 0.05$).

Global path analysis

A lower amount of fox and stone marten scats lead to significantly less number of *P. bourgaeana* dispersed seeds under lynx presence, which translated into lesser surviving seeds, lesser emerged seedlings, and ultimately lesser surviving seedlings (Fig. 2 and S4; Tables S2-S4). In the case of stone marten, only 2 seeds were dispersed in lynx presence which indicates the collapse of its contribution of seed-dispersal for the Iberian pear. Forest habitats were mostly selected by the stone marten for faecal-marking, and therefore also to disperse *P. bourgaeana* seeds (Fig. S3A). The coverage degree of the seed-arrival microsite (the lowest was ‘open’, intermediate was ‘rock’ and the highest was ‘shrub’ type) negatively affected to the number of scats of both mesocarnivore species (Fig. 2A). Shrub microsite showed lower number of surviving seeds and emerging seedlings, in comparison to more open microsites (Fig. 2A). No direct lynx effect was found on post-dispersal seed predation by rodents (Fig. 2A). Lynx indirect effects accounted up to 36% of the variation in seedling survival respect to other exogenous variables (Fig. 4). The type of habitat and microsite of seed dispersal explained a 43% and 21% of the overall variation in seedling survival (Fig. 4).

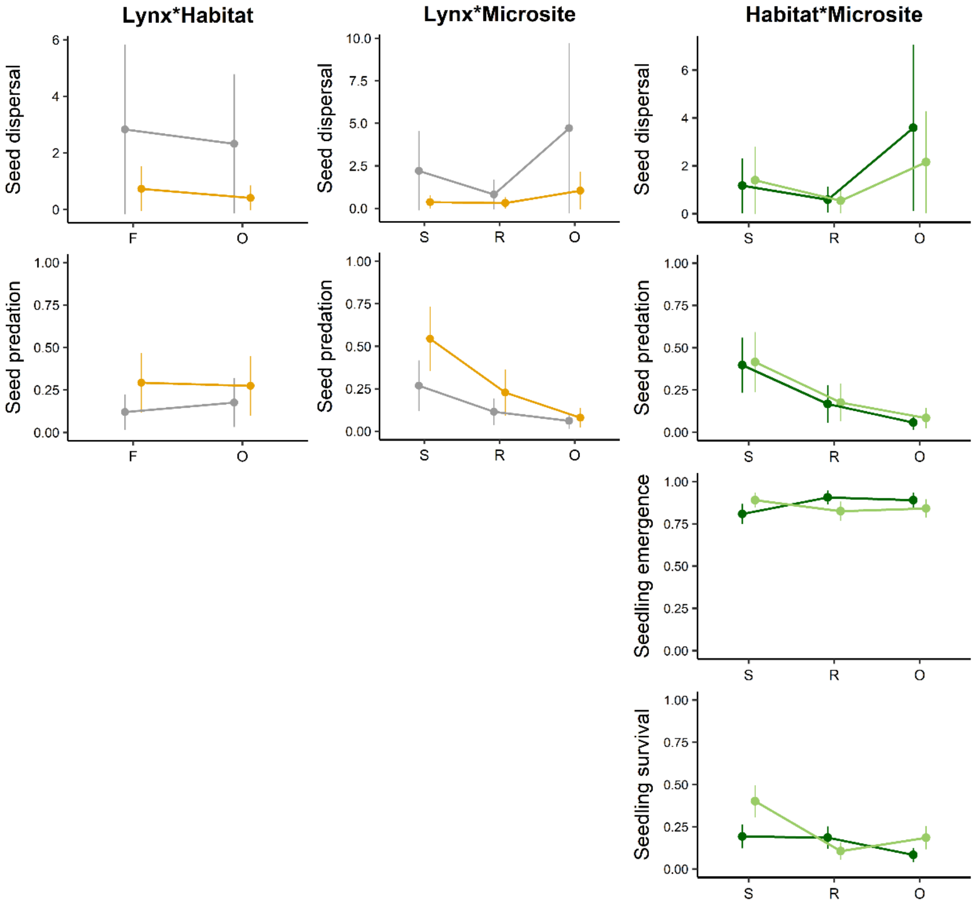

Figure 1. Number of seeds dispersed by mesocarnivores (*V. vulpes* and *M. foina* jointly), seed predation probability by rodents, and emergence and survival probabilities of Iberian pear (*P. bourgaeana*) seedlings. Circles and bars denote back-transformed means and delta standard errors estimated by GLMMs for each single field experiment, with Poisson distribution (log link) for seed dispersal and binomial distribution (logit link) for the rest of models (see Appendix S1: Table S1). Panels for Lynx*Habitat and Lynx*Microsite interactions show lynx absence (grey) and lynx presence (orange) scenarios. Panel for Habitat*Microsite interaction show forest (dark green) and open (light green) habitat types. Abbreviations: Habitat (F = forest, O = open); Microsite (S = shrub, R = rock, O = open).

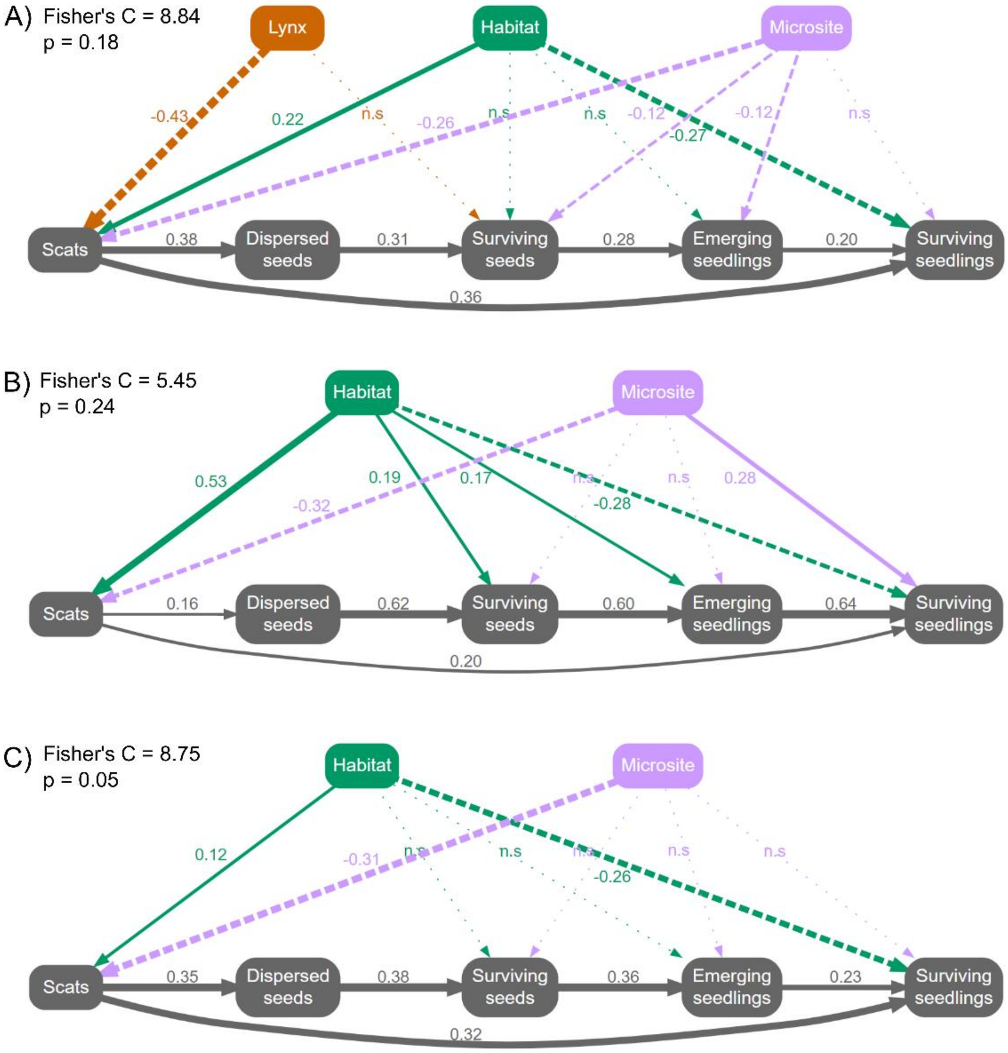

Figure 2. Global (A), lynx presence (B) and lynx absence (C) path analysis of causal relationships between exogenous variables (lynx presence, habitat and microsite) and the vital phases of the life-cycle of an Iberian pear (*P. bourgaeana*) considering seeds dispersed by the red fox (*V. vulpes*) and the stone marten (*M. foina*) jointly. Exogenous variables were included as ordinal: lynx (0 = absence, 1 = presence), habitat (0 = open, 1 = forest), microsite (0 = open, 1 = rock, 2 = shrub). Arrow widths are proportional to the value of standardised path coefficients. Solid arrows show significant and positive direct effects, dashed arrows significant and negative effects and dotted arrows non-significant effects (p > 0.05).

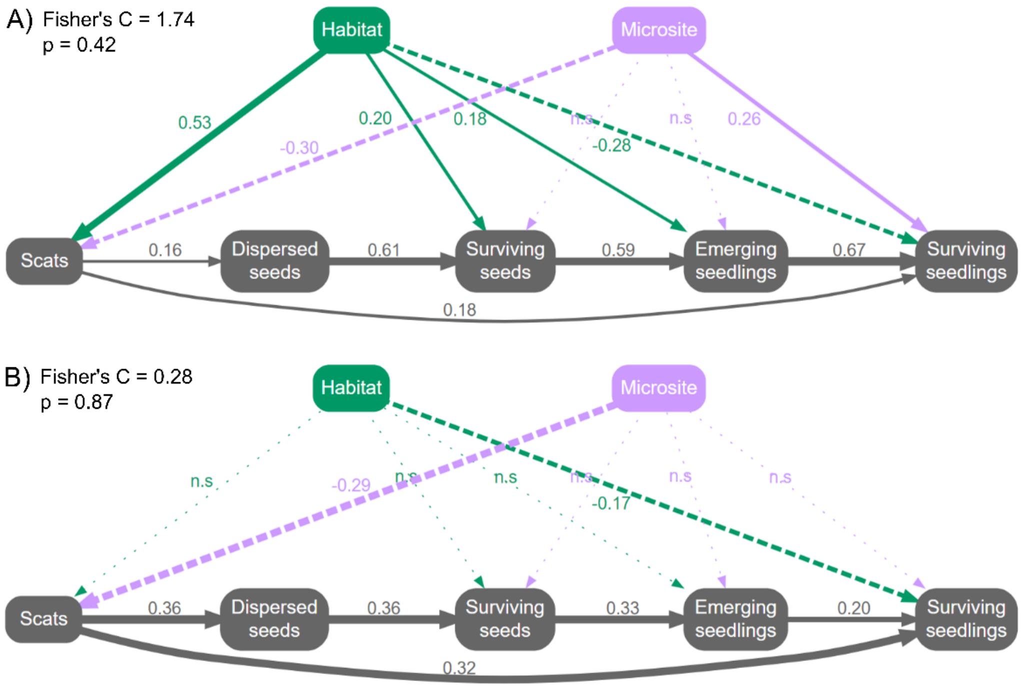

Figure 3. Path analysis of lynx presence (A) and lynx absence (B) scenarios of causal relationships between exogenous variables (habitat and microsite) and the vital phases of the life-cycle of an Iberian pear (*P. bourgaeana*) considering seeds dispersed by the red fox (*V. vulpes*). Exogenous variables were included as ordinal: habitat (0 = open, 1 = forest), microsite (0 = open, 1 = rock, 2 = shrub). Arrow widths are proportional to the value of standardised path coefficients. Solid arrows show significant and positive direct effects, dashed arrows significant and negative effects and dotted arrows non-significant effects ($p > 0.05$).

