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Doctoral thesis:
Prescribed burning to reduce fire severity: effects on pine forests in the Iberian System

Ph. D. student:
Juncal Espinosa Prieto
to be eligible for the doctor degree by University of Valladolid

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Prescribed burning to reduce fire severity:
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“En dos palabras puedo resumir cuanto he aprendido acerca de la vida: sigue adelante”

Robert Frost

“¿Has leído algo sobre navegación? Klaus había leído exactamente quince libros de navegación y dos de meteorología, pero una cosa es la teoría y otra, la práctica. Poco, les podría haber preparado para la travesía a la cueva sombría en manos de un furioso y malhumorado lago. Pero al finalizar la tormenta y calmarse las aguas, los Baudelaire no pudieron evitar sentir una ligera sensación de logro, un extraño momento de alegría en sus tristes vidas: lo habían conseguido”

Lemony Snicket’s A Series of Unfortunate Events

A mis padres
A Lorenzo

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Y por si alguien no se ha enterado, soy de Palencia.

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List of Acronyms

ICP Forests: International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests
LAI: Leaf area index
MLC: Mass loss calorimeter
PB: Prescribed burning
SMP: Strategic management points
SNFI3: Third Spanish National Forest Inventory
UNECE: United Nations Economic Commission for Europe
WUI: Wildland-urban interface

asl: Above sea level
Bmt: Bark thickness
D30: Diameter at 30 cm from the base
D60: Diameter at 60 cm from the base
D130: Diameter at 130 cm from the base
DPn: Density of *Pinus nigra*
DPp: Density of *Pinus pinaster*
Dt: Total density
FLc: Percentage of fuel load consumed
G: Basal area
GLM: General linear model
GPS: Global positioning system
H1lb: Height to the first live branch
HmMS: Mean maximum scorch height
Ht: Total height
ICP Forests: International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests
INIA: National Institute for Agriculture and Food Research and Technology
LAI: Leaf area index
PB: Prescribe burning
Pn: Percentage of *Pinus nigra*
PN: Plot number
Pp: Percentage of *Pinus pinaster*
PT: Plot type

RH:	Relative humidity
S LAI:	Standard deviation of LAI
T:	Air temperature
TmM Litter:	Mean maximum temperature in litter horizon
TmM Soil:	Mean maximum temperature in soil
TMxB:	Absolute maximum bark temperature
TMxC:	Absolute maximum cambium temperature
WS:	Wind speed
Z:	Zone

AB:	Autumn-burned plot
asl:	Above sea level
BAR:	Bark
BRA:	Branch
BT:	Bark thickness
C:	Carbon
Ca:	Calcium
CON:	Cone
D60:	Diameter at 60 cm from the base
D130:	Diameter at 130 cm from the base
Dt:	Total density
F:	Fraction
G:	Basal area
H1:	Height to the first live branch
Ht:	Total height
ICP Forests:	International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests
ICP-MS:	Inductively coupled plasma optical mass spectrometry
INF:	Inflorescence
K:	Potassium
Mg:	Magnesium
N:	Nitrogen
NB:	Non-burned plot
NEN:	*Pinus nigra* needle
NEP:	*Pinus pinaster* needle
NT:	Nutrient
P:	Phosphorus
PB:	Prescribe burning
Pn:	Percentage of *Pinus nigra*
Pp:	Percentage of *Pinus pinaster*
PT:	Plot treatment
RH:	Relative humidity
RS:	Fire rate of spread

SB: Spring-burned plot
SMx: Mean maximum scorch height
T: Air temperature
TmMxB: Mean maximum bark temperature
TmMxC: Mean maximum cambium temperature
TMxB: Absolute maximum bark temperature
TMxC: Absolute maximum cambium temperature
TRMxB60: Percentage of trees in which the maximum temperature in the bark surface (air temperature) was higher than 60 °C
TRMxC60: Percentage of trees in which the maximum temperature in the cambium was higher than 60 °C
UNECE: United Nations Economic Commission for Europe
WS: Wind speed
Z: Zone

AB: Autumn-burned plots
AIC: Akaike information criterion
AUC: Area under curve
asl: Above sea level
C: Carbon
CPO: Conditional predictive ordinate
D60: Diameter at 60 cm from the base
D130: Diameter at 130 cm from the base
DIC: Deviance information criterion
Dt: Total density
GLMM: Generalized linear mixed model
H1lb: Height to the first live branch
HSmMn: Mean minimum scorch height
HSmMx: Mean maximum scorch height
Ht: Total height
ICP Forests: International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests
INLA: Integrated nested Laplace approximation
LCPO: Mean logarithmic score
LMM: Linear mixed effect model
MS: Mixed stand
N: Nitrogen
NB: Non-burned plots
PB: Prescribe burning
Pm: Monthly precipitation
Pn: Percentage of *Pinus nigra*
Pp: Percentage of *Pinus pinaster*

PS: Pure stand
PT: Plot treatment
RH: Relative humidity
RS: Fire rate of spread
S: Type of stand
SB: Spring-burned plots
StD: Days with storm
SwD: Days with snow
T: Air temperature
tB40C: Time during which temperature was higher than 40 °C in bark surface
tC40C: Time during which temperature was higher than 40 °C in cambium
tB60C: Time during which temperature was higher than 60 °C in bark surface
tC60C: Time during which temperature was higher than 60 °C in cambium
THMxB60: Maximum bark thickness
THMnB60: Minimum bark thickness
TmMxB: Mean maximum bark temperature
TmMxC: Mean maximum cambium temperature
TmMx: Mean maximum temperature
TmMn: Mean minimum temperature
TMxB: Absolute maximum bark temperature
TMxC: Absolute maximum cambium temperature
TRMxB40: Percentage of trees in which the maximum temperature in the bark surface (air temperature) was higher than 40 °C
TRMxC40: Percentage of trees in which the maximum temperature in the cambium was higher than 40 °C
TRMxB60: Percentage of trees in which the maximum temperature in the bark surface (air temperature) was higher than 60 °C
TRMxC60: Percentage of trees in which the maximum temperature in the cambium was higher than 60 °C
UNECE: United Nations Economic Commission for Europe
WAIC: Watanabe-Akaike information criterion
WS: Wind speed

asl: Above sea level
Bpo: Percentage of bark burned
BT: Bark thickness
C60%: Percentage of trees in which the maximum temperature in the cambium was higher than 60 °C
CV: Coefficient of variation of bark thickness
EDA: Exploratory data analysis
FH: Flame height
FL: Flame length
FLI: Fire-line intensity

GLM:	Generalized linear model
H:	Moisture content
HF:	Heat flux
HT:	Heating rate in the cambium area
INLA:	Integrated nested Laplace approximation
MLC:	Mass loss calorimeter
PB:	Prescribe burning
PT:	Plot treatment
RH:	Relative humidity
RS:	Fire rate of spread
SD:	Standard deviation
SMn:	Mean minimum scorch height
SMx:	Mean maximum scorch height
T:	Air temperature
t60:	Time during which temperature was higher than 60 °C in cambium
te300:	Time during which temperature was higher than 300 °C in bark surface
Temx:	Absolute maximum bark temperature
ti:	Time to ignition
tf:	Flame residence time
Tmx:	Absolute maximum cambium temperature
W:	Percentage of weight consumed
WAIC:	Watanabe-Akaike information criterion
WS:	Wind speed
Z:	Zone

AB:	Autumn-burned plot
asl:	Above sea level
BAI:	Basal area increment
DBH:	Diameter at breast height
FH:	Flame height
FL:	Flame length
FLI:	Fire-line intensity
H:	Tree height
Hc:	Percentage of live crown height
ICP Forests:	International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests
L16:	Litterfall biomass from May 2016 to May 2017
L17:	Litterfall biomass from November 2016 to November 2017
NB:	Non-burned plot
P:	Plot treatment
PB:	Prescribe burning
PT:	Plot treatment
RH:	Relative humidity

RS: Fire rate of spread
S: Type of stand
SB: Spring-burned plot
SE: Standard error
SMx: Maximum scorch height
T: Air temperature
t60: Time during which the temperature in the cambium area was higher than 60 °C
t300: Time during which the temperature in the bark surface was higher than 300 °C
UNECE: United Nations Economic Commission for Europe
WS: Wind speed

Bpo: Percentage of bark burned
C: Carbon
Ca: Calcium
DBH: Diameter at breast height
HT: Heating rate in the cambium area
ICP Forests: International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests
K: Potassium
LAI: Leaf area index
Mg: Magnesium
MLC: Mass loss colorimeter
N: Nitrogen
P: Phosphorus
PB: Prescribed burning
t60: Time during which temperature was higher than 60 °C in cambium
te300: Time during which temperature was higher than 300 °C in bark surface
Temx: Absolut maximum bark temperature
tf: Flame residence time
Tmx: Absolute maximum cambium temperature
W: Percentage of weight consumed

AEMET: State Meteorological Agency of Spanish Government
Bpo: Percentage of bark burned
INIA: National Institute for Agriculture and Food Research and Technology
PB: Prescribed burning

Abstract

Wildfires are a global phenomenon, occurring on most continents and affecting a wide range of ecosystems. To the extent that predictive climate models indicate a more frequent occurrence of extreme meteorological episodes, more intense and severe fires are expected. Overall, these high-intensity wildfires are characterized by negative ecological and socio-economic effects, involving the loss of different ecosystem services, goods and even human lives, and also contributing substantially to carbon emissions. In addition, socioeconomic changes that occurred during the 20th century (e.g. rural land abandonment and the extension of wildland-urban interface areas) have exacerbated the problem. There is therefore a need to develop effective forest management systems to address these new scenarios.

There is currently a general consensus, both in the scientific community and among forest managers - and more specifically among agents responsible for fire management - that fire prevention measures should be augmented, particularly those measures related to fuel management. In this context, prescribed burning under canopy vegetation strata, usually carried out with low to moderate intensity, may increase wildfire protection, reducing the horizontal and vertical fuel continuity and promoting landscape heterogeneity. Despite its benefits, use of this fuel management tool in Spain remains limited and is often only carried out in strategic areas at a very local scale. However, efforts are being made to include prescribed burning in forest management plans, and the total area treated has increased in recent years.

Planning under-canopy prescribed burning involves preparing burn prescriptions in relation to meteorological variables, season and ignition patterns, as these factors determine fire behaviour. At the same time, the negative impacts of the treatment must be minimized and the positive effects maximized. Interactions between the aforementioned factors and some species traits might accentuate or decrease the stress on ecosystems, although in many cases, the way in which species respond to the perturbation is uncertain. Some pine species, defined as fire resisters (e.g. *Pinus nigra* and *Pinus pinaster*), possess traits that enable survival after low- to moderate-intensity fires, e.g. thick bark, self-pruning strategies, high crown height, large protected buds, relatively thick needles and deep rooting habit. However, while prescribed burning seems to be a potentially valuable management tool in these forest ecosystems, increasing stand resilience with apparently minimal effects, some data gaps have been identified regarding application of the treatments.

In this context, the goal of this doctoral thesis was **to examine the integrated effect of two seasons of low-intensity prescribed burning in the medium-term at both tree and stand level**. For this purpose, a pure stand of *Pinus nigra* and a mixed stand of *Pinus nigra* and *Pinus pinaster* in the Cuenca Mountains (Iberian System) were chosen. In the study area, these species generate an ecotone of stable stands, which should be taken into account in prescribed burning treatment programmes. Three treatments were carried out in both types of stand: prescribed burning in autumn, prescribed burning in late spring and no burning.

Investigation of the effects of the joint disturbance on crown, via the amount and patterns of litterfall, cambium area and tree growth enabled us to understand, for the first time, the responses of this kind of stands to perturbation. Research on the response of trees to prescribed burning in Europe is disperse, and the data obtained in this doctoral research may provide new information, or expand on existing knowledge, enabling the design, implementation and testing of more effective prescriptions. This doctoral thesis includes **five scientific articles**, four of them published in international scientific journals and which analyze different aspects related to the main objective. The articles are presented in their original form and are organized in **three chapters**.

Litterfall plays a crucial role in the biogeochemical cycle of forest species and is used as an indicator in climate change projections. Despite its importance, no previous studies have been conducted to assess the effects of prescribed burning on the total amount of litterfall or of the different fractions or nutrient contents in pine forests; thus, the data obtained may be an initial reference. In addition, the use of sample collection guidelines from ICP Forests enables comparison of the data obtained with its wide database. **Chapter 2** (comprising **Subchapter 2.1** and **Subchapter 2.2**) describes the short- medium-term impact of prescribed burning, which decreased steadily throughout the study period. The species composition and stand characteristics appeared to dampen the negative effect of fire treatment. The impact on the total amount of litterfall was particularly notable after spring burning, although the recovery rate was faster than after autumn burning. Given the specific characteristics of fructification in *Pinus nigra*, special consideration should be given to the effects of burning on inflorescences and cone production, which should be taken into account in prescribed burning plans. Although there were differences in nutrient concentrations between stands and between fractions, the burning treatments appeared to have little effect on nutrient content of litterfall. In addition, another new insight into variables involved in litterfall process during and after burning was obtained by using a Bayesian approach (**Subchapter 2.3**). Bayesian analysis considers an entire distribution of possible values of parameters and also allows numerous variables to be analyzed together (e.g. meteorological variables; stand characteristics and tree traits that influence fire protection; and fire prescription and behaviour). Use of Bayesian analysis showed that meteorological variables must be considered in burning plans and that the adaptations of the species under study were sufficient to provide complete protection against low-intensity fire.

When a tree is exposed to a surface fire, the probability of survival varies widely, depending on the extent to which the cambium is protected from lethal temperatures. Although fire may not lead to death of the tree, the stress caused may be sufficient to deplete annual ring growth or decrease the photosynthetic activity of the crown. Several studies have assessed the insulating capacity of **bark**, in both the laboratory and field, by monitoring cambial surface temperatures. However, no previous studies have considered bark traits, fire intensity and flammability (sustainability, ignitability and consumability) together as predictor variables. In addition, most existing studies have been conducted in non-Mediterranean ecosystems with non-standardized methods, making comparison of the results difficult. In **Chapter 3**, a mass loss calorimeter (MLC) was used, for first time in a vertical configuration (for more realistic simulation of field burning), to evaluate the protective role of bark. A wide range of heat fluxes were established to simulate prescribed burning and also a wildfire condition, and the results were compared with those obtained in a field burning. The role of bark thickness in protecting against cambium damage was confirmed, especially at low heat flux at which other variables, such as flame residence time, are more important. A minimum bark thickness of 17 mm can be considered a threshold for providing protection from cambium death in prescribed burning plans. Although previous studies have yielded contradictory results regarding the role of bark roughness, the findings revealed that it had a slightly positive effect in protecting the cambium at low heat exposure. The flame was extinguished after the end of radiant heat exposure, although smouldering fire continued to increase the temperature in the cambium, highlighting the role of bark flammability. Comparison of these findings with field data confirmed that stands are not vulnerable at low-intensity prescribed burning and that only local conditions at tree level can generate burn intensities that can potentially cause damage in the cambium.

Comparison of **tree growth** after prescribed burning can be used as a proxy for tree resilience and resistance. Prescribed burning can deplete tree vitality, as trees allocate stored carbohydrates to replenish tissues damaged by fire. However, it can also have positive effects on tree growth, increasing resource availability by removing understory and releasing nutrients. Few studies have modelled individual growth responses as a function of tree and stand characteristics or as direct and indirect effects of fire. Furthermore, although burn season can be an easily controlled feature in prescribed burning plans, little is known about how burn season affects post-fire tree growth. In **Chapter 4**, *Pinus nigra* trees were selected in the study area, and dendrochronological methods were used to investigate whether tree growth responses are influenced by tree and stand characteristics, fire season or fire severity variables. The findings suggest that low-intensity prescribed burning (spring and autumn) is a potentially valuable management tool for reducing fire hazard and does not affect tree growth in the short-term. Nevertheless, special consideration should be given to fire severity variables such as exposure of outer and inner bark to critical temperatures or maximum scorch height in burn prescriptions.

Resumen

Los incendios forestales son un fenómeno global, afectando a un amplio rango de ecosistemas. En la medida en que los modelos de predicción climática auguran una mayor incidencia de episodios meteorológicos extremos, se prevén incendios más intensos y severos. En general, este tipo de incendios se caracterizan por sus importantes efectos negativos desde un punto de vista ecológico y socioeconómico, implicando desde la pérdida de los servicios ecosistémicos hasta la pérdida de bienes e incluso vidas humanas; incluyendo también el aumento de las emisiones de carbono. Además, los cambios socioeconómicos que han tenido lugar a lo largo del siglo XX (por ejemplo, el abandono del territorio rural o el incremento de las áreas de interfaz urbano-forestal) han exacerbado el problema. En consecuencia, es necesario el desarrollo de una gestión forestal adecuada a este nuevo escenario.

Actualmente existe un consenso generalizado, tanto en la comunidad científica como entre los gestores forestales, y más concretamente en los responsables de la gestión del fuego, de que las medidas preventivas contra incendios deben verse incrementadas, particularmente las relativas a la gestión del combustible. La quema prescrita bajo el dosel arbóreo, normalmente, se ejecuta en condiciones de baja a moderada intensidad, con el fin de incrementar la protección frente a incendios forestales al reducir la continuidad horizontal y vertical del combustible, promoviendo, a su vez, una mayor heterogeneidad del paisaje. A pesar de sus beneficios, el uso de esta herramienta de gestión del combustible, en España, es muy puntual y suele verse reducida a áreas estratégicas a escala muy local. Sin embargo, se está haciendo un esfuerzo importante para incluir esta herramienta en el manejo forestal y el número de hectáreas tratadas con quema prescrita se ha visto incrementado en los últimos años.

La planificación de la quema prescrita bajo dosel arbóreo implica el diseño de prescripciones precisas, en función de las variables meteorológicas, la época del año y los patrones de ignición, ya que estos factores determinan el comportamiento del fuego, al mismo tiempo que se persigue reducir al mínimo los impactos negativos y maximizar los efectos positivos del tratamiento. Las interacciones entre dichos factores y determinadas características de las especies forestales pueden acentuar o reducir el estrés en los ecosistemas, aunque en ocasiones, la respuesta de las especies a la perturbación es incierta. Algunas especies del género *Pinus* se consideran como resistentes al fuego (es el caso de *Pinus nigra* y *Pinus pinaster*). Estas especies poseen rasgos que permiten garantizar con mucha probabilidad su supervivencia a los incendios de intensidad entre baja y moderada: corteza gruesa, mecanismos de autopoda de ramas bajas, altura de copa considerable, yemas grandes y protegidas, acículas relativamente gruesas o enraizamiento profundo, entre otras. Sin embargo, si bien las quemas prescritas parecen ser un instrumento de gestión potencialmente valioso en las masas forestales de estas especies, aumentando su resistencia con efectos negativos aparentemente escasos, se siguen planteando algunas dudas importantes relativas a su aplicación.

En este contexto, el objetivo de esta tesis doctoral es **comprender el efecto integrado de dos estaciones de quema prescrita de baja intensidad a medio plazo, tanto a escala de árbol como de rodal**. Para ello, se eligió una masa pura de *Pinus nigra* y una masa mixta de *Pinus nigra* y *Pinus pinaster* en la serranía de Cuenca (sistema Ibérico). En esta zona las dos especies generan un ecotono de rodales estables, que no debe obviarse en los programas de tratamiento con quema prescrita. En cada tipo de masa se estableció una red de parcelas y se llevaron a cabo tres tratamientos: parcelas quemadas en otoño, parcelas quemadas a finales de primavera y parcelas no quemadas que actuaron como testigo. El estudio de la perturbación conjunta en la copa del árbol, a través del análisis de la cantidad y patrones de desfronde, el área cambial y el crecimiento del árbol, permitió explorar, por primera vez, las respuestas de este tipo de masas. Además, dado que la investigación respecto a la respuesta del arbolado a la quema prescrita en Europa se ha descrito como dispersa, los datos obtenidos en esta tesis doctoral pueden generar nuevo conocimiento, o según el caso expandir el ya existente, para diseñar, implementar y testar prescripciones más eficaces. La tesis doctoral incluye **cinco artículos científicos,** cuatro de ellos publicados en revistas científicas internacionales que analizan distintos aspectos relacionados con el objetivo general. Los artículos se presentan en su forma original y están organizados en **tres capítulos**.

El **desfronde** tiene un papel crucial en el ciclo biogeoquímico de las especies forestales y se utiliza como un indicador en las proyecciones de cambio climático. A pesar de su importancia, no se han realizado estudios para evaluar los efectos de la quema prescrita en la cantidad total, las fracciones o el contenido de nutrientes del desfronde en pinares, por lo que los resultados obtenidos aquí pueden constituir una referencia inicial. Además, el uso de las directrices de recolección utilizadas por el ICP Forests, ha permitido la comparación de los resultados obtenidos con su amplia base de datos. En el **Capítulo 2** (que comprende los **Subcapítulos 2.1 y 2.2**) se muestra un impacto limitado a corto y medio plazo de la quema prescrita, que disminuyó a lo largo del período de estudio. La composición específica y las características de la masa parecieron amortiguar el efecto negativo del fuego. El impacto de la quema en la cantidad total de hojarasca fue más evidente tras la quema de primavera, aunque la tasa de recuperación fue más rápida en esta época que durante la quema de otoño. Dadas las peculiaridades de la fructificación de las masas de *Pinus nigra*, los efectos de la quema prescrita en las fracciones de inflorescencias y piñas deben ser seriamente considerados en los planes de quema. Aunque se registraron diferencias en las concentraciones de nutrientes entre masas y fracciones, en general el efecto de la quema prescrita en el contenido de nutrientes del desfronde fue escaso. Utilizando un enfoque Bayesiano, se analizaron las variables involucradas en el proceso de desfronde durante y después del tratamiento de quema prescrita (**Subcapítulo 2.3**). El análisis bayesiano no se basa únicamente en una estimación puntual de los parámetros, sino más bien, en una distribución completa de los posibles valores, permitiendo que puedan ser analizadas conjuntamente muchas variables (por ejemplo, variables meteorológicas, características de la masa y rasgos del árbol que influyen en la protección contra los incendios, prescripción y comportamiento del fuego). Esto reveló que las variables meteorológicas debían considerarse seriamente en los planes de quema y que las adaptaciones de las especies estudiadas eran suficientes para proporcionar una protección completa en el caso de fuegos de baja intensidad.

Cuando un árbol está expuesto a un fuego de superficie, la probabilidad de sobrevivir varía ampliamente, dependiendo de su capacidad para proteger el cambium de temperaturas letales. Si el fuego no causa la muerte del árbol, puede estresarlo lo suficiente como para mostrar una disminución del crecimiento anual o de la actividad fotosintética de la copa. Varios estudios han evaluado la capacidad aislante de la **corteza**, monitorizando las temperaturas alcanzadas en el cambium, tanto en laboratorio como en campo. Sin embargo, ninguno de ellos ha considerado conjuntamente como variables predictoras: los rasgos de la corteza, la intensidad del fuego y la inflamabilidad (incluidas variables relacionadas con la ignitabilidad, la sostenibilidad y la consumibilidad). Además, la mayoría de los estudios actuales se han realizado en especies de ecosistemas no mediterráneos y con distintas metodologías al no existir un método normalizado, lo que dificulta la comparación de los resultados. En el artículo del **Capítulo 3,** se utilizó un calorímetro de pérdida de masa (MLC), por primera vez en su configuración vertical (con la que se consigue una simulación más realista de la quema en el campo) con el fin de evaluar el papel protector de la corteza. Se estableció una amplia gama de flujos de calor, simulando condiciones similares a las de una quema prescrita pero también a las que podrían suscitarse en un incendio forestal, y se compararon los resultados con los obtenidos en las quemas de campo. Se confirmó el papel del espesor de la corteza en la protección del cambium, especialmente trabajando con flujos de calor bajos donde otras variables, como el tiempo de residencia de la llama, podrían ser más importantes. En los planes de quema prescrita podría incluirse un límite de 17 mm de espesor de corteza, como punto crítico para la letalidad del cambium. Aunque estudios previos han mostrado resultados contradictorios sobre el papel de la rugosidad de la corteza, las conclusiones obtenidas revelaron un ligero efecto positivo en la protección del cambium a baja exposición de calor. Se demostró que la llama se extinguía poco después de cesar la exposición a la fuente de calor, aunque la combustión interna continuaba aumentando la temperatura en la zona del cambium; lo que puso de relieve el papel que juega la inflamabilidad de la corteza. La comparación con los datos de campo confirmó que los rodales no son vulnerables a las quemas prescritas de baja intensidad y que sólo las condiciones locales pueden generar intensidades suficientes como para causar un daño potencial al árbol.

La comparación del **crecimiento** de los árboles después de una quema prescrita puede utilizarse como un indicador de la resistencia y la capacidad de recuperación de los mismos. La quema prescrita puede agotar la vitalidad del árbol, ya que este asigna los carbohidratos almacenados para reponer los tejidos dañados por el fuego. En cambio, también puede tener efectos positivos en el crecimiento, aumentando la disponibilidad de recursos al eliminar el sotobosque o liberar nutrientes después del fuego. Pocos estudios han utilizado modelos de respuesta de crecimiento en función de variables de árbol y masa o en función de efectos directos e indirectos del fuego. Asimismo, aunque la época de quema es un parámetro que puede controlarse fácilmente en los planes de quema prescrita, se sabe poco sobre cómo afecta al crecimiento de los árboles. En el **Capítulo 4** se seleccionaron varios árboles de *Pinus nigra* en el área de estudio de la quema prescrita y se utilizaron métodos dendrocronológicos para investigar si el crecimiento está influenciado por las propias características del árbol y de la masa, la época de la quema prescrita y la severidad del fuego. Los resultados sugieren que el tratamiento de quema prescrita de baja intensidad (en

la época de primavera y otoño de manera diferente) es una herramienta de gestión potencialmente valiosa para reducir el peligro de incendio sin efecto sobre el crecimiento arbóreo a corto plazo. Debe prestarse especial atención a las variables de severidad del fuego, como la exposición a temperaturas críticas en la corteza exterior e interior o a la altura máxima de chamuscado en las prescripciones diseñadas.

Chapter 1

General introduction

Doctoral thesis, ***Prescribed burning to reduce fire severity: effects on pine forests in the Iberian System,*** **2021**

Juncal Espinosa Prieto

1.1. Forest fires

Although evolutionary and palaeoecological studies suggest that fire is a natural phenomenon in the Mediterranean basin (Pausas et al., 2009), the large increase in the number of fires and in the area burned during the last century has led to a disastrous situation. Fire destroys more trees than all other types of natural disturbance together (Alexandrian et al., 2000), as well as causing socio-economic and ecological damage and loss of human lives.

Industrialization and rural exodus have led to land abandonment, increasing the cover by early succession species (many of which are very flammable) (Moreira et al., 2001). Indeed, the Third Spanish National Forest Inventory (SNFI3) data highlight a notable expansion of the forest area (MITECO, 2007). Furthermore, the increase in tourism and recreational activities has resulted in greater numbers of visitors to forests, thus contributing to the risk of fire ignitions (Ganteaume et al., 2013). Similarly, large urban areas have expanded into neighbouring wildland areas leading to extension of the wildland-urban interface (WUI) (Chas-Amil et al., 2013).

In addition to changes in forest areas, future climate change scenarios predict an increase in temperature of 1.5 °C between 2030 and 2052 if global warming continues to increase at the current rate (IPCC, 2018). Nineteen of the 20 warmest years on record have occurred since 2001, with 2016 and 2020 ranked as the warmest since record-keeping began in 1880 (NASA, 2020). In this context, the Mediterranean region has been described as a climate change "hot spot". The area is likely to warm at a rate about 20% faster than the global annual mean surface temperature, with particularly high values in summer, in continental areas north of the basin and generally during the day rather than at night (Lionello and Scarascia, 2018).

These projections of more severe climate along with the new landscape are changing fire behaviour in the Mediterranean basin. The expected consequences include more intense, severe fires as well as a longer fire season period (Westerling et al., 2006). In fact, in the last five-year period a new type of fire is occurring in different parts of the world, defined by some authors as "6th generation fires or fire storms" (Castellnou et al., 2018) and by others as "extreme wildfire events" (Tedim et al., 2018). This type of fire can spread faster and across larger areas, and e.g. Castellnou et al. (2018) reported an average area of 10000 ha burned per hour in Portugal in October 2017. Under these conditions, the collapse of fire suppression systems is comprehensible, with fires beyond the capacity of control, causing devastating consequences. In addition, the emission and atmospheric transport of greenhouse gasses released by forest fires as a product of the combustion of vegetation and the loss of associated carbon sequestration and storage, are contributing significantly to climate change (Sommers et al., 2014). As a consequence, the forest landscape is suffering a complete change which has created the need to develop effective forest management plans adequate to these new scenarios.

1.1.1. Changes in forest fires in Spain and in the Mediterranean basin

The latest data on forest fires in Spain published by the Ministry for the Ecological Transition and the Demographic Challenge (López and López, 2019) show a decreasing trend in the number of fire events in the decade 2006-2015, although with variable values throughout the entire study period (1968-2015). Analysis of the data reveals that since 1994 there has been a significant decrease in the area affected, reaching slightly more than 100000 ha year^{-1}, with the second lowest value of the entire series (48717 ha) reached in 2014. This negative trend can be at least partly explained by an increased effort in fire management and prevention after the extensive fires that occurred in the 1980s (Turco et al., 2016). Likewise, the increase of awareness actions, preventive measures financed under the European rural development policy and the progressive increase in knowledge of the causes of forest fires have contributed to this trend (López and López, 2019). Regarding large forest fires, including those exceeding 500 ha, it has been shown that the total area affected has generally decreased in the last few decades, except in 2012. Among the conifers, the most affected species during the decade 2006-2015 were *Pinus halepensis* (22.02%), *Pinus pinaster* (21.26%), *Pinus canariensis* (9.79%) and *Pinus nigra* (3.55%), while among the broadleaved species, *Eucalyptus globulus* (12.31%) and *Quercus ilex* (6.25%) are the most affected.

Despite the decreasing trend in the number of fires and surface area affected during the last decade (after a period of high rates in the 1970s, 1980s and mid-1990s), Spain continues to be one of the countries with the highest incidence of forest fires in Europe (second after Portugal). In Portugal, Spain, the Mediterranean area of France, Italy, Greece and Turkey, forest fires represent more than 80% of the total forest area burned on the continent (San-Miguel-Ayanz et al., 2020). The combination of meteorological factors (e.g. droughts and heatwaves), rough topography, flammable vegetation types, undermanaged forest and high ignition rates drive fires that affect extensive areas (megafires), even beyond national boundaries, especially in southern Europe (e.g. ~200 kha burned in Portugal in mid-October 2017 or 63 kha in Greece in August 2007) (Castellnou et al., 2018). In this respect, extreme fire seasons could affect multiple countries simultaneously (Turco et al., 2016).

1.2. Fuel management

Rural exodus, land abandonment, fire exclusion policies and other socio-economic changes in the last decade have promoted a transition from mosaic-managed type landscapes to a high fuel load, continuous and highly hazardous vegetation complexes (Palahí et al., 2008; Fernandes et al., 2014). Moreover, the substantial increase in fire suppression costs has limited investments in fuel management and fire prevention. The general consensus among the scientific community and forest managers is that fire prevention and fuel management actions must be increased and that old approaches based primarily on fire suppression should be abandoned (Marino et al., 2014).

Intense vegetation removal in strategic locations is used to create fuel breaks, where the potential fire intensity is decreased to a level where suppression resources succeed in containing the fire. In addition, fuel breaks can be used to anchor indirect control lines using backfires (Oliveira et al., 2016). Fuel modification strategies include different options for controlling fuel build-up. Pruning affects canopy base height and thus reduces vertical fuel continuity (e.g. Scott and Reinhardt, 2001), while thinning alters canopy bulk density and decreases the horizontal fuel continuity (e.g. Prichard et al., 2010). Prescribed fire is effective at reducing surface fuel and it can also increase canopy base height by scorching the lower crown of the stand. It is generally less effective at reducing canopy bulk density (Agee and Skinner, 2005). Different methods (e.g. chemical, biological, manual, mechanical and fire-based methods) are used depending on factors such as vegetation type and characteristics, extend of the fire problem, available funds, experience and expertise, tradition and social concerns. Chemical treatments are sometimes applied locally, although they are much less common, as they increase flammability in the short-term and generate environmental concern (Rigolot et al., 2009). Livestock grazing is another option for maintaining less flammable landscapes by controlling fuel cover and load (Jauregui et al., 2009; Ruiz-Mirazo et al., 2011), although the effect can be negative when the carrying capacity of the land is exceeded. In some cases, livestock is actively used to control vegetation re-growth in combination with other fuel treatments. Manual methods generally cover only limited areas and are expensive (Xanthopoulos et al., 2006). Mechanical methods are widely used for their effectiveness, although they may also be very expensive, especially for small forest properties (Marino et al., 2014). The use of fire as a management tool must be carefully planned in order to achieve the pursued objectives (Fernandes, 2015). Combined treatments, such mechanical treatments followed by prescribed burning, produce resilient forest structures more rapidly than only using prescribed burning, which should be repeated over time (Schwilk et al., 2009). In particular, thinning and prescribed burning are considered good management options in *Pinus nigra* forests in the Mediterranean basin (Piqué and Domènech, 2018).

Nevertheless, the new forest scenario in which large forest fires are increasingly present requires proactive tools. The use of new geographic information technologies, remote sensors and fire simulators provide support for identifying strategic management points (SMP). Defining areas according to a specific methodology that considers the fire risk, fire behaviour in the study area and the vulnerability of natural, rural or urban values enables spatio-temporal planning of fuels and infrastructures to limit fire potential. This in turn enables detection of extinction opportunities and the design of safe, effective strategies for large forest fires. Thus, implementation of SMPs in the field involves a series of preliminary preparation actions, such as fuel treatment, construction of water points and access, among others.

1.3. Prescribed burning (PB) treatment

The idea of establishing a different fire regime by allowing the spread of low-intensity unplanned fires and extensive application of PB (Piñol et al., 2007; Regos et al., 2014) has been promoted as an opportunity to suppress large fires in adverse climate conditions.

Prescribed burning is defined as the planned use of fire to meet clear management objectives under suitable environmental conditions (Wade, 1989). Using this forest treatment may reduce the horizontal and vertical continuity and the high fuel load accumulated in forest stands (Fernandes and Botelho, 2003). It can also increase the height to the live crown, decrease crown density, retain large trees of fire-resistant species (Agee and Skinner, 2005), make it easier to extinguish the fire and even decrease tree mortality (Piqué and Domènech, 2018). However, the treatment effectiveness can vary depending on type, amount, size, spatial distribution and intensity of treatment, time since implementation, ecosystem type, topography, geographic location and meteorological conditions at the time of fire (Cochrane et al., 2012). Beyond fire prevention, PB has beneficial effects on the vitality of stands by recovering the distribution of the most balanced vegetation layers. Occasionally, it enables recovery of certain habitats and improves biodiversity (e.g. Fernandes et al., 2013; Shakesby et al., 2015). From a social point of view, in rural areas PB can also reduce conflicts regarding the use of fire as a tool to favour controlled grazing or other traditional activities, involving different stakeholders and local interests. Towards society prescribed burning could have a significant pedagogical effect, by allowing "explain" the role of fire as a natural disturbance of ecosystems. The success of prescribed burning depends on suitable planning, which distinguishes between PB and traditional fire use. Monitoring pre-fire, fire and post-fire variables is essential to determine whether the pursued objectives have been met (Fernandes and Botelho, 2004).

Many countries face strong public opposition to the use of PB as a preventive method, which hinders the large scale application of the treatment for efficient fuel reduction (Regos et al., 2014). In Europe, PB is only occasionally used in Portugal, Spain and France, and its use is still questioned in other countries (Montiel and Kraus, 2010). In the Cuenca Mountains (the study area), PB is generally carried out in strategic zones and at landscape scale. However, an important effort is being made to extend the implementation of this technique, especially below canopy. Hence, research on the effects of PB is fundamental for fire management, to determine the parameters required to design, test and implement more effective prescriptions. In this respect, the intensity (energy released by fire), season, frequency, extent and severity (magnitude of fire effects) define the prescribed fire regime, which aims to minimize the negative effects of fire on trees and maximize stand resistance and resilience.

1.3.1. Ignition pattern

During prescribed burning, fire intensity can be controlled by the ignition pattern (Keeley, 2009). Selection of a particular type of ignition pattern varies depending on type of fire required to achieve the burning goals, ranging from the least intense fires (backing fires) to the most intense (heading fires) or intermediate fires (flanking fires or point source fires). Fire can be applied by strip ignition technique or by spot fires. The fire intensity, the area covered and smoke dispersal can be altered by varying the space of front lines or modifying the interaction between fronts. A rapid advancement of the front and a shorter residence time of the fire in the soil may prevent overheating, excessive consumption of organic matter and high temperature (Vega, 2001).

In the study area, low-intensity PB is indicated for reducing the under-canopy vegetation strata. Low-intensity fire, adequate meteorological, fuel and soil conditions together determine the likelihood that the fire will be confined to the area of interest and conducted with the intensity that will meet the forest management goals (Grebner et al., 2012).

1.3.2. Prescribed burning season

Burning season is a controllable feature in planning PB; however, few studies have investigated the effect of season on tree traits, post-fire tree growth or mortality (Valor, 2018). Fire season may impact ecosystems differently through differences in fire intensity, mainly due to variations in meteorological conditions (Hamman et al., 2008), phenological status of the tree and the tree carbohydrate storage during burning (Harrington, 1993; Thies et al., 2005; Knapp et al., 2009).

In the Mediterranean basin, particularly in the Cuenca Mountains, PB is generally performed in the early (spring) and late (early autumn) seasons (Fig. 1). Some studies have shown that early season burning, at the beginning of the annual growth period, is likely to be more disruptive to trees (Hough, 1968; Garrison, 1972). Carbohydrate reserves are at their lowest levels shortly after breaking dormancy, and it may be more difficult for plants to recover from tissue loss due to fire under such conditions (Garrison, 1972). In addition, the tender, early-season tissues may be more sensitive to heat (Bond and van Wilgen, 1994); a higher level of damage to fine roots (abundant during this period) has also been pointed out (Harrington, 1993). Likewise, late season burning is likely to be of greater intensity because the fuels are drier (Skinner and Chang, 1996), which may exacerbate tree mortality (Thies et al., 2005). However, other studies have shown little or no effect of burning season on different ecological variables (Knapp et al., 2009).

