Belén Acuña Míguez
Tesis doctoral

Funcionalidad y resiliencia frente a la sequía de los bosques en expansión de sabina albar

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Rey Juan Carlos

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Resumen

Antecedentes

La región Mediterránea es considerada un punto caliente en el contexto de cambio global. Por un lado, en esta zona se está produciendo un incremento de la temperatura media mayor que el promedio global y las tendencias en precipitaciones señalan una alteración en la frecuencia e intensidad de las mismas. Junto con el cambio climático, en la región Mediterránea nos encontramos con el cambio en los usos del suelo. El principal cambio en los usos que se está produciendo en esta zona es el abandono rural que está provocando una expansión de los bosques hacia campos de cultivo abandonados. La interacción entre el cambio climático y el abandono rural puede llegar a provocar cambios con profundas implicaciones ecológicas en los sistemas forestales ya que alteran su estructura y funcionalidad y, por tanto, pueden llegar a comprometer las funciones y servicios que éstos proporcionan. La expansión forestal que se produce por la colonización de árboles en antiguos campos de cultivo produce un gradiente de variación en la edad de los árboles desde bosques maduros a frentes de expansión, en el efecto de los legados de los usos del suelo anteriores y de adversidad ambiental. Entender cómo todos estos factores se relacionan entre sí, promoviendo cambios en las funciones ecosistémicas, y cómo a su vez esto afecta a la resiliencia de los árboles frente al cambio climático es clave para determinar la importancia y vulnerabilidad de estos nuevos sistemas.

Objetivos

El objetivo principal de esta tesis doctoral es entender la variación de funciones ecosistémicas a lo largo de un gradiente de expansión forestal y su efecto en la respuesta de los árboles a la sequía. Los objetivos específicos están organizados en los 4 capítulos: i) cuantificar el contenido y stocks de carbono, nitrógeno y fósforo en el suelo orgánico y suelo mineral a lo largo del gradiente; ii) entre los grupos microbianos del suelo y su funcionamiento a lo largo de un gradiente de expansión forestal; iii) examinar el reciclado de nutrientes a lo largo del gradiente a través de dos años de caída de hojarasca y la descomposición de la misma y iv) evaluar la asignación de biomasa y resiliencia de los árboles en dos etapas del gradiente de expansión forestal a través de cronologías de anillos de crecimiento de raíces secundarias y troncos de árboles.

Métodos

Esta tesis doctoral se ha desarrollado en tres localidades (Maranchón, Huertahernando y Ribarredonda) del Parque Natural del Alto Tajo (Guadalajara, España), área que ha experimentado un abandono rural considerable durante las últimas décadas. Como especie de estudio se ha utilizado la sabina albar (*Juniperus thurifera* L.) debido a la expansión de sus bosques en la zona durante dicho periodo.

En cada localidad se establecieron un total de 18 parcelas representativas del gradiente de expansión de la sabina albar. El gradiente de expansión de dicha especie se caracterizó identificando tres etapas: bosque maduro, zona de transición y frente de avance. La clasificación de cada una de las parcelas en una etapa u otra del gradiente se llevó a cabo mediante la comparativa de imágenes aéreas de 1957 y 2021. Esta división en etapas del gradiente de expansión nos ha permitido abordar las posibles diferencias existentes entre el núcleo de bosque maduro desde donde la especie se expande hacia zonas de reciente colonización que fueron campos de cultivos.

Resultados

Capítulo 1:

Se encontró menor concentración de nitrógeno total y mayor ratio C/N en la capa orgánica del suelo del frente de avance que en la del bosque maduro y en la de la zona de transición. En suelo mineral bajo copa, la concentración de carbono orgánico total y fósforo inorgánico fue mayor en el bosque maduro que en el frente de avance con valores intermedios en la zona de transición. Además, la concentración de nitrógeno total en el suelo mineral fue mayor en el bosque maduro que en el frente de avance sin diferencias entre los suelos bajo copa y fuera de copa. La ratio C/N fue mayor bajo copa que fuera de copa. Por último, los stocks de carbono orgánico, nitrógeno total y fósforo inorgánico no variaron a lo largo del gradiente de expansión.

Capítulo 2:

El gradiente de expansión forestal (de bosque maduro a frente de avance) tuvo un efecto negativo directo sobre el ciclo del fósforo y un efecto positive indirecto, mediado por la cantidad de material orgánica en el suelo y el pH del mismo, sobre la abundancia de microorganismos y las actividades enzimáticas. El microhábitat (bajo copa y fuera de copa) tuvo un efecto positivo directo sobre la abundancia de actinomicetos y hongos micorrícicos arbusculares y sobre los ciclos de carbono y fósforo. Además, la abundancia de bacterias tuvo efectos directos sobre las actividades enzimáticas relacionadas con los ciclos de carbono y fósforo.

Capítulo 3:

Se encontraron diferencias en la caída de hojarasca (*litter*) entre años, siendo ésta mayor en 2021 que en 2020 sin diferencias entre etapas. Se observó que la principal fracción de hojarasca en ambos años fue la fracción foliar. La fenología de la producción de la fracción foliar no varió entre etapas, mostrando en ambos años picos en verano. Sin embargo, la cantidad fue mayor en el frente de avance que en el bosque maduro y en la zona de trancisión. En cuanto a la tasa de descomposición, esta fue menor bajo copa que fuera de copa sin diferencias entre etapas.

Capítulo 4:

El crecimiento secundario del tronco y la raíz, así como el exponente alométrico fue mayor en la zona de transición que en el bosque maduro. Ambos órganos mostraron un *trade-off* entre resistencia y recuperación a la sequía en los árboles del bosque maduro mientras que la raíz y el tronco de los árboles de la zona de transición mantuvieron altos valores en ambos índices. La resiliencia no mostró diferencias entre órganos siendo mayor en la zona de transición que en el frente de avance. Sin embargo, la resiliencia relativa en las raíces fue mayor que en las del bosque maduro sin diferencias en el tronco. Por último, el evento de sequía de 2012 mostró un mayor impacto sobre los componentes de resiliencia que el evento de 2005.

Conclusión

Esta tesis muestra la importancia de conservar los nuevos bosques establecidos en antiguos campos de cultivo dado su capacidad de almacenamiento de carbono en suelo la cual es equiparable a la del bosque maduro. Además, los resultados indican que los suelos de estos nuevos bosques podrían alcanzar el funcionamiento y la abundancia de microorganismos de los bosques maduros. Sin embargo, cabe destacar que los árboles del bosque maduro presentaron una menor resiliencia a los eventos de sequía. Por lo tanto, la presencia de gradientes de expansión, caracterizados por la heterogeneidad de etapas de desarrollo del bosque, es una estrategia de gestión que incrementaría la multifuncionalidad de los bosques de *J. thurifera* en el contexto actual de cambio global.

Summary

Background

Mediterranean region is considered a global change hotspot. This area is experiencing a higher increase in average temperature than the global average, and climate models showed a change in frequency and intensity of precipitation. Climate change is co-occurring with land use changes being, being rural abandonment one of the main changes in Mediterranean region which leads a forest expansion into abandoned agricultural lands. The interaction between climate change and land use change can potentially induce profound ecological implications in forests by altering their structure and functionality and, potentially, compromising the functions and services they provide. Forest expansion due to tree colonisation in past agricultural lands creates a gradient of tree age, land-use legacies and environmental adversity from mature forests to expanding fronts. Understanding how all these factors are connected and promote changes in ecosystem functions, and how this affects tree resilience to climate change is crucial for determining the importance and vulnerability of these new systems.

Objectives

The main objective of this thesis is to understand the variation of ecosystem functions along a forest expansion gradient and its impact on tree response to drought. The specific objectives are organized into four chapters: i) to quantify carbon, nitrogen, and phosphorus content and stocks in organic and mineral soil along the gradient; ii) to assess soil microbial groups and their functioning along the expansion gradient; iii) to examine nutrient cycling across the gradient through two years of litterfall and its decomposition; and iv) to evaluate biomass allocation and resilience of trees at two stages of the expansion gradient using growth ring chronologies of secondary roots and tree stems.

Methods

This thesis was conducted in three sites (Maranchón, Huertahernando, and Ribarredonda) within Alto Tajo Natural Park (Guadalajara, Spain), an area that has experienced rural abandonment in recent decades. The thesis was focused on *Juniperus thurifera* L. (Spanish juniper) due to the expansion of its forests in the area during this period.

In each site, 18 plots representative of *J. thurifera* forest expansion were established. Forest expansion gradient was characterised by identifying three stages: mature forests, transition zone and expanding front. Each plot was classified in each stage by comparing aerial photographs of 1957 versus 2021. This categorisation into stages of the expansion gradient allowed us to explore potential differences existing between the core of mature forest, from which the species expands into recently colonised areas that were agricultural fields.

Results

Chapter 1

Forest floor at expanding front showed lower total nitrogen concentration and higher C/N ratio than in mature forests and transition zone. In mineral soils under canopy, total organic carbon and inorganic phosphorus concentrations were higher in mature forests than at expanding front. Moreover, total nitrogen concentration in mineral soil was higher in mature forest than at expanding front without differences between microhabitats (under canopy and open areas). Contrary, C/N ratio was higher under canopy than open areas without differences along the forest expansion gradient. Finally, we did not find differences in element stocks in mineral soils along the forest expansion gradient.

Chapter 2

Forest expansion gradient (from mature forest to expanding front) had a negative direct effect on P-cycling and a positive indirect effect on soil microbial abundance and enzymatic activities through its direct effect on organic matter and pH. Microhabitat (under canopy and open areas) showed a positive direct effect on the abundance of actinomycetes and arbuscular mycorrhizal fungi abundances and on C and P cycling. Moreover, bacterial abundance had direct effects on enzymatic activities related to carbon and phosphorus cycling.

Chapter 3

Litterfall quantity was higher in 2021 than in 2020 without differences among stages of forest expansion gradient. The main litter fraction both years was foliar fraction. Foliar phenology did not vary among stages, showing two peaks in summer for both years. However, the foliar fall quantity was higher at expanding front than in mature forests and transition zone. Regarding decomposition rate, it was lower under canopy than in open areas, without differences among stages of forest expansion gradient.

Chapter 4

Stem and root growth as well as its allometric exponent were higher in the transition zone than in mature forests. Both organs exhibited a trade-off between resistance and recovery in mature forests but maintenance of higher values in the transition zone. Resilience did not show differences between organs being higher in the transition zone than in mature forests. However, relative resilience in roots in the transition zone was higher than in mature forests, without differences in stems between stages. Finally, the 2012 drought event showed a higher impact on the components of resilience than the 2005 drought event.

Conclusion

This thesis highlights the importance of conserving new forests established on abandoned agricultural lands due to their carbon storage capacity of soils, comparable to that of mature forests. Furthermore, results indicate that these new forests could achieve soil functioning and microbial abundance comparable to mature forests. However, it is remarkable that trees in mature forests showed lower resilience to drought events. Therefore, the presence of expansion gradients, characterised by heterogeneity in forest development stages, is a convenient management strategy to increase *J. thurifera* forest multifunctionality in the current global change context.

Introducción general

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Antecedentes

Cambio global

Los seres humanos somos parte integral de los ecosistemas y nos beneficiamos de los servicios que estos nos proporcionan (servicios de aprovisionamiento, de regulación, culturales y de soporte) (Millennium Ecosystem Assesment, 2005). El impacto cada vez mayor de las personas en los ecosistemas ha motivado el desarrollo de investigaciones dirigidas a comprender cómo los ecosistemas modificarán las funciones y, por tanto, los servicios que proporcionan en diferentes escenarios de cambio global (Naeem et al., 2012).

El cambio global reúne todos los impactos de la actividad humana en la composición y funcionamiento de la biosfera (Duarte et al., 2006) y está compuesto por diferentes motores de cambio. Un motor de cambio directo es cualquier factor que altera un aspecto del ecosistema, afectando por tanto a los procesos ecosistémicos y que puede así ser identificado y medido. Según el *Millenium Ecosystem Assessment* (2005) nos encontramos con cinco motores principales de cambio global: cambio en los usos del suelo, cambio climático, proliferación de especies invasoras, sobreexplotación de recursos naturales y contaminación. Los motores de cambio global no ocurren de manera aislada y la combinación entre ellos provoca efectos aditivos (sin interacción), antagónicos (amortiguan los efectos mutuos) o sinérgicos (amplificando los efectos de cada uno) (Tylianakis et al., 2008; Leuzinger et al., 2011). Así, los estudios que examinan múltiples motores de cambio global son esenciales para predecir la totalidad de sus efectos en la estructura y funcionamiento de los ecosistemas (Turner et al., 2020; Avolio et al., 2020).

Cambio climático y cambio en los usos del suelo en la región Mediterránea

La región Mediterránea, debido a sus particularidades climáticas y a las profundas transformaciones antrópicas que ha sufrido durante milenios, es considerada un punto caliente en el contexto de cambio global (Matesanz & Valladares, 2014). Los registros hasta la fecha muestran un aumento de las temperaturas medias anuales de entre 0.1 y 0.5 °C por década durante el siglo XX, especialmente si se analizan los registros desde

1980 (Fig 1 A y B; MedECC, 2020). Este incremento de las temperaturas en la región Mediterránea se produce a un ritmo mayor que el aumento promedio global (Lionello & Scarascia, 2018). Además, se ha observado un aumento en la intensidad, cantidad y duración de las temperaturas extremas y de las olas de calor (MedECC, 2020). En cuanto a las precipitaciones, los registros muestran una disminución en las precipitaciones de invierno especialmente en el sur y centro de la cuenca mediterránea. La confianza en la tendencia de las precipitaciones debido a la emisión de gases de efecto invernadero es baja debido a que las tendencias de precipitaciones muestran un variabilidad espacial y temporal (por estaciones y por décadas) que podría enmascarar las tendencias durante el periodo desde que hay registros (Fig 1. D y F; Vicente-Serrano et al., 2021). En cuanto a las predicciones futuras, las tendencias señalan que en la región Mediterránea se seguirá produciendo un aumento de las temperaturas y una disminución de las precipitaciones, además de una mayor variabilidad y frecuencias de eventos climáticos extremos como olas de calor y sequía (Zittis et al. 2016; Lionello & Scarascia, 2020). Los cambios producidos hasta la fecha y las predicciones futuras aumentan la vulnerabilidad de los ecosistemas mediterráneos donde se han observado impactos en ecosistemas forestales, marinos, de agua dulce, pastizales, etc., que provoca una disminución de los suministros de servicios ecosistémicos, produciendo así impactos en la salud humana (Ali et al., 2022).

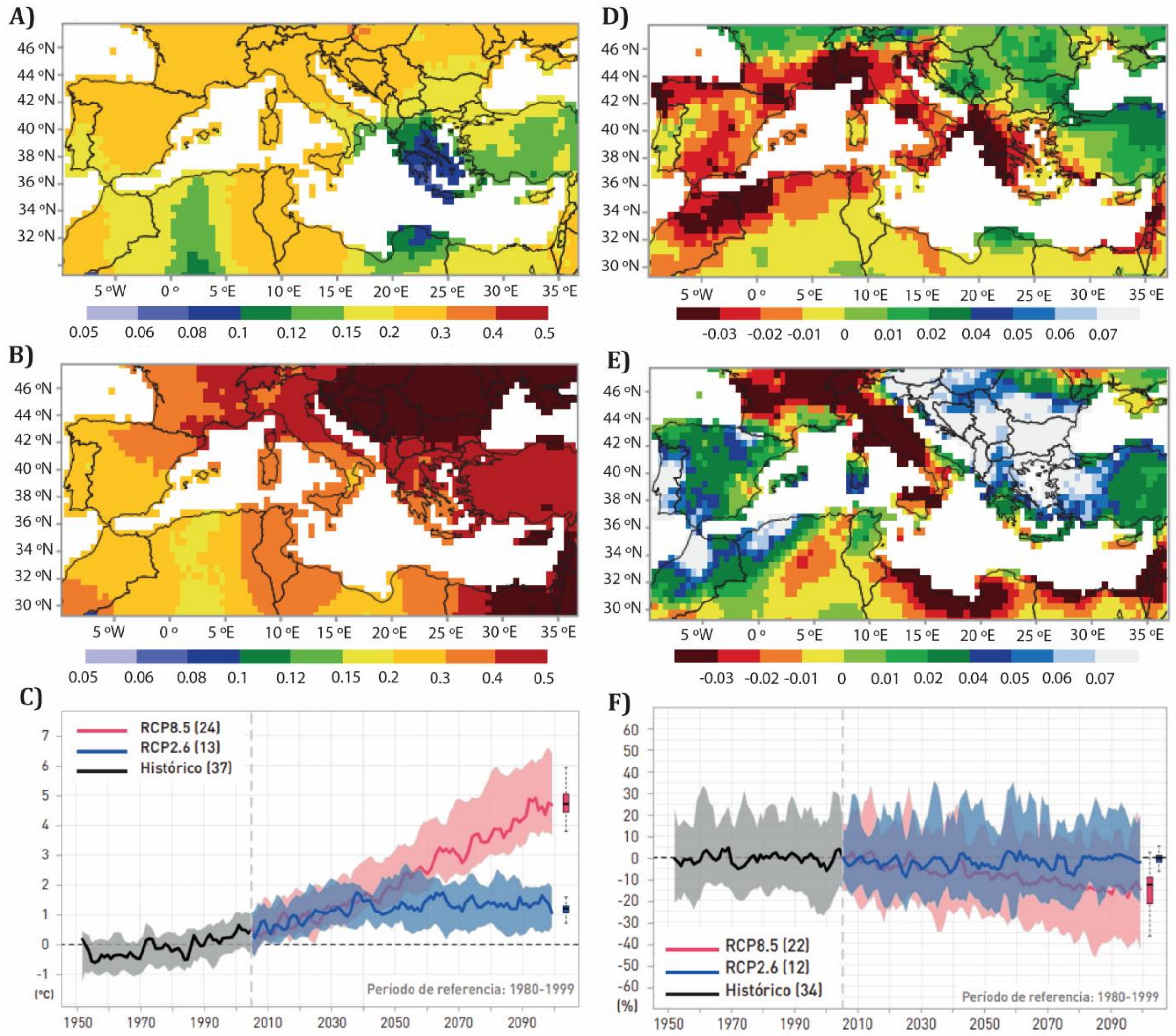


Figura 1. Cambios observados y proyecciones en la temperatura (a, b y c) y las precipitaciones (d, e y f). Tendencias recientes en la temperatura (a y b, °C década-1) y en las precipitaciones (d y e, mm día-1 década-1) en la cuenca mediterránea en la tierra. Mapas a y d, medias para el periodo 1950-2018, mapas b y e para el período 1980-2018. Las gráficas c y f muestran los cambios proyectados para los escenarios RCP2.6 y RCP 8.5 en la cuenca mediterránea para la temperatura anual (c) y precipitación anual (f) en relación con el período de referencia pasado reciente (1980 -1999) basados en la media del conjunto EURO-CORDEX, 0.11º. Figuras modificadas y extraídas de MedECC (2020).

Junto con el cambio climático, los cambios en los usos del suelo son otro de los principales motores de cambio global (Vitousek, 1994; Verburg et al., 2011). Los usos del suelo de un territorio son dinámicos y los ritmos y tipos de cambio dependen de diferentes factores como son la accesibilidad del territorio y el aumento o disminución de la población debido a las migraciones de los seres humanos (Gallant et al., 2004). Se estima que los cambios en los usos del suelo han afectado a una tercera parte de la superficie global entre 1960 y 2019 (Winkler et al., 2021). Estos cambios en los usos del suelo difieren entre regiones a nivel mundial, de forma que en el norte global los cambios mayoritarios son aforestación y abandono de tierras de cultivo y en el sur global la deforestación y la expansión agrícola (Winkler et al., 2021). En Europa, cabe destacar el abandono rural como uno de los principales cambios en los usos del suelo (Keenan, 2015). El éxodo rural promueve el cese de las actividades agrícolas y ganaderas lo cual facilita la expansión y densificación de los bosques. Europa ha mostrado ser el continente con un mayor crecimiento proporcional de área forestal. Las estimas varían desde 8000 km^2 por año desde 1990 (FAO, 2011) a 28300 km^2 por año entre 1982 y 2015 (Song et al., 2018). Estudios más recientes han mostrado que, aunque el incremento en área forestal está ampliamente distribuido por Europa, dos terceras partes de los nuevos bosques se producen en campos de cultivo abandonados, especialmente en el este y sur de Europa (Fig 2; Palmero-Iniesta et al., 2021). Por ejemplo, en España, la cobertura forestal de "bosques nuevos" (bosques que han colonizado tierras de cultivo abandonadas) ha aumentado un 14% entre los años 1956 y 1999 (Vilà-Cabrera et al., 2017; SISPARES database, www.sispares.com).

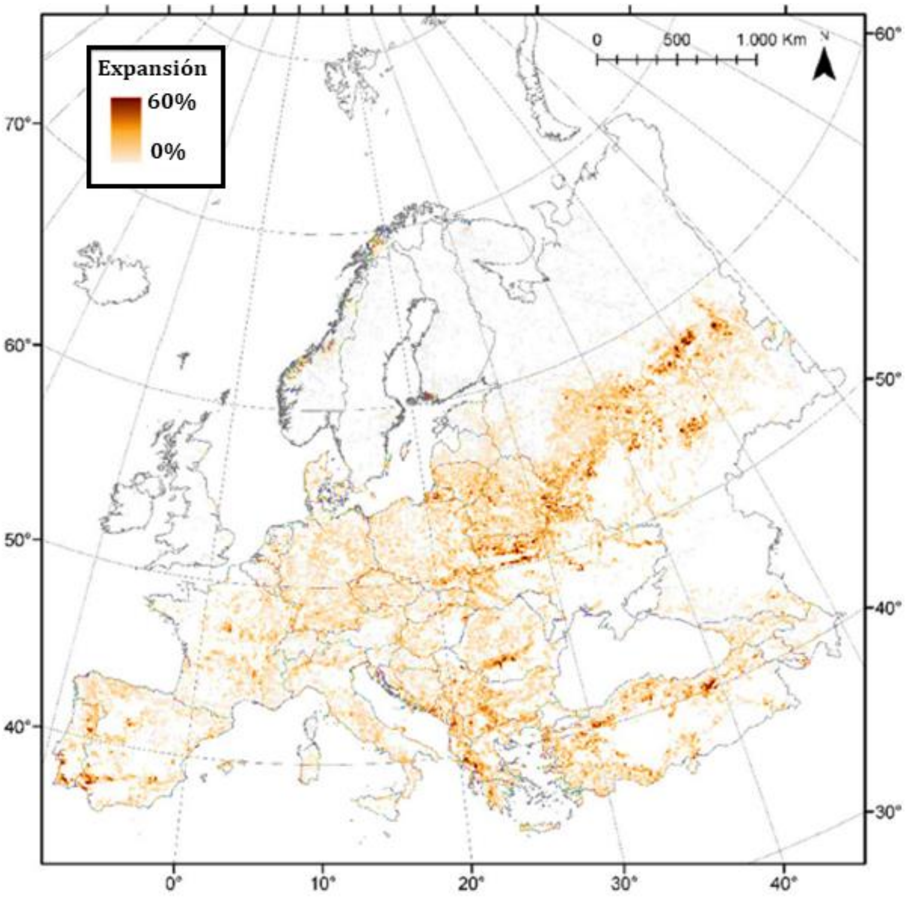


Figura 2. Expansión forestal en Europa entre los años 1992 y 2015. Cada píxel muestra el porcentaje de expansión relativa a 10-km de celda de red. Extraída de Palmero-Iniesta (2021).

Impacto en los sistemas forestales

La interacción entre el cambio climático y el abandono rural pueden llegar a provocar cambios con profundas implicaciones ecológicas en los sistemas forestales ya que alteran su estructura y funcionalidad y, por tanto, pueden llegar a comprometer las funciones y servicios que éstos proporcionan (Peñuelas et al., 2017). Los bosques son considerados como uno de los principales reguladores de la multifuncionalidad ecosistémica, ya que proveen de múltiples funciones y servicios para los seres humanos (van der Plas et al., 2019; Lucas-Borja & Delgado-Baquerizo, 2019) que incluyen los ciclos de nutrientes (disponibilidad de nutrientes y mineralización), secuestro de carbono, regulación del clima y agua, descomposición de materia orgánica y producción de madera (Ushio et al., 2010; Aponte et al., 2013; Byrnes et al., 2014). Así, entender cómo el abandono rural y la expansión forestal afecta a la multifuncionalidad de esos nuevos sistemas forestales en un contexto de cambio climático es clave para entender posibles relaciones de compensación o *trade-offs* entre diferentes funciones (Garland et al., 2021).

Diferentes estudios han mostrado que el ensamblaje de comunidades vegetales en campos de cultivo abandonados produce patrones repetibles asociados a la teoría de la sucesión cuando el legado de los usos del suelo en los campos de cultivo no ha superado los umbrales bióticos y abióticos que produzcan la degradación de esas zonas (Cramer et al., 2008). Así, la colonización de antiguos campos de cultivo es un tipo de sucesión secundaria que produce una cronología de edades de la vegetación. Muchos estudios han mostrado que las diferencias de edades promueven diferencias en funciones como ratio de descomposición, reciclado de nutrientes o secuestro de carbono (Entry & Emmingham, 1998; Hedo et al., 2015; Lucas-Borja & Delgado-Baquerizo, 2019).

Sin embargo, la diferencia de edades no es lo único que puede afectar a las diferencias en la funcionalidad a lo largo de la expansión natural de los bosques hacia tierras de cultivo abandonadas. Estas tierras presentan una historia de usos del suelo definido por la secuencia compleja de los usos que los humanos han producido en un sitio concreto (Arce-Nazario, 2007; Ewers et al., 2013). Cuando se produce el cese de las actividades humanas en un lugar determinado, hay una serie de propiedades bióticas y abióticas que permanecen durante cientos e incluso miles de años tras el abandono que se conocen como legado de los usos del suelo (Foster et al., 2003; Plue et al., 2008). Por lo tanto, los árboles que se establecen en tierras de cultivo abandonadas tienen que hacer frente a legados como suelos más profundos, con pH más altos, mayor contenido de nutrientes (Compton & Boone, 2000; Fraterrigo et al., 2005) y una mayor tasa de descomposición (Freschet et al., 2014). Estos legados se han relacionado con mayor crecimiento secundario de los árboles (Vilà-Cabrera et al., 2017; Alfaro-Sánchez et al., 2019) y una mayor producción de biomasa total de los mismos (Freschet et al., 2014). Además de los legados en los usos del suelo, cuando los árboles se establecen en zonas de cultivo abandonadas podrían tener que hacer frente a condiciones ambientales más adversas (por ejemplo, una mayor irradiancia debido a una menor cobertura forestal que aumenta la evapotranspiración). Esas condiciones en las zonas de reciente colonización se han relacionado con árboles con un fenotipo más eficiente, por ejemplo, con una mayor eficiencia en el uso del agua y un incremento de la resiliencia comparado con los árboles del bosque maduro (Acuña-Míguez et al., 2020; Benavides et al., 2023). Entender cómo todos estos factores se relacionan entre sí, promoviendo cambios en las funciones ecosistémicas, y cómo a su vez esto afecta a la resiliencia de los árboles frente al cambio climático es clave para determinar la importancia y vulnerabilidad de estos nuevos sistemas.

Carbono, nitrógeno y fósforo en el suelo

Entre las diferentes funciones de los bosques, una de las más principales es la fijación de carbono atmosférico lo cual contribuye a la regulación del clima y a mitigar los efectos del cambio climático (Ruiz-Peinado et al., 2017). El abandono rural y la reforestación de esas áreas ha sido considerado uno de los principales contribuidores de secuestro de carbono entre 1950 y 2010 (Fuchs et al., 2016). Cabe destacar que, aunque la fijación de carbono en biomasa aérea es más estudiada que la fijación en los suelos, el almacenamiento en estos últimos representa un 44% del carbono total almacenado en los sistemas forestales (Pan et al., 2011) y es considerado el segundo mayor sumidero de carbono después de los océanos (Stockmann et al., 2013). Entender el almacenamiento de carbono en suelos en un contexto de cambio global es clave para evaluar su potencial de secuestro de carbono y reducir los niveles de dióxido de carbono atmosférico (Rumpel & Kögel-Knabner, 2011). Numerosos estudios llevados a cabo en ambientes mediterráneos han mostrado que el abandono de campos de cultivo y la posterior recolonización por vegetación natural ha aumentado la cantidad de carbono orgánico en el suelo (Acín-Carrera et al., 2013; De Baets et al., 2013; Van Hall et al., 2017).

Los cambios en los usos del suelo además de afectar a la fijación de carbono, afectará a la cantidad de diferentes nutrientes en él. Junto con el carbono, el nitrógeno y fósforo son claves para el funcionamiento de los ecosistemas. Los suelos en los ecosistemas mediterráneos en general y en los bosques en particular, se caracterizan por su escasez en estos elementos (Mayor & Rodà, 1994; Sardans et al., 2004). Diversos estudios proponen que actividades agrícolas como el uso de fertilizantes favorece la presencia de nutrientes en estos suelos tras su abandono (Koerner et al. 1997; Falkengren-Grerup et al., 2006), lo cual podría beneficiar a los árboles que colonizan esas tierras. Sin embargo, estos resultados no son del todo claros ya que otros estudios han mostrado como el nitrógeno disminuye y el fósforo no presenta grandes cambios (Verheyen et al. 1999; Maloney et al. 2008).

El papel de las comunidades microbianas

La cantidad de esos nutrientes dependerá de los efectos de los legados en los usos del suelo sobre las comunidades microbianas (tanto en su abundancia como en su funcionamiento) ya que estas son actores claves en los sistemas forestales como mediadores directos de los ciclos de carbono y nutrientes en el suelo (van der Heijden et al., 2008; Graham et al., 2016). Se ha estimado que los hongos y bacterias son responsables de aproximadamente el 90% de la descomposición de la materia orgánica (McGuire et al., 2010) por lo que el estudio de la abundancia de ambos grupos es clave para analizar la funcionalidad del bosque

Los usos agrícolas (como el uso de fertilizantes o el arado) pueden producir efectos a largo plazo sobre las propiedades del suelo como, por ejemplo, en su fertilidad (Flores-Rentería et al., 2015) lo cual afecta a la diversidad o abundancia de las comunidades microbianas (Compton & Boone, 2000; van der Heijden et al., 2008). El abandono de esas tierras agrícolas y la posterior expansión del bosque sobre ellas, promueve cambios en la estructura y cobertura vegetal que afectan a las propiedades fisicoquímicas y microbianas del suelo. El cambio de la composición de la hojarasca de comunidades vegetales de tipo herbáceas a hojarasca más recalcitrante característica de los árboles aumenta el tiempo de residencia de la materia orgánica y reduce la movilización de nutrientes en el suelo mediada por microorganismos (Roberts & Jones, 2000; Guidi et al., 2014). Además, el establecimiento de los árboles puede modificar las condiciones microclimáticas del suelo a través de la provisión de sombra y aislamiento térmico (Hagedorn et al., 2014). La actividad microbiana del suelo en los ecosistemas forestales mediterráneos se ha visto afectados tanto por los legados del uso como por el tipo de cobertura forestal (García-Morote et al., 2012; Correia et al., 2021). Esto se debe a que las comunidades microbianas del suelo son muy sensibles y por tanto un rápido indicador de perturbaciones y cambios en los usos del uso (Zelles et al., 1994).

Por lo tanto, la expansión forestal hacia campos de cultivo abandonados puede modificar la estructura y/o actividad de las comunidades microbianas del suelo. Así entender cómo los legados de los usos del suelo junto con el establecimiento de los árboles modifican la abundancia y actividad microbiana es clave para entender los cambios en los procesos biogeoquímicos del suelo.

Reciclado de nutrientes

Como se ha mencionado anteriormente el uso de los campos de cultivo abandonados puede afectar a la abundancia y actividad microbiana (Freschet et al., 2014), lo cual puede afectar al reciclado de nutrientes. El reciclado de nutrientes engloba entre otros procesos a la caída de hojarasca y a su descomposición. Ambos procesos forman la primera vía por la cual la materia orgánica y nutrientes son suministrados a la comunidad del suelo, vinculando los productores primarios y los procesos edáficos (Aber & Melillo 1980). La producción primaria neta, es decir, la tasa de formación de biomasa que es usada para crear estructuras orgánicas en las plantas, puede ser medida a través de la producción de hojarasca (Malhi et al., 2011) debido a su papel como principal fuente de carbono orgánico del suelo y ciclado de nutrientes (Vitousek, 1982). Seguido de la caída de la misma, su descomposición contribuye a la estructura y funcionamiento de los ecosistemas ya que sirve a dos funciones principales: i) regulación del ciclo de nutrientes mediante el control de las tasas y momento de liberación de los mismos (Blair, 1988) y ii) formación de la materia orgánica del suelo (Swift et al. 1979).

Ambos procesos son claves en la funcionalidad de los bosques y está controlado tanto por factores bióticos como por las condiciones ambientales (Gauquelin et al., 2018). Además de los legados de los usos del suelo y las edades de los árboles, a lo largo de un gradiente de expansión forestal encontramos diferencias en la cobertura arbórea (Acuña-Míguez et al., 2020). Las zonas recientemente colonizadas presentarán una menor cobertura forestal lo cual podría afectar a la cantidad de hojarasca que se produce y a las condiciones microclimáticas que regulan la descomposición de esta (Bravo-Oviedo et al., 2017).