Path analysis by predation risk scenario

The habitat and microsite type where seeds landed strongly interacted with the presence of lynx in seed dispersal models, especially for fox-dispersed seeds (Table S1); thus, we obtained coefficients separately for lynx presence and absence scenarios (Table S5-S8; Fig 2BC). A higher proportion of fox scats deposited in forest habitats in the presence of the apex predator, led to more dispersed seeds in comparison with open habitats (Fig. 1B and 3A). We found a subtle habitat effect (Std. coeff. = 0.12; $p = 0.02$) on faecal-marking in the lynx absence scenario considering both carnivores jointly (Fig. 2C), in contrast with the non-significant effect on fox model (Fig. 3B). Open habitats favoured seedling survival in both predation risk scenarios directly (Fig. 3 and 5), but resulted in opposite indirect effects due to the larger fox-seed dispersal in forest habitats under lynx presence (Fig. S4). We also found a differential direct effect of the type of microsite on seedling survival between predation risk scenarios (Fig. 2BC and 3). In lynx presence, coverage degree at microsite level highly favoured seedling survival (Fig. 4), in spite of faecal-marking occurred mostly in open microsites (Fig. 5). Meanwhile in lynx absence, we did not found differences between microsites in seedling survival (Fig. 2BC and 3). In absence of lynx, foxes dispersed seeds mostly to open and also shrub microsites (Fig. 5). Habitat of seed arrival explained a 45% and 72% of the variation in seedling survival in lynx presence and absence scenarios, respectively (Fig. 4). The microsite of seed dispersal was the most important effect in lynx presence scenarios (Fig. 4).

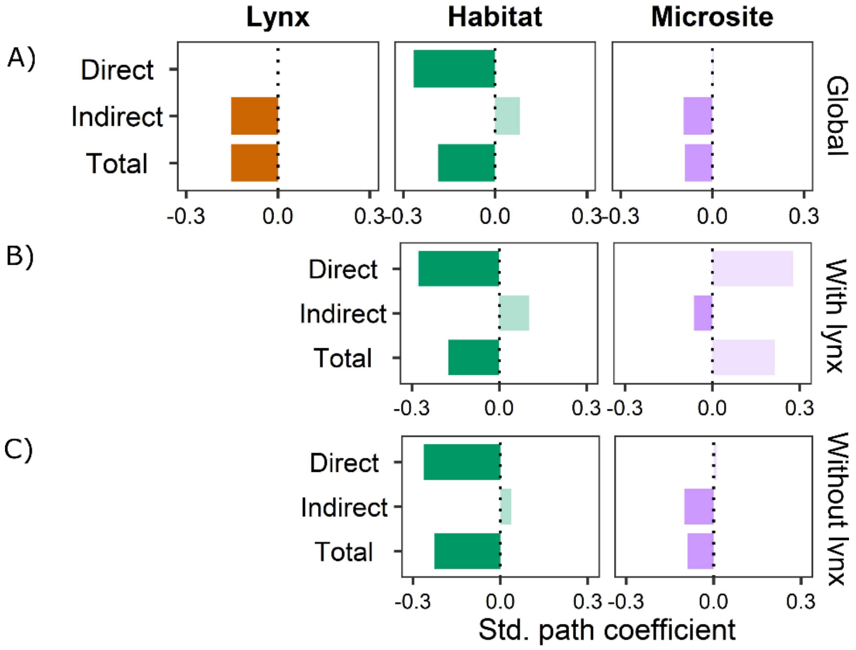

Figure 4. Direct, indirect and total effects of lynx, habitat and microsite on seedling survival from the global SEM (A), the lynx presence SEM (B), and the lynx absence SEM (C) considering Iberian pear seeds dispersed by the red fox (*V. vulpes*) and the stone marten (*M. foina*) jointly. Direct effects are the standardised path coefficients directly linking seedling survival to the predictor variables. Indirect effects are the product of direct paths linking seedling survival to predictors through at least one intermediate variable. Total effects are the sum of direct and indirect effects for each predictor.

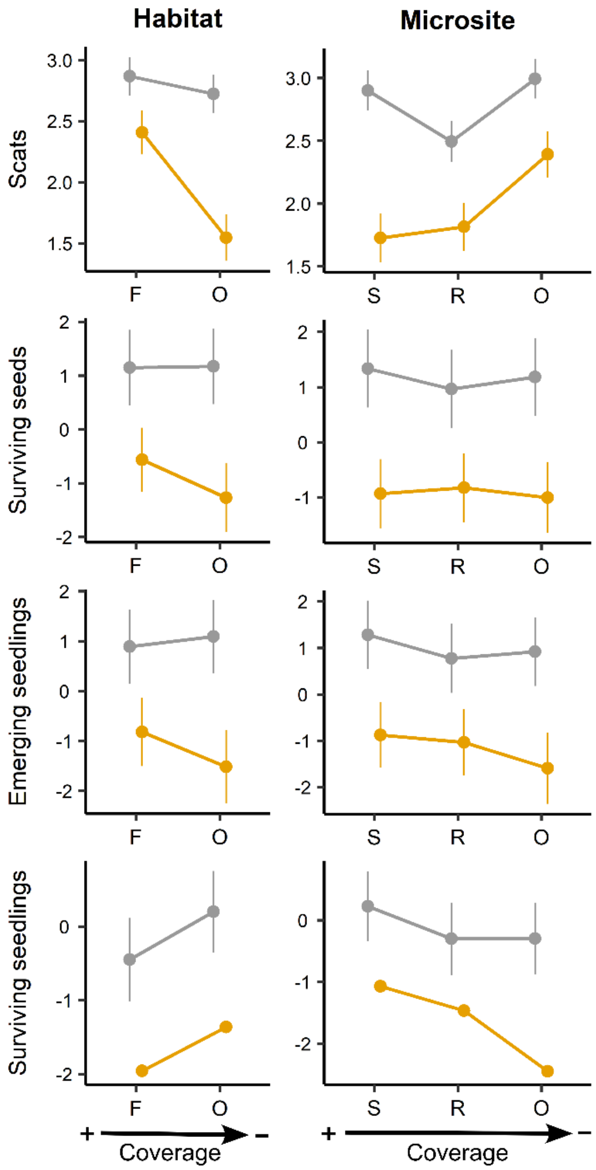

Figure 5. Linear predictions of the number of mesocarnivore scats (*V. vulpes* and *M. foina* jointly), surviving seeds, emerging seedlings and surviving seedlings of Iberian pear (*P. bourgaeana*) by habitat (F: forest, O: open) and microhabitat type (S: shrub, R: rock, O: open) in each predation risk scenario (grey refers to lynx absence and orange to lynx presence scenarios). The circles are the marginal means after Tukey post-hoc tests for path analysis models by predation risk scenario. Bars denote the respective delta standard errors (see Appendix S1: Tables S5-S8). The arrows represent how the coverage decreases from forest to open habitats and from shrub to open microsites.

Discussion

Although previous studies have documented how anthropic disturbances on carnivore communities can cascade down to primary producers (Beschta and Ripple 2012, Cancio et al. 2016, Fedriani et al. 2020a), our research is pioneer on revealing cascading effects on multiple key vital processes for plants until they are established as seedlings. Through the combination of field experiments across the different life-cycle stages of a seed and structural equations modelling, we demonstrated for the first time that a *rewilding* scenario can trigger trophic cascades altering tree establishment. According our predictions, subordinate carnivores living in a landscape of fear created by the Iberian lynx proportionally dispersed less seeds in open and high-risky habitats, leading to less seedling recruitment and ultimately less establishment. However, contrary to our expectations, open habitats favoured seedling survival especially in shrubs, despite this microsite which had the highest post-dispersal seed predation rates by rodents. These findings highlight that, in the current global change scenario, is important to investigate the cascading impacts of apex predators rewilding across whole food webs (Estes and Duggins 1995, Wilmers et al. 2012).

Mesopredator suppression effects on seed dispersal

The Iberian pear is mostly dispersed by medium-sized mammalian carnivores in Mediterranean ecosystems lacking of other frugivore megafauna (Fedriani and Delibes 2009a), whose abundance is often limited by larger predators such as the Iberian lynx (Jiménez et al. 2019, Burgos et al. 2023). Mesocarnivores mobilised a great number of pear seeds outside lynx territories, but mesopredator suppression and the creation of a landscape of fear caused the collapse of the seed-dispersal service provided by martens in areas inhabited by lynx. In the case of seed dispersal by the red fox, it was reduced in lynx presence, but foxes still continued dispersing Iberian pear seeds. Such results are in line with previous research that situated the stone marten as the most sensitive species to predation risk in scenarios of apex predator restoration (Virgós et al. 2020, Burgos et al. 2023).

The spatial segregation respect to top-order predators is driven by behaviourally-mediated trophic cascades, leading subordinate carnivores to avoid costly encounters by altering their habitat-use and foraging behaviour (Schmitz et al. 1997, Fedriani et al. 2000). Iberian pear trees often grow up in open landscapes lacking of forest coverage, resulting in risky areas for mesocarnivores. Accordingly, Burgos et al. (2022) found that the visitation rate to fruiting pear trees by foxes decreased in presence of the apex predator and several previous research have found similar outcomes for other fearful frugivorous species (Fedriani and Boulay 2006, Carreira et al. 2020, Selwyn et al. 2020). In this line, path analysis revealed a negative effect of open habitats on the faecal-marking rate of foxes when co-existed with the Iberian lynx, indirectly altering the overall seed-rain patterns (see Fig. 3). Foxes mobilised less seeds in open and highly exposed habitats under predation risk as we predicted, what could affect qualitatively the seed-dispersal effectiveness (Schupp et al. 2010).

Additionally, we found that the faecal patterns of mesocarnivores were biased to open microsites, a common territorial-marking behaviour of carnivores (Martínez et al. 2008, Fedriani and Delibes 2009a, Suárez-Esteban et al. 2013). Although selection of microsites could vary respect to their availability, García-Cervigón et al. (2018) showed that the proportion of dispersed seeds by carnivores to vacant microsites can be twice higher than expected according to their availability. However, foxes intensified their faecal-marking over or beneath shrubs outside lynx territories, probably due to they selected more conspicuous microsites to place their scats in open habitats where scent-marking increased due to the risk-free scenario (Gosling and Roberts 2001). Plant recruitment processes have been traditionally more related to seed rain rather than microsite limitations (García et al. 2005a, b). However, the seed-arrival microsite has been demonstrated to be an important factor determining plant demography attributes (Escribano-Ávila et al. 2013, González-Varo et al. 2014, Donoso et al. 2016). Microhabitat selection by frugivores frequently depends on landscape structure (García-Cervigón et al. 2018) and varies temporal and spatially (Fedriani and Delibes 2009a, Suárez-Esteban et al. 2013), but studying the most common deposition sites is paramount to know the local environmental conditions which could affect plant life stages after seed dispersal.