Fig. 1. Prescribed burning executed in a mixed stand of *Pinus nigra-Pinus pinaster* in May 2016 (left) and in a pure stand of *Pinus nigra* in November 2016 (right).

1.3.3. Longevity of the prescribed fire effect

The rate of fuel recovery defines the longevity of the treatment effect (Fernandes, 2015), which probably varies with ecosystem type and geographic region (Parks et al., 2014). The benefit of fuel reduction burning (in terms of fire suppression) decreases over time (Enright and Fontaine, 2014). Numerous studies have explored the effect of time between burning. Prichard and Kennedy (2014) suggested that effectiveness is more important after 20-30 years after burning in semi-arid landscapes with low primary productivity. Nevertheless, in more productive ecosystems (with rapid regeneration of the understory), treatments may need to be repeated at shorter intervals of 2-10 years (Finney et al., 2005; Stephens et al., 2012). Collins et al. (2009) investigated successive naturally-occurring fires in mixed conifer forest and established that the probability of the latter type of fire affecting the previous fire area is extremely low when the time between fires is less than 9 years and when meteorological conditions are not extreme. Teske et al. (2012) suggested that large fires are less likely to occur if the area has burned in the last 5 years. Espinosa et al. (2019), who evaluated the effectiveness of PB in Europe, suggested a generic interval of 4 years between consecutive treatments in *Pinus pinaster* stands, depending on site productivity and stand age and structure. The high level of variability indicates the many parameters involved in determining the duration of effectiveness of the treatment. Therefore, an integrated short to medium-term analysis is absolutely necessary for evaluating the treatment efficacy.

1.4. Main characteristics of the study site

The PB treatments under study in this doctoral research were carried out in two different sites in the northwest of the Cuenca Mountains (Iberian System): a mixed stand of *Pinus nigra* and *Pinus pinaster* (El Pozuelo) and a pure stand of *Pinus nigra* (Beteta). The main characteristics of the two sites are briefly described in Table 1.

Table 1. Main characteristics of El Pozuelo and Beteta sites (mean and standard deviation).

--	El Pozuelo	Beteta
Longitude	40° 33′ 36′′ N	40° 33′ 06′′ N
Latitude	002° 15′ 56′′ W	002° 06′ 32′′ W
Main species	*Pinus nigra* (89±11%) *Pinus pinaster* (11±11%)	*Pinus nigra* (100%)
pH of topsoil *	7.3 (clay texture)	6.9 (loamy-sand texture)
Elevation	1015±5 m asl	1232±7 m asl
Slope	3-8%	3-10%
Stand density	627±238 trees ha^{-1}	1286±339 trees ha^{-1}
Stand basal area	25.4±9.7 m^2 ha^{-1}	36.6±10.7 m^2 ha^{-1}
Dominant tree height	18.6±0.8 m	17.0±1.6 m
Tree height	12.2±2.0 m	13.2±2.7 m
First live branch	6.4±1.8 m	8.2±2.5 m
Diameter at breast height	19.8±2.6 cm	18.8±4.1 cm
Bark thickness	1.7±0.3 cm	1.7±0.4 cm
Fuel Model **	TU1	TU1

(*) Data from Plaza-Álvarez et al. (2017). (**) Data from Scott and Burgan (2005).

1.5. Mediterranean pine species: *Pinus nigra* Arn. subsp. *salzmannii* (Spanish black pine) and *Pinus pinaster* Ait. (maritime pine)

Pines subjected to a low-severity fire regime are typically fire resisters, possessing traits that enable survival after fires of low to moderate intensity (Fernandes et al., 2008). These traits are observed in *Pinus nigra*, which has been able to persist over several centuries under a surface fire regime, as indicated by the presence of a relict and multi-aged forest in Eastern Spain (Fulé et al., 2008). The traits also occur in *Pinus pinaster*, which has historically been subjected to surface fires (Tapias et al., 2001).

The EU Natura 2000 Directive (European Union, 1992) has classified some sub-Mediterranean endemic populations of *Pinus nigra* as priority habitats for conservation. Similarly, the convention for Conservation of European Wildlife and Natural Habitats (European Union, 1996) has classified stands of this species as "a habitat of European interest". Furthermore, the area covered by *Pinus nigra* forests in mountainous areas of the Mediterranean region has decreased in last decades, and these forests are among those predicted to be seriously affected by climate change within the Central Iberian Peninsula (López-Serrano et al., 2009). In Spain, the sub-species *salzmannii* is mainly present in the Pyrenees, the Iberian System, and the Sierra of Cazorla and Segura (Alía et al., 2009). The species exhibits some limiting characteristics concerning the use of prescribed fire as a management tool: montane communities of Spanish black pine do not produce serotinous cones (Tapias et al., 2001) and do not maintain a canopy or soil seed bank (Ordoñez et al., 2005). Trees less than 15 years old do not produce significant numbers of fruiting cones (Tapias et al., 2001; Alía et al., 2009). Furthermore, it is common to find only a few highly productive trees in a stand (Tiscar and Linares, 2011). In addition, difficulties in initial seedling recruitment may occur in the regeneration phase after PB (Lucas-Borja et al., 2016). However, *Pinus nigra* has a thick bark and a high crown base height (Pausas et al., 2009), a self-pruning strategy (Tapias et al., 2001; Fulé et al., 2008) and is a long-lived tree with a relatively open structure (Fulé et al., 2008). Therefore, careful study of PB-induced disturbance should be conducted prior to the implementation of treatment in these stands considering these particular aspects. In the Cuenca Mountains, Spanish black pine has traditionally been managed by the shelterwood method, with a shelter-phase of 20-25 years and a rotation period of 100-125 years, although in recent decades the method has changed to an uneven-aged system (Tiscar and Linares, 2011).

The area of distribution of *Pinus pinaster* is the Western Mediterranean region, and the species mainly occurs in the Iberian Peninsula. *Pinus pinaster* is frequently affected by wildfires, and was the second most affected species in Spain between 2006 and 2015 (López and López, 2019). Perhaps because of this, maritime pine has evolutionary adaptations that help it to survive in fire-prone environments (Fernandes and Rigolot, 2007). Its bark can be classified as moderately to very thick (although this varies between populations) (Fernandes et al., 2008). The higher crown base height of large trees reduces exposure to heat and hence increases the probability of surviving fire (Ryan, 1998). Tree growth is not notably damaged until 25–50% of the crown length is scorched (Fernandes et al., 2008). The bark is laminated, which contributes to expulsion of heat from the bole (Fernandes and Rigolot, 2007). Relative to other conifers, needle necrosis occurs after long exposure to a certain temperature (Fernandes et al., 2008). The large buds (shielded by scales and by long needles) may endure very high levels of defoliation. A seed store in serotinous cones facilitates reproduction after fire (Fernandes and Rigolot, 2007). Despite these traits, climate change scenarios predict drier conditions that will decrease the resilience of maritime pine ecosystems (Fernandes and Rigolot, 2007), and adequate fuel management is therefore essential to decrease the impact of fire in these stands.

In the Cuenca Mountains, the *Pinus nigra-Pinus pinaster* ecotone generates stable stands, which should be taken into account in forest management programmes (Fig. 2). Depending on the taxonomic composition and the vegetation structure (height, stem density, age, etc.), recovery rates of ecosystem may or may not be altered, i.e. the resilience of the forest ecosystem may or may not be overcome. Exploration of the resilience of pure and mixed stands to perturbations such as PB is therefore essential to establish recommendations for management and fire prevention strategies for these stands.

Fig. 2. (Right) Mixed stand of *Pinus nigra-Pinus pinaster* and (left) pure stand of *Pinus nigra*, both before prescribed burning.

1.6. Fire effects

Forests can be conceived as complex, self-organizing systems with multiple natural processes that respond autonomously to internal and external drivers (Thompson et al., 2009). In this context, PB can impact forest systems, particularly in the crown, nutrient cycling, vascular tissue in the stem and the growth capacity. Crown damage can be measured by traditional methods such as visual estimation of the volume scorched (Martinson and Omi, 2013) or crown scorch height. However, other new tools may help to determine the level of damage, such as monitoring of litterfall biomass and estimation of the LAI from hemispherical photographs (Montes et al., 2007; Rodríguez-García et al., 2014). Tree damage can be estimated by direct and indirect variables of cambium damage, such as scorch height (Regelbrugge and Conard, 1993), exposure time to a critical temperature (Hare, 1965), heating rate and temperature reached in the inner bark, flame residential time (Madrigal et al., 2019), bark consumption or a combination of these. Similarly, disrupted tree growth may be an ecophysiological response, and in some cases may predict tree mortality (Bigler and Bugmann, 2004). Hence, evaluating the integral effect when formulating PB plans and programmes is essential to forest ecosystem dynamics.

1.6.1. Effect of prescribed burning on litterfall biomass

1.6.1.1. Total litterfall

Litter dynamics form an essential part of nutrient cycling and energy transfer in forest stands (Kavvadias et al., 2001; Lado-Monserrat et al., 2016). In addition, litterfall biomass may provide an insight into the effects of climate change on forests (Hansen et al., 2009). The relationship between litterfall and decomposition determines the depth of the forest floor layer, which protects the soil against erosion and improves water infiltration (Roig et al., 2005). Litter is also an important source of carbon (Sayer et al, 2006) and it has been proposed as a good indicator of stand productivity (Kunhamu et al., 2009). Overall, the amount of litterfall depends on environmental variables, stand and tree individual characteristics, phenology of the species, disturbance of forest management practices (e.g. prescribed burning) and other external variables (e.g. insects or pests).

Although some authors have suggested the importance of understanding the effects of silvicultural treatments on litterfall and litter decomposition processes in different forest species and under different geoclimatic conditions (Blanco et al., 2006; Lado-Monserrat et al., 2016), no previous studies have evaluated the effect of PB on total litterfall biomass, litterfall fractions or nutrient content of litterfall in pine forests. Thus, the findings reported here can be used as initial reference data on litterfall dynamics.

As part of the research reported in this doctoral thesis, a litterfall collection system was designed in accordance with the recommendations outlined in the Manual of the United Nations Economic Commission for Europe (UNECE) (ICP Forests - Ukonmaanaho et al., 2016), in order to guarantee the quality and quantity of the samples (Fig. 3). In addition, the use of these guidelines allowed the results comparison with the wide ICP Forests data base.

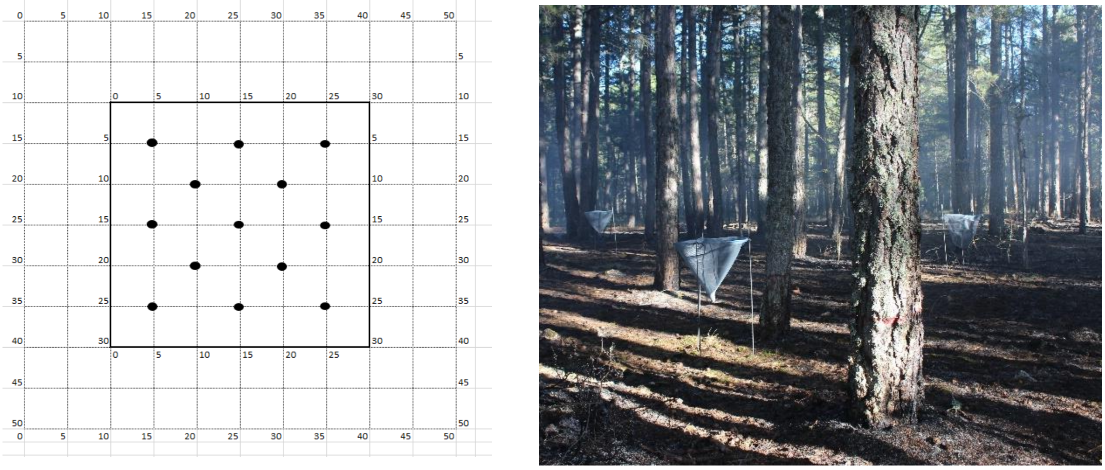

Fig. 3. (Left) Example of plot design (distance in m) and standard distribution of collectors (black dots) in the sampling plot. (Right) Collectors installed in the pure stand.

1.6.1.2. Litterfall fractions

For studying the litterfall fractions, samples from each plot were separated into six groups: needles, cones, inflorescences, branches of diameter less than 2 cm, bark and miscellaneous material (unclassified material, seeds, lichens and leaves of other species) (Fig. 4). Overall, needles usually represent the largest fraction. A severe impact of PB on the crown may imply an increase in needle fall thus leading to a higher risk of a potential future fire and a short-lived treatment efficacy. Fluctuations in reproductive organs (cones and inflorescences) should be carefully evaluated in PB planning to take into account the particular fructification characteristics of each species. Branch patterns vary considerably, although some peaks may appear due to meteorological events. In addition, heat pruning may cause an increase in this fraction, particularly in species with low crown height. The bark fraction exhibits great variability. In species such as maritime pine, the bark is laminated and the outer layers are exfoliated during combustion (Fernandes and Rigolot, 2007). The miscellaneous fraction varies and several peaks can be observed. Some winter peaks may be due to leaves of other deciduous species. Peaks in February and May–July may correspond to seeds. Miscellaneous peaks may also be associated with meteorological events.

Fig. 4. (Left and right) Examples of classification in different fractions of the total litterfall collected.

1.6.1.3. Nutrient cycle

Prescribed burning not only disrupts the amount and distribution of litterfall, but can alter the carbon and macronutrient content of litterfall. The nutrient pool in soils is crucial to the recovery potential and post-fire health status of the ecosystem (regrowth, characteristics and functioning) (Näthe et al., 2018). Indeed, determining the capacity of Mediterranean species to adapt to variable nutrient supply levels in the context of global change may be essential for predicting their future survival capacity (Sardans et al., 2005). In this doctoral thesis a complete annual analysis of the chemical content of the litterfall fractions was carried out.

1.6.1.4. Variables influencing litterfall

Identifying the variables that influence litterfall can help to improve PB prescriptions and reduce the vulnerability of stands managed by this technique.

Overall, the amount and quality of litterfall depend on meteorological variables, geographic factors, stand and tree individual characteristics, disturbance of forest management practices, and to a lesser extent, other external variables. The relationship between litterfall and the duration of drought conditions has been demonstrated in several studies (e.g. Roig et al., 2005). Furthermore, much of the variation in litterfall response to burning can be attributed to stand characteristics (age, structure, species, etc.) (Blanco et al., 2006) and to the variable heat sensitivity of different tissues and species (Catry et al., 2010). Low-intensity PB may have scarce effects on tree crowns. However, intense PB may lead to an increase in litterfall, thereby increasing the risk of future fire. The litterfall process is complex, and mathematical models can be used to simplify and understand the process. Use of new approach involving a Bayesian statistic could solve this problem, because Bayesian analysis does not only rely on a point estimate of the parameter, but rather considers an entire distribution of possible values.

1.6.2. Effect of prescribed burning on vascular tissues

The variation in plant response to burning can be attributed to the diverse heat sensitivity of tissues and species (Catry et al., 2010), as well as temperature and combustion time of fire (Dickinson and Johnson, 2004; Michaletz and Johnson 2007), tree size and stand structure (Fernandes et al., 2008).

Bark thickness is a morphological variable which has been suggested to contribute to fire resistance (e.g. Rigolot, 2004; Brando et al., 2012); indeed, small differences in bark thickness may produce large differences in fire resistance (Bond and van Wilgen, 1994; Moreira et al., 2007). In addition, species occurring in fire-prone habitats characteristically have thicker bark (e.g. Poorter et al., 2014; Rosell et al., 2014), although substantial gaps in data prevent generalization of this relationship at a global scale (Schubert et al., 2016). Other bark properties such as flammability and physical structure (e.g. bark roughness) may also influence the vulnerability of the living tissues in the trunk (e.g. Vines, 1968; Frejaville et al., 2013). Overall, most of the lethal situations during surface fires occur when the temperature of the cambium rises above 60 °C (Bauer et al., 2010), although prolonged exposure of the cambium to temperatures below 60 °C may be sufficient to cause necrosis of the cambium (Dickinson and Johnson, 2004). Damage to the cambium may involve disruption of transportation of photosynthate and water to the crown, which may affect photosynthetic production or lead to death of the tree. Although several studies have been carried out to assess the insulating capacity of bark, by monitoring cambial and surface temperatures during experimental burning conducted in laboratory or in the field, very few of these studies have considered bark properties, fire intensity, sustainability, ignitability and consumability together as predictor variables. This is the first step required to enable the results to be extended to other conifers.

To quantify cambium damage and fire resistance in *Pinus nigra*, a mass loss calorimeter (MLC) device was used in a vertical configuration (more suitable for simulating heating and bark position in the field) (Fig. 5). In addition, data were compared with the findings obtained in the low-intensity PB conducted in the field.

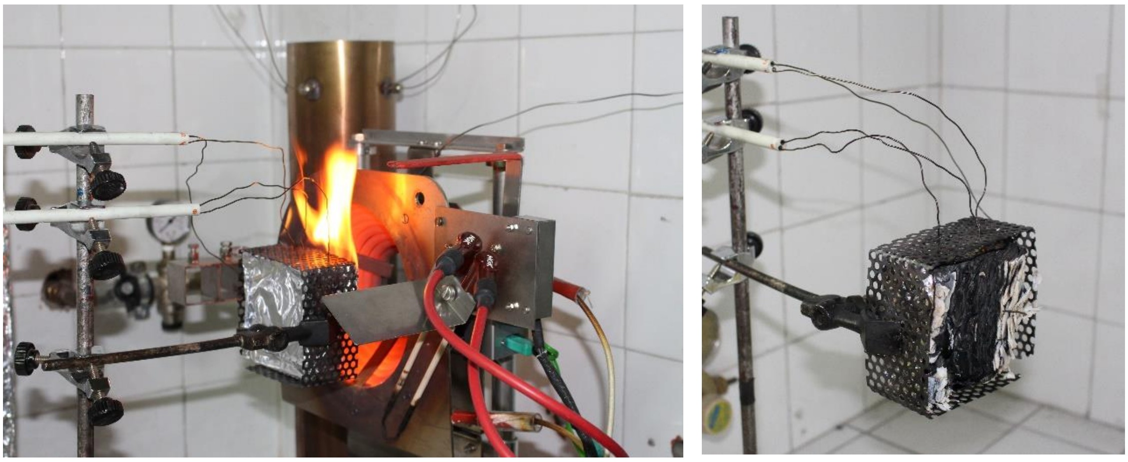

Fig. 5. (Left) Vertical configuration of the MLC device during burning. (Right) The same sample after burning.

1.6.3. Effect of fire disturbance on tree growth

Tree growth is regulated by a combination of exogenous and endogenous factors (Lucas-Borja and Vacchiano, 2018). Overall, it is affected by climate conditions (Ames et al., 2015) and water and carbon balance (Candel-Pérez et al., 2012). Factors such as phenotypic plasticity, genetic variability and interactions with site factors and disturbances (Lucas-Borja and Vacchiano, 2018) also explain the responses. Needle abundance is also strongly associated with wood production (Vanninen and Mäkelä, 2000).

Prescribed burning may improve tree efficiency by eliminating the unproductive lower branches (Villarrubia and Chambers, 1978), increasing light and soil nutrient availability or reducing tree competition. Conversely, fire-surviving trees can be compromised in their physiological functionality, show reduced growth and be more likely to succumb to delayed death (e.g. Maringer et al., 2016; Thompson et al., 2017). Most studies of post-PB growth response produce highly variable, often contradictory, conclusions which can be attributed to differences in type and season of fire, burning conditions and post-burn stresses (Botelho et al., 1998). Thus, study and modelling of these variables may be necessary in order to clarify post-burning tree growth processes.

After low-intensity PB was carried out, 15 *P. nigra* trees in each plot were selected for study, and dendrochronological methods and mixed modelling were used to investigate whether tree growth responses are influenced by the environmental conditions, stand and tree characteristics, fire season and fire severity variables.

1.7. Justification and innovative aspects

Forest fires are the main threat to many Mediterranean forests and have significant environmental and socioeconomic impacts. Climate change processes, alterations in traditional land management practices and the uncontrolled increase in urban sprawl into the wildland-urban interface (WUI) are exacerbating the problem. In addition, a new phenomenon of large and catastrophic fires, "megafires", is occurring in southern Europe. These extraordinary wildfires tend to overwhelm suppression capabilities, often causing civilian and firefighter fatalities.

Altogether, this has created more complex scenarios for forest management. Thus, it is essential to have available tools for protecting forest ecosystems from fire. Prescribed burning acts on forest fuels by modifying the behaviour of a potential fire, reducing its severity and associated impacts and also facilitating extinction activities. In addition, PB can also increase biodiversity and ecological resiliency by creating patchy landscape mosaics. However, the use of PB may alter the functional conditions of the ecosystem. This dissertation contributes to filling the gaps in general knowledge and technical information about integrated fire effects in the context of sustainable forest management.

Despite the importance of understanding the effect of PB on litterfall biomass in different pine forests, this aspect has not been investigated so far. Existing studies regarding litterfall mainly focus on the effect of different thinning regimes and there remain uncertainties regarding the effects of fire in nutrient content of litterfall. A new method was used to measure crown damage by examining hemispherical photographs, obtained with a ForeStereo® device, which could simplify the field work. Given the complexity of the litterfall process, a Bayesian approach was used, for the first time, for a better understanding of the variables that influence on litterfall. This approach integrated the analysis of the previous information, the data from studies carried out in the experimental design and consideration of the accumulated empirical experience. The present study provides a valuable source of litterfall data to evaluate the disturbance caused by PB on the total amount, fractions, carbon and macronutrient contents.

Although the key role of bark properties in protecting vital tissues from fire damage is recognized, most current studies have been conducted in non-Mediterranean ecosystems and have used different approaches and non-standardized methods; in addition, very few of the studies have jointly considered variables regarding bark traits, fire intensity and flammability (e.g. residence time above a lethal temperature, maximum exposure time or bark consumed). A methodology was developed in which the loss mass calorimeter was used, for the first time, in a vertical configuration, thus realistically simulating the heating conditions and bark position in the field. This novel method demonstrated that bark flammability significantly affects the potential damage to the cambium.

The physiological functions of trees that survive fire can be negatively affected, with the trees exhibiting reduced growth and being more likely to die. Measuring post-fire growth is essential to understanding treatment effectiveness. In this research, the tree growth response was assessed, and variables such as maximum scorch height, time to exposure of a critical temperature and litterfall biomass were included for the first time in growth models.

This type of information is key to fire management, enabling the longevity of fuel reduction treatment to be determined and providing more accurate prescriptions for fire prevention plans, guiding the procedures adopted by land managers.

1.8. Objectives

The fundamental role of scientific research regarding PB management is to establish relationships between burning conditions, fire behaviour and fire impacts.

The goal of this doctoral research was to obtain an understanding of the medium-term combined effects of low-intensity PB in two seasons at both tree and at stand level. A pure stand of *Pinus nigra* and a mixed stand of *Pinus nigra* and *Pinus pinaster* in the Cuenca Mountains (Iberian System, Spain) were chosen for study. In this area, the two species generate an ecotone of stable stands and low-intensity PB is typically carried out as a preventive tool. Two levels of fire severity are determined by variations in fire season (spring and autumn). The study of integrated effects on litterfall-cambium area-tree growth enables clarification of the complex interactions between the post-fire dynamics. The proposed hypothesis is that fire traits of *Pinus nigra* and *Pinus pinaster* and adaptation of these pure and mixed stands to surface fires enable development of low-intensity PB under canopy as a fuel reduction treatment, to reduce fire severity with scarce damage at tree and stand level. The specific objectives of the research were as follows:

1. To analyze the effect of prescribed burning in two seasons (spring and autumn) on the quantity, patterns and fractions of litterfall biomass and on the carbon and macronutrient contents (Chapter 2 - Subchapter 2.1 and 2.2).

2. To ascertain the influence of meteorological variables, stand and tree traits, fire prescription and fire behaviour on litterfall biomass, through easily measurable variables (Chapter 2 - Subchapter 2.3).

3. To explore the relationship between the resistance of the cambium to fire and bark properties (i.e. thickness and the coefficient of variation of bark thickness), fire intensity, ignitability, sustainability and consumability variables (Chapter 3).

4. To compare the previous results obtained in the laboratory with those obtained in experimental low-intensity prescribed burns conducted in the field (Chapter 3).

5. To determine any variations in post-burning growth of *P. nigra* (Chapter 4).

1.9. Structure of the doctoral thesis

The doctoral thesis is organized in chapters, written in the format of scientific articles, four of which have been published in international, peer-reviewed, scientific journals, another one is in the review phase. This introduction (**Chapter 1)** provides the conceptual framework of the thesis. **Chapter 2** (*Effect of prescribed burning on litterfall biomass*) is divided into three subchapters, which examine the effects of two seasons of low-intensity PB on total litterfall biomass, litterfall fractions and nutrient content, in a pure *Pinus nigra* stand and in a mixed *Pinus nigra-Pinus pinaster* stand (**Subchapter 2.1**, *Short-term effects of prescribed burning on litterfall biomass in mixed stands of Pinus nigra and Pinus pinaster and pure stands of Pinus nigra in the Cuenca Mountains - Central-Eastern Spain* and **Subchapter 2.2**, *The effect of low-intensity prescribed burns in two seasons on litterfall biomass and nutrient content*). In addition, Bayesian modelling was used to assess the effect of a combination of variables affecting litterfall dynamics (**Subchapter 2.3**, *Use of Bayesian modeling to determine the effects of meteorological conditions, prescribed burn season, and tree characteristics on litterfall of Pinus nigra and Pinus pinaster stands*). In **Chapter 3** (*Predicting potential cambium damage and fire resistance in Pinus nigra Arn. ssp. Salzmannii*) cambial damage and fire resistance of *Pinus nigra* samples were tested in a mass loss calorimeter in a vertical configuration. The results obtained using the device were also compared with those obtained during execution of the aforementioned low-intensity PB. In **Chapter 4** (*Tree growth response to low-intensity prescribed burning in Pinus nigra stands: effects of burn season and fire severity*) the impact of the same low-intensity PB on tree growth was examined. **Chapter 5** includes the general discussion and the management implications. The thesis conclusions are listed in **Chapter 6**. Then, in **Chapter 7** is exposed the future research perspectives and finally an **Appendix I** is included, with the main characteristics of the scientific articles of the doctoral thesis.

A conceptual diagram of the doctoral thesis, including the five studies, is shown in Fig. 6.

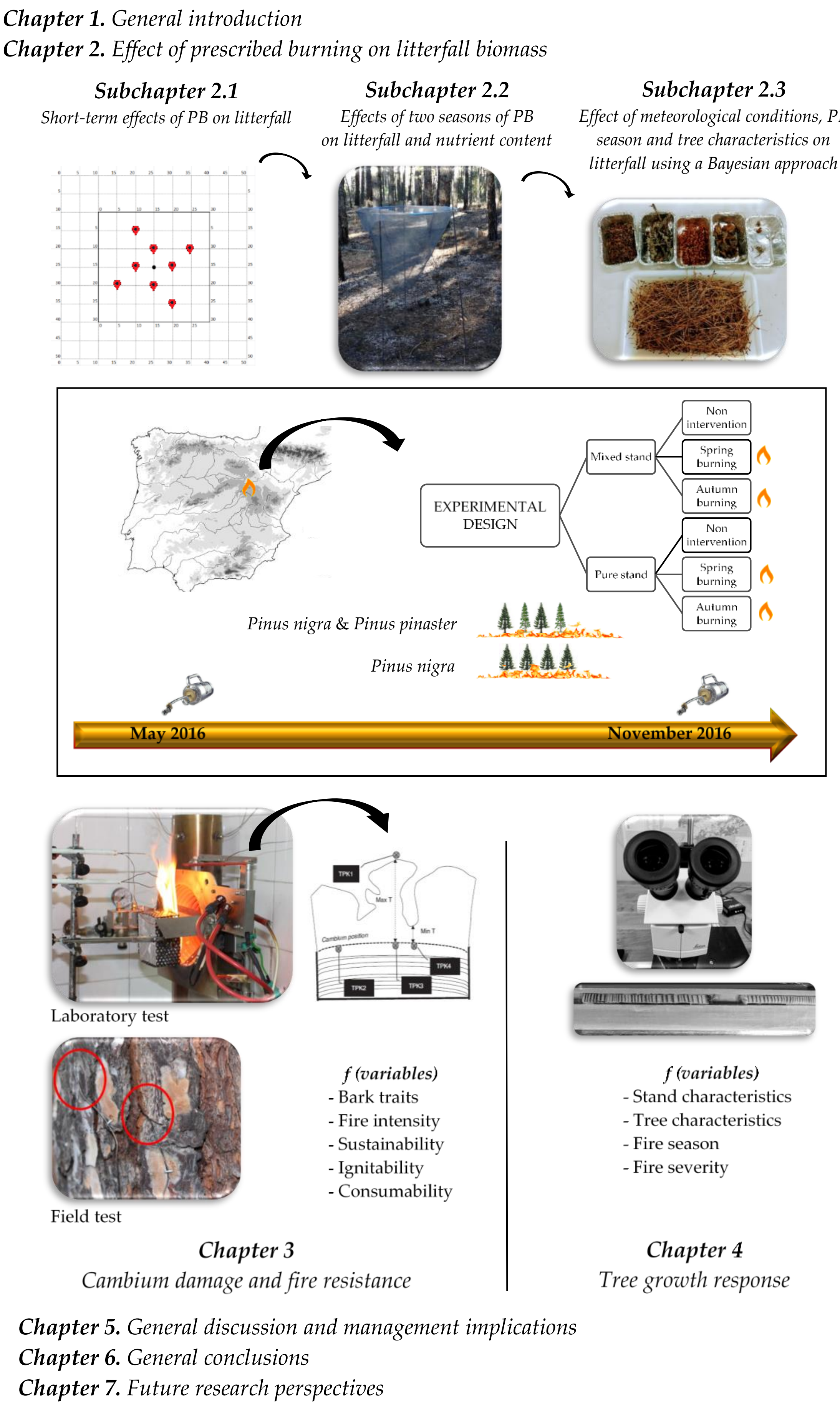

Fig. 6. Conceptual diagram of the doctoral thesis.

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Chapter 2

Effect of prescribed burning on litterfall biomass

Subchapter 2.1

Short-term effects of prescribed burning on litterfall biomass in mixed stands of *Pinus nigra* and *Pinus pinaster* and pure stands of *Pinus nigra* in the Cuenca Mountains (Central-Eastern Spain)

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Abstract

Fire severity, defined as the magnitude of fire effects in an ecosystem, is a key factor to consider in planning management strategies for protecting forests against fire. Although prescribed burning has been used as a fuel reduction tool in forest ecosystems, it is quite limited in the Mediterranean region. Furthermore, little is known about how tree crowns are affected by prescribed underburning aimed at reducing fire severity in conifer stands. As part of an ongoing study to assess the effects of prescribed burning on the tree canopy, litterfall is currently being monitored in a network of experimental plots located in mixed (*Pinus nigra* and *Pinus pinaster*) and pure (*P. nigra*) conifer stands in the Cuenca Mountains (Castilla-La Mancha, Spain). A total of 12 study plots (30 m × 30 m) were established in a completely randomized experimental design to determine the effect of burning, with two treatments: no burning (control) and burning (i.e. with three replicate plots for each treatment and site). Burning was conducted in May 2016. In each plot, eight litterfall collectors were installed at regular intervals, according to international protocols (ICP Forests), and all biomass falling into the collectors is being monitored monthly. The specific objective of this study is to assess how prescribed burning affects the rate of generation of foliar and non-foliar litterfall biomass due to the fire. In addition, the Leaf Area Index was estimated before burning and one year later to verify possible changes in the structure of the stands. This information could be used to help minimize the negative impacts of prescribed underburning on litterfall. To our knowledge, this study represents the first attempt to evaluate the effect of prescribed burning on litterfall biomass in Europe.

Keywords: tree crown; fire severity; ICP Forests; LAI; defoliation.

Introduction

Forest fires represent one of the main types of disturbance in ecosystems in many parts of the world. Indeed, along with climate, fire is considered the main causal agent of vegetation changes, acting as a "global herbivore" (Bond and Keeley, 2005). Increased numbers of fires have been reported in some countries in the Mediterranean Europe, including Spain (San-Miguel-Ayanz et al., 2012; Rodrigues et al., 2013). Nonetheless, huge spatial and temporal variability in fire frequency trends have been suggested, especially in Spain, where increasing and decreasing trends have been detected depending on the analysis period and scale considered (Turco et al., 2016). Fire regimes are related to climate and environmental changes (Pausas and Keeley, 2009) and are also dependent on human activity (Archibald et al., 2013; Salis et al., 2014). High-intensity fires threaten important ecological and social functions of pine species and their economic value (Pausas et al., 2008). The negative impact of forest fires on forests and their associated functions is expected to increase in the future as model predictions indicate increased frequency, intensity and severity of forest fires due to land use change and climate change (Flannigan et al., 2009).

The importance of intensifying preventive measures is highlighted in order to minimize the forest fire risk, which is mainly due to the conditions of horizontal and vertical continuity and a high fuel load in forest stands in the Iberian Peninsula. Use of prescribed burning as a fuel management tool facilitates fire suppression efforts by reducing the intensity and size of wildfires and the damage that they cause (Fernandes and Botelho, 2003). However, the use of prescribed fire may alter the structural and functional conditions of the ecosystem. Such alterations can affect the tree crowns, especially under intense burning processes, which can have repercussions in changes in the patterns and regimes of the litterfall biomass.

For the implementation of prescribed fire as a management tool, it is essential to know the processes that determine the dynamics of forest ecosystems. One of these processes is the nutrient cycle, i.e. the flow of organic and inorganic matter through the ecosystem components. The main route of transfer of these nutrients to forest soil generally occurs via litterfall (leaves, buds, flowers, fruits, barks, twigs, etc.) (Bray and Gorham, 1964; Vitousek et al., 1995; Berg and Meentemeyer, 2001), although the volume of fine roots may play a more important role (Vogt et al., 1986). The balance between biomass production and decomposition controls the amount of carbon available in the soil and, therefore, site productivity. In Mediterranean forest ecosystems, the role of litter decomposition in nutrient cycling becomes even more important when considering the degradation of forest vegetation and soils by wildfire, long destructive cultivation and overgrazing (Kavvadias et al., 2001). Several studies have examined how litterfall is associated with climatic and site variables, such as those relating litterfall to climatic characteristics (Kouki and Hokkanen, 1992) and humidity (Hennessey et al., 1992). Furthermore, on a European scale, shedding of pine needles can also be related with relatively good precision to latitude (Berg et al., 1993, 1999), amplitude of the senescence period and nitrogen re-displacement (Del Arco et al., 1991), forest production (Bray and Gorham, 1964; Albrektson, 1988; Bellot et al., 1992) and soil fertility (Hernández et al., 1992). Scarce information has been reported regarding the effect of silviculture treatments (Roig et al., 2005) and the same happens with information regarding the effects of prescribed burning treatments on litterfall biomass.

Leaf Area Index (LAI) is another factor strongly related to forest productivity and stand structure (Innes et al., 2005). Accurate estimation of LAI is fundamental to understand the functioning of ecosystem processes, including rainfall, radiation and CO_2 interception, as well as quantification of ecosystem productivity (Montes et al., 2007), a factor closely associated with litterfall. Although several studies report little change in physiology, growth or stand structure after a low severity prescribed burning (e.g. Valor et al., 2015; Battipaglia et al., 2016), the impact of this treatment via direct effects on the canopy (heat from the flames) and possible stress by cambium heating (which could reduce LAI) are uncertain.

Pure stands of *Pinus nigra* Arn. ssp. *salzmannii* (Spanish black pine) and mixed stands of *Pinus pinaster* Ait. (maritime pine) and *Pinus nigra* Arn. ssp. *salzmannii* have been chosen. Spanish black pine is one of the most widely distributed species in Central and Eastern Spain and Southeast France. The convention for Conservation of European Wildlife and Natural Habitats (European Union, 1996) has classified stands of this species as "habitat of European interest" (Lucas-Borja et al., 2016). Spanish black pine has disappeared from some regions, mainly as a result of wildfires or interspecific competition (mostly with *Pinus pinaster*) (Barbero et al., 1998). Spanish black pine is a Mediterranean tree species that is resistant to low-intensity surface fires due to the thick bark characteristic of the species (Fulé et al., 2008; Touchan et al., 2012; Pausas, 2015). However, recurrent large wildfires are threatening the permanence of *P. nigra* forests in some Mediterranean areas as a result of the almost total lack of capacity of this non serotinous pine to regenerate after fire (Espelta et al., 2003; Ordóñez et al., 2005; Fernandes et al., 2008; Christopoulou et al., 2013; Lucas-Borja et al., 2017). In addition, the species releases its seeds early in the year, at the beginning of spring, and therefore no seed bank is maintained after summer wildfires (Ordóñez et al., 2005). According to Lucas-Borja et al. (2016), little is known about the impact of prescribed burning on the natural regeneration of Spanish black pine. On the other hand, maritime pine is a conifer from the Western Mediterranean basin that covers more than 1200000 ha of land in Spain as a dominant species, under different elevation, climate and soil conditions, resulting in a high level of genetic variation (Alía et al., 1996). Important reforestation projects carried out during the 20th century, motivated by different factors, led to the wide expansion of this species. Fire is an important threat to maritime pine, but also plays a crucial role in the perpetuation of natural stands (Fernandes and Rigolot, 2007; Cruz and Fernandes, 2008). Some provenances of this species have a thick bark (that allows survival of the trees after low-intensity fire) and also produce serotinous cones (Fernandes and Rigolot, 2007). The provenance under study (from the Cuenca Mountains) does not produce serotinous cones (Alía et al., 1996). In Spain, the total area occupied by maritime pine that was burned between 1974 and 2010 was 674055 ha, representing 31% of the total burned forest area. The area of Spanish black pine burned in the same period represents 4% of the total (Vázquez de la Cueva, 2016). Several authors have reported higher stability of mixed species stands than of monocultures (e.g. Schütz et al., 2006; Felton et al., 2010). However, others argue that damage to mixed stands will only be reduced in the same proportion of the stable species (Lupke and Spellmann, 1997). Exploration of the resilience of pure and mixed stands to perturbations such as prescribed burning is therefore essential to establish recommendations for management and fire prevention strategies for these stands.