Resiliencia de los árboles

Todos los posibles cambios asociados a los legados de los usos del suelo y expansión y densificación de los bosques vienen acompañados por el incremento de la adversidad ambiental debido al cambio climático. La interacción entre ambos motores de cambio global puede afectar a la resiliencia de los bosques (entendida como la habilidad de un bosque de mantener su estado y funciones después del impacto de una perturbación; Holling, 1973). El estudio del impacto de la sequía en la resiliencia de los bosques usando técnicas dendrocronológicas en el crecimiento secundario ha aumentado en las últimas décadas (Kannenberg et al., 2020). Sin embargo, esta metodología no tiene en cuenta otros procesos como la asignación de carbono hacia el crecimiento de las raíces (Doughty et al., 2014). Se estima que el sistema radical forma entre el 20 y el 40 % de la biomasa de los árboles (Jackson et al., 1997) y se puede dividir en dos tipos: raíces finas (<2mm y responsables de la absorción de agua y nutrientes) y raíces gruesas (>2mm y responsables del anclaje de los árboles al suelo y el transporte de agua desde capas más profundas) (Brunner & Godbold, 2007). Aunque el estudio de la estructura y crecimiento de las raíces en relación con eventos de sequía ha aumentado en ambos tipos, las raíces gruesas son menos estudiadas (aunque cabe destacar algunos estudios como los de Pretzsch et al., 2012a; Pretzsch et al., 2012b; Nikolova et al., 2021). Sin embargo, el estudio de las dinámicas de crecimiento de las raíces y la asignación diferencial de biomasa entre los troncos y raíces es fundamental para una compresión integral de los efectos de la sequía y el abandono de los usos agrícolas en la resiliencia y funcionalidad de los bosques teniendo en cuenta todo el árbol.

Objetivos de la tesis doctoral

El objetivo principal de esta tesis doctoral es entender la variación de funciones ecosistémicas a lo largo de un gradiente de expansión forestal y su efecto en la respuesta de los árboles a la sequía.

Para ello, utilizamos como especie de estudio la sabina albar (*Juniperus thurifera* L.) debido a la expansión de sus bosques durante las últimas décadas (Gimeno et al., 2012; Velázquez et al., 2022). Consideramos un gradiente de expansión de la especie divido en tres etapas: bosque maduro, zona de transición y frente de avance. Esta división nos permite abordar las posibles diferencias existentes entre el núcleo de bosque maduro desde donde la especie se expande hacia zonas de reciente colonización que fueron campos de cultivos (Fig 3.).

Se abordaron los efectos de dos motores de cambio global (cambios en los usos del suelo y cambio climático) en el funcionamiento de los bosques de sabina albar. Por una parte, se analizó el contenido y stock de carbono orgánico, nitrógeno total y fósforo inorgánico a lo largo del gradiente, considerando además posibles diferencias en dichos elementos bajo la copa de los árboles y fuera de ellas (capítulo 1). Posteriormente se examinaron los posibles cambios en las comunidades microbianas, así como en las actividades enzimáticas del suelo (capítulo 2). Además de analizar la funcionalidad del suelo mediante las actividades enzimáticas, llevamos a cabo un estudio durante dos años del aporte de nutrientes (mediante la caída de hojarasca y su posterior descomposición) (capítulo 3). Por último, para dos etapas del gradiente, estudiamos la respuesta resiliente del crecimiento secundario en tronco y raíces frente a la sequía (capítulo 4) (Fig 3).

Todos los capítulos de esta tesis doctoral han sido escritos en inglés para su publicación en revistas internacionales. A continuación, se detalla el título y los objetivos específicos de cada capítulo

Capítulo 1: Influencia de los usos agrícolas en el contenido y stock de carbono, nitrógeno y fósforo a lo largo de un gradiente de expansión forestal.

En este capítulo cuantificamos el contenido y *stock* de carbono orgánico total, nitrógeno total, ratio C/N y fósforo inorgánico en capa orgánica del suelo (donde unimos las capas LFH, por sus siglas en inglés, *litter, fragmented y* hummus) y en el suelo mineral a lo largo de un gradiente de expansión forestal desde bosques maduros hacia frente de avance. Además, en el análisis del suelo mineral diferenciamos entre suelos bajo copa y suelos en zonas abiertas.

Capítulo 2: Regeneración natural de los bosques como una estrategia de manejo para restauras el funcionamiento del suelo de los campos de cultivo abandonados.

En este capítulo examinamos la relación entre los grupos microbianos del suelo y su funcionamiento a lo largo de un gradiente de expansión forestal de bosque maduro a frente de avance considerando dos microhábitats bajo copa y fuera de copa.

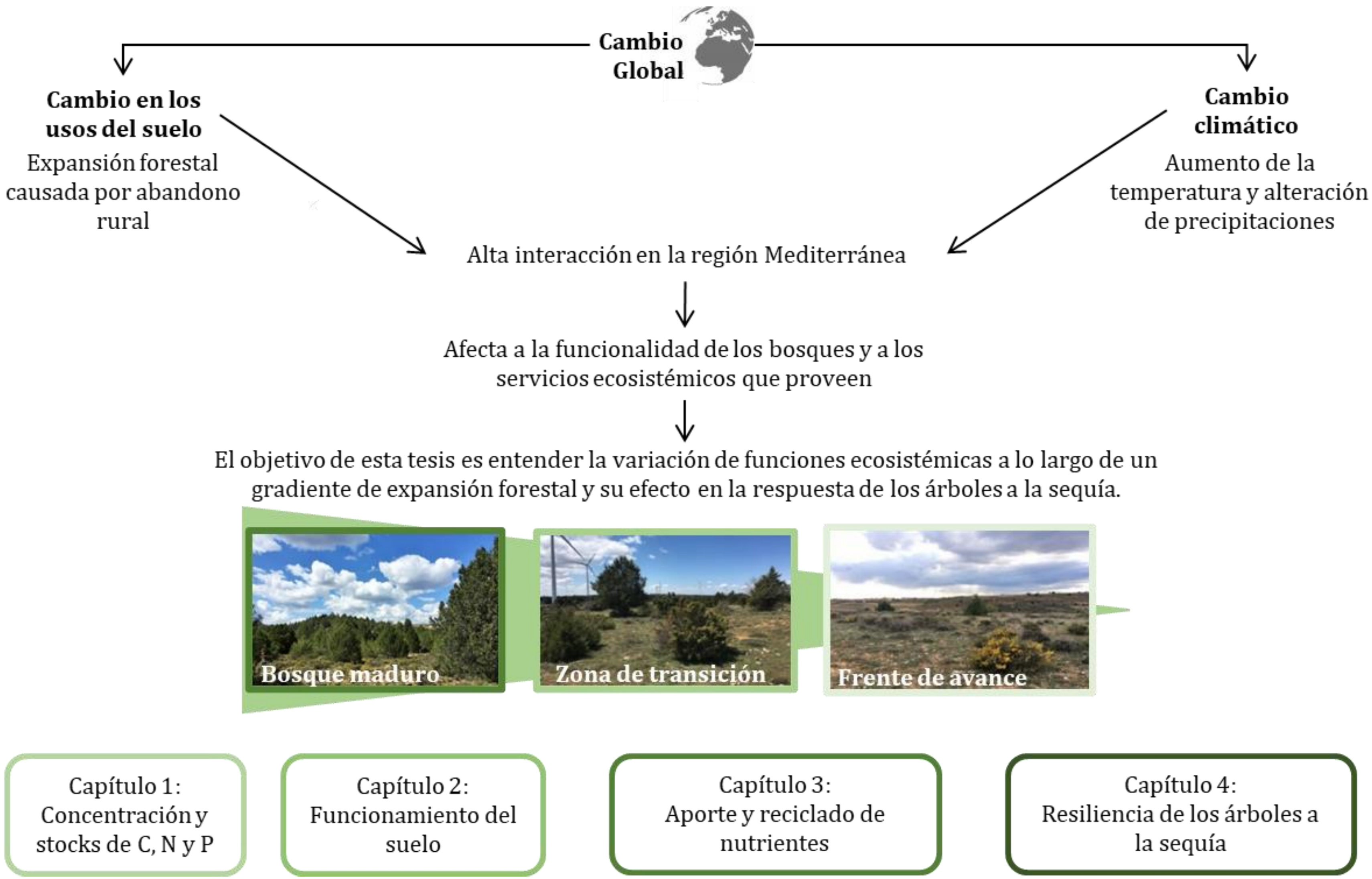
Específicamente, nuestros objetivos fueron: i) cuantificar la variación en las propiedades del suelo (pH, materia orgánica, abundancia de los grupos microbianos y las actividades enzimáticas) a lo largo del gradiente de expansión forestal y teniendo en cuenta los dos microhábitats) y ii) descifrar las vías por las cuales las propiedades del suelo (físico-químicas y microbianas) ligadas a la expansión forestal y a los dos microhábitats controlan los ciclos de carbono y nutrientes en el suelo.

Capítulo3: Producción de hojarasca y descomposición a lo largo de un gradiente de expansión forestal.

En este estudio examinamos dos años de caída de hojarasca y la descomposición de la misma a lo largo de un gradiente de expansión forestal considerando dos microhábitats en la descomposición (fuera de copa y bajo copa) para entender si: i) la fenología en la productividad, en términos de cantidad de hojarasca depende de la etapa del gradiente de expansión, ii) la pérdida intranual de masa durante la descomposición es más dependiente de los legados del uso del suelo o de diferencias en condiciones microclimáticas, y iii) la pérdida interanual de masa durante dos años de descomposición bajo la copa de los árboles muestra un patrón diferencial a lo largo del gradiente de expansión forestal.

Capítulo 4: El aumento de la recuperación del crecimiento en las raíces secundarias mejora la resiliencia a la sequía en los árboles de la zona de transición.

En este trabajo utilizamos cronologías de anillos de crecimiento de raíces secundarias y troncos de árboles de sabina albar para evaluar la asignación de biomasa y la resiliencia de los árboles en dos etapas del gradiente de expansión forestal (bosque maduro y zona de transición). Específicamente, nuestros objetivos fueron analizar: i) la variación del crecimiento secundarios de raíces y troncos entre las dos etapas del gradiente, ii) alometría raíz-tronco entre esas dos etapas; y iii) diferencias entre la resistencia, recuperación, resiliencia y resiliencia relativa entre ambos órganos, etapas del gradiente de expansión y eventos de sequía.



Cambio Global

Cambio en los usos del suelo
Expansión forestal causada por abandono rural

Cambio climático
Aumento de la temperatura y alteración de precipitaciones

Alta interacción en la región Mediterránea

Afecta a la funcionalidad de los bosques y a los servicios ecosistémicos que proveen

El objetivo de esta tesis es entender la variación de funciones ecosistémicas a lo largo de un gradiente de expansión forestal y su efecto en la respuesta de los árboles a la sequía.

Capítulo 1: Concentración y stocks de C, N y P

Capítulo 2: Funcionamiento del suelo

Capítulo 3: Aporte y reciclado de nutrientes

Capítulo 4: Resiliencia de los árboles a la sequía

Figura 3. Esquema conceptual de la tesis doctoral y aspectos abordados en cada capítulo

Metodología de la tesis doctoral

En cada capítulo de la tesis doctoral se aplicó una metodología específica que serán detalladas en la sección “Material y Métodos” del capítulo correspondiente. A continuación, se describe el sistema de estudio, tanto el área como la especie.

Área de estudio

La tesis doctoral se ha desarrollado en el Parque Natural del Alto Tajo (Guadalajara, España). Esta zona ha sufrido un éxodo rural significativo desde los años 1960 (Fig 4), que ha provocado el cese de actividades agrícolas y ganaderas.

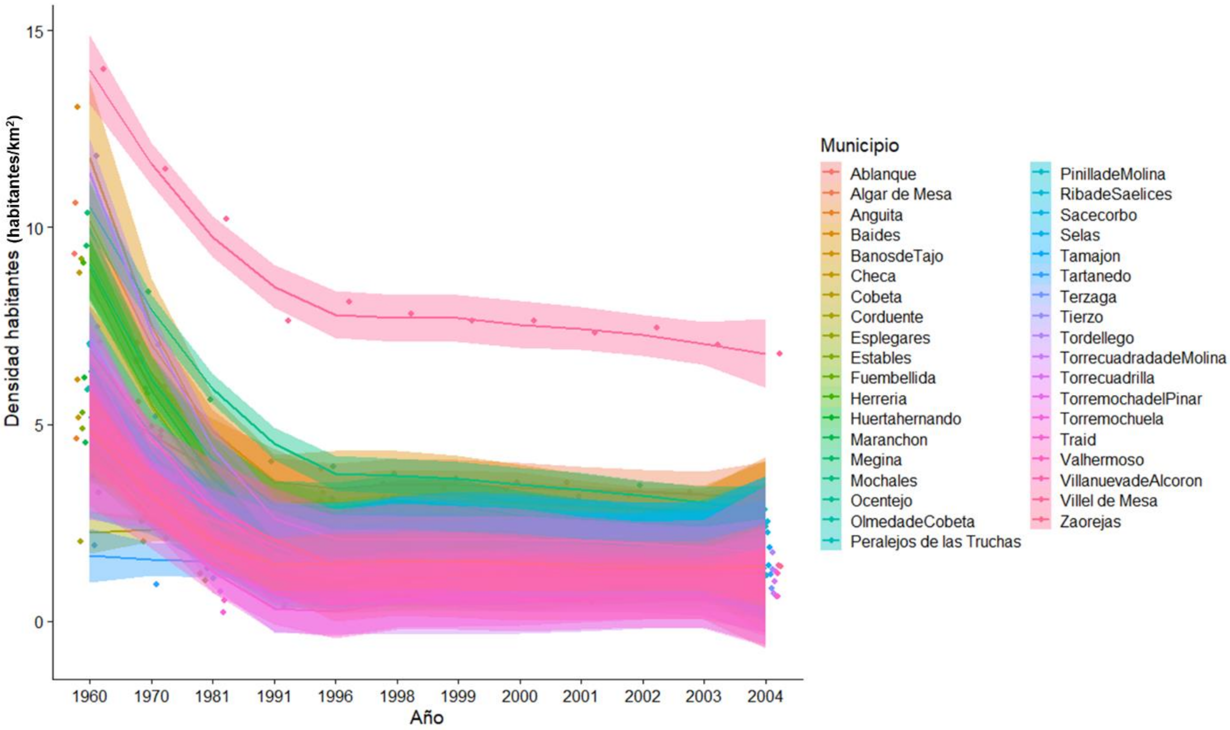


Figura 4. Densidad de habitantes (habitantes por km^2) desde 1960 al 2004 en los municipios pertenecientes al Parque Natural del Alto Tajo y alrededores. Los datos han sido extraídos del Instituto Nacional de Estadística (INE; www.ine.es)

El Parque Natural del Alto Tajo se caracteriza por un sustrato calizo y suelos pobres, poco profundos y básicos con origen Jurásico y Cretácico. El clima es continental mediterráneo con veranos calurosos y secos e inviernos fríos. La temperatura media anual es de 10.3 °C, siendo enero el mes más frío (8.22 °C y -3.19 °C de temperaturas máxima y mínima medias respectivamente) y julio (29.1 °C y 10.3 °C de temperatura máxima y mínima medias respectivamente) y agosto (28.7 °C y 10.3 °C de temperatura máxima y mínima medias respectivamente) los más cálidos (Fig 5). La precipitación total anual media es de 485.9 mm, siendo los meses más secos julio y agosto (24.5 mm y 24.8 mm, respectivamente) (Fig 5) (Agencia Estatal de Meteorología, estación de Molina de Aragón, 40° 50’ 40’’ N, 1° 53’ 07’’O, 1063 m.s.n.m, periodo 1951-2018).

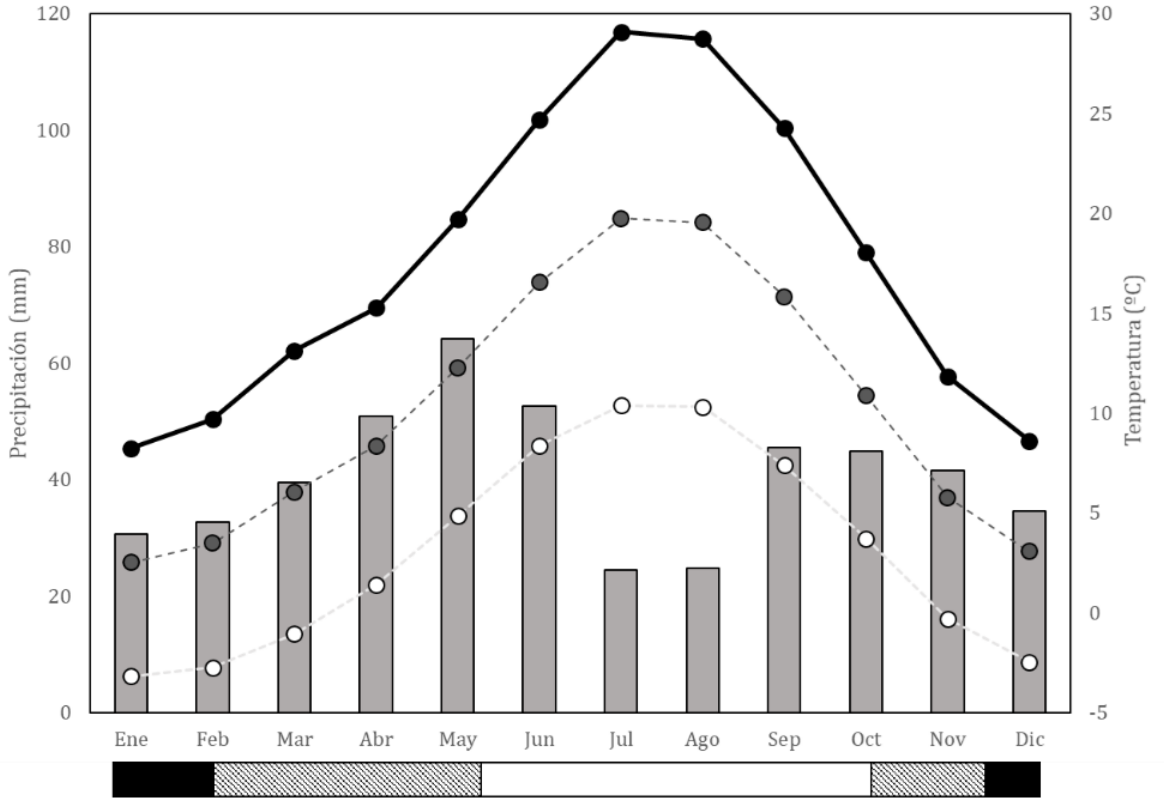


Figura 5. Climodiagrama de la estación de Molina de Aragón (Guadalajara, 40° 50’ 40’’ N, 01° 53’ 07’’ O, 1063 m s.n.m., 1951-2018). Las barras corresponden a la precipitación total mensual media y las líneas y puntos negros, grises y blancos a las temperaturas máximas, medias y mínimas mensuales, respectivametne, para el período 1951-2018. Las porciones rellenas y ralladas de la barra horizontal inferior indican los periodos en los que se producen heladas de forma segura y probable, respectivamente. Datos de la Agencia Estatal de Meteorología (AEMET).

Entre los municipios de la zona, esta tesis doctoral se ha desarrollado en tres localidades: Huertahernando (40°49′28″N, 2°17′08″OW), Ribarredonda (40°52′30″N 2°18′29″O) and Maranchón (41°02′55″N 2°12′13″O). En cada localidad se establecieron un total de 18 parcelas representativas del gradiente de expansión de la sabina albar. El gradiente de expansión de dicha especie se caracterizó identificando tres etapas: bosque maduro, zona de transición y frente de avance. La clasificación de cada una de las parcelas en una etapa u otra del gradiente se llevó a cabo mediante la comparativa de imágenes aéreas de 1957 y 2021 (Fig 6).

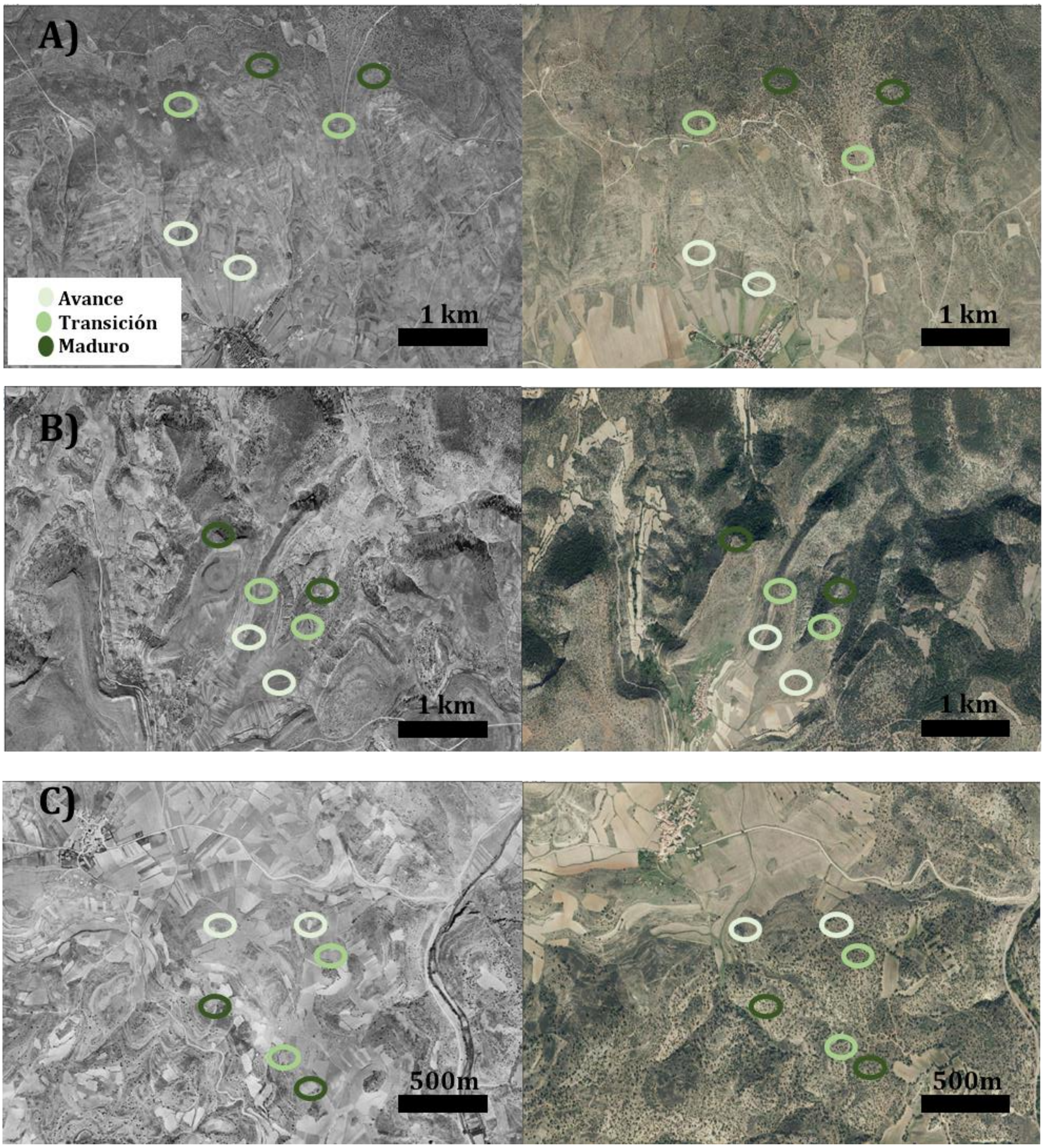


Figura 6. Fotografías áreas de las tres localidades de estudio: Maranchón (A), Huertahernando (B) y Ribarredonda (C) en 1957 (izquierda) y 2021 (derecha). En cada una de las imágenes se muestra el gradiente de expansión forestal. El tamaño de los círculos que señalan las parcelas no están en escala. Las imágenes han sido proporcionadas por el Instituto Geográfico Nacional (IGN; https://www.ign.es/web/ign/portal).

Especie de estudio

La sabina albar (*Juniperus thurifera* L.), es una especie arbórea de la familia Cupressaceae. Es una especie dioica con dimorfismo sexual. Sus ramas son más o menos patentes y forman una copa de aspecto piramidal. Sus hojas son todas decusadas y difieren entre juveniles y adultas. Las hojas juveniles miden 3-6 mm y son aciculares mientras que las adultas miden entre 1,5-2 mm y están imbricadas pero libres en el ápice. El gálbulo (fructificación carnosa) mide entre 7-8 mm y madura a los 2 años (Castroviejo et al., 1986).

Actualmente, su área de distribución se ve restringida al suroeste del Mediterráneo; concretamente existen poblaciones de sabina albar en España, Marruecos, Francia (incluyendo Córcega), Argelia y los Alpes italianos. La mayor parte del área de extensión de esta especie, aproximadamente un 90%, se encuentra en España (Costa et al., 1997), donde la sabina ocupa unas 600 000 ha del país, de las cuales aproximadamente 117 000 ha son de masas forestales puras de *J. thurifera* (Alonso Ponce et al., 2010).

Normalmente, es una especie dominante en bosques de baja densidad sobre suelos pobres y poco fértiles, generalmente rocosos y de escasa profundidad. *J. thurifera* puede establecerse en un amplio rango altitudinal, y se da en climas de tipo mediterráneo continental tanto árido como subhúmedo (Gauquelin et al., 1999). *J. thurifera* tolera un amplio rango de temperaturas (altas temperaturas en verano y temperaturas muy bajas y heladas frecuentes en invierno); por tanto, está adaptada a la sequía estival típica de ambientes mediterráneos, en los que las precipitaciones son moderadas o bajas y se concentran en invierno normalmente en forma de nieve, por lo que la precipitación útil es mucho menor que la nominal (Montesinos et al., 2009).

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Chapter 1

Carbon, nitrogen and inorganic phosphorus concentration and stock along a forest expansion gradient

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Abstract

Land-use changes are considered as one of the main drivers of global change. One of the main land-use changes in land abandonment which has promoted forest expansion in Southern and Western Europe. Past activities such as agriculture and farmland has shown land-use legacies that can endure for decades to centuries. Some legacies are related to concentration and stocks of C, N and P which can vary along a gradient of forest expansion into past agricultural lands. Our objective is to quantify the amount of soil organic carbon, total nitrogen, C/N ratio and inorganic phosphorous in forest floor and mineral floor along a gradient of forest expansion - from mature forest to expanding front on abandoned agricultural lands, considering two microhabitats – under the canopy and open areas. This study was carried out in Alto Tajo Natural Park (Guadalajara, Spain) where we established in three sites a gradient of forest expansion from mature forest to expanding front with a intermediate stage called transition zone. In each site, we selected one plot of each stage and established sampling points in three trees based on frequency of diameter class in each plot. We sampled forest floor and mineral soils under tree canopies and outside them. We found lower total nitrogen concentration and higher C/N ratio in forest floor at expanding front than in mature forests and transition zone. In mineral soils under canopy, total organic carbon and inorganic phosphorus concentrations were higher in mature forests than at expanding front. Moreover, total nitrogen concentration in mineral soil was higher in mature forest than at expanding front without differences between microhabitats (under canopy and open areas). Contrary, C/N ratio was higher under canopy than open areas without differences along the forest expansion gradient. Finally, we did not find differences in element stocks in mineral soils along the forest expansion gradient. Thus, past agricultural uses reduced C, N, and P concentration in forest floor and mineral soils. However, our study showed that forest established in past agricultural lands are as important as mature forests in terms of carbon and nutrient storage. For instance, we suggest the conservation of these new forest as a possible management strategy to global change mitigation.

Keywords: forest floor, *Juniperus thurifera*, land abandonment, land-use legacies, mineral soil

Introduction

Land-use history is the series of human uses, such as agriculture and grazing, that a site has experienced (Arce-Nazario, 2007; Ewers et al., 2013). These uses can promote, in part, land-use legacies that are abiotic and biotic properties found at a site due to the influence of human activities (Foster et al., 2003). Land-use changes are considered as one of the main drivers of global change. Agriculture and farmland practices have shown to promote changes in physical, chemical and biological properties that can endure for decades to centuries after land abandonment (Dupouey et al., 2002; Flinn & Vellend, 2005; Plue et al., 2008).

In many parts of Europe, the main land-use change is land abandonment, i.e., the cessation of agricultural and farmland activities, which has promoted natural forest expansion (Keenan, 2015). Land abandonment also occurs in areas with a long history of intense cultivation such as Mediterranean region (Kosmas et al., 2000). Agricultural practices such as soil tillage promote a degradation of soil quality (i.e., decrease aggregate stability and water holding capacity) (Gardi et., 2002). It has been shown that when agricultural practices cease and trees colonise these areas, a recover of soil quality and, associated ecosystem services (i.e. carbon sequestration, water regulation, and nutrient recycling) (Novara et al., 2017; Romero-Díaz et al., 2017). After natural revegetation of past agricultural lands, soil properties are improved, like organic matter content, soil structure and infiltration rate resulting in an effective protection against soil erosion (Kosmas et al., 2000).

Agricultural practices also include the use of fertilisers that promotes changes in nutrient content in soils due to fertilisation (Edmeades, 2003). A higher nutrient content due to past land uses has been associated to an improvement of tree performance in past agricultural lands compared to mature forests (Alfaro-Sánchez et al., 2019; Acuña-Míguez et al., 2020; Benavides et al., 2023). However, there is no consensus of the effect (positive or negative) of past land uses in carbon and nutrient contents. Several studies have found higher levels in carbon (C), nitrogen (N), C/N ratio and available phosphorus (P) in soils of formerly cultivated areas while other studies have found no differences, or even reduced levels of them (Koerner et al. 1997; Falkengren-Grerup et al. 2006; Verheyen et al. 1999; Maloney et al. 2008).

Therefore, during forest expansion into past agricultural lands, land use legacies related to C, N and P in soils could influence tree performance as well as tree establishment could also affect elemental composition by absorbing or releasing different elements (Bautista-Cruz & del Castillo, 2005). Soil C, N and P are important for the functioning of ecosystems (Vitousek et al., 2010; Du et al., 2020). Soil organic carbon maintains soil structure, increases water infiltration rates and water holding capacity, regulates nutrient supply, and stabilizes soils against erosion (Lorenz et al., 2019; Loveland & Webb, 2003). Nitrogen and phosphorus are the main constraints to net primary production in most terrestrial ecosystems (Du et al., 2020, LeBauer & Treseder, 2008). Thus, additional SOC accumulation depends on enough available N and P (Du et al., 2020, Vitousek et al., 2010). It is also interesting to analyse C/N, which is a useful indicator of soil organic matter decomposition (higher value of the ratio indicates that organic matter is accumulating faster than it is decomposing) (Brady & Weil, 2017) and inorganic phosphorus, which it is available for plants.

Forest floor is an important structural and functional component of forests since it determines soil nutrient dynamics by inputs via litterfall and outputs via litter decomposition (Covington et al., 1981). It is expected that nutrient concentration in forest floor varies along successional stages since chemical composition of leaves vary. Most studies that analyse this variation focus on studying forest floor of deciduous broadleaf versus coniferous trees (McGroddy et al., 2004; Augusto et al., 2015). However, it has been shown that also along a gradient of forest expansion of the same species, trees established in each successional stage also showed different leave chemical characteristics (Acuña-Míguez et al., 2020; Guerrieri et al., 2021). Thus, to understand differences in soil nutrient concentrations along the expansion gradient it is key to analyse past land use legacies in mineral soils but also analyse nutrients inputs related to forest floor.

This study focused on *Juniperus thurifera* L. a species of Mediterranean continental climate regions (Gauquelin et al., 1999; Montesinos et al., 2009). This species is colonising past agricultural lands over the past forty years (Gimeno et al., 2012; Velázquez et al., 2022). Our objective is to quantify the amount of soil organic carbon, total nitrogen, C/N ratio and inorganic phosphorous in forest floor and mineral floor along a gradient of forest expansion - from mature forest to expanding front on abandoned agricultural lands, considering two microhabitats - under the canopy and open areas. We expected greater soil organic carbon in mature forests than at expanding front due to soil maturity. In contrast, we expected that the use of fertilisers along time increases the quantity of inorganic phosphorous and total nitrogen at expanding front. Moreover, we expected that differences in quantity of different elements in mineral soils along the gradient are higher in open areas than under canopies because of the effect of tree establishment in soil C, N and P content.

Material & Methods

Experimental design

We established a gradient of *J. thurifera* forest expansion divided into three stages: mature forest, transition zone and expanding front in three sites in Alto Tajo Natural Park (Guadalajara, Spain). In each site and stage, we setup two plots (3 sites x 3 stages x 2 =18 plots). Thirty adult trees were measured in each plot (Villellas et al., 2020; Acuña-Míguez et al., 2020) We selected four trees in each plot based on frequency of diameter classes and established a subplot of 12 meters of radius around each tree to measure all trees inside it. We calculated tree density (nº tree / ha), quadratic sum diameter (cm; as the square root of the sum of the square of diameter/diameter squared of all stems of each tree) and basal area (m^2/ha) of each subplot.

Soil samples

Soil sampling was carried out in autumn 2021. In one stage of each site, we selected three of the four trees selected previously and we established sampling points under tree canopies and outside them and sampled forest floor and mineral soil.

Forest floor was collected using a frame of 0.25 x 0.25 m^2. Due to the low quantity of forest floor outside canopies, we analysed this fraction only under canopies. Forest floor samples did not differentiate among forest floor layers (litter, fragmented and hummus) and samples were treated as one layer. Samples were oven-dried at 65ºC in the laboratory and weighted.

At the same points where we collected forest floor, we collected samples of mineral soils at 30 cm depth when it was possible. All the soil samples were dried (65 °C) and sieved through a 2 mm sieve. Stone fraction was determined gravimetrically and was considered as the fraction greater that 2 mm. Due to the high stone content in this soil, we used sand cone method to estimate bulk density (DeLong et al., 2012). All samples were finely ground to analyse total organic carbon (TOC), total nitrogen (TN) and inorganic phosphorus. TOC and TN contents were analysed by dry combustion method using a total analyser (LECO HCN-600). Inorganic phosphorus was analysed by Olsen method (Olsen & Sommers, 1982)

Stock of organic carbon, nitrogen and inorganic phosphorus in mineral soils was determined using the following formula:

$$Stock = \sum Coni \cdot BDi \cdot di \ \cdot 1 \cdot STi \cdot 10$$

Where stock is the element stock (Mg ha^{-1}), $Coni$ is the element concentration at point i (kg Mg^{-1} soil), BDi is bulk density at point i (Mg m^{-3}), di is the thickness of depth at point i(m), STi is the stone fraction at point i and 10 is needed to express results in the correct units.