Post-dispersal seed predation in a landscape of fear

Post-dispersal seed predation is often affected by the relative abundance or the activity of seed predators (Fedriani and Manzaneda 2005, Garrote et al. 2019). This fact could explain the high removal rates found in our experiment (~30%) respect to previous studies with *P. bourgaeana* and other large seeded species (1-10%; Dirzo et al. 2007, Suárez-Esteban et al. 2018). Although no direct causal effect of the Iberian lynx presence was found on seed removal, we could assign the subtle higher proportion of removed seeds in lynx territories (57%) to the increased relative abundance of seed predators found in previous studies (Pender et al. 2013, Burgos et al. 2023). Hence, the reduced predation pressure driven by mesopredator suppression can release the small prey abundance in a context of apex predator restoration (Miller et al. 2012, Jiménez et al.

2019), with potential consequences for seed survival and ultimately for plant establishment. Similar subtle increases in seed predation have been documented in defaunated tropical ecosystems, attributable to lower competition (Wright et al. 2000, Galetti et al. 2015), and in other predator-free systems (Maron and Pearson 2011). Nevertheless, the experimental nature of this study allowed us to demonstrate for the first time how cascading effects can alter at the same time mutualistic and antagonistic plant-animal interactions with a strong impact on plant dynamics.

Differences in seed predation rates among habitats have been frequently related to ecological preferences of seed predators (Diaz et al. 1999, García et al. 2011). Accordingly, we found evidence from large-scale habitat effects in our seed predation experiment, but only in lynx presence scenarios. The positive effect of forest habitats on the abundance of remaining seeds (i.e. non-predated), in comparison to open landscapes, seemed to be an indirect consequence of the intensive faecal-marking of foxes in forests under predation risk. These results support the existence of strong context-dependency on post-dispersal processes and highlight the importance of well-replicated local and regional studies (Fedriani and Manzaneda 2005, Suárez-Esteban et al. 2018, Garrote et al. 2019).

Defleshed seeds found in frugivore depositions are usually vulnerable to seed predators such as rodents (Fedriani and Delibes 2011, 2013; but see Enders and Vander Wall 2012). Nonetheless, seed predation often vary among microsites (Hulme 1994). For instance, Rey et al. (2002) found that in a Mediterranean forest the preferred seed species was mostly consumed by rodents under shrubs, as we found for *P. bourgaeana* in our study. Vegetation cover often decreases the perceived predation risk by small prey such as rodents (Rosalino et al. 2011, Loggins et al. 2019) and hence can favour seed predation (Rey et al. 2002, Matías et al. 2009). Mesocarnivores mainly dispersed seeds to open microsites which kept low seed-predation rates, similarly to other Mediterranean plants dispersed by mammal carnivores (e.g. *C. album* and *J. phoenicea;* Suárez-Esteban et al. 2018). Although by depositing dispersed seeds in open microsites carnivores assist escape from seed predators (Fedriani and Delibes 2009a), the low quality of open microsites for seedling survival in Mediterranean ecosystems may not to compensate this fitness advantage.

Trophic cascades triggered by apex predators

The loss of key mutualistic interactions in plant-frugivore networks can trigger trophic cascades across the different vital phases of plants (Traveset et al. 2012, Rogers et al. 2021). Thus, the functional loss of the dispersal service that the stone marten provides to *P. bourgaeana* could ultimately compromise the persistence and spreading of this scarce tree in lynx restoration scenarios. Fedriani et al. (2020) demonstrated that defaunation scenarios affecting key seed dispersers, such as foxes and badgers, altered the demography of *P. bourgaeana*, by limiting seed arrival to vacant habitats (e.g. old fields). Thus, although we found that foxes continued dispersing seeds under predation risk, subtle functional differences among frugivores (e.g. habitat-use patterns or dispersal distances; Jordano et al. 2007, Escribano-Ávila et al. 2014) can be crucial for post seed-dispersal stages. Lynx presence determined that *P. bourgaeana* seeds mostly reached less favourable habitats for recruitment (i.e. forests; Fig 5), contrary our expectations that forest habitats would provide more suitable conditions for seedling survival. However, this biased seed dispersal towards forests explains the indirect positive effect of forest habitat on seedling survival respect to open habitats. This finding highlights the importance of trophic cascades for the most limiting stage on the early life-cycle of *P. bourgaeana,* the first summer survival (Fedriani and Delibes 2009b). This fleshy-fruited tree is often growing in open landscapes not only due to a mammal-dispersal syndrome (Fedriani and Delibes 2009b, Fedriani et al. 2010), but also to this habitat type provides more suitable conditions for plant establishment. Thus, the impact of post-dispersal processes are expected to spatially vary within population in a landscape of fear, being highest where recruitment is seed limited by the presence of an apex predator (Calviño-Cancela 2007).

Seed–seedling conflicts can be also relevant on plant establishment processes in a trophic cascade context (Schupp 1995, Garrote et al. 2022b). For instance, local environmental conditions may favour seed survival and emergence after seed dispersal but disfavour at the same time seedling survival, as we found in our study. The combination of a reduced seed rain in open habitats and a high rate of seed predation in the seed-arrival microsite most favourable for seedling survival (i.e. beneath shrubs in open habitats) may also contribute to reduce the potential of *P. bourgaeana* to expand beyond its current distribution (Fedriani et al. 2018, 2020a) in a top-predator restoration context. Variation in the seed rain occurred at different spatial scales (habitat and microsite), but the low spatial scale seemed to be especially important for seedling survival in presence of an apex predator. Seed arrival to nurse plants often increases plant recruitment (Gómez-Aparicio et al. 2004), due to reduced insolation and herbivory (Mendoza et al. 2009, Perea et al. 2020). However, habitat and microsite suitability can vary in space but also in time, for example according to climate change or neighboring plants (Schupp et al. 2010, García-Cervigón et al. 2018, Garrote et al. 2022b). Thus, in spite of lynx may be currently limiting the establishment of certain plant species, predicting cascading impacts in long-term at community level may be challenging, given the variation on habitat requirements among plant species and the differential habitat use by seed dispersers (Maron and Pearson 2011, Donoso et al. 2017, Fedriani et al. 2020a).

Although the potential of compensation by other seed-disperser species (e.g. *Cyanopica cooki*) seems to be limited for our study plant species mainly dispersed by carnivores (Fedriani et al. 2020a), other key seed-dispersers whose abundance is not limited by the Iberian lynx (e.g. badger) could partially compensate the reduced seed dispersal service in areas with lynxes. Several plants highly dispersed by carnivores in our study area, are also mobilised by birds, as is the case of *A. unedo* or *Juniperus sp.* (Herrera 1998, Santos et al. 1999, García 2001)*,* but these groups of seed dispersers show important functional differences, in the same way that occurs among mesocarnivore species (González-Varo et al. 2015, Fedriani et al. 2020a). In this line, Escribano-Ávila et al. (2014) found that foxes and martens played a key role in colonization processes for *Juniperus thurifera*, due to the major seed dispersal away from the maternal surrounding, in contrast with the local seed-dispersal of thrushes. Thus, since frugivores differ in subtle but key aspects of the seed dispersal process (Escribano-Ávila et al. 2013, Fedriani et al. 2020a), the potential for intra-guild compensation to ameliorate the loss of a certain species is variable and difficult to predict.

Concluding remarks

Our findings highlight the need for integrating cascading effects on plant-dynamics, especially in the current context of global change. Considering habitat-specific seed dispersal when assessing the quality of the ecosystem service that frugivores provide to plants is crucial to evaluate the potential effects of ecological disturbances across heterogeneous landscapes. Our results indicate that relatively subtle behavioural differences among seed dispersers can lead to substantial functional variations, paramount to predict the impact of the frugivore loss on plant demography dynamics. Reintroduction programs of apex predators in defaunated ecosystems lacking of frugivore megafauna should consider trophic cascades as powerful ecological mechanisms that can alter mutualistic and antagonistic interactions across food webs in contrasting ways.

Supporting information – Chapter 4

Cascading effects triggered by apex predators can ultimately affect plant recruitment: a study case on a Mediterranean fruit tree

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Unpublished

Appendix S1. Tables

Table S1. Chi-square scores and significance of predictor variables (., $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) of generalized linear mixed models performed for each experiment separately. Poisson errors (log link) were used for the number seeds dispersed and binomial errors (logit link) for seed predation, seedling emergence and seedling survival rates. The variance of the models explained by the fixed effects is indicated by the marginal pseudo-R^2 (R^2_m), and the variance explained by both the fixed and random effects is indicated by the conditional pseudo-R^2 (R^2_c). Hyphen symbol refers to non-relevant variables in such models.

Response	Lynx	Habitat	Microsite	Lynx*Habitat	Lynx*Microsite	Habitat*Microsite	R^2_m	R^2_c
Seed dispersal (overall)	1.52	**33.09*** **	**290.51*** **	**5.46* **	**11.25** **	**29.04*** **	0.19	0.98
Seed dispersal (*V. vulpes*)	0.83	**20.54*** **	**263.37*** **	**7.63** **	**16.44*** **	**27.72*** **	0.15	0.99
Seed dispersal (*M. foina*)	3.11 .	**25.34*** **	**9.05** **	-	-	**-**	0.39	0.97
Seed predation	0.97	0.07	**20.24** **	0.08	2.90	0.56	0.14	0.95
Seedling emergence	-	0.63	2.88	-	-	3.72	0.18	0.51
Seedling survival	-	2.36	3.09	-	**-**	**6.01* **	0.38	0.70

Table S2. Standardized coefficients, standard errors and significance values of global SEM. Lynx (Absence, Presence), Habitat (Forest, Open) and Microsite (Shrub, Rock, Open) were included as categorical variables. Poisson errors were used. Fisher's C = 3.55, df = 4, p = 0.47, n = 120. R^2_m and R^2_c were 0.54 and 0.78 for the number of scats, 0.16 and 0.98 for the number of seeds, 0.22 and 0.89 for the number of surviving seeds, 0.12 and 0.88 for emerging seedlings and 0.09 and 0.26 for surviving seedlings, respectively.