To our knowledge no previous studies have evaluated the effect of prescribed burning on litterfall biomass. This study is a first attempt to compare litterfall patterns in pure stands of *P. nigra* and mixed stands of *P. nigra* and *P. pinaster* in the short-term, after prescribed burning, and one year after the disturbance. The hypothesis proposed in this study is that low-intensity prescribed burning in pure stands of *Pinus nigra* and mixed stands of *Pinus nigra* and *Pinus pinaster* does not affect crown trees.

To corroborate this hypothesis the specific aims of this study were as follows: (i) to analyze the effect of prescribed burning on the quantity, patterns and fractions of the litterfall in pure *P. nigra* and mixed *P. nigra* and *P. pinaster* stands in the Cuenca Mountains; (ii) to compare changes in LAI one year after treatment.

This study is part of a more comprehensive research study on the impacts of prescribed underburning, in which other treatment (autumn burning) and effects (soil, vegetation, tree heating of trunks and growing) are being investigated.

Materials and methods

Study site

As mentioned, the study is part of a wider study being carried out in the community of Castilla-La Mancha (Central-Eastern Spain). Two sites in the North zone of the Cuenca Mountains (Iberian System), El Pozuelo (40° 33′ 36″ N / 002° 15′ 56″ W) and Beteta (40° 33′ 06″ N / 002° 06′ 32″ W), were chosen for study. The average altitude is 1015 and 1294 m above sea level (asl) in El Pozuelo and Beteta respectively. Both areas have slopes between 3 and 10%. Both stands are natural; the Beteta stand was managed in 1971, but the El Pozuelo stand has not been managed. Spanish black pine forests in the Cuenca Mountains have traditionally been managed using the shelterwood method, with a shelter-phase of 20-25 years and a rotation period of 100-125 years (Tiscar Oliver et al., 2011). Controlled grazing and hunting-based management are also carried out in the stands.

The soils in the Cuenca Mountains are calcareous: shallow and rendzina soils predominate in the steepest areas and calcimorphic brown soils in the flat areas and troughs (Lucas-Borja et al., 2017). In both study areas the soil is classified in the order Inceptisol according to the Soil Taxonomy classification (USDA, 1987) used in the National Soil Atlas of Spain (1:200000). The climate is classified as humid Mediterranean (Allué, 1990). The mean annual temperature is 10.7 °C (the warmest month is July with an average of 19.8 °C and the coldest month, December with 2.0 °C), and the mean annual precipitation is 537 mm (56 mm summer months).

The area of El Pozuelo is included in the public utility forest CU217 and is characterized by being a mixed stand of *P. nigra* and *P. pinaster*. The mean tree density is 627 trees ha^{-1}, with *P. nigra* comprising 89% and *P. pinaster* 11% of the trees (Table 1). The mean tree height is 12.2 m with the first live branch appearing at a mean height of 6.4 m. The mean breast diameter is 19.8 cm. The understory vegetation is mainly dominated by *Cistus laurifolius* L., *Genista scorpius* Sibth. & Sm. ex Boiss, *Prunus spinosa* L., *Arrhenatherum bulbosum* and *Bupleurum rigidum* L. Beteta is included within in public utility forest CU179 and is a pure stand of *P. nigra*, with *Genista scorpius* Sibth. & Sm. ex Boiss. and *Rosa canina* L. occurring as the main understory species. The mean density of trees is 1286 trees ha^{-1}, of mean height 13.2 m, and first live branch appearing at a mean height of 8.2 m. The mean breast diameter is 18.8 cm. Both zones have an irregular distribution of pine regenerated under canopy, with density ranging from 78 to 11611 seedlings ha^{-1}. The mean shrub cover in all plots is in a range of 5-20%, and the fuel model is TU1 (El Pozuelo and Beteta) according to the classification of Scott and Burgan (2005).

Table 1. Main parameters measured in the treatment plots (control and burned) in the study areas (El Pozuelo and Beteta).

Z	PN	PT	Dt	DPn	DPp	Pn	Pp	Ht	H1lb	D30	D60	D130	G	Bmt
--	--	--	Trees ha^{-1}	Trees ha^{-1}	Trees ha^{-1}	%	%	m	m	cm	cm	cm	m^2 ha^{-1}	cm
El Pozuelo	P2C P5C P9C	Control	563 (74)	526 (90)	37 (17)	93 (4)	7 (4)	11.5 (3.2)	5.1 (1.9)	20.8 (4.2)	19.7 (3.8)	18.7 (3.7)	22.5 (7.5)	1.4 (0.5)
El Pozuelo	P3B P4B P6B	Burned	881 (227)	770 (148)	111 (89)	89 (8)	11 (8)	12.3 (0.8)	7.1 (0.6)	22.6 (1.2)	21.8 (1.0)	19.8 (1.0)	33.7 (9.7)	1.9 (0.7)
Beteta	B1C B3C B9C	Control	1456 (507)	1456 (507)	0 (0)	100 (0)	0 (0)	10.1 (3.2)	6.1 (3.3)	16.3 (1.8)	15.1 (1.7)	13.8 (1.6)	25.5 (2.1)	1.3 (0.4)
Beteta	B2B B5B B6B	Burned	1215 (209)	1215 (209)	0 (0)	100 (0)	0 (0)	12.7 (0.8)	7.5 (1.3)	21.5 (2.1)	20.0 (1.9)	18.2 (2.0)	39.1 (6.7)	1.9 (0.7)

Z: zone; PN: plot number; PT: plot type; Dt: total density; DPn: density of *Pinus nigra*; DPp: density of *Pinus pinaster*; Pn: percentage of *Pinus nigra*; Pp: percentage of *Pinus pinaster*; Ht: total height; H1lb: height to the first live branch; D30: diameter at 30 cm from the base; D60: diameter at 60 cm from the base; D130: diameter at 130 cm from the base; G: basal area; Bmt: bark thickness. Standard deviation in brackets.

Experimental design

Plot establishment and collection of pre-burn data

A randomized design was applied in both study areas (El Pozuelo and Beteta). Triplicate 50 m × 50 m (2500 m^2) plots were established for the burning and control treatments in each study site, yielding a total of 12 plots (n = 6 in El Pozuelo and n = 6 in Beteta). For data collection, a subplot of 30 m × 30 m (900 m^2) was established in the centre of each plot, to avoid the edge effect (Fig. 1a). The plots are representative of the study area and are fairly homogeneous in terms of vegetation, density, orientation, etc. Plots P2C, P5C, P9C represent the control plots and plots P3B, P4B, P6B represent the burned plots in El Pozuelo. Plots B1C, B3C, B9C represent the control plots and B2B, B5B, B6B represent the burned plots in Beteta.

All the trees in each of the 12 plots were identified, and the following measurements were made: total height (Ht, m), height to the first live branch (H1lb, m), diameter at heights of 0.3, 0.6 and 1.3 m from the base (D30, D60, D130, cm), maximum and minimum bark thickness at 0.6 m from the base.

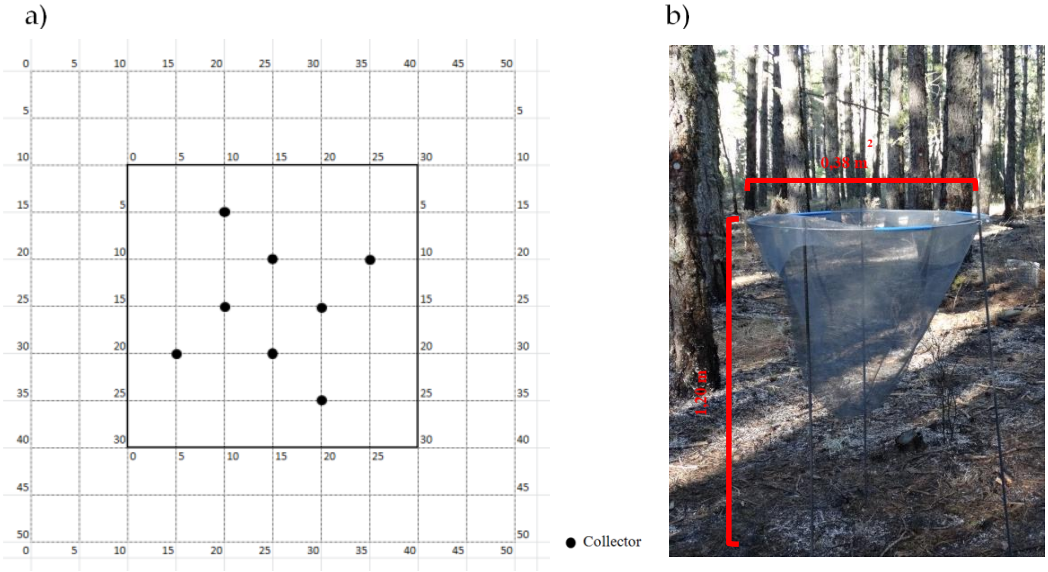

Fig.1. (a) Example of plot design (distances in m) and standard distribution of collectors in the sampling plots. (b) Collector installed in El Pozuelo.

Burning

The prescribed burning was conducted in May 2016 on the same day in each of the sites. The strip ignition technique was applied at distances of 1-2 m, downhill and with upslope wind. This method favors the rapid advancement of the front and a shorter residence time of the fire in the soil, thus avoiding overheating, excessive consumption of organic matter and high temperature (Vega, 2001). This gradual form of burning facilitates control of the operation and is also the most widely used method in the area.

Precipitation (Ortrat, S.L.; KW3-02), wind speed (Casella; 178031C-3), temperature and relative humidity (Geonica; STH-5031) were recorded every 10 min at a meteorological station located adjacent to the study. During the burning, the temperature of the cambial (inner bark) and bark region (outer bark) of 15 randomly selected trees was monitored at a height of 0.6 m (height of maximum heating according to prescription) with type K 1 mm diameter Inconel-sheathed thermocouples (0.3 s of response time). The thermocouples were connected to data loggers (DT-USB TCDirect®), which recorded the data with a frequency of 1 s. Maximum and minimum scorch height was measured after burning in all trees.

Litterfall

The litterfall collection system was designed in accordance with the recommendations outlined in the Manual of the United Nations Economic Commission for Europe under the project entitled "International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests" (ICP Forests) (Ukonmaanaho et al., 2016), to guarantee the quality and quantity of the sample. The system consisting of 8 collectors per replicate plot was installed immediately after the prescribed burning. The spatial distribution is systematic and covers the entire working area in the 30 m × 30 m plot, to guarantee the representativeness of the sample (Fig. 1a). The catchment area of the collectors is always horizontal, to correct for the effect of the slope in collecting material. The catchment area is 0.38 m^2. The bag is 0.75 m deep, to prevent the sample from being lost as a result of wind action. The collectors were placed at a height of 1.2 m from the ground to enable adequate drainage by gravity and to prevent capture of biomass from shrub strata. They were anchored firmly to the ground to provide greater resistance to the inclement conditions (snow, wind or rain) or other types of disturbance (cattle, wildlife, etc.). The fiberglass mesh (pore size, 2 mm) (Fig. 1b) was firmly attached to the structure. Fiberglass provides resistance against external meteorological conditions and also ensures drainage while preventing loss of smaller elements, such as needles. The material was collected monthly to prevent decomposition of the biomass or the chemical leachate. This frequency of collection also ensures the easy identification of, for example, the fine elements of the flower and terminal shoots, which are rapidly compressed. Samples were taken to the laboratory on the same day and oven-dried at 65 °C to constant weight (i.e. for at least 48 h). The samples from each plot were then combined and the fractions separated. For the purposes of this study, these fractions were established: needles, branches of diameter < 2 cm, bark, cones, seeds, inflorescences, lichens, leaves of other species and miscellaneous.

Leaf Area Index (LAI)

LAI is defined as the projected leaf area per unit ground area (Gower and Norman, 1991; Beheraa et al., 2015). Although this definition is clear for flat broad leaves, the meaning of one-sided area is not so clear for coniferous needles, which may be cylindrical or close to hemicylindrical (Chen and Black, 1992). In numerous studies, LAI has been defined on the basis of projected leaf area (Lopes et al., 2016). For temporal study of the variation in the LAI, we used the MU2005-01739 "ForeStereo®" system, patented by the Spanish Forest Research Centre of the Spanish National Institute for Agriculture and Food Research and Technology (INIA) and implemented for LAI measurements (Rodríguez-García et al., 2014). This system provides hemispherical stereoscopic images of the stand that are transformed into 3D projections in which the existing trees are identified. It has two optical systems for capturing "fish eye" images with the parallel optical axes. Because the trees appear as complete images, the stereoscopic system enables calculation of distances from the device to significant points into the trees in the 3D scene, measurement of diameters along the stem, heights and crown dimensions, and establishment of the position of the trees. For each plot, one pair of images (one per camera) were taken of the plot centre identified by GPS. The images were taken immediately before the prescribed burning (May 2016) and one year later (April 2017) in order to assess differences due to treatment.

Statistical analysis

The data were tested for normality and when necessary were transformed (logarithmic and angular transformation). The General Linear Model (GLM) repeated measures procedure was used to perform an impact analysis of burning and control treatments, to identify the effect of prescribed burning on the above parameters. The between-subjects factor (treatment) included two levels (control and burning) and the within-subjects factor was the date (12 levels). Comparison of parameters between treatments was conducted using the Bonferroni test.

ForeStereo®-estimation of LAI is based on the inverse Poisson model (Weiss et al., 2004), which establishes the gap fraction as a function of the zenith direction. Image segmentation is aimed at separating the visible sky from the foliage elements (Ishida, 2004; Nobis and Hunziker, 2005; Schwalbe et al., 2009). The hierarchical classification method developed by Herrera et al. (2009) and Sánchez-González et al. (2016) for ForeStereo® images and based on the intensity, anisotropic variances of visible bands and greenness ratio, better segmentation of foliage elements in high light variability environments. The stems are also matched in the stereoscopic hemispherical images captured with ForeStereo® (Sánchez-González et al., 2016) and can be excluded from LAI estimation. The Wilcoxon paired test was used to assess differences between LAI before burning and one year after burning. All above analyses were carried out with STATISTICA 10.0 (Statsoft Inc., Tulsa, USA).

Results

Burning

The mean daily air temperature during prescribed burning was 21.5 °C in El Pozuelo and 20.4 °C in Beteta; the relative humidity was 48% in El Pozuelo and 33% in Beteta. In relation to fire behavior, rate of spread in El Pozuelo was 0.65±0.10 m min^{-1} and 0.76±0.09 m min^{-1} in Beteta. The flame height was 53.7±15.6 cm and 43.7±7.8 cm and the flame length 30±6 cm and 38±8 in El Pozuelo and Beteta, respectively. The characteristics of the prescribed burnings in each plot are shown in Table 2. The maximum flame temperature reached during burning was high (between 559 and 787 °C), but only small proportion of monitored trees (6-16%) were affected by cambial heating. It was generated a moderate maximum scorched height in the leeward side of trunks (70 cm in El Pozuelo and 160 cm in Beteta), but in most cases lower than the height of the first living branch (Table 1). Crown damage caused by the effects of fire, was observed in different proportions of trees in each plot generating a moderate scorched crown range of 0-13% in El Pozuelo and 1-17% in Beteta however, no trees were completely scorched, except seedlings under canopy.

Table 2. Main parameters measured during and after prescribed burning in El Pozuelo and Beteta.

Z	PN	PT	T	RH	WS	FLc	TmM Litter	TmM Soil	HmMS	TMxB	TMxC
--	--	--	°C	%	m s^{-1}	%	°C	°C	cm	°C	°C
El Pozuelo	P3B	Burned	20.17	53.12	0.17	59.31	157.25	37.80	32	559.5	151.5
El Pozuelo	P4B	Burned	21.97	47.47	0.81	61.19	261.87	31.10	66	668.0	43.0
El Pozuelo	P6B	Burned	22.42	42.61	1.32	75.21	372.13	37.20	113	787.0	67.0
Beteta	B2B	Burned	18.80	34.77	0.79	77.22	394.30	42.00	151	688.5	81.5
Beteta	B5B	Burned	20.65	33.10	0.71	65.16	384.57	40.30	150	754.5	82.0
Beteta	B6B	Burned	21.69	30.15	0.82	62.23	303.96	30.00	178	605.5	61.5

Z: zone; PN: plot number; PT: plot type; T: air temperature; RH: relative humidity; WS: wind speed; FLc: percentage of fuel load consumed; TmM Litter: mean maximum temperature in litter horizon; TmM Soil: mean maximum temperature in soil; HmMS: mean maximum scorch height; TMxB: absolute maximum bark temperature; TMxC: absolute maximum cambium temperature.

Litterfall

The mean accumulated litterfall collected in the study period (from May 2016, immediately after burning, to April 2017) in control plots in El Pozuelo was 3171±649 kg ha^{-1} $year^{-1}$ and in burned plots, 3257±598 kg ha^{-1} $year^{-1}$ (Fig. 2a). In Beteta, the mean accumulated litterfall in control plots was 2028±530 kg ha^{-1} $year^{-1}$ and in burned plots, 3520±135 kg ha^{-1} $year^{-1}$ (Fig. 2b). There was no significant difference in accumulated litterfall ($F = 0.03$, $p = 0.8743$) between control and burned plots in El Pozuelo (Fig. 2a), but significant differences ($F = 22.35$, $p = 0.0091$) in accumulated litterfall were observed in Beteta (Fig. 2b).

In both study areas and both types of plot, litterfall accumulation peaked between August and September and was minimal in December, although it decreased significantly (El Pozuelo: $F = 15.47$, $p = 0.0001$; Beteta: $F = 24.99$, $p = 0.0001$) in the spring months (February to April) (Fig. 3). Two minor peaks also occurred in November and January in both, control and burned in El Pozuelo and Beteta. The percentage of annual litterfall in summer months (July, August and September) was similar in control and burned plots, 47% and 46%, respectively. The mean values were not significantly different in unburned and burned plots ($F= 1.47$, $p = 0.2917$) (Fig. 3a). In Beteta (Fig. 3b), the percentage accumulation increased to 53% in control plots and 45% in the burned plots. The mean amount of litterfall in burned plots increased significantly ($F = 30.61$, $p = 0.0052$) in the months immediately after the burning (second half of May and all of June). After the summer, the mean values became more similar and the curves were almost equal, although differences (36% higher burned plots) were observed in April 2017 in the unburned and burned plots in Beteta. In both zones, unburned and burned plots were different, but the curves were similar (Fig. 3).

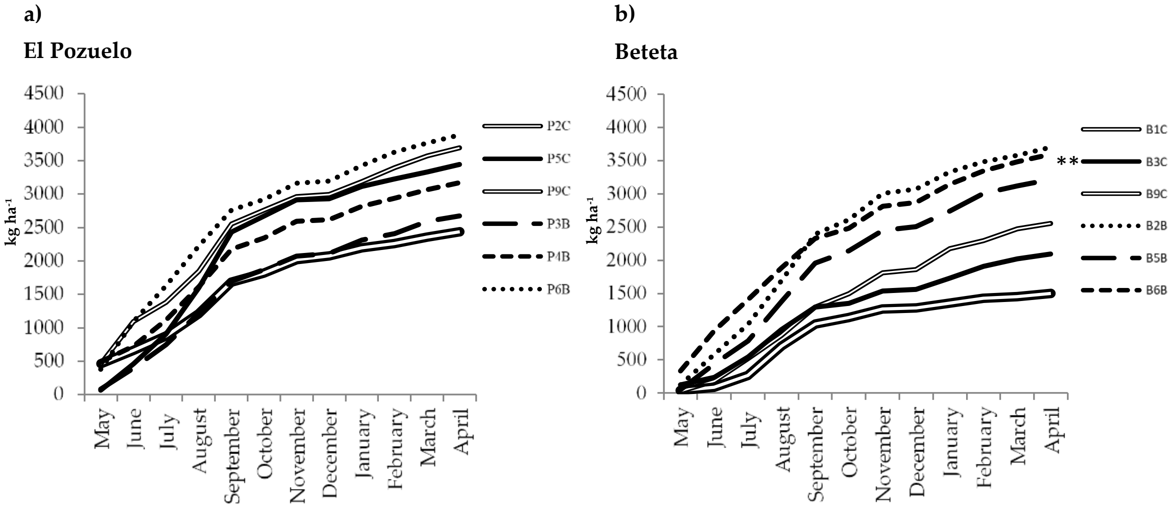

Fig. 2. Total litterfall accumulated per plot (control and burned) during the period May 2016 – April 2017 in (a) El Pozuelo and (b) Beteta. Control in solid line and burned in dashed line. Statistical significance of difference between treatments (control vs burned) is indicated as (**) $p < 0.05$.

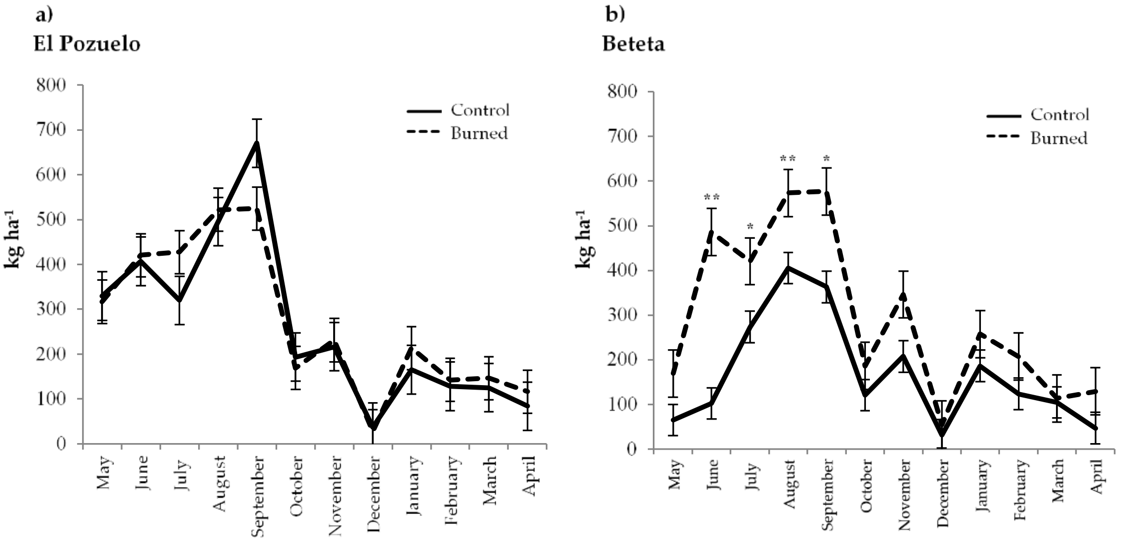

Fig. 3. Variation in mean litterfall in all control and burned plots, collected during the period May 2016 – April 2017 in (a) El Pozuelo and (b) Beteta. Control in solid line and burned in dashed line. Litterfall accumulation is expressed as the mean ± standard error, n = 6. Statistical significance of difference between treatments (control vs burned) is indicated as (*) $p < 0.1$, (**) $p < 0.05$.

Litterfall main fractions

Needles were the most important litterfall fraction at both sites in both types of plots. The mean proportions of needles in the litterfall collected in the period were 44 and 43% in the control and burned plots in El Pozuelo and 66 and 65% in the respective plots in Beteta.

The needle litterfall fraction varied following a seasonal pattern (El Pozuelo: F = 34.51, $p < 0.0001$; Beteta F = 79.67, $p < 0.0001$). In El Pozuelo, the maximum value was reached faster in burned plots than in the control plots (August and September, respectively). Peaks also occurred in November and January (174 and 123 kg ha^{-1} in control plots; 200 and 146 kg ha^{-1} in burning plots). In Beteta, another two peaks were observed in November and January (162 and 80 kg ha^{-1} in control plots; 250 and 158 kg ha^{-1} in burned plots). The minimum values occurred in both sites in December (13 and 12 kg ha^{-1} in control and burned plots in El Pozuelo; 25 and 41 kg ha^{-1} in control and burned plots in Beteta).

In El Pozuelo, two months immediately after the burning (July) the amount of needles was significantly higher (little significance) in the burned than in the control plots (F = 6.97, p = 0.0576) and from September onwards the differences were not significant (Fig. 4a). This highlights the main short-term effect of burning on the needle fraction, which was masked in the analysis of total litterfall (Fig. 3a). In Beteta, the maximum was reached in August and in the burned remains in September. The amount of needles was always higher in the burned plots than in control plots confirming the significant differences in accumulate (Fig. 2b) and total litterfall (Fig. 3b) but only significant differences were detected from July (F = 149.17, p = 0.0002) to September (F = 5.37, p = 0.0532) and during winter (January, F = 25.35, p = 0.0073).

The maximum amount of cones was recorded in May in control (299 kg ha^{-1}) and burned (264 kg ha^{-1}) plots in El Pozuelo. In Beteta the maximum was recorded in January (39 kg ha^{-1}) and June (28 kg ha^{-1}) in control plots and June (141 kg ha^{-1}) in burned plots. In El Pozuelo (Fig. 4b) during the month immediately after the burning the amount of cones was highest in the control plots. In Beteta the opposite was observed, but significant difference between the two treatments in May and June were not detected (F = 1.93, p = 0.2363; F = 3.90, p = 0.1195; respectively).

In both areas (Fig. 4c, 5c), the amount of inflorescences was higher in the control plots than in burned plots (185 and 100 kg ha^{-1} $year^{-1}$ in control and burned plots of El Pozuelo; 66 and 38 kg ha^{-1} $year^{-1}$ in control and burned plots of Beteta). The differences were significant only in El Pozuelo for July (F = 11.97, p = 0.0258).

The remains of unclassified material are referred to as "miscellaneous" (Fig. 4d and 5d). In El Pozuelo little significant differences (F = 5.33; p = 0.0821) between control and burned plots were only observed at the end of the study period (Fig. 4d). In Beteta the differences between treatments were visible throughout the year (Fig. 5d) showing a significant interaction between month (intra-subject factor) and treatment (F = 4.56, p = 0.0001).

Branches and barks were the least important fractions, in terms of quantity, in both zones. In El Pozuelo, the amount of branches remained below 5 kg ha^{-1} between August and December (Fig. 4e) and reached a peak (23 kg ha^{-1} in control plots and 25 kg ha^{-1} in burned plots) at the end of the study period. Similar observations were made in Beteta, and the amount remained below 10 kg ha^{-1} between July and December, reaching a peak in winter (48 kg ha^{-1} in control plots and 41 kg ha^{-1} in burned plots) (Fig. 5e).

In both study sites, the bark fraction was not significantly higher in the burned than in control plots (Fig. 4f and 5f), and the intra-annual variability was high, with various peaks being observed.

The fractions of pine seeds, lichens and leaves of species other than *Pinus* spp. contributed least to the total biomass. The amounts of these fractions varied annually in both treatments and study areas, often yielding zero values.

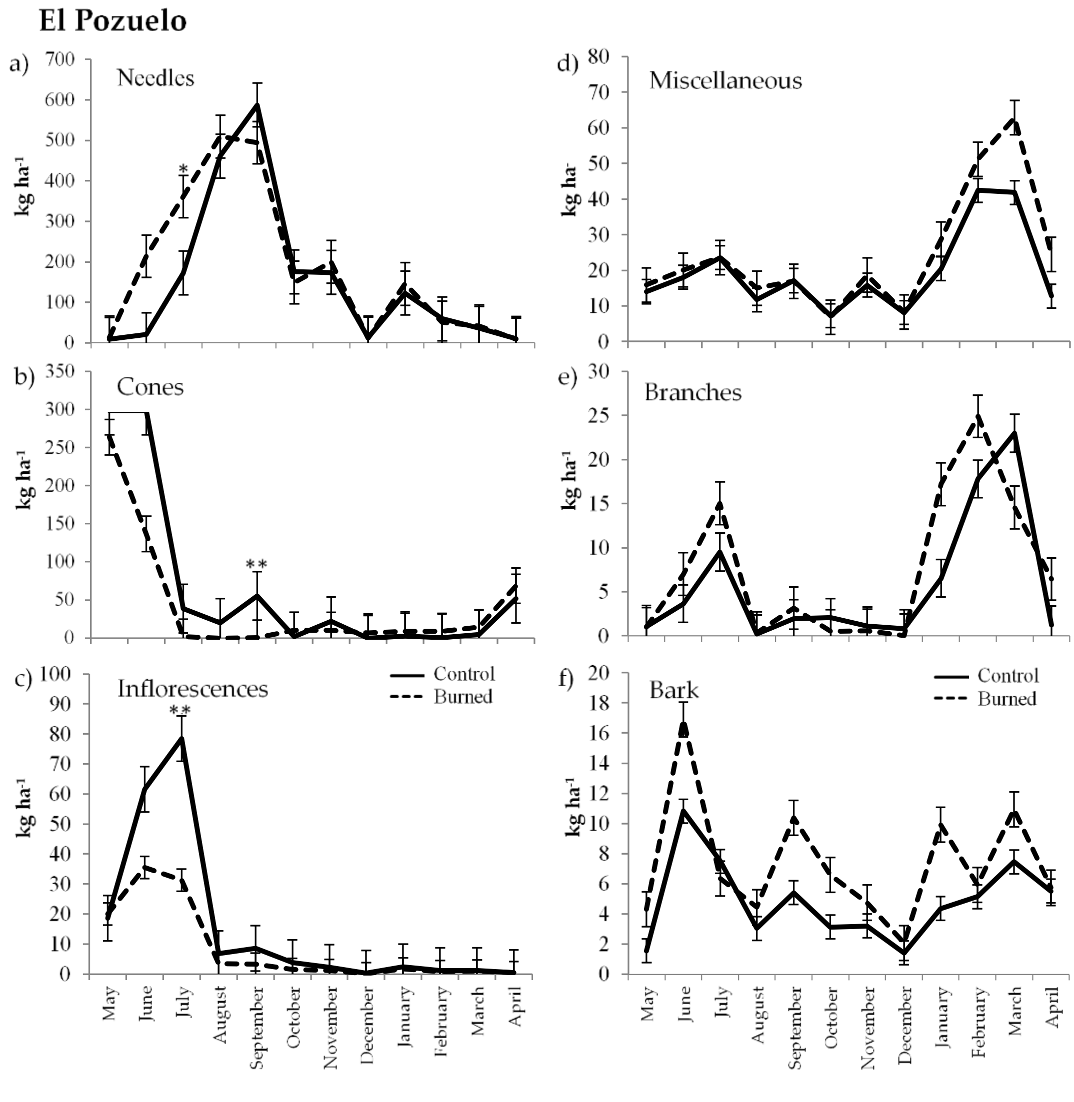

Fig. 4. Changes in the main litterfall fractions (a) needles, (b) cones, (c) inflorescences, (d) miscellaneous, (e) branches and (f) bark, collected during the period May 2016-April 2017 in El Pozuelo. Control in solid line and burned in dashed line. Litterfall production is expressed as the mean ± standard error, n = 6. Statistically significant difference between treatments (control and burned) is indicated as (*) $p < 0.1$, (**) $p < 0.05$.

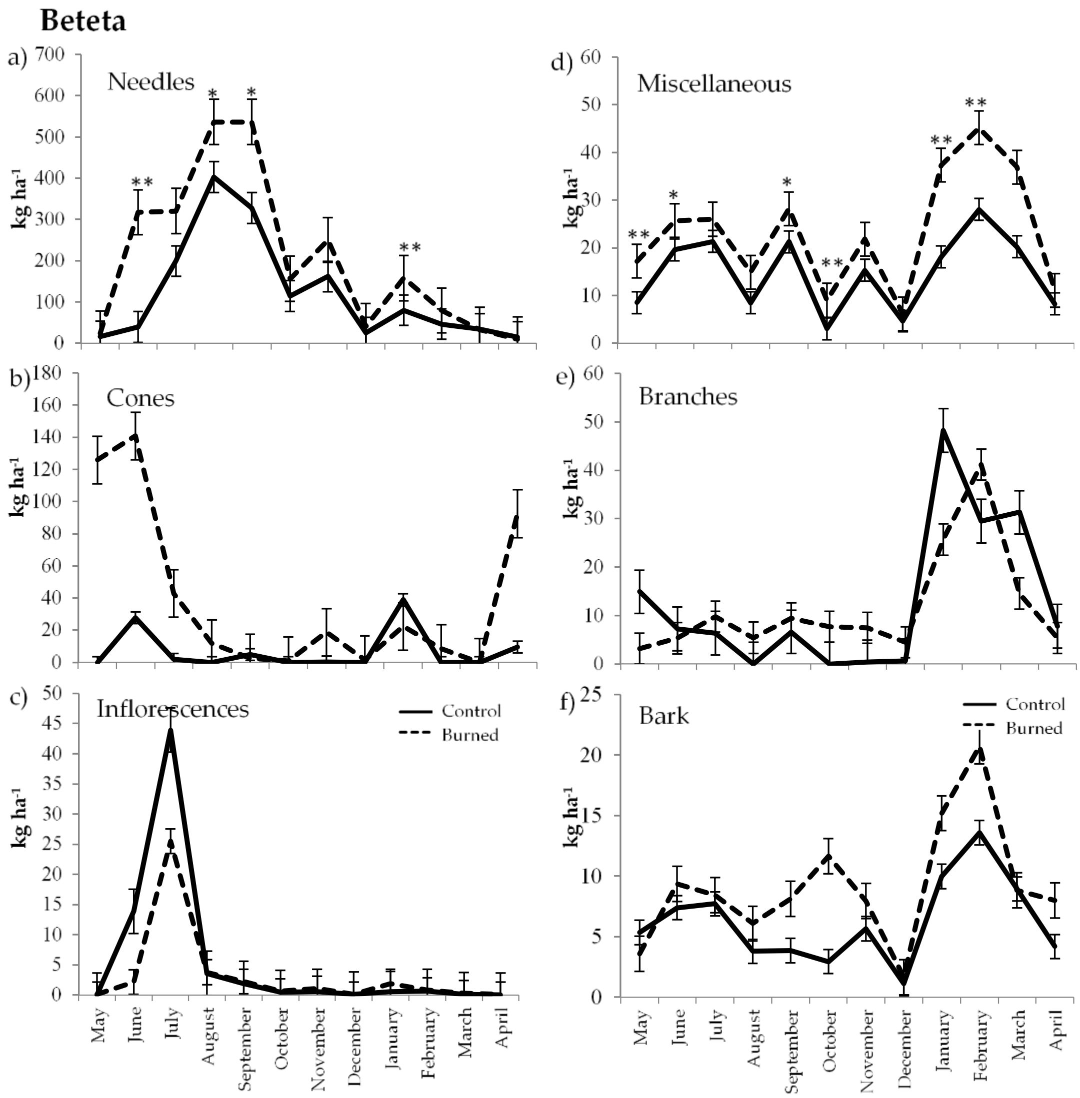

Fig. 5. Variation in the main litterfall fractions (a) needles, (b) cones, (c) inflorescences, (d) miscellaneous, (e) branches and (f) bark, collected during the period May 2016-April 2017 in Beteta. Control in solid line and burned in dashed line. Litterfall production is expressed as the mean ± standard error, n = 6. Statistically significant difference between treatments (control and burned) is indicated as (*) $p < 0.1$, (**) $p < 0.05$.

Leaf Area Index (LAI)

In El Pozuelo, the LAI decreased in all plots (control and burned), except P6B. The most important decrease occurred in plot P3B (decrease of 0.4 points), as also observed in one of the control plots (P9C, 0.38). In Beteta, the LAI only increased in two burned plots (B2B and B6B). Nevertheless, there were no significant differences in LAI one year after burning in any of the plots (Table 3) (Wilcoxon paired test $p = 0.2393$).

Table 3. Results of LAI measurement before and one year after the prescribed burning.

Z	PT	LAI	LAI	S LAI	S LAI
--	--	April 2016	April 2017	April 2016	April 2017
El Pozuelo	Control	2.0	1.8	0.5	0.6
El Pozuelo	Burned	2.7	2.6	0.1	0.2
Beteta	Control	2.9	2.7	0.2	0.0
Beteta	Burned	2.8	3.0	0.5	0.8

Z: zone; PT: plot type; S LAI: standard deviation of LAI.

Discussion

The parameters monitored during prescribed burning (PB) showed that the effects on soil and vegetation were of low-intensity and severity. The efficacy of burning for reducing dead forest fuel was moderate-high (reduction between 59 and 77% of litter biomass), and the main objective of the treatment, i.e. reduction of fire hazard, was therefore successfully achieved. Several studies have shown the efficacy of prescribed underburning for reducing fire risk and preventing forest fires, with associated benefits to ecosystems or, at least, a low impact in forest systems (e.g. Schwilk et al., 2009). Recent studies in Europe highlight that low-intensity PB increases growth of *P. nigra* stands (Valor et al., 2015). Conifers such as *P. pinea*, with adaptation traits to low-intensity fires, show higher photosynthetic activity and stomatal conductance after PB (Battipaglia et al., 2016). Jiménez et al. (2017) demonstrated that physiological activity in *P. pinaster* is only significantly affected when fire generates simultaneous damage to the living tissues of tree trunk (cambium) and crown (scorched needles). The findings of the present study indicate a low level of damage to tree trunk (Table 2) and canopy and therefore it is reasonable to expect little physiological change at stand level (Jiménez et al., 2017).

In the present study, accumulated biomass litterfall during the first year after PB (Fig. 2) was within the range of natural litterfall in Spanish conifer stands. Comparison of the annual litterfall data in a nearby sample plot with latitudinal similarity in Mora de Rubielos (Teruel), obtained with data provided by ICP Forests (Level II plots), showed that the values are within the range of data recorded in the six years before the prescribed burning (2558 kg ha^{-1} $year^{-1}$ period 2010/2011 and 4870 kg ha^{-1} $year^{-1}$ period 2013/2014). In another two Level II plots of *Pinus nigra*, mean litterfall accumulation during the 11 years was 3724±705 and 7583±1403 kg ha^{-1} $year^{-1}$ in Cuellar (Segovia) and Dodro (La Coruña), respectively. Altogether, the mean litterfall accumulation in all 8 Level II conifer plots in Spain was 4750±2100 kg ha^{-1} $year^{-1}$. In general, litterfall production is low at high latitudes where a short growing season limits plant growth, but increases towards equatorial latitudes, where plants can grow throughout the year (Albrektson, 1988).