Statistical analysis

We used linear mixed models (LMMs) to analyse the effect of forest expansion gradient on tree density, quadratic sum and basal area, total organic carbon, inorganic phosphorus, total nitrogen and C/N ratio concentration in forest floor. We set as fixed effect the microhabitat and as random effect was the plot. Models of total organic carbon, total nitrogen, inorganic phosphorus and C/N ratio were fitted linearly. Total organic carbon and inorganic phosphorus were log transformed to conform normality,

Total organic carbon, inorganic phosphorus, total nitrogen and C/N ratio concentration and stocks in mineral soil and bulk density were analysed using LMMs. We set as fixed effect the microhabitat, the forest expansion gradient and the interaction between them and as random effect the plot. C/N ratio was log transformed to conform normality.

We finally selected the model with the lowest Akaike Information Criterion AIC (the best or most parsimonious models) using the dredge function from the *MuMIn* R package (Barton, 2020). The method was set to maximum likelihood (ML) during the fixed-effect model selection phase, although the final models are presented using restricted maximum likelihood (REML; Kuznetsova et al., 2017). Models fit was visually checked to ensure model assumptions. We calculated marginal and conditional R^2 with the *sjPlot* R package (Lüdecke et al., 2021).

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2021).

Results

Basal area was higher in mature forest than expanding front with intermediate value in transition zone (Table 1). Tree density was higher in mature forest and transition zone than in expanding front and quadratic sum was higher in mature forest than in transition zone and expanding front (Table 1).

Table 1. Mean ± standard deviation of tree density, quadratic sum and basal area in each stage. Different letters indicate significant differences.

	Tree density (tree/ha)	Quadratic sum (cm)	Basal Area (m^2/ha)
Mature forest	281 ± 57 a	13.36 ± 1.08 a	5.60 ± 0.98 a
Transition zone	205 ± 67 a	9.80 ± 0.83 b	2.33 ± 0.54 b
Expanding front	58 ± 13 b	9.54 ± 1.44 b	0.90 ± 0.30 c

We did not find differences among stages of forest expansion gradient in soil organic carbon, nor phosphorus concentration in forests soils (Fig 1A, B). Nitrogen content was lower in expanding front than transition zone and mature forests (Fig 1C) meanwhileC/N ratio was higher at expanding front than in the other two stages (Fig 1D) (Table S1).

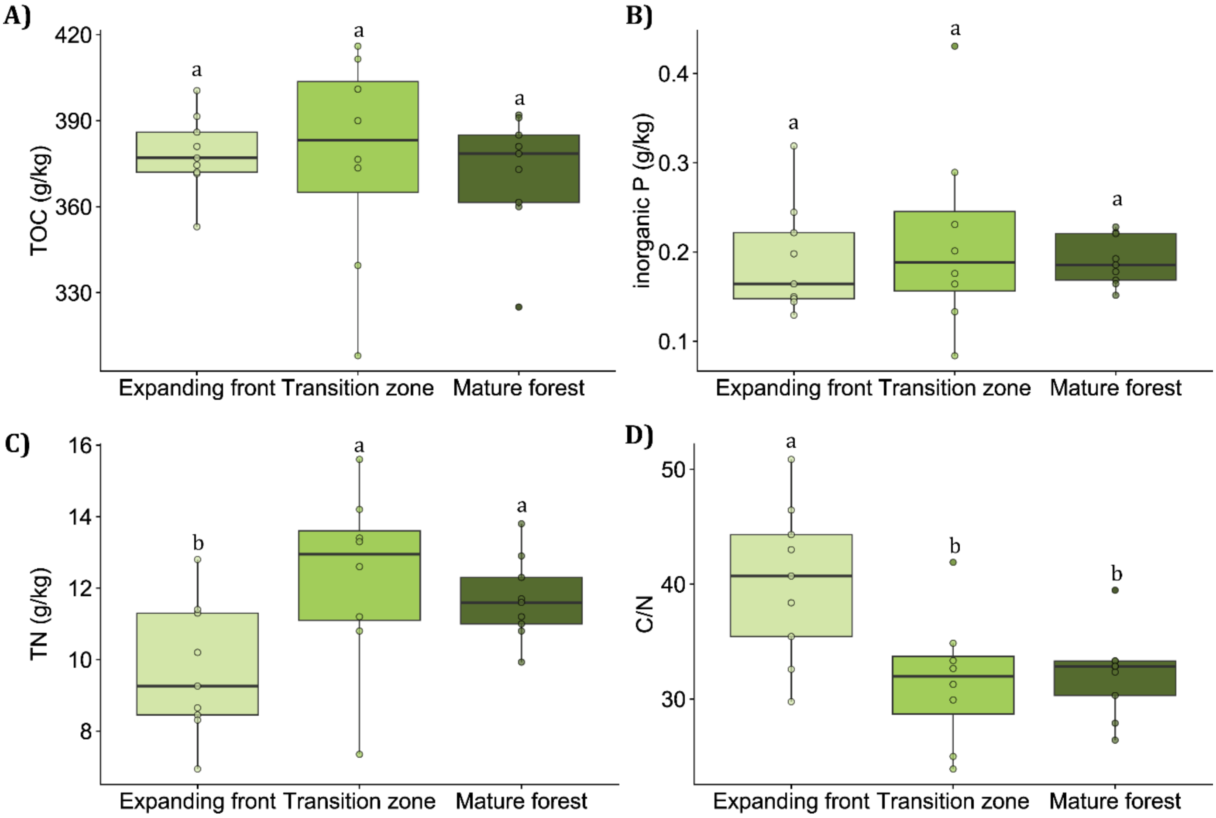

Figure 1. Effect of stages of forest expansion gradient on (A) total organic carbon (TOC), (B) inorganic phosphorous (inorganic P), (C) total nitrogen content (TN), and (D) C/N ratio in forest floor. Different letters indicate significant differences ($p<0.05$).

In mineral soils, carbon and phosphorus content increased across forest expansion gradient stages, but only under canopy, being significantly higher in mature forests compared with the expanding front, while no differences due to the expansion gradient were found in open areas (Fig 2A, B; Table S2). Nitrogen content also increased across forest expansion gradient without differences between microhabitats (Fig 2C, D; Table S2). C/N ratio did not vary along the gradient but was higher under canopy than in open areas (Fig 2E, F; Table S2).

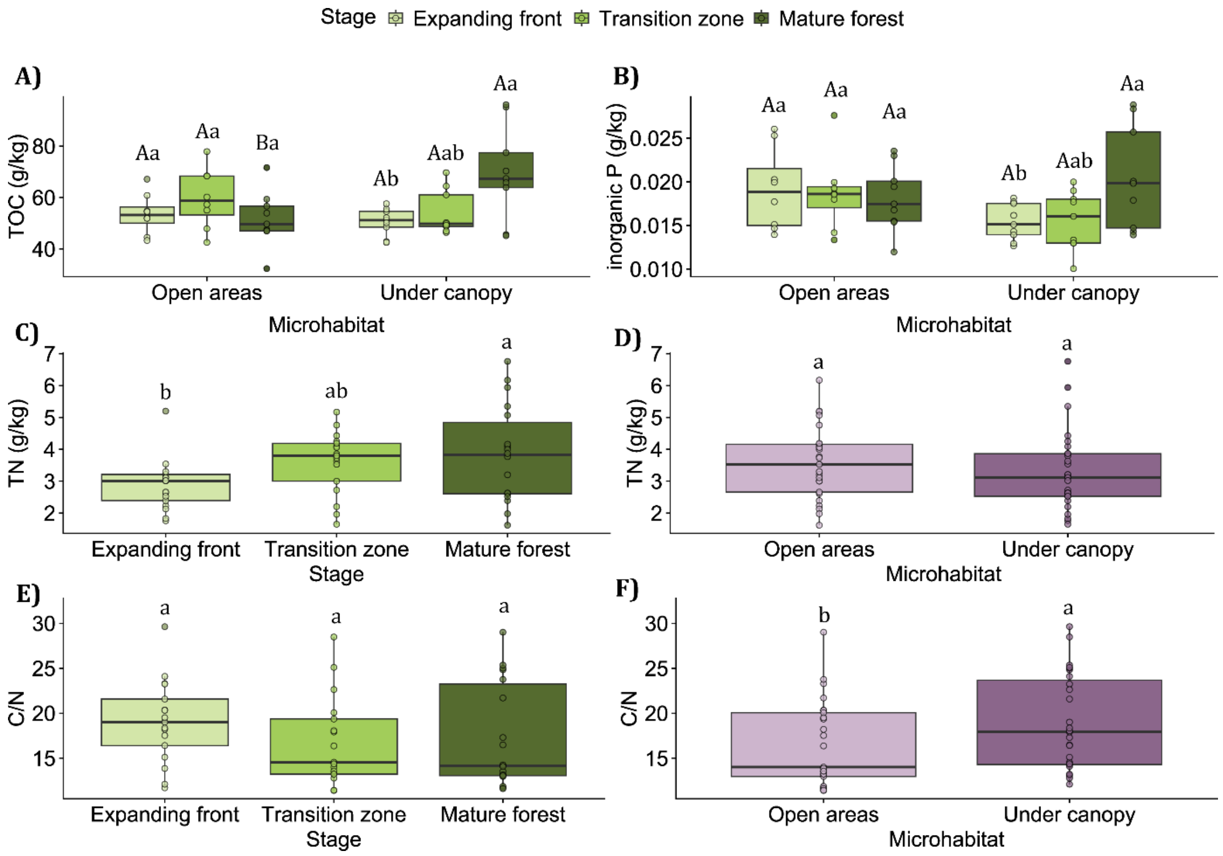

Figure 2. Effect of stages of forest expansion gradient (stages in green) and the microhabitat (purple) on (A) total organic carbon (TOC) (interaction between factors), (B) inorganic phosphorous content (inorganic P) (interaction between factors), (C, D) total nitrogen content (TN) and (E, F) C/N ratio in mineral soil. In graphic A and B, capital letters show differences between microhabitats for a given forest expansion stage, and lowercase show differences among forest expansion stages for each microhabitat ($p < 0.05$). From panel C to panel F different letters indicate significant differences between treatments of each factor ($p < 0.05$).

We did not find differences in bulk density, stone fraction and thickness of depth among stages of forest expansion gradient but bulk density was higher in open areas than under canopy (Table 2)

Table 2. Mean ± standard deviation of bulk density (Mg/m^3), stone fraction and thickness of depth (m) in each stage and microhabitat. Different letters indicate significant differences.

		Bulk Density	Stone fraction	Depth
Stage	Mature forest	0.22 ±0.03 a	0.67 ± 0.03 a	0.099 ± 0.002 a
	Transition zone	0.23 ± 0.03 a	0.64 ± 0.04 a	0.096 ± 0.003 a
	Expanding front	0.22 ±0.03 a	0.65 ± 0.03 a	0.103 ± 0.003 a
Microhabitat	Under canopy	0.17 ± 0.01 b	0.67 ± 0.02 a	0.100 ± 0.002 a
	Open areas	0.28 ± 0.03 a	0.65 ± 0.03 a	0.099 ± 0.003 a

We did not find differences in carbon, nitrogen and phosphorus stocks among stages or between microhabitats (Table. 3).

Table 3. Mean ± standard deviation of C stock (Mg/ha), N stock (Mg/ha) and inorganic P stock (Mg/ha) in each stage and microhabitat. Different letters indicate significant differences.

		C stock	N stock	P stock
Stage	Mature forest	12.85 ± 1.75 a	0.76 ± 0.11 a	0.005 ± 0.001 a
	Transition zone	14.07 ± 2.45 a	0.89 ± 0.17 a	0.004 ± 0.001 a
	Expanding front	16.35 ± 4.03 a	0.94 ± 0.24 a	0.005 ± 0.001 a
Microhabitat	Under canopy	13.22 ± 3.03 a	0.75 ± 0.10 a	0.004 ± 0.0004 a
	Open areas	15.66 ± 3.03 a	0.98 ± 0.18 a	0.005 ± 0.001 a

Discussion

Our study shows greater differences in element content in mineral soil than forest floor along the gradient. Total organic carbon, inorganic phosphorus and total nitrogen were higher in mature forests than expanding fronts. However, differences in element content in mineral soil do not promote differences in element stocks. Thus, we demonstrate that land-use legacies, including agricultural practices such as fertilization, did not lead to an increase in nitrogen and inorganic phosphorus content in soils at the expanding front. Moreover, soil maturity did not contribute to an increase in nutrient stocks in mature forests.

We found greater total nitrogen content and lower C/N ratio in mature forest and transition zone than expanding fronts in forest floor without differences in organic carbon and inorganic phosphorus content along the forest expansion gradient. Previous studies have found differences in leave nutrient content along a gradient of forest expansion that are related to tree establishment in open areas (Guerrieri et al., 2021, Benavides et al., 2023). These differences could be maintained in litterfall that would promote differences in forest floor along the gradient. Our study showed that differences found in C/N ratio are related to nitrogen and not to organic carbon because we did not find differences in organic carbon content along the expansion gradient (Fig 1). Higher C/N ratio in forest floor of expanding front than mature forests and transition zone indicate a low litter quality in this stage (Wang et al., 2015). C/N ratio is related to nitrogen immobilization and mineralization during decomposition (Swift et al., 1979). Thus, an increase in C/N ratio produce an acceleration in mineralization of soil organic matter, rapidly leading to an N limitation (Wang et al., 2015). Our results suggest that mature forests and transition zone are less nitrogen limited environment contrary to our expectations of nitrogen rich soils at expanding fronts due to past uses. However, future studies should analyse nitrogen bioavailability content and not only total nitrogen content since it could be more related to fertilisation.

Regarding mineral soil, mature forest showed greater nutrient content than expanding fronts in terms of organic carbon and inorganic phosphorous (only under canopy) and total nitrogen. Agricultural practices are related to the use of fertilisers to avoid N and P limitation and increase productivity. However, previous studies have found that cropping also increase vulnerability to soil erosion resulting in losses of organic carbon and total nitrogen (Li et al., 2020, Tan et al., 2015) that also increase due to harvested agricultural products. Forests do not typically experience significant nutrient drains as biomass is either not harvested, or, if it is harvested, involves mostly woody biomass with low nutrient content. Therefore, our results showed that mature forests can maintain a higher nutrient content due to non-previous land use. Differences found on C/N ratio between microhabitats could be due to the type of vegetation in each microhabitat that has different litter quality that promote differences in decomposition and mineralization processes. Inorganic phosphorus content was greater in mature forests that expanding front which is contrary to previous studies that showed a decrease in inorganic P content along a forest expansion gradient (De Schrijver et al., 2012). It has been described that soils of mature forests are more acid than soils of recent forests (Falkengren-Grerup et al., 2006; Abadie et al., 2018). In more acidic soils, P tends to react with cations such as Al^{3+} and Fe^{3+} to form compounds which are not bioavailable (Seubert et al., 1977; Korning et al., 1994). This could explain why we found a decrease in inorganic P content in expanding front although a greater content would be expected due to past land uses.

Carbon, nitrogen and inorganic phosphorus stocks in mineral soils did not vary along the gradient or microhabitats. Bell et al., (2021) showed that mean annual temperature plays a more relevant role in soil carbon stocks in past agricultural lands than previously thought. In our area mean annual temperature is 10.7 °C (AEMET data) meanwhile the most favourable mean annual temperature to increase soil carbon stocks in during secondary succession between 13-17 °C. In addition, type of past crop (woody versus annual) (Bell et al., 2021) and intensity of past land uses could influence soil capacity to soil organic carbon and nutrient accumulation. Thus, future studies should investigate which type of past uses happen in this area to better understand this pattern during secondary succession. Moreover, it has been showed that the capacity of soil to store carbon in N-limited ecosystems, such Mediterranean ecosystems, is mainly governed by changes in total N stocks (Alberti et al., 2011) which could be our case during *J. thurifera* secondary succession due to total N stocks did not vary along the gradient Notice that has to be consider that the high variability in our data (element content, bulk density, stone fraction and thickness of depth) own of soil heterogeneity has to be consider and it could disguise possible differences in element stocks.

Conclusions

Our study highlighted the importance of past agricultural uses in carbon, nitrogen and inorganic phosphorus content in forest floor and mineral soils. We showed that during secondary succession of *Juniperus thurifera* forests -from mature forest to expanding fronts- nitrogen concertation in forest floor and total organic carbon, total nitrogen and inorganic phosphorus concentration in mineral soils decreased. We suggest that forest floor differences are related to the process of tree establishment in past agricultural lands meanwhile soil mineral differences are related to soil maturity and legacies of agricultural practices. We did not find differences among stocks along the forest expansion gradient suggesting that new forests establish in past agricultural lands have the potential of mature forests to store carbon, nitrogen and inorganic phosphorus. For this reason, we suggest the conservation of these new forest as a possible management strategy to global change mitigation.

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Supplementary information chapter 1

Table S1. Model predictions of total nitrogen and C/N ratio in forest floor. *** *p*-value <0.001, ***p*-value<0.01 * *p*-value <0.05

	Estimate	Std. Error	R2
Total nitrogen			
(Intercept)	9.701***	0.635	
Transition zone	1.991*	0.898	
Mature forest	2.605**	0.925	
			0.262
C/N			
(Intercept)	40.170***		
Transition zone	-8.081**		
Mature forest	-8.559**		0.346

Table S2. Estimated fixed effects and random effects for total organic carbon, inorganic phosphorus and total nitrogen concentration and C/N ratio in mineral soils. C/N ratio estimates are log transformed. *** *p*-value <0.001, ***p*-value<0.01 * *p*-value <0.05

	Estimate	Std. Error	R2m	R2c
Total organic carbon				
Fixed effects				
(Intercept)	53.619***	3.957		
Microhabitat [Under canopy]	-3.052	5.439		
Stage[Transition zone]	6.094	5.596		
Stage [Mature forest]	-1.930	5.439		
Microhabitat [Under canopy]*Stage [Transition zone]	-2.594	7.692		
Microhabitat [Under canopy]*Stage [Mature forest]	21.024**	7.578		
Random effects	Std. Dev.			
Site	0.001			
Residuals	0.025			
Whole model			0.253	0.263
Inorganic phosphorus				
Fixed effects				
(Intercept)	0.0188***	0.0019		
Microhabitat [Under canopy]	-0.0034	0.0017		
Stage[Transition zone]	-0.0004	0.0018		
Stage [Mature forest]	-0.0007	0.0018		
Microhabitat [Under canopy]*Stage [Transition zone]	0.0006	0.0025		
Microhabitat [Under canopy]*Stage [Mature forest]	0.0057*	0.0024		
Random effects	Std. Dev.			
Site	0.0025			
Residuals	0.0035			
Whole Model			0.147	0.427
Total nitrogen				
Fixed effects				
(Intercept)	2.832**	0.469		
Stage[Transition zone]	0.714*	0.343		
Stage [Mature forest]	0.987**	0.338		
Random effects	Std. Dev.			
Site	0.695			
Residuals	0.999			
Whole Model			0.106	0.398
C/N ratio				
Fixed effects				
(Intercept)	2.877***	0.144		
Microhabitat [Under canopy]	0.112*	0.055		
Stage[Transition zone]	-0.132	0.068		
Stage [Mature forest]	-0.126	0.067		
Random effects	Std. Dev.			
Site	0.229			
Residuals	0.199			
Whole Model			0.071	0.599

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Chapter 2

Natural forest regeneration as a management strategy to restore soil functioning of abandoned lands

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Abstract

1. Land abandonment is promoting forest expansion into abandoned agricultural lands in Europe. This process leads to changes in ecosystems that affect several ecosystem services. Soil microbial communities play a key role in forest functioning. However, the relationship among forest expansion, microbial communities and soil functioning is still unknown.

2. In this study, we used a gradient of *Juniperus thurifera* expansion on abandoned agricultural lands to examine the relationship between soil microbial groups and soil functioning along a gradient of forest expansion - from mature forest to expanding front - on abandoned agricultural lands, considering two microhabitats - under the canopy and open areas. Specifically, our objectives were 1) to evaluate how soil physico-chemical and microbial properties varied along the forest expansion gradient and between microhabitats also considering their interaction; and 2) to decipher the pathways by which soil properties (physical-chemical, microbial), linked to the forest expansion gradient and the microhabitat, control the carbon and nutrient cycling in soils. In Alto Tajo Natural Park (Spain), we replicated in three sites a gradient of forest expansion (18 plots). We selected four trees in each plot and sampled soil under the canopy and outside it and analysed soil properties, microbial community structure and abundance (using PLFAs-NFLAs) and seven potential enzymatic activities related to C, N and P cycling.

3. Forest expansion gradient had a negative direct effect on P-cycling and a positive indirect effect on soil microbial groups and functioning through its direct effect on OM and pH. The microhabitat showed a positive direct effect on the abundance of actinomycetes and arbuscular mycorrhizal fungi abundances and on C and P cycling. Moreover, bacterial abundance played a key role *in J. thurifera* soil functioning.

4. Synthesis and applications: Despite the lower soil activity and microbial abundance of expanding areas compared to mature forests, soils of recently colonised areas have the potential to reach mature forest soil functioning. Therefore, forest management should consider preserving advancing fronts to promote ecosystem recovery.

Keywords: agricultural lands, enzymatic activity, *Juniperus thurifera,* land-use change, Mediterranean forests, NFLAs, PLFAs

Introduction

The abandonment of agricultural lands has been considered the main land-use change in several European landscapes for decades (Macdonald et al., 2000). Because of this process, the forest area is progressively increasing in Eastern and Southern Europe (Palmero-Iniesta et al., 2021). In Spain, forest expansion on past agricultural lands is an ecological process which has increased approximately a 15% during the last decades (Vilà et al., 2017). Natural regeneration promotes substantial changes in ecosystem structure, species composition, resource availability and functioning (Espelta et al., 2020; Benavides et., 2022; Acuña-Míguez et al., 2023), and a range of positive and/or negative effects on ecosystem services have been reported (Martín-Forés et al., 2020). It is essential to evaluate forest expansion and its associated management because it may impact biodiversity, as well as carbon and nutrient stocks and dynamics (Vilà-Cabrera et al., 2017; Ameztegui et al., 2021).

Microbial communities are key actors in forest ecosystems as direct mediators of carbon and nutrient cycling in soils (van der Heijden et al., 2008; Graham et al., 2016). In Mediterranean forests constrained by drought, heat waves and poor soil fertility, soil microorganisms are crucial for ecosystem functioning (Pérez-Izquierdo et al., 2017; Castaño et al., 2018; Flores-Rentería et al., 2018; Prieto-Rubio et al., 2023). Bacteria and fungi are responsible for about the 90% of all organic matter decomposition in soils (McGuire et al., 2010). These microbial groups have contrasted phenotype and phylogeny, which are at the origin of their divergent colonisation and functional abilities. For example, fungi are thought to be more tolerant to drought than bacteria and are mainly involved in the degradation of recalcitrant organic matter, while bacteria mainly regulate the fast carbon cycling pathways in soil (Rousk & Bååth, 2007; Kirchman, 2012). However, recent co-occurrence network studies point towards much more complex scenarios to explain the response of bacterial and fungal communities to environmental variations (Gao et al., 2022).

Agriculture may promote long term effects (i.e. land-use legacies) on soil properties, such as increased soil fertility (Flores-Rentería et al., 2015) and pH (Falkengren-Grerup et al., 2006; Abadie et al., 2018) and decreased organic matter content (Zornoza et al., 2009) and microbial biomass abundances or microbial diversity (Compton and Boone, 2000; van der Heijden et al., 2008). In turn, forest colonisation of abandoned lands promotes important changes in aboveground factors (vegetation structure and cover) and belowground ones (soil physical-chemical and microbial properties). For example, increased vegetation cover in former agricultural lands causes changes in carbon cycling and CO_2 sequestration through the accumulation of carbon in the tree biomass (Vilà-Cabrera et al., 2017). Tree establishment can impact the storage and residence time of soil organic matter due to changes in the quality and quantity of litter that enters the soil and associated changes in edaphic properties (Guidi et al., 2014). Moreover, tree establishment can also modify soil microclimatic conditions via provision of shade and soil temperature insulation (Hagedorn et al., 2014). In forest expansion, shifts in leaf litter composition from herbaceous debris to the more recalcitrant characteristic of trees lead to increased litter residence times and reduced microbial-mediated mobilisation of nutrients in soil (Roberts and Jones, 2000). Both, land-use legacies together with increased vegetation cover have been shown to exert effects on soil biological activity in Mediterranean forest ecosystems (García-Morote et al., 2012; Correia et al., 2021). In fact, soil microbial communities are often the most sensitive and rapid indicator of perturbation and land use changes (Zelles et al., 1994). Similar to trees, microbial communities colonise abandoned agricultural lands, a progressive settlement that is likely regulated by resource availability, environmental stress and biological interactions (Sun et al., 2017). Forest expansion into former agricultural lands can thus modify the structure and/or activity of soil microbial communities and, consequently induce changes in soil biogeochemical processes. However, the mechanisms and extent to which forest expansion affects soil abiotic and biotic properties remains largely unknown.

In this study, we used *J. thurifera* as the study species due to the substantial expansion of its forests over the past forty years (Gimeno et al., 2012; Velázquez et al., 2022). We examine the relationship between soil microbial groups and soil functioning along a gradient of forest expansion - from mature forest to expanding front - on abandoned agricultural lands, considering two microhabitats - under the canopy and open areas. Specifically, our objectives were 1) to evaluate how soil physico-chemical and microbial properties varied along the forest expansion gradient and between microhabitats also considering their interaction; and 2) to decipher the pathways by which soil properties (physical-chemical, microbial), linked to the forest expansion gradient and the microhabitat, control the carbon and nutrient cycling in soils. We hypothesised that (i) mature forests will show greater organic matter content and total soil microbial biomass and fungal abundance than areas recently colonised due to land use legacies and soil maturity. (ii) As *J. thurifera* forests are open woodlands, we expected large differences in organic matter content and microbial communities between soil microhabitats, with greater OM, microbial abundance and microbial activity under canopy than in open areas. (iii) These variations in OM and microbial communities were expected to prompt direct changes in the soil functioning, affecting enzymatic activities related to the cycling of labile and reluctant organic compounds.

Materials and Methods

Experimental design and sampling

This study was conducted in the Alto Tajo Natural Park (Guadalajara, Spain). We sampled across a gradient of forest expansion consisting of three stages: mature forest, transition zone and expanding front, which was replicated at three sites: Huertahernando (40°49′28″N, 2°17′08″OW), Ribarredonda (40°52′30″N 2°18′29″O) and Maranchón (41°02′55″N 2°12′13″O) (see Benavides et al., 2022 for further details). In each site, there were two plots at each stage of forest expansion (18 plots in total). Based on the frequency of tree diameter class within each plot, we selected four trees with average diameter in each plot and we sampled under the canopy and outside it in each of the selected trees (8 samples per plot) (Table S1). The litter layer of forest floor weas removed and then three soil subsamples (10-15 cm depth) where collected and pooled into a single composite sample. Once in the lab, roots were manually removed and soil was homogenized and sieved (2 mm mesh). A fresh soil portion was used to determine soil gravimetric moisture content by lost weight of samples oven-dried at 105ºC for 48h. Two soil subsamples were preserved at -20ºC and -80ºC for enzymatic and PLFAs/NLFAs analyses, respectively. The remaining soil was air-dried to determine pH (1:5, w:v in H2O) and organic matter (OM) content by loss on ignition at 400°C for 4h.

Measurement of extracellular enzymatic activities

We determined seven potential enzymatic activities in soils related to carbon, nitrogen and phosphorous cycling (Pierre-Emmanuel et al., 2016; Pérez-Izquierdo et al., 2017) under optimal controlled conditions: β-glucosidase (EC 3.2.1.3) and cellobiohydrolase (EC 3.2.1.91) that are cellulose-hydrolysing enzymes (named labile-C); β-xylosidase (EC 3.2.1.37) and β-glucuronidase (EC 3.2.1.31) that are hemicellulose-hydrolyzing enzymes (named moderately labile-C); alkaline phosphatase activity (EC 3.1.3.2) that hydrolyzes

P-containing organic compounds; and chitinase (EC 3.2.1.14) and leucine-aminopeptidase (EC 3.4.11.1), that respectively cleaving chitin and polypeptides, and hence mobilizing N-enriched residues. We followed the experimental procedure described previously to measure these enzymes (Pérez-Izquierdo et al., 2017). Briefly, 1 g of soil was incubated (100 rpm, at 25 ºC overnight) in the corresponding buffer (Tris-maleate 40 mM, pH 8 for leucine aminopeptidase; Tris-acetate 10 mM, pH 11 for alkaline phosphatase; and Tris-acetate 10 mM, pH 4.5 for the rest of enzymes). After soil incubation, all enzymes were determined by fluorogenic assays that were performed by using the substrates methylcoumarin (AMC) for leucine aminopeptidase and methylumbelliferone (MU) for the rest of enzymes. Fluorescence was measured with a Victor microplate reader (Perkin-Elmer Life Sciences, Massachusetts, USA), at wavelengths of excitation/emission of 355/460 nm, and results were expressed as pmol mg soil^{-1} h^{-1}.

Soil microbial community: fatty acids analyses

We extracted phospholipids (PLFAs) and neutral lipids (NLFAs) fatty acids from 0.5 g of freeze-dried soil using the procedure described by Frostegård et al. (1993) with the modifications proposed by Buyer and Sasser (2012). Firstly, internal standards were prepared for PLFAs and NLFAs, by respectively dissolving either 40.9 mg of PLFA C19 standard (1,2-dinonadecanoyl-sn-glycero-3-phosphocholine) or 22.7 mg of NLFA C13 standard (Glyceril tritridecanoate) in 20 ml of 1:1 chloroform:methanol. Lipids was extracted from the soil with a citrate buffer:methanol:chloroform 1:2.5:1.25 mixture (Bligh-Dyer extractant), with the corresponding internal standard (added at a rate of 1 µl ml-1). Lipids were separated by solid phase extraction using chloroform to elute NFLAs and an eluting solution of methanol:clhroform:water, 5:5:1 for PLFAs. Fatty acids were transesterified and the resulting fatty acid methyl esters (FAMEs) were dissolved in hexane before analysis. Samples were measured on a gas chromatograph fitted with a flame ionization detector (Agilent 7890A Gas chromatograph, Santa Clara, CA, USA). PLFAs and NLFAs abundances were expressed in nmol g-1 dry soil. The resulting chromatography peaks were integrated, and the area transformed to concentration with the internal standard. Peaks were grouped by microbial groups: 10Me 16:0 and 10Me 18:0 for Actinomycetes; i14:0, a15:0, i15:0, i16:0, a17:0 and i17:0 for Gram-positive (Gram^{+}) bacteria; 16:1ω7c, 16:1ω7t, 16:1ω9, cy17:0, 18:1ω7 and cy19:0 for Gram-negative (Gram$^-$) bacteria; 15:0, 17:1 ω8 and 17:0 used as non-specific bacterial markers; 18:2ω6,9 for fungi; and NLFA 16:1ω5 for arbuscular mycorrhizal fungi (AMF) as indicated in the literature (Frostegård & Bååth, 1996; Kramer & Gleixner, 2006; Dungait et al., 2011; Williams et al., 2014; Lekberg et al., 2022). Total bacteria were calculated as the sum of all the peaks assigned to bacteria (also non-specific bacterial markers) and total fungi were calculated as the sum of all the peaks (fungi and AMF peaks). Total microbial biomass was calculated as the sum of these peaks plus the unspecific peaks (i.e., general markers, not specific of any microbial group): 14:0, Br16:0, 16:1ω5, 16:0, Br18:0, 18:1ω9, 18:1, 18:0, 20:0. Fungal to bacterial and Gram+ to Gram- bacteria ratios were calculated by dividing the sum of the respective markers.

Statistical analyses

We used linear mixed models (LMMs) to analyse the effect of the forest expansion gradient, microhabitat and the interaction between both factors on soil organic matter, pH, enzymatic activities, and microbial biomass (total and by group: bacteria, Gram$^+$, Gram$^-$, Fungi, AMF). We set as fixed effects the forest expansion gradient stages, the microhabitat and the interaction between both and tree nested within site as random factor. β-glucosidase, cellobiohydrolase, chitinase, β-glucuronidase and β-xylosidase were log-transformed to meet normality. We reduced all models to those with the lowest Akaike Information Criterion AIC (the best or most parsimonious models) using the dredge function from the *MuMIn* R package (Barton, 2020). The method was set to maximum likelihood (ML) during the fixed-effect model selection phase, although the final models are presented using restricted maximum likelihood (REML; Kuznetsova et al., 2017). Models fit was visually checked to ensure model assumptions. We calculated marginal and conditional r^2 with the *sjPlot* R package (Lüdecke et al., 2021)

We grouped potential soil enzymatic data on labile C cycling (β-glucosidase, cellobiohydrolase), moderated-labile C cycling (β-glucuronidase, β-xylosidase), P cycling (alkaline phosphatase) and N cycling (chitinase, leucine-aminopeptidase). These groupings are well-established biological indicators of the microbial nutrient demand and linked to carbon and nutrient cycling in soils (Sinsabaugh et al., 2008; Waring et al., 2014; Zuccarini et al., 2022). The correlations among organic matter, pH, abundance of different soil microbial groups and enzymatic activities were firstly explored by Spearman's test.

We used Structural Equation Modelling (piecewise SEM; *piecewiseSEM* package (Lefcheck, 2016) to analyse the effects of the forest expansion gradient stage, the microhabitat (under canopy/open areas), soil properties and biomass of microbial groups on carbon and nutrient cycling (grouped soil enzymatic activities) (Fig S1). The main difference between piecewise SEM and traditional SEMs is that the first allows the incorporation of hierarchical structures (random factors). The model goodness-of-fit was assessed through direct separation tests (d-sep; Shipley, 2000) of conditional independence expressed on Fisher's C statistic terms (Lefcheck, 2016).