Response	Predictor	Estimate	SE	p	Std. Estimate
Scats	Lynx	-	-	**0***	-
Scats	Lynx = Presence	2.07	0.14	**0***	-
Scats	Lynx = Absence	2.78	0.13	**0***	-
Scats	Habitat	-	-	**0***	-
Scats	Habitat = Open	2.25	0.10	**0***	-
Scats	Habitat = Close	2.62	0.10	**0***	-
Scats	Microsite	-	-	**0***	-
Scats	Microsite = Rock	2.17	0.11	**0***	-
Scats	Microsite = Shrub	2.43	0.11	**0***	-
Scats	Microsite = Open	2.70	0.10	**0***	-
Dispersed seeds	Scats	0.07	0.01	**0***	0.38
Surviving seeds	Dispersed seeds	0.02	0.00	**0***	-
Surviving seeds	Lynx	-	-	0.32	-
Surviving seeds	Lynx = Presence	0.36	0.65	0.56	-
Surviving seeds	Lynx = Absence	1.13	0.63	0.07	-
Surviving seeds	Habitat	-	-	0.25	-
Surviving seeds	Habitat = Open	0.71	0.51	0.17	-
Surviving seeds	Habitat = Close	0.80	0.51	0.12	-
Surviving seeds	Microsite	-	-	**0***	-
Surviving seeds	Microsite = Rock	0.47	0.52	0.37	-
Surviving seeds	Microsite = Shrub	0.84	0.51	0.10	-
Surviving seeds	Microsite = Open	0.93	0.51	0.07	-

Emerging seedlings	Surviving seeds	0.02	0.00	**0*** **	-
Emerging seedlings	Habitat	-	-	0.77	-
Emerging seedlings	Habitat = Close	0.60	0.55	0.27	-
Emerging seedlings	Habitat = Open	0.63	0.55	0.25	-
Emerging seedlings	Microsite	-	-	**0*** **	-
Emerging seedlings	Microsite = Rock	0.30	0.55	0.59	-
Emerging seedlings	Microsite = Shrub	0.77	0.55	0.16	-
Emerging seedlings	Microsite = Open	0.78	0.55	0.16	-
Surviving seedlings	Scats	0.03	0.01	**0.01***	-
Surviving seedlings	Emerging seedlings	0.01	0.00	**0*** **	-
Surviving seedlings	Habitat	-	-	**0.002****	-
Surviving seedlings	Habitat = Close	-0.92	0.44	**0.03***	-
Surviving seedlings	Habitat = Open	-0.33	0.43	0.44	-
Surviving seedlings	Microsite	-	-	0.11	-
Surviving seedlings	Microsite = Rock	-0.80	0.46	0.08	-
Surviving seedlings	Microsite = Open	-0.75	0.45	0.09	-
Surviving seedlings	Microsite = Shrub	-0.33	0.44	0.45	-

Table S3. Standardized coefficients, standard errors and significance values of red fox (*V. vulpes*) global SEM. Lynx (Absence, Presence), Habitat (Forest, Open) and Microsite (Shrub, Rock, Open) were included as categorical variables. Poisson errors were used. Fisher's C = 5.59, df = 6, p = 0.47, n = 120. R^2_m and R^2_c were 0.39 and 0.75 for the number of scats, 0.16 and 0.98 for the number of seeds, 0.18 and 0.90 for the number of surviving seeds, 0.12 and 0.89 for emerging seedlings and 0.09 and 0.25 for surviving seedlings, respectively.

Response	Predictor	Estimate	SE	p	Std. Estimate
Scats	Lynx	-	-	**0.01***	-
Scats	Lynx = Presence	2.05	0.16	**0*****	-
Scats	Lynx = Absence	2.59	0.16	**0*****	-
Scats	Habitat	-	-	**0*****	-
Scats	Habitat = Open	2.19	0.11	**0*****	-
Scats	Habitat = Close	2.45	0.11	**0*****	-
Scats	Microsite	-	-	**0*****	-
Scats	Microsite = Rock	2.02	0.12	**0*****	-
Scats	Microsite = Shrub	2.39	0.12	**0*****	-
Scats	Microsite = Open	2.55	0.11	**0*****	-
Dispersed seeds	Scats	0.08	0.01	**0*****	0.39
Surviving seeds	Dispersed seeds	0.01	0.01	**0*****	-
Surviving seeds	Lynx	-	-	0.52	-
Surviving seeds	Lynx = Presence	0.33	0.69	0.62	-
Surviving seeds	Lynx = Absence	0.87	0.67	0.19	-
Surviving seeds	Habitat	-	-	0.51	-
Surviving seeds	Habitat = Open	0.58	0.53	0.28	-
Surviving seeds	Habitat = Close	0.62	0.53	0.24	-
Surviving seeds	Microsite	-	-	**0.001****	-
Surviving seeds	Microsite = Rock	0.32	0.54	0.54	-
Surviving seeds	Microsite = Open	0.72	0.54	0.17	-
Surviving seeds	Microsite = Shrub	0.75	0.54	0.16	-

Emerging seedlings	Surviving seeds	0.01	0.01	**0*****	-
Emerging seedlings	Habitat	-	-	0.66	-
Emerging seedlings	Habitat = Close	0.43	0.57	0.45	-
Emerging seedlings	Habitat = Open	0.46	0.57	0.41	-
Emerging seedlings	Microsite	-	-	**0*****	-
Emerging seedlings	Microsite = Rock	0.09	0.58	0.86	-
Emerging seedlings	Microsite = Open	0.57	0.57	0.31	-
Emerging seedlings	Microsite = Shrub	0.66	0.57	0.24	-
Surviving seedlings	Scats	0.02	0.01	0.05	-
Surviving seedlings	Emerging seedlings	0.01	0.01	**0*****	-
Surviving seedlings	Habitat	-	-	**0.007****	-
Surviving seedlings	Habitat = Close	-1.01	0.43	**0.01***	-
Surviving seedlings	Habitat = Open	-0.51	0.42	0.22	-
Surviving seedlings	Microsite	-	-	**0.02***	-
Surviving seedlings	Microsite = Rock	-1.00	0.46	**0.03***	-
Surviving seedlings	Microsite = Open	-0.93	0.44	**0.03***	-
Surviving seedlings	Microsite = Shrub	-0.35	0.43	0.41	-

Table S4. Standardized coefficients, standard errors and significance values of stone marten (*M. foina*) global SEM. Lynx (Absence, Presence), Habitat (Forest, Open) and Microsite (Shrub, Rock, Open) were included as categorical variables. Poisson errors were used. Fisher's C = 3.25, df = 4, p = 0.52, n = 120. R^2_m and R^2_c were 0.48 and 0.55 for the number of scats and 0.04 and 0.41 for the number of seeds, respectively.

Response	Predictor	Estimate	SE	p	Std. Estimate
Scats	Lynx	-	-	**0*****	-
Scats	Lynx = Presence	-1.95	0.40	**0*****	-
Scats	Lynx = Absence	0.36	0.30	0.23	-
Scats	Habitat	-	-	**0*****	-
Scats	Habitat = Open	-1.56	0.31	**0*****	-
Scats	Habitat = Close	-0.02	0.26	0.92	-
Scats	Microsite	-	-	**0*****	-
Scats	Microsite = Rock	-1.56	0.33	**0*****	-
Scats	Microsite = Shrub	-0.67	0.29	**0.02***	-
Scats	Microsite = Open	-0.13	0.27	0.62	-
Dispersed seeds	Scats	0.25	0.03	**0*****	0.35

Table S5. Standardized coefficients, standard errors and significance values of lynx presence SEM. Habitat (Forest, Open) and Microsite (Shrub, Rock, Open) were included as categorical variables. Poisson errors were used. Fisher's C = 5.77, df = 4, p = 0.21, n = 60. R^2_m and R^2_c were 0.53 and 0.77 for the number of scats, 0.13 and 0.96 for the number of seeds, 0.45 and 0.86 for the number of surviving seeds, 0.42 and 0.90 for emerging seedlings and 0.08 and 0.10 for surviving seedlings, respectively.

Response	Predictor	Estimate	SE	p	Std. Estimate
Scats	Habitat	-	-	**0*****	-
Scats	Habitat = Open	1.55	0.19	**0*****	-
Scats	Habitat = Close	2.41	0.18	**0*****	-
Scats	Microsite	-	-	**0*****	-
Scats	Microsite = Shrub	1.73	0.19	**0*****	-
Scats	Microsite = Rock	1.81	0.19	**0*****	-
Scats	Microsite = Open	2.39	0.18	**0*****	-
Dispersed seeds	Scats	0.09	0.01	**0*****	0.16
Surviving seeds	Dispersed seeds	0.15	0.01	**0*****	-
Surviving seeds	Habitat	-	-	**0.005****	-
Surviving seeds	Habitat = Open	-1.27	0.64	0.05	-
Surviving seeds	Habitat = Close	-0.57	0.59	0.34	-
Surviving seeds	Microsite	-	-	0.85	-
Surviving seeds	Microsite = Open	-1.00	0.64	0.12	-
Surviving seeds	Microsite = Shrub	-0.93	0.62	0.14	-
Surviving seeds	Microsite = Rock	-0.82	0.62	0.19	-
Emerging seedlings	Surviving seeds	0.22	0.02	**0*****	-
Emerging seedlings	Habitat	-	-	**0.01***	-
Emerging seedlings	Habitat = Open	-1.52	0.73	**0.03***	-
Emerging seedlings	Habitat = Close	-0.82	0.69	0.23	-
Emerging seedlings	Microsite	-	-	0.14	-
Emerging seedlings	Microsite = Open	-1.59	0.76	**0.03***	-

Emerging seedlings	Microsite = Rock	-1.03	0.71	0.15	-
Emerging seedlings	Microsite = Shrub	-0.88	0.70	0.21	-
Surviving seedlings	Scats	0.03	0.01	**0*****	-
Surviving seedlings	Emerging seedlings	0.17	0.01	**0*****	-
Surviving seedlings	Habitat	-	-	**0*****	-
Surviving seedlings	Habitat = Close	-1.96	0.05	**0*****	-
Surviving seedlings	Habitat = Open	-1.36	0.05	**0*****	-
Surviving seedlings	Microsite	-	-	**0*****	-
Surviving seedlings	Microsite = Open	-2.44	0.05	**0*****	-
Surviving seedlings	Microsite = Rock	-1.46	0.05	**0*****	-
Surviving seedlings	Microsite = Shrub	-1.07	0.05	**0*****	-

Table S6. Standardized coefficients, standard errors and significance values of lynx absence SEM. Habitat (Forest, Open) and Microsite (Shrub, Rock, Open) were included as categorical variables. Poisson errors were used. Fisher's C = 5.25, df = 2, p = 0.08, n = 60. R^2_m and R^2_c were 0.26 and 0.72 for the number of scats, 0.14 and 0.99 for the number of seeds, 0.20 and 0.93 for the number of surviving seeds, 0.19 and 0.93 for emerging seedlings and 0.22 and 0.50 for surviving seedlings, respectively.

Response	Predictor	Estimate	SE	p	Std. Estimate
Scats	Habitat	-	-	**0.01***	-
Scats	Habitat = Open	2.72	0.16	**0*****	-
Scats	Habitat = Close	2.87	0.16	**0*****	-
Scats	Microsite	-	-	**0*****	-
Scats	Microsite = Rock	2.49	0.16	**0*****	-
Scats	Microsite = Shrub	2.90	0.16	**0*****	-
Scats	Microsite = Open	2.99	0.16	**0*****	-
Dispersed seeds	Scats	0.08	0.01	**0*****	0.35
Surviving seeds	Dispersed seeds	0.02	0.00	**0*****	-
Surviving seeds	Habitat	-	-	0.75	-
Surviving seeds	Habitat = Close	1.15	0.70	0.10	-
Surviving seeds	Habitat = Open	0.17	0.70	0.09	-
Surviving seeds	Microsite	-	-	**0.01***	-
Surviving seeds	Microsite = Rock	0.96	0.71	0.17	-
Surviving seeds	Microsite = Open	1.18	0.70	0.09	-
Surviving seeds	Microsite = Shrub	1.33	0.70	0.06	-
Emerging seedlings	Surviving seeds	0.02	0.00	**0*****	-
Emerging seedlings	Habitat	-	-	**0.02***	-
Emerging seedlings	Habitat = Close	0.89	0.74	0.23	-
Emerging seedlings	Habitat = Open	1.09	0.74	0.14	-
Emerging seedlings	Microsite	-	-	**0*****	-
Emerging seedlings	Microsite = Rock	0.77	0.74	0.29	-

Emerging seedlings	Microsite = Open	0.92	0.74	0.22	-
Emerging seedlings	Microsite = Shrub	1.28	0.74	0.08	-
Surviving seedlings	Scats	0.02	0.01	0.09	-
Surviving seedlings	Emerging seedlings	0.01	0.00	**0*** **	-
Surviving seedlings	Habitat	-	-	**0.002** **	-
Surviving seedlings	Habitat = Close	-0.45	0.56	0.42	-
Surviving seedlings	Habitat = Open	0.20	0.55	0.72	-
Surviving seedlings	Microsite	-	-	0.11	-
Surviving seedlings	Microsite = Rock	-0.30	0.59	0.61	-
Surviving seedlings	Microsite = Open	-0.29	0.58	0.61	-
Surviving seedlings	Microsite = Shrub	0.22	0.57	0.69	-

Table S7. Standardized coefficients, standard errors and significance values of red fox (*V. vulpes*) lynx presence SEM. Habitat (Forest, Open) and Microsite (Shrub, Rock, Open) were included as categorical variables. Poisson errors were used. Fisher's C = 1.72, df = 2, p = 0.42, n = 60. R^2_m and R^2_c were 0.53 and 0.76 for the number of scats, 0.13 and 0.96 for the number of seeds, 0.44 and 0.86 for the number of surviving seeds, 0.41 and 0.89 for emerging seedlings and 0.08 and 0.11 for surviving seedlings, respectively.