Similarities between control and burned plots were found in relation to the temporal pattern of litterfall (Fig. 3). Although annual litterfall patterns may vary slightly from year to year (Cañellas et al., 1996; Pausas, 1997), is accepted a maximum in the summer months in conifer stands. Maximum amounts were observed in September in El Pozuelo and in

August in Beteta. Blanco et al. (2006) reported peaks in two stands of *P. sylvestris* in the Western Pyrenees in September and October. Peaks have also been described for *P. sylvestris* in September-October (Guerrero et al., 1998) and in August-October in *P. pinea* and *P. sylvestris* (Hernández et al., 1992). Other peaks were observed in November and January in both areas (June-July by Blanco et al., 2006). A winter peak in litterfall in conifer stands was also reported by Roig et al. (2005). Litterfall was minimal in December in both areas.

Litterfall accumulation appears to be related to the annual climatic characteristics. The litterfall data (Fig. 3) were related to the temperature, precipitation and wind data from a meteorological station located in Mariana, Cuenca (40° 09′ 09″ N / 002° 08′ 29″ W), close to the study area (Table S1, Supplementary material 1). Litterfall seems to be maximal in the months with physiological drought. A relationship between dry conditions during the growth period and the timing of needle fall (main fraction) has been reported for coniferous forests by different authors (Hennessey et al., 1992; Pausas, 1997). The gradual leaf-fall may also depend on the duration of light period (Wareing and Thompson, 1975). The Mediterranean area is characterized by an irregular distribution of rainfall in the leaf growing season, which results in some degree of variability in the litterfall (Roig et al., 2005). Precipitation and wind tend to increase in November in the zone, which may accelerate the shedding of needles, branches and other biomass elements, thus leading to an increase in the amount of litterfall collected (Table S1, available in Supplementary material 1). The similar patterns obtained for control and burned plots for both locations (Fig. 3) suggest that ecophysiological tree processes have not been altered by prescribed burning (Battipaglia et al., 2016; Jiménez et al., 2017).

In El Pozuelo, the increase in total litterfall was not significant during the study period (Fig. 3a); nevertheless, a significant increase in needle fall was detected in the two months after PB (July) relative to that in control plots (Fig. 4a). In the Beteta stand the amount of litterfall intercepted immediately after the burning was higher than the amount captured in the control plots (Fig. 3b). The differences were significant for total biomass and the needle fraction until September (5 months after PB) (Fig. 5a).

Although there are no studies comparing the effects of prescribed burning on litterfall biomass, the study findings were compared with those of studies relating this factor to treatments representing a disturbance in forest ecosystems, such as different thinning regimes. For example, Roig et al. (2005) did not find significant changes in amount of litterfall immediately after thinning, such effects were seen two and three years later and then disappeared five years after the disturbance. In a study of *P. sylvestris* stands of age 40 and 52 years, Cousens (1988), observed an immediate response after thinning, with no lag in litter production. Agren and Knecht (2001) proposed that silvicultural works could produce an increase of litterfall production one year after thinning, stabilizing to normal values in subsequent years.

Cone production (Fig. 4b and 5b) may vary in relation to natural variation (cycles), but the largest amounts are collected at the end of spring and beginning of the summer following the pine flowering season (Blanco et al., 2006). In El Pozuelo (Fig. 4b), the amount of cones is higher in the unburned plots than in the burned plots after the burning; in Beteta the opposite occurs suggesting a possible short-term increase (Fig. 5b). In El Pozuelo differences were not significant from October to April.

There is a marked seasonality in the phenology of needle fall and there is also a marked seasonality in inflorescence shedding, which occurs almost exclusively in the months of June and August (Pausas et al., 1994). Special attention must be paid to this point because an increase in shedding of inflorescences in control plots in both locations (Fig. 4c and 5c) suggests a decrease in the number of inflorescences due to the heat during burning. This could enhance the negative effects on emergence and seedling mortality (Lucas-Borja et al., 2016) and have an important impact on regeneration process that must be taken into account in the management of such stands.

In El Pozuelo, the distribution of the miscellaneous fraction (Fig. 4d) remained constant until February and March when it reached maximum values of around 40-70 kg ha^{-1}. The distribution of the miscellaneous fraction in Beteta was irregular (Fig. 5d), registering a peak in the same months as El Pozuelo. Although the miscellaneous fraction followed the same trend in unburned and burned plots of the two zones, the most important differences between the two plot types occurred in the months of maximum collection (February and March).

Branch biomass was irregularly distributed (Fig. 4e and 5e) with maximum in winter months, as Blanco et al. (2006) highlight in a previous study. El Pozuelo presents another peak from June to July in burned and unburned plots due to the increase of the winds and the precipitations in those months.

The bark fraction is affected by sporadic occurrence of storm or snowfall and like the branch fraction, did not follow a clear pattern (Blanco et al., 2006). There were many months with higher bark biomass in burned plots for both locations (Fig. 4f and 5f) suggesting a direct effect of scorch of tree trunk that generates an easier detachment of outer bark. Nevertheless, these differences were not significant in any month.

In El Pozuelo, given the total amount of litterfall fractions, only cones and inflorescences (Fig. 4b and 4c) have decreased after burning, the remaining fractions have increased or remained practically the same (branches and bark, Fig. 4e and 4f). In Beteta (Fig. 5), prescribed burning increased the production of litterfall for all fractions, except the inflorescences (Fig. 5c) and branches (Fig. 5e) that have practically not varied along the monitoring.

In El Pozuelo, the amount of needles in the total litterfall was 44 and 43% (control and burned plots), and in Beteta it was 66 and 65%. There is no difference in percentage between untreated and treated plots, although in Beteta, the total amount of litterfall was greater in burned than in control plots. In El Pozuelo, there was scarcely any difference in the annual mean and the proportions of the fractions are still maintained. Differences were observed in the two zones possibly due to the different stand characteristics. Blanco et al. (2006) reported that the needle fraction was 50-60% of the litterfall, similar to values reported by

Gallardo et al. (1995). Several authors have proposed a mean needle fall value estimate on a global basis of about 70% (Meentemeyer et al., 1982; Albrektson, 1988).

The different response of the two stands studied immediately after the prescribed burning can be explained by the mixed nature of the stand of El Pozuelo. Recent comparative studies of mixed and pure forests have reported that mixed forests may be expected to demonstrate higher levels of resilience and resistance to environmental hazards (Bravo-Oviedo et al., 2014). The "insurance hypothesis" suggests that their response to disturbance will be less intense and their recovery will be quicker than that of monocultures (Loreau, 2001; Jactel et al., 2009).

In El Pozuelo and Beteta stands the LAI values were between 1.8 and 3.0. There were no significant differences before and one year after the burning in the plots, and there were also no significant differences between control and burned plots. Some authors (Montes et al., 2007) have used the same method to studied LAI in *P. sylvestris* stands in the Central Mountain Range of Spain and have reported values ranging from 4.30 to 6.78 for this species. The absence of changes in this parameter one year after burning assess that physiological processes and biomass cycles were not altered by PB at stand level. Long-term monitoring is required to confirm this finding.

Conclusions

Prescribed burning is a forestry tool used to prevent forest fires. Implementation of this method must take into account the characteristics of each zone and the potential effects on ecosystems. This study is a first attempt to explore the short-term effects on litterfall biomass after prescribed fire. The findings show an initial increase in the amount of litterfall collected in the burned plots, especially in the pure stand at Beteta, until the end of summer. In mixed stands (El Pozuelo) an initial increase was also observed in the needle fraction after two months (July), although the variation in the amounts for the different treatments varied more widely thereafter. After the season of maximum leaf-fall, litterfall production in the plots with different treatments stabilized and the effect of the disturbance was scarcely noticeable. Despite differences in production between unburned and burned plots, litterfall patterns remain the same in both types of treatments and were mainly determined by meteorological factors such as temperature, rainfall, storms and snowfall, as well as by the marked phenological character of some of the biomass fractions. The collected material was divided into fractions and slightly larger quantities were collected in the burned plots except for the inflorescences for which a significant reduction was observed in burned plots, suggesting that reproductive organs may be affected by heat. Exploration of this aspect by implementation of PB during autumn would be one way of addressing this possibility. The ForeStereo® system was used to compare the LAI of the plots from the initial state and one year after the disturbance, and no differences were recorded. Therefore, main conclusion from management point of view is that conservative prescribed burning aimed at generating fire of low-intensity and severity in pure and mixed *Pinus nigra* and *Pinus pinaster* stands in the Central-Eastern Spain did not affect the short-term stand stability. Despite the increase of the litterfall biomass following the burning, little or no differences relative to control plots were observed 2-5 months after burning. The accumulated annual biomass was also within the range reported for natural stands in the study area. However, long-term monitoring should be conducted to enable the stand condition to be checked over time.

Acknowledgements

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Supplementary material 1

Table S1. Mean litterfall collected between May 2016 and March 2017 in the burned and control plots in El Pozuelo and Beteta in relation to data recorded at the Mariana weather station (40° 09' 09'' N / 002° 08' 29'' W).

	Unit	Zone	Plot type	May	June	July	August	September	October	November	December	January	February	March
Rainfall	mm	--	--	52.91	10.19	6.96	5.39	13.87	48.21	65.00	6.50	15.07	38.07	35.40
Mean temperature	°C	--	--	11.9	18.2	22.5	21.3	16.2	11.6	5.4	2.8	1.0	4.9	7.0
Wind	$m\ s^{-1}$	--	--	0.88	0.75	0.66	0.67	0.59	0.52	0.66	0.48	0.95	1.06	1.00
Mean litterfall	$kg\ ha^{-1}$	El Pozuelo	Control	330	407	320	495	670	193	216	37	165	128	125
Mean litterfall	$kg\ ha^{-1}$	El Pozuelo	Burned	317	420	427	522	525	169	231	28	213	143	146
Mean litterfall	$kg\ ha^{-1}$	Beteta	Control	65	102	273	405	363	121	208	31	186	123	105
Mean litterfall	$kg\ ha^{-1}$	Beteta	Burned	169	486	420	574	577	186	346	55	258	207	113

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Chapter 2

Effect of prescribed burning on litterfall biomass

Subchapter 2.2

The effect of low-intensity prescribed burns in two seasons on litterfall biomass and nutrient content

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Abstract

Litterfall production and composition, fall pattern and nutrient content were studied in a mixed stand of *Pinus nigra* and *Pinus pinaster* (El Pozuelo), as well as in a pure stand of *Pinus nigra* (Beteta) in the Cuenca Mountains in order to determine the effect of two-season prescribed burning treatments. Needles were the most abundant fraction. Pinecone fraction decreased after burning in the mixed stand and the opposite occurred in the pure stand. The inflorescence fraction showed a decrease in the spring-burned plots at El Pozuelo and Beteta. Bark, branch and miscellaneous fractions were affected mainly by meteorological events. Low-intensity prescribed burning was not found to cause significant perturbations. The perturbation was mitigated over the years. An immediate effect of prescribed burning in spring was seen at El Pozuelo and Beteta, although it was more significant for the pure stand. The effect of prescribed burning in autumn at Beteta had a delayed response. As regards nutrient contents, no differences in carbon concentrations were detected. Overall, an increasing trend in N, P, K concentrations in needles after the burning treatment was found. Calcium was not a limiting factor. Magnesium content exhibited no clear trend.

Keywords: Cuenca Mountains; defoliation; forest fire; *Pinus nigra*; *Pinus pinaster*; tree crown.

Introduction

Current climate change projections predict increased temperature and droughts in fire-prone regions such as the Mediterranean (Ferreira et al., 2016), which may mean an increase in the area affected by forest fire. Prescribed burning (PB) can be useful to reduce surface fuels, increase height to the live crown, decrease crown density, retain large trees of fire-resistant species (Agee and Skinner, 2005) and thus, mitigate high-intensity wildfires (Boer et al., 2009).

Pinus nigra Arn. ssp. *salzmannii* (Spanish black pine) and *Pinus pinaster* Ait. (maritime pine) have been suggested to be adapted to fire-prone habitats through several strategies (Fernandes et al., 2008). Montane communities of Spanish black pine show adaptations such as thick bark, high crown base height (Pausas et al., 2008; Valor et al., 2015) and self-pruning ability (Tapias et al., 2001; Fulé et al., 2008). The same applies to *P. pinaster* (Ryan, 1998; Fernandes and Rigolot, 2007; Fernandes et al., 2008). However, *P. nigra* does not produce serotinous cones (Tapias et al., 2001) and it also does not maintain a canopy or soil seed bank (Ordóñez et al., 2005). Thus, although PB seems to be a suitable fuel reduction treatment, an adequate prescription must be carefully planned to avoid decreasing resilience in these stands. In addition, the Cuenca Mountains are a typical area where the *P. nigra*-*P. pinaster* ecotone generates stable stands with high ecosystem services and ecological values. It is recognised that interactions between species may reduce the impact of disturbances over time (Thompson et al., 2009). Exploring the resilience of mixed and pure stands to perturbations such as PB is therefore essential in order to establish recommendations for management and fire prevention strategies in these stands.

In a preliminary, short-term study carried out in the same study area (Espinosa et al., 2018), spring PB appeared to alter litterfall biomass patterns. A trend to stabilisation of the amount of litterfall was also observed 1 year after burning, with some variations in the responses of the different types of stands (mixed or pure). In addition, some slight differences in inflorescences in control and spring-burned plots occurred during the first year. However, the short period of time during which the litterfall was studied raised doubts about the efficacy of the treatment and the duration of the impact. Therefore, a longer-term study is needed to achieve a deeper knowledge of the efficacy and impacts of PB. In this sense, the present study integrates the results of three periods of monitoring in order to analyse the effects of PB in the medium-term, adding valuable information to our previous study.

The impact of PB depends greatly on how effective it is in reducing fuel and the extent to which it disturbs the litterfall (Espinosa et al., 2019). Indeed, if PB negatively affects the crown, this could lead to an increase in litterfall, which could increase fire risk, thus reducing the effectiveness of the treatment. Regarding the impact of PB, no studies have been conducted to assess the effects on litterfall biomass in pine forests. Thus, the findings reported here expand on existing information about litterfall dynamics after thinning. While some authors observed effects during 1–3 years after thinning (e.g. Agren and Knecht, 2001; Roig et al., 2005), another noted alterations in litterfall beyond 5 years (e.g. Navarro et al., 2013). These contrasting results suggest that the response of forest stands is determined by multiple factors. Although burning season can be controlled in PB programs, no studies have investigated how burning season affects litterfall patterns in pine forests. The fire season may have different impacts on ecosystems through differences in fire intensity and meteorological conditions (Hamman et al., 2008), phenological status and carbohydrate storage of the trees during burning (Harrington, 1993; Thies et al., 2005; Knapp et al., 2009). Thus, the present study included spring and autumn PBs in order to compare medium-term changes in litterfall in two seasons. This adds an important component relative to our previous research in which only the short-term effects of spring PB were analysed (Espinosa et al., 2018).

Prescribed burning not only disrupts the amount and distribution of litterfall, but can alter its carbon and macronutrient contents. Litter dynamics are an essential part of nutrient cycling and energy transfer in forest stands (Kavvadias et al., 2001). The nutrient pool in soils is crucial for the recovery potential and health status of the ecosystem after fire (regrowth, characteristics and functioning) (Näthe et al., 2018). Thus, the nutrient content of the litterfall fractions must be analysed to detect any changes after treatment, adding new information to our previous results. Although some authors have suggested the importance of understanding the effects of silvicultural treatments on litterfall and litter decomposition processes in different forest species and under geoclimatic conditions (Blanco et al., 2006; Lado-Monserrat et al., 2016), such information is scarce and generally focuses on the effect of different thinning regimes on litterfall. As already mentioned, the effects of PB have not previously been evaluated in pine forests.

The hypothesis proposed in the present study is that PB in a mixed *P. nigra* and *P. pinaster* stand and in a pure *P. nigra* stand will have significant impacts on litterfall dynamics (biomass fractions and nutrient content) and that the PB season will affect litterfall patterns.

To test this hypothesis, the specific research objectives of this work were as follows: (i) to analyse the effect of seasonal PB (spring and autumn) on the quantity, patterns and fractions of litterfall in these types of stand; (ii) to evaluate the effect of disturbance caused by PB on the carbon and macronutrient content of the litterfall. In addition, as needle fall is used as a sensitive indicator in climate change projections, the present study provides a valuable source of litterfall data relative to mixed *P. nigra* and *P. pinaster* stands and pure *P. nigra* stands.

Materials and methods

Study sites

Two sites in the Cuenca Mountains (Iberian System, Central-Eastern Spain) separated by a straight-line distance of 14 km, El Pozuelo (40° 33′ 36″ N / 002° 15′ 56″ W) and Beteta (40° 33′ 06″ N / 002° 06′ 32″ W), were selected for study (Fig. 1). In both areas, mean slope varies between 3 and 10%, and elevation ranges between 1015 and 1294 m above sea level (asl). According to data provided by the State Meteorological Agency of Spanish Government (AEMET, 2018) obtained from the nearest weather station, located in Cañizares (940 m asl), the mean annual temperature over the last 21 years was 12.18 °C (13.28 °C during the period from 2016 to 2018) with average precipitation of 717 mm (599 mm in the period from 2016 to 2018). During the maximum litterfall season (June-September), the mean temperature was 19.78 °C and mean precipitation 129 mm. There is a high degree of daily and also seasonal oscillation. The soil is calcareous (Lucas-Borja et al., 2017; Plaza-Álvarez et al., 2017). The mean shrub cover in both zones ranges from 5 to 20% and pine regeneration is irregularly distributed (from 78 to 11611 seedlings ha^{-1}). The main characteristics of the stands under study are shown in Table 1.

Experimental design

Data were collected in nine plots (30 × 30 m) per experimental site following a random block design. A total of three treatments per experimental site (non-burned plots, spring-burned plots and autumn-burned plots) with three replicates per block were established. The plots were representative of the study area and homogeneous in terms of structure and composition of stand. To avoid edge effects, a 20-m strip adjacent to each plot was delimited.

All trees in plots were identified and the following measurements were made: total height (Ht, m), height to the first live branch (H1, m), diameter at heights of 0.6 and 1.3 m from the base (D60 and D130, cm), and maximum and minimum bark thickness at 0.6 m from the base (leeward and windward).

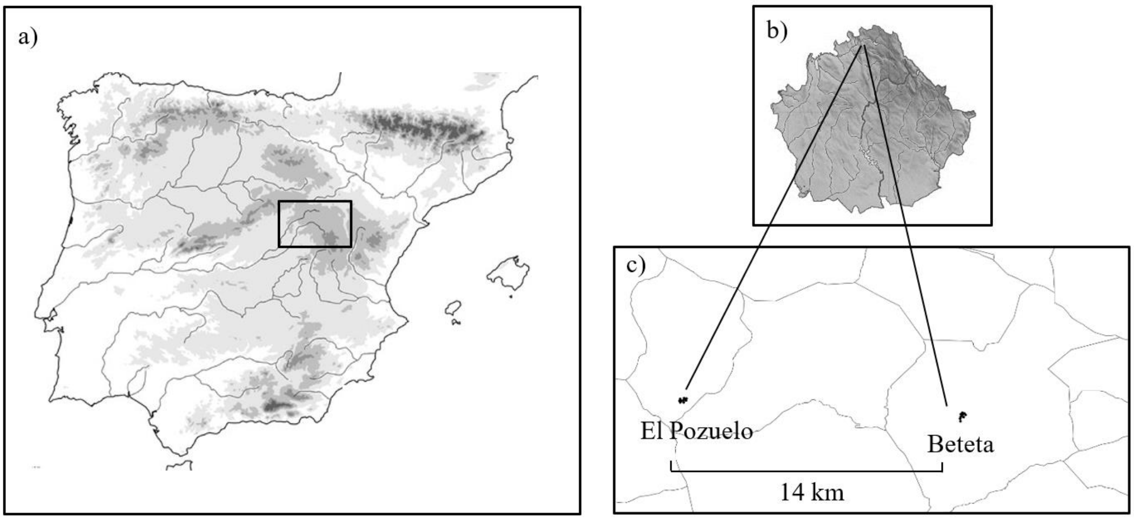

Fig. 1. Location of the study plots in (a) the Iberian Peninsula; (b) the province of Cuenca; (c) locations of El Pozuelo (mixed *P. nigra*-*P. pinaster* stand) and Beteta (pure *P. nigra* stand).

Table 1. Main parameters measured in burned and non-burned plots at El Pozuelo and Beteta.

Z	PT	Dt	Pn	Pp	Ht	H1	D60	D130	BT	G
--	--	Trees ha^{-1}	%	%	m	m	cm	cm	cm	m^2 ha^{-1}
* El Pozuelo	Non-burned	563 (74)	93 (4)	7 (4)	11.5 (3.2)	5.1 (1.9)	19.7 (3.8)	18.7 (3.7)	1.4 (0.5)	22.5 (7.5)
* El Pozuelo	Spring-burned	881 (227)	89 (8)	11 (8)	12.3 (0.8)	7.1 (0.6)	21.3 (1.0)	19.8 (1.0)	1.9 (0.7)	33.7 (9.7)
El Pozuelo	Autumn-burned	437 (110)	85 (20)	15 (20)	12.9 (2.1)	6.9 (2.4)	22.6 (2.9)	21.0 (2.8)	1.8 (0.5)	20.0 (0.7)
* Beteta	Non-burned	1456 (507)	100 (0)	0 (0)	10.1 (3.2)	6.1 (3.3)	15.1 (1.7)	13.8 (1.6)	1.3 (0.4)	25.5 (2.1)
* Beteta	Spring-burned	1215 (209)	100 (0)	0 (0)	12.7 (0.8)	7.5 (1.3)	20.0 (1.9)	18.2 (2.0)	1.9 (0.7)	39.1 (6.7)
Beteta	Autumn-burned	1274 (335)	100 (0)	0 (0)	14.1 (2.2)	9.4 (1.6)	21.9 (4.9)	20.0 (4.4)	2.0 (0.2)	45.2 (0.9)

Z: zone; PT: plot treatment; Dt: total density; Pn: percentage of *Pinus nigra*; Pp: percentage of *Pinus pinaster*; Ht: total height; H1: height to the first live branch; D60: diameter at 60 cm from the base; D130: diameter at 130 cm from the base; BT: bark thickness; G: basal area. Standard deviation in brackets. (*) Data from Espinosa et al. (2018).

Prescribed burning

Spring burns were conducted by the Regional Forest Service in May 2016 (on the same day at each site) whereas autumn burnings were carried out in November 2016 (same day at each of the sites). The strip ignition technique was applied at distances of 1-2 m, downhill and with a head wind. This burning technique is the most widely used in the study area for low-medium-intensity fire behaviour. Precipitation, wind speed, temperature and relative humidity were recorded every 10 min at a meteorological station located adjacent to the study plots. During the burning, the temperature of the bark (outer bark) and at the depth of the cambium (inner bark) of 15 randomly selected trees per plot was monitored at a height of 0.6 m with type K 1-mm-diameter Inconel-sheathed thermocouples (0.3-s response time). The thermocouples were connected to data loggers (DT-USB TCDirect®) that recorded the data at a frequency of 1 s. The threshold value of 60 °C in the cambium area corresponds to the commonly accepted lethal temperature for tree cells (Hare, 1965). Maximum scorch height was measured after burning. The results are shown in Table 2.

Table 2. Main parameters measured during prescribed burnings at El Pozuelo and Beteta.

Z	PT	T	RH	WS	RS	SMx	TmMx B	TmMx C	TMx B	TMx C	TRMx B60	TRMx C60
--	--	°C	%	m s^{-1}	m min^{-1}	cm	°C	°C	°C	°C	%	%
* El Pozuelo	Spring-burned	21.5	47.7	0.8	0.65	70	209	51	787	521	82	16
El Pozuelo	Autumn-burned	11.9	67.0	0.3	0.59	34	127	49	605	77	60	18
* Beteta	Spring-burned	20.4	32.7	0.8	0.76	160	279	41	755	315	96	11
Beteta	Autumn-burned	12.0	43.5	0.1	0.72	59	93	40	702	75	57	5

Z: zone; PT: plot treatment; T: air temperature; RH: relative humidity; WS: wind speed; RS: fire rate of spread; SMx: mean maximum scorch height; TmMxB: mean maximum bark temperature; TmMxC: mean maximum cambium temperature; TMxB: absolute maximum bark temperature; TMxC: absolute maximum cambium temperature; TRMxB60: percentage of trees in which the maximum temperature in the bark surface (air temperature) was higher than 60 °C; TRMxC60: percentage of trees in which the maximum temperature in the cambium was higher than 60 °C. (*) Data from Espinosa et al. (2018).

Litterfall

After the prescribed burns had been carried out, a total of eight litterfall collectors per plot (n = 18) were installed (the catchment area was 0.38 m^2). In order to ensure representativeness of the sample, the collectors were distributed such that they covered the entire working area (in the 30 × 30 m plot). The litterfall collection system was designed in accordance with the recommendations and parameters outlined in the manual of the United Nations Economic Commission for Europe (UNECE) under the project entitled "International co-operative program on assessment and monitoring of air pollution effects on forests" (ICP Forests, 2011) (Ukonmaanaho et al., 2016) to guarantee the quality and quantity of the sample (see details in Espinosa et al., 2018). On each collection day, the samples were taken to the laboratory and oven-dried at 65 °C to constant weight. The samples from each plot were weighed and separated into different fractions: needles, cones, inflorescences, branches of diameter < 2 cm, bark and miscellaneous (unclassified material, seeds, lichens and leaves of other species).

The study was carried out between May 2016 and October 2018 in the spring-burned and non-burned plots, and from November 2016 to October 2018 in the autumn-burned plots (a total of 4032 samples).

Nutrient content analyses

Needles, cones, inflorescences, branches of diameter < 2 cm and bark corresponding to the same period (first period: May 2016 to April 2017 for the non-burned and spring-burned plots and second period: May 2017 to April 2018 for the non-burned, spring-burned and autumn-burned plots) were pooled to obtain a composite sample per fraction, period and plot, as in the ICP Forests guidelines (Ukonmaanaho et al., 2016). The annual pool enabled comparison with data obtained by ICP Forests, Level II Plots for 2005-2014, in control *Pinus nigra* plots (ICP Forests, 2011).

Finally, the carbon (C) and nitrogen (N) contents of samples were determined using a LECO analyser, in solid milled material. The concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) in samples (temperature 65 °C) were determined by inductively coupled plasma optical mass spectrometry (ICP-MS) after wet digestion with HNO_3 (8 ml, 69%) and H_2O_2 (2 ml) at 190 °C for 15 min in a microwave.

Statistical analysis

For the statistical analysis of the litterfall results, total litterfall and different fractions in kilograms per hectare were selected as target variables. A linear mixed model of repeated measurements analysis of variance with two between-subjects factors was chosen: site (two levels: El Pozuelo and Beteta) and burning treatment (three levels: spring-burned, autumn-burned and non-burned plots) and their interactions. One within-subjects factor (repeated measurements) was selected (30 and 26 levels corresponding to months in temporal periods of spring and autumn PB respectively). A first-order autoregressive variance structure was considered for the errors in the linear mixed model.

With respect to the statistical analysis of the nutrient contents (target variables C, N, P, K, Ca and Mg), between-site differences in nutrient concentration varied depending on the nutrient and fraction considered. Therefore, the zones (El Pozuelo and Beteta) were considered independently. We used a linear mixed model with one between-subjects factor (three levels: spring-burned, autumn-burned and non-burned plots) and two within subjects factors (litterfall fractions and the two defined periods; see Nutrient content analyses section) was applied. The variance-covariance matrix for the model errors was defined as an unstructured type for the fractions and a compound symmetry type for the years.

The statistical analysis was segmented by site. The statistical analyses were performed using SAS 9.4 software (SAS, 2013) and pairwise *t*-tests were used for all the comparisons of the treatments. A significance level of 95% to detect differences between treatments was established.

Results

Total litterfall

Annual litterfall biomass collected in the non-, spring- and autumn-burned plots in the mixed (El Pozuelo) and pure (Beteta) stands are shown in Table 3.

At El Pozuelo, although a slight increase in the litterfall in spring-burned plots was observed 4 months after PB, the differences were not significant (Fig. 2a) (Espinosa et al., 2018). In the second period, the biomass was greater in the non-burned plots than in the spring-burned plots. After autumn PB (i.e. in November), the differences between the non-burned and autumn-burned plots were highly significant, the biomass being greater in the non-burned plots. At Beteta (Fig. 2b), an increase in the litterfall in the spring-burned plots was observed immediately after spring PB and in subsequent periods (Espinosa et al., 2018). Treatment effects were observed after the autumn PB, with significant differences between November and February in the first period and also between November and January in the second period (Fig. 2b).

For all treatments, the months from June to September were those when the maximum litterfall was collected, accounting for a mean percentage of 63±1 and 52±6% of annual litterfall at El Pozuelo and Beteta respectively.

Intra-annual variability in litterfall was observed in both areas for all periods (Fig. 2a and 2b). In both stands, litterfall reached maximum levels in August (775±217 kg ha^{-1} at El Pozuelo and 660±135 kg ha^{-1} at Beteta) (Fig. 2a and 2b). At El Pozuelo, the lowest amount of litterfall was collected during winter months (December, January and February). The same occurred at Beteta, except in the autumn-burned plots, in which more variable amounts of litterfall were collected. Some secondary minimum peaks were also recorded in spring (March, April and May) in both areas (Fig. 2a and 2b).

Main litterfall fractions

Annual litterfall production per treatment and fraction is shown in Table 4. Needles comprised the largest fraction in both stands, accounting for 58% of the biomass at El Pozuelo and 57% at Beteta. The mean percentage of *Pinus nigra* needles in all plots at El Pozuelo was 76±7%. The needle fraction reached similar maximum and minimum levels to the total litterfall at El Pozuelo (Fig. 3a) and Beteta (Fig. 3b). At El Pozuelo, the pattern after spring burning was similar to that observed for the total fraction. In the second period, the needle fraction was higher in the non-burned plots than in the spring-burned plots. After the autumn burning, the pattern was again similar to that observed for the total fraction, with the needle fraction being higher in the non-burned plots (Fig. 3a). At Beteta, the needle fraction was higher in the spring-burned plots than in the non-burned plots during the first period, confirming the significant differences between the plots (Fig. 3b). Although the differences were maintained, the effect was clearer at the start of the maximum litterfall period. After the autumn PB, significant differences between treatments were detected from November to February (Fig. 3b).

Cone fall peaked typically in May-July (Fig. 3c and 3d). Inflorescences differed significantly between zones (p = 0.0051), although not between treatments (Table 5). Significant differences were observed after spring burning at El Pozuelo and after autumn burning in both stands (Fig. 3e and 3f).

Branch fall was significantly higher at Beteta than at El Pozuelo. Overall, there was no significant difference between treated and untreated plots (Table 5). The inter- and intra-annual variability was high in both stands, with many peaks throughout the year. Some significant differences (Fig. 4a and 4b) were observed in winter months (particularly December, January and February).

The bark fraction differed significantly between areas (p = 0.0384). No differences between treatments were detected (Table 5). As with branches, high annual variability was observed in this fraction, with many peaks (Fig. 4c and 4d).

The miscellaneous fraction accounted for 10 and 12% of total biomass at El Pozuelo and Beteta respectively for all years and treatments. Seeds comprised 0.4% of total litterfall in both areas. No clear pattern (Fig. 4e and 4f) or any differences between zones and treatments (Table 5) were detected.

Table 3. Annual litterfall collected at El Pozuelo and Beteta per treatment.

Z	PT	* May 2016 - April 2017	May 2017 - April 2018	May 2018 - October 2018
--	--	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
El Pozuelo	Non-burned	3171 (649)	3537 (583)	1965 (426)
El Pozuelo	Spring-burned	3257 (599)	2991 (191)	1879 (197)
Beteta	Non-burned	1989 (519)	2585 (762)	1504 (152)
Beteta	Spring-burned	3482 (129)	3120 (327)	1762 (415)
Z	PT	November 2016 - October 2017	November 2017 - October 2018	--
--	--	kg ha^{-1}	kg ha^{-1}	--
El Pozuelo	Non-burned	3532 (585)	2726 (459)	--
El Pozuelo	Autumn-burned	2732 (325)	2640 (771)	--
Beteta	Non-burned	2393 (739)	2376 (472)	--
Beteta	Autumn-burned	3629 (527)	3495 (792)	--

Z: zone; PT: plot treatment. Standard deviation in brackets. (*) Data from Espinosa et al. (2018).

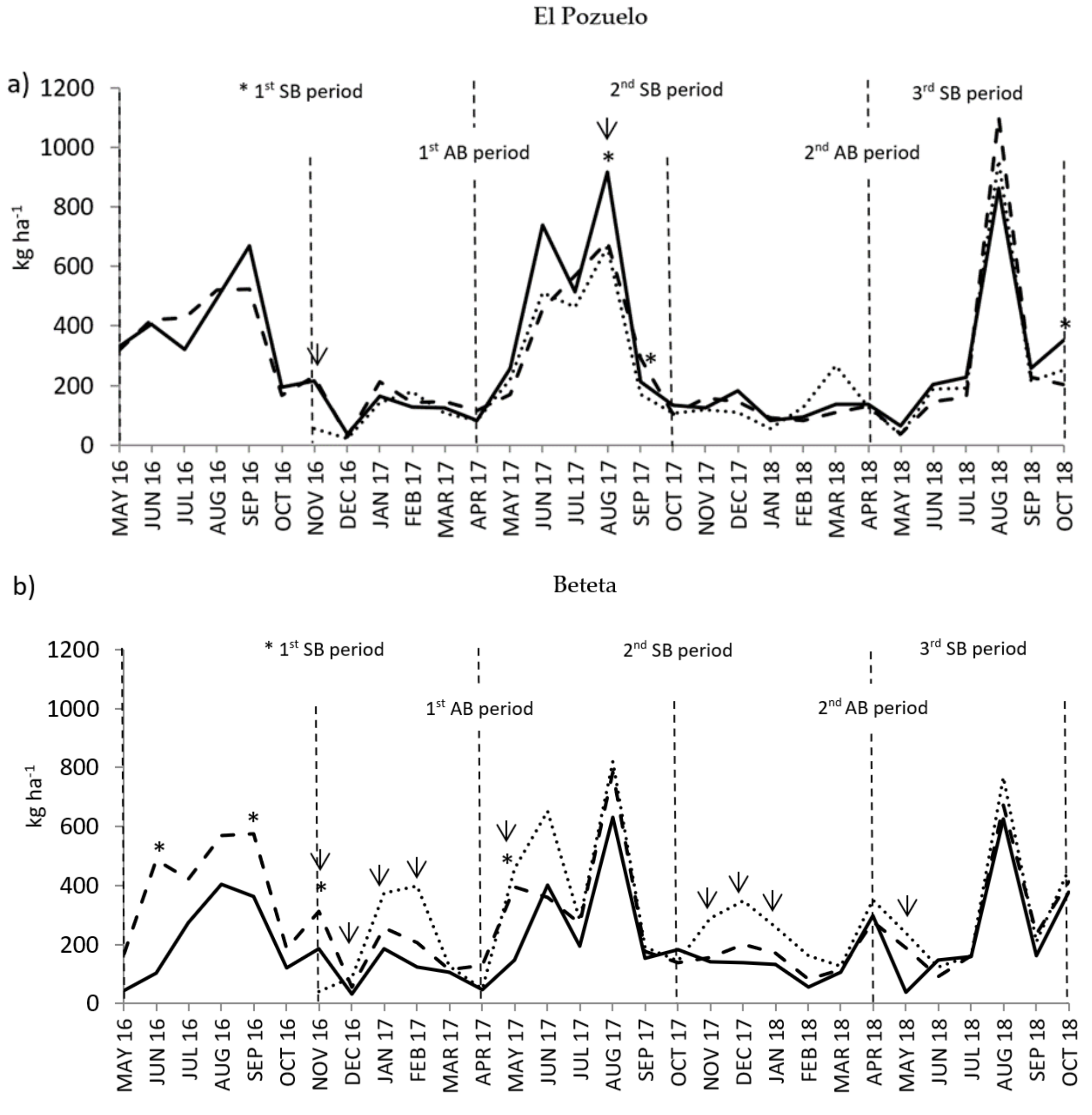

Fig. 2. Evolution of monthly collected litterfall biomass (kg ha^{-1}) in burned (spring and autumn burns) and non-burned plots, from May 2016 to October 2018 in (a) El Pozuelo and (b) Beteta. Non-burned (NB) plots in solid line, spring-burned (SB) plot in dashed line and autumn-burned (AB) plots in dotted line. (*) Represents significant differences in *t*-test of pairwise comparisons non-burned and spring-burned plots $p < 0.05$ and (arrow) represents significant differences in *t*-test of pairwise comparisons non-burned and autumn-burned plots $p < 0.05$.

Table 4. Annual litterfall production (kg ha^{-1} year^{-1}) per treatment and fraction at El Pozuelo and Beteta.

Z	PT	Needles	Cones	Inflorescences	Branches	Bark	Miscellaneous
--	--	kg ha^{-1} year^{-1}	kg ha^{-1} year^{-1}	kg ha^{-1} year^{-1}	kg ha^{-1} year^{-1}	kg ha^{-1} year^{-1}	kg ha^{-1} year^{-1}
El Pozuelo	Non-burned	1947 (152)	657 (195)	179 (9)	211 (201)	73 (20)	350 (102)
El Pozuelo	Spring-burned	2077 (161)	347 (260)	98 (3)	207 (164)	115 (38)	360 (79)
El Pozuelo	Autumn-burned	1480 (101)	351 (134)	177 (66)	266 (105)	95 (12)	339 (6)
Beteta	Non-burned	1423 (52)	169 (120)	55 (15)	274 (172)	98 (33)	334 (184)
Beteta	Spring-burned	2066 (556)	483 (20)	37 (1)	304 (232)	142 (47)	470 (204)
Beteta	Autumn-burned	1840 (20)	373 (354)	83 (29)	603 (30)	154 (20)	570 (217)

Z: zone; PT: plot treatment. Standard deviation in brackets.

Table 5. Mean ± standard error for total and fractions of litterfall biomass (kg ha^{-1} month^{-1}) at El Pozuelo and Beteta.