We performed a SEM for each carbon or nutrient cycle, where each one was modelled by forest expansion, microhabitat, soil properties and microbial group abundances using LMMs. We set out forest gradient stages and microhabitats to directly affect carbon and nutrient cycles, as well as, indirectly through soil properties and soil microbial groups (Fig S1). Moreover, soil physico-chemical properties could affect directly and indirectly, through soil microbial groups, to carbon and nutrient cycles. Finally, soil microbial groups could affect carbon and nutrient cycles directly or by regulating the abundance of other microbial groups (indirectly) (Fig S1). Firstly, all models were run with the site as random effect to consider the autocorrelation within sites. When no random effect of site was detected (i.e., those with response variable fungi, AMF and Gram$^-$ abundances and moderately-labile C, N-cycling and P-cycling), linear and not mixed models were used.

Given that stage of forest expansion gradient and microhabitat were categorical variables, we recoded the values of both factors (stages: expanding front=1, transition zone=2 and mature forest=3; and microhabitat: open area=1 and under canopy=2) and then model as numeric. Thus, the coefficient represented the expected change in the response variable as going from the expanding area to mature forests and from open to under canopy (Lefcheck, 2021). Moreover, we normalised all variables using the mean value and standard deviation (i.e. scaling).

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2021).

Results

Soil physicochemical properties

Soil organic matter gradually increased across forest expansion gradient stages, but only under canopy, being significantly higher in mature forests compared with the expanding front, while no differences due to the expansion gradient were found in open areas (Fig 1A, Table S2). Contrary to OM, in the case of pH, no interaction among factors was found, and significantly less alkaline soils were found under canopy than in open areas (Fig 1B, Table S2), and in the transition zone and mature forests compared with the expanding front (Fig 1C, Table S2).

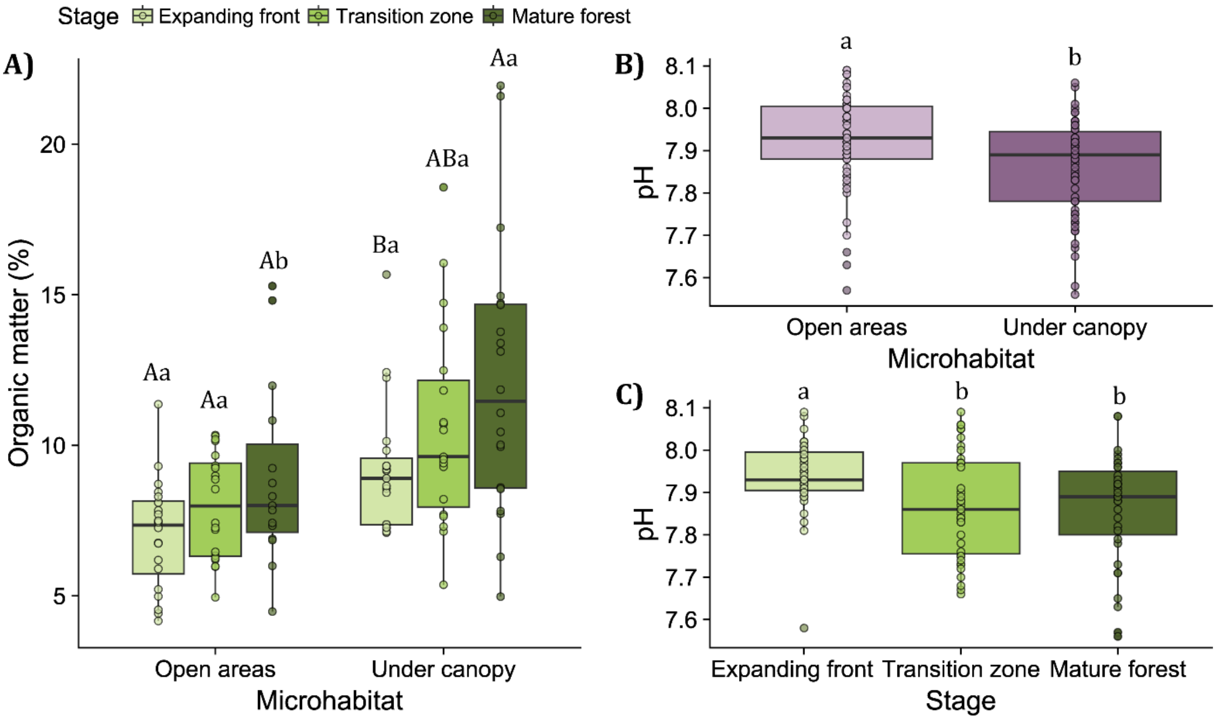

Figure 1. Effect of forest expansion gradient (stages in green) and the microhabitat (purple) (fix factors) on (A) soil organic matter (interaction between factors), and (B, C) pH, analysed by LMMs with the site as random factor. In graphic A, capital letters show differences among forest expansion stages for each microhabitat, while lowercase letters show differences between microhabitats for a given forest expansion stage ($p < 0.05$). In graphics B and C, different letters indicate significant differences among treatments ($p < 0.05$).

Microbial Biomass and enzymatic activities

A significant interaction of the factors forest expansion gradient and microhabitat was observed for total soil microbial biomass (Fig 2A, Table S3). In open areas, total soil microbial biomass did not differ among forest expansion stages, while under canopy it was significantly higher in mature forest than at the expanding front (Fig 2A). Total soil microbial biomass was significantly higher under canopy than in open areas but only at the mature forest stage, while no differences between microhabitat treatments were found in the transition zone or at the expanding front (Fig 2A).

The biomass of $Gram^+$ and $Gram^-$ bacteria, Actinomycetes and Fungi in soils was significantly higher in the mature forest than at the expanding front (Fig 2 B, C, D, F). Regarding the microhabitat, the biomass of $Gram^+$ and $Gram^-$ bacteria, Fungi and AMF, as well as the fungi to bacteria ratio were significantly higher under canopy (Fig 2 B, C, E, F, H) than in open areas, while the $Gram^+$ to $Gram^-$ ratio was lower (Fig 2G).

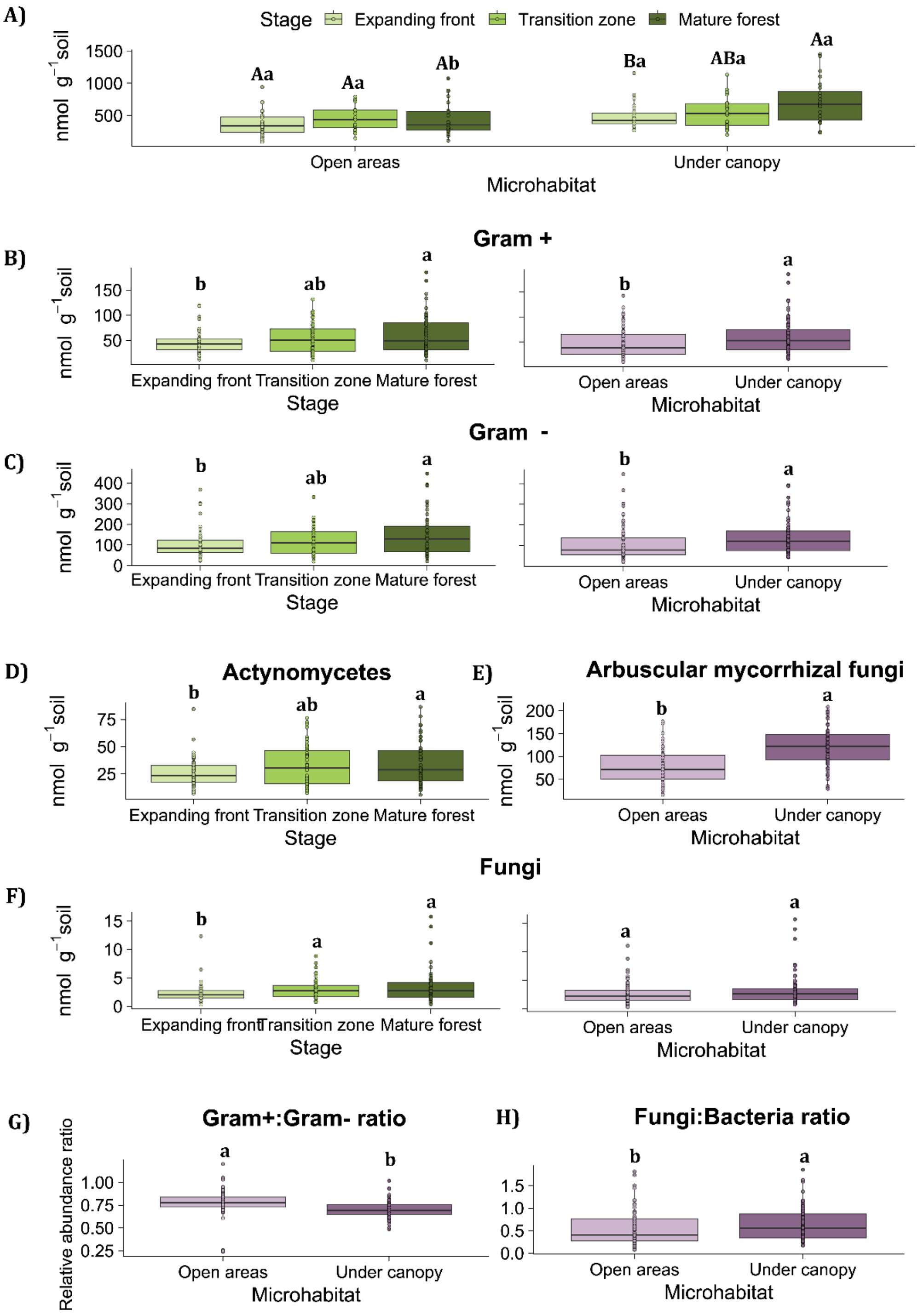

Figure 2. Effect of the forest expansion gradient (stages in green) and the microhabitat (purple) (fix factors) on (A) Total Soil Microbial Biomass (interaction between factors), (B) Gram$^+$ bacteria, (C) Gram$^-$ bacteria, (D) Actinomycetes, (E) Arbuscular mycorrhizal fungi (AMF), (F) Fungi (G) Gram$^+$ to Gram$^-$ ratio, and (H) Fungi to Bacteria ratio, analysed by LMMs with the site as random factor. In graphic A, capital letters show differences among forest expansion stages for each microhabitat, and lowercase letters show differences between microhabitats for a given forest expansion stage ($p < 0.05$). From graphic B to panel G different letters indicate significant differences between treatments of each factor ($p < 0.05$).

The forest expansion gradient stages did not differ on their soil enzymatic activity, except in the case of leucine (N-cycle) that was significantly higher in the mature forest and transition zone compared with the expanding front (Fig 3, Table S4). The enzymatic activity of soils was significantly higher under canopy than in open areas (Fig 3), except for leucine-aminopeptidase that was similar in both microhabitats.

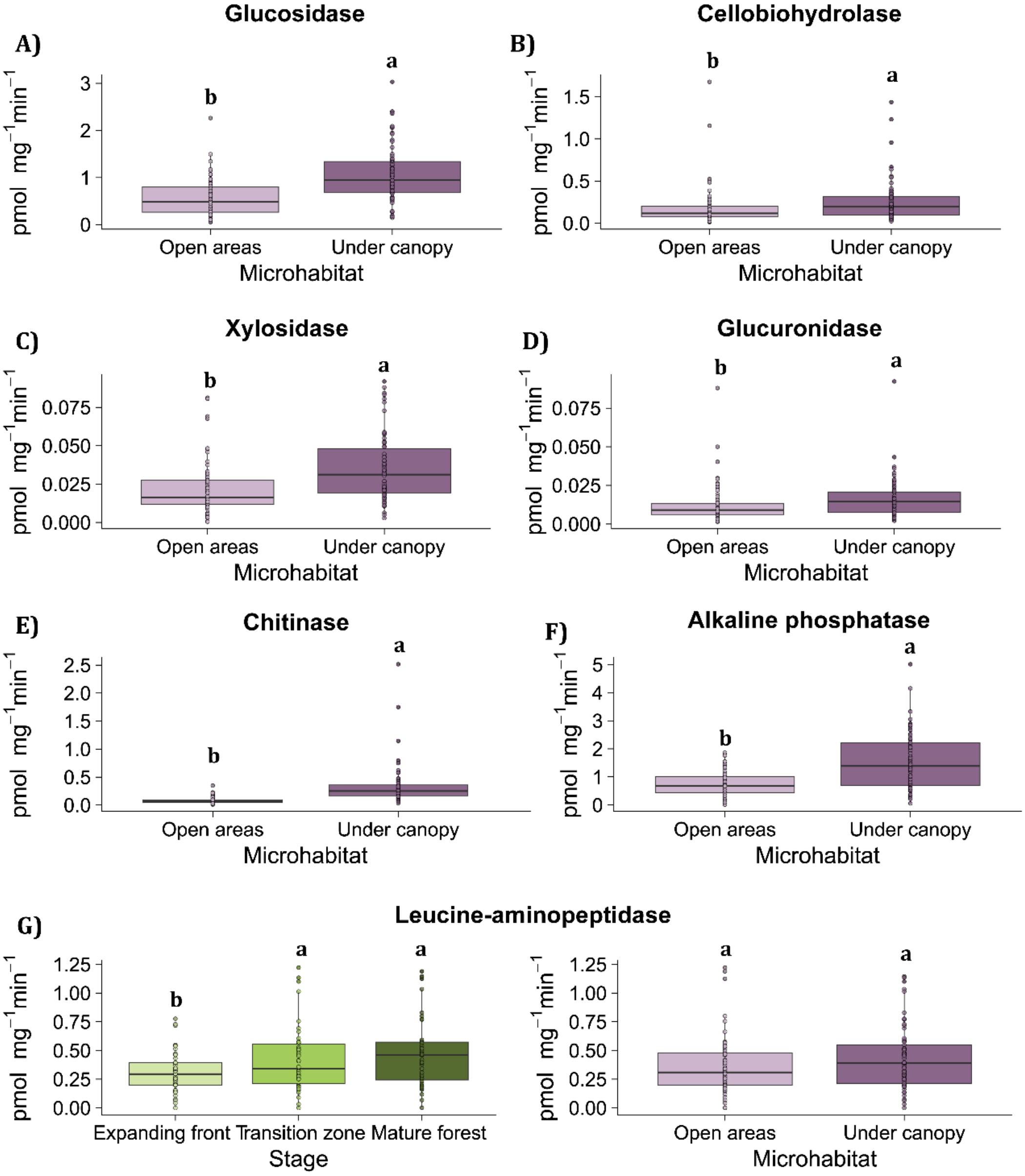

Figure 3. Effect of the microhabitat (purple) and forest expansion gradient (stages in green) (fix factors) on (A) β-glucosidase, (B) cellobiohydrolase, (C) β-xylosidase, (D) β-glucuronidase, (E) chitinase, (F) alkaline phosphatase and (G) leucine, analysed by LMM with the site as random factor. In graphics, different letters indicate significant differences between treatments of each factor ($p < 0.05$).

Relationships among physicochemical, microbial soil properties and soil functions

Total and by group microbial biomass correlated positively with soil organic matter and negatively with pH (Fig S2). Significant positive correlations were found among the microbial biomass of the different microbial groups, and among these with soil enzymatic activities (Fig S2).

The forest expansion gradient and microhabitat showed direct effects on soil properties. Accumulation of organic matter and acidification of soil occurred in mature forests and under tree canopies, and both variables were negatively related (Fig 4).

Soil OM directly favoured the abundance of $Gram^+$ bacteria and AMF (Fig 4). Although the forest expansion gradient was not related to microbial abundances, microhabitat was directly related to AMF (positive) and Actinomycetes (negative) biomass. Moreover, $Gram^+$ biomass was directly and positively related to $Gram^-$, Actinomycetes and Fungi abundances (Fig 4).

The forest expansion gradient had a direct effect on P-cycling and most of its effects were mediated through changes in soil properties (i.e., land use legacies). On the other hand, canopy cover has a significant positive effect on labile C cycling, moderately-labile C cycling and P cycling. The activity of labile C enzymes increased with $Gram^+$ bacteria abundance and decreased with that of Actinomycetes. Moreover, soil OM had a direct positive effect on these enzymes. Moderately-labile carbon cycling (hemicellulose) increased with $Gram^-$ and fungi abundances and decreased with $Gram^+$ bacteria abundance. P cycling was positively related to fungi abundance and negatively with pH.

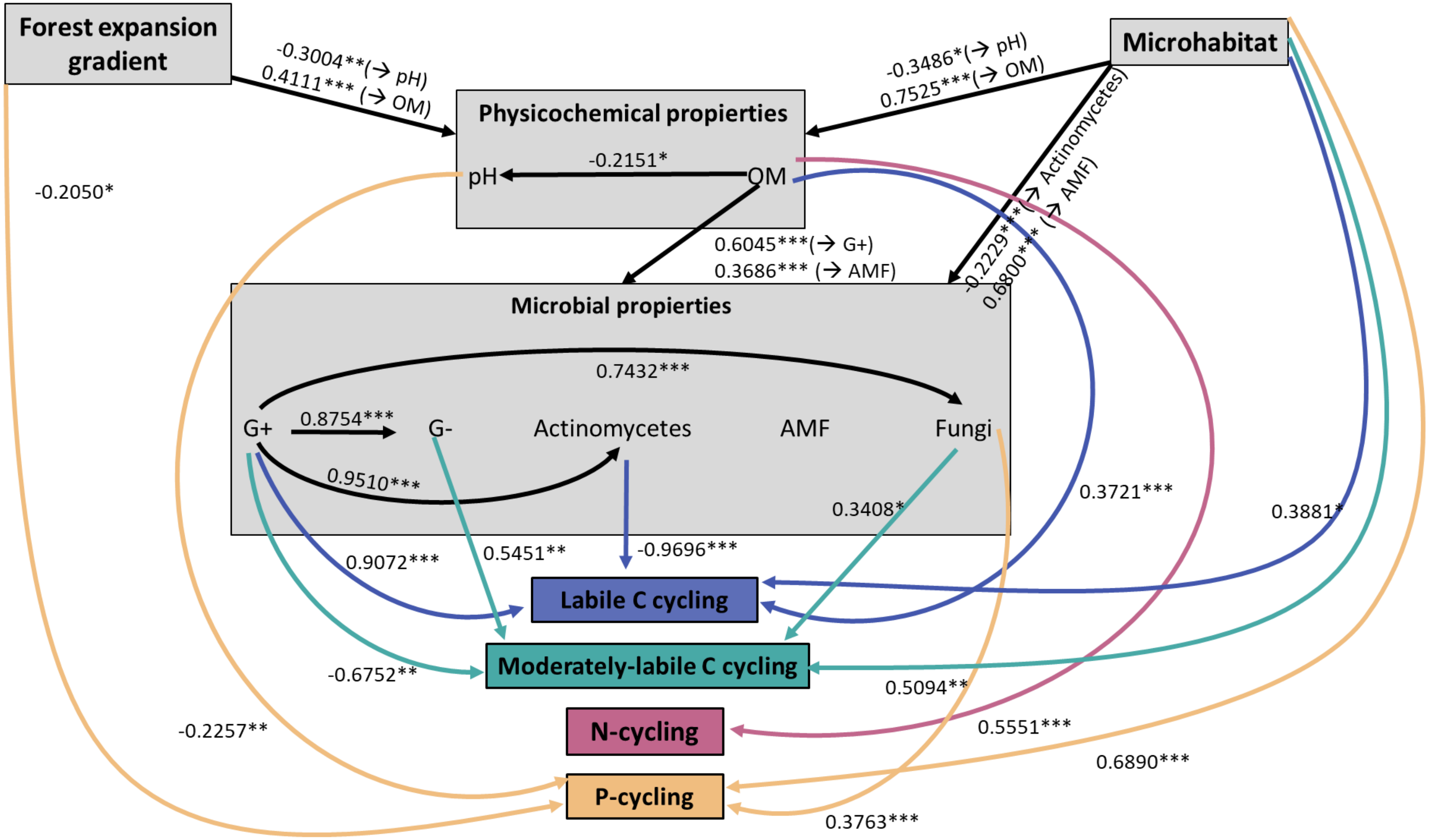

Figure 4. Structural equation models analysing causal relationships among the forest expansion gradient (stage, from expanding to mature), microhabitat (under canopy microhabitat), soil abiotic variables, microbial functional groups and soil nutrient cycling (enzymatic activities). Colour lines indicate enzymatic activities related to C-labile (purple; Fisher's C 45.956 with p-value= 0.829; marginal r^2= 0.37, conditional r^2=0.53), Moderately-labile C (blue; Fisher's C 46.676 with p-value 0.808; r^2= 0.19), nitrogen (pink; Fisher's C 55.513 with p-value 0.707; r^2= 0.31) and phosphorous (yellow; Fisher's C 61.239 with p-value 0.294; r^2= 0.43) cycling in soil. Estimates and p-values (***P < 0.001, **P <0.01, *P <0.05) are given on the respective lines. OM = organic matter; G+ = Gram$^+$ bacteria; G- = Gram$^-$ bacteria; AMF = arbuscular mycorrhizal fungi.

Discussion

Our study demonstrates that forest expansion gradient and microhabitat have an impact on soil characteristics, microbial community and soil functioning. While forest expansion gradient had a direct effect on P-cycling and indirect effect on soil microbial groups and soil functioning through its direct effect on OM and pH, microhabitat showed a direct effect on actinomycetes and AMF abundances and on labile C, non-labile C and P cycling.

The study of a forest expansion gradient (from mature forest to expanding front) into past agricultural lands allows to understand the effects of soil use legacies in soil functionality. Soil legacies of past agricultural practices are widespread, but studies that show the mechanisms driving such legacies are still scarce (de la Peña et al., 2006). Our results showed differences in soil properties along the gradient of forest expansion (greater soil organic matter in mature forests than in the expanding front under canopies, but not in open areas along the gradient and a decrease in pH in mature forests and under canopies). Our results indicate that OM is not recovered after land abandonment under canopies in recently colonised areas. This agrees with previous studies that showed that soil organic matter formation due to accumulation of litter in forests after land abandonment needs time, and OM content recovers slowly even though trees have already colonised these areas (Correira et al., 2021). Moreover, the pH decreased observed in our study has also been previously described (i.e., soils of mature forests are more acid than soils of recent forests; Falkengren-Grerup et al., 2006; Abadie et al., 2018).

Similar to OM, we found an increase in total soil microbial biomass under canopies in mature forests but no differences along the gradient in open areas. This result suggests that there is a land use legacy effect that is reflected in a lower microbial biomass found in expanding fronts. In agreement with this, it has been previously detected less abundant soil microbial communities in agricultural lands than in undisturbed soils (Kulmatiski & Beard, 2008; Zornoza et al., 2009). Along the gradient, Gram$^+$ and Gram$^-$ bacteria, fungi and actinomycetes abundances were greater in mature forests than at the expanding front with intermediate abundance in the transition zone, likely reflecting the succession of different microbial groups probably. AMF varied between microhabitat being higher under canopies, but we did not find differences among stages which could be explained by the fact that land use legacies affect greater to AMF composition rather than AMF abundance (Stover et al., 2012). Previous studies had shown that conventional agricultural management (i.e., tillage) promote bacterial abundance because these practices disrupt fungal mycelium (Helgason et al., 2009). Even though, we did not find any differences in fungi/bacteria ratio along the gradient, we found greater ratio values under canopy than in open areas. These results suggest the recovery of fungal abundance due to tree establishment in past agricultural lands and the cessation of agricultural management (Kulmatiski & Beard 2008, Ortiz et al., 2022). Tree establishment effect is shown in the present study because canopy cover had a positive effect in microbial abundance in all stages of the forest expansion gradient. Moreover, we found greater gram-positive/gram-negative ratio in open areas, which could be reflecting harsher climatic conditions in open areas than under canopies (gram-positive are more resistance to drought than gram-negative bacteria; Curiel Yuste et al., 2014).

The effect of forest expansion gradient and microhabitat on microbial community and soil functioning is mainly due to their effect on organic matter. Our results are in accordance with García-Morote et al. (2012) that showed that maturity, determined by accumulation of organic matter along time, is a relevant factor influencing soil activity and microbial abundance in *Juniperus thurifera* forests (i.e. we found that organic matter was not recovered after tree colonisation of expanding fronts). Accumulation of organic matter promotes changes in soil properties such as the formation of stable aggregates that decrease soil erosion and increase soil-water content (Barthes & Roose, 2002). These changes could be stimulating the proliferation of soil microbial biomass and enhance soil activity. Along the gradient, patches under vegetation in these areas are “islands of soil activity” (García-Morote et al., 2012). Tree density in *J. thurifera* forests is low and soils are expose to high irradiation. Thus, vegetation cover provides shadow, decreasing soil evapotranspiration and irradiation and increases organic matter.

Previous studies in a gradient from grasslands to *J. thurifera* forests showed the key role of fungal communities due to their ability to consume low quality organic matter characteristic of this species (Lloret et al., 2015). However, although we showed a direct effect of fungi abundance on moderately-labile C cycling, we found that C cycling is mediated predominantly by bacterial abundances (labile C cycling by Gram$^+$ and moderately-labile C cycling by Gram$^-$ abundance). Moreover, Gram$^+$ abundance promotes an increase of Gram$^-$, Actinomycetes and fungi abundance. Thus, we proposed that in this system the regulation of microbial abundance mediated by Gram$^+$ abundance could be due to i) its adaptation to extreme Mediterranean climate conditions during summer due to the thickness of their wall (Curiel Yuste et al., 2014) and ii) its participation in the final step of degradation of cellulose that release glucose, a major C source for microbial communities (Michel and Matzner, 2003).

In Mediterranean ecosystems, high values of phosphatase activity have been related to various hypothesis. Sclerophyll woody vegetation in these ecosystems requires more energy to be degraded (Margalef et al., 2014), which could explain our results that show more phosphatase activity under canopies than in open areas. Moreover, we found more activity of this enzyme in expanding areas. Land-use legacies have the potential to support greater nutrient availability in these areas (de la Peña et al., 2016) reducing P limitation and improving phosphatase activity (Janes-Bassett et al., 2022). Moreover, our results are in accordance with Pérez-Izquierdo et al. (2017) that showed the importance of fungal communities with P-mobilization. We did not expect any effects of AMF on P and N-cycling as previously studies have shown (Hodge & Fitter, 2010). Further studies that analyse the biodiversity and specific composition of soil microbial community are needed to better understand the role of fungal communities in soil functioning along gradients of forest expansion.

Conclusions

Our study highlighted the effects of land-use legacies and tree establishment on microbiological and physicochemical variables of soils and this trend seems to depend on the soil organic matter quantity. As a general result, we draw the following conclusions: (i) soil functioning is not fully recovered in forest expanding front yet, (ii) patches under tree canopy are islands of soil functioning and microbial abundance; iii) soils in mature forests, due to their higher OM quantity, harbour a greater abundance of bacterial communities that are the main drivers of soil functioning in these systems. Despite the slower soil functioning of expanding fronts compared to mature forests, soils of recently colonised areas could have the potential to reach forest soil functioning. Therefore, the preservation of areas recently colonised by trees is crucial to the ecological recovery of agricultural abandoned soils.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

B.A.M., A.R. & A.B.O. conceived the study. B.A.M & A.B.O. contributed to field work and B.A.M., A.R. & I.C. contributed to laboratory work. B.A.M. & A.B.O. analysed the data. B.A.M. wrote the initial manuscript. All authors commented on and edited versions of the manuscript.

Data Availability

Data will be made available on request.

Supplementary information chapter 2

Table S1. Biometric characteristics of trees in each plot.

Site	**Stage of forest expansion**	**Plot**	**DBH (cm)**	**Heigh (m)**	**Average crown diameter (m)**
Ribarredonda	Mature forest	RR_MF1	20.9 ± 4.0	5.3 ± 0.3	5.83 ± 0.4
Ribarredonda	Mature forest	RR_MF2	26.8 ± 4.5	6.1 ± 0.7	6.3 ± 1.0
Ribarredonda	Transition zone	RR_TZ1	14.2 ± 1.2	4.8 ± 0.2	3.51 ± 0.3
Ribarredonda	Transition zone	RR_TZ2	13.5 ± 30.5	4.7 ± 0.	3.9 ± 0.2
Ribarredonda	Expanding front	RR_EF1	10.4 ± 0.3	4.5 ± 0.2	3.7 ± 0.1
Ribarredonda	Expanding front	RR_EF2	16.4 ± 2.2	5.2 ± 0.6	4.8 ± 0.3
Huertaherndo	Mature forest	HH_MF1	27.5 ± 8.0	6.9 ± 0.2	6.0 ± 0.3
Huertaherndo	Mature forest	HH_MF2	27.6 ± 5.6	5.2 ± 0.7	6.9 ± 0.6
Huertaherndo	Transition zone	HH_TZ1	23.0 ± 3.1	5.6 ± 0.7	5.9 ± 0.6
Huertaherndo	Transition zone	HH_TZ2	16.4 ± 3.4	5.2 ± 0.7	5.0 ± 0.5
Huertaherndo	Expanding front	HH_EF1	12.1 ± 3.0	3.9 ± 0.4	3.7 ± 0.3
Huertaherndo	Expanding front	HH_EF2	18 ± 4.2	4.1 ± 0.6	4.9 ± 0.6
Maranchón	Mature forest	MM_MF1	22.6 ± 2.4	4.7 ± 0.5	6.6 ± 0.2
Maranchón	Mature forest	MM_MF2	29.4 ± 8.4	7.2 ± 0.9	6.2 ± 0.5
Maranchón	Transition zone	MM_TZ1	18.5 ± 4.4	4.3 ± 0.4	5.1 ± 0.4
Maranchón	Transition zone	MM_TZ2	30.2 ± 3.0	5.6 ± 0.9	5.4 ± 0.5
Maranchón	Expanding front	MM_EF1	10.9 ± 0.8	3.5 ± 0.3	3.4 ± 0.3
Maranchón	Expanding front	MM_EF2	15.5 ± 2.7	3.9 ± 0.4	4.6 ± 0.6

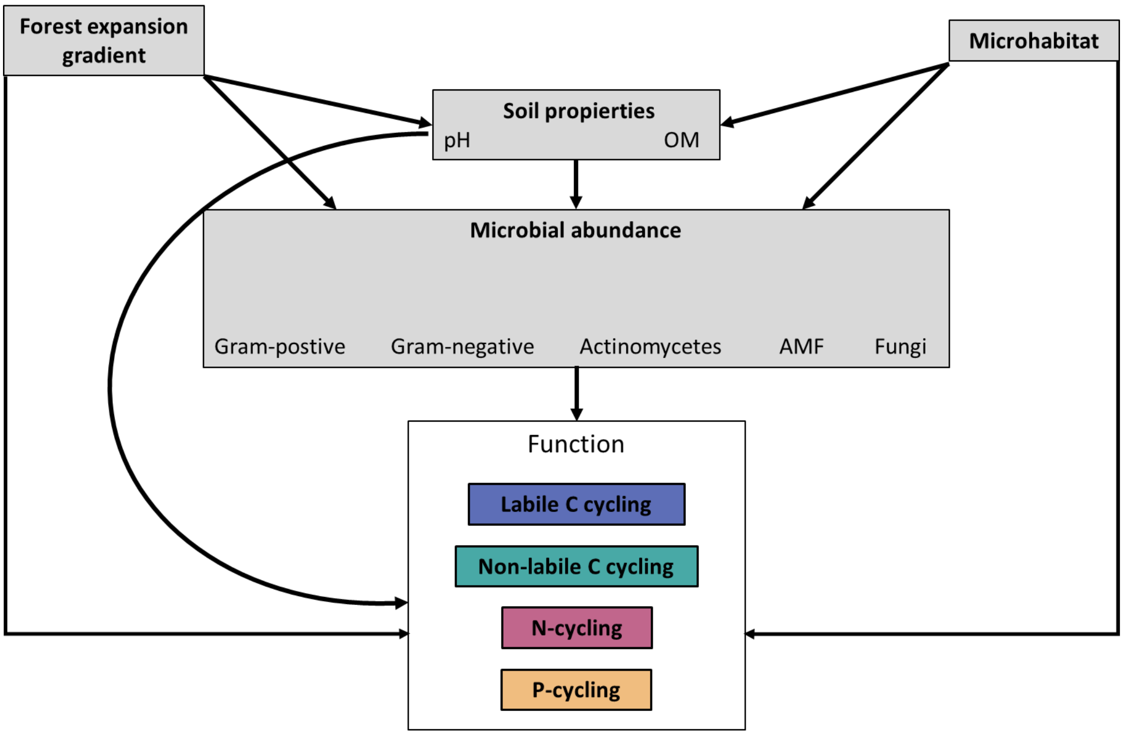

Figure S1. *A priori* structural equation model (SEM)

Table S2. Estimated fixed effects and random effects for soil characteristics in relation to microhabitat and stages of forest expansion gradient. *** *p*-value <0.001, ***p*-value<0.01 * *p*-value <0.05

	Estimate	Std. Error	Df	t-value	R2m	R2c
Organic matter						
Fixed effects						
(Intercept)	7.040**	0.918	4.042	7.667		
Microhabitat [Under canopy]	1.753**	0.601	67.478	2.879		
Stage[Transition zone]	1.116	0.813	111.522	1.372		
Stage [Mature forest]	1.326	0.819	113.013	1.617		
Microhabitat [Under canopy]*Stage [Transition zone]	0.640	0.868	68.057	0.738		
Microhabitat [Under canopy]*Stage [Mature forest]	2.351**	0.874	69.257	2.691		
Random effects	Variance	Std. Dev.				
Site	1.537	1.240				
Sample point:Site	3.486	1.867				
Residuals	4.449	2.109				
Whole model					0.255	0.649
pH						
Fixed effects						
(Intercept)	7.986***	0.038	2.821	207.538		
Microhabitat [Under Canopy]	-0.083***	0.014	72.022	-5.859		
Stage[Transition zone]	-0.074**	0.023	66.994	-3.166		
Stage [Mature forest]	-0.078**	0.024	67.015	-3.313		
Random effects	Variance	Std. Dev.				
Site	0.004	0.059				
Sample point:Site	0.003	0.055				
Residuals	0.007	0.085				
Whole model					0.183	0.569

Table S3. Estimated fixed and random effects for soil microbial groups in relation to microhabitat and stages of forest expansion gradient. *** *p*-value <0.001, ***p*-value<0.01 * *p*-value <0.05

	Estimate	Std. Error	Df	t-value	R2m	R2c
Total microbial biomass						
Fixed effects						
(Intercept)	308.394*	76.902	3.715	4.010		
Microhabitat [Under Canopy]	64.040	57.625	66.230	1.111		
Stage[Transition zone]	60.225	63.104	124.619	0.954		
Stage [Mature forest]	51.705	63.743	125.337	0.811		
Microhabitat [Under Canopy]*Stage [Transition zone]	6.222	81.518	66.207	0.076		
Microhabitat [Under Canopy]*Stage [Mature forest]	164.351*	81.288	67.309	2.022		
Random effects	Variance	Std. Dev.				
Site	11769	108.5				
Sample point:Site	10060	100.3				
Residuals	37725	194.2				
Whole model					0.124	0.445
Gram $^{+}$ bacteria						
Fixed effects						
(Intercept)	40.076*	9.758	3.121	4.107		
Microhabitat [Under Canopy]	13.480**	4.579	70.829	2.944		
Stage[Transition zone]	8.418	6.508	66.397	1.294		
Stage [Mature forest]	15.571*	6.509	66.445	2.392		
Random effects	Variance	Std. Dev.				
Site	207.357	14.400				
Sample point:Site	130.756	11.430				
Residuals	727.160	29.970				
Whole model					0.077	0.370
Gram $^{-}$ bacteria						
Fixed effects						
(Intercept)	92.263*	23.673	3.489	3.897		
Microhabitat [Under Canopy]	28.701*	11.847	65.920	2.423		
Stage[Transition zone]	14.916	17.617	61.503	0.847		
Stage [Mature forest]	38.757*	17.621	61.548	2.199		
Random effects	Variance	Std. Dev.				
Site	1117.000	33.420				
Sample point:Site	1200.460	34.650				
Residuals	4855.900	69.680				
Whole model					0.062	0.365
Fungi						
Fixed effects						
(Intercept)	2.073*	0.626	3.931	3.313		
Microhabitat [Under canopy]	0.647	0.346	65.631	1.868		
Stage[Transition zone]	0.708	0.509	62.193	1.392		
Stage [Mature forest]	1.204*	0.506	61.448	2.377		
Random effects	Variance	Std. Dev.				
Site	0.706	0.840				
Sample point:Site	0.938	0.969				

	Estimate	Std. Error	Df	t-value	R2m	R2c
	Variance	Std. Dev.				
Residuals	4.114	2.028				
Whole model					0.059	0.328
Actinomycetes						
Fixed effects						
(Intercept)	25.370*	5.731	2.708	4.427		
Stage[Transition zone]	8.090	3.703	66.879	2.184		
Stage [Mature forest]	9.390*	3.703	66.879	2.536		
Random effects	Variance	Std. Dev.				
Site	77.770	8.819				
Sample point:Site	44.276	6.654				
Residuals	231.710	15.222				
Whole model					0.047	0.375
Arbuscular mycorrhizal fungi						
Fixed effects						
(Intercept)	76.810***	4.635	131.187	16.574		
Microhabitat [Under Canopy]	42.326	5.886	70.085	7.191		
Random effects	Variance	Std. Dev.				
Sample point:Site	339.401	18.423				
Residuals	1187.304	34.462				
Whole model					0.228	0.400
Gram + : Gram - ratio						
Fixed effects						
(Intercept)	0.773***	0.015	136.785	52.279		
Microhabitat [Under Canopy]	-0.070**	0.021	63.773	-3.368		
Random effects	Variance	Std. Dev.				
Site	3.233e-11	5.686e-06				
Sample point:Site	5.874e-04	2.424e-02				
Residuals	1.495e-02	1.223e-01				
Whole model					0.073	0.118
Fungi : bacteria ratio						
Fixed effects						
(Intercept)	0.551*	0.092	2.311	5.989		
Microhabitat [Under Canopy]	0.152*	0.050	70.089	1.634		
Random effects	Variance	Std. Dev.				
Site	0.020	0.141				
Sample point:Site	0.045	0.212				
Residuals	0.087	0.294				
Whole model					0.011	0.434

Table S4. Estimated fixed and random effects for enzymatic activities in relation to microhabitat and stages of forest expansion gradient. *** *p*-value <0.001, ***p*-value<0.01 * *p*-value <0.05.