Response	Predictor	Estimate	SE	p	Std. Estimate
Scats	Habitat	-	-	**0*****	-
Scats	Habitat = Open	1.52	0.18	**0*****	-
Scats	Habitat = Close	2.38	0.17	**0*****	-
Scats	Microsite	-	-	**0*****	-
Scats	Microsite = Rock	1.72	0.18	**0*****	-
Scats	Microsite = Shrub	1.79	0.18	**0*****	-
Scats	Microsite = Open	2.34	0.17	**0*****	-
Dispersed seeds	Scats	0.08	0.011	**0*****	0.16
Surviving seeds	Dispersed seeds	0.15	0.01	**0*****	-
Surviving seeds	Habitat	-	-	**0.004****	-
Surviving seeds	Habitat = Open	-1.25	0.64	0.05	-
Surviving seeds	Habitat = Close	-0.53	0.59	0.37	-
Surviving seeds	Microsite	-	-	0.52	-
Surviving seeds	Microsite = Rock	-1.04	0.64	0.10	-
Surviving seeds	Microsite = Open	-0.93	0.62	0.13	-
Surviving seeds	Microsite = Shrub	-0.69	0.6	0.26	-
Emerging seedlings	Surviving seeds	0.20	0.02	**0*****	-
Emerging seedlings	Habitat	-	-	**0.008****	-
Emerging seedlings	Habitat = Close	-1.47	0.71	**0.03***	-
Emerging seedlings	Habitat = Open	-0.76	0.66	0.24	-
Emerging seedlings	Microsite	-	-	0.36	-
Emerging seedlings	Microsite = Rock	-1.35	0.72	0.05	-

Emerging seedlings	Microsite = Open	-1.15	0.70	0.10	-
Emerging seedlings	Microsite = Shrub	-0.85	0.68	0.20	-
Surviving seedlings	Scats	0.02	0.01	**0*****	-
Surviving seedlings	Emerging seedlings	0.17	0.01	**0*****	-
Surviving seedlings	Habitat	-	-	**0*****	-
Surviving seedlings	Habitat = Close	-1.94	0.05	**0*****	-
Surviving seedlings	Habitat = Open	-1.37	0.05	**0*****	-
Surviving seedlings	Microsite	-	-	**0*****	-
Surviving seedlings	Microsite = Rock	-2.38	0.05	**0*****	-
Surviving seedlings	Microsite = Open	-1.49	0.05	**0*****	-
Surviving seedlings	Microsite = Shrub	-1.09	0.05	**0*****	-

Table S8. Standardized coefficients, standard errors and significance values of red fox (*V. vulpes*) lynx absence SEM. Habitat (Forest, Open) and Microsite (Shrub, Rock, Open) were included as categorical variables. Poisson errors were used. Fisher's C = 1.68, df = 2, p = 0.43, n = 60. R^2_m and R^2_c were 0.24 and 0.76 for the number of scats, 0.15 and 0.99 for the number of seeds, 0.19 and 0.94 for the number of surviving seeds, 0.17 and 0.94 for emerging seedlings and 0.20 and 0.52 for surviving seedlings, respectively.

Response	Predictor	Estimate	SE	p	Std. Estimate
Scats	Habitat	-	-	0.28	-
Scats	Habitat = Open	2.55	0.18	**0*****	-
Scats	Habitat = Close	2.62	0.18	**0*****	-
Scats	Microsite	-	-	**0*****	-
Scats	Microsite = Rock	2.22	0.19	**0*****	-
Scats	Microsite = Shrub	2.73	0.18	**0*****	-
Scats	Microsite = Open	2.80	0.18	**0*****	-
Dispersed seeds	Scats	0.08	0.01	**0*****	0.36
Surviving seeds	Dispersed seeds	0.01	0.01	**0*****	-
Surviving seeds	Habitat	-	-	0.45	-
Surviving seeds	Habitat = Open	0.88	0.76	0.25	-
Surviving seeds	Habitat = Close	0.94	0.76	0.22	-
Surviving seeds	Microsite	-	-	**0*****	-
Surviving seeds	Microsite = Rock	0.69	0.77	0.36	-
Surviving seeds	Microsite = Open	0.87	0.77	0.25	-
Surviving seeds	Microsite = Shrub	1.15	0.76	0.13	-
Emerging seedlings	Surviving seeds	0.02	0.01	**0*****	-
Emerging seedlings	Habitat	-	-	**0.02***	-
Emerging seedlings	Habitat = Close	0.58	0.82	0.47	-
Emerging seedlings	Habitat = Open	0.79	0.82	0.33	-
Emerging seedlings	Microsite	-	-	**0*****	-
Emerging seedlings	Microsite = Rock	0.44	0.83	0.59	-

Emerging seedlings	Microsite = Open	0.56	0.83	0.49	-
Emerging seedlings	Microsite = Shrub	1.05	0.82	0.20	-
Surviving seedlings	Scats	0.01	0.01	0.37	-
Surviving seedlings	Emerging seedlings	0.01	0.00	**0***	-
Surviving seedlings	Habitat	-	-	**0.008****	-
Surviving seedlings	Habitat = Close	-0.67	0.62	0.27	-
Surviving seedlings	Habitat = Open	-0.12	0.61	0.83	-
Surviving seedlings	Microsite	-	-	**0.03***	-
Surviving seedlings	Microsite = Rock	-0.66	0.66	0.31	-
Surviving seedlings	Microsite = Open	-0.65	0.65	0.31	-
Surviving seedlings	Microsite = Shrub	0.11	0.63	0.85	-

Appendix S2. Figures

Figure S1. (a-f) Photographs illustrating the landscape structure of forest and open habitat types selected for this study. (a-b) Show the panoramic landscape perspective of a forest transect, (c-d) show the landscape transition between forest and open habitats and (e-f) illustrate transects along open landscapes lack of arboreal vegetation except isolated individuals or clusters of Iberian pear (*Pyrus bourgaeana*) or oaks (*Quercus sp.*). (g-h) Examples of Iberian pear seeds dispersed by mesocarnivores, (h) one of these had germinated inside of a red fox scat. (i) Seed manipulation under lens at the laboratory. (j-o) Post-dispersal seed predation experiment: (j) Example of manual extraction of pear seeds from a wild Iberian pear fruit; (k) Field work to install Petri dishes with pear seeds for small mammals; (l-m) Predated Iberian pear seeds by rodents in our experiment; (n) Natural predation of Iberian pear seeds by rodents found in the wild; (o) Wood mouse (*A. sylvaticus)* predating pear seeds in our experiment (image taken by camera-trapping). (p-r) Seedling emergence and survival experiment: (p) Pear seeds sowed in our experiment; (q) Iberian pear seedlings in the microsite ‘shrub’, under a rosemary (*R. officinalis*); (r) 1st year Iberian pear seedlings that survived at the end of the experiment.


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Figure S2. Photographs of the post-dispersal seed predation experiment by small mammals. (a-b) Installation of camera-traps (Scoutguard SG562-C; white led) in front of the Petri dishes with Iberian pear seeds dispensed during the experiment. Camera-traps were placed at the height of 20–60 cm above the ground and 1.5-m apart from the Petri dish, operated 24 h/day and were programmed with a trigger delay of 1 s, to take three consecutive images. Camera-traps worked during 5 consecutive days coinciding with the seed-predation experiment. (c-d) Images taken by camera-trapping of wood mouse (*A. sylvaticus*) eating Iberian pear seeds in our experiment. The 100% of seed predation events recorded by camera-trapping were assigned to rodent species (*A. sylvaticus* and *M. spretus*).

Figure S3. Global path models (A) for the stone marten (*M. foina*) and (B) for the red fox (*V. vulpes*) of causal relationships between exogenous variables (lynx presence, habitat and microsite) and the vital phases of the life-cycle of an Iberian pear (*P. bourgaeana*) until seed dispersal for the stone marten and seedling survival for the red fox. Exogenous variables were included as ordinal: lynx (0 = absence, 1 = presence), habitat (0 = open, 1 = forest), microsite (0 = open, 1 = rock, 2 = shrub). Arrow widths are proportional to the value of standardised path coefficients. Solid arrows show significant and positive direct effects, dashed arrows significant and negative effects and dotted arrows non-significant effects (p > 0.05). The global model A (Fisher's C = 4.02, df = 4, p = 0.40, n = 120) and B (Fisher's C = 5.28, df = 6, p = 0.51, n = 120) fitted the data.

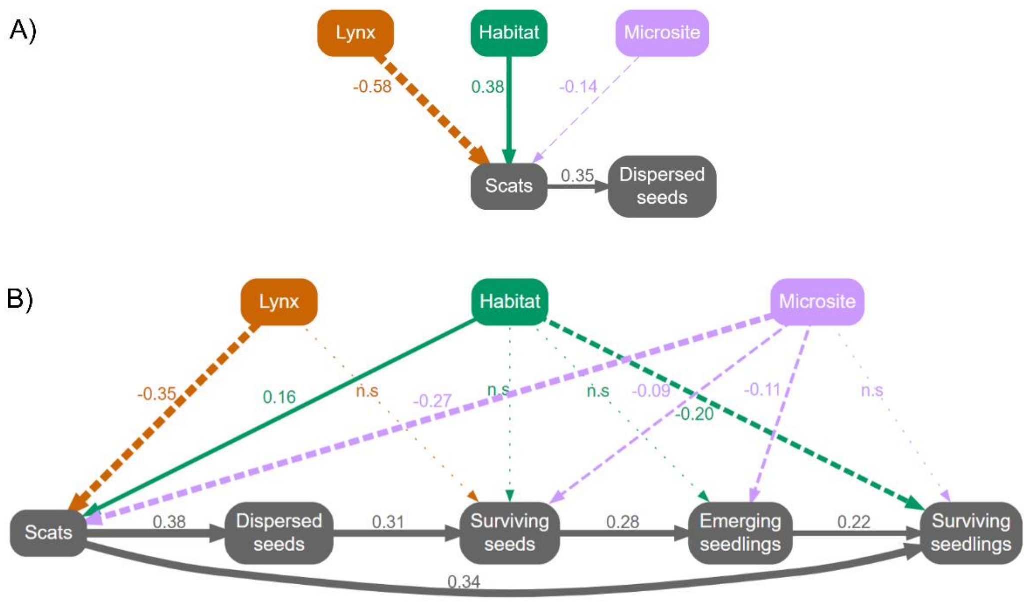
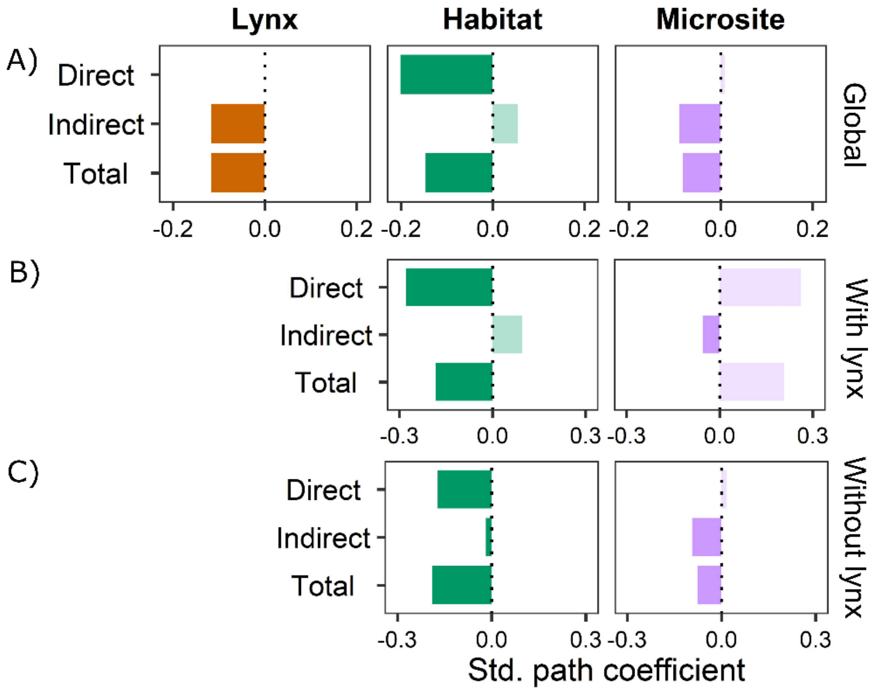