F	Z	PT			--
--	--	Non-burned	Spring-burned	Autumn-burned	--
Total	El Pozuelo	**291.02±14.57 a**	**273.12±14.57 a**	**224.39±15.31 b**	265.59±8.56 A
	Beteta	**206.17±14.57 a**	**285.80±14.57 b**	**300.40±15.31 b**	261.53±8.56 A
Needles	El Pozuelo	172.16±17.84 a	182.57±17.84 a	122.04±15.92 a	161.56±10.09 A
	Beteta	**130.98±17.84 ab**	**174.24±17.84 a**	**151.64±15.92 b**	152.33±10.09 A
Inflorescences	El Pozuelo	19.47±3.30 a	11.60±3.30 a	14.75±3.28 a	**15.31±1.91 A**
	Beteta	6.36±3.30 a	5.10±3.30 a	6.94±3.28 a	**6.07±1.91 B**
Cones	El Pozuelo	47.63±14.03 a	24.21±14.03 a	29.24±12.38 a	34.01±7.92 A
	Beteta	11.83±14.03 a	32.76±14.03 a	31.19±12.38 a	24.84±7.92 A
Branches	El Pozuelo	15.27±5.27 a	16.67±5.27 a	22.21±6.57 a	**17.75±3.26 A**
	Beteta	**21.77±5.27 a**	**24.05±5.27 a**	**49.69±6.57 b**	**30.56±3.26 B**
Bark	El Pozuelo	6.21±1.37 a	9.36±1.37 a	7.94±1.64 a	**7.83±0.84 A**
	Beteta	8.47±1.37 a	11.38±1.37 a	12.20±1.64 a	**10.57±0.84 B**
Miscellaneous	El Pozuelo	30.29±6.66 a	28.72±6.66 a	28.22±8.16 a	29.14±4.09 A
	Beteta	26.77±6.66 a	38.29±6.66 a	48.74±8.16 a	37.16±4.09 A

F: fraction; Z: zone; PT: plot treatment. Different letters indicate significant differences ($p < 0.05$) with *t*-test. Comparisons were made for all pairwise combinations of treatments and sites for each row and between sites in the final column. Significant differences are indicated in bold.

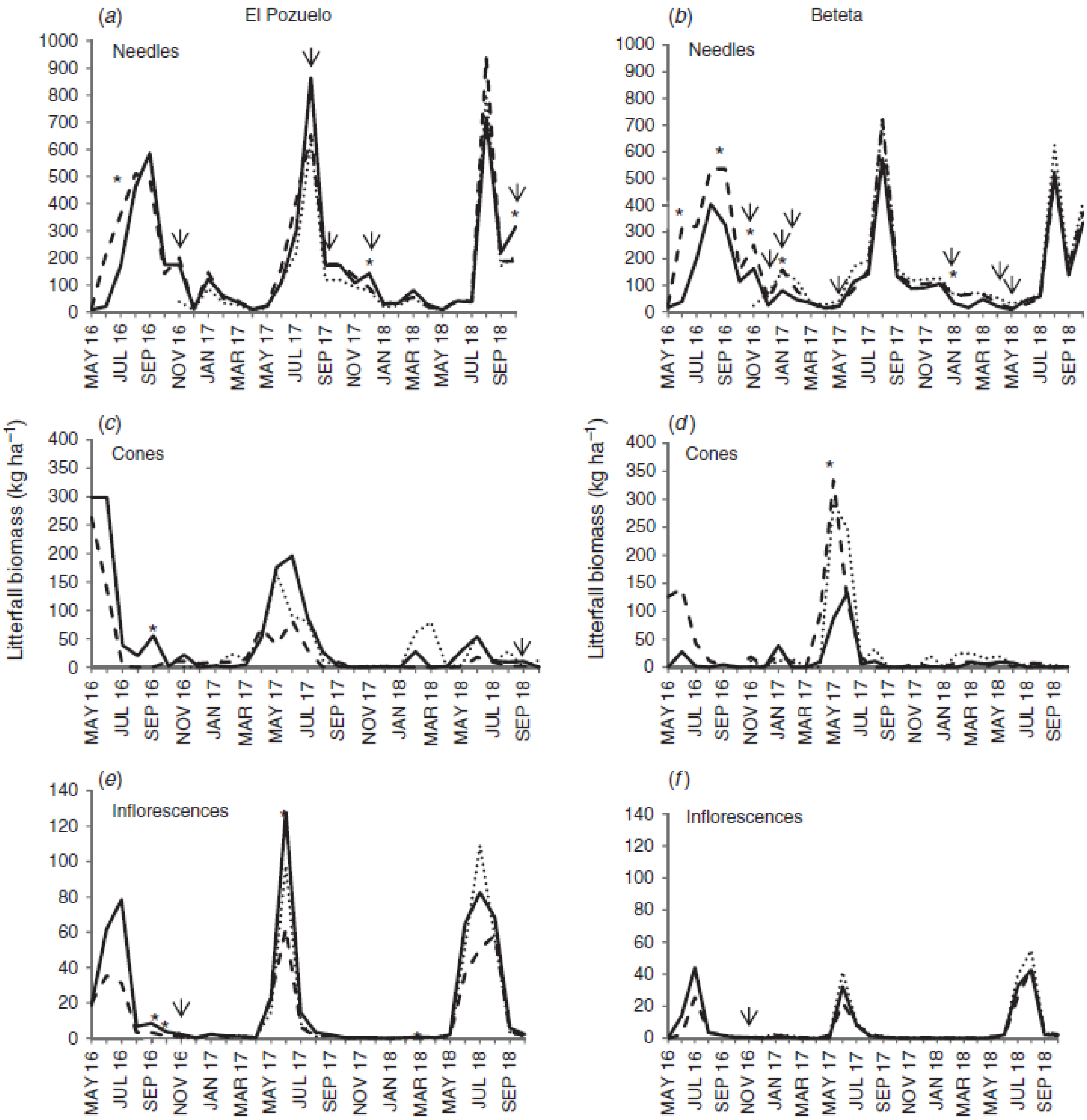

Fig. 3. Evolution of mean monthly collected litterfall (kg ha^{-1}) in (a) needles, (c) cones and (e) inflorescences at El Pozuelo and (b) needles, (d) cones and (f) inflorescences at Beteta in burned (spring and autumn burns) and non-burned plots, from May 2016 to October 2018. Non-burned plots in solid line, spring-burned plots in dashed line and autumn-burned plots in dotted line. (*) Represents significant differences in *t*-test of pairwise comparisons non-burned and spring-burned plots $p < 0.05$ and (arrow) represents significant differences in *t*-test of pairwise comparisons non-burned and autumn-burned plots $p < 0.05$.

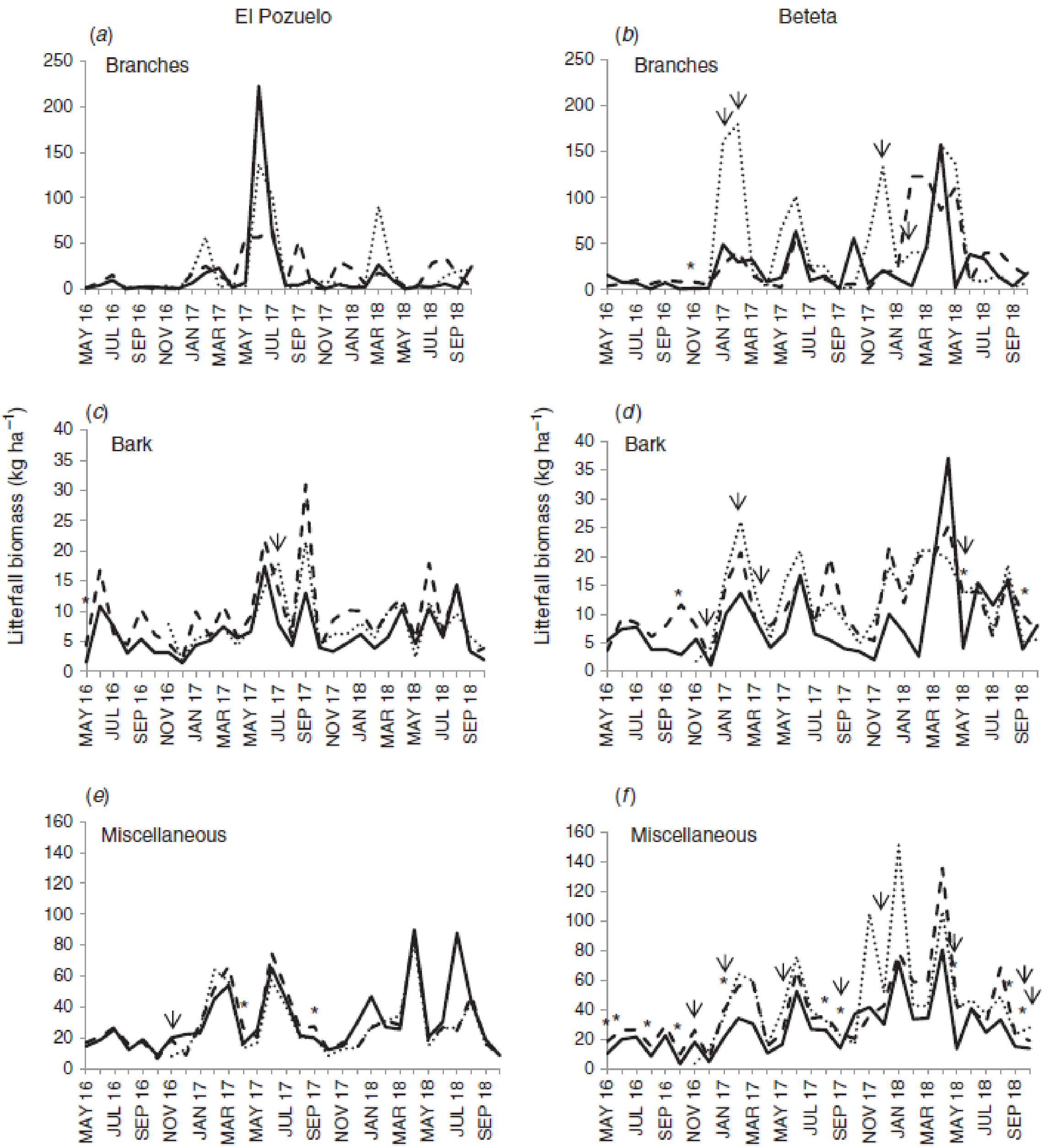

Fig. 4. Evolution of mean monthly collected litterfall (kg ha^{-1}) in (a) branches, (c) bark and (e) miscellaneous at El Pozuelo and (b) branches, (d) bark and (f) miscellaneous at Beteta in burned (spring and autumn burns) and non-burned plots, from May 2016 to October 2018. The solid line corresponds to non-burned plots, the dashed line to spring-burned plots and the dotted line to autumn-burned plots. (*) Represents significant differences in *t*-test of pairwise comparisons non-burned and spring-burned plots $p < 0.05$ and (arrow) represents significant differences in *t*-test of pairwise comparisons non-burned and autumn-burned plots $p < 0.05$.

Nutrient content

Carbon and macronutrients (N, P, K, Ca and Mg) were analysed in this study. The concentrations for each nutrient, fraction, period and treatment at El Pozuelo and Beteta are presented in Table S1 (available in Supplementary material 1) along with the mean and standard error in Table 6.

The mean concentration of C did not differ between El Pozuelo and Beteta. The only significant differences ($p = 0.0132$) detected were between *Pinus nigra* needles in non-burned and autumn-burned plots at El Pozuelo. Significant differences in N content of *Pinus nigra* needles were observed between non-burned and spring-burned plots in both stands, and differences between *Pinus pinaster* needles in non-burned and autumn-burned plots ($p = 0.0293$) were observed at El Pozuelo. In general, the P concentration was higher in needles from burned plots. As regards K, the only differences detected were between *Pinus nigra* needles in non-burned and autumn-burned plots ($p = 0.1140$) at El Pozuelo. Differences in Ca concentration between treatments (spring and autumn-burned plots) were detected in the cone and inflorescence fractions and also in Mg concentrations in *Pinus nigra* needles at Beteta.

Table 6. Mean ± standard error of nutrient content for C and macronutrients in fractions of litterfall biomass for the three treatments at El Pozuelo and Beteta. C, N, K, Ca, Mg (mg g^{-1}) and P (mg kg^{-1}).

NT	F	El Pozuelo			Beteta		
--	--	Non-burned	Spring-burned	Autumn-burned	Non-burned	Spring-burned	Autumn-burned
C	NEN	**513±1 a**	**515±1 ab**	**519±2 b**	523±2 a	523±2 a	521±2 a
	NEP	504±2 a	507±2 a	505±3 a	--	--	--
	CON	491±2 a	493±2 a	485±4 a	487±2 a	493±2 a	493±2 a
	INF	494±2 a	493±2 a	497±3 a	497±2 a	499±2 a	504±2 a
	BRA	494±1 a	493±1 a	492±2 a	502±2 a	501±2 a	498±2 a
	BAR	485±2 a	486±2 a	482±2 a	488±2 a	490±2 a	488±2 a
N	NEN	**4.0 ±0.2 a**	**4.6±0.2 b**	**4.4±0.2 ab**	**4.3±0.1 a**	**5.2±0.1 b**	**4.8±0.2 ab**
	NEP	**3.0±0.1 a**	**3.1±0.1 a**	**3.4±0.1 b**	--	--	--
	CON	2.8±0.3 a	2.5±0.3 a	2.5±0.4 a	2.3±0.3 a	2.8±0.3 a	2.3±0.3 a
	INF	7.5±0.5 a	7.1±0.5 a	7.0±0.6 a	7.7±0.3 a	8.2±0.3 a	8.2±0.4 a
	BRA	4.1±0.3 a	3.8±0.3 a	4.2±0.5 a	4.3±0.3 a	4.3±0.3 a	3.8±0.4 a
	BAR	**4.3±0.1 ab**	**4.1±0.1 b**	**4.6±0.1 a**	4.4±0.1 a	4.4±0.1 a	4.5±0.1 a
P	NEN	**175±17 a**	**248±17 b**	**260±20 b**	**317±31 ab**	**312±31 b**	**420±40 a**
	NEP	**141±30 ab**	**283±30 b**	**197±35 a**	--	--	--
	CON	187±41 a	193±41 a	117±48 a	161±42 a	236±42 a	125±55 a
	INF	**218±27 a**	**220±27 a**	**312±31 b**	301±33 a	368±33 a	373±43 a
	BRA	143±39 a	168±39 a	181±46 a	241±42 a	190±41 a	129±54 a
	BAR	157±18 a	162±18 a	163±21 a	222±40 a	209±40 a	161±52 a
K	NEN	**1.58±0.22 a**	**2.15±0.22 ab**	**2.53±0.27 b**	1.80±0.10 a	1.75±0.10 a	1.86±0.14 a
	NEP	0.98±0.36 a	1.33±0.36 a	1.43±0.44 a	--	--	--
	CON	1.54±0.29 a	1.37±0.29 a	1.29±0.36 a	2.61±0.88 a	2.71±0.88 a	1.51±1.21 a
	INF	0.88±0.21 a	0.87±0.21 a	0.97±0.26 a	1.77±0.52 a	2.25±0.52 a	1.05±0.74 a
	BRA	0.59±0.18 a	1.12±0.18 a	0.53±0.23 a	2.40±0.69 a	2.11±0.69 a	0.42±0.98 a
	BAR	0.63±0.21 a	0.89±0.21 a	0.48±0.25 a	1.97±0.74 a	1.46±0.74 a	0.50±1.05 a
Ca	NEN	7.99±0.70 a	8.19±0.70 a	8.34±0.96 a	5.22±0.40 a	4.56±0.40 a	5.37±0.49 a
	NEP	8.95±0.32 a	8.14±0.32 a	8.38±0.44 a	--	--	--
	CON	**1.78±0.93 a**	**5.23±0.93 b**	**0.54±1.28 a**	1.62±0.85 a	1.76±0.85 a	0.43±1.02 a
	INF	**4.95±0.39 a**	**4.87±0.39 a**	**1.00±0.54 b**	2.12±0.65 a	1.76±0.65 a	1.68±0.78 a
	BRA	8.22±0.63 a	7.51±0.63 a	6.42±0.86 a	4.33±0.87 a	3.20±0.87 a	4.83±1.06 a
	BAR	8.12±0.66 a	8.50±0.66 a	7.77±0.91 a	6.05±0.46 a	5.89±0.46 a	6.52±0.56 a
Mg	NEN	0.93±0.17 a	1.08±0.17 a	0.84±0.28 a	**1.41±0.19 ab**	**1.21±0.19 b**	**1.87±0.26 a**
	NEP	1.25±0.12 a	1.35±0.12 a	1.67±0.19 a	--	--	--
	CON	0.61±0.12 a	0.55±0.12 a	0.36±0.18 a	0.58±0.09 a	0.58±0.09 a	0.50±0.12 a
	INF	0.74±0.20 a	0.78±0.20 a	0.59±0.30 a	0.69±0.08 a	0.75±0.08 a	0.75±0.10 a
	BRA	0.60±0.15 a	0.61±0.15 a	0.39±0.23 a	0.64±0.07 a	0.59±0.07 a	0.51±0.10 a
	BAR	0.48±0.13 a	0.76±0.13 a	0.34±0.20 a	0.58±0.05 a	0.58±0.05 a	0.47±0.10 a

NT: nutrient; F: fraction; NEN: *Pinus nigra* needles; NEP: *Pinus pinaster* needles; CON: cones; INF: inflorescences; BRA: branches; BAR: bark. Different letters indicate significant differences ($p < 0.05$) with *t*-test. Comparisons were made for all pairwise combinations of treatments and sites for each row. Significant differences are indicated in bold.

Discussion

The PB treatments were of low-intensity (typical type of burning conducted in the *Pinus nigra* stands in the study area). Overall, scorch height (Table 2) was lower than the height of the first living branch (Table 1). No trees were completely scorched, except seedlings under the canopy and small dominated trees. The highest scorch marks on stems were mainly due to lichens present on the stem, which burned briefly, possibly explaining the occasional peaks in maximum temperature in the cambium area (Table 2). The mean bark thicknesses (Table 1) protected the cambium from temperatures higher than the critical threshold of 60 °C (Hare, 1965) and from a long flame residence time. Indeed, the temperature of the cambium was higher than 60 °C in only a small percentage of trees (below 18%) (Table 2). Thus, neither transport of photosynthate to the crown nor nutrient and water storage were expected to be disrupted, and burning scarcely affected photosynthetic production in the upper part of the crown, explaining the negligible amount of live needles collected in plots.

The previous short-term study of spring PB (Espinosa et al., 2018) revealed an immediate effect in both areas (the total litterfall accumulated was greater in the spring-burned plots than in the non-burned plots). At El Pozuelo, this difference was only 3% whereas at Beteta, there was 75% more in the spring-burned plots. In the second period, the difference decreased at El Pozuelo and by the end of the study, the results were reversed (i.e. litterfall was greater in the non-burned plots than in the spring-burned plots). In this context, the significant differences observed may be explained by the fact that the fire advanced the needle fall cycle in spring-burned plots during the first year, which meant that the quantity of needles in non-burned plots was greater in subsequent years. According to Nárovec and Nárovcová (2012), natural senescence of needles begins in the third to fifth year depending on the area. By comparison, at Beteta, the amount of litterfall was higher in the spring-burned plots for all period studied, although the differences decreased steadily over time (a difference of 75% first period, 21% second period and 17% remaining period). Thus, although the differences between burned and non-burned plots initially appeared to stabilise after the summer months in the first year (Espinosa et al., 2018), the impact of the treatment was maintained until the third year of study (at least in the pure stand).

After autumn PB at El Pozuelo, no effect was observed. In fact, higher amounts of biomass were collected in the non-burned plots than in the autumn-burned plots (23% more first period and 3% more second period). Conversely, the effect of autumn burns was detected at Beteta. Three to four months after burning, significant differences existed, more evident in months of maximum litterfall (Espinosa et al., 2018) and even in the second period from November to January. The latter results obtained in winter months may be explained by a late perturbation effect, which was manifested in the snowfall or storm season. Blanco et al. (2006) also reported this delayed effect of perturbation. Indeed, although the influence of physiological drought on litterfall is recognised (García-Plé et al., 1995; Roig et al., 2005), it seems that snowfall and storms (mainly during winter months) may negatively impact litterfall. The differences observed decreased from one year to the next (51% more first period and 47% second period) although the rate of decrease was lower than in spring.

Although no significant differences were observed (Table 5) between the two stands (which may be due to all treatments being considered together), the contrasting results following spring and autumn burns may be explained by the composition of the stands. Several authors have reported that mixed stands are stabler than pure stands (e.g. Felton et al., 2010), with a lower response after disturbance and a faster recovery process (Loreau et al., 2001; Jactel et al., 2009).

No previous studies comparing the effects of PB on litterfall biomass have been conducted in pine forests. Thus, the findings of the present study were compared with previous data on disturbance caused by different thinning regimes. Although no trend of increasing litter production following treatment was found by Roig et al. (2005), the effects were perceived 2-3 years later and disappeared 5 years after thinning. Similarly, Agren and Knecht (2001) suggested an increase in litterfall production 1 year after thinning, with values returning to normal in subsequent years. By contrast, data provided by Jiménez and Navarro (2016) indicate continuing differences in litterfall production after 8 years. This finding is further supported by Lado-Monserrat et al. (2016). These contrasting results highlight the fact that multiple factors condition the response of forest stands, making predictions difficult (Blanco et al., 2006) and highlighting the need for longer-term studies such as the present study.

Despite the disturbance to litterfall caused by burning treatments, the mean total litterfall for all treatments (3054 kg ha^{-1} year^{-1} at El Pozuelo and 3050 kg ha^{-1} year^{-1} at Beteta) is similar to those reported by ICP Forests (3337±841 kg ha^{-1} year^{-1}) (ICP Forests, 2011) after an 11-year-long study (2005-2014) of a *Pinus nigra* stand in Mora de Rubielos (Teruel), in which the altitude and latitude (1410 m asl and 40° 19′ 00′′ respectively) were similar to those of the present study area. Studies conducted in other areas of Europe, also focusing on *Pinus nigra*, reported values of 2500 kg ha^{-1} year^{-1} (Kavvadias et al., 2001). Roig et al. (2005) obtained values of 3284 kg ha^{-1} year^{-1} for *Pinus pinaster* in central Spain. Overall, the findings are within the range (3000-11000 kg ha^{-1} year^{-1}) reported for different types of forest worldwide (Zhang et al., 2014). However, the slight differences highlight the difficulty in comparing litterfall values due to the number of variables involved (Pausas et al., 1994; García-Plé et al., 1995; Berg and Meentemeyer, 2001).

Litterfall patterns often show a seasonal distribution. In the present study, the maximum levels of litterfall in summer months (June-September) are consistent with the results obtained by other authors in Mediterranean ecosystems (Roig et al., 2005; Bueis et al., 2017). Litterfall usually peaked in August, which is consistent with results reported by Bueis et al. (2017) and Martínez-Alonso et al. (2007). Minor discrepancies may be mainly due to the phenology of the species (Bueis et al. 2017) and the relationship between litterfall and months with physiological drought (García-Plé et al., 1995; Santa Regina and Tarazona, 2000; Roig et al., 2005; Blanco et al., 2006). Insect pests and other external variables may also be important. Minimum litterfall was recorded in winter months in both areas, although another minimum was observed at Beteta in spring. Blanco et al. (2006) also reported minimal production in winter and spring.

The most abundant fraction was needles, accounting for 58 and 57% of litterfall at El Pozuelo and Beteta, respectively. These results are closer to the values observed in a similar ecosystem, (62%) obtained at Mora de Rubielos (ICP Forests, 2011) and the values reported by Pausas et al. (1994) and Martínez-Alonso et al. (2007) for *Pinus sylvestris* (~ 50%). Slightly higher values were reported by Kurz et al. (2000) for *Pinus pinaster* (60-80%) and by Blanco et al. (2006) for *Pinus sylvestris* (50-70%). The trend for needles is similar to that observed for total litterfall biomass (Pausas, 1997; Blanco et al., 2006; Hansen et al., 2009).

The cone fraction accounted for 14% of total litterfall biomass at El Pozuelo and 10% at Beteta. A peak was almost always distinguishable in May and July, following pine flowering season (Blanco et al., 2006). Fluctuations in cone production were observed over the years considered, as also reported by Ordóñez et al. (2005) and Del Cerro Barja et al. (2009). Different responses between sites were found, although none were significant. At Beteta, there were more cones in the burned plots than in the non-burned plots, whereas at El Pozuelo, the opposite was found. Inflorescences account for a mean value of 2 and 5% of total litterfall for all periods and treatments at El Pozuelo and Beteta respectively. Pausas et al. (1994) pointed to a marked seasonality in shedding of inflorescences, with variation in peaks between May and August, which may also be due to meteorological conditions. The higher amount of inflorescences in control plots compared with burned plots at El Pozuelo and Beteta following spring burns is an effect that requires longer-term study. If fructification was similar among plots, inflorescence fall would not be a good index of fructification potential, probably because prescribed burns do not affect the female flowers mainly found in the apical fast-growing shoots at the top of the tree (Shmida et al., 2000). However, if the number of cones was higher in the control plots, we could assume that PB significantly affects the regeneration potential in *Pinus nigra* stands. Although it is difficult to determine the scope of fire effects on the cone and inflorescence fractions given the particular fructification characteristics of each species as well as other external factors (Del Cerro Barja et al., 2009), the findings are nonetheless important for forest managers, who will be able to plan PB in non-masting years or initial seedling recruitment to ensure periodic fructification and natural regeneration, thus contributing towards the persistence of black pine forests (Lucas-Borja and Vacchiano, 2018).

As regards branches, this fraction accounted for a mean value of 7% at El Pozuelo and 12% at Beteta with respect to the total litter for all years and treatments. In a *Pinus nigra* stand at Mora de Rubielos, branches accounted for a mean of 9% of total litter biomass (ICP Forests, 2011), this value being within the range recorded in the study plots. Certain peaks are due to snowfall in winter months and may also be associated with storms or episodes of high wind as well as with drier conditions (June-September). Self-pruning (Piqué and Domènech, 2018) and high crown base height are characteristics of *Pinus nigra* that allow the species to adapt to low-intensity burning and also to prevent the possible effects of thermal pruning. Bark production accounted for 3% of total litter production at El Pozuelo and 4% at Beteta for all treatments in all years. Blanco et al. (2006) obtained slightly higher values, between 9 and 11%. This fraction exhibited inter-annual variability with no clear trend (Pausas, 1997). As with branches, meteorological events may influence bark litter production (Blanco et al., 2006). Bark in maritime pine is laminated and the outer layers are exfoliated during combustion (Fernandes and Rigolot, 2007). This fact may explain the significant differences between zones, although this cannot be confirmed given the irregularity of the patterns. Certain peaks at the end of the summer may be due to stem growth (García-Plé et al., 1995). The miscellaneous fraction shows several peaks. Some winter peaks may be due to leaves of other deciduous species, mainly *Quercus* sp., although there is a low presence of this species. Peaks in February and May-July may correspond to seeds, but the amount of seed is very small. Miscellaneous peaks may also be associated with heavy rain and storms (Navarro et al., 2013).

Litterfall influences post-fire recovery processes, providing a pathway for nutrient release through leaching, mineralisation or immobilisation. Differences in nutrient concentrations between sites and fractions were recorded. These differences were found to reflect the particular characteristics at each site. Needles constitute an important nutrient sink through which most nutrients return to the forest soil in both areas, as also highlighted by Blanco et al. (2008).

The burning treatments had little effect on the concentration of C in the different fractions at both sites. The results provided by ICP Forests in a *Pinus nigra* stand (ICP Forests, 2011) reveal a mean concentration of carbon of 546 mg g^{-1} in the needle fraction, which is close to the mean concentrations obtained at El Pozuelo (499 mg g^{-1}) and Beteta (500 mg g^{-1}) for all periods and treatments. The findings for the branch fraction are similar.

A slight overall increase in N concentration in the needle fraction was detected in the burned plots in comparison with the non-burned plots; this was also reported by Tahmasbian et al. (2019) for suburban eucalyptus stands. However, other authors reported that foliar N concentration was not affected by prescribed fire (Landsberg et al., 1984). The needle fraction N values obtained at El Pozuelo and Beteta (4.49 and 5.06 mg g^{-1} mean value for all treatments and periods) were not within the range reported by ICP Forests (between 8.42 and 21.18 mg g^{-1}) (Rautio et al., 2016). This may be explained by the fact that the ICP Forests needle fraction included fresh needles, which can be assumed to contain more N. Thus, the N foliar values coincide with the N values in old needles, probably close the time of natural abscission, although nutrient concentrations in the needle fraction are also affected by factors such as nutrient availability in soil, climatic variables and site conditions (e.g. Kavvadias et al., 2001; Roig et al., 2005). However, N from needles is recycled back to the stem just before abscission, and the N concentration in fallen needles is therefore lower than in fresh needles (Bueis et al., 2017). Notable differences were observed in P concentrations between non-burned and burned plots in both stands. An increase in P concentration after the first year in all fractions was detected (notable at El Pozuelo). A similar increase in P in the foliar fraction after PB was also detected by Gillon et al. (1999) in Aleppo pine forests. However, Lado-Monserrat et al. (2016) observed little variation in P concentrations in response to different rates of tree removal. The P concentrations in the needle fraction were higher in the Beteta stand than in the El Pozuelo stand. Phosphorus is an essential nutrient for the generation of new tissues, so higher concentrations might be expected following a disturbance. The K concentration increased in spring-burned plots during the first period in all fractions at El Pozuelo and in the needle fraction at Beteta. A similar increase was also detected by Kuechler et al. (2006) after PB treatment in a long-term study. Foliar K fell beneath the range of natural concentrations in the area (1.53 and 1.79 mg kg^{-1} at El Pozuelo and Beteta respectively), 2.36 mg kg^{-1} at Mora de Rubielos in the needle fraction (ICP Forests, 2011). Despite the increase, Lado-Monserrat et al. (2016) observed little variation in K concentrations in a study of nutrient fluxes in response to different rates of tree removal.

Calcium was the most abundant element, particularly in needles, as they have the greatest Ca requirement (higher mean values at El Pozuelo than Beteta) although also in branches and bark. Calcium is abundant in the soils of the study area and was expected to be a non-limiting element. A general reduction in Mg concentration in litterfall after burning was observed, mainly as a consequence of competition with other cations (Lado-Monserrat et al., 2016; Bueis et al., 2018). This decrease in Mg was not detected in the needle fraction at El Pozuelo, where there was a 53% increase in the case of *Pinus nigra* and 45% for *Pinus pinaster* in first year, although it was found in the other fractions. At Beteta, however, the decrease in Mg was detected in the needle fraction.

Conclusions

Overall, the study findings (biomass and nutrient content) suggest that low-intensity PB in both spring and autumn has a limited medium-term influence (i.e. 3 years after treatment) on litterfall dynamics in a mixed *Pinus nigra*-*Pinus pinaster* stand and a pure *P. nigra* stand.

The season in which the PB was carried out (spring or autumn) affected litterfall biomass, mainly when certain meteorological phenomena (snowfall or storms) occurred after the treatment. Stand composition (mixed or pure) and characteristics may also have influenced the results. As shown in a previous study with the same experimental design (Espinosa et al., 2018), the findings indicated a short-term effect on litterfall biomass following spring PB, particularly in the pure stand. The effect of the treatment decreased gradually over time. The effects of autumn PB were scarcely noticeable in the mixed stand and were delayed in the pure stand. The rate of recovery was faster after spring PB than after autumn PB in the pure stand.

Concentrations of carbon and macronutrients were not significantly modified by the burn treatment. Differences in nutrient concentrations between sites and fractions were detected and may be due to characteristics of the sites and stands. The concentrations of N, P and K increased in the needle fractions after burning, these nutrients being key to stand recovery following disturbance. It was found that the values obtained for N were similar to old needles, so no greater damage as regards crowns is expected. Calcium was not found to be a limiting factor. No competition between Mg and other cations following fire was detected, at least at El Pozuelo.

The litterfall data obtained during the study may provide information for present and future projections of climate change as needle fall is a sensitive indicator of this phenomenon.

Information about the influence of silvicultural treatments on litterfall biomass, litter dynamics and nutrient cycling processes remains limited, particularly in Mediterranean forests. Such knowledge is key to fire management, allowing us to determine the longevity of fuel reduction treatments and providing parameters to design, test and implement more effective prescriptions.

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Supplementary material 1

Table S1. Concentrations of C, N, K, Ca, Mg (mg g^{-1}) and P (mg kg^{-1}) in fractions of litterfall biomass for the three treatments at El Pozuelo and Beteta.

NT	F	El Pozuelo					Beteta				
--	--	May 2016 – April 017		May 2017 – April 2018			May 2016 – April 2017		May 2017 – April 2018		
--	--	Non-burned	Spring-burned	Non-burned	Spring-burned	Autumn-burned	Non-burned	Spring-burned	Non-burned	Spring-burned	Autumn-burned
C	NEN	510.67	512.43	514.33	518.73	518.63	522.23	518.97	524.70	526.67	521.37
	NEP	500.73	502.07	507.40	512.13	505.07	--	--	--	--	--
	CON	492.93	487.9	489.47	497.37	484.80	487.10	492.23	489.50	493.20	493.13
	INF	493.00	489.80	494.50	4950.20	496.57	493.63	495.27	500.23	502.20	504.17
	BRA	491.20	493.30	496.20	492.60	491.63	506.30	501.27	499.47	500.50	498.40
	BAR	488.83	487.47	481.63	484.27	482.17	488.67	490.77	487.50	489.13	488.20
N	NEN	3.93	4.57	4.07	4.64	4.43	4.19	5.22	4.51	5.19	4.87
	NEP	2.55	2.87	3.30	3.37	3.45	--	--	--	--	--
	CON	2.7	1.9	2.9	3.2	2.5	2.50	2.85	2.29	2.89	2.32
	INF	6.93	7.15	8.26	7.14	7.01	7.10	7.60	8.38	8.99	8.21
	BRA	4.18	3.93	4.14	3.79	4.24	4.26	4.02	4.41	4.60	3.80
	BAR	3.95	3.67	4.80	4.55	4.59	4.00	3.81	4.80	5.01	4.52
P	NEN	115.23	183.45	234.19	313.14	260.24	279.66	297.92	354.32	326.31	420.09
	NEP	129.65	249.01	152.78	317.32	197.04	--	--	--	--	--
	CON	157.66	186.41	217.99	198.99	116.99	330.62	329.25	92.57	143.56	124.92
	INF	115.93	154.55	319.19	284.54	312.17	244.62	362.47	358.06	374.09	372.99
	BRA	145.80	205.95	140.41	129.51	181.55	326.76	233.91	155.57	145.38	128.87
	BAR	132.29	173.46	182.34	150.20	162.77	299.18	257.93	143.94	159.86	160.91
K	NEN	0.77	1.68	2.39	2.61	2.53	1.78	1.86	1.81	1.63	1.86
	NEP	0.80	1.58	1.15	1.07	1.42	--	--	--	--	--
	CON	1.04	1.16	2.03	1.57	1.29	4.84	3.67	1.56	1.73	1.50
	INF	0.85	0.98	0.90	0.76	0.97	2.25	3.51	1.28	0.98	1.04
	BRA	0.72	1.74	0.45	0.48	0.53	4.28	3.69	0.50	0.52	0.42
	BAR	0.72	1.35	0.53	0.42	0.47	3.45	2.44	0.48	0.47	0.50
Ca	NEN	7.65	8.01	8.31	8.31	8.34	5.21	3.96	5.22	5.14	5.36
	NEP	10.07	7.63	7.82	8.63	8.37	--	--	--	--	--
	CON	2.99	9.87	0.56	0.58	0.54	4.13	3.08	0.42	0.43	0.43
	INF	7.94	7.52	1.95	2.22	1.99	2.60	3.85	1.64	1.79	1.68
	BRA	9.10	7.78	7.32	7.22	6.41	3.31	1.52	5.34	4.86	4.83
	BAR	8.53	8.52	7.69	8.48	7.76	5.30	4.94	6.78	6.82	6.51
Mg	NEN	0.79	1.21	1.06	0.93	0.83	0.96	0.95	1.84	1.47	1.87
	NEP	0.64	0.93	1.86	1.75	1.66	--	--	--	--	--
	CON	0.78	0.66	0.43	0.43	0.35	0.84	0.71	0.41	0.43	0.49
	INF	0.89	0.97	0.58	0.57	0.58	0.61	0.80	0.76	0.69	0.75
	BRA	0.73	0.85	0.46	0.36	0.38	0.77	0.70	0.49	0.48	0.51
	BAR	0.59	1.18	0.35	0.32	0.33	0.67	0.68	0.45	0.47	0.47

NT: nutrient; F: fraction; NEN: *Pinus nigra* needles; NEP: *Pinus pinaster* needles; CON: cones; INF: inflorescences; BRA: branches; BAR: bark.

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Chapter 2

Effect of prescribed burning on litterfall biomass

Subchapter 2.3

Use of Bayesian modeling to determine the effects of meteorological conditions, prescribed burn season, and tree characteristics on litterfall of *Pinus nigra* and *Pinus pinaster* stands

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Abstract

Research Highlights: Litterfall biomass after prescribed burning (PB) is significantly influenced by meteorological variables, stand characteristics, and the fire prescription. Some of the fire-adaptive traits of the species under study (*Pinus nigra* and *Pinus pinaster*) mitigate the effects of PB on litterfall biomass. The Bayesian approach, tested here for the first time, was shown to be useful for analyzing the complex combination of variables influencing the effect of PB on litterfall. *Background and Objectives*: The aims of the study focused on explaining the influence of meteorological conditions after PB on litterfall biomass, to explore the potential influence of stand characteristic and tree traits that influence fire protection, and to assess the influence of fire prescription and fire behavior. *Materials and Methods*: An experimental factorial design including three treatments (control, spring, and autumn burning), each with three replicates, was established at two experimental sites (n = 18; 50 × 50 m^2 plots). The methodology of the International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) was applied and a Bayesian approach was used to construct a generalized linear mixed model. *Results*: Litterfall was mainly affected by the meteorological variables and also by the type of stand and the treatment. The effects of minimum bark thickness and the height to the first live branch were random. The maximum scorch height was not high enough to affect the litterfall. Time during which the temperature exceeded 60 °C (cambium and bark) did not have an important effect. *Conclusions*: Our findings demonstrated that meteorological conditions were the most significant variables affecting litterfall biomass, with snowy and stormy days having important effects. Significant effects of stand characteristics (mixed and pure stand) and fire prescription regime (spring and autumn PB) were shown. The trees were completely protected by a combination of low-intensity PB and fire-adaptive tree traits, which prevent direct and indirect effects on litterfall. Identification of important variables can help to improve PB and reduce the vulnerability of stands managed by this method.