	Estimate	Std. Error	Df	t-value	R2m	R2c
Glucosidase						
Fixed effects						
(Intercept)	-0.870***	0.087	133.519	-9.998		
Microhabitat [Under canopy]	0.770***	0.109	71.309	7.101		
Random effects	Variance	Std. Dev.				
Sample point:Site	0.124	0.352				
Residuals	0.414	0.644				
Whole model					0.217	0.471
Cellobiohydrolase						
Fixed effects						
(Intercept)	-2.149**	0.178	2.939	-12.089		
Microhabitat [Under canopy]	0.480**	0.147	137.013	3.261		
Random effects	Variance	Std. Dev.				
Site	0.062	0.249				
Residuals	0.764	0.874				
Whole model					0.067	0.136
Xylosidase						
Fixed effects						
(Intercept)	-4.155***	0.107	128.652	-39.026		
Microhabitat [Under canopy]	0.586***	0.141	64.057	4.161		
Random effects	Variance	Std. Dev.				
Sample point: Site	0.052	0.228				
Residuals	0.640	0.800				
Whole model					0.111	0.177
Glucuronidase						
Fixed effects						
(Intercept)	-4.714***	0.097	133.124	--48.606		
Microhabitat [Under canopy]	0.420***	0.122	72.136	3.441		
Random effects	Variance	Std. Dev.				
Sample point: Site	0.150	0.387				
Residuals	0.519	0.720				
Whole model					0.06	0.272
Phosphatase						
Fixed effects						
(Intercept)	0.733 *	0.162	2.971	4.522		
Microhabitat [Under canopy]	0.799***	0.129	65.389	6.211		
Random effects	Variance	Std. Dev.				
Sample point: Site	0.074	0.273				
Site	0.049	0.222				
Residuals	0.53	0.733				
Whole model					0.195	0.346

	Estimate	Std. Error	Df	t-value	R2m	R2c
Chitinase						
Fixed effects						
(Intercept)	-2.706***	0.1426	2.645	-18.980		
Microhabitat [Under canopy]	1.315***	0.102	72.347	12.940		
Random effects	Variance	Std. Dev.				
Sample point:Site	0.076	0.275				
Site	0.042	0.205				
Residuals	0.371	0.609				
Whole model					0.471	0.599
Leucine						
Fixed effects						
(Intercept)	0.318***	0.045	6.291	7.107		
Stage [Transition zone]	0.104*	0.053	132.006	1.969		
Stage [Mature forest]	0.150**	0.054	132.125	2.791		
Random effects	Variance	Std. Dev.				
Site	0.002	0.044				
Residuals	0.065	0.255				
Whole model					0.056	0.100

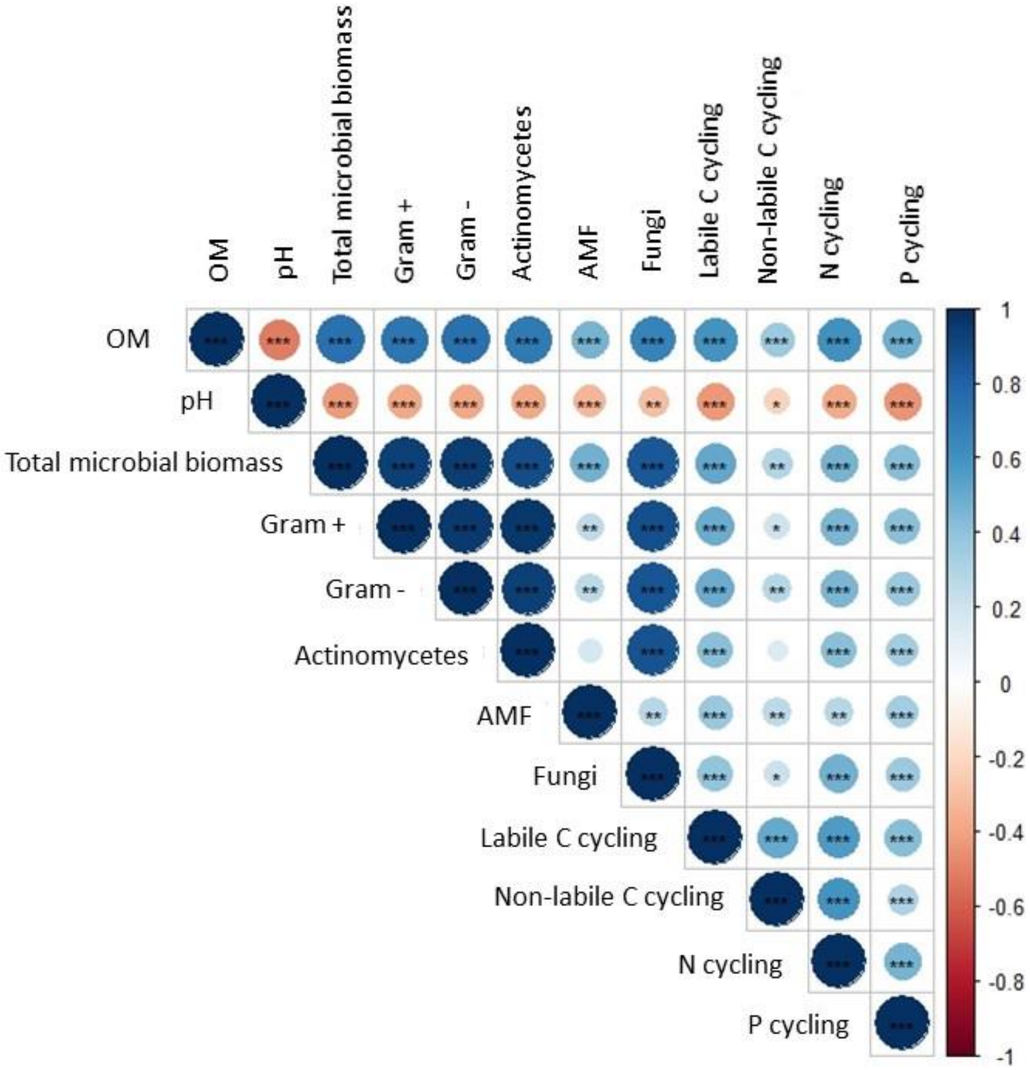

Figure S2. Correlations among physical-chemical soil properties, enzymatic activity and soil microbial biomass (total and "by guild"). Blue and red circles indicate positive and negative correlations respectively. Size of the circle and intensity of the colour indicate the magnitude of the correlation, being higher with longer size and more intense colour. Significant Spearman's correlations are indicated with asterisks: P<0.001 (***); P<0.01 (**); P<0.05 (*).

Table S5. Standarised coefficients for each level of factor in Structural Equation Model. Letters denote groupings via post-hoc tests.

Response	Predictor	Estimate ± SE
OM	Stage: Mature forest	0.437 ± 0.267 a
	Stage: Transition zone	-0.022 ± 0.264 ab
	Stage: Expanding front	-0.388 ± 0.260 b
	Microhabitat: Under canopy	0.385 ± 0.250 a
	Microhabitat: Open areas	-0.367 ± 0.251 b
pH	Stage: Mature forest	-0.184 ± 0.322 b
	Stage: Transition zone	-0.190 ± 0.317 b
	Stage: Expanding front	0.394 ± 0.316 a
	Microhabitat: Under canopy	-0.166 ± 0.309 b
	Microhabitat: Open areas	0.180 ± 0.310 a
Actinomycetes	Microhabitat: Under canopy	0.341 ± 0.111 a
	Microhabitat: Open areas	-0.362 ± 0.114 b
AMF	Microhabitat: Under canopy	0.330 ± 0.118 a
	Microhabitat: Open areas	-0.350 ± 0.122 b
Labile C cycling	Microhabitat: Under canopy	0.194 ± 0.261 a
	Microhabitat: Open areas	-0.194 ± 0.263 b
Non-labile C cycling	Microhabitat: Under canopy	0.247 ± 0.129 a
	Microhabitat: Open areas	-0.262 ± 0.133 b
P-cycling	Stage: Mature forest	-0.280 ± 0.140 a
	Stage: Transition zone	0.085 ± 0.135 a
	Stage: Expanding front	0.130 ± 0.132 a
	Microhabitat: Under canopy	0.327 ±0.109 a
	Microhabitat: Open areas	- 0.370 ± 0.112 b

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Chapter 3

Litterfall production and foliar decomposition along a gradient of forest expansion

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Abstract

Agricultural land abandonment in Southern and Western Mediterranean areas has promoted forest expansion into these abandoned lands. In these areas where ecosystems are characterised by poor nutrient soils, the amount and quality of plant material returning to the soil (i.e. litterfall) and its rate of decomposition are critical factors. In this study, we examine two year litter fall phenology and leaf litter decomposition along a gradient of *Juniperus thurifera* forest expansion (from mature forests to expanding fronts) to analyse if: i) phenology of tree productivity, in terms of amount of litter, depends on stage of forest expansion gradient, ii) intra-annual mass loss during varied among stages or between microsites (is decomposition more dependent of land-use legacies or microclimatic conditions) and iii) mass loss during two years under canopies show a different pattern along the gradient of forest expansion. In Alto Tajo Natural Park (Spain), we replicated in three sites a gradient of forest expansion (18 plots). We selected five trees in each plot to measure litterfall and four trees to measure litter decomposition under their canopies and outside them. We found that litterfall quantity was higher in 2021 than in 2020 without differences among stages of forest expansion gradient. The main litter fraction both years was foliar fraction. Foliar phenology did not vary among stages, showing two peaks in summer for both years. However, the foliar fall quantity was higher at expanding front than in mature forests and transition zone. Regarding decomposition rate, it was lower under canopy than in open areas, without differences among stages of forest expansion gradient. Thus, organic matter accumulation at expanding fronts could be favoured due to a greater foliar fall than in mature forests without differences in decomposition rates. Moreover, our results suggest the importance of photodegradation in *J. thurifera* litter decomposition due to a greater decomposition rate in open areas than under canopies.

Keywords: *Juniperus thurifera*; Mediterranean forests; nutrient cycling; productivity

Introduction

Agricultural land abandonment has been a major trend in several European landscapes for decades and occurs particularly in areas of low productive agriculture, such as mountain areas, poor soils or hard climatic conditions (MacDonald et al., 2000; Estel et al., 2015). Land abandonment is often followed by natural forest expansion, which has contributed to a general increase of forest area in Europe since the mid of the 20th century, especially in South and Western Mediterranean (Palmero-Iniesta et al., 2021). For this reason, land abandonment has been considered the most important land-use change in the Mediterranean region (Weissteiner et al., 2011). In these areas where ecosystems are characterised by poor nutrient soils, the amount and quality of plant material returning to the soil (i.e. litterfall) and its rate of decomposition are critical factors (Fioretto et al., 2003).

Litterfall and its decomposition is the primary pathway by which organic matter and nutrients are supplied to the soil community, linking primary producers and soil processes (Aber & Melillo 1980). Net primary production, i.e. rate of formation of biomass that is used to create organic structures in plants, is usually evaluated through litter production (Malhi et al., 2011) due to its role as the main source of soil organic carbon (SOC) and plant nutrient cycling (Vitousek, 1982). Following literfall, decomposition pathway contributes to the ecosystem structure and functioning by serving two primary functions: i) regulation of nutrient cycling by control rates and timing of nutrient release (Blair, 1988) and ii) formation of soil organic matter (Swift et al. 1979). Agricultural land abandonment and its colonisation by trees could change ecosystem functioning by promoting changes in carbon and nutrient inputs in soils. Shifts in vegetation cover, from cropland to forest cover, potentially affect soil properties through fundamental changes in organic matter and promote changes in nutrient cycling (van Hall et al., 2017). Several studies have shown differences in litter production and decomposition along forest succession, comparing species characteristics of each stage of succession (Cortez et al., 2017). However, little is known about changes in primary production and its decomposition of the same tree species at different stages of succession.

Trees established in abandoned lands have to cope with hard environmental conditions, e.g. greater irradiance due to lower canopy cover that increase water evapotranspiration. These conditions at expanding fronts have shown a more efficient tree phenotype that have changed tree growth allometries and increased resilience compared to trees established in mature forests (Benavides et al., 2023; Acuña-Míguez et al., 2023). For instance, trees at expanding fronts which are exposed to harder environmental conditions, could change the timing of leaf senesce according to these climatic conditions (Andivia et al., 2018). Besides environmental properties, structural properties (i.e. stand age, basal area or canopy cover) determine the amount and phenology of litterfall and its decomposition in forests (Roig et al., 2005; Berg & Meentemeyer, 2011). Structural properties varied along a gradient of forest expansion, from mature forests to expanding fronts (Acuña-Míguez et al., 2020) which could influence in primary production (measured as amount of litterfall). Litterfall under canopies play other important roles such as buffering changes in soil water content and temperature, reducing erosion, and increasing soil microbial biodiversity (Giweta et al., 2020). Thus, differences in litterfall quantity along the forest expansion gradient could impact decomposition rates.

Litter decomposition is a complex phenomenon led by both biotic, e.g. soil biota or species composition that alter litter quality (Hättenschwiler et al., 2005) and abiotic factors such as UV radiation, temperature and water availability (Liski et al., 2003, Austin & Vivanco, 2006, Ferreira et al., 2023). Climate and soil properties can affect litter decomposition depending on the scale. Climatic conditions have been considered as the main factor affecting decomposition rates at global and regional levels (Liski et al., 2003), whereas it is expected that in low tree density forests, microclimatic conditions (soil temperature and soil moisture) could vary at small scales promoting changes in litter decomposition under canopies or in open areas. In Mediterranean forests, soil properties are one of the main determinants of litter decomposition rates by promoting specific microclimate conditions and microbial activity (Romanyá et al. 2000). Moreover, in the Mediterranean region, litter photodegradation is a key process due to recalcitrant nature of vegetation litter in these areas (Dirks et al., 2010; Gilksman et al., 2018). All these factors together with past agriculture practices that could promote land-use legacies can affect decomposition rates differently along a gradient of forest expansion (Bergès et al., 2017).

In this study, we examine two year litter fall phenology and leaf litter decomposition along a gradient of *J. thurifera* forest expansion (from mature forests to expanding fronts) to analyse if: i) phenology of tree productivity, in terms of amount of litter, depends on stage of forest expansion gradient, ii) intra-annual mass loss during varied among stages or between microsites (is decomposition more dependent of land-use legacies or microclimatic conditions) and iii) mass loss during two years under canopies show a different pattern along the gradient of forest expansion. We hypothesise that litterfall quantity is greater in mature forests than expanding fronts due to higher tree size in this stage. On the other hand, we expect an early peak of litterfall in expanding fronts due to the harsher climatic conditions compared to mature forests. Decomposition rate between microhabitats will vary depending on the stage of forest expansion gradient. Thus, in mature forests we expect a greater decomposition rate under canopies than in open areas due to the greater total microbial biomass found previously in this stage (Acuña-Míguez et al., 2023). In contrast, in expanding front because of the reduction of microbial biomass, we expect that UV radiation in open areas plays the key role.

Materials & Methods

Study area and species

Study species was *J. thurifera*, a species that forms low-density forests and exhibit the ability to establish in harsh conditions (poor and rocky soils) and tolerate a wide range of climatic conditions (Gauquelin et al., 1999; Montesinos et al., 2009). Its distribution is limited to Western Mediterranean basin.

We conducted the study in Alto Tajo Natural Park, where *J. thurifera* forests are progressively expanding into abandoned lands (Gimeno, Pías, et al., 2012). The climate is categorised as continental Mediterranean, characterised by hot and arid summers as well as cold and snowy winters. In this area, we selected three sites (Huertahernando (40°49′28″N, 2°17′08″OW), Ribarredonda (40°52′30″N 2°18′29″O) and Maranchón (41°02′55″N 2°12′13″O)) which contains well-conserved *J. thurifera* forests that are colonising abandoned agricultural areas (Villellas et al., 2020). Thus, we divided a gradient of forest expansion from mature forests to expanding front with a transition zone where trees exhibited intermediate characteristics in terms of tree density, tree size and age (more details in Villellas et al., 2020 and Acuña-Míguez et al., 2020).

Microclimatic conditions

In spring 2020, we deployed climatic sensors in 9 plots representing a forest expansion gradient in each site (i.e. three plots per site representing each stage). In Ribarredonda, we established two soil temperature sensors (HOBO UX120-006M, MX1100), two soil moisture sensors (ECH2O Dielectric Aquameter). In Maranchón and Huertahernando, we established two soil temperature sensors (SparkFun DS18B20 Waterproof Temperature Sensor) and two soil moisture sensors (Capacitive Soil Moisture Sensor SKU SEN0193-DFRobot). Sensors was established at 30 cm depth, one under canopy and other outside it. Readings of each sensor in Ribarredonda were recorded every 10 minutes with a data logger (HOBO® H21-00I; all components from HOBO® Onset Computer Corporation, Bourne, MA, USA) and readings of each sensor in Huertahernando and Maranchón were recorded every 10 minutes with a data logger (Adafruit Feather M0 Adalogger).

Litterfall data

In winter 2019, we selected 5 trees based on frequency of diameter class in each plot (Table S1). One 0.049 m^2 littertrap was established under canopy of each tree, positioned at a distance from the trunk equal to the half the crown radius (Fig 1). We collected samples monthly from December 2019 to December 2021 and oven dried at 65 °C during two days. Once oven dried, we separated in different fractions (leaves, wood, reproductive and miscellaneous) and weight. We calculated also total litterfall as the sum of all fractions except reproductive fraction due to its high dependence of tree sex. We grouped fractions per season each year to analyse litterfall phenology and total litterfall each year.

Figure 1. Littertrap established under canopy of each tree.

Decomposition

In late autumn 2019, we established a litter decomposition experiment in each plot. We selected four trees based on frequency of diameter class (Table S1). Each tree was our sampling point and four litterbags were incubated under and outside canopy of each tree (Fig 2). Each litterbag contained 4.045 ± 0.001 g of air-dried foliar material from *J. thurifera* shed in August 2019. The total number of incubated bags were 4bags/canopy effect x 2 microhabitats (under/outside) x 4 trees/plot x 18 plots = 576. Collection dates differed between microhabitats. Under canopy, we collected at 3, 9, 12 and 24 months since November 2019 and in open areas, we collected at 3, 6, 9 and 12 months since started date. In each collection, we transported into a bag each litter bag in order to avoid any material loss. Differences in collection dates was due to a photodegradation of litterbags during the first year in open areas.

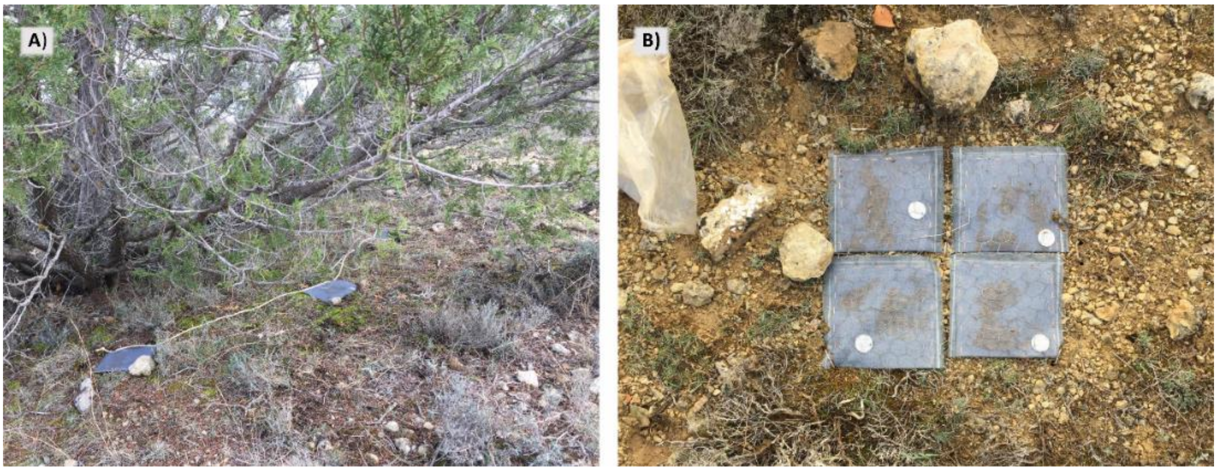

Figure 2. Decomposition bag experiment under canopy (A) and in open areas (B).

Statistical analysis

We used linear mixed models (LMMs) to analyse the effect of microhabitat and years on microclimatic conditions (mean, maximum and minimum soil moisture and soil temperature). We set as fixed effect the microhabitat and year (2020 and 2021) and the interaction between them. Plot was selected as random effect.

Total littterfall and foliar fall were analysed using LMMs and we set in both cases tree nested site as random effect. To analyse litterfall response, we set as fixed effects stages of the gradient, year and the interaction between them. To analyse foliar fall response, we first grouped the variable by season each year and set as fixed effects stages of the gradient, season and the interaction between them. Both variables were log transformed + 1 to conform linear model assumption.

Decomposition rates (1 and 2 years after incubation) were calculated using a single exponential decay model (Olson, 1963):

$$\ln\left(\frac{w_t}{w_0}\right) = -k \cdot t$$

Where w_0 is the original litter mass (g) and w_t is the remaining mass after time t which is the number of months since incubation and k is the decomposition rate (month^{-1}).

Litter decomposition ($\ln(\frac{w_t}{w_0})$) during one and two years were analyse using LMMs. In both models we set the tree as random effect. To analyse litter decomposition, we set as fixed effects stages of forest expansion gradient, microhabitat, collection month and the interaction among them.

We reduced all LMMs to those with the lowest Akaike Information Criterion AIC (the best or most parsimonious models) using the dredge function from the MuMIn R package (Barton, 2020). The method was set to maximum likelihood (ML) during the fixed-effect model selection phase, although the final models are presented using restricted maximum likelihood (REML; Kuznetsova et al., 2017). Models fit was visually checked to ensure model assumptions. We calculated marginal and conditional r2 with the *sjPlot* R package (Lüdecke et al., 2021).

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2021).

Results

Microclimatic conditions

LMMs (according to AIC), which better explained variation in mean temperature and mean, maximum and minimum soil were those with microhabitat and year without interaction between them as fixed effects. Maximum temperature and minimum temperature were better explained by LMMs with microhabitat and year, respectively.

Mean and maximum temperature and mean, maximum and minimum soil moisture was significant higher in open areas than under canopies (Table 1). Mean and minimum temperature was higher in 2020 than in 2021 meanwhile mean and maximum soil moisture was higher in 2021 than in 2020 (Table 1).

Table 1. Differences of Mean temperature (ºC), Maximum temperature (ºC), Minimum temperature (ºC), Mean soil moisture (%), Maximum soil moisture (%) and Minimum soil moisture (%) between microhabitats. Different letters indicate significant differences between microhabitats and between years (p<0.05).

		Microhabitat		**Year**	
		Open areas	**Under Canopy**	**2020**	**2021**
Temperature (ºC)	Annual Mean	15.61 ± 0.66 a	13.68 ± 0.49 b	16.08 ± 0.64 a	13.65 ± 0.53 b
	Maximum	21.89 ± 0.80 a	18.48 ± 0.79 b	20.86 ± 0.76 a	19.72 ± 0.81 a
	Minimum	10.38 ± 0.58 a	9.61 ± 0.49 a	11.77 ± 0.60 a	8.77 ± 0.47 b
Soil Moisture (%)	Annual Mean	11.99 ± 0.60 a	8.59 ± 0.68 b	7.83 ± 0.59 b	11.74 ± 0.62 a
	Maximum	23.62 ± 1.40 a	18.68 ± 1.44 b	16.58 ± 1.46 b	23.85 ± 1.33 a
	Minimum	5.84 ± 0.47 a	4.25 ± 0.42 b	4.29 ± 0.44 a	5.49 ± 0.43 a

Litterfall

We did not find differences in litter production among stages for each year (Fig 4). However, the amount of litter was higher during 2021 than 2020 (Fig 4; Table S2). We also found that leaves were the main fraction of the total litter fall in both years (Table 2).

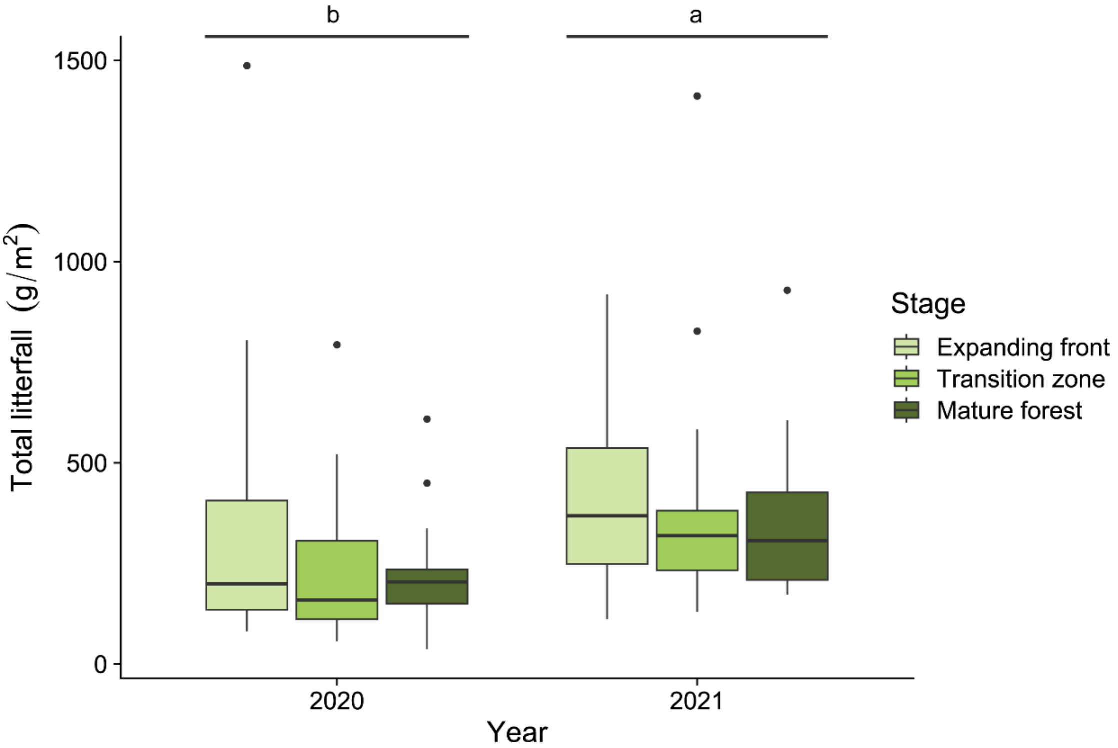

Figure 4. Effect of stages of forest expansion gradient and years on total litterfall production. Different letters indicate significant differences (p<0.05).

Table 2. Mean annual values for different litterfall fractions in g m^{-2} ± standard error.

Year	Stage	Leaves	Wood	Reproductive	Miscellaneous
2020	Expanding front	220.77 ± 26.38	25.12 ± 3.62	64.79 ± 40.74	22.40 ± 4.25
	Transition zone	163.62 ± 22.97	22.29 ± 2.92	50.35 ± 12.96	20.09 ± 4.59
	Mature forest	166.22 ± 13.05	28.50 ± 6.96	21.26 ± 3.74	16.38 ± 2.24
2021	Expanding front	344.22 ± 34.34	29.87 ± 4.13	18.30 ± 7.28	9.93 ± 1.35
	Transition zone	295.47± 40.68	31.38 ± 4.11	23.71 ± 7.32	11.61 ± 3.19
	Mature forest	273.21 ± 26.55	31.91 ± 4.54	18.91 ± 5.05	11.50 ± 1.84

Two peaks in summer of each year showed the phenology of foliar production without differences among stages (Fig 5A). We also found greater foliar fall in expanding front tan in mature forests or transition zone (Fig 5B) (Table S3).

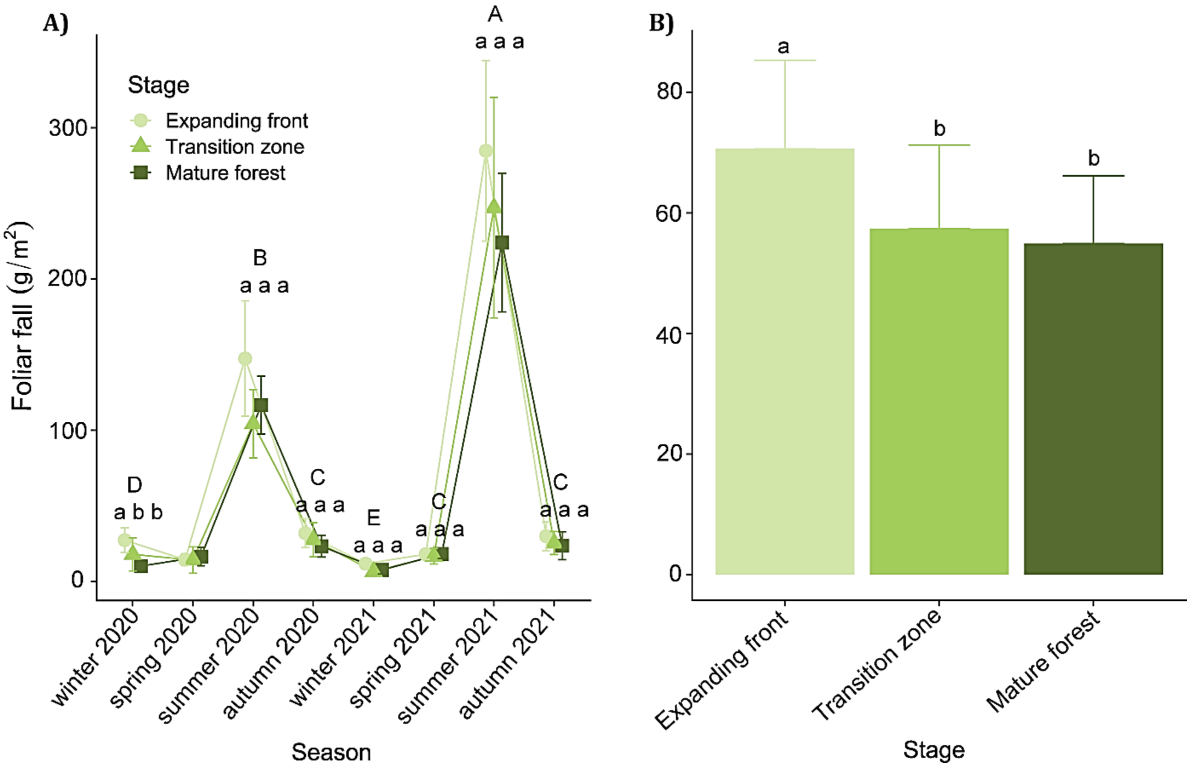

Figure 5. Effect of stages of forest expansion gradient and seasons (A) and stages (B) on foliar litterfall. In panel A, capital letters show significant difference among seasons and lowercase letters show significant difference among stages for each season (p<0.05). In panel B, different letters show significant differences among stages of forest expansion gradient (p<0.05).