Figure S4. Direct, indirect and total effects of lynx, habitat and microsite on seedling survival from the global SEM (A), the lynx presence SEM (B), and the lynx absence SEM (C) considering Iberian pear seeds dispersed only by the red fox (*V. vulpes*). Direct effects are the standardised path coefficients directly linking seedling survival to the predictor variables. Indirect effects are the product of direct paths linking seedling survival to predictors through at least one intermediate variable. Total effects are the sum of direct and indirect effects for each predictor.



General discussion

In this thesis, we have investigated the multi-trophic interactions among an apex predator, several frugivorous carnivores and the community of fleshy-fruited plants in Mediterranean ecosystems of Southern Spain. Although previous research has determined how human-mediated disturbances on carnivore communities can cascade down to primary producers (Ripple and Beschta 2012a, Cancio et al. 2016, Fedriani et al. 2020a), this study is pioneer on revealing cascading effects triggered by apex predators on key multiple vital processes for plants simultaneously, from seed dispersal to seedling recruitment and establishment. Thus, through the combination of diverse analytical techniques and field experiments, we have demonstrated for the first time that a top-predator *rewilding* scenario can trigger trophic cascades on the recruitment of a Mediterranean large-fruited plant (*P. bourgaeana*), whose seed dispersal is mainly driven by mammal carnivores in defaunated ecosystems. Therefore, this thesis supports our predictions that the Iberian lynx can reduce mesopredator abundances and alter their behaviour, affecting a key plant-animal mutualism. This top-down direct effect results in negative indirect effects on the number of dispersed seeds as well as the diversity of dispersed species. However, the trophic cascade triggered by the Iberian lynx on the quality of the seed dispersal service provided by mesocarnivores can result in species-specific net effects for plant establishment.

Specifically, in chapter 1, we showed how two sympatric mesopredators (*M. foina* and *G. genetta*) living in a landscape of fear decreased in abundance, as result of the top-down control exerted by the Iberian lynx in a natural experiment. Nevertheless, the suppression strength and the spatio-temporal avoidance strategies developed by subordinate predators markedly varied among species, which led to contrasting ecological implications for seed dispersal. Bottom-up effects contributed to favour the coexistence among the Iberian lynx and the common genet, owing the high availability of the main prey of the latter in lynx territories. However, the stone marten practically disappeared from lynx range. In chapter 2, we demonstrated that frugivorous carnivores modified their foraging behaviour in response to predation risk, turning into less efficient seed dispersers for *P. bourgaeana*, a mammal-dispersed tree whose spreading opportunities could be limited by the loss or sharp reduction of their main seed dispersers. In chapter 3, we evaluated the quantitative contribution of frugivorous carnivores to seed rain of the entire community of Mediterranean fleshy-fruited plants among habitats and predation risk scenarios. We found a lower plant diversity was dispersed and proportionally a reduced seed dispersal in open and high-risky habitats under predation risk, owing the lower overall abundance of frugivorous carnivores. Finally, in chapter 4, we showed how the seed limitation in scenarios of lynx presence translated into lower seedling recruitment and establishment for a Mediterranean tree mainly dispersed by frugivorous carnivores. However, contrary to our expectations, open habitats favoured seedling survival for *P. bourgaeana*, especially when seeds were mobilised beneath shrubs. Furthermore, this seed-arrival site (shrubs) showed the highest post-dispersal seed predation rates by rodents. Thus, our outcomes highlight the importance of investigating the cascading impacts of reintroducing apex predators across the whole food web, considering the current global change scenario (Estes and Duggins 1995, Wilmers et al. 2012).

Apex predators as top-down regulators in trophic webs

The loss of apex predators as consequence of local extinctions worldwide has revealed extensive cascading effects at different levels of the ecosystems (Schmitz 2006, Estes et al. 2011, Ripple et al. 2014a). In the Iberian Peninsula, the successive rewilding actions involving the restoration of the Iberian lynx populations have saved this endangered species from extinction, providing glimmers of hope for large carnivore conservation (Simón et al. 2012). However, the recovery of this feline species in our study area is recent and still spatially heterogeneous, primarily due to the patchy distribution of its main prey across the Natural Park, the wild rabbit. The human-assisted spread of epizootic diseases affecting rabbit populations worldwide, is still one of the main conservation concerns for dietary specialist carnivores such as the Iberian lynx, limiting their range and abundance (Ferrer and Negro 2004, Monterroso et al. 2016).

Where the Iberian lynx was present, the abundance of most frugivorous mesopredators inhabiting the study area drastically decreased, except the European badger (see Chapter 3: Appendix S2, Table S4; Palomares et al. 1996, Jiménez et al. 2019). Badger abundance even increased inside lynx territories meanwhile the red fox and the stone marten, reduced their relative abundance more than 5 and 50 times respectively, due to the stone marten disappeared from several lynx territories. In terms of population density (i.e. individuals by surface), we only obtained abundance estimates based on capture-recapture for the stone marten and the common genet, due to the rest of mesocarnivore species were hardly individually identifiable (Chapter 1). We found that the Iberian lynx led these two sympatric mesopredators to decrease the average population size to very low levels (0.04 and 0.15 individuals per km^2, respectively). Similar mesopredator suppression has been found by several apex predators across the world, such as the grey wolf, the dingo or the Eurasian lynx (Ritchie et al. 2012, Newsome et al. 2017). However, the Iberian lynx has been demonstrated to be an exemplary adversary for mesopredators, spending a great effort looking actively for competitors and killing them whenever is possible (Palomares et al. 1996, Fedriani et al. 1999, Jiménez et al. 2019).

The restoration of this top-down regulation in large carnivore *rewilding* scenarios may considerably differ in strength among subordinate species. Despite the similar dietary habits and habitat-usage patterns of the community of mesopredators, genets showed a much lower sensitivity in terms of abundance in the presence of the apex predator, in comparison to other sympatric species such as the red fox or the stone marten (see Chapter 3: Appendix S2, Table S4). Although ecologically similar species could be expected to develop analogous strategies to cope with apex predators in a context of high prey availability (Karanth et al. 2017, Monterroso et al. 2020), we found the stone marten and the genet showed different coexistence patterns with lynx. Martens spatially avoided the apex predator and genets temporally, but spatial encounters only occurred among genets and lynx in hot-spots of high rodent availability (Chapter 1), as would be predicted by the optimal foraging theory (MacArthur and Pianka 1966). A possible explanation could be related to subordinate species use different behavioural mechanisms in order to avoid agonistic encounters or develop tolerance to predation risk. For example, both genets and martens are able to climb trees easily to escape from a lynx, but the perception of predation risk may variable among species and be related to habitat preferences and the presence of refuges (Santos-Reis et al. 2004, Finke and Denno 2006). Nevertheless, fine-scale and long-term monitoring studies on habitat usage patterns of the entire carnivore community are still insufficient for a comprehensive understanding of species-specific anti-predatory responses, especially in highly diverse ecosystems such as Mediterranean forests.

Similarly to previous research found for other prey species (Meserve et al. 2003, Gordon et al. 2015, Jiménez et al. 2019), a reduced overall predation pressure inside lynx territories seemed to cause a small mammal release (i.e. rabbits and rodents), increasing their abundance as we predicted in Chapter 1 (see Appendix S3, Table S3). Specifically, the garden dormouse (*Eliomys quercinus*) reached a remarkable peak of abundance in areas inhabited by the apex predator, being more than 8 times higher than outside lynx range. Therefore, as we expected for a micromammal specialized consumer (Virgós et al. 1999, López-Martín 2006), genets synchronized their activity patterns with rodents, probably because they offer energetically richer food intake than other resources, such as fruits (Santos et al. 2020, Vilella et al. 2020). In contrast, mesopredators with a wider trophic niche breadth were not forced to co-exist with lynx because were able to feed on a broader spectrum of food resources outside lynx territories. However, martens overlapped temporally with rodents but only inside lynx territories, when the availability of this prey was maximum because the ending of hibernation of the garden dormouse (Moreno 1988). Thus, considering the described differential top-down effect among mesopredator species, the recovery of apex predators in areas where had disappeared, probably has contrasting indirect impacts on lower trophic levels.

Since *rewilding* scenarios of large carnivores (e.g. wolves, bears, lynx) have gaining importance due to the threatened state of carnivore populations worldwide (Ripple et al. 2014a, Wolf and Ripple 2018), trophic web science is essential to assess how apex predator recovery can restore the ecosystem functioning prior to their extinction. The still heterogeneous spatial distribution of the Iberian lynx across the Iberian Peninsula (MITECO 2023) may be paramount to create a variable landscape of fear where apex predators, mesopredators and prey have variable local abundances and complementary ecological roles (Laundré et al. 2014, Gaynor et al. 2019). Therefore, conservation practitioners should consider to promote a locally patchy distribution of apex predators, where a variable landscape of fear may result in balanced community net effects at regional scale. This knowledge will drive more effective conservation strategies of carnivores, by taking evidence-based decisions and sowing the seed for future research. Although our study is mainly focused on the community of frugivorous carnivores, considerations prior reintroductions of apex predators should be also taken for the whole carnivore guild and the wide spectrum of ecosystem services that they provide (Hoeks et al. 2020, Marneweck et al. 2022).