Keywords: *Pinus nigra*; *Pinus pinaster*; Cuenca Mountains; integrated nested Laplace approximation (INLA); vulnerability.

Introduction

The projected changes in climate are likely to affect fire regime and increase the fire risk in some Mediterranean countries (including Spain) (Moriondo et al., 2006). Fuel reduction treatments such as prescribed burning (PB) reduce surface continuity and ladder fuels, increase the height of live crown, decrease crown density, retain large trees of fire-resistant species (Agee and Skinner, 2005), improve success of fire control (Fernandes and Botelho, 2003), and may even be effective in creating fire-resilient stands. Nevertheless, fire often has complex effects on different parts of individual trees, microsite conditions, microclimate, and regeneration (Harmon, 1984). In this study, special attention is given to the effect of PB on tree crowns through the subsequent litterfall.

The relationship between litterfall and decomposition determines the depth of the forest floor layer, which acts as a nutrient reservoir in forest ecosystems (Lado-Monserrat et al.,

2016) and plays a key role in protecting the soil against erosion and improving water infiltration (Roig et al., 2005). Litter is also an important source of carbon (C) input to the soil (Sayer, 2006). In addition, litterfall biomass has been proposed as a good indicator of stand productivity (Kunhamu et al., 2009) and it may provide information about the effects of climate change on forests (Hansen et al., 2009). Litterfall patterns undergo inter- and intra-annual variation (e.g. Pausas et al., 1994; Kurz et al., 2000) including spatial variation. Overall, the amount and quality of litterfall depend on climate variables, geographic factors, stand and tree individual characteristics, disturbance of forest management practices, and to a lesser extent, other external variables.

The relationship between litterfall and the duration of drought conditions has been demonstrated in several studies (Wieder and Wright, 1995; Santa-Regina and Tarazona, 2000; Roig et al., 2005; Blanco et al., 2006). In addition, drought conditions may have stronger impacts on Mediterranean ecosystems where rainfall is irregularly distributed (Gracia et al., 2001). Overall, litterfall production has been reported to be related to latitude, mainly involving temperature and water availability (Pausas et al., 1994). Indeed, (Zhang et al., 2014) defined a wide range of 3000-11000 kg ha^{-1} $year^{-1}$ of litterfall in different forest types studied worldwide (over a very wide latitudinal range).

Much of the variation in litterfall response to burning can be attributed to stand characteristics (age, structure, species, etc.) (García-Plé et al., 1995; Blanco et al., 2006) and to the variable heat sensitivity of different tissues and species (Catry et al., 2010). *Pinus nigra* Arn. ssp. *salzmannii* (Spanish black pine) and *Pinus pinaster* Ait. (maritime pine), the species considered in this study, have evolutionary adaptations that help them to persist in fire-prone environments (Fernandes and Rigolot, 2007; Fulé et al., 2008). Thus, *Pinus nigra* has a thick bark, a high crown base height (Pausas et al., 2009), and a self-pruning strategy (Tapias et al., 2001; Piqué and Domènech, 2018). Bark is considered as the main protective tissue of the cambium (Lawes et al., 2011) and is necessary for photosynthate transport to the crown and nutrient and water storage (Romero, 2007). In addition, low-intensity PB will burn the less productive part of the crown in larger trees, but scarcely affect the photosynthetic production in the upper part of the crown (Helms, 1970; Wyant et al., 1986). Maritime pine also has thick bark and high crown base height. In addition, the large buds (shielded by scales and by long needles) may enable very high levels of defoliation, and the storage of seed in serotinous cones facilitates reproduction after fire (Fernandes and Rigolot, 2007). In the study area, Spanish black pine often appears mixed with maritime pines. Species diversity has been suggested to promote ecosystem resilience in response to changes through diversity (Elmqvist et al., 2003).

The use of prescribed fire can influence the patterns and regimes of the litterfall biomass (Espinosa et al., 2018). Indeed, the immediate impact of PB on litterfall and the subsequent recovery of fuel load and structure are considered critical for evaluating the treatment (Mirra et al., 2017; Espinosa et al., 2019). To apply PB safely and minimize the impacts on the ecosystem, weather and fuel related variables must be within the established threshold limits. The burning season is the most controllable feature of the PB regime from a management point of view. However, few studies have investigated the effect of burning season on litterfall. Low-intensity PB may have scarce effects on tree crowns. However, if the PB is very intense, it may cause an increase in litterfall, thereby increasing the risk of a future fire.

The variable scorch height is frequently measured in the field and may be associated with total or partial cambial death or stress, caused by temperatures above the critical threshold (Hare, 1965) or long flame residence times (Seifert et al., 2017). This could damage phloem and xylem tissues, thus disrupting translocation of photosynthates to the roots (Rozas et al., 2011). This effect becomes more important as tree height increases because living tissues are less protected by the bark than they are at the base (Madrigal et al., 2019).

In a previous study (Espinosa et al., 2018), we evaluated the short-term effect of PB in a mixed stand of *Pinus nigra* and *Pinus pinaster* and in a pure stand of *Pinus nigra*. The main aim of the PB conducted was to reduce the accumulated fuel far beyond 50%, which was achieved. The findings showed an increase in the amount of litterfall biomass immediately after PB in spring, especially in the pure stand. The impact of the disturbance was clearly mitigated in the mixed stand. The effect of the treatment decreased over time. In a second study (Espinosa et al., 2020a), the effects on litterfall of autumn prescribed burning were integrated and results of all treatments in the medium-term were analyzed; in addition, information on the nutrient content of litterfall was included. In this regard, the effects (increment in the amount of litterfall) of PB in autumn were scarcely noticeable in the mixed stand and the effects were delayed in the pure stand. The complexity of the litterfall process requires the application of mathematical models to simplify and understand it. The main approach used in the aforementioned study was frequentist inference. However, some of the assumptions of *p*-values and maximum likelihood estimators are not appropriate for the complex functioning of the litterfall process. A new approach through a Bayesian statistic could solve this problem because Bayesian analysis does not rely solely on a point estimate of the parameter, but rather an entire distribution of possible values. In addition, the Bayesian model may perform better for small sample sizes (also, Bayesian approaches can incorporate more parameters than data) (Clark, 2005). In this study, this is a key point as litterfall dynamics are strongly dependent on geoclimatic and species characteristics and usually the sample size is small to apply a frequentist approach.

From the prior knowledge of post-PB litterfall dynamics, we hypothesized that litterfall may be affected by three broad types of variables: meteorological conditions; stand characteristics (mixed and pure stand) and tree traits that influence fire protection; and fire prescription and behavior. The Bayesian approach involved the analysis of the prior information (Espinosa et al., 2018), data from the other studies cited (e.g. Roig et al., 2005; Blanco et al., 2006) and consideration of the accumulated empirical experience.

The aims of the present study were as follows: (i) to ascertain the influence of meteorological conditions after PB on litterfall biomass and to explore the most important variables describing the process; (ii) to explore the potential influence of stand characteristic (mixed and pure stands) and tree traits that influence fire protection (bark thickness and crown base height); and (iii) to assess the influence of fire prescription (season) on litterfall biomass dynamics and fire behavior through easily measurable field variables.

Materials and Methods

Study Site

Two different sites in the northwest of the Cuenca Mountains (Iberian System) were selected for this study: a mixed stand (MS) of *Pinus nigra* and *P. pinaster* in El Pozuelo and a pure stand (PS) of *P. nigra* in Beteta. According to the data for the last 21 years recorded at the nearest weather station (Cañizares: 940 m asl, provided by the Spanish Meteorological Agency; AEMET, 2018), the mean annual temperature was 12.1 °C (13.2 °C for the period 2016 to 2018) and the average precipitation was 717 mm (599 mm for the period 2016 to 2018). The main characteristics of the two sites were described in (Espinosa et al., 2018; Espinosa et al., 2020a) and a brief summary is shown in Table 1.

Table 1. Main characteristics of El Pozuelo and Beteta (mean and standard deviation).

--	El Pozuelo	Beteta
Coordinates—Longitude	40° 33′3 6′′ N	40° 33′ 06′′ N
Coordinates—Latitude	002° 15′ 56′′ W	002° 06′ 32′′ W
Main species	*Pinus nigra* (89±11%) *Pinus pinaster* (11±11%)	*Pinus nigra* (100%)
pH of topsoil *	7.3 (clay texture)	6.9 (loamy-sand texture)
Elevation	1015±5 m asl	1232±7 m asl
Slope	3-8%	3-10%
Stand density	627±238 trees ha^{-1}	1286±339 trees ha^{-1}
Stand basal area	25.4±9.7 m^2 ha^{-1}	36.6±10.7 m^2 ha^{-1}
Dominant tree height	18.6 ± 0.8 m	17.0 ±1.6 m
Tree height	12.2±2.0 m	13.2±2.7 m
First live branch	6.4±1.8 m	8.2±2.5 m
Diameter at breast height (DBH)	19.8±2.6 cm	18.8±4.1 cm
Bark thickness	1.7±0.3 cm	1.7±0.4 cm
Main understory vegetation	*Cistus laurifolius* L.; *Genista scorpius* Sibth. & Sm. ex Boiss; *Prunus spinosa* L.; *Arrhenatherum bulbosum* (Willd.) C.Presl; *Bupleurum rigidum* L.	*Genista scorpius* Sibth. & Sm. ex Boiss.; *Rosa canina* L.
Fuel Model **	TU1	TU1

(*) Data from Plaza-Álvarez et al. (2017). (**) According to the classification established by Scott and Burgan (2005).

Experimental Design

A total of nine square plots of 50 m × 50 m were chosen at each site by applying a randomized complete block design (n = 18). For data collection, subplots of 30 m × 30 m were established to prevent the edge effect (Fig. 1). A total of three treatments were established per experimental site (NB: no burning, SB: spring burning, and AB: autumn burning), with three replicates per block.

All trees in the plots were identified, and the following parameters were measured in each tree: total height (Ht, m), height to the first live branch (H1lb, m), diameter at heights of 0.6

and 1.3 m from the base (D60 and D130, cm), and maximum and minimum bark thickness at 0.6 m from the base (THMxB60, THMnB60 cm). The main stand characteristics of no burning, spring burning, and autumn burning plots in mixed and pure stands are shown in Table 2.

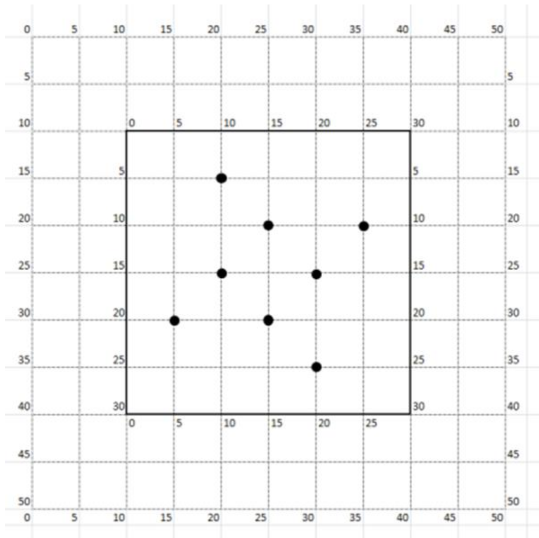

Fig. 1. Example of plot design (distances in m) and standard distribution of collectors in the sampling plots (Espinosa et al., 2018).

Table 2. Characteristics of no burning (NB), spring burning (SB), and autumn burning (AB) plots in mixed and pure stands (mean and standard deviation).

S	PT	Dt	Pn	Pp	Ht	H1lb	D60	D130	THMxB60	THMnB60
--	--	Trees ha^{-1}	%	%	m	m	cm	cm	cm	cm
Mixed *	NB	563	93	7	11.5	5.1	19.7	18.7	1.82	0.99
		(74)	(4)	(4)	(3.2)	(1.9)	(3.8)	(3.7)	(0.58)	(0.37)
Mixed *	SB	881	89	11	12.3	7.1	21.3	19.8	2.49	1.34
		(227)	(8)	(8)	(0.8)	(0.6)	(1.0)	(1.0)	(0.13)	(0.18)
Mixed **	AB	437	85	15	12.9	6.9	22.6	21.0	2.43	1.17
		(110)	(20)	(20)	(2.1)	(2.4)	(2.9)	(2.8)	(0.21)	(0.19)
Pure *	NB	1456	100	0	10.1	6.1	15.1	13.8	1.64	0.92
		(507)	(0)	(0)	(3.2)	(3.3)	(1.7)	(1.6)	(0.10)	(0.09)
Pure *	SB	1215	100	0	12.7	7.5	20.0	18.2	2.46	1.23
		(209)	(0)	(0)	(0.8)	(1.3)	(1.9)	(2.0)	(0.18)	(0.20)
Pure **	AB	1274	100	0	14.1	9.4	21.9	20.0	2.71	1.45
		(335)	(0)	(0)	(2.2)	(1.6)	(4.9)	(4.4)	(0.24)	(0.23)

S: type of stand; PT: plot treatment; Dt: total density; Pn: percentage of *Pinus nigra*; Pp: percentage of *Pinus pinaster*; Ht: total height; H1lb: height to the first live branch; D60: diameter at 60 cm from the base; D130: diameter at 130 cm from the base; THMxB60: maximum bark thickness at 60 cm from the base; THMnB60: minimum bark thickness at 60 cm from the base. (*) Data from Espinosa et al. (2018). (**) Data from Espinosa et al. (2020a).

Prescribed Burning

Prescribed burning was conducted by the Castilla-La Mancha Regional Forest Service in order to reduce the vertical and horizontal continuity of fuel. The spring PB was carried out in May 2016, while the autumn PB was carried out in November 2016. The strip ignition technique was applied at a distance of 1-2 m downhill and with a head wind. This ignition pattern favors rapid advancement of the front and a shorter residence time of the fire in the soil, thus preventing overheating, excessive consumption of organic matter, and high temperatures being reached (Vega, 2001). This technique is the most widely used in the study area to produce low- medium-intensity fire. The efficacy of the burnings was moderate-high (reduction of 59-77% of litter biomass). The main goal of the treatment was therefore successfully achieved (Espinosa et al., 2018). Precipitation (Ortrat, S.L.; KW 3-02), wind speed (Casella; 178031C-3), temperature, and relative humidity (Geonica; STH-5031) were recorded every 10 min at a meteorological station adjacent to the study plots. During the burning, the temperature of the bark (air-surface) and the cambial region (inner bark) of 15 randomly selected trees per plot was monitored at a height of 0.6 m with type K-1 mm-diameter inconel-sheathed thermocouples (0.3 s of response time). The thermocouples were connected to dataloggers (DT-USB TCDirect®), which recorded the data every second. Variables concerning temperature reached and residence time of flame in bark surface and cambium area were calculated. The threshold value of 60 °C in the cambium area corresponds to the commonly accepted lethal temperature for tree cells (Hare, 1965), although long duration of temperatures of 40-50 °C may cause stress in trees (Lawes et al., 2011). Maximum and minimum scorch heights were measured after burning. Data collected during and after PB are shown in Table 3.

Litterfall Biomass

Litterfall was collected in fiber glass collectors installed in each plot (eight per plot) after the PB (Fig. 1). In order to guarantee the quality and quantity of the samples obtained, the litterfall collection system was designed following the recommendations and parameters outlined in the manual published by the United Nations Economic Commission for Europe (UNECE) under the project entitled "International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests" (ICP Forests, Level II plots) (Ukonmaanaho et al., 2016). The spatial distribution of the design covered the entire working area in the 30 m × 30 m plot to ensure the representativeness of the sampling. The specific characteristics of the collectors are detailed in Espinosa et al. (2018). The litterfall was collected monthly to prevent decomposition of the materials or the chemical leachate. All samples were transported to the laboratory on the same day that they were collected and were oven-dried at 65 °C to constant weight, before being separated into different fractions: needles, cones, inflorescences, branches, bark, and miscellaneous material. The study was carried out between May 2016 and April 2018 in SB plots and between November 2016 and October 2018 in AB plots.

Table 3. Variables measured during and after prescribed burning in mixed and pure stands (mean and standard deviation).

--	--	Mixed	Mixed	Pure	Pure
--	--	SB	AB	SB	AB
T	°C	21.5 (1.2)	11.9 (0.4)	20.4 (1.5)	12.0 (0.9)
RH	%	47.7 (5.3)	67.0 (1.3)	32.7 (2.3)	43.5 (0.8)
WS	m s^{-1}	0.8 (0.6)	0.3 (0.3)	0.8 (0.1)	0.1 (0.1)
RS	m min^{-1}	0.6 (0.1)	0.6 (0.2)	0.8 (0.1)	0.7 (0.0)
HSmMx	cm	70 (41)	34 (11)	160 (16)	59 (26)
HSmMn	cm	16 (9)	5 (5)	40 (18)	4 (3)
TmMxB	°C	209 (200)	126 (118)	279 (208)	94 (108)
TmMxC	°C	51 (80)	49 (11)	41 (45)	40 (13)
TMxB	°C	787 (--)	605 (--)	755 (--)	702 (--)
TMxC	°C	521 (--)	77 (--)	315 (--)	75 (--)
TRMxB40	%	93 (7)	93 (0)	100 (0)	91 (11)
TRMxC40	%	33 (31)	76 (4)	31 (10)	45 (9)
TRMxB60	%	82 (89	60 (8)	96 (8)	57 (26)
TRMxC60	%	16 (15)	18 (10)	11 (4)	5 (8)
tB40B	s	562 (1057)	179 (163)	617 (550)	301 (657)
tC40C	s	284 (212)	47 (128)	666 (545)	7 (15)
tB60B	s	404 (1121)	134 (91)	369 (524)	174 (268)
tC60C	s	170 (120)	6 (12)	251 (206)	2 (0)

SB: spring burning; AB: autumn burning; T: air temperature; RH: relative humidity; WS: wind speed; RS: fire rate of spread; HSmMx: mean maximum scorch height; HSmMn: mean minimum scorch height; TmMxB: mean maximum bark temperature; TmMxC: mean maximum cambium temperature; TMxB: absolute maximum bark temperature; TMxC: absolute maximum cambium temperature; TRMxB40: percentage of trees in which the maximum temperature in the bark surface (air temperature) was higher than 40 °C; TRMxC40: percentage of trees in which the maximum temperature in the cambium was higher than 40 °C; TRMxB60: percentage of trees in which the maximum temperature in the bark surface (air temperature) was higher than 60 °C; TRMxC60: percentage of trees in which the maximum temperature in the cambium was higher than 60 °C; tB40C: time during which temperature was higher than 40 °C in bark; tC40C: time during which temperature was higher than 40 °C in cambium; tB60C: time during which temperature was higher than 60 °C in bark; tC60C: time during which temperature was higher than 60 °C in cambium. For calculating tB40B, tC40C, tB60B, and tC60C, only the trees that exceeded 40 and 60 °C, respectively, were selected.

Data Analysis

The Bayesian methodology specifies a probability model that applies prior knowledge about a research parameter, consequently, it is conditioning to the model to perform the adjustment of assumptions. In ecology and environmental analysis, the response variable is commonly measured more than once for each subject across levels of one or more factors, referred to as repeated measures. These types of data cannot be analyzed by linear regression because the residuals for each variable are correlated. The linear mixed effects model (LMM) is commonly used to analyze repeated measures data. This model combines both fixed and random effects on a linear scale. As the subjects are expected to vary independently in the random effect, correlations between observations within a subject are allowed. Generalized linear mixed models (GLMM) are popular because of their ability to directly recognize multiple levels of dependency and model different types of data. Likelihood-based inference may be unreliable, particularly for small sample sizes, with variance components being particularly difficult to estimate.

In the present study, a generalized linear mixed model approach was used with the aim of establishing the relationship between litterfall and the different variables considered.

The mixed model included 18 plots, each with 24 successive litterfall seizures (μ_{jk}). The month (k) indicated the time since the PB was carried out (adding the month of the year would only be an unnecessary autocorrelation). There was a lag in the prescribed burnings (spring-autumn) as they were not conducted in the same month. Considering the meteorological variables enabled indirect inclusion of the phenological component. Furthermore, the model considers the order of data collection (i.e. it is a "repeated measures models" application).

We assumed the seizures followed a conditionally independent Gaussian likelihood function:

$$y_{i,j} \sim \text{Gaussian}\left(\mu_{jk}\right), \; i = 1,\ldots,18; \; k = 1,\ldots,24 \tag{1}$$

Thus, for the log(μ_{ij}), a linear model was specified by including the different covariates, x_j:

$$\log\left(\mu_{ij}\right) = \sum_{m=1}^{M} \alpha_m x_j + u_j + v_j \tag{2}$$

$\alpha_m \sim \text{Normal}(0, \tau_\alpha)$, $\tau_\alpha \sim$ known
$u_j \sim \text{Normal}(0, \tau_u)$, $\tau_u \sim \text{Gamma}(a_u, b_u)$
$v_j \sim \text{Normal}(0, \tau_v)$, $\tau_v \sim \text{Gamma}(a_v, b_v)$

Where:

α_m indicates a set of "fixed" effects for the relevant covariates
u_j is a "random" stand effect
v_j is a "random" effect treatment

The statistical inference was carried out by integrated nested Laplace approximation (INLA) implemented in the R-INLA package (Cosandey-Godin et al., 2014). INLA uses Bayesian inference on latent Gaussian models by combining Laplace approximations and numerical integration in a very effective manner (Rue et al., 2009).

The input variables (Table 4) were divided into three groups: variables related to meteorological conditions; stand characteristics (mixed and pure stand) and traits that influence tree protection against fire damage; and fire prescription and behavior. The climatic variables considered (TmMx, TmMm and Pm) were obtained one month before the litterfall samples were collected (Roig et al., 2005). The effect of these variables is not expected to be immediate and the stand response time may be delayed.

Table 4. Variables (fixed effects) combined in the models and variables selected by the best fit model.

--	Abbreviation	Variable	Unit	Variables Selected
Meteorological variables	TmMx	Mean maximum temperature	°C	*
	TmMn	Mean minimum temperature	°C	-
	Pm	Monthly precipitation	mm	*
	SwD	Days with snow	days	*
	StD	Days with storm	days	*
	WS	Wind speed	m s^{-1}	-
Stand and tree trait variables	THMxB60	Maximum bark thickness at 60 cm from the base	cm	-
	THMnB60	Minimum bark thickness at 60 cm from the base	cm	*
	H1lb	Height to the first live branch	m	*
Fire prescription and behavior variables	HSmMx	Mean maximum scorch height	cm	*
	HSmMn	Mean minimum scorch height	cm	-
	TmMxB	Mean maximum bark temperature	°C	-
	TmMxC	Mean maximum cambium temperature	°C	-
	tB40C	Time during which temperature was higher than 40 °C in bark-surface	s	-
	tC40C	Time during which temperature was higher than 40 °C in cambium	s	-
	tB60C	Time during which temperature was higher than 60 °C in bark-surface	s	*
	tC60C	Time during which temperature was higher than 60 °C in cambium	s	*

(-) Indicates variables not selected. (*) Indicates variables selected.

Cross-validation is commonly used to estimate the out-of-sample prediction error (Geisser and Eddy, 1979; Vehtari and Lampinen, 2002). However, researchers require alternative measures such as cross-validation, which involves repeated model fitting and can run into trouble when data are scarce (Gelman and Shalizi, 2013). The index most commonly used for purposes of comparison (e.g. to evaluate which model best fits the data) is the deviance information criterion (DIC) (Spiegelhalter et al., 2003; Van Der Linde, 2005). Similarly to the Akaike information criterion (AIC), the DIC has two components: a term that measures goodness of fit and a penalty term for increasing model complexity. The Watanabe-Akaike information criterion (WAIC) (Watanabe, 2010) has recently been suggested as a suitable alternative for estimating the out-of-sample expectation in a fully Bayesian approach. This method begins with the computed log pointwise posterior predictive density and then adds a correction for the effective number of parameters to adjust for overfitting (Gelman and Shalizi, 2013). The Watanabe-Akaike information criterion works on the predictive probability density of detected variables rather than on the model parameters and it can therefore be applied in statistical models with non-identifiable parameterization (Li et al., 2016). We also used the conditional predictive ordinate (CPO) (Pettit, 1990), which is based on leave-one-out cross-validation to evaluate model assessment. CPO estimates the probability of detecting a value after the others have already been observed. The mean logarithmic score (LCPO) was calculated as a measure of the predictive quality of the model (Gneiting and Raftery, 2007; Roos and Held, 2011). High LCPO values suggest possible outliers, high-leverage, and influential observations. Finally, we used an area under operating curve score (AUC) approach to calculate the predictive accuracy of each method by comparing the validation data with the predicted presence value. The AUC score, a commonly used and adequately performing measure of predictive accuracy (Huang and Ling, 2005), calculates the relative numbers of correctly and incorrectly identified predictions across all possible classification threshold values of the binomial response. An AUC value equal to or below 0.5 indicates a predictive ability equal to random expectation, and a value of one indicates a perfect predictive ability (Qiao et al., 2015).

Results

Litterfall

Mean total litterfall production for all treatments was 3054±339 kg ha^{-1} year^{-1} in the MS and 3050±674 kg ha^{-1} year^{-1} in the PS (Table 5). Intra- and inter-annual variability was observed in both stands (Fig. 2). Maximum amounts of litterfall were collected between June and September, representing 63% of the total biomass in the MS and 51% in the PS (mean values for all treatments). Maximum amounts were mainly collected in August in both areas, while minimum amounts were collected in winter months (December, January, and February). Needles were the most important fraction, representing 58% of the total biomass in the MS and 57% in the PS (mean values of all treatments) (Espinosa et al., 2020a). There was a short-term effect (3-4 months) on litterfall after SB in the MS and the PS, although the differences were greater in the PS (Table 5). In the following months, no differences were observed in the MS (the litterfall biomass was actually greater in the NB plots). In the PS, the differences remained throughout the study period, although they decreased steadily over time. No effect was observed after autumn-prescribed burning in the MS, but the effect of autumn burning was detected in the PS.

Table 5. Litterfall collected in mixed and pure stands per treatment.

S	PT	May 2016-April 2017	May 2017-April 2018	May 2018-October 2018
--	--	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Mixed	NB	3171	3537	1965
Mixed	SB	3257	2991	1879
Pure	NB	1989	2585	1504
Pure	SB	3482	3120	1762
S	**PT**	**November 2016-October 2017**	**November 2017-October 2018**	**--**
--	--	kg ha^{-1}	kg ha^{-1}	--
Mixed	NB	3532	2726	--
Mixed	AB	2732	2640	--
Pure	NB	2393	2376	--
Pure	AB	3629	3495	--

S: type of stand; PT: plot treatment; NB: no burning; SB: spring burning; AB: autumn burning. Data from Espinosa et al. (2020a).

Bayesian Model

Graphical representation of INLA estimation for the fixed effects is presented in Fig. 3. This chart presents nine of the 17 variables finally selected by the model (Table 4) and how they are related to litterfall. Variables distributed on the positive side are positively related to litterfall and those on the negative side are negatively related to litterfall. The variables that appear in both positive and negative areas have random effects on litterfall. This distribution is summarized in more detail in Table 6, with mean, standard deviation, and 95% credibility interval for the different variables. The mean values (disks) and 95% credible intervals (lines) highlight the estimated effect of the coefficients of variation. Credible intervals crossing zero (dashed line) suggest that the absence of any effect of the corresponding covariate cannot be ruled out, given the data and the model assumptions. The distribution of random effects is also shown in Table 6. Random effects are often used to account for over dispersion in Poisson models, and high values of random effects show that covariate explains most of the over dispersion in the data. However, if the scale of the estimated random effects is similar to that of the estimated covariates, both the covariates and the random effects will explain the overdispersion.

Meteorological variables (mainly mean maximum temperature, TmMx; days with snow, SwD; days with storm, StD) were the most important variables influencing litterfall biomass. The proposed model provides a positive response concerning the variability in litterfall due to two types of stands. The findings showed that minimum bark thickness and the height to the first live branch had random effects on the response variable. Although the treatment also had a positive effect on litterfall, it had a slightly weaker effect than the type of stand. Scorch height on the leeward side did not affect litterfall.

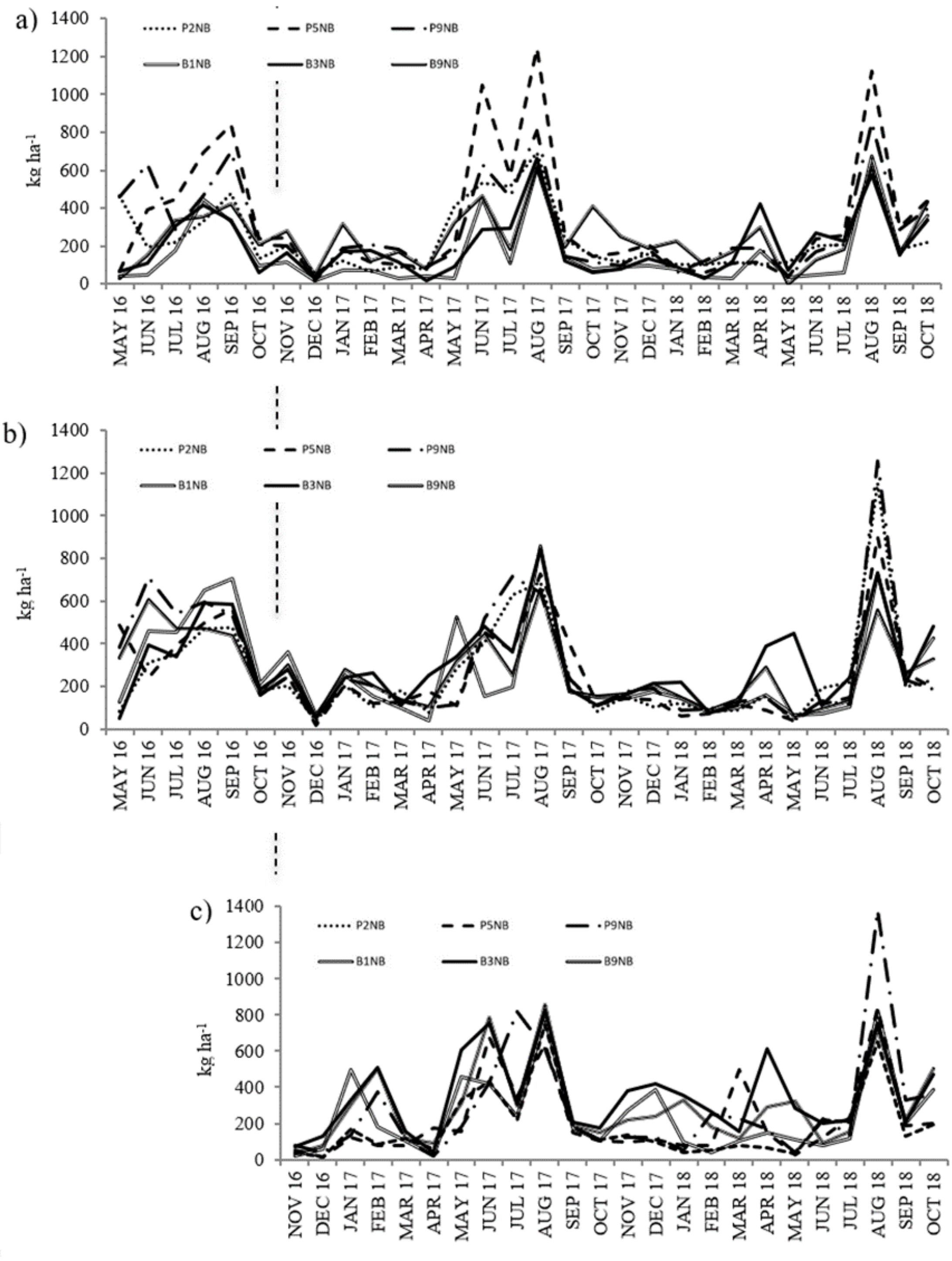

Fig. 2. Monthly changes in the amounts of litterfall collected (kg ha^{-1}) in (a) unburned plots in the mixed stand (P2NB, P5NB and P9NB) and the pure stand (B1NB, B3NB and B9NB); (b) plots burned in spring in the mixed stand (P3SB, P4SB and P6SB) and the pure stand (B2SB, B5SB and B6SB); and (c) plots burned in autumn in the mixed stand (P1AB, P7AB and P8AB) and the pure stand (B4AB, B7AB and B8AB).

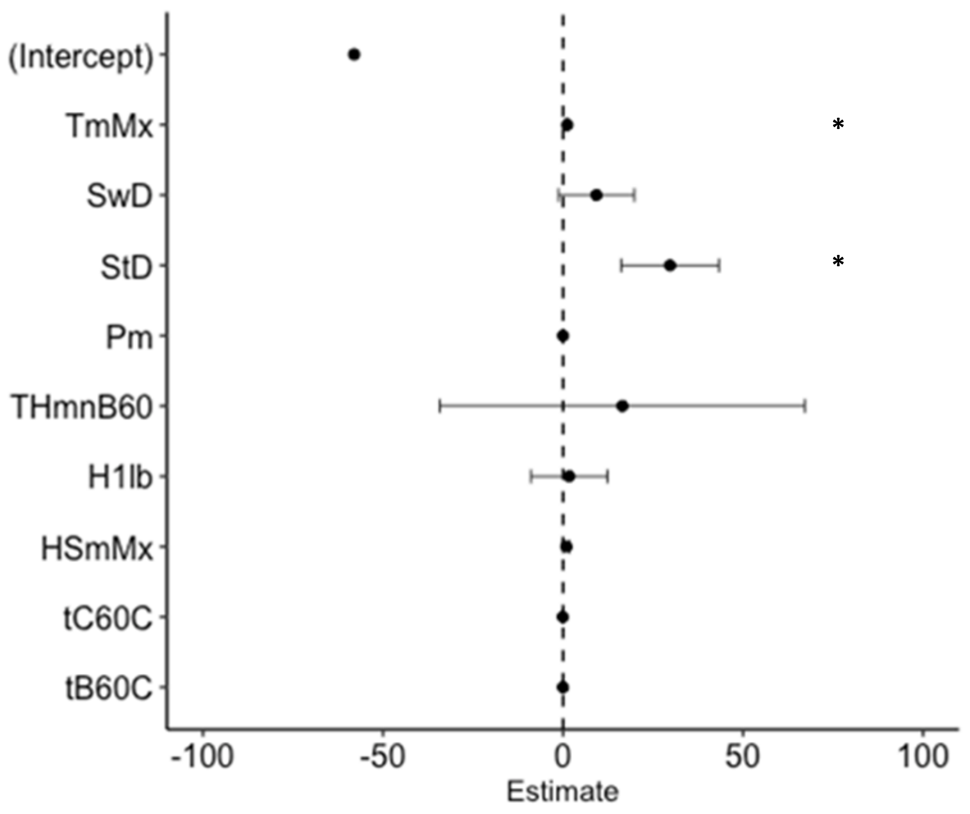

Fig. 3. Estimation of fixed effects. TmMx: mean maximum temperature; SwD: days with snow; StD: days with storm; Pm: monthly precipitation; THMnB60: minimum bark thickness at 60 cm from the base; H1lb: height to the first live branch; HSmMx: mean maximum scorch height; tC60C: time during which temperature was higher than 60 °C in cambium; tB60C: time during which temperature was higher than 60 °C in bark. (*) Indicates significant effects.

Table 6. Fixed and random effects, mean, standard deviation, and 95% credibility interval in the Bayesian model.

--	Variables	Type of Effect	Mean	Standard Deviation	0.025quant	0.975quant
Meteorological variables	**TmMx**	**Fixed**	**1.1701**	**0.1190**	**0.9363**	**1.4036**
	SwD	Fixed	9.6835	5.2434	−0.6092	19.9739
	StD	**Fixed**	**29.3036**	**6.7296**	**16.0870**	**42.5045**
	Pm	Fixed	0.0004	0.0191	−0.0372	0.0379
Stand and tree trait variables	Stand	Random	1.00×10^{-03}	3.50×10^{-03}	0.0000	6.90×10^{-03}
	THMnB60	Fixed	8.4226	25.4134	−41.2572	58.4590
	H1lb	Fixed	2.2874	5.1195	−7.7954	12.3090
Fire prescription and behavior variables	Treatment	Random	1.02×10^{-02}	5.44×10^{-02}	0.000	7.10×10^{-02}
	HSmMx	Fixed	0.8115	0.4748	−0.1175	1.7459
	tC60C	Fixed	−0.0511	0.0403	−0.1302	0.0279
	tB60C	Fixed	−0.0150	0.0500	−0.1135	0.0827
--	Intercept	--	−44.6160	92.7082	−228.3046	129.4254
--	Precision for the Gaussian observations	--	0.0000	0.0000	0.0000	0.0000

TmMx: mean maximum temperature; SwD: days with snow; StD: days with storm; Pm: monthly precipitation; THMnB60: minimum bark thickness at 60 cm from the base; H1lb: height to the first live branch; HSmMx: mean maximum scorch height; tC60C: time during which temperature was higher than 60 °C in cambium; tB60C: time during which temperature was higher than 60 °C in bark. Values shown in bold indicate significant effects.

Discussion

Although the importance of litterfall in the dynamics of the recovery of forest soils is recognized (Berg and Meentemeyer, 2001), scarce information about the effects of PB treatments on litterfall has been published. When planning prescribed burns, forest managers must reach decisions in the face of uncertain conditions, as litterfall patterns depend on several variables that often interact in a complex way. The Bayesian approach seems to be a good statistical model for dealing with complex models (it enables the inclusion of multiple variables) and for implementing prior knowledge (Espinosa et al., 2018; Espinosa et al., 2020a), thus enabling more robust conclusions to be obtained.

An immediate impact on litterfall was detected after spring and autumn PB. The effect was stronger in the pure stand. Over time, the effect of the treatment decreases. The different effects of PB on the two types of stand strengthen the results obtained in the statistical analysis where the random variable “stand” directly influenced the litterfall. A gradual decline in the effect of the disturbance has also been described by (Agren and Knecht, 2001; Roig et al., 2005) in different thinning regimes. Despite this immediate effect, mean total litterfall collected was within the range of natural litterfall in Spanish stands (e.g. Roig et al., 2005; Blanco et al., 2006) and close to those obtained (3337±841 kg ha^{-1} $year^{-1}$) by ICP Forests, 2020 in a *Pinus nigra* stand in Mora de Rubielos (Teruel).