Litter decomposition

Best linear effects models for intra-annual litter decomposition decay rate included interactions between microhabitat and months (Table S4). Decay rate was higher in open areas than under canopies (Table 3) and stage of forest expansion gradient did not have a significant effect on decomposition decay rate.

Table 2. Model predictions of decomposition rate for 1 year. Different letters indicate significance differences ($p<0.01$)

	Estimate	Std. Error	marginal R2	conditional R2
Fixed effects				
(Intercept)	4.5560	0.0046		
Microhabitat [Open areas]:months	-0.0254 a	0.0008		
Microhabitat [Under canopy]:months	-0.0294 b	0.0007		
Random effects	Standard deviation			
uij	0.0021			
			0.7192	0.7330

We found that the effect of microhabitat on mass loss was dependent on each month (Fig 6A). During the first 9 months, mass loss was higher under canopy than open areas. However, at time 12 months, mass loss in open areas was higher. Moreover, the effect of forest expansion did not change along time (Fig 6B) (Table S4).

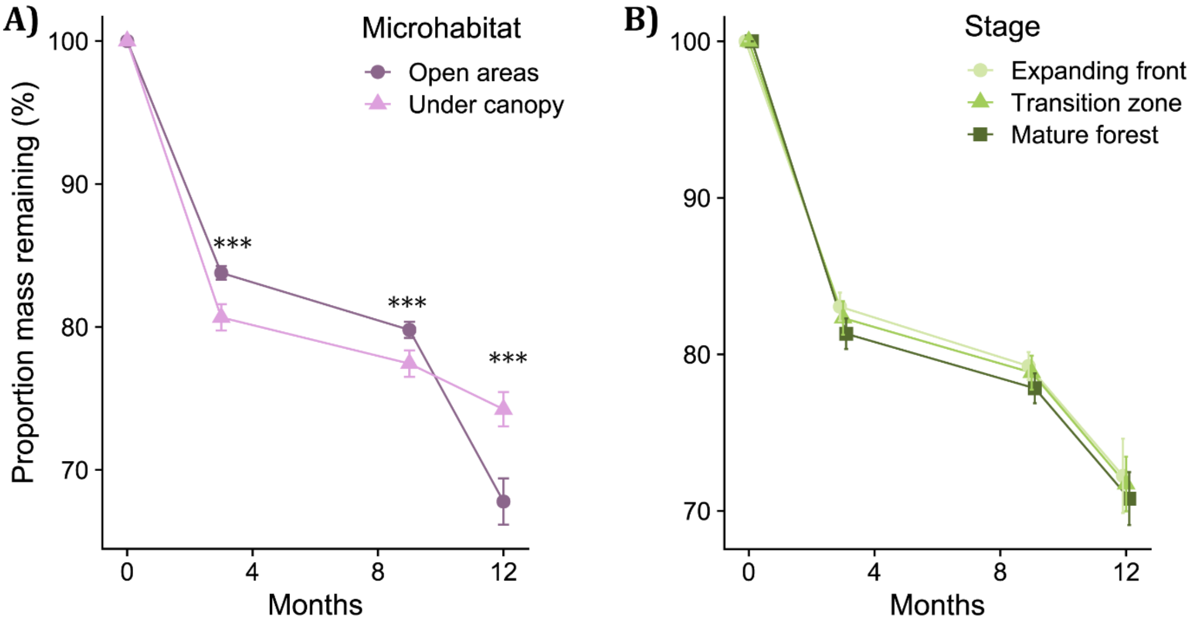

Figure 6. Effect of microhabitat (A) and forest expansion gradient (B) on mass loss (%) for 12 months. (*** $p < 0.001$)

Best linear effects models for litter decomposition decay rate under canopy for two years included interactions between stages and months (Table S5). Under canopies, we did not find differences in decay rates neither mass loss after two years (Table 3; Fig 7).

Table 3. Model predictions of decomposition rate for 2 years under canopies.

	Estimate	Std. Error	marginal R2	conditional R2
Fixed effects				
(Intercept)	4.5680	0.0036		
Stage [Expanding front]:months	-0.0191	0.0008		
Stage [Transition zone]:months	-0.0205	0.0008		
Stage [Mature forest]:months	-0.0195	0.0008		
Random effects	Standard deviation			
uij	0.0025			
			0.8179	0.8388

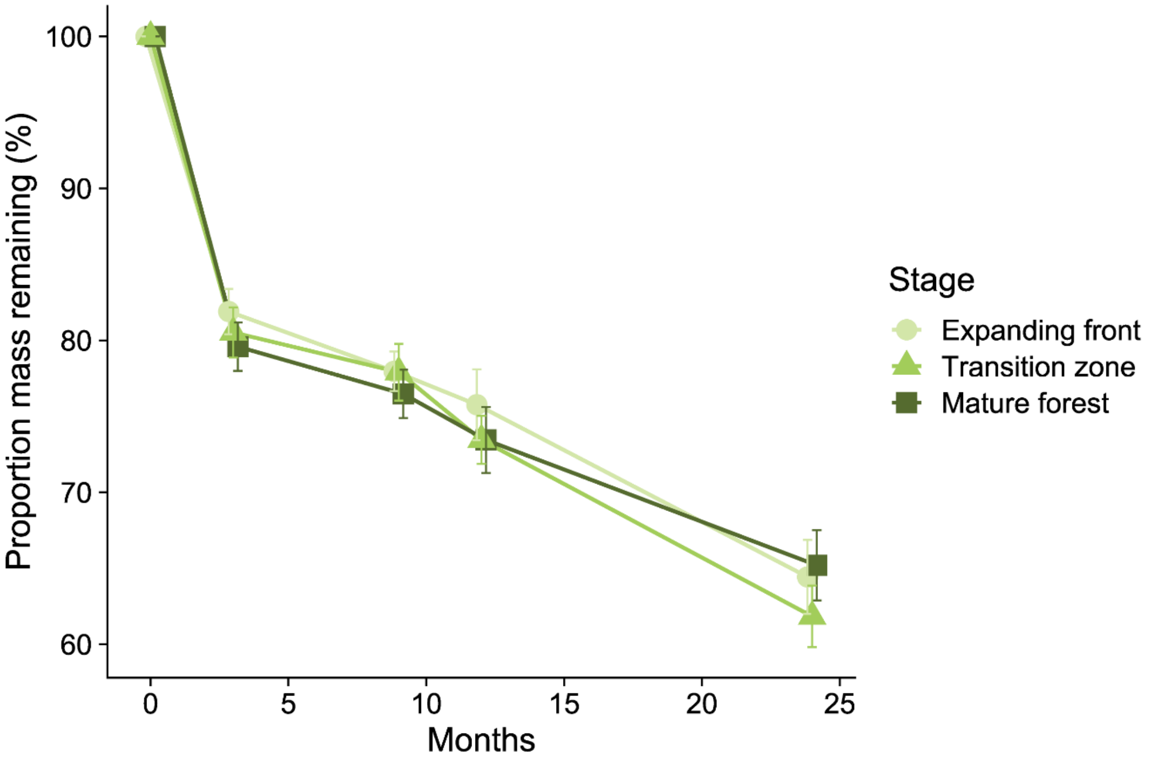

Figure 7. Effect of forest expansion gradient on mass loss (%) for 24 months under tree canopies.

Discussion

We found that productivity (measured by foliar litterfall) in expanding front was greater than in transition zone and mature forests. However, decomposition rates and mass loss were different between microhabitats without effects of forest expansion gradient.

Studies in Mediterranean ecosystems showed that canopy or shrub effect is related to amelioration of microclimate such as lower air and soil temperatures, radiation and vapour pressure deficit but with no effect on soil moisture (Gómez-Aparicio et al., 2005). Canopy effect on soil moisture is less clear in mediterranean ecosystems but most studies showed a reduction on soil moisture under canopies (Prévosto et al., 2020) specially during dry periods (Valladares et al., 2008). In our study we showed that canopy effects described previously is constant along the gradient of forest expansion (i.e., tree canopy reduces soil temperature as well as soil moisture). Thus, canopy effect prevails over land-use legacy effect on microclimatic conditions.

Total litterfall did not vary among stages but we found greater litterfall in 2021 than 2020. This could be explained by better climatic conditions in 2021 than in 2020 that promote a higher foliar renewal in this year (Andivia et al., 2018). In our study, we focused on foliar fall phenology because litterfall seasonality is determined by plant vegetative phenology (i.e., leaf emergence, development, ageing and abscission) and litterfall is mainly composed by leaves (Chave et al. 2009). Temporal patterns of leaves deposition did not differ among stages of forest expansion, finding peaks in each summer of each year. Foliar cover renewal of Mediterranean evergreen species in early summer is interpreted as an evolutionary adaptation to water deficit characteristic of Mediterranean climate (Escudero & Del Arco 1987; Caritat et al. 2006). However, we found greater leaf fall mass at expanding front than in mature forests or transition zone during the experiment. Previous studies have found that an increase of stand age led to a larger productivity, in terms of litterfall quantity (Lucas-Borja et al., 2016; Lucas-Borja et al., 2019). However, it has been showed that mature *J. thurifera* forests are less productive than young ones (Garcia-Morote et al. 2012) which is in accordance with our results. An increase in productivity at expanding fronts could be related to additional available resources due to past land uses that ultimately results in a greater foliage production (Gillespie et al., 1994, Albaugh et al., 2006) or a result of a mechanism in *J. thurifera* that favours exposure to light due to low tree density in this stage (Chan et al., 2005; García-Morote et al., 2012).

We found that mass loss after one year of decomposition was approximately 20 % greater than previous studies in Mediterranean ecosystems (Almagro et al., 2012), being higher in open areas than under canopies. Availability of moisture in plant litter has been identify as an important mechanism of litter decomposition in Mediterranean ecosystems that promotes microbial activity (Dirks et al., 2010; Jacobson et al., 2015). We found that soil moisture content is greater in open areas than under canopies, suggesting a more water limited environment under canopies. However, Acuña-Míguez et al. (2023) showed lower microbial biomass and lower microbial activity in open areas than under *J. thurifera* canopies. Thus, we suggest that greater mass loss after one year in open areas is more related to photodegradation than differences found in soil moisture. In Mediterranean ecosystems, photodegradation is an important mechanism in decomposition of recalcitrant litter type characteristics of Mediterranean vegetation (Austin & Vivanco 2006; Henry et al. 2008; Dirks et al. 2010). Our study agrees with other studies showing a decrease in decomposition rates under canopies due to the interception of solar radiation to the soil surface (Almagro et al., 2012).

We did not find differences in mass loss or decomposition rates among stages after either one or two years. These results are in accordance with previous results in different ecosystems which found either not differences in different successional stages or different response depending on species studied (Wardle et al., 2009). The lack of variation observed in this study suggest that processes that promote decomposition in each stage are different. Differences in tree density have been found along a gradient of forest colonisation into past agricultural lands being greater in mature forest than in expanding fronts (Acuña-Míguez et al., 2020). In mature forest, an increase in tree density could reduce forest floor temperature which is associated with an increase in litter moisture content and an increase in microbial activity (Zhang and Zak 1995). In contrast, at expanding fronts, a decrease in tree density could promote an increase of the effect of photodegradation as it occurs in our study.

Conclusions

Litterfall phenology did not vary among stages of *J. thurifera* forest expansion showing the early summer characterisct foliar cover renewal of Mediterranean evergreen species. However, foliar fall quantity was higher in trees established at expanding fronts than those established in mature forest. This difference could be due to an adaptation to increasing solar radiation in this new forests. Decomposition rate did not vary among stages but we found greater decomposition rate in open areas than under canopies, suggesting the importance of photodegradation on *J. thurifera* litter decomposition. The increase in foliar fall quantity at the expanding fronts at the same decomposition rate tht mature forest suggest a high organic matter accumulation in this stage. Further studies are needed in order to asses foliar fall quality and nutrient realese during decomposition process to better understand differences in nutrient cycling along the *J. thurifera* expansion gradient

Acknowledgments

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Supplementary information chapter 3

Table S1. Biometric characteristics of trees in each plot

Site	**Stage of forest expansion**	**Plot**	**DBH (cm)**	**Heigh (m)**	**Average crown diameter (m)**
Ribarredonda	Mature forest	RR_MF1	20.9 ± 4.0	5.3 ± 0.3	5.83 ± 0.4
Ribarredonda	Mature forest	RR_MF2	26.8 ± 4.5	6.1 ± 0.7	6.3 ± 1.0
Ribarredonda	Transition zone	RR_TZ1	14.2 ± 1.2	4.8 ± 0.2	3.51 ± 0.3
Ribarredonda	Transition zone	RR_TZ2	13.5 ± 30.5	4.7 ± 0.	3.9 ± 0.2
Ribarredonda	Expanding front	RR_EF1	10.4 ± 0.3	4.5 ± 0.2	3.7 ± 0.1
Ribarredonda	Expanding front	RR_EF2	16.4 ± 2.2	5.2 ± 0.6	4.8 ± 0.3
Huertaherndo	Mature forest	HH_MF1	27.5 ± 8.0	6.9 ± 0.2	6.0 ± 0.3
Huertaherndo	Mature forest	HH_MF2	27.6 ± 5.6	5.2 ± 0.7	6.9 ± 0.6
Huertaherndo	Transition zone	HH_TZ1	23.0 ± 3.1	5.6 ± 0.7	5.9 ± 0.6
Huertaherndo	Transition zone	HH_TZ2	16.4 ± 3.4	5.2 ± 0.7	5.0 ± 0.5
Huertaherndo	Expanding front	HH_EF1	12.1 ± 3.0	3.9 ± 0.4	3.7 ± 0.3
Huertaherndo	Expanding front	HH_EF2	18 ± 4.2	4.1 ± 0.6	4.9 ± 0.6
Maranchón	Mature forest	MM_MF1	22.6 ± 2.4	4.7 ± 0.5	6.6 ± 0.2
Maranchón	Mature forest	MM_MF2	29.4 ± 8.4	7.2 ± 0.9	6.2 ± 0.5
Maranchón	Transition zone	MM_TZ1	18.5 ± 4.4	4.3 ± 0.4	5.1 ± 0.4
Maranchón	Transition zone	MM_TZ2	30.2 ± 3.0	5.6 ± 0.9	5.4 ± 0.5
Maranchón	Expanding front	MM_EF1	10.9 ± 0.8	3.5 ± 0.3	3.4 ± 0.3
Maranchón	Expanding front	MM_EF2	15.5 ± 2.7	3.9 ± 0.4	4.6 ± 0.6

Table S2. Model predictions of total litterfall.

	Estimate	Std. Error	marginal R2	conditional R2
Fixed effects				
(Intercept)	5.3072**	0.2047		
Year [2021]	0.4724***	0.047		
Random effects	Standard deviation			
ui	0.3427			
uij	0.3853			
Whole model			0.1331	0.7642

Table S3. Model predictions of leaves fall.

	Estimate	Std. Error	marginal R2	conditional R2
Fixed effects				
(Intercept)	3.00805***	0.21905		
Stage [Mature forest]	-0.93223***	0.1851		
Stage [Transition zone]	-0.85833***	0.1851		
Season [Spring 2020]	-0.52699***	0.15403		
Season [Summer 2020]	1.75393***	0.15403		
Season [Autumn 2020]	0.20602	0.15403		
Season [Winter 2021]	-0.73391***	0.15403		
Season [Spring 2021]	-0.18711	0.15403		
Season [Summer 2021]	2.4707***	0.15403		
Season [Autumn 2021]	0.09569	0.15403		
Stage [Mature forest]*Season [Spring 2020]	0.95377***	0.21783		
Stage [Trnasition zone]*Season [Spring 2020]	0.57902**	0.21783		
Stage [Mature forest]*Season [Summer 2020]	0.80309***	0.21783		
Stage [Transition zone]*Season [Summer 2020]	0.57364**	0.21783		
Stage [Mature forest]*Season [Autumn 2020]	0.63572**	0.21783		
Stage [Transition zone]*Season [Autumn 2020]	0.60897**	0.21783		
Stage [Mature forest]*Season [Winter 2021]	0.53905*	0.21783		
Stage [Transition zone]*Season [Winter 2021]	0.28957	0.21783		
Stage [Mature forest]*Season [Spring 2021]	0.96722***	0.21783		
Stage [Trnasition zone]*Season [Spring 2021]	0.67983**	0.21783		
Stage [Mature forest]*Season [Summer 2021]	0.74692***	0.21783		
Stage [Transition zone]*Season [Summer 2021]	0.71992**	0.21783		
Stage [Mature forest]*Season [Autumn 2021]	0.71184**	0.21783		
Stage [Transition zone]*Season [Autumn 2021]	0.77757***	0.21783		
Random effects	Standard deviation			
ui	0.3042			
uij	0.3976			
Whole model			0.6763	0.8100

Table S4. Model selection table of mass loss for one year introducing as fixed effect the interaction between forest expansion gradient and time (months) and the interaction between microhabitat and time.

Model	(Intercept)	Microhabitat:Time	Stages:Time	df	AIC	weight
2	4.556	+		5	-1361.8	0.661
4	4.556	+	+	7	-1360.4	0.339

Table S5. Model selection table of mass loss for two years under canopies introducing as fixed effect the interaction between forest expansion gradient and time (months).

Model	(Intercept)	Stages:Time	df	AIC	weight
2	4.568	+	6	-1341.7	1

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Chapter 4

Increased recovery in coarse-root secondary growth improves resilience to drought in transition forests

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Abstract

1. Interaction of global change drivers affects forest resilience. Land-use changes (land abandonment) and climate change (a higher frequency and intensity of droughts) are interacting in the Mediterranean Region. Components of resilience in secondary stem growth have been widely studied but, despite the importance of root systems in forest functionality and resilience, non-previous studies have assessed them in coarse roots.

2. In this study, we use Juniperus thurifera tree-ring chronologies in coarse roots and stems to assess biomass allometry and tree resilience to drought events comparing two stages of a forest expansion gradient (mature forests and transition zone) in Alto Tajo Natural Park. We extracted cores of stems and coarse roots in 48 trees distributed in different developmental stages and calculated cross-sectional area increments, root-stem allometric relationship and resilience components for both organs in each individual for two drought events (2005 and 2012).

3. Stem and root growth as well as its allometric exponent were higher in the transition zone than in mature forests. Both organs exhibited a trade-off between resistance and recovery in mature forests but maintenance of higher values in the transition zone. Resilience did not show differences between organs being higher in the transition zone than in mature forests. However, relative resilience in roots in the transition zone was higher than in mature forests, without differences in stems between stages. Finally, the 2012 drought event showed a higher impact on the components of resilience than the 2005 drought event.

4. Synthesis. This study extends the knowledge of root response to drought events and highlights the potential of land-use legacies to reduce the negative impact of climate change by promoting increased root recovery after drought events in trees established in past agricultural lands.

Keywords: biomass allocation, components of resilience, dendrochronology, expanding forests, global change ecology, *Juniperus thurifera,* land abandonment

Introduction

Land-use changes and, particularly, land abandonment are the main factors that have influenced the increase of forest areas in Europe. Almost two-thirds of recent European forests occur in former agricultural land, especially in Eastern and Southern Europe (Palmero-Iniesta et al., 2021). Several positive and negative effects on ecosystem services have been described because of forest expansion (Martín-Forés et al., 2020). The process of expansion and densification of forest area is accompanied by the current increase in environmental adversity due to climate change, especially the growing mean temperatures and in the frequency and intensity of drought periods (Vicente-Serrano et al., 2014). Thus, the interaction between land-use changes and more frequent droughts associated with climate change, particularly in the Mediterranean region, affects forest functioning (Guerrieri et al., 2021) and forest resilience. Forest resilience is the ability of a forest to maintain its state and functions after the impact of a perturbation (Holling, 1973). The study of the impact of drought on forest resilience using tree-ring widths has increased in the last decade (Kannenberg et al., 2020). Tree ring data provide long-term records of the radial growth of individual stems and can be used to study tree response before, during and after drought events. This approach assumes that tree cores collected at breast height and the resilience indices derived from them, those developed by Lloret et al. (2011), reveal the whole-tree response to drought without considering other processes like carbon allocation to root growth (Doughty et al., 2014).

Roots are estimated to make up 20%–40% of the biomass of trees (Jackson et al., 1997). Root systems of woody species are composed of two types of roots: fine roots (<2 mm in diameter), which function are water and nutrients uptake and coarse roots (>2 mm) which are responsible for anchoring trees to the soil and serve as organs to transport water from deeper soil horizons (Brunner et al., 2015). Although there is an increasing interest to study drought effects in the structure and growth of both coarse and fine roots (Kozlowski & Pallardy, 2002), coarse roots are less studied due to the difficulty of sample extractions. The analysis of root growth dynamics and the biomass allocation between roots and stems is, therefore, fundamental to a comprehensive understanding of drought and land abandonment effects in whole-tree drought resilience and forest functionality (Brunner & Godbold, 2007).

Biomass allocation response to a perturbation can be classified following two theories: the optimal partitioning theory (OPT) and the allometric biomass partitioning theory (APT; Pretzsch, Biber, et al., 2012). According to APT, resource allocation patterns between different organs change with plant size and it is not affected by variation in the local environmental conditions (Enquist & Niklas, 2001; Müller et al., 2000). In contrast, OPT states that a plant always in- vests in improving the access to the current limiting factor. Thus, in drought events, plants would invest in root growth to uptake water (Bloom et al., 1985). Field and laboratory manipulation experiments, particularly in seedlings, have supported OPT, showing that many tree species respond to dry conditions with an increase in root-shoot ratio (Poorter et al., 2012). However, it has been shown that this in- crease is influenced by the severity of the stress and that only severe droughts promote C allocation to roots (Poorter et al., 2012). Other studies have analysed biomass allocation by studying the root-stem growth allometry relationship, showing unclear results: some studies have supported APT (Nikolova et al., 2021), OPT (Pretzsch, Uhl, et al., 2012) or none of them (Pretzsch, Biber, et al., 2012).

Juniperus is an evergreen gymnosperm genus with broad geo- graphical distribution, occurring in the Arctic, Africa, East Africa, Central Asia, Central America and South Asia, and it has significant ecological and socioeconomic importance in the Northern Hemisphere (Farjon, 2010; Tavankar, 2015). Moreover, adaptative architectural features in the species of this genus, such as extensive lateral root systems, large root/shoot ratios and tracheids with small diameters (Krämer et al., 1996; Martínez-Vilalta et al., 2002), confer them high resistance to drought and enhance their ability to persist in dry environments. In certain areas, juniper encroachment has been considered a negative process because of the invasive role of the genus over other species (*Juniperus virginiana* L. in USA; Torquato et al., 2020). In other areas, juniper expansion is at the expense of crop abandonment and contributes to increasing ecosystem services (*Juniperus thurifera* L. in Spain; Martín-Forés et al., 2020). In the last years, the number of works that study the ecological processes underlying the expansion of *Juniperus* forest in Mediterranean ecosystems has increased (Alfaro-Sánchez et al., 2021; Gimeno, Escudero, et al., 2012), but there are no studies that have analysed how this expansion affects to below-ground organs.

Due to the adaptative characteristics to drought of *Juniperus* species roots (Krämer et al., 1996) and the expansion that has been shown in previous studies (Gimeno, Escudero, et al., 2012), we use *J. thurifera* tree-ring chronologies both in coarse roots and stems to assess the biomass allometry and tree resilience to drought events in different stages of forest expansion gradient. Specifically, we analyse the following: (i) variation in secondary growth of stems and roots between two stages of gradient expansion forests, (ii) root-stem allometry between these two stages and (iii) differences in resistance, recovery, resilience and relative resilience between organs, stages of forest expansion gradient and drought events. We hypothesised that stems and roots of *J. thurifera* individuals show greater annual growth in the transition zone than in mature forests as previous studies of the species showed in stems (Alfaro-Sánchez et al., 2021). Thus, we expect a greater allometric exponent of trees in the transition zone than in mature forests due to the greater annual growth in the two organs in the transition zone. We hypothesize that components of resilience will vary between organs and stages in *J. thurifera* forest expansion. Drought will affect less the growth rates of trees established in past agricultural lands than in mature forests due to ecological mechanisms associated with past land uses (i.e. location of agricultural lands in flat areas and deeper soils, continuous amelioration of soil structure by agricultural practices and higher soil nutrient concentration) that could buffer new forests from severe drought stress (Vilà-Cabrera et al., 2017). Thus, we propose that higher intensity of drought in mature forests will promote OPT strategy (Poorter et al., 2012), whereas APT will prevail in the transition zone. Finally, adaptive characteristics acquired by trees that colonise open areas together with land-use legacies in the transition zone could promote an increase in resilience in trees established in this stage.

Materials and methods

Study area and species

This study focused on *J. thurifera* that is a dominant species forming low-density forests that can establish on poor, shallow, rocky soils and tolerates broad temperature range (high temperatures in summer, and low ones and frost in winter) typical of Mediterranean continental climate regions (Gauquelin et al., 1999; Montesinos et al., 2009). Its distribution is restricted in the Western Mediterranean basin.

We conducted this study in Alto Tajo Natural Park where *J. thurifera* forests are expanding towards abandoned lands (Gimeno, Pías, et al., 2012). The climate in this area is continental Mediterranean, characterised by hot and dry summers and cold and snowy winters (mean annual total rainfall ± standard error (SE): 477.1 ± 15.6 mm, mean annual temperature: 10.4 ± 0.2 C, Molina de Aragón 40°50'40" N, 1°53'07" W, 1063 m a.s.l., 1951–2017 period; Agencia Estatal de Meteorología). We selected three sites (Huertahernando, Ribarredonda and Maranchón) of well-conserved forests where previous studies have shown a forest expansion gradient (Villellas et al., 2020). These studies classified the gradient in three stages: expanding front (areas of recent abandoned lands), mature forests (well-conserved forest) and transition zone between the other stages with intermediate characteristics related to tree density, tree size and age (for further information see Villellas et al., 2020; Acuña-Míguez et al., 2020). Due to the reduced tree size in the expanding front, we conducted this study in mature forests and transition zone (two plots per site). Previous studies showed differences between these two stages at the forest expansion gradient in tree size, age and tree density (Acuña-Míguez et al., 2020).

Sample collection and tree-ring data

In each plot, we selected four trees based on frequency of diameter class within each plot. We extracted one core from the stem of each tree at breast height according to existing methodology (Preztsch, Biber, et al. 2012; Nikolova et al. 2021). Then, we excavated and selected two coarse roots of each tree considering the growth variability due to soil characteristics (i.e., stoniness or compaction), and extracted two cores of each coarse root in perpendicular orientation at 30-50 cm distance from the trunk using a Haglöf increment borer (Fig S1).

We measured the diameter at breast height of each tree (DBH) and the quadratic mean diameter (QMD, calculated as the square root of the sum of square diameter at breast height of each stem of a tree; Stewart & Salazar, 1992) of all neighbouring trees in a radius of 6 meters for calculating the local basal area as a measure of competition of each tree. Moreover, we measured the diameter of the cored roots at the position of coring (Table 1; Table S1)

Table 1. Mean ± SD of diameter at breast height (DBH), DR1 (root diameter 1), DR2 (root diameter 2) and local basal area (Local BA) of trees selected in each stage of forest expansion gradient and in each site.

Site	Stage of forest expansion gradient	DBH (cm)	DR1 (cm)	DR2(cm)	Local BA (m^2 ha^{-1})
Huertahernando	Mature forest	15.86 ± 4.33	7.24 ± 2.05	5.83 ± 1.59	25.06 ± 23.90
	Transition zone	17.34 ± 6.05	7.36 ± 2.06	7.31 ± 1.51	2.48 ± 4.50
Ribarredonda	Mature forest	21.69 ± 6.53	6.18 ± 1.38	6.54 ± 2.08	6.24 ± 9.68
	Transition zone	16.60 ± 5.15	6.93 ± 2.39	6.69 ± 2.47	19.03 ± 10.60
Maranchón	Mature forest	17.49 ± 4.20	5.46 ± 1.22	5.08 ± 1.65	20.87 ± 22.15
	Transition zone	16.16 ± 4.05	7.05 ± 3.15	6.88 ± 2.79	8.29 ± 9.23

We longitudinally cut each core using a microtome and scanned them at 2400 dpi. We measured ring widths to an accuracy of 0.01 mm using the CooRecorder v9.3 software (Cybis Elektronik 2018). The cross-dating of individual series was checked using the CDendro v9.1 software and COFECHA program (Holmes 1983). We calculated the mean growth of the two radii of each core. Then, we calculated cross-sectional area increment (CSAI, cm2) for each core using *bai.out* function of dplR package (Bunn, 2010). For the following analysis, we used the temporal series from 2000 to 2018 to keep most of the trees in the study. We calculated back-wards diameter time series for each root and stem using cross-sectional area increments of each one. With these time series, we applied the basic allometric equation (Huxley and Tessier, 1936) which describes how the studied plant organs main root and stem change with plant size:

$$lndri \ = \alpha 0 + \alpha 1 \ln \left(dsi\right) \qquad \text{(eq. 1)}$$

Where $\alpha 0$ is the allometric factor, $\alpha 1$ corresponds with the allometric exponent and dri and dsi are the root and stem diameter for year i. $\alpha 1$ is the biologically relevant term which covers both radial increment and plant proportions in the long term. The allometric exponent is 1 when plant growth is in a steady state (Nikolova et al., 2021; Poorter et al., 2012)

Drought events and resilience indices

We calculated the 6-month SPEI (standardized precipitation-evaporation index) from 1901 to 2018 and used 6-month SPEI from 2003-2015 (Fig 1) because it allowed us to have three years before and after the drought event in our temporal ring series. SPEI represents a multiscale drought index that accounts for both temperature and evapotranspiration effects on the water balance. Negative SPEI values indicate dry periods, while positive SPEI values indicate wet periods (Vicente-Serrano et al., 2010).

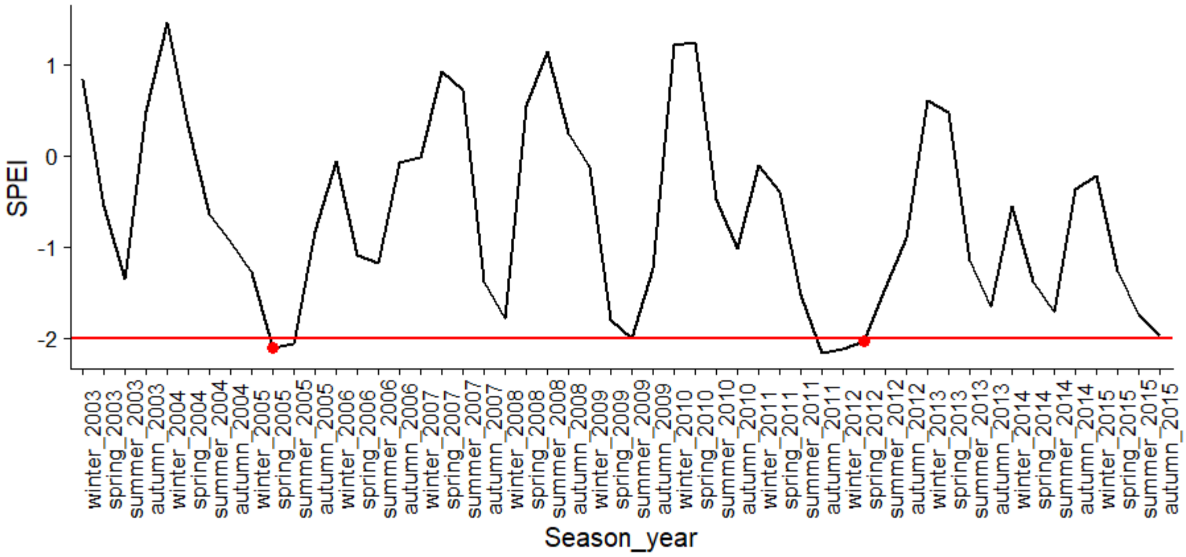

Figure 1. 6-month SPEI calculated for each season from 2003 to 2015. Red points show the lowest values in spring.

These values were downloaded from the SPEIbase (https://spei.csic.es/database.html, SPEIbase v2.6, (Beguería et al., 2014)). To choose drought events that could impact in J. thurifera growth, we focused on the most negative values in spring. Secondary growth initiates in April-May in this species (Camarero et al., 2010) and it has been shown that spring SPEI has an impact in this species (Alfaro-Sánchez et al., 2021). Thus, we chose 2005 and 2012 as drought events because spring SPEI showed the lowest values of all the study period (SPEI<-2; Fig2).

Then, we calculated the components of resilience described by Lloret et al. (2011): resistance (Rt; eq. 2), recovery (Rc; eq. 3), resilience (Rs; eq. 4) and relative resilience (RRs; eq. 5) for each drought event (2005 and 2012) and for each organ (stem and root) using the cross-sectional area increment (CSAI). For roots, we considered components of resilience value as the mean between the indices measured in the two roots of each tree.