Mesocarnivores represent a key guild in top-down and bottom-up regulation processes in trophic webs (Meserve et al. 2003, Brashares et al. 2010, Tambling et al. 2018). Despite small carnivores are globally widespread, still suppose an unknown group of mammals with scarce representation in research (Bencatel et al. 2018). For instance, the European wild cat (*Felis silvestris*) or the polecat (*Mustela putorius*) populations are probably decreasing in the Iberian Peninsula, but monitoring programs are insufficient to know their true conservation status. These dietary-strict carnivores feed mainly on small mammal prey species in the Mediterranean basin (Moleón and Gil-Sánchez 2003, Santos et al. 2009), thus the increase of rabbit's and rodent's abundance following the recovery of the Iberian lynx, could potentially favour the coexistence of these species, as we found for the common genet. Furthermore, the invasion of species as the racoon (*Procyon lotor*) or the recent natural arrival of the golden jackal (*Canis aereus*) in the Iberian Peninsula supposes the addition of one more mesopredator species to the extant community (Krofel et al. 2017, Valdez et al. 2022). Thus, similar potential top-down effects and coexistence patterns to those described in Chapter 1 are expected. For instance, since golden jackal shows highly trophic overlap with the red fox, especially in habitats with high availability of rodents (Tsunoda 2022), it would be expected that competition for this abundant food resource decreased inside Iberian lynx territories. Nevertheless, further studies are necessary to know the impact of apex predators on novel or sensible species whose population trend is uncertain and thus, reintroduction programs should manage the priorities of conservation relative to each species.

Predation risk drives more fearful frugivores

Join to the top-down suppression in abundance, apex predators can promote a landscape of fear where elusive subordinate species also alter their spatial-usage and foraging patterns (Ripple and Beschta 2004, Carreira et al. 2020, Selwyn et al. 2020). Frugivorous mesocarnivores search for fruits actively because these are a predictable and valuable resource, mostly during the autumn and winter in Mediterranean forests (Herrera 1989, Rosalino et al. 2010). However, predation risk led to less frequent visits of mesocarnivores to *P. bourgaeana* trees (see Chapter 2), in line with previous research suggesting subordinate frugivores avoid fruit producing trees in presence of larger predators (Garrote et al. 2018, Carreira et al. 2020). Behaviourally-mediated trophic cascades can also vary among species (Schmitz et al. 2000, 2004), leading to contrasting alterations in the foraging behaviour of fearful frugivores, and ultimately affecting frugivory and seed dispersal patterns (Carreira et al. 2020). In this line, we found a lack of visitation and fruit consumption of stone martens to *P. bourgaeana* trees inside lynx territories, thus promoting the virtual extinction of the seed dispersal service provided by this mesocarnivore in lynx *rewilding* scenarios (only 2 *P. bourgaeana* seed dispersal events; Chapter 3). Conversely, we found badgers visited 57% more and consumed 43% more fruits inside lynx territories, accordingly to the lack of sensitivity also found in the abundance estimates (Fedriani et al., 1999). In a middle point, foxes reduced visitation rates and fruit consumption in *P. bourgaeana* trees placed inside lynx territories (57% and 38% less visits and fruits, respectively). The smaller size of martens and foxes relative to the badger could lead them to be easily predated by a bigger competitor (Virgós et al. 2020), which may explain their low abundance and then, their low frugivory rates in areas inhabited by lynx. By last, no visits of genets were detected in fruiting pear trees either inside and outside lynx territories, which is probably due to their less frugivore diet and the rare use of deforested areas such as where *P. bourgaeana* grows up (Virgós et al. 1999, Santos-Reis et al. 2004).

Foxes and badgers avoided high-production trees in lynx presence (Chapter 2), behavioural alteration probably linked to the cost-benefit balance stated by the optimal foraging theory (Charnov 1976). Low-production patches could be safer for foraging in spite of food resources are potentially more challenging to obtain (Brown et al. 1999). However, in lynx absence scenarios, carnivores invested foraging efforts towards bigger cropsize trees, in accordance with other similar studies found respect fruit availability (Virgós et al. 2010, García et al. 2011, Selwyn et al. 2020). In addition, the stone marten seemed to specialize its diet under predation risk towards the preferred food resource (Chapter 3; 99% of seeds corresponded to *A. unedo*), similarly to Gazzola and Balestrieri (2020) found for this species in a scenario of intra-guild competition. Despite other fruiting species were even abundant (e.g. *J. oxycedrus* or *O. europaea* var. *sylvestris*) in lynx presence scenarios, the strawberry tree is a rich-sugar and protein fruit (Herrera 1987), highly available and predictable for frugivores under predation risk (see Chapter 3: Appendix S3, Fig. S2). Foraging in safe fruit patches, as the dense forests of strawberry tree, could be less risky for frugivores which live in a landscape of fear (Laundré et al. 2010). Accordingly, we found in Chapter 3 that the faecal-marking patterns of the stone marten were biased to forest refuge habitats with high abundance of *A. unedo* (Virgós et al. 2010, 2020). However, this potential dietary specialization may be a product of the low sample size of stone marten scats collected inside lynx territories (n = 15).

The contribution to effective seed dispersal (Schupp et al. 2010) for *P. bourgaeana* is practically limited to carnivore mammals due to their large body size, and because the rest of the assemblage of frugivores (e.g. ungulates or lagomorphs) act largely as seed predators or pulp feeders (Fedriani & Delibes, 2009b; Fedriani & Delibes, 2013). Some large bird species such as the Iberian magpie (*Cyanopica cooki*) could also contribute to seed dispersal of *P. bourgaeana,* but extremely low fruit consumption was found in our experiment for this group (~1%; see Chapter 2: Table S1). Accordingly, we found that the main disperser for this tree in our study area was the red fox, in spite of the reduced fruit consumption found in predation risk scenarios (Chapter 2). Foxes consumed 32% less *P. bourgaeana* fruits per time (i.e. lower efficiency) and their visits to pear trees were 45% shorter in time in lynx presence scenarios (Chapter 2), probably related to an anti-predatory response (Haswell et al. 2018, Carreira et al. 2020, Selwyn et al. 2020). Since *P. bourgaeana* trees often grow up in open landscapes lacking of forest coverage, the surrounding of fruiting trees represented risky areas where the perceived predation risk increases. Contrastingly, the badger is the most important disperser for this plant in other Mediterranean areas (e.g. Doñana National Park; Fedriani and Delibes 2009a), where *P. bourgaeana* dispersal services may not be influenced by lynx presence. However, seed dispersal patterns are different among frugivores (Jordano et al. 2007, Fedriani et al. 2010) and variable behavioural adaptations in the use of space can occur in response to the perceived predation risk (Schmitz et al. 1997, Ferreiro-Arias et al. 2021), thus the spatial distribution of *P. bourgaeana* could be altered in a *rewilding* scenario. Accordingly, we found that the red fox reduced the faecal-marking in open habitats when co-existed with the apex predator (Chapter 3 and 4), probably as consequence of an alteration in the use of space to avoid agonistic encounters with lynx in risky habitats. Thus, this habitat-use adaptability shown by the red fox, biased the seed-arrival patterns for *P. bourgaeana* and the rest of plant species dispersed by foxes under predation risk, with species-specific consequences for plant establishment (García-Cervigón et al. 2018).

Functional decay of the seed dispersal service

Our predictions that the quantity and the diversity of the seed dispersal could be altered due to the sudden presence of an apex predator in a *rewilding* scenario are confirmed in Chapter 3 and 4, especially for plants mostly dispersed by frugivorous carnivores, as is the case of large-fruited plants. Similarly to what happen in other defaunated ecosystems (Kurten 2013, Rogers et al. 2021), the overall lower abundance of frugivorous carnivores in lynx presence led to a drastic reduction in the quantitative component of seed dispersal effectiveness for the whole community of plant species consumed by this guild (49% lower seed rain). We found a reduced diversity of plant species dispersed by martens in lynx presence scenarios respect to lynx absence (~85% lower). The loss of species dispersed (e.g. *M. communis* or *J. oxycedrus*) by the stone marten under predation risk explains the high dissimilarity of the plant species assemblage (i.e. beta diversity) dispersed between predation risk scenarios. However, seed dispersal dissimilarity was lower for the red fox, mainly due to the replacement of plant species in its diet. That is, some plant species dispersed by foxes outside lynx territories were replaced by other in sites with lynx (e.g. *F. carica* and *R. ulmifolius*). Thus, the smaller abundance and array of frugivorous carnivores in lynx *rewilding* scenarios (e.g. lacking of stone martens; see Chapter 1), may promote the consumption and mobilisation of a less diverse assemblage of fruiting species (García and Martínez 2012), especially when no functional complementarity exists among seed dispersers (García-Rodríguez et al. 2022). This is especially important for the largest fruit species of mesocarnivores in our study area (*A. unedo* and *P. bourgaeana*), which may be the most affected by the trophic cascade triggered by lynx.

Large-fruited plants usually depend on the seed-dispersal service provided by large frugivores, mainly attributable to mesocarnivores in defaunated Mediterranean ecosystems (Fedriani and Delibes 2009a, Escribano-Ávila et al. 2013). Thus, the reduced diversity and quantity of seeds dispersed by mesocarnivores in presence of an apex predator, could determine the spatial distribution and genetic structure of large-fruited plant communities at landscape-scale (Choo et al. 2012, Traveset et al. 2014). For instance, Fedriani et al. (2020) demonstrated that defaunation scenarios affecting key mammalian seed dispersers, such as foxes and badgers, altered the demography of *P. bourgaeana*, by limiting seed arrival in open landscapes and thus, recolonization processes. It is true that other key large seed-dispersers whose abundance and behaviour are not affected by the presence of the Iberian lynx (e.g. badger), could potentially compensate the reduced seed dispersal service of foxes and martens for large-fruited plant communities, at least in a quantitative way. Nevertheless, functional diversity exists among mammalian seed dispersers, and thus the quality of the seed dispersal service is variable among species (González-Varo et al. 2013). For instance, food habits and seed dispersal patterns are variable among frugivorous carnivores, thus differential species-specific top-down regulation would suppose contrasting effects on the seed dispersal function. Fruit can represent up to 90% of the diet of the stone marten, contrasting with the more specialised diet of the common genet on micromammals (Virgós et al. 1999, López-Martín 2006). Moreover, foxes are important seed dispersers in old-field colonization processes, transporting seeds away from the maternal surrounding (Fedriani and Delibes 2009a), in contrast with the markedly clustered faecal-marking behaviour of badgers (Fedriani et al. 2020a). However, badgers are key seed dispersers in scrublands, meanwhile foxes contribute to natural restoration processes (Fedriani et al. 2018, 2020a, Isla et al. 2023). Thus, in case of fox defaunation scenarios were completely compensated with badgers, functional heterogeneity would be reduced and thus, response diversity within the plant-frugivore assemblage to ecosystem alterations (García et al. 2013, González-Varo et al. 2017). Since disperser species differ in subtle but key aspects of the seed dispersal process (Escribano-Ávila et al. 2013, Fedriani et al. 2020a), the potential for intra-guild compensation to ameliorate the loss of a certain seed disperser is variable and potentially difficult to predict.