Meteorological variables were the most important variables influencing litterfall biomass. Indeed, the marked inter-annual variability in climate in most Mediterranean areas drives the inter- and intra-annual variability in litterfall (Pausas et al., 1994; Kurz et al., 2000; Blanco et al., 2006). Litterfall dynamics may be more sensitive and increase when low-intensity prescribed burning is followed by snow (not significant variable) or storm events. Snowfall and storms have been suggested to be the cause of peaks in the abundance of some fractions of litter such as green needles, branches, and bark (e.g. Blanco et al., 2006; Martínez-Alonso et al., 2007; Espinosa et al., 2018). Data provided by AEMET (1997-2018) showed that a range of months with the most snowy days occurred mainly between December and March. During this period, for all treatments, branches represented on average 40% and 52% (MS and PS, respectively) of the total litterfall collected (Espinosa et al., 2020a). Branches may break as a result of the weight of accumulated snow, especially branches that are partly affected by fire. In addition, radiation or convection processes may increase thermal pruning. However, the low-intensity burning carried out in this study does not allow us to confirm the existence of this effect. The months with the most stormy days occurred between May and August. Nonetheless, findings on the influence of storms in litterfall biomass may interact with the time of natural abscission of needles (Espinosa et al., 2018).

Prescribed burning must be planned carefully, especially if carried out after months of high temperatures and consecutive months of low rainfall. The mean maximum temperature is a significant variable affecting litterfall in the proposed model. However, the model showed that the effects of mean precipitation varied at random, in contrast to previous findings on the influence of physiological drought on litterfall (García-Plé et al., 1995; Roig et al., 2005). This may be because the precipitation considered corresponded to the month prior to collecting the litterfall, and its influence on litterfall may be due to periods of extended drought when the accumulated precipitation is below the tree requirements. It is widely known that temperature and water regulate biological processes. Indeed, litterfall biomass is most abundant in summer (June-September) (Fig. 2) in the stands under study, representing mean percentages of 63% and 52% of the annual biomass in the MS and in the PS, respectively. Likewise, the combination of increased temperature and longer duration of light period in warmer months increases the activity of growth hormones that cause the abscission of old needles (Wareing and Thompson, 1975). Information about the ecosystem response to warming is essential for determining the impacts of perturbation in forest systems. An increase in temperature of 1.5 °C is predicted to occur between 2030 and 2052, if temperatures continue to increase at the current rate (IPCC, 2018). The effect of meteorological conditions must therefore be taken into account in considering the impact of PB on litterfall. Other variables indirectly related to meteorological conditions such as phenology may influence the litterfall dynamics, although to a lesser extent (Bueis et al., 2017).

The proposed model provides a positive response concerning the variability in litterfall due to type of stand (mixed or pure). As mentioned, a slight damping of PB effects in the mixed stand was already observed in the previous study (Espinosa et al., 2018); the present results, which cover a longer period of time, reinforce these findings. In the Cuenca Mountains, the *Pinus nigra*-*Pinus pinaster* ecotone generates stable stands, which should be taken into account in PB. Exploration of the resilience and vulnerability of mixed and pure stands to perturbations such as PB is essential to establish recommendations for management and fire prevention strategies.

Minimum bark thickness had a random effect in litterfall. In the present study, the PB was of low-intensity (the typical type of burning conducted in these *Pinus nigra* stands). The bark thickness was therefore probably sufficiently thick to protect trees against thermal damage (even the minimum values measured) (Espinosa et al., 2020b), thus guaranteeing supply of water and nutrients to the needles (and preventing premature fall). The thick bark of *Pinus nigra* is considered an adaptation to surface fire (Tapias et al., 2004; Pausas et al., 2009), and this species can persist after being affected by surface fires during several centuries (Fulé et al., 2008). The same applies to maritime pine populations (Tiscar and Linares, 2011). As bark thickness is an easily measurable variable and is correlated with the time required to reach lethal temperatures in the cambium (Gashaw et al., 2002; Van Mantgem and Schwartz, 2003), it can help to predict the resistance of bark to a surface fire and to prevent the crown being indirectly affected.

Although high crown base height may enable trees to escape the effects of surface fires such as those induced by PB, the study findings showed a random relationship between the amount of litterfall biomass and the height to the first live branch. In MS, the first live branch appeared at 6.4±1.8 m from the base, and in PS, at 8.2±2.5 m (Table 1). In most cases, scorch height was lower than the height of the first living branch, which may explain the observed responses in Bayesian models (for low crown base height or more variable values, the relationship may be stronger). It is widely accepted that *Pinus nigra* exhibits a self-pruning strategy (Tapias et al., 2001; Fulé et al., 2008) with high crown base heights (Pausas et al., 2009) that enable adult trees to resist fire. The present data are consistent with the previously reported crown base height of 7.3 m for the same species in stands in Northeastern Spain (Piqué and Domènech, 2018). Nevertheless, an average base height of 4.00±0.15 m has been reported for *Pinus nigra* stands in Eastern Spain (Fulé et al., 2008). A low height to the first live branch must be taken into account in planning prescribed burning as it will likely affect litterfall biomass.

Along with type of stand, the proposed model provided a positive response concerning the variability of litterfall due to treatment (NB, SB, and AB). However, type of stand had a slightly stronger effect on litterfall. This may be due to the low-intensity of prescribed burning and because the immediate effect of PB (3-4 months after PB) decreased over time (24 months), thus the accumulated litterfall may disguise these immediate effects produced by burning. However, if PB has a severe and immediate impact, it may cause an increase in accumulated fallen biomass, thus leading to a higher risk of future fire and a short-lived treatment efficacy (Espinosa et al., 2019).

Scorch height on the leeward side had a random effect on litterfall. The maximum scorch height was reached on the leeward side of the trunk (63±70 cm in MS and 107±110 cm in PS, Table 3), although below the mean height to the first live branch. Differences in scorch height on the leeward and windward sides (12±20 cm in MS and 21±40 cm in PS, Table 3) were due to the so-called "chimney effect" caused by the incidence of tree on the flame geometry (Hernando and Guijarro, 1998). Consequently, there are differences between the expected (prescribed) and real height of the flame at which the stem will be heated. Overall, a high scorch is caused by the effect of flame height, which could translate into stronger effects of radiation and convection processes negatively affecting the crown. Nevertheless, scorch marks on the highest parts of the stems in the stands under study were mainly due to the presence of lichens, which burned briefly. Furthermore, no trees were completely scorched, except seedlings under canopy and small dominated trees. In this respect, analysis of the nutrient contents of needle fraction collected reinforced the findings, showing nitrogen (N) concentrations corresponding to old needles in abscission time (4.5 and 5.1 mg g^{-1} mean value for all treatments and periods in El Pozuelo and Beteta, respectively) (Espinosa et al., 2020a).

The time during which the temperature was higher than 60 °C in bark and cambium had random effects on litterfall. In PB, in both spring and autumn, the temperature in the cambium area only exceeded 60 °C in 17% of trees in MS and in 8% of trees in PS (Table 3). As Bayesian models are based on an iterative learning process and the findings depend on previous knowledge about the system and new evidence (from the data), it is reasonable to consider that the study variables will have random effects on litterfall. This may also be explained by the fact that the burning was not completely homogeneous over the whole plot, but depended on fuel accumulation close to the trees. The process of cambial damage may be especially important in the context of PB, in which relatively low-intensity fire does not generally result in large amounts of crown damage (Van Mantgem and Schwartz, 2003). However, more data on moderate- high-intensity fires are needed to clarify the effects of these variables.

Conclusions

The effects of meteorological variables on litterfall were more significant and even more important than variables associated with stand characteristics or with prescription burning. Mean maximum temperature in the months prior to prescribed burning must be considered in decision making processes, because high temperatures may enhance the accumulation of fallen biomass, decreasing the duration of treatment effects and finally enhancing the fire risk. The final model did not indicate a close relationship between mean precipitation and litterfall. Presumably, an extended period of drought conditions is required to induce stress in trees and increase litterfall.

The variability in litterfall was affected by stand characteristics and tended to dampen the effect of perturbation in the mixed stand. Adaptation of bark thickness and high first live branch height in the species studied was sufficient to provide complete protection against low-intensity fire.

Although the treatment affected the variability in litterfall, the stand characteristic had a slightly stronger effect on litterfall. The results suggested that burning in spring (when the mean temperature is generally higher than in autumn) may have a greater impact on litterfall. In addition, the maximum litterfall production begins immediately afterward and may reinforce the effect. However, one-off episodes of snowfall and storms during autumn-winter may increase the abundance of litterfall, and the effect may even be maintained throughout the year until the time of maximum leaf-fall. Maximum scorch height (leeward size) did not have effects on litterfall. This variable is easily measurable after the passage of fire, and it can help in adjusting prescriptions and in estimating the effects on litterfall. The cambial temperature reached did not affect the live cells, and it therefore had no indirect impact on litterfall.

This is the first report of the use of a Bayesian approach to describe the complex litterfall dynamics after PB, with good results. However, the model should be tested with a larger database including information from more moderate-severe fires in order to improve PB planning.

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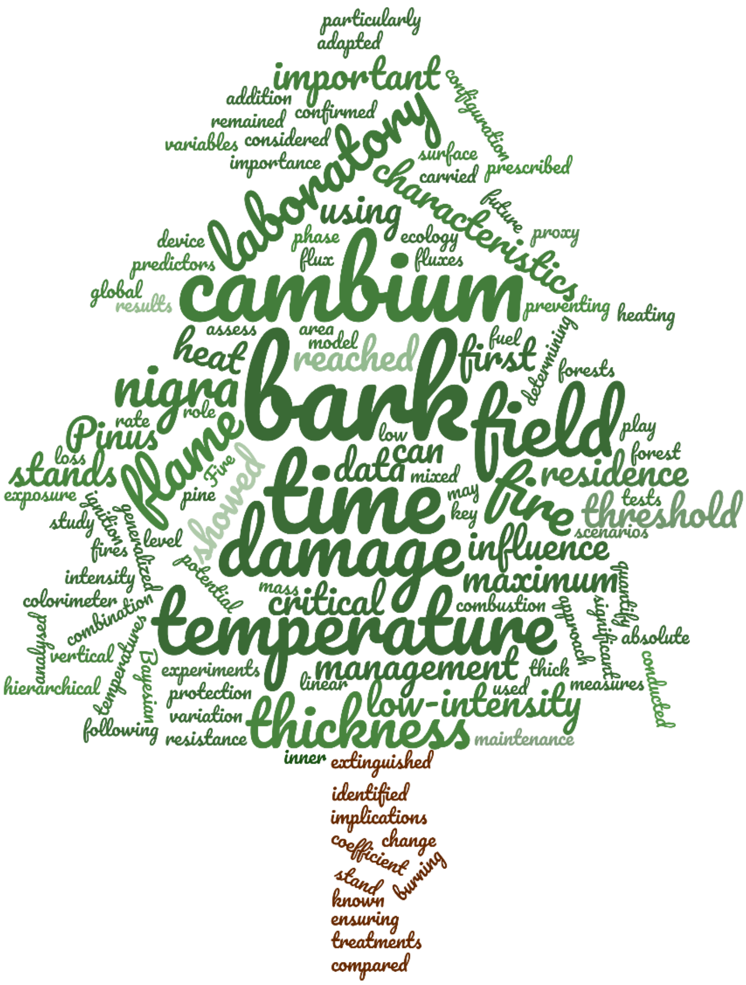
Chapter 3

Predicting potential cambium damage and fire resistance in *Pinus nigra* Arn. ssp. *salzmannii*



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Predicting potential cambium damage and fire resistance in *Pinus nigra* Arn. ssp. s*alzmannii*.

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Abstract

Fire management can play a key role in ensuring stand maintenance in future scenarios of global change, particularly in *Pinus nigra* stands, which are known to be adapted to low-intensity surface fires through characteristics such as thick bark. In this study, laboratory tests were carried out to quantify cambium damage and fire resistance in *P. nigra*, by using a mass loss colorimeter device in a vertical configuration for the first time. In addition, low-intensity prescribed burning treatments were conducted in the field, and the field and laboratory data were compared. The following variables were used as proxy measures to assess cambium damage: time that temperature remained above 60 °C, heating rate and absolute maximum temperature in the inner bark area. The data were analysed using a Bayesian hierarchical approach (generalized linear mixed model). A threshold heat flux (25 kW m^{-2}) for the time to ignition of bark was identified. A critical temperature of 60 °C was reached in the cambium during the combustion phase, after the flame was extinguished. The laboratory experiments showed, for the first time, the influence of flame residence time on the potential cambium damage. A bark thickness of 17 mm can be considered the threshold level for preventing critical temperatures being reached in *Pinus nigra* stands. The influence of bark thickness on protection against fire was confirmed, as was the importance of the coefficient of variation of bark thickness. The field results showed that flame characteristics (maximum temperature and residence time) were the most significant predictors of cambium damage. The combination of fire intensity and exposure time at low heat fluxes is more important than bark in determining cambium damage and may have important implications in the field of forest fuel management and in the ecology of pine forests.

Keywords: prescribed burning; wildfire; fire adaptation; fire ecology; tree mortality; bark thickness.

Introduction

Future scenarios of climate change project a longer period of fire season (Westerling et al., 2006) and an increase in burnt areas (Mann et al., 2016). Prescribed burning (PB) may be a tool to buffer the impact of wildfires on forest ecosystems properties (Plaza-Álvarez et al., 2017), mainly diminishing fuel loads. However, the way in which species display strategies to respond to perturbations is an important uncertainty in predicting the reaction of ecosystems (Pellegrini et al., 2017). Hence, forest managers require knowledge about the damage that fires cause in trees to enable the effective implementation of prescribed burning and for planning post-fire management.

Bark thickness is regarded as vital in stem resistance to fire (e.g. Harmon, 1984; Lawes et al., 2011a; Pausas, 2017), even in juvenile trees and saplings (Jackson et al., 1999; Midgley and Lawes, 2016). Furthermore, species that live in fire-prone habitats, particularly those maintained by frequent low-intensity surface fires, generally have thicker bark (Rosell and Olson, 2014). Mostly, it protects underlying tissues from temperatures above 60 °C that is commonly accepted to cause necrosis of the cambium (e.g. Hare, 1965; Dickinson and Johnson, 2004; Bauer et al., 2010). High-intensity fires may cause the death of the cambium around the entire bole circumference of stem, disturbing water and nutrient supply to the leaves and translocation of photosynthates to the roots (Rozas et al., 2011), which may cause death of the tree. If the fire does not cause the tree death, it may stress enough to the tree to show a systemic reaction which is reflected in a depletion of annual ring widths of the stem (Seifert et al., 2017). Other properties also influence fire resistance: specific gravity, moisture content, bark flammability and physical structure (Vines, 1968; Hengst and Dawson, 1993; Gignoux et al., 1997), although to a much lesser extent than bark thickness (VanderWeide and Hartnett, 2011; Odhiambo et al., 2014).

The area of distribution of *Pinus nigra* Arn. ssp. *salzmannii* (Spanish black pine) in the Mediterranean region has been reduced in the last decade, partly due to the incidence of more intense wildfires (López-Serrano et al., 2009). In addition, montane communities of Spanish black pine do not produce serotinous cones (Tapias et al., 2001), and the fact that they do not maintain a canopy or soil seed bank (Ordóñez et al., 2005) exacerbates the problem. Nonetheless, *Pinus nigra* is adapted to surface fire regimes by its thick bark and high crown base height (Pausas et al., 2008), as indicated by the presence of a relict, multi-aged forest in Eastern Spain (Fulé et al., 2008). It is also a long-lived tree species with a relatively open structure (Fulé et al., 2008), in which post-surface fire growth remains unaffected (Valor et al., 2015). Fuel reduction treatments with low-intensity prescribed burning may, therefore, be a good option for this species.

Several studies have been carried out to assess the insulating capacity of bark by monitoring cambial and surface temperatures, by experimental burning conducted in the field (Hengst and Dawson, 1993; Pinard and Huffman, 1997; Lawes et al., 2011a); in laboratory tests (Bauer et al., 2010; Odhiambo et al., 2014); or in both, field and laboratory, determining the distribution of temperature inside a tree under fire conditions (Costa et al., 1991). Other studies have assessed forest fuel flammability in forest species (Madrigal et al., 2009) or the relationship between flammability properties and the chances of survival after a fire (Frejaville et al., 2013). However, very few studies have considered together as predictor variables: bark traits, fire intensity and flammability (sustainability, ignitability and consumability) (Anderson, 1970; Martin et al., 1993; Pausas, 2017). In addition, most of the current studies have been conducted in non-Mediterranean ecosystems (Rosell, 2016) with different approaches and non-standardized methods, making it difficult to compare the results (Dehane et al., 2015). The established protocols by Della Rocca et al. (2018) and Madrigal et al. (2019) with the mass loss calorimeter (MLC), in a horizontal configuration, were used to characterize the resistance of bark to fire. Nevertheless, a vertical configuration (Dehane et al., 2015) seemed more suitable for simulating heating conditions and bark position in the field (Molina et al., 2018). The heat fluxes selected in the laboratory test covered a wide temperature range, simulating conditions observed in prescribed burning or in a wildfire and the bark thickness range sampled allowed tested the resistance threshold of *Pinus nigra* bark. However, because laboratory assessment of bark fire resistance is limited by the scale of the experiment, data from prescribed burning trials were required for comparison with data obtained in laboratory (Molina et al., 2018). Known the effectiveness of *Pinus nigra* bark against cambium damage due to overheating after the passage of fire, this study addressed the influence of additional variables that had not been sufficiently clarified to date. In this context, we explored the potential influence of coefficient of variation of bark thickness as there was not sufficient data to support its role in stem protection (Graves et al., 2014).

Because of live tissue in the inner bark is particularly sensitive to fire (Frejaville et al., 2013), and the degree to which the trunk resists fire depends on the flammability and thermal conductivity of the bark (Dehane et al., 2015), variables such as time of exposure to a critical temperature (60 °C), heating rate and temperature in inner bark were used as a good proxy measures for determining the potential damage to the cambium (Madrigal et al., 2019). Similarly, variables related to heat exposure, bark flammability - and the interactions between these – can potentially affect the resistance of bark to fire, although the available information is scarce (Frejaville et al., 2013; Varner et al., 2015), especially regarding thin barks. Thus, determining the most suitable variables for predicting tree damage caused by thermal injury may be key to understanding and making management decisions prior to fire (e.g. adjustment of fire prescriptions) and after fire for guiding the procedures adopted by land managers.

The novelty of this study is to address, for the first time, the influence of these variables both in the laboratory and in the field, being a first step to expand the results to other conifers. The aims of the present study were as follows: (i) to analyze some properties of *Pinus nigra* bark, such as thickness and the coefficient of variation of bark thickness, to determine the fire induced cambium damage; (ii) to relate external variables of fire behaviour (temperature and heat exposure) and variables associated with bark flammability (sustainability, ignitability and consumability) to the resistance of the cambium to fire; and (iii) to compare the results obtained in the laboratory with those obtained in experimental low-intensity prescribed burning treatments conducted in the field.

We hypothesized that heat exposure and bark flammability should have an important effect on bark resistance to wildfires and prescribed fires under canopy.

Materials and methods

Fire resistance tests in the laboratory

A mass loss calorimeter device was used to determine how bark resists fire in laboratory tests. Protocols have previously been established for determining bark flammability and fire resistance with a mass loss calorimeter (Dehane et al., 2015; Madrigal et al., 2019). However, this is the first time that an MLC device has been used in a vertical position. In this configuration, the radiation is distributed over the samples in a more realistic way, i.e. as in the field (Fig. 1a). Four heat fluxes were used, covering a wide temperature range, simulating conditions observed in prescribed burning or in a wildfire.

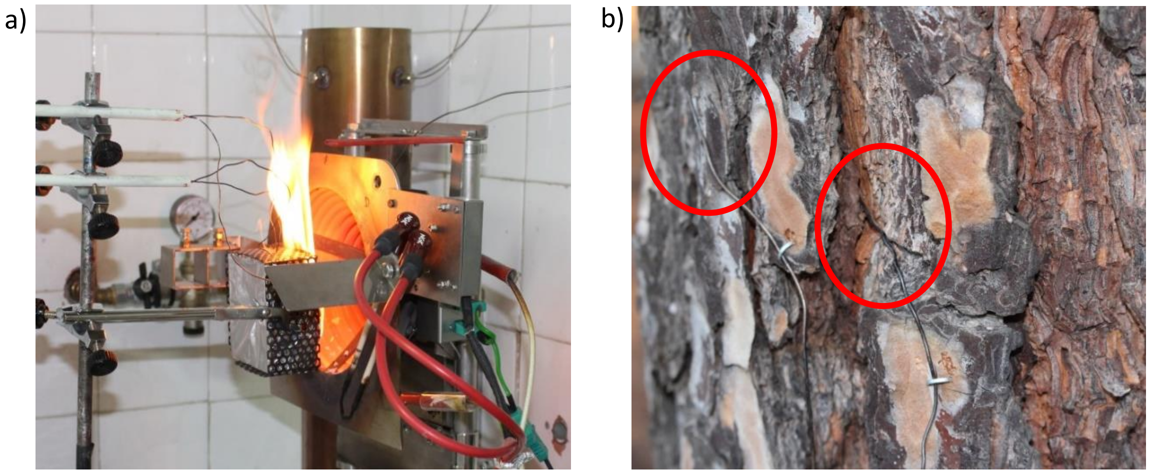


Fig. 1. (a) Laboratory test: mass loss calorimeter (MLC) device in a vertical configuration. (b) Field prescribed burning: thermocouples located in inner and outer bark.

Pinus nigra trees were extracted from a pure stand located in El Pozuelo (Central Spain; 40° 34′ 45″ N / 002° 14′ 30″ W) with similar characteristics to those described in section *"Field experiments"* and in Espinosa et al. (2018). A total of 40 trees were selected to represent the absolute ranges of bark thickness previously measured in the study areas (Espinosa et al., 2018). Two circular samples of thickness 10 cm were obtained at a height of 0.5-0.7 m from the ground in each tree. Graves et al. (2014) recommended sampling at a height of 0.5 m from the ground because this is an ecologically relevant height for trees in low-intensity fire-prone habitats. In the laboratory, each circular sample was divided into four subsamples of 10 × 10 cm to adapt to the dimensions of the MLC. The samples were stabilized at room temperature (20 °C) until constant weight. The absolute bark thickness (BT, mm) was measured (before and after the tests) in three positions in each sample (maximum, minimum and mean bark thickness) on the most representative face. The coefficient of variation of bark thickness was also calculated (CV, expressed in times 1), as the ratio between the standard deviation and the mean value for the samples. This coefficient was used as proxy for bark roughness. The thermocouples were placed in three holes drilled between the outer and inner bark. Another thermocouple was located on the surface of the bark, in the centre of the sample (for more details, see Madrigal et al., 2019). All samples were weighed before and after the test, and the weight loss was calculated (W, %). The distance between the MLC and the sample was 23 mm (Madrigal et al., 2019). Four heat flux rates (HF, kW m^{-2}) of 20, 25, 50 and 70, corresponding to air temperatures of 567, 620, 813 and 896 °C (calibrated with a radiometer: Madrigal et al., 2009), were selected. These temperatures are within the range of those measured or estimated in low and moderate-high-intensity fires (Cheney, 1981), which can easily reach 1000 °C (Wotton et al., 2012). Thirty replicate samples were tested for each heat flux, except HF = 20 kW m^{-2}, for which a total of 15 replicates were tested (n = 105). A pilot ignition system was used in the tests. Once the samples were exposed to the radiant heat, the delay between the beginning of the test and sample ignition was recorded (ti, s). Any event or anomaly (e.g. multiple ignitions) that occurred during the test was also recorded. Each test was continued until extinction of the flame (flame residence time; tf, s) or for up to 5 min (maximum exposure time). Finally, the samples were stored at room temperature until the temperature at the depth of the cambium fell below 60 °C. The maximum period of time chosen for simulation of the experimental surface fire is consistent with the typical duration of surface fires, i.e. 2-9 min (Vines, 1968; Hengst and Dawson, 1993). In order to characterize fire resistance, the time during which the temperature of samples was higher than 60 °C (t60, s) and the maximum temperatures reached in the cambium (Tmx, °C) were recorded. The heating rate at the depth of the cambium (HT, °C s^{-1}) was also calculated. Kaloustian et al. (1996) established the temperature at which some Mediterranean plants lose weight by volatilization to be 300 °C, which is within the range of temperature at the flame tip (200-400 °C) reported by Wotton et al. (2012). Thus, the time during which the temperature of the samples was higher than 300 °C (te300, s) and the absolute maximum temperature (Temx, °C) were also recorded, by the surface thermocouple. The moisture content of the samples (H, %) was measured before each experiment with an electric resistance xylohygrometer equipped with insulated electrodes, graduated up to 30% in fractions of 1% (maximum). Corrections were made regarding the species and the air temperature (UNE-EN 13183–2). Prior to the experiment, each sample was scanned (scan type, 600 ppp) on the face on which the thermocouples were placed, to determine the area of bark and wood. The sample was rescanned after the test to establish the percentage of bark that had burned (Bpo, %). The images were processed with ImageJ® software. A complete summary of the variables used is shown in Tables 1 and 2.

Table 1. Summary of variables.

	Abbreviation	Variable	Unit
RESPONSE VARIABLES	t60	Time during which the temperature of the sample was higher than 60 °C in the cambium area	s
	HT	Heating rate in the cambium area	°C s^{-1}
	Tmx	Maximum absolute temperature reached in the cambium area	°C
PREDICTOR VARIABLES		**Bark traits**	
	BT	Bark thickness	mm
	CV	Coefficient of variation of bark thickness	times 1
		Fire intensity	
	HF	Heat flux	kW m^{-2}
	te300	Time during which the temperature of the sample was above 300 °C at the surface	s
	Temx	Maximum absolute temperature reached at the surface	°C
		Sustainability	
	tf	Flame residence time	s
		Ignitability	
	ti	Time to ignition	s
		Consumability	
	W	Weight loss	%
	Bpo	Bark burned	%

Table 2. Mean and standard deviation (in brackets) values of variables studied.

	--	--	Heat flow rates			
	Variable	Unit	H20	H25	H50	H75
RESPONSE VARIABLES	t60	s	548 (538)	880 (601)	1708 (736)	1907 (290)
	HT	°C s^{-1}	0.0876 (0.0382)	0.1027 (0.0713)	0.1227 (0.0967)	0.1139 (0.0755)
	Tmx	°C	74 (37)	87 (47)	135 (112)	133 (80)
PREDICTOR VARIABLES			**Bark traits**			
	BT	mm	16.4 (9.6)	17.9 (10.7)	17.4 (9.1)	16.4 (8.7)
	CV	Times 1	0.49 (0.20)	0.50 (0.13)	0.44 (0.17)	0.51 (0.17)
			Fire intensity			
	te300	s	218 (123)	289 (129)	329 (84)	325 (18)
	Temx	°C	645 (175)	695 (158)	798 (190)	899 (83)
			Sustainability			
	tf	s	234 (98)	285 (82)	339 (59)	323 (8)
			Ignitability			
	ti	s	58 (18)	49 (33)	19 (49)	4 (2)
			Consumability			
	W	%	4.8 (2.0)	6.0 (2.0)	10.1 (2.7)	10.6 (2.9)
	Bpo	%	28 (24)	35 (17)	40 (24)	48 (16)

Field experiments

The study was conducted in two areas in Central Spain (El Pozuelo and Beteta). El Pozuelo is characterized by a mixed stand of *Pinus nigra* (89%) and *Pinus pinaster* Ait. (maritime pine) (11%) and Beteta by a pure stand of *Pinus nigra*. The mean tree density was 627 and 1286 trees ha^{-1} in El Pozuelo and Beteta, respectively. The mean height and diameter at breast height were 12.2 m and 19.8 cm respectively in El Pozuelo and 13.2 m and 18.8 cm in Beteta. Both stands are natural. Spanish black pine forests in the Cuenca Mountains have traditionally been managed using the shelterwood method, with a shelter-phase of 20-25 years and a rotation period of 100-125 years. Although this method changed in the last decades to an uneven-aged system due to the difficulties in achieving successful natural regeneration (Tiscar Oliver et al., 2011). The topographic features of the study site correspond to an elevation of 1093±122 m asl and flat ground, with a slope of between 3 and 10%. More information and details are reported in Espinosa et al. (2018). In order to carry out the PB, 12 experimental plots (n = 6 in El Pozuelo and n = 6 in Beteta) of 30 × 30 m were established. Two types of treatments were applied in each zone: spring burning (conducted in May 2016) and autumn burning (conducted in November 2016). Triplicate samples were included in each treatment. The strip ignition technique was applied at distances of 1-2 m downhill and with upslope wind. This technique is the most widely used in the study area to produce low- medium-intensity fire (Vega, 2001). The main characteristics of the PB treatments are shown in Table 3.

Bark thickness was measured (to the nearest mm) with a bark gauge. The gauge was inserted to the point of resistance of the cambium. Bark thickness was defined as the distance from the cambium to the bark surface. When the bark was uneven, the maximum thickness was measured at the highest point and the minimum thickness at the lowest point of the bark surface. However, because of the irregularity in the bark surface and the difficulty in estimating the exact measurement location, bark thickness measurements are estimated to be accurate to approximately 0.5 mm (Butler et al., 2005).

Table 3. Main parameters measured during and after prescribed burning treatments.

Z	PT	T	RH	WS	RS	FLI*	FH	FL*	SMx	SMn	C60%
--	--	ºC	%	m s^{-1}	m min^{-1}	kW m^{-1}	cm	cm	cm	cm	%
El Pozuelo	Spring burning	21.5 (1.2)	47.7 (5.3)	0.8 (0.6)	0.65 (0.10)	20.0 (8.8)	53 (15)	30 (6)	70 (41)	16 (9)	16 (15)
El Pozuelo	Autumn burning	11.9 (0.4)	67.0 (1.3)	0.3 (0.3)	0.59 (0.24)	11.2 (6.6)	17 (10)	23 (6)	34 (11)	5 (5)	18 (10)
Beteta	Spring burning	20.4 (1.5)	32.7 (2.3)	0.8 (0.1)	0.76 (0.09)	32.6 (13.3)	43 (8)	38 (8)	160 (16)	40 (18)	11 (4)
Beteta	Autumn burning	12.0 (0.9)	43.5 (0.8)	0.1 (0.1)	0.72 (0.03)	13.8 (10.7)	26 (13)	25 (9)	59 (26)	4 (3)	5 (8)

Z: zone; PT: plot treatment; T: air temperature; RH: relative humidity; WS: wind speed; RS: fire rate of spread; FLI: fire-line intensity; FH: flame height; FL: flame length; SMx: mean maximum scorch height; SMn: mean minimum scorch height; C60%: percentage of trees in which the maximum temperature in the cambium was higher than 60 °C. Standard deviation in brackets. (*) Byram (1959).

A total of 15 trees per plot were chosen on the basis of the diameter class. In each prescribed burning, two thermocouples were placed in each selected tree, one beneath the bark at the cambium and other in the outer area of bark at 0.6 m from the base (Graves et al., 2014) (Fig. 1b). At each location, a hole was drilled through the tree to insert the corresponding thermocouple at the cambium level. The hole was then covered with aluminum foil to prevent heat from entering during the PB. The thermocouples (type K 1 mm diameter inconel-sheathed, with response time of 0.3 s) were connected to data loggers (DT-USB TCDirect®), which recorded the temperatures every second. The data loggers were buried to prevent them being destroyed by the fire.

Data analysis

Three types of response variables were chosen to determine the damage to the cambium for each heat flux: time during which the temperature of cambium was above 60 °C, heating rate of cambium and predictor variables were related to bark traits, fire severity, sustainability, ignitability and consumability (White and Zipperer, 2010).

Initially, exploratory data analysis (EDA) was carried out in order to summarize the main characteristics of the data set. EDA was a critical first step to determine what the data might indicate beyond the Bayesian approach or starting assumptions. Scatter plots were constructed in order to map each variable to an x- or y-axis coordinate, and relationships between explanatory variables were established.

Generalized linear models (GLMs) are commonly used to model relationships between variables. The response and variable parameters are assumed to be random in the Bayesian approach, and a distribution must therefore be assigned to the parameters, i.e. the "prior distribution". Any parameters that define the prior distribution are called hyperparameters. A Bayesian version of the Gaussian GLM with an identity link can be described as follows. Let $(x_i, y_i), i = 1, \dots, n$ be a set of observations. It is assumed that y_i follows a Gaussian distribution with mean μ_i and variance σ^2, the identity link function (i.e. $g(\mu_i) = \mu_i$) and linear predictor $\eta_i = \beta_0 + \beta_1 x_1$. That is,

$$y_i \sim N(\mu_i, \sigma^2)$$

$$\mu_i \sim \beta_0 + \beta_1 x_{i1} + \cdots + +\beta_n x_{in}$$
$$\beta_0 \sim f_1$$
$$.$$
$$.$$
$$.$$
$$\beta_n \sim f_n$$
$$\sigma^2 \sim f_m$$

Note that the model is the same as in the frequentist approach, but that it is now assumed that $\beta_0, \dots, \beta_n$ and σ^2 are realizations from distributions $f_0, \dots, f_n$ and f_m respectively. To implement this type of model in R (statistical software version 3.6.0), the integrated nested Laplace approximation (INLA) was used via INLA function in the INLA package (Rue et al., 2009).

To evaluate the model fit, we applied the Watanabe-Akaike information criterion (WAIC) (Watanabe, 2010). WAIC has been suggested to be appropriate for estimating the out-of-sample expectation in a fully Bayesian approach. This method starts with the computed log pointwise posterior predictive density and then adds a correction for the effective number of parameters, to adjust for overfitting (Gelman and Shalizi, 2013). The Watanabe-Akaike information criterion works on a predictive probability density of detected variables rather than on the model parameter, and it can therefore be applied in particular statistical models (i.e. models with nonidentifiable parameterization) (Li et al., 2016).

Results

Laboratory tests of fire resistance

The thickness of the bark samples (n = 315) ranged from 1.8 to 52.3 mm (standard deviation 9.5 mm). The samples can be considered dry, according to the mean moisture content (7.3±1.5%). The relationship between maximum bark thickness and flame residence time is shown in Fig. 2a. In samples subjected to heat fluxes of 50 and 70 kW m^{-2}, the flame was extinguished shortly after the maximum exposure time (300 s) in most cases. The relationship between maximum bark thickness and time to ignition (Fig. 2b) revealed a threshold at around 20-23 s, with different responses for the two lowest heat fluxes HF20 and HF25 (ti ranged from 23 to 185 s) and the two highest heat fluxes HF50 and HF70 (ti ranged from 2 to 20 s with three peaks). The lowest ti variability was related to higher HF. The heating rate in the cambium is shown in Fig. 3. The exponential trend suggests that HT tended to be constant for bark of thickness > 17-20 mm, showing higher variability with bark thickness lower than 17 mm.

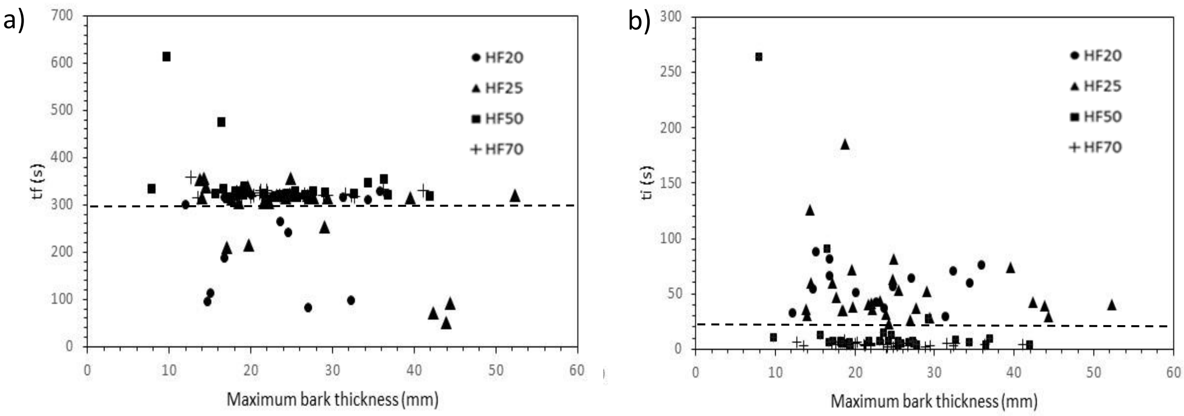


Fig. 2. (a) Relation between maximum bark thickness and flame residence time (tf) for the different heat fluxes (HF). (b) Relation between maximum bark thickness and time to ignition (ti) for the different heat fluxes (HF).

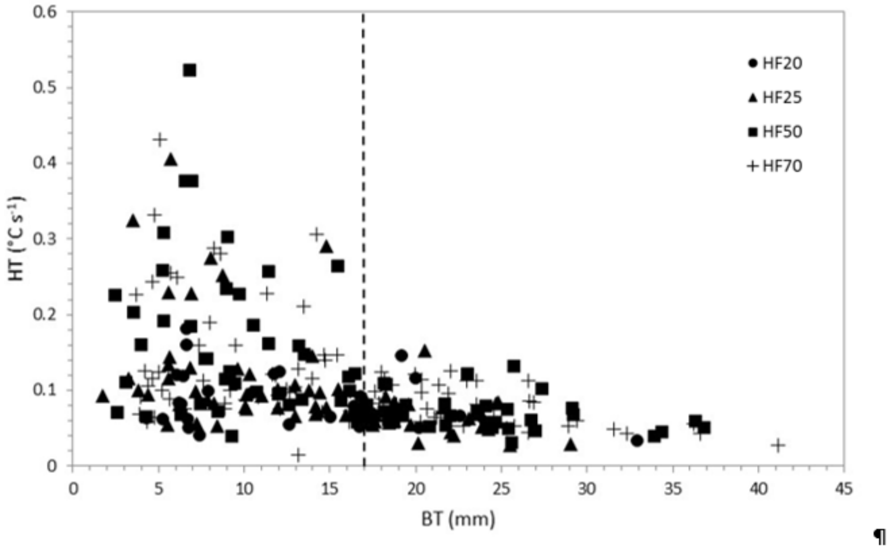


Fig. 3. Relation between bark thickness (BT) and heating rate in the cambium area (HT) at the different heat fluxes (HF) tested.