$Rt = CSAI_{\text{drought event}} / CSAI_{\text{3-years pre-drought}}$ (eq. 2)
$Rc = CSAI_{\text{3-years post-drought}} / CSAI_{\text{drought event}}$ (eq. 3)
$Rs = CSAI_{\text{3-years post-drought}} / CSAI_{\text{3-years pre-drought}}$ (eq. 4)
$RRs = (CSAI_{\text{3-years post-drought}} - CSAI_{\text{drought event}}) / CSAI_{\text{3-years pre-drought}})$ (eq. 5)

Statistical analyses

We used linear mixed models (LMMs) for each organ to analyse how cross-sectional area increment varied among stages. As fixed effect, we introduced stages of forest expansion gradient, years, the basal area, and the interaction among stage and year. For the stem, the random effect was the tree nested in site (eq. 6) and for the root, the random effect was the root nested in tree and nested in site (eq. 7). Cross-sectional area increments and basal area were log-transformed to conform model assumptions.

$$yijl = \alpha 0 + \alpha 1 Stageij + \alpha 2 Yearij + \alpha 3 BasalAreaijl + ui + uij + \beta 1 (StageixYearij) + \varepsilon ijl \qquad \text{(eq. 6)}$$

where $yijl$ is cross-sectional area increments for stem, for the tree *j* in the site *i* of the year *l*, $Stage$ of the forest expansion gradient, $Year$ is year where CSAI was measured and $BasalArea$ of each individual in each year.

$$yijkl = \alpha 0 + \alpha 1 Stageijk + \alpha 2 Yearijk + \alpha 3 BasalAreaikl + ui + uij + uijk + \beta 1 (StageijkxYearijk) + \varepsilon ijkl \qquad \text{(eq. 7)}$$

where $yrijkl$ is cross-sectional area increments for root, for the tree root k of tree j in the site I of the year l, $Stage$ of the forest expansion gradient, $Year$ is year where CSAI was measured and $BasalArea$ of each root in each year.

We used a LMM to analyse the differences in root-stem allometry between two stages of forest expansion gradient. We introduced root diameter as response variable root and stem diameter, forest expansion stage and its interaction as fixed factors. We introduced root nested in tree as random intercept and stem diameter as random slope (eq. 7). Root and stem diameter were log-transformed to conform model assumptions.

$$\ln(Drootijk) = \alpha 1 + \alpha 2Stage + ui + uij + (\beta 1 + \beta 2Stage + vi + vij) \cdot \ln(Dstemijk) + \varepsilon ijk \qquad \text{(eq. 8)}$$

where $Drootijk$ is root diameter, for the tree root *i* of tree *j* in the year *k*, $Dstemijk$ is the stem diameter for the tree root *i* of the tree *j* in the year *k* and $Stage_j$ is the stage of forest expansion of the tree *j.*

We conducted four LMMs (one per component of resilience) to analyse how components of resilience varied between organs, drought events, and stages of forest expansion gradient. We set as fixed effects the interaction among stages of forest expansion gradient, organs and drought events, and a plot and tree nested in plot as random effects. We analysed if there were differences between two models that differed in random effects, as there were no differences, we selected the simplest one (it means plot as random effect; eq. 8). All components of resilience were log-transformed to conform to normality. In the case of relative resilience, it was transformed with natural logarithm +1 to remove negative values. In case of interactions were significant, we conducted post-hoc Tukey analyses.

$$yij = \alpha 0 + \alpha 1Organ + \alpha 2Stage + \alpha 3Drought + ui + \beta 1Organ\ x\ Stage + \beta 2Stage\ x\ Drought + \beta 3Organ\ x\ Drought + \gamma 1Organ\ x\ Stage\ x\ Drought + \varepsilon ij \qquad \text{(eq. 9)}$$

where yij is the log-transformed response variable (Rt, Rc, Rs, RRs) for the tree *i* in the plot *j*, $Organ_j$ (is either root or stem), *Stage* of forest expansion of the plot *j*, and *Drought* is the drought event (2005 or 2012).

We reduced all models to those with the lowest Akaike Information Criterion AIC (the best or most parsimonious models) using the dredge function from the *MuMIn*

R package (Barton, 2020). The method was set to maximum likelihood (ML) during the fixed-effect model selection phase, although the final models are presented using restricted maximum likelihood (REML) (Kuznetsova et al., 2017). Models fit was visually checked to ensure model assumptions. We calculated marginal (i.e. the proportion of variance explained by fixed effects) and conditional (i.e. the proportion of variance explained by fixed and random effects) r^2 with the *sjPlot* R package (Lüdecke 2021). All statistical analyses were performed using R version 3.5.1 (R Core Team 2019).

Results

Cross-sectional area increment

Linear mixed effects model (according to AIC) which better explained variation in stem and root CSAI were those with year, stages of forest expansion gradient and basal area as fixed effects. We found that basal area had a significant positive effect on stem and root Ig (Table S2 and Table S3, respectively). Stem CSAI (Fig 2; marginal r^2= 0.736; conditional r^2= 0.932) and root CSAI (Fig 2; marginal r^2= 0.760; conditional r^2= 0.941) were higher in transition zone than in mature forests.

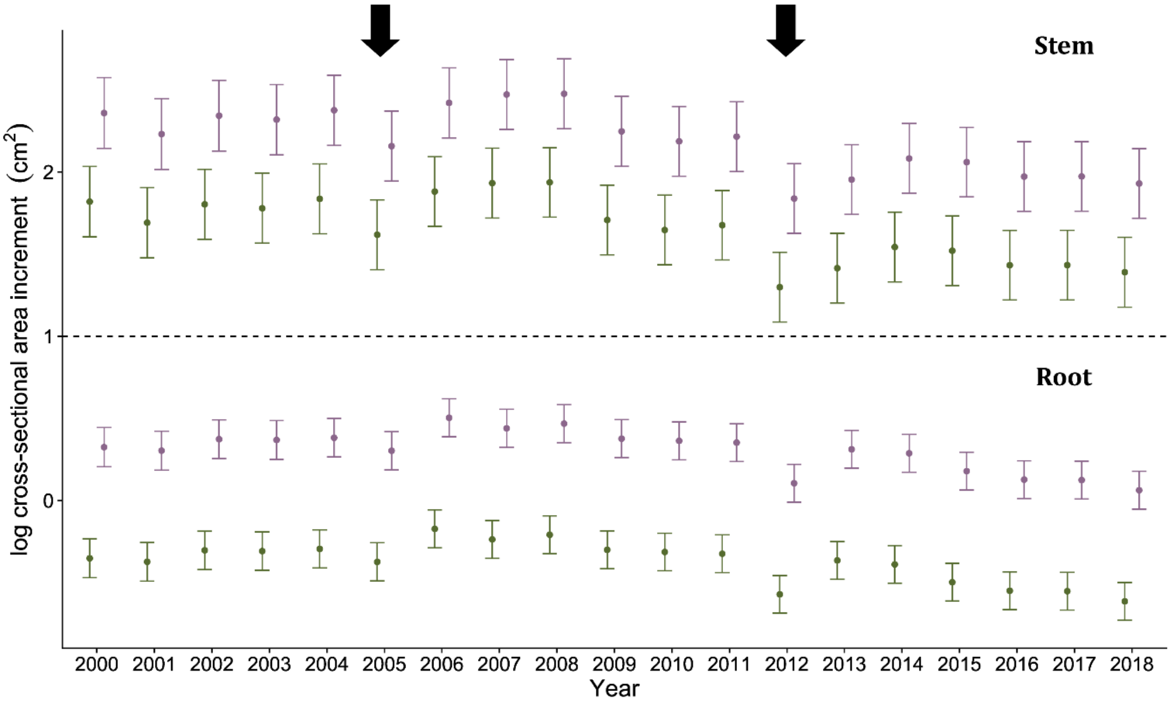

Figure 2. Mean ± SE for cross sectional area increment of stem and roots in mature forest (green points and bar) and transition zone (purple points and bar) along the studied temporal series. Values are log-transformed. Arrows indicate both drought events occurrence.

Root-stem allometry

We found that root-stem allometry varied between stages of forest expansion gradient (marginal $r^2 = 0.476$; conditional $r^2 = 0.995$; Figure 3). Allometric exponent was higher in the transition zone than in mature forest (1.619 and 1.306, respectively; Table 2).

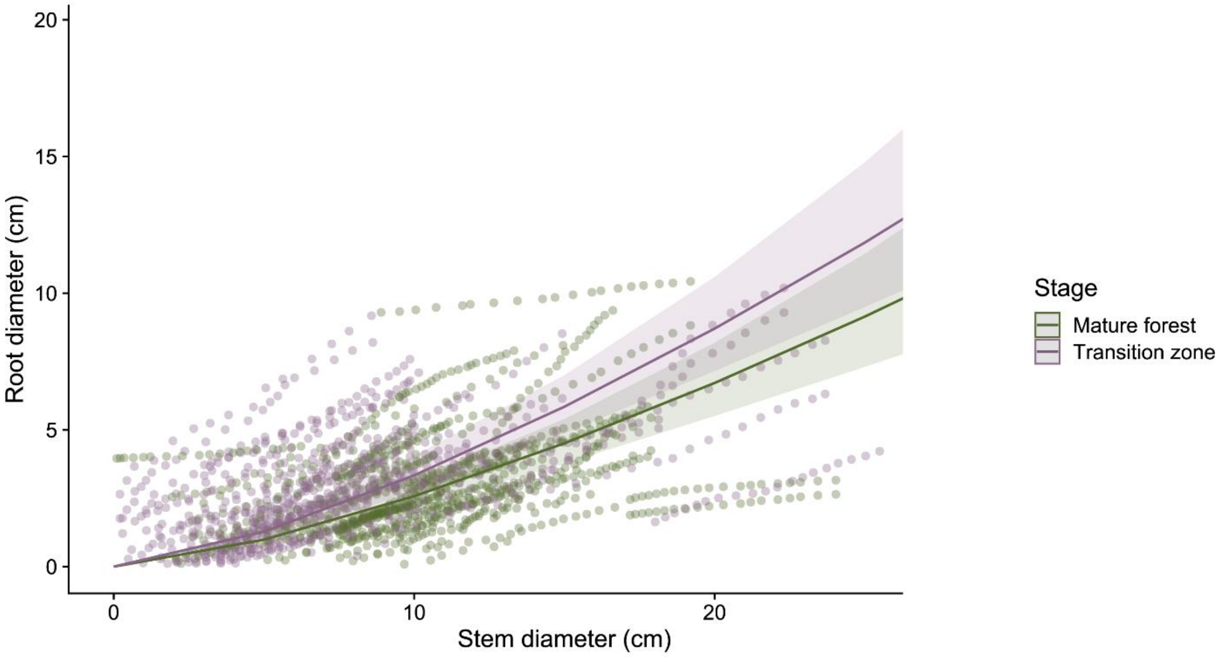

Figure 3. Relationship between root diameter and stem diameter in mature forest (green) and transition zone (purple). Coefficients of model predictions were transformed to arithmetic scale.

Table 2. Estimated fixed effects for allometry model (eq. 8) for root diameter (ln dr) in relation to stem diameter (ln ds) and stage of forest expansion, being ds root and stem diameter respectively.

Fixed effects	Estimate	Std. Error
$Intercept$	-2.500***	0.347
$\ln(ds)$	1.619*	0.124
$Mature\ forest$	-0.312***	0.141
Random effects	Standard deviation	
ui	1.810	
uij	2.047	
vi	0.669	
vij	0.731	

Components of resilience

Best linear-mixed effects model (according to AIC) that explained variation in resistance included year and the interaction between stages of forest expansion and organs (Table S4). We found that resistance was significantly lower in 2012 than in 2005 (Figure 4a). In mature forests resistance was higher in roots than in stems, but no differences between organs were found in the transition zone (Figure 4b). Trees in the transition zone showed higher stem resistance than in mature forests (Figure 4b; marginal $r^2 = 0.225$; conditional $r^2 = 0.367$). Best linear model for recovery included the interaction between years and organs and the interaction between stages and organs (Table S4). Recovery was lower in stems in 2012 than in stems in 2005 (Figure 4c). Contrary to resistance, stems showed a higher recovery in mature forest than roots in this stage (Figure 4d). Moreover, roots of trees established in the transition zone showed a higher recovery than those established in mature forests (Figure 4d; marginal $r^2 = 0.146$; conditional $r^2 = 0.264$).

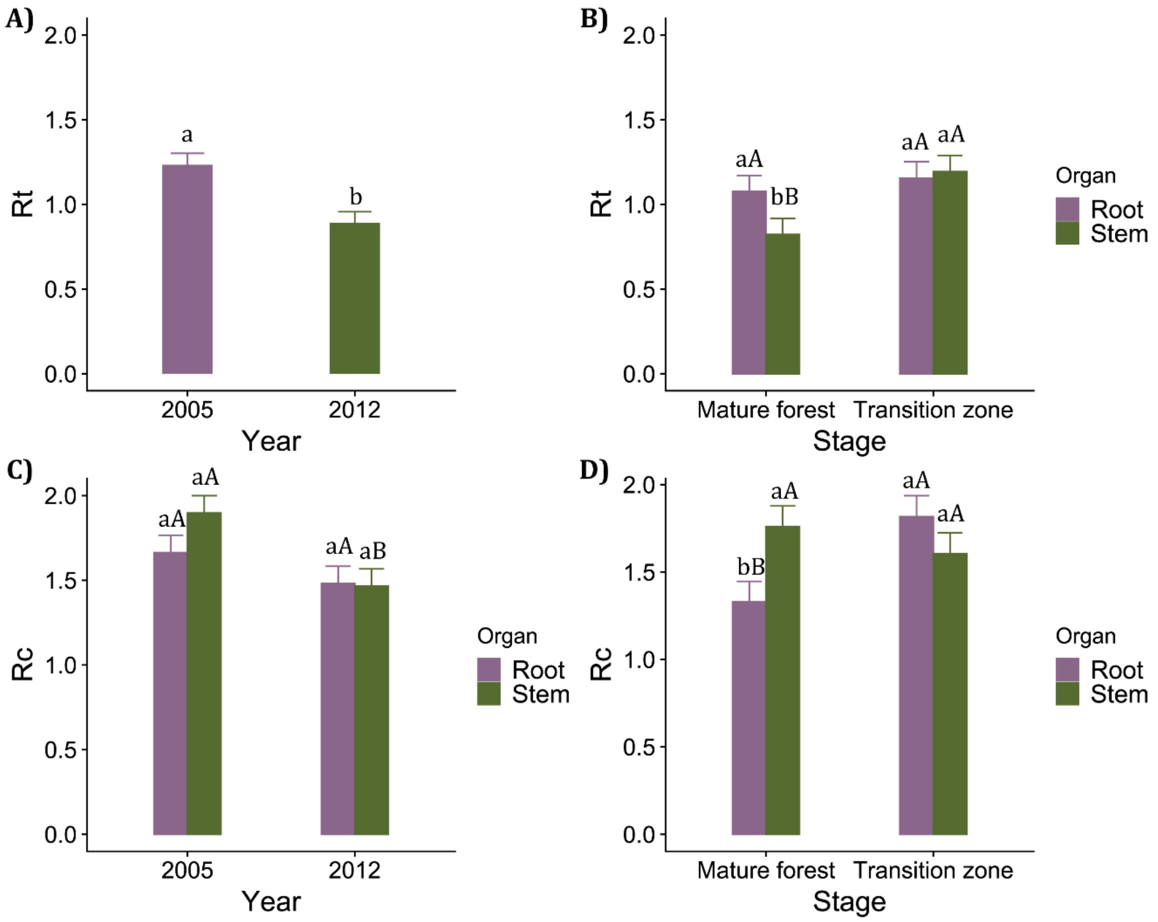

Figure 4. Model prediction of resistance (a, b) and recovery (c, d) for (a) the effect of the year, (b) the effect of the interaction between the stage of forest expansion gradient and organ, (c) the effect of the interaction between year and organs and (d) the effect of the interaction between stage of forest expansion gradient and organs. In panel a different letters indicate significant differences ($p < 0.05$). In panels b, c and d different lowercase letters show differences between organs for each level of the factor ($p < 0.05$) and different capital letters show differences between each level of the factor for each organ ($p < 0.05$).

Best linear effect model that explained variation in resilience included stages of forest expansion gradient and the interactions between organs and years (Table S4). Resilience was significantly higher in the transition zone than in mature forest (Figure 5b). There were no significant differences between organs in 2005. However, resilience was significantly higher in roots than in stems in 2012. In both organs, resilience decreased significantly in 2012 compared with 2005 (Figure 5a; marginal r2 = 0.311; conditional r2 = 0.423).

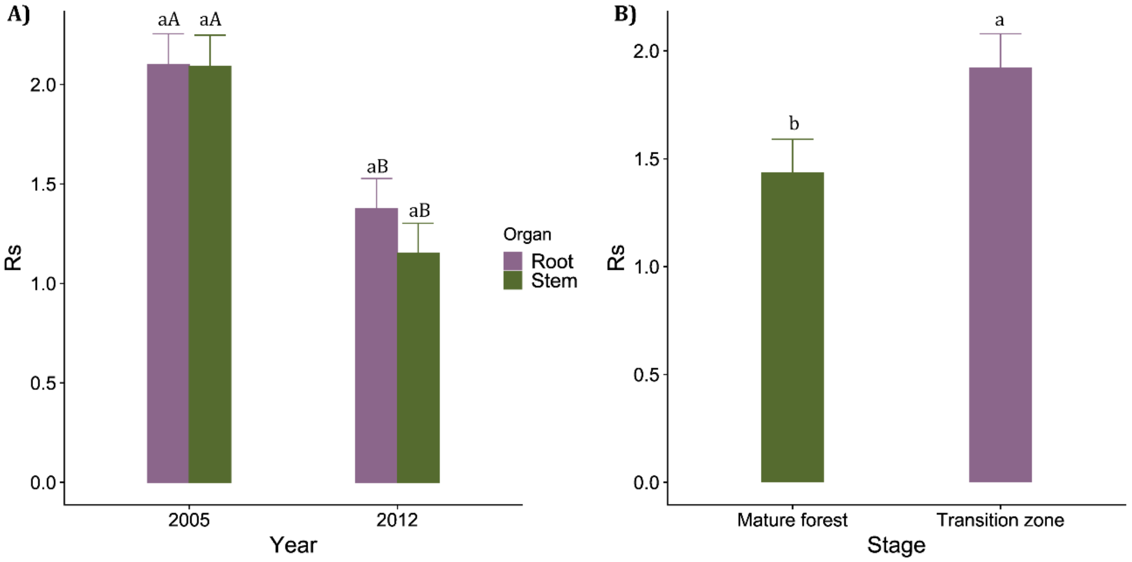

Figure 5. Model prediction of resilience for (a) the effect of the interaction between year and organs, (b) the effect of the stage of forest expansion gradient. In panel a, different lowercase letters show differences between organs for each year ($p < 0.05$), and different capital letters show differences between year for each organ ($p < 0.05$). In panel b, different letters indicate significant differences ($p < 0.05$).

Best linear effects models for relative resilience included interactions between organs and years and between organs and stages of forest expansion (Table S4). Relative resilience measured in stems was higher in 2005 than in 2012 (Figure 6a). There were no differences between stems of trees established in different stages (Figure 6b) However, roots of trees established in the transition zone showed higher relative resilience than roots of trees established in mature forests (Figure 6b; marginal $r^2 = 0.143$; conditional $r^2 = 0.182$).

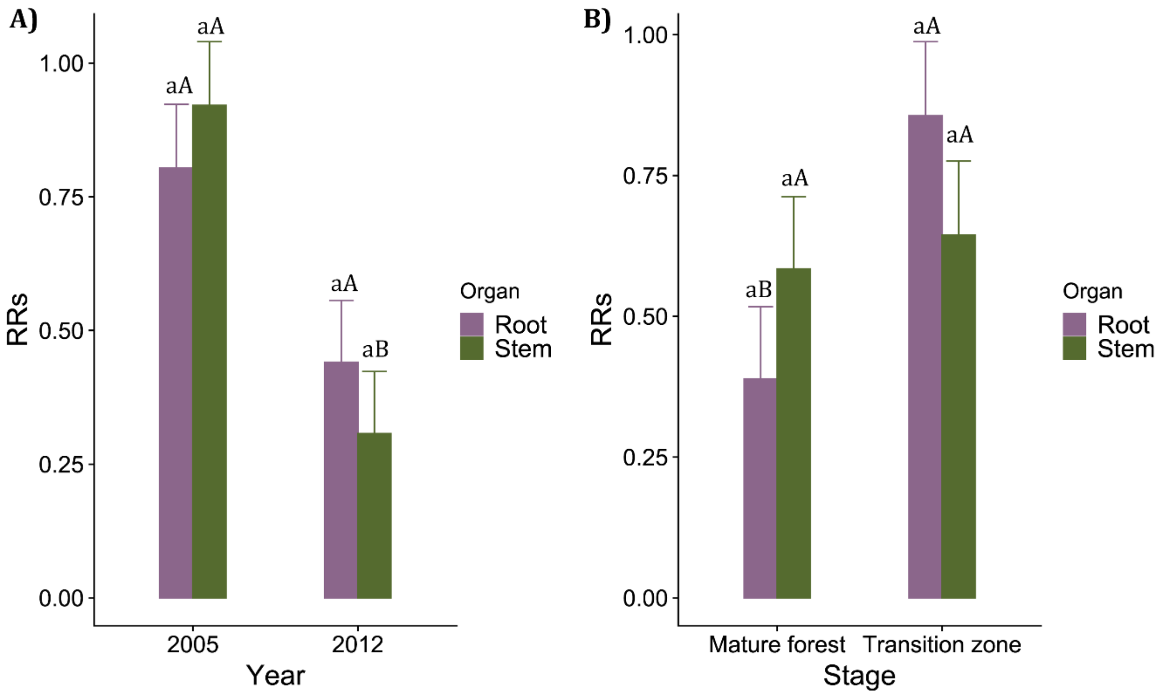

Figure 6. Model prediction of relative resilience for (a) the effect of the interaction between year and organs, (b) the effect of the interaction between the stage of forest expansion gradient and organs. In panel a, different lowercase letters show differences between organs for each year ($p < 0.05$), and different capital letters show differences between year for each organ ($p < 0.05$). In panel b, differentlowercase letters show differences between organs for each stage ($p < 0.05$), and different capital letters show differences between stage for each organ ($p < 0.05$).

Discussion

The stage of forest expansion gradient affects secondary growth of roots and stems as well as biomass allocation to roots, being higher in trees established in the transition zone in both cases. The strategies of the trees to face drought events were different between the stages. In mature forests, we found a trade-off between resistance and recovery (higher resistance of roots and higher recovery of stems) while roots and stems of trees established in the transition zone maintained high values of both indices. We did not find differences between organs in resilience, but tree resilience was higher in the transition zone than in mature forests. However, relative resilience measured in stems was not different between stages, we found that relative resilience of roots was higher in the transition zone suggesting a greater relative resilience of trees in this stage.

Cross-sectional area increments and root-stem allometry between stages of forest expansion gradient

Lower stem growth in *J. thurifera* trees established in mature forests has been previously observed in this system (Alfaro-Sánchez et al., 2021). Some studies have associated this decrease to competition (Alfaro-Sánchez et al., 2021; Gimeno, Camarero, et al., 2012) while others have not found this relationship (Granda et al., 2013). In this study, we show a decrease in root and stem growth in mature forests without an effect of local basal area around each individual in the growth of both organs (Tables S5 and S6). Forests that colonise abandoned fields can benefit from previous land uses (i.e. agricultural uses increase soil nitrogen levels; Nadal- Romero et al., 2018). Thus, we proposed that land-use legacies could reduce nutrient limitation and increase growth of roots and stems.

Biomass allocation to roots with size has been related previously to a decrease in competition (Nikolova et al., 2021). In our case, this pattern was not related to local basal area around each tree (Table S7). We found that the increase in biomass allocation to roots was higher in the transition zone than in mature forests. The establishment in open areas in Mediterranean climate implies greater water stress during summer than other regions, triggered by high irradiance due to lower canopy cover. In water-limited environments, the allocation to fine roots tends to be strongly related to aboveground size (Magnani et al., 2002). In contrast, conifers increase stem growth at the expense of root growth under favourable soil moisture conditions (Thurm et al., 2016). Moreover, previous studies in young trees have shown that biomass allocation to roots increases to capture water and nutrients to support the growth facilitated by high light availability (Annighöfer et al., 2022). Although, we did not find an effect of local basal area in allometric relationship, we propose that the greater allometric exponent in the transition zone could be reflecting the effect of higher light availability in this stage during the process of forest colonisation that promote an increase in belowground biomass (Pretzsch, Biber, et al., 2012).

Resistance and recovery: Different strategies of trees between stages of forest expansion gradient

Our results support the idea that trees of *J. thurifera* established in different stages of forest expansion gradient displays different strategies to cope with drought. Although, this species is among the most drought-resistant conifers known (Olano et al., 2017), the difference between root and stems indices in each stage indicates that the resistance and recovery to drought is organ and stage dependent.

Gazol et al. (2017) showed that there is a global trade-off between resistance and recovery being the most resistant trees those that established in the driest areas. In the present study, we have shown that the trade-off between these two components of resilience can be found between organs in the same individual, at least in mature forests. Roots of trees in mature forests showed higher resistance, but lower recovery than stems. During drought events, trees in mature forests allocated more resources to roots (higher resistance). After drought events, mature forests trees showed higher stem growth than before the drought event (values of recovery >1). Thus, we suggest that OPT is occurring in mature forests with a greater investment in roots than in stems during drought that enables the opposite investment after drought due to a more developed below-ground hydraulic system that improves water acquisition. In the transition zone, we found that both organs maintained high rates of resistance and recovery without differences between organs, suggesting that APT was acting during and after drought.

We suggest that allometric partitioning in trees established in the transition zone is a plastic response of tree growth acquired through the process of colonisation of past agricultural lands. *J. thurifera* juvenile trees of this species show a more efficient phenotype with greater water-use efficiency and greater growth rates in new forests than in mature forests (Benavides et al., 2022) and these differences are maintained in adult trees (Acuña-Míguez et al., 2020; Alfaro-Sánchez et al., 2021). Higher efficiency in trees established in past agricultural lands could be related to amelioration of drought effects due to land-use legacies (Vilà-Cabrera et al., 2017), as previously shown for the same ecosystem, region and species (Gimeno, Camarero, et al., 2012). Thus, we propose that, although colonisation process of past agricultural lands could be limited by the higher irradiation due to less tree cover, land-use legacies (i.e. higher nutrient concentration and deeper soils) allow that trees established in the transition zone invest more in both organs to increase the likelihood to overcome the first summer droughts by improving the acquisition and use of the most limiting factor (water). On the other hand, trees established in mature forest would benefit from a greater tree cover, which reduces excessive irradiation. However, negative effects of tree cover during dry years could be happening in mature forests (Valladares et al., 2008) promoting OPT strategy in trees of this stage due to a greater impact of drought events (Poorter et al., 2012).

The different strategies followed by trees at each stage (APT in the transition zone and OPT in mature forests) caused changes in resilience among stages. In mature forests, the trade-off between resistance and recovery and the higher investment in roots than in stems during drought decrease the resilience of trees established in this stage without differences between organs. Higher recovery found in stems in mature forests might reflect a compensation strategy, that is, preferential carbon allocation to stem after drought to rebuild damaged xylem. However, OPT strategy of trees in this stage, and particularly, stems growth suppression during drought events, indicates a greater drought growth sensitivity that might led to chronic stress in the long-term (Zoblin, 2022). Thus, this strategy might cause higher sensitivity to drought in trees established in mature forests under a scenario of climate change that increase frequency and intensity of drought events (Gessler et al., 2020). Moreover, roots in the transition zone showed higher recovery than roots in mature forests. Hagedorn et al. (2016) showed that trees prioritise the investment in roots after drought to restore trees' capability to acquire water and nutrients. Thus, it seems that the optimal strategy to cope with drought is to maintain high rates of resistance and recovery in both organs, in other words, APT strategy.

Moreover, resilience components were dependent of drought events identity (2012 and 2005). The main difference between these two drought events was the duration (Figure 1). 2012 drought was longer, with autumn and winter of 2011 showing low SPEI values, depleting soil water reserves before the start of the growing season. Previous studies of *J. thurifera* in this area has shown that tree-ring formation was strong dependent on previous autumn environmental conditions (Granda et al., 2013) what was associated to the capacity of conifers to store carbohydrates that can be used to grow the following year (DeSoto et al., 2016; Kagawa et al., 2006). Thus, *J. thurifera* trees could be more resistant in 2005 than in 2012 due to milder conditions in autumn 2004 than in autumn 2011 (Figure 1). The greater recovery in stems in 2012 could be due to the better environmental conditions in 2012 autumn (Granda et al., 2018).

The importance of measuring both organs in relative resilience

Although components of resilience described by Lloret et al. (2011) have been widely used, most studies have not shown their results of relative resilience. Relative resilience is the component of resilience that considers the damage during the drought event (Equation 4). When the value of this index is lower than 1 the drought effect persists after the disturbance (Lloret et al., 2011).

We found differences in relative resilience between the two stages of forest expansion in roots without differences in stems. Previous studies in different species did not find differences in relative resilience between species (Granda et al., 2018), maybe due to the use of this component only in stems. Although, both organs in both stages showed that the drought effect persists after the event, roots in the transition zone showed the lowest drought legacy effect. Higher relative resilience can be reflecting a higher buffer capacity to recover or compensating positive effects of the impact due to a higher neighbour mortality that increase resource availability to surviving trees (Lloret et al., 2011). In this case, we showed that there is a higher buffer capacity to recover of roots in the transition zone (Figure 4d). Our results showed the importance of measuring this component of resilience in both organs to avoid underestimation (in the transition zone) and overestimation (in mature forests) of the relative resilience of trees if only stems are used (Figure 6).

Conclusions

Forest growth and dynamics are increasingly driven by drought, which are raising their frequency and intensity in many already dry regions of the world. Moreover, forests are expanding and becoming denser in the Northern Hemisphere, mainly due to land abandonment. Hence, there is a need to understand the mechanisms of forest responses to drought in this type of ‘new forests’. This study focused on the different responses of roots and stems in trees to drought events in different stages of *Juniperus thurifera* expansion gradient. Our results show that secondary growth is greater in both organs in the transition zone which could be causing a higher relative investment in roots in this stage. Moreover, our findings reveal that tree strategy to cope with drought differs between organs and stages. In mature forests, a trade-off between organs in resistance and recovery promotes a decrease in resilience whereas high values in these traits in both organs in the transition zone promote an increase. These differences could be due to the benefits of land-use legacies. Our results show the importance about measuring all secondary growth resilience components at stem and root levels since differences between stages only appear in relative resilience of roots. Stems do not show differences in relative resilience between stages but roots in mature forests are less relatively resilient which could cause a high vulnerability of trees established in this stage. Thus, our study extends the knowledge of the resilience in forests undergoing expansion and highlights the potential of land-use legacies to reduce the negative impact of climate change by promoting increased root recovery after drought events in trees established in past agricultural lands.

Author contributions

Belén Acuña-Míguez contributed to the methodological approach, performed the field and laboratory work, led the analysis and the writing of the manuscript; Jose Miguel Olano contributed to analysis and to the manuscript production; Fernando Valladares contributed to the methodological approach and to the manuscript production; Miguel García-Hidalgo contributed to laboratory work and analysis and manuscript production; Andrés Bravo-Oviedo led the methodological approach and contributed to field work, data analysis and the writing of the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.14024.

Data availability statement

Datasets generated in the current study are archived on DIGITAL. CSIC https://doi.org/10.20350/digitalCSIC/14764 (Acuña-Míguez et al., 2022).