Several plants highly dispersed by carnivores in our study area, are also dispersed by birds, as is the case of *A. unedo* or *Juniperus sp.* (Herrera 1998, Santos et al. 1999, García 2001). However, most frugivore bird species of Europe (e.g. *Erithacus rubecula* and *Sylvia* sp.) partially consume certain soft large fruits with small seeds (e.g. *A. unedo* or *F. carica)* but are unable to feed on the whole fruit, swallowing only some seeds with part of pulp content (Herrera 1984, Debussche and Isenmann 1989). Birds show also important functional differences respect to mammal carnivores, in the same way that occurs among carnivore species (González-Varo et al. 2015). Despite migratory birds can be important drivers of seeds towards cooler latitudes in the current climate change scenario (González-Varo et al. 2021), and dominant avian frugivores drive active seed dispersal in the colonization front of range expansion processes (Isla et al. 2023), seed dispersal by birds keeps frequently restricted to the maternal plant surrounding. In this line, Escribano-Ávila et al. (2014) showed that foxes and stone martens played a key role in land abandonment colonization processes for *Juniperus thurifera*, due to the major contribution of carnivores to disperse seeds away from the adult plants, in contrast with the local movements of thrushes. Thus, carnivores contribute to seed flow among contrasting type of habitats (González-Varo et al. 2015), where open or disturbed landscapes play a central role, meanwhile open landscapes lacking of tree vegetation are frequently avoided by frugivore birds (Garcia et al. 2010). Alternatively, top-order frugivorous carnivores could also compensate the decreased seed-dispersal service produced after apex predator reintroductions (Vidal et al. 2013, García-Rodríguez et al. 2022), but the current *rewilding* context for the Iberian lynx in Mediterranean ecosystems represents still a defaunation scenario, lacking in megafauna of seed dispersers such as the extinct brown bear (*Ursus arctos*). This type of potential compensation has been documented not only among native seed dispersers (Cordeiro and Howe 2003), but also by exotic and domestic species (García et al. 2014, Muñoz-Gallego et al. 2019), and even by humans (Spengler 2019). Several megafaunal-dispersed trees which still exist today have overcome extinction by implementing vegetative sprouting and relying on human seed dispersal (Van Zonneveld et al. 2018). Therefore, understanding the cascading effects of rewilding apex predators could be particularly relevant in defaunated ecosystems, where mesocarnivores maintain a key mutualism with fleshy-fruited plants, especially for those of large fruit size.

Seed dispersal effectiveness in a landscape of fear

Habitat and microhabitat selection by frugivores frequently depends on landscape structure and habitat preferences (García-Cervigón et al. 2018) and varies temporal and spatially (Fedriani and Delibes 2009a, Suárez-Esteban et al. 2013). Thus, studying the most common seed-arrival sites is paramount to know the local environmental conditions which could affect plant life stages after seed dispersal, especially in a seed dispersal limited scenario (Reid et al. 2021). Post-dispersal seed predation may act contrastingly among habitats and microsites (Hulme 1994), depending on the ecological preferences of seed predators (Diaz et al. 1999, García et al. 2011). In this line, we found that mesocarnivores mainly dispersed seeds to open microsites which kept low seed-predation rates, similarly to previous studies (Suárez-Esteban et al. 2018). However, open microsites in Mediterranean ecosystems are probably low-quality emplacements for seeds. Perea et al. (2020) showed that herbivory intensity by wild ungulates on plant recruits was highest in open microsites, contributing to decrease the likelihood of plant recruitment in spite of the low seed-predation rates. Vegetation cover often decreases the perceived predation risk by small prey such as rodents (Rosalino et al. 2011, Loggins et al. 2019) and hence can favour seed predation (Rey et al. 2002, Matías et al. 2009), as we found for *P. bourgaeana* seed predation by rodents beneath shrubs (Chapter 4).

Our results suggest that the refuge coverage is a more determinant factor at local level (i.e. microsite) than at landscape scale (i.e. habitat) for seed predation by rodents (Sanguinetti and Kitzberger 2010). Furthermore, post-dispersal seed predation often depends on the relative abundance or the activity of seed predators (Fedriani and Manzaneda 2005, Garrote et al. 2019). However, no direct causal effect of the Iberian lynx presence was found on *P. bourgaeana* seed removal rates by rodents in Chapter 4 (but see Meserve et al. 2003, Maron and Pearson 2011). This remarkable finding contrasts with the higher rodent abundance found in lynx presence scenarios in Chapter 1. The most plausible explanation is that the most abundant seed predator species in lynx presence scenarios (i.e. the garden dormouse) was hibernating during our seed predation experiment, and thus was inactive during the post-dispersal peak of our study plant species (Moreno 1988). Nevertheless, further research focusing on post-dispersal processes could shade light on how relevant seed predation may be to the regeneration dynamics of plants in a *rewilding* scenario of apex predators.

Apex predator *rewilding* scenarios are limiting the quantity of seeds dispersed for large-fruited plants, but could also be favouring those plant species more sensitive to insolation and water-stress (e.g. *A. unedo*, *V. tinus*) in early stages of the life-cycle, due to the biased mobilisation towards forest habitats (Santiso 2015, Tribulato et al. 2019). In Mediterranean ecosystems, where the shortage of water supply and herbivory pressure are often the most important filters for plant establishment (Mendoza et al. 2009, Perea et al. 2020), landing into forest habitats could affect positively seedling survival and ultimately enhance plant persistence, as we predicted in our hypotheses (Fedriani and Delibes 2009a, Fedriani et al. 2018). Thus, although carnivores are less efficient seed dispersers in a predation risk scenario, they could be moving seeds towards favourable habitats for the establishment of Mediterranean plants (Bustamante et al. 1992). However, the findings of Chapter 4 respect to the first year seedling survival of *P. bourgaeana* revealed opposite findings for this plant species. Open habitats provided the most suitable environmental conditions for the first summer survival of *P. bourgaeana* seedlings, especially beneath shrubs (Gómez-Aparicio et al. 2004). Thus, the role of transporting seeds to open landscapes played by the red fox may be key for the recruitment of this plant species, not only considering land abandonment colonization processes (Fedriani et al. 2018) but also apex predator *rewilding* scenarios.

Despite the overall low seedling survival of woody species in the limiting water-stressful and insolation conditions typical of Mediterranean ecosystems (Mendoza et al. 2009, Martínez-Baroja et al. 2022), we found *P. bourgaeana* seedling survival was even lower in lynx presence scenarios (1%) in comparison to lynx absence scenarios (8%). Thus, the combination of a reduced seed rain in open landscapes in lynx presence and a high level of seed predation in the seed-arrival microsite most favourable for seedling survival (i.e. beneath shrubs in open habitats) may contribute to reduce the potential of *P. bourgaeana* to expand beyond its current distribution in a top-predator *rewilding* context (Fedriani et al. 2018, 2020a). The Iberian pear is a pioneer plant species after land abandonment, which grows most often in substrates lacking or scarce of vegetation (Fedriani et al. 2010, Castro 2012). Therefore, the clumped distribution of adult *P. bourgaeana* trees in open landscapes detected in our study area (see Chapter 2) can be not only due to the mammal-generated seed rain, but also to the suitable environmental conditions that this habitat type provides for the establishment of this plant species. However, predicting cascading impacts in long-term at community level may be difficult given the variation on habitat requirements among plant species and the differential habitat use by the most important seed dispersers, especially in heterogeneous landscapes and highly diverse ecosystems (Maron and Pearson 2011, Donoso et al. 2017, Fedriani et al. 2020a).

Future challenges for trophic web ecology

The presence of the Iberian lynx may be limiting the colonization of certain plant species towards open landscapes, affecting plant-community structure and their potential of colonization in response to global change (Schleuning et al. 2020). However, apex predators could also contribute to maintain the valuable 'mosaic landscapes' in their natural distribution ranges (Curveira-Santos et al. 2017, Brotons et al. 2018). Biodiversity hot-spots are often linked to well-preserved forests, but recent research has pointed that the landscape heterogeneity including forests, grasslands or even managed open landscapes such as *Dehesas* in Mediterranean ecosystems, can be key for biodiversity (Moreno et al. 2016). Therefore, despite the reduced seed rain and diversity of plants dispersed by mesocarnivores inside lynx territories, the heterogeneous landscape of fear in scenarios of apex predator *rewilding* could ultimately cascade on more diverse communities of plants at regional scale. The exclusion of key frugivores such as foxes and martens from rodent-rich patches inhabited by lynx, could trigger a two-told effect on the ecosystem functioning by limiting quantitatively seed dispersal and plant establishment inside lynx territories, but boosting it outside. However, the human-favoured overpopulation current context of game ungulates in Mediterranean ecosystems of central and southern Spain supposes an extra limiting factor for plant recruitment (Pulido et al. 2010, Velamazán et al. 2020). Seedlings and saplings of several fleshy-fruited plant species are often highly damaged by ungulates in our study area due to their high palatability (e.g. *A. unedo*, *M. communis*), thus seed dispersal in dense tree cover patches as occurs in lynx presence scenarios, would facilitate seedling survival against herbivory (Perea and Gil 2014, Perea et al. 2020). In a wolf *rewilding* context, Ripple and Beschta (2003) found that browsing intensity by ungulates on riparian ecosystems was reduced in high-risk sites in response to the perceived predation risk, but such top-down effects have not been documented yet for the case of the Iberian lynx. The main findings of this thesis provide a novel framework which lays the foundations to study alternative cascading effects structuring ecosystems across food webs. However, more research is needed on the ecological consequences of *rewilding* apex predators regarding the current context of global change.

General conclusions

General conclusions

1. The *rewilding* scenario of large predator populations worldwide can trigger trophic cascades on lower trophic levels across food webs, ultimately affecting the community of large-fruited plants mainly dispersed by frugivorous mesocarnivores. Thus, reintroduction programs of apex predators should consider trophic cascades as powerful mechanisms, able to alter plant-animal interactions and key ecosystems functions.

1. Iberian lynx recovery across the Iberian Peninsula can reverse the potential mesopredator release scenario, but the strength of this top-down suppression is species-specific and coexistence can be favoured for species with a narrow trophic niche breadth.

2. The recovery of lynx populations can alter the foraging behaviour of key mammal frugivores in Mediterranean ecosystems. Specifically, foxes visited fruiting trees less often, consumed less fruit and spent less time searching for fruits under predation risk, resulting in less efficient seed dispersers.

3. Cascading effects triggered by apex predators caused a reduction on the seed rain and plant diversity of the entire assemblage of large-fruited plants mobilised by the red fox and the stone marten living under predation risk.

4. Large fleshy-fruited plants mainly dispersed by medium- and large- sized seed dispersers can experience limitations of dispersal and recruitment in ecosystems lacking of frugivore megafauna. This is the case of the Iberian pear, a Mediterranean fruit tree whose seed dispersal is drastically reduced and biased towards non-favourable habitats for seedling survival (i.e. forests) in presence of lynx.

5. Relatively subtle behavioural differences among frugivores can lead to important functional variations and contrasting seed dispersal patterns among seed-arrival sites, paramount to predict the impact of seed disperser loss on plant demography dynamics. However, seed dispersal compensation effects could balance the lack of the seed dispersal service of key frugivores quantitatively but not qualitatively, due to functional diversity.

6. Bottom-up forces were also important in the trophic web by facilitating coexistence among predators in a context of high-availability of food resources.

However, the release of rodents in lynx presence did not seem to increase post-dispersal seed predation.

7. Seed predation by rodents was microsite-mediated (i.e. low spatial scale) instead of habitat-mediated (i.e. high spatial scale), resulting in a seed-seedling conflict due to the high seed predation rates in the microsite more suitable for seedling survival.

8. Despite apex predator *rewilding* scenarios limited the quantity of seeds dispersed across the landscape of fear, lynx presence could be favouring those plant species more sensitive to insolation and water-stress in early stages of the life-cycle.

9. Conservation practitioners should consider to promote a heterogeneous distribution of apex predators, likely resulting in balanced net effects for the different trophic levels at regional scale. Furthermore, complex community interactions should be accounted for when planning future large predator reintroductions, mainly in defaunation scenarios.

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