Supplementary information chapter 4

Figure S1. Photographs of roots cores collection

Table S1. Complete information about trees studied

Plot	Stage of forest expansion gradient	Site	Tree	QMD (cm)	DR1 (cm)	DR2 (cm)	Local BA (m^2 ha^{-1})
MM_MA1	Mature forest	Maranchon	MM_MA1_112	16.10	4.40	3.80	27.22
MM_MA1	Mature forest	Maranchon	MM_MA1_115	15.20	7.00	4.50	26.52
MM_MA1	Mature forest	Maranchon	MM_MA1_130	16.70	4.50	6.40	8.76
MM_MA1	Mature forest	Maranchon	MM_MA1_141	21.40	7.00	4.50	25.97
MM_MA2	Mature forest	Maranchon	MM_MA2_09	14.60	5.10	5.10	2.40
MM_MA2	Mature forest	Maranchon	MM_MA2_13	23.60	4.30	2.50	68.44
MM_MA2	Mature forest	Maranchon	MM_MA2_23	21.20	4.70	6.00	2.57
MM_MA2	Mature forest	Maranchon	MM_MA2_24	11.10	6.70	7.80	5.06
MM_IN1	Transition zone	Maranchon	MM_IN1_41	14.00	2.50	5.35	12.48
MM_IN1	Transition zone	Maranchon	MM_IN1_BN	15.20	2.50	5.40	0.00
MM_IN1	Transition zone	Maranchon	MM_IN1_BR	13.50	7.40	4.50	0.77
MM_IN2	Transition zone	Maranchon	MM_IN2_10	20.45	6.60	5.70	1.07
MM_IN2	Transition zone	Maranchon	MM_IN2_12	12.70	9.10	9.00	19.87
MM_IN2	Transition zone	Maranchon	MM_IN2_15	24.30	8.60	11.10	8.52
MM_IN2	Transition zone	Maranchon	MM_IN2_16	14.30	8.20	3.80	22.80
MM_IN2	Transition zone	Maranchon	MM_IN2_39	14.80	11.50	10.20	0.78
HH_MA1	Mature forest	Huertahernando	HH_MA1_05	14.00	6.10	3.50	8.76
HH_MA1	Mature forest	Huertahernando	HH_MA1_17	14.60	6.40	5.90	11.03
HH_MA2	Mature forest	Huertahernando	HH_MA2_22	12.50	7.90	6.60	55.51
HH_MA1	Mature forest	Huertahernando	HH_MA1_29	10.30	4.70	4.00	2.60

Plot	Stage of forest expansión gradient	Site	Tree	QMD (cm)	DR1 (cm)	DR2 (cm)	Local BA (m^2 ha^{-1})
HH_MA2	Mature forest	Huertahernando	HH_MA2_23	15.90	7.80	4.80	31.73
HH_MA2	Mature forest	Huertahernando	HH_MA2_28	18.60	10.20	6.70	28.04
HH_MA2	Mature forest	Huertahernando	HH_MA2_37	16.40	5.00	7.00	62.79
HH_MA2	Mature forest	Huertahernando	HH_MA2_38	24.60	9.80	8.10	0.00
HH_IN1	Transition zone	Huertahernando	HH_IN1_06	18.70	4.50	8.40	0.00
HH_IN1	Transition zone	Huertahernando	HH_IN1_27	14.40	9.00	8.30	0.00
HH_IN1	Transition zone	Huertahernando	HH_IN1_29	19.60	7.50	6.70	0.00
HH_IN1	Transition zone	Huertahernando	HH_IN1_38	22.50	9.60	9.00	0.00
HH_IN2	Transition zone	Huertahernando	HH_IN2_08	11.80	8.00	6.00	2.30
HH_IN2	Transition zone	Huertahernando	HH_IN2_20	10.50	8.80	6.10	12.76
HH_IN2	Transition zone	Huertahernando	HH_IN2_25	28.20	7.50	8.90	0.00
HH_IN2	Transition zone	Huertahernando	HH_IN2_48	13.00	4.00	5.10	4.80
RR_MA1	Mature forest	Ribarredonda	RR_MA1_02	22.20	6.10	7.30	0.00
RR_MA1	Mature forest	Ribarredonda	RR_MA1_17	21.50	7.50	4.50	21.85
RR_MA1	Mature forest	Ribarredonda	RR_MA1_26	15.60	8.00	4.20	0.00
RR_MA1	Mature forest	Ribarredonda	RR_MA1_45	19.10	5.60	6.40	1.69
RR_MA2	Mature forest	Ribarredonda	RR_MA2_05	22.20	4.50	5.50	0.00
RR_MA2	Mature forest	Ribarredonda	RR_MA2_13	24.30	6.00	5.50	21.52
RR_MA2	Mature forest	Ribarredonda	RR_MA2_25	35.10	7.40	10.10	0.00
RR_MA2	Mature forest	Ribarredonda	RR_MA2_39	13.50	4.30	8.80	4.88
RR_IN1	Transition zone	Ribarredonda	RR_IN1_16	24.10	5.40	8.00	31.93
RR_IN1	Transition zone	Ribarredonda	RR_IN1_17	15.70	7.50	7.20	24.19
RR_IN1	Transition zone	Ribarredonda	RR_IN1_53	11.00	4.30	5.10	18.03
RR_IN1	Transition zone	Ribarredonda	RR_IN1_67	14.00	5.70	5.00	34.45
RR_IN2	Transition zone	Ribarredonda	RR_IN2_20	12.10	5.90	3.50	13.22
RR_IN2	Transition zone	Ribarredonda	RR_IN2_24	14.90	5.60	5.20	10.23
RR_IN2	Transition zone	Ribarredonda	RR_IN2_30	16.20	11.00	8.30	16.54
RR_IN2	Transition zone	Ribarredonda	RR_IN2_42	24.80	10.00	11.20	3.64

Table S2. Model predictions of cross-sectional area increments of stems

	Estimate	Std. Error	marginal R2	conditional R2
Fixed effects				
(Intercept)	-1.3510***	0.2166		
Stage [Transition zone]	0.5397***	0.1003		
Year [2001]	-0.1281*	0.0605		
Year [2002]	-0.0165	0.0604		
Year [2003]	-0.0402	0.0603		
Year [2004]	0.0169	0.0604		
Year [2005]	-0.2017***	0.0610		
Year [2006]	0.0613	0.0617		
Year [2007]	0.1127	0.0626		
Year [2008]	0.1175	0.0630		
Year [2009]	-0.1119	0.0638		
Year [2010]	-0.1724**	0.0645		
Year [2011]	-0.1436*	0.0652		
Year [2012]	-0.5208***	0.0657		
Year [2013]	-0.4051***	0.0662		
Year [2014]	-0.2765***	0.0669		
Year [2015]	-0.2989***	0.0675		
Year [2016]	-0.3870***	0.0680		
Year [2017]	-0.3861***	0.0685		
Year [2018]	-0.4294***	0.0691		
Basal area [log]	0.7126***	0.0149		
Random effects	Standard deviation			
ui	0.3382			
uij	0.3396			
Whole model			0.7355	0.9320

Table S3. Model predictions of cross-sectional area increments of roots

	Estimate	Std. Error	marginal R2	conditional R2
Fixed effects				
(Intercept)	-1.9974***	0.1156		
Stage [Transition zone]	0.6766***	0.1300		
Year [2001]	-0.0212	0.0467		
Year [2002]	0.0488	0.0464		
Year [2003]	0.0439	0.0462		
Year [2004]	0.0573	0.0464		
Year [2005]	-0.0217	0.0465		
Year [2006]	0.1792***	0.0473		
Year [2007]	0.1151*	0.0477		
Year [2008]	0.1434**	0.0481		
Year [2009]	0.0518	0.0488		
Year [2010]	0.0385	0.0496		
Year [2011]	0.0279	0.0500		
Year [2012]	-0.2195***	0.0506		
Year [2013]	-0.0129	0.0513		
Year [2014]	-0.0372	0.0520		
Year [2015]	-0.1457**	0.0526		
Year [2016]	-0.1976***	0.0531		
Year [2017]	-0.2002***	0.0536		
Year [2018]	-0.2621***	0.0540		
Basal area [log]	0.6970***	0.0108		
Random effects	Standard deviation			
u_i	0.1082			
u_{ij}	0.3851			
u_{ijk}	0.3134			
Whole model			0.7608	0.9410

Table S4. Models predictions for resistance, recovery, resilience and relative resilience

	Estimate	Std. Error	marginal R2	conditional R2
Resistance				
Fixed effects				
(Intercept)	0.1794*	0.0833		
Organ [Stem]	-0.2985***	0.0672		
Stage [Transition zone]	0.0052	0.1134		
Year [2012]	-0.2853***	0.0484		
Organ [Stem] * Stage [Transition zone]	0.3288***	0.0966		
Random effects	Standard deviation			
ui	0.1563			
Whole model			0.2249	0.3674
Recovery				
Fixed effects				
(Intercept)	0.2886***	0.0748		
Organ [Stem]	0.3011***	0.0778		
Year [2012]	-0.0841	0.0643		
Stage [Transition zone]	0.2916**	0.0963		
Organ [Stem] * Year [2012]	-0.1362	0.0908		
Organ [Stem] * Stage [Transition zone]	-0.3483***	0.0908		
Random effects	Standard deviation			
ui	0.1238			
Whole model			0.1457	0.2637
Resilience				
Fixed effects				
(Intercept)	0.4609***	0.0880		
Stage [Transition zone]	0.2874*	0.1062		
Organ [Stem]	0.0154	0.0761		
Year [2012]	-0.3476***	0.0749		
Organ [Stem] * Year [2012]	-0.1792	0.1059		
Random effects	Standard deviation			
ui	0.1590			
Whole model			0.3111	0.4233
Relative resilience				
Fixed effects				
(Intercept)	0.3328***	0.0828		
Organ [Stem]	0.2276*	0.1044		
Stage [Transition zone]	0.2896**	0.1012		
Year [2012]	-0.1556	0.0863		
Organ [Stem] * Stage [Transition zone]	-0.2890*	0.1219		
Organ [Stem] * Year [2012]	-0.2107	0.1219		
Random effects	Standard deviation			
ui	0.0910			
Whole model			0.1430	0.1823

Table S5. Model selection table of cross-sectional area increment of stems introducing as fixed effects local basal area (Local BA), basal area (BA), stage of forest expansion gradient (Stage) and year.

Model	(Intercept)	Local BA	BA	Stage	Year	df	AIC	weight
15	-1.359		0.7124	+	+	24	467.3	0.719
16	-1.378	0.0011	0.7125	+	+	25	469.2	0.281

Table S6. Model selection table of cross-sectional area increment of roots introducing as fixed effects local basal area (Local BA), basal area (BA), stage of forest expansion gradient (Stage) and year.

Model	(Intercept)	Local BA	BA	Stage	Year	df	AIC	weight
15	-1.997		0.6970	+	+	25	1008.0	0.702
16	-1.960	0.0021	0.6968	+	+	26	1009.8	0.298

Table S7. Model selection table of root-stem allometric relationship introducing as fixed effects stem diameter (Ln(Dstem)), stage of forest expansion gradient (Stage), interaction between them and local basal area (Local BA), basal area (BA).

Model	(Int)	Ln(Dstem)	Stage	Ln(Dstem)*Stage	Local BA	df	AIC	weight
7	-2.498	1.619	-0.3134			10	-2002.0	0.692
15	-2.297	1.544	-0.7164	0.1504		11	-2000.4	0.308

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Discusión general

Discusión general

El objetivo principal de esta tesis doctoral es analizar la variación de funciones y, por tanto, de servicios ecosistémicos de soporte y regulación, de los bosques mediterráneos a lo largo de un gradiente de expansión forestal en un contexto de cambio global. Los resultados de esta tesis muestran que existen diferencias entre las etapas del gradiente de expansión de la sabina albar en contenido C, N y P, en las actividades enzimáticas y abundancia microbiana del suelo y en la productividad de los bosques que tienen un efecto final sobre la resiliencia de los árboles (Fig 1).

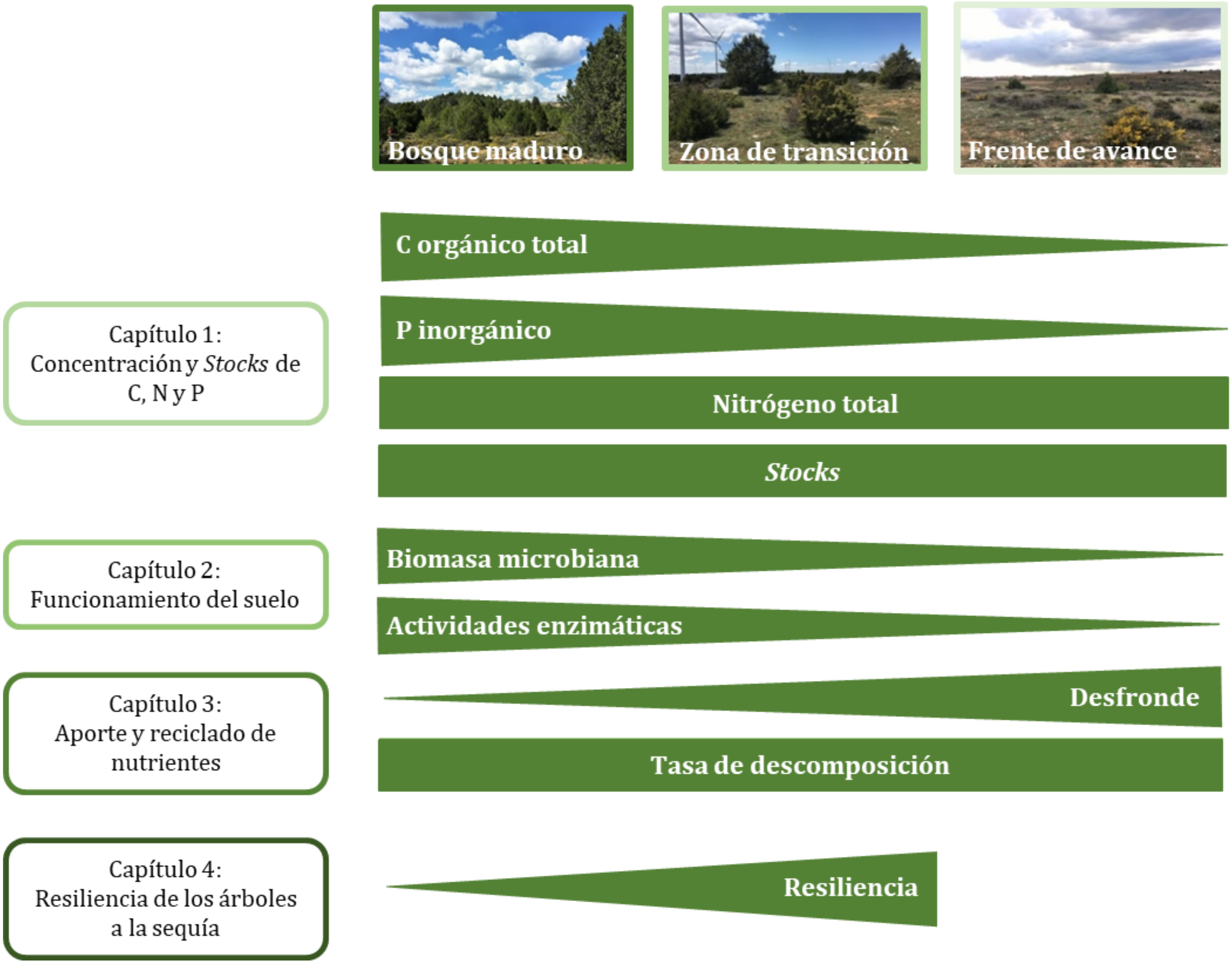

Figura 1. Esquema de la variación de funciones y resiliencia de los árboles a lo largo de un gradiente de expansión de *J. thurifera*

Las prácticas agrícolas convencionales son conocidas por disminuir los stocks de carbono orgánico en el suelo a lo largo del tiempo (Carlson et al., 2017). La recuperación del carbono orgánico del suelo durante la sucesión secundaria que se produce tras el cese de actividades agrícolas depende de la zona, de la intensidad de las actividades o del tipo de cultivo. Por ejemplo, en España, país que según la FAO (2019) ha reducido a la mitad el porcentaje de tierras agrícolas durante las seis últimas décadas, se ha observado que el incremento de carbono orgánico tras el abandono es lento o imperceptible en el sureste bajo condiciones semiáridas (Segura et al., 2020), en las estepas de yesos en el centro del país (Martínez-Duro et al., 2010) o en el clima húmedo del Pirineo (Nadal-Romero et a., 2016). Se ha observado que este incremento es principalmente dependiente de la temperatura media anual viéndose favorecido en una ventana de entre 13 - 17 °C (Bell et al., 2021). Así, en nuestra área de estudio donde tenemos temperaturas medias anuales por debajo de los 13 °C (AEMET), la acumulación de carbono orgánico del suelo es más lenta y aún observamos una menor concentración de carbono orgánico en el suelo mineral del frente de avance. Además, durante la expansión de la sabina albar hacia zonas de cultivo abandonadas encontramos diferencias en la concentración de nitrógeno total y fósforo inorgánico en el suelo mineral, siendo mayor en el bosque maduro que en el frente de avance. Esperábamos que el uso de fertilizantes en tierras agrícolas incrementara la cantidad de nitrógeno y fósforo en antiguos campos de cultivo. El hecho de no observar dicha tendencia sugiere que el uso de fertilizantes y/o la intensidad de las prácticas agrícolas en nuestra zona de estudio fue baja. Es por ello que, en los bosques de sabina albar, la madurez y desarrollo del suelo es lo que proporcionaría mayor concentración de nutrientes (capítulo 1) además de efectos sobre el funcionamiento y abundancia microbiana (capítulo 2). Los efectos de las prácticas agrícolas en las concentraciones de nutrientes no son del todo claros y, por lo tanto, futuras investigaciones deberían centrarse en estudiar el tipo de cultivo y prácticas agrícolas que se llevaron a cabo en esas zonas, así como otras características físicas y químicas del suelo donde sí que podría observarse un legado de los usos agrícolas. Por ejemplo, la tendencia de un aumento del pH y una disminución de la materia orgánica se ha observado en numerosos estudios (Flinn & Marks, 2007; Abadie et al., 2018), incluso en el capítulo 2 de la presente tesis doctoral. Como posibles efectos positivos del legado de los usos agrícolas del suelo podría estar el aumento de la profundidad en antiguos campos de cultivo que podrían favorecer un incremento de la disponibilidad hídrica (Abadie et al., 2018) o posibles cambios en la estabilidad de los micro o macroagreados (Ortiz et al., 2022).

Esta tesis además aporta información sobre el potencial de los bosques nuevos en campos de cultivo abandonados como sumideros de C, ya que no mostraron diferencias con los bosques maduros en cuanto a stocks de C. Nuestros resultados van en la línea de los de Vilà-Cabrera et al. (2017) que mostraron que el carbono almacenado en la parte aérea de los bosques nuevos es similar al almacenado en los bosques maduros. Estos autores observaron además que la tasa de crecimiento de los nuevos bosques era mayor que la de los bosques maduros, lo que indica un descenso de la productividad con la edad (Tang et al., 2014) y signos de saturación en almacenamiento de carbono (Nabuurs et al., 2013). Por lo tanto, en el contexto de cambio global estos nuevos bosques son importantes en la provisión de los servicios ecosistémicos asociados a la fijación de dióxido de carbono atmosférico tanto en la parte aérea (Vilà-Cabrera et al., 2017) como en el suelo (capítulo 1 de la presente tesis doctoral). Sin embargo, cabe destacar la importancia del establecimiento de estrategias de seguimiento y manejo forestal que permitan detectar signos de saturación en el almacenamiento de carbono y poder llevar a cabo prácticas de manejo que retrasen dicha saturación en estos nuevos bosques (Nabuurs et al., 2013).

Los usos históricos del suelo han mostrado ser más determinantes en las comunidades microbianas del suelo que en la composición de la vegetación o en las propiedades del suelo (Jangid et al., 2011). Sin embargo, en esta tesis, la disminución de la biomasa microbiana y funcionamiento del suelo en el frente de avance vino determinada principalmente por la disminución de materia orgánica (capítulo 2), la cual es considerada un indicador de madurez del suelo, importante en los bosques de *J. thurifera* (García-Morote et al., 2012). Se ha observado que la formación de materia orgánica debido a la acumulación de hojarasca en los bosques tras el abandono se produce lentamente, aunque los árboles colonicen dichas zonas (Correira et al., 2021). La recuperación de las comunidades microbianas en bosques de antiguos campos de cultivos a comunidades de bosques maduros puede ocurrir a escalas de tiempo cortas de 50 años (Jangid et al., 2011). En nuestra zona de estudio los árboles del frente de avance tienen una edad media aproximada de 30 años (Acuña-Míguez et al., 2020), por lo que podemos decir que aún no ha pasado el tiempo suficiente tras el abandono para ver si las comunidades microbianas se han recuperado. Aún así, nuestros resultados muestran una mayor producción de hojarasca en el frente de avance (capítulo 3) lo cual favorecería la formación de materia orgánica del suelo en dicha etapa y, por lo tanto, alcanzar niveles de abundancia microbiana y funcionamiento del suelo similar al bosque maduro.

Aunque la sucesión secundaria se ha estudiado más en las comunidades vegetales, dicho proceso afecta también a las comunidades microbianas del suelo. La sucesión secundaria de las comunidades del suelo parece producirse una vez establecidas las comunidades vegetales (Lozano et al., 2014). Las prácticas agrícolas pueden disminuir la ratio hongo/bacteria debido a que las prácticas agrícolas tradicionales como, por ejemplo, arar el suelo, perjudican el micelio fúngico (Helgason et al., 2009; Ortiz et al., 2022). Contrario a lo esperado, no observamos diferencias en dicha ratio a lo largo del gradiente. Sin embargo, si observamos diferencias entre bajo copa y fuera de copa, sugiriendo que es el establecimiento de los árboles de sabina albar en campos de cultivo abandonados lo que promueve la recuperación de la abundancia de hongos en dichas zonas (Kulmatiski & Beard, 2008; Lozano et al., 2014; Ortiz et al., 2022). Cabe destacar en este sistema la importancia de las bacterias Gram-positivas en la regulación de la abundancia del resto de microorganismos lo cual puede ser debido tanto a su adaptación a las condiciones climáticas extremas de la región Mediterráneo debido a su pared celular (Curiel Yuste et al., 2014) como a su participación en el paso final de la degradación de celulosa que libera glucosa, la principal fuente de carbono de las comunidades microbianas (Michel & Matzner, 2003). Futuros estudios que analicen la biodiversidad y la composición específica de las comunidades microbianas del suelo son necesarios para entender el rol de las comunidades fúngicas a lo largo del gradiente, las cuales solo parecieron estar involucradas en el ciclo del fósforo (Pérez-Izquierdo et al. 2017).

Las diferencias mencionadas a lo largo del gradiente en cuanto a la abundancia de microorganismos y actividades enzimáticas (capítulo 2) no se traducen en una variación de la tasa de descomposición de hojarasca a lo largo del mismo (capítulo 3). Nuestros resultados sugieren que podría existir una compensación de los procesos que promueven la descomposición en los extremos del gradiente. En el bosque maduro, la descomposición de la hojarasca viene determinada por el incremento de biomasa microbiana y su actividad observada en el capítulo 2, mientras que la disminución de densidad arbórea en el frente de avance observada por Acuña-Míguez et al. (2020) y en el capítulo 1 de la presente tesis, incrementaría la fotodegradación de la hojarasca, un proceso especialmente importante en la región Mediterránea debido al tipo de hojarasca recalcitrante de la vegetación de estas zonas (Austin & Vivanco, 2006; Henry et al., 2008; Dirks et al., 2010; Gliksman et al., 2018). Además, en el capítulo 1 observamos un incremento de la ratio C/N en el suelo orgánico en el frente de avance que indicaría una menor calidad de la hojarasca en esta etapa. Estos resultados junto con los resultados del capítulo 2 donde observamos una menor abundancia de microorganismos en el frente de avance, reforzaría nuestra hipótesis de que en esta etapa la descomposición viene determinada por la fotodegradación. La importancia del proceso de fotodegradación en la descomposición de la hojarasca de la sabina albar se observa también en las diferencias encontradas entre microhábitats (bajo copa y zonas abiertas). La mayor radiación solar en las zonas abiertas promueve la fotodegradación de la hojarasca mediante la mineralización de compuestos fotorreactivos y la fotooxidación de enlaces químicos (King et al. 2012; Barnes et al. 2015) además del aumento de la temperatura que promueve la degradación térmica de la hojarasca (capítulo 3, tabla 1) (Lee et al. 2012; Van Asperen et al. 2015).

Nuestros resultados además muestran que la caída de la hojarasca, como *proxy* de la productividad primaria neta, del frente de avance fue mayor que en el bosque maduro (capítulo 3) lo cual había sido descrito previamente por García-Morote et al. (2012.). Este incremento podría estar relacionado con una mayor disponibilidad de recursos debido a los pasados usos del suelo que fomentaran una mayor productividad (Gillespie et al., 1994, Albaugh et al., 2006) o ser el resultado del efecto positivo a una mayor exposición a la luz solar debido a la disminución de la densidad arbórea en el frente de avance (Chan et al., 2005; García-Morote et al., 2012). Como se ha mencionado anteriormente, hace falta un estudio más en profundidad de la estructura del suelo o de los tipos de cultivos que se han llevado a cabo en esa zona, ya que no se ha observado un incremento en los nutrientes en el frente de avance, por lo que los efectos positivos deben estar relacionado con otros legados. Por lo tanto, el aumento de la producción de hojarasca en el frente de avance en comparación con el bosque maduro teniendo una misma tasa de descomposición en ambas etapas fomentaría la acumulación de materia orgánica en las zonas de cultivo abandonadas, limitante en estas zonas de la actividad enzimática y actividad microbiana.

Esta tesis además muestra que la estrategia de los árboles para hacer frente a la sequía depende de la etapa donde se encuentre (capítulo 4). Los árboles de las zonas de transición fueron más resilientes a la sequía que los árboles de los bosques maduros principalmente porque siguieron estrategias de alocación de recursos diferentes durante la sequía entre el tronco y la raíz. Hemos mostrado que los árboles que se encuentran en el bosque maduro decidan más recursos a las raíces durante la sequía (mayor resistencia en el crecimiento de este órgano) mientras que después de la sequía se produce una mayor recuperación del crecimiento del tronco (teoría del reparto óptimo, OPT por sus siglas en inglés). Por el contrario, los árboles en la zona de transición muestran altos valores de resistencia y recuperación en ambos órganos (teoría del reparto alométrico, APT por sus siglas en inglés). Así sugerimos que el reparto alómetrico de los árboles la zona de transición es una respuesta plástica del crecimiento de los árboles al proceso de colonización de antiguos campos de cultivo que promueve la inversión en el crecimiento de ambos órganos. Los juveniles de *J. thurifera* mostraron un fenotipo más eficiente en el frente de avance con un mayor uso eficiente del agua y tasa de crecimiento (Benavides et al., 2023), lo cual se mantiene en los árboles adultos (Acuña-Míguez et al., 2020). Esta mayor eficiencia se ha relacionado con una disminución de los efectos de la sequía debido al legado de los usos del suelo (Gimeno, et al., 2012; Vilà-Cabrera et al., 2017) aunque como hemos mostrado en esta tesis dichos legados no están relacionados con una mayor concentración de nutrientes (capítulo 1). El incremento de cobertura forestal en el bosque maduro podría tener un impacto negativo durante los años secos (Valladares et al., 2008) lo cual promueve el reparto óptimo de los árboles (Poorter et al., 2012). El conjunto de los resultados del capítulo 4 muestran que la estrategia óptima para hacer frente a la sequía es mantener altos valores de resistencia y recuperación en ambos órganos y, por tanto, la estrategia APT de los árboles de la zona de transición.

Los legados ecológicos derivados del abandono de tierras agrícolas en España y su potencial, tanto para evitar la degradación del suelo como para mitigar los efectos del cambio climático, deben ser tenidos en cuenta en la planificación de políticas de desarrollo rural (van Leeuwen et al., 2019; Bell et al., 2021) y han de ser acompañados de un seguimiento que minimice los efectos negativos de una expansión forestal descontrolada la cual podría dar lugar a una homogenización del paisaje, pérdida de biodiversidad y mayor probabilidad de incendios (Viedma et al., 2006). Aún teniendo en estos impactos negativos, ponen de manifiesto la importancia de los nuevos bosques como proveedores de servicios ecosistémicos en el contexto actual de cambio global, así como su mayor resiliencia a los, cada vez más frecuentes, eventos de sequía.

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Conclusiones generales

General conclusions

Conclusiones generales

1. A lo largo del gradiente de expansión forestal de *J.* thurifera hacia campos de cultivo abandonados -desde bosques maduros a frentes de avance- se observa una disminución de la concentración de nitrógeno total en la capa orgánica y suelo mineral, así como una disminución de carbono orgánico total y fósforo inorgánico en el suelo mineral.
2. A pesar de las diferencias encontradas en las concentraciones de carbono, nitrógeno y fósforo a lo largo del gradiente, no encontramos diferencias en los *stocks* de estos elementos en el suelo mineral. De este modo, los resultados de esta tesis indican que los bosques establecidos en antiguos campos de cultivo son sumideros de carbono, nitrógeno y fósforo, al menos tan importantes como los suelos de los bosques maduros.
3. El suelo en el frente de avance presentó una menor abundancia de microorganismos y actividades enzimáticas relacionadas con los ciclos de carbono y nitrógeno que en el bosque maduro. Estas diferencias se debieron a la menor cantidad de materia orgánica en los suelos de los nuevos bosques establecidos en antiguos campos de cultivo. Sin embargo, la cantidad de hojarasca caída en el frente de avance fue mayor que en el bosque maduro sin diferencias en la tasa de descomposición entre etapas. Todo ello sugiere que estas zonas pueden llegar a alcanzar el contenido en materia orgánica de los bosques maduros y por tanto su funcionamiento y abundancia microbiana.
4. Las comunidades bacterianas, especialmente las bacterias Gram-positivas, son claves en el funcionamiento del suelo de los bosques de sabina albar. Por un lado, las bacterias Gram-positivas tuvieron un efecto positivo sobre la abundancia de bacterias Gram-negativas, actinomicetos y hongos, y por otro lado las bacterias Gram-positivas y Gram-negativas tuvieron un efecto positivo sobre los ciclos de carbono lábil y moderadamente lábil, respectivamente.
5. Los suelos bajo copa son islas de actividad enzimática y abundancia microbiana del suelo en los sabinares independientemente de la etapa del gradiente de expansión forestal. Sin embargo, la tasa de descomposición fuera de la copa de los árboles fue mayor que bajo copa, mostrando así la importancia de la fotodegradación en la descomposición de la hojarasca de *J. thurifera*.
6. Los resultados de esta tesis sugieren que los procesos que determinan la tasa de descomposición en los extremos del gradiente de expansión forestal son diferentes.

Mientras que en el bosque maduro la descomposición vendría determinada por la actividad microbiana, en el frente de avance vendría determinada por la fotodegradación debido a la menor cobertura forestal.

7. La mayor productividad en los árboles del frente de avance, en términos de producción de hojarasca, podría deberse al efecto de una mayor radiación solar debido a una menor cobertura forestal en dicha etapa del gradiente y una mayor disponibilidad de recursos debido a los usos agrícolas del suelo.
8. En la zona de transición, etapa entre los dos extremos del gradiente de expansión forestal, el crecimiento secundario de raíces y troncos y la inversión en el crecimiento de las raíces fue mayor que en el bosque maduro. Además, las estrategias de los árboles para hacer frente a la sequía difieren entre órganos y entre etapas. Mientras que el bosque maduro las raíces son más resistentes y los troncos se recuperan más tras la sequía, en la zona de transición ambos órganos mostraron una alta recuperación y resistencia a la sequía lo cual hacía a los árboles de esa etapa más resilientes.
9. La resiliencia relativa es la resiliencia teniendo en cuenta el impacto producido sobre el crecimiento secundario de los árboles durante la sequía. Los árboles de la zona de transición presentan una mayor resiliencia relativa que los árboles del bosque maduro debido a las diferencias en dicho componente entre las raíces. Por lo tanto, los resultados de esta tesis muestran que el estudio de la resiliencia relativa en ambos órganos es clave para no infraestimar o sobrestimar la respuesta en el crecimiento secundario de los árboles tras un episodio de sequía.
10. Esta tesis muestra la importancia de conservar los nuevos bosques establecidos en antiguos campos de cultivo dado su capacidad de almacenamiento de carbono en suelo la cual es equiparable a la del bosque maduro. Además, los resultados indican que los suelos de estos nuevos bosques podrían alcanzar el funcionamiento y la abundancia de microorganismos de los bosques maduros. Sin embargo, cabe destacar que los árboles del bosque maduro presentaron una menor resiliencia a los eventos de sequía. Por lo tanto, la presencia de gradientes de expansión, caracterizados por la heterogeneidad de etapas de desarrollo del bosque, es una estrategia de gestión que incrementaría la multifuncionalidad de los bosques de *J. thurifera* en el contexto actual de cambio global

General conclusions

1. Along *J. thurifera* expansion gradient into abandoned agricultural lands - from mature forests to expanding fronts - a decrease in total nitrogen concentration is observed in forest floor and mineral soil, as well as a decrease in total organic carbon and inorganic phosphorus in mineral soil.
2. Despite differences found in carbon, nitrogen and inorganic phosphorus concentrations along the gradient, we did not find differences in stocks of these elements in the mineral soil. Therefore, forests established in past agricultural lands act as carbon, nitrogen, and phosphorus sinks, at least as important as soils in mature forests.
3. Soils at expanding fronts exhibited lower microbial abundance and enzymatic activities related to carbon and nitrogen cycling compared to mature forest due to lower soil organic matter content in past agricultural lands. However, the amount of foliar fall at expanding front was higher than in mature forests, without differences in decomposition rates. Thus, the results of this thesis indicate that forest established in past agricultural lands eventually reach the organic matter content of mature forests and, therefore, their functioning and microbial abundance.
4. Bacterial communities, mainly Gram-positive, play a key role in *J. thurifera* soil functioning. Gram-positive bacteria had a positive effect on Gram-negative bacteria, actinomycetes and fungi abundances. Moreover, both Gram-positive and Gram-negative bacteria had a positive effect on the cycles of labile and moderately labile carbon, respectively.
5. Soils under the tree canopy act as islands of enzymatic activity and microbial abundance in *J. thurifera* forests regardless of the stage of the forest expansion gradient. However, decomposition rate in open areas was higher than under tree canopy, highlighting the importance of photodegradation in the decomposition of *J. thurifera* litter.
6. Results of this thesis suggest that the processes determining decomposition rate at the extremes of the forest expansion gradient are different. While microbial activity would predominantly determine litter decomposition in the mature forest, photodegradation would be an essential process at the expanding front due to reduced forest cover.

7. An increase in productivity in trees at the expanding front, in terms of foliar production, could be attributed to the effect of increased solar radiation due to lower forest cover compared to mature forest and an increased in resource availability due to previous agricultural land use.
8. In transition zone, stage between the two extremes of the forest expansion gradient, secondary growth of roots and stems, and relative investment in root growth were higher than in the mature forest. Additionally, tree strategies to cope with drought differed between organs and stages. While in mature forest roots are more resistant, and stems recover better after drought, in transition zone, both organs showed high recovery and drought resistance, making trees in this stage more resilient.
9. Relative resilience considers the damage to secondary growth of trees during drought. Trees in transition zone exhibit higher relative resilience than trees in mature forest due to differences in this component of resilience between roots. Therefore, results of this thesis show that studying relative resilience in both organs is crucial to avoid underestimation or overestimation on the response in the secondary growth of trees after a drought episode.
10. This thesis highlights the importance of conserving new forests established on abandoned agricultural lands due to their carbon storage capacity of soils, comparable to that of mature forests. Furthermore, results indicate that these new forests could achieve soil functioning and microbial abundance comparable to mature forests. However, it is remarkable that trees in mature forests showed lower resilience to drought events. Therefore, the presence of expansion gradients, characterised by heterogeneity in forest development stages, is a convenient management strategy to increase *J. thurifera* forest multifunctionality in the current global change context.

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