The median temperature curves at the cambium level for each HF are shown in Fig. 4. The critical temperature of 60 °C was not reached at HF = 20 kW m^{-2}. The temperature of the cambium continued to increase 5 min after of maximum exposure time, indicating that the bark continues to provide heat to the cambium during combustion. After 6-7 min, the cambium temperature exceeded 60 °C at almost all the HF (except 20 kW m^{-2}), reaching a maximum of 100 °C at the highest HF tested.

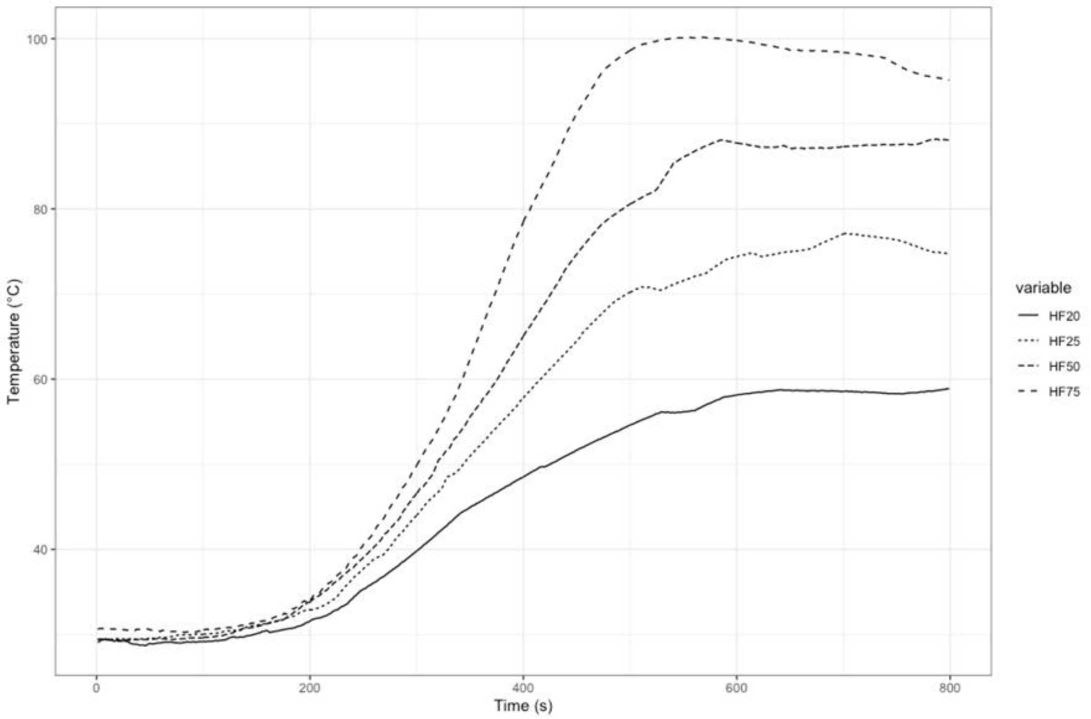


Fig. 4. Median temperature curves at the cambium level for each heat flux (HF) tested.

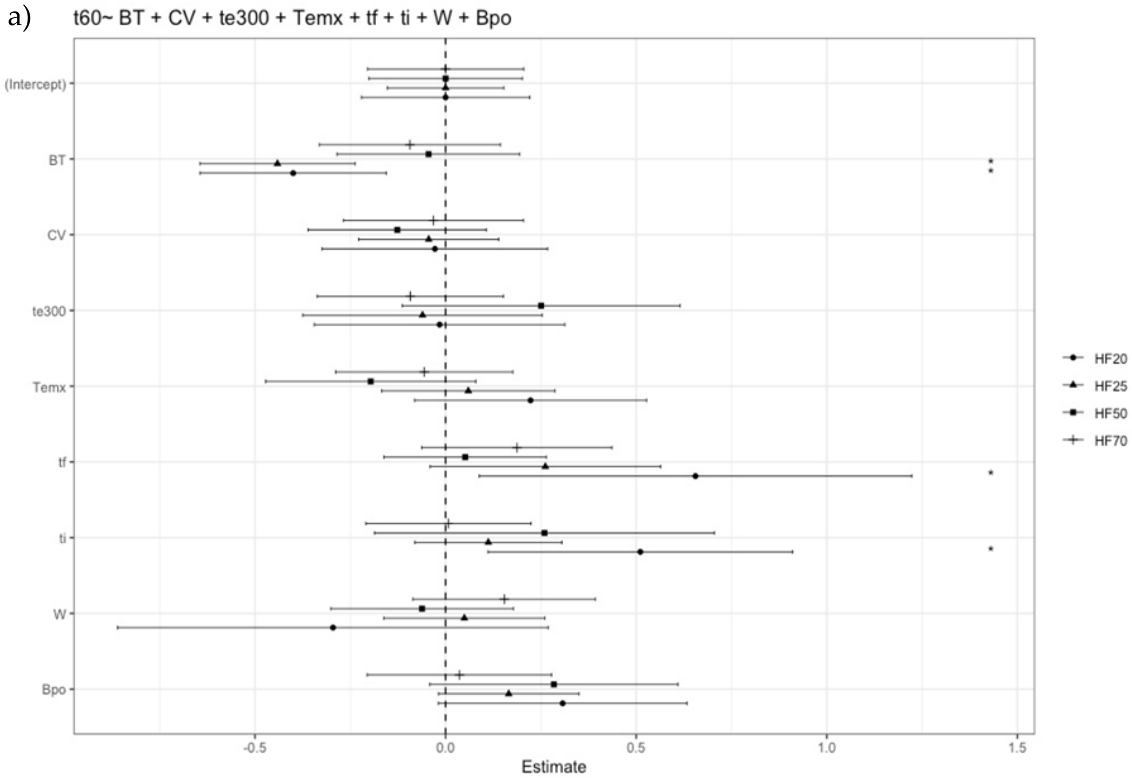
Bayesian models

The estimated fixed effects in the laboratory data are graphically represented in Fig. 5, in which the variables and their relationships with t60 (Fig. 5a), HT (Fig. 5b) and Tmx (Fig. 5c) are shown. The variables distributed on the positive side of each figure contribute to higher response values; by contrast, variables distributed on the negative side in each figure contribute to negative response values. This distribution is summarized in more detail in Table S1 (supplementary material) with mean, standard deviation and 95% credible interval for the different variables. Variables in both areas (positive and negative) in Table S1 are not clearly related to the response variable.

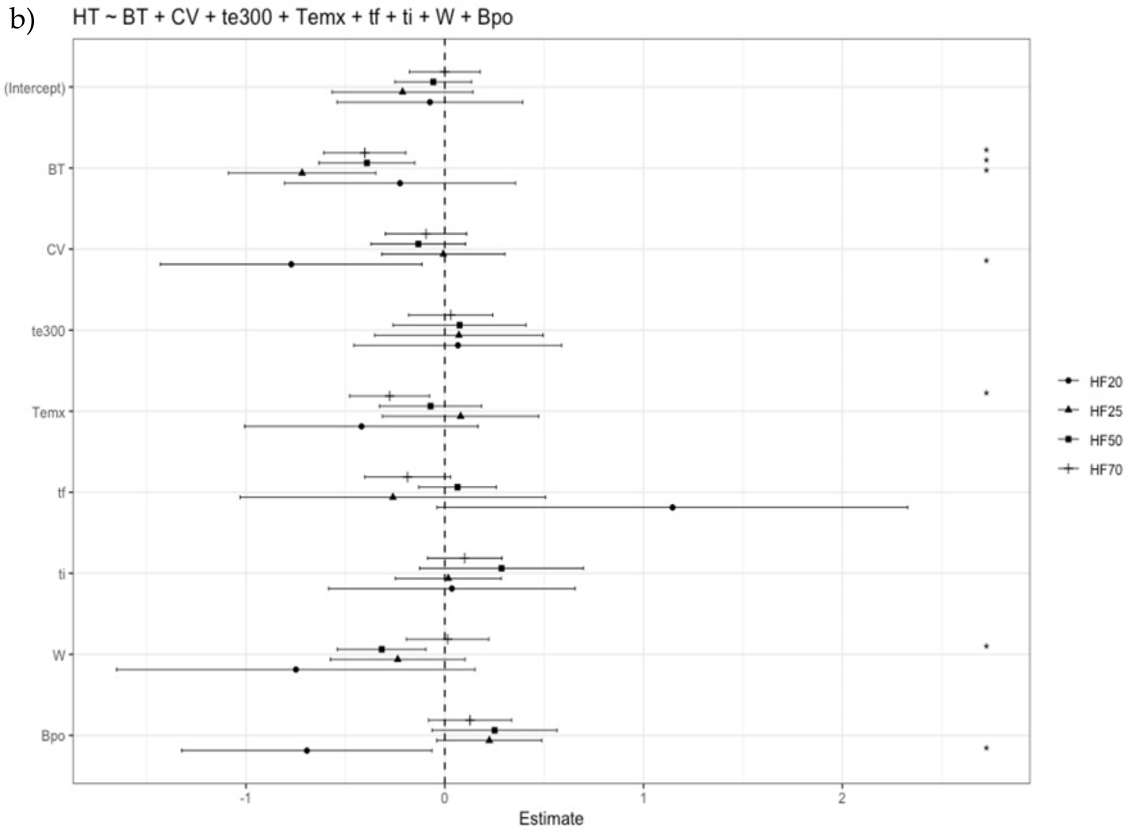
The different variables behaved differently depending on the heat flux applied. Analysis of the relationships between t60 and the covariates BT, CV, te300, Temx, tf, ti, W and Bpo (Fig. 5a) revealed a negative relationship between BT and t60, but which was only significant for HF20 and HF25. A positive trend was also observed for tf, ti and Bpo, although only tf and ti in HF20 were significant.

Fig. 5b shows the relationships between HT and the same covariates as in Fig. 5a. In this case, there was a significant relationship between BT for all HF, except 20 kW m^{-2}. However, CV and Bpo were significant variables at this lowest HF. No significant relationship was observed for tf and ti. Similarly, Fig. 5c shows the relationship between BT and Bpo.

a)


t60~ BT + CV + te300 + Temx + tf + ti + W + Bpo
(Intercept)
BT
CV
te300
Temx
tf
ti
W
Bpo
-0.5
0.0
0.5
1.0
1.5
Estimate
HF20
HF25
HF50
HF70

b)


HT ~ BT + CV + te300 + Temx + tf + ti + W + Bpo
(Intercept)
BT
CV
te300
Temx
tf
ti
W
Bpo
-1
0
1
2
Estimate
HF20
HF25
HF50
HF70

c)

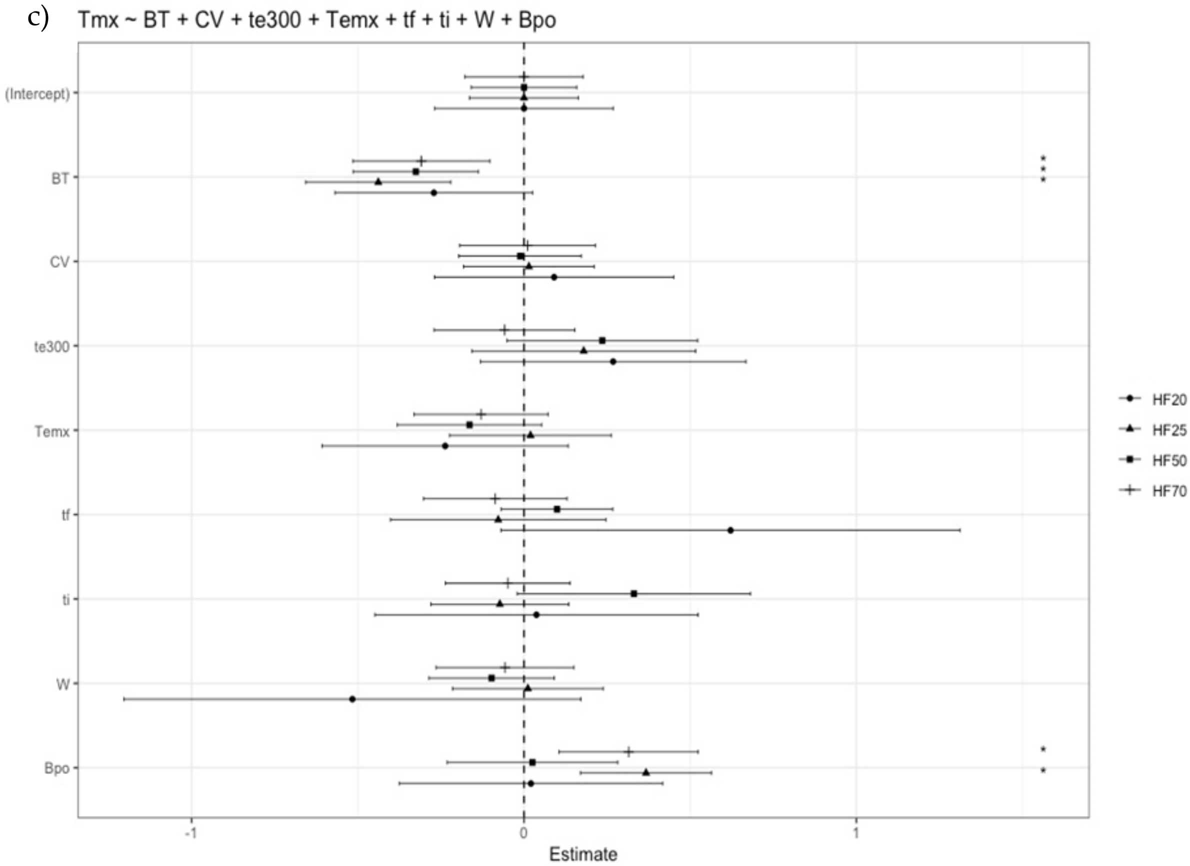
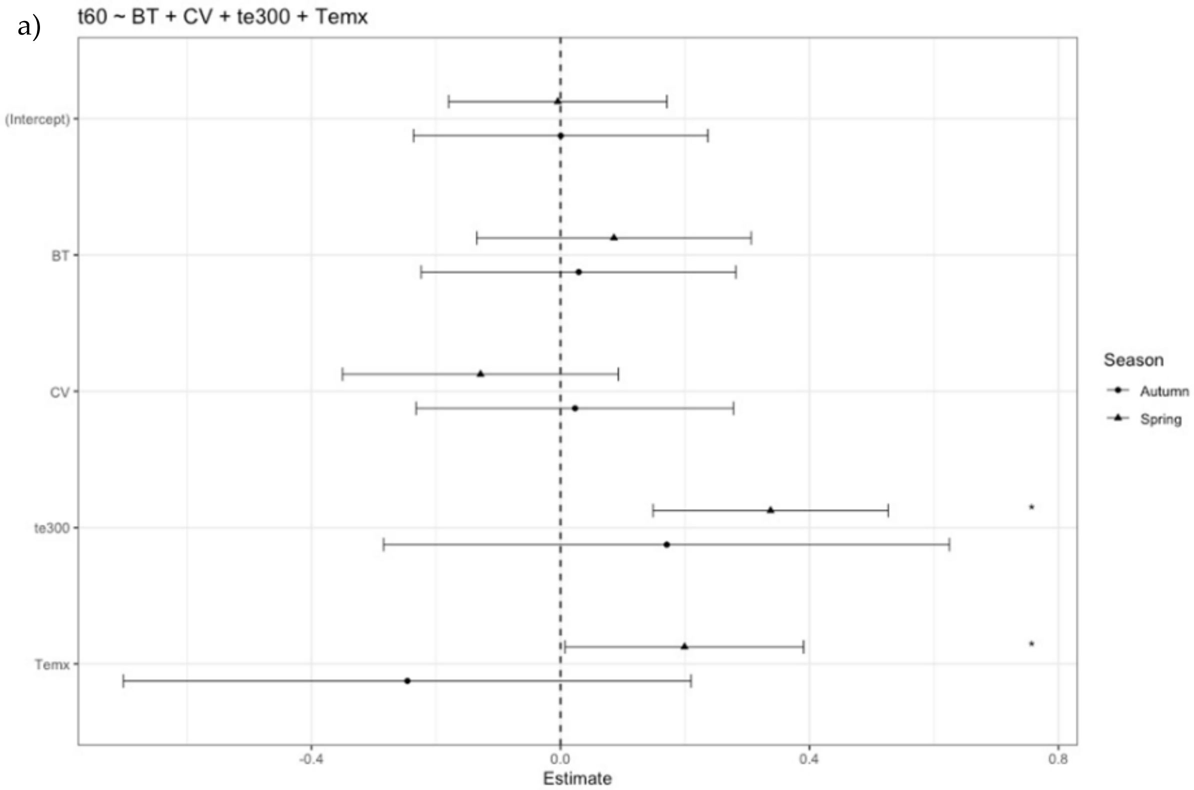
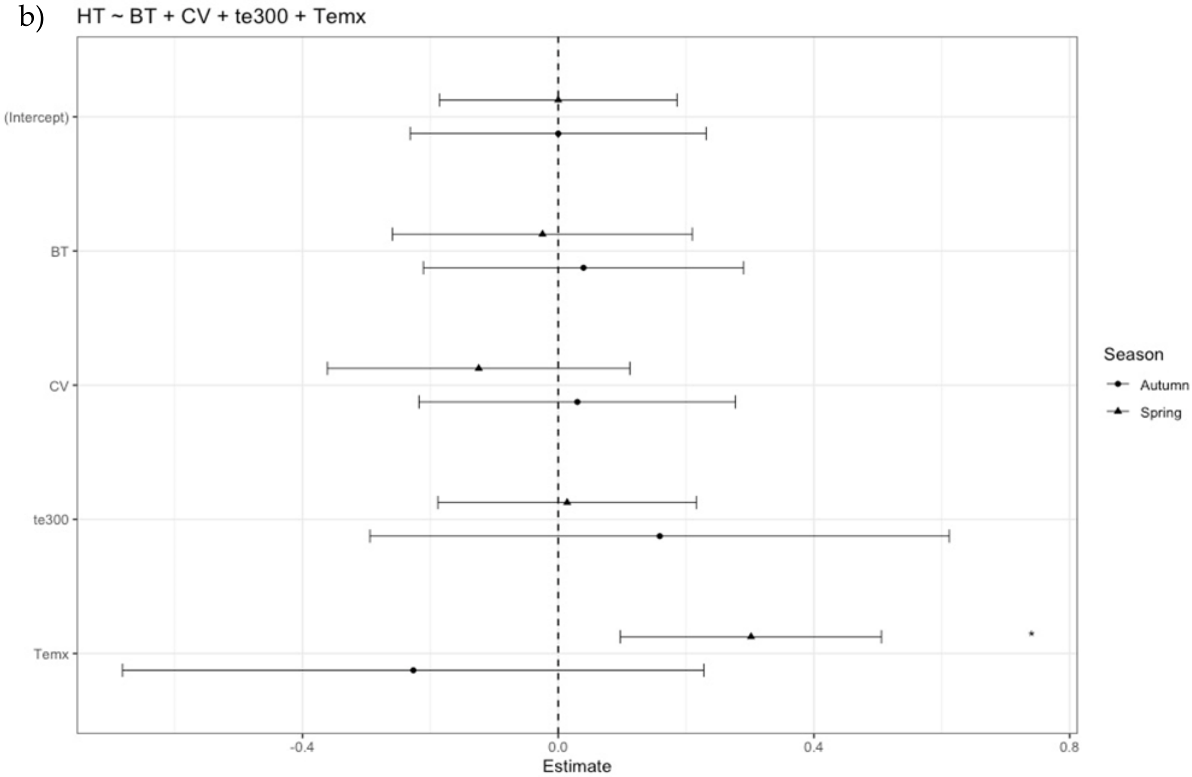


Fig. 5. Graphical representation of fixed effects of (a) time during which temperature of the samples was higher than 60 °C (t60); (b) heating rate (HT); (c) absolute maximum temperature reached in the cambium area (Tmx) for laboratory data. (*) Indicates a significant difference. The abbreviations are explained in Table 1.

a)


t60 ~ BT + CV + te300 + Temx
(Intercept)
BT
CV
te300
Temx
-0.4
0.0
0.4
0.8
Estimate
Season
Autumn
Spring
*
*

b)


HT ~ BT + CV + te300 + Temx
(Intercept)
BT
CV
te300
Temx
-0.4
0.0
0.4
0.8
Estimate
Season
Autumn
Spring
*

c)

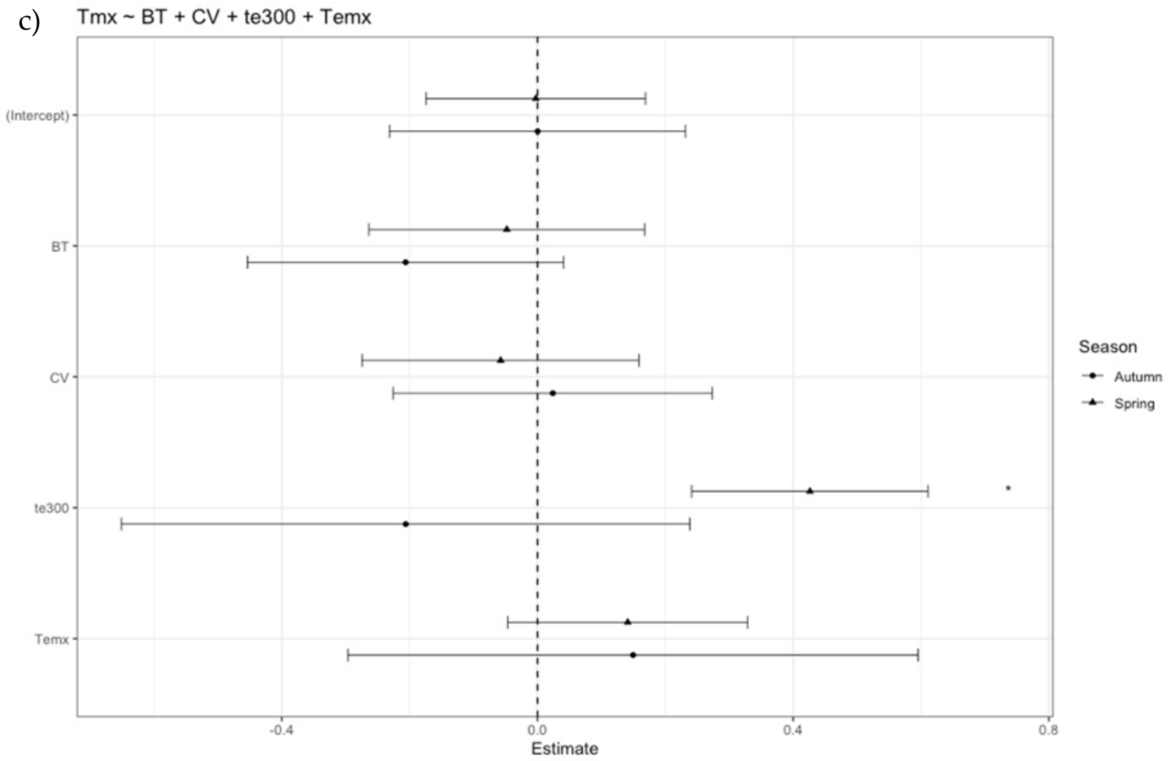


Fig. 6. Graphical representation of fixed effects of (a) time during which temperature of the samples was higher than 60 °C (t60), (b) heating rate (HT) and (c) absolute maximum temperature reached in the cambium area (Tmx) for field data. SB, spring burning; AB, autumn burning. (*) Indicates significant difference. The other abbreviations are explained in Table 1.

In order to compare the laboratory and field results, a graphical representation of the estimated fixed effects in the field data is presented in Fig. 6 and Table S2 (supplementary material). Common descriptor variables were used (BT, CV, te300 and Temx), as some variables cannot be reproduced in the field. Data from spring burning and autumn burning were analysed separately. Bark thickness and CV were randomly related to t60, HT and Tmx in both seasons. Significant results were obtained for te300 (in response variables t60 and Tmx) and Temx (in response variables t60 and HT), especially in spring burnings, in which the fire intensity was highest.

Discussion

Information about the way in which bark properties and fire behavior interact is important in relation to fire management decisions. Previous analysis of data showed that once the samples were no longer exposed to radiant heat, the flame tended to be extinguished within a short period of time (Fig. 2a). In addition, the time to ignition indicated a threshold heat flux of 25 kW m^{-2} (Fig. 2b). Two types of responses were observed. For the two lowest heat fluxes (≤ 25 kW m^{-2}), the time to ignition was longer than 20 s, while for the highest heat fluxes (> 25 kW m^{-2}), the time to ignition was shorter than 20 s. This limit of heat radiance has been proposed as representative of a low- moderate-intensity fire (Cruz et al., 2006; Madrigal et al., 2019). Madrigal et al. (2019) observed (by cluster analysis) two different types of response in samples of *Pinus pinea*, with BT higher than 20 mm and less than 20 mm. However, this was not observed in the present study, and a good fit to the curve was not achieved, partly due to the different sample sizes and the wide range of bark thicknesses selected in this study. Nevertheless, a limit of around 17-20 mm can be seen in Fig. 3, where the curve tends to stabilize. Thus, a bark thickness of 17 mm can be considered the critical threshold at which the probability of cambium death decreases. Both of these values are remarkably similar to those reported for deciduous trees in Bolivia (≥ 18 mm) (Pinard and Huffman, 1997), for three species of *Eucalyptus* (> 20 mm) (Wesolowski et al., 2014) and for tropical eucalypt species (≥ 24 mm) (Lawes et al., 2011b). In the series of tests carried out, heating flux increased from 20 to 70 kW m^{-2}, conditions that can be reached in prescribed burning or in wildfire.

Considering that a maximum exposure time of 300 s can be considered a high level of exposure (corresponding to a high-intensity fire or a very low rate of spread), the median temperatures in the cambium were reached after an exposure time of 300 s. These findings show that (i) the bark was exposed to a high level of radiation, but the damage to the cambium is negligible or low for all heat fluxes tested, at least during 300 s; (ii) the highest level of cambium damage (temperatures above 60 °C) was detected after radiant exposure corresponding to the bark combustion phase: bark burns slowly sustained by the heat without flame (smouldering). Furthermore, the intensity and exposure time at radiant source were proposed as a critical combination for establishing cambium damage (especially in thin bark). To our understanding, this is the first experimental evidence that the potential damage to the cambium caused by the combination of heat and exposure is exacerbated by bark flammability.

The study findings confirmed the key role of bark thickness in protecting the cambium against fire for all variables considered as predictive of cambium damage (time that the cambium is above 60 °C, heating rate and absolute maximum temperature in the cambium). Other studies have also shown the role of bark in protecting the stem (e.g. Lawes et al., 2011a; Brando et al., 2012; Pausas, 2017). This relationship was not significant at HF20 for HT and Tmx, possibly because the insulating capacity of bark provides complete protection at lower HF (Fig. 4) and thus other variables, such as the flame residence time (tf), may be more meaningful. These findings are consistent with those reported by Seifert et al. (2017), confirming that the time of exposure to fire is a determining factor in causing damage to the cambium.

Coefficient of variation of bark thickness seemed to have a slightly positive effect on protection of the cambium via two response variables, t60 and HT (although the relationship was only significant for HT at low heat flux). Although there is not sufficient data to support the role of bark roughness in protecting the stem, it has been suggested that it provides protection against sun scald, extreme diurnal temperature fluctuations, pathogens, nutrient losses and the adaptation of dead cells to the increase in diameter (Glitzenstein and Harcombe, 1979). Bauer et al. (2010) and Odhiambo et al. (2014) explained that the spaces within the dead outer bark layers are filled with air, which enhance the resistance to heat. The same explanation was given by Pinard and Huffman (1997), who stated that the air trapped in void spaces of structured bark increases heat insulation due to low heat conductivity. Furthermore, habitat has been considered a predictor of roughness, with fire-prone areas having rougher bark than fire sensitive species (Graves et al., 2014). However, Frejaville et al. (2013) did not observe any significant influence of bark roughness in fire resistance in trees in an Alpine ecosystem. Despite having positive effects, roughness, density and the moisture content of bark have been shown to have little effect relative to bark depth (Hengst and Dawson, 1993; Pinard and Huffman, 1997; Brando et al., 2012). The present findings show that roughness is a significant variable in reducing cambium damage only at low heat flux, which is consistent with ecological evidence of species adapted to low-intensity fires (Pausas, 2017).

The time during which the temperature was above 300 °C in the outer bark (air surface) appeared to have different effects for temperatures above 60 °C, heating rate and absolute maximum temperature in the cambium, varying among heat fluxes, but with no clear relationship. As thermocouples were located on the outer bark (at 23 mm from radiant flux) and radiation fluxes ranged from 20 to 70 kW m^{-2}, it is reasonable to expect temperatures above 300 °C during the time that thermocouples were exposed to heat. This does not therefore seem to be a good indicator of cambium damage, at least in laboratory tests. Similarly, the absolute maximum air temperature was not a good predictor of cambium damage, producing random responses for the different variables (t60, HT and Tmx) and heat fluxes. Temperature peaks may not affect the cambium if they are not prolonged. This is due to the insulating capacity of bark (Graves et al., 2014), described as a combination of thermal conductivity and the ease at which a temperature pulse moves through a given thickness of bark (thermal diffusivity). Thus, one-off heating of bark does not imply a response in the cambium area. Costa et al. (1991) reported that temperature at a specific location inside a tree is actually influenced by the distribution of temperature over the whole bark surface, which is very irregular under fire conditions.

A longer flame residence time was expected to have negative effects on the cambium area. According to experimental results (Fig. 4) flame residence time higher than 5-6 min could be critical for cambium damage. In this context, a significant relationship between tf and t60 was observed at HF20 (Fig. 5a) and although not significant for HT and Tmx, it was notable (Fig. 5b and 5c). As already mentioned, at lower heat fluxes (HF20), bark thickness completely protects the cambium cells, and the findings indicate that variables related to flame residence time may be more important. Madrigal et al. (2019) reported that the duration of heating required to damage or kill the cambium is proportional to the squared value of bark thickness. Likewise, the findings showed that a longer time to ignition (what implied a longer exposure time in laboratory test) may produce higher values of t60 and HT. Although not clear, it is possible that there is a positive relationship between tf and ti for HF20 (correlation coefficient of -0.2) (Fig. 5a). Frejaville et al. (2013) suggested that flammable bark would increase the heat transfer to the cambium due to a reduction in bark thickness and blackening of the bark surface. Likewise, other studies have emphasized that high flammability may potentially generate an increase in fire vulnerability (Dehane et al., 2015). Information about the relationship between flammability and thermal conductivity of bark is scarce despite being important for characterizing the vulnerability of living tissues in the trunk (Dehane et al., 2015).

Two variables were selected in order to measure the consumability: percentage weight and bark consumed. There did not seem to be a clear relationship between weight and time that the cambium remained above 60 °C, heating rate and absolute maximum temperature in the cambium for any heat flux. However, a greater reduction in bark surface had a negative effect on the cambium. The percentage weight loss is probably related to water loss and volatilization of gases. However, reduction in bark surface would be related to the loss of bark thickness and therefore to less protection of the cambium. In fact, small differences in bark thickness produce large differences in fire resistance (Bond and van Wilgen, 1994; Moreira et al., 2007).

The results obtained in the mass loss calorimeter were compared with those obtained in PB conducted in the field (Fig. 6) for a more realistic representation of how bark is affected by fire (Molina et al., 2018). No significant results were observed in relation to the cambium damage and BT or CV. During spring burnings, temperatures of 60 °C in the cambium were only exceeded in 16% of the trees in El Pozuelo and 11% of those in Beteta. In the autumn burnings the percentages were 18 and 5% respectively in El Pozuelo and Beteta (Table 3). These values may be indicative of low-intensity fires, and thus the bark (near the ground level) presumably protects the cambium (Graves et al., 2014), particularly in species with thick bark, such as *Pinus nigra*, which are more tolerant of this type of fire. The lack of a significant relationship therefore makes sense. However, time during which the temperature of the sample was above 300 °C and absolute maximum temperature at the surface had significant effects on t60 and Tmx and on t60 and HT respectively. Variables such as te300 or Temx showed some capacity to predict cambium damage as a value of punctual severity at tree level. These variables indicate individual overheating of the tree, which did not occur in laboratory tests, as the trials are more homogeneous. Higher heating was mainly due to the natural accumulation of branches fall around the tree or lichens growing along the stem, which led to a longer residence time of the fire. However, radiation from intense, more distant flames may have the same effect (Vines, 1968). Therefore, the choice of the prescribed burning technique (e.g. strip ignition) should favour rapid advancement of the fire front, which should prevent the trunk overheating and excessive consumption of organic matter (Vega, 2001), even in a low-intensity fire. In addition, the removal of fuel accumulations at the base of the trunk is recommended to reduce the residence time of the flames. Additional data from more intense PB trials are needed in order to confirm these findings and for comparison with laboratory-acquired data. Nevertheless, the findings for the *Pinus nigra* stands and the additional values obtained in the laboratory confirm the idea that these stands are not very vulnerable to low-intensity fires, because of the high resistance of bark to fire (Valor et al., 2015) and because only local conditions at tree level can generate local burn intensities that can potentially cause damage to the cambium (Jiménez et al., 2017, Madrigal et al., 2019).

Conclusions

In this study, a mass loss calorimeter device was used in a vertical configuration for the first time in laboratory tests. A threshold at 25 kW m^{-2} was detected as representative of low-moderate-intensity fires, so that below this value, negligible cambium damage is expected.

This could be considered a reference value for improving fire prescriptions or evaluating potential mortality rates after surface wildfires.

The flame tends to become extinguished shortly after the end of radiant heat exposure (300 s), even at high heat fluxes, although smouldering fire on bark increases the temperature of cambium. The process described demonstrates for the first time that bark flammability (duration of flame and bark surface burned) significantly affects the potential damage to the cambium. These findings lead the way to new studies relating ecological and combustion properties of tree bark and the potential influence of these properties in protecting living tissues after fire. The study findings confirm the influence of bark thickness in preventing cambium damage during the passage of fire in *Pinus nigra* stands. A bark thickness of 17 mm was found to be critical regarding cambium death. There is no consensus about how bark roughness contributes to protecting against low-intensity fire; however, the results obtained for two variables representative of cambium damage indicate that roughness may have a positive effect in protecting the cambium during low heat exposure.

The results of laboratory tests and field burning suggest that, at low heat flux, other variables, such as flame residence time, may be more important than bark thickness for protecting the cambium from the effects of fire. Thus, the combination of intensity and time of heat exposure appear to be decisive.

The time during which temperature remains above 300 °C at the bark surface and absolute maximum temperature in the cambium were not good predictors of cambium damage in laboratory tests, in which the burning was homogeneous. However, they were good indicators in field trials, as the PB generated different levels of heating in the tree. These findings show the importance of confirming laboratory findings with additional field data

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Supplementary material 1

Table S1. Fixed effects. Mean, standard deviation (SD) and 95% and credibility interval for laboratory data.

	t60 - HF = 20 kW m^{-2}				HT - HF = 20 kW m^{-2}				Tmx - HF = 20 kW m^{-2}			
Fixed effects:	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q
(Intercept)	0.0000	0.1096	-0.2166	0.2164	0.0000	0.1688	-0.3351	0.3346	0.0000	0.1414	-0.2794	0.2792
BT	-0.4311	0.1229	-0.674	-0.1884	-0.1419	0.2096	-0.5580	0.2734	-0.2738	0.1586	-0.5871	0.0393
CV	-0.0441	0.1431	-0.3268	0.2384	-0.7273	0.2955	-1.3138	-0.1416	0.0804	0.1845	-0.2842	0.4448
te300	-0.1625	0.2044	-0.5663	0.2410	-0.1391	0.3252	-0.7846	0.5055	0.1378	0.2636	-0.3830	0.6582
Temx	0.2439	0.1406	-0.0340	0.5215	-0.3844	0.3359	-1.0511	0.2812	-0.1943	0.1813	-0.5526	0.1637
tf	0.7098	0.2830	0.1506	1.2687	0.9669	0.4899	-0.0054	1.9376	0.6607	0.3650	-0.0606	1.3814
ti	0.3595	0.2020	-0.0396	0.7583	-0.1408	0.3514	-0.8382	0.5554	-0.055	0.2605	-0.5696	0.4593
W	-0.1988	0.2780	-0.7482	0.3502	-0.5824	0.4058	-1.3877	0.2217	-0.4353	0.3586	-1.1438	0.2727
Bpo	0.2235	0.1797	-0.1316	0.5783	-0.9093	0.3653	-1.6343	-0.1854	-0.0523	0.2318	-0.5102	0.4053
	t60 - HF = 25 kW m^{-2}				HT - HF = 25 kW m^{-2}				Tmx - HF = 25 kW m^{-2}			
Fixed effects:	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q
(Intercept)	0.0000	0.0784	-0.1543	0.1542	0.0000	0.1122	-0.2211	0.2209	0.0000	0.0843	-0.1660	0.1658
BT	-0.4270	0.1055	-0.6346	-0.2195	-0.4363	0.1196	-0.6720	-0.2007	-0.4393	0.1134	-0.6626	-0.2162
CV	-0.0342	0.0942	-0.2197	0.1512	0.0068	0.1274	-0.2441	0.2576	0.0188	0.1014	-0.1807	0.2181
te300	-0.0908	0.1623	-0.4103	0.2285	0.0979	0.2034	-0.3028	0.4982	0.1577	0.1746	-0.1859	0.5011
Temx	0.0963	0.1169	-0.1338	0.3263	0.0319	0.2115	-0.3848	0.4484	0.0556	0.1257	-0.1919	0.3029
tf	0.2879	0.1558	-0.0189	0.5944	-0.1188	0.1752	-0.4639	0.2261	-0.0673	0.1676	-0.3973	0.2623
ti	0.1071	0.0982	-0.0862	0.3003	0.0197	0.1510	-0.2779	0.3170	-0.0793	0.1057	-0.2872	0.1285
W	0.0392	0.1068	-0.1710	0.2493	-0.1812	0.1372	-0.4517	0.0890	0.0041	0.1149	-0.2220	0.2300
Bpo	0.1777	0.0946	-0.0085	0.3639	0.2207	0.1310	-0.0374	0.4786	0.3754	0.1018	0.1750	0.5756
	t60 - HF = 50 kW m^{-2}				HT - HF = 50 kW m^{-2}				Tmx - HF = 50 kW m^{-2}			
Fixed effects:	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q
(Intercept)	0.0000	0.1075	-0.2116	0.2114	0.0000	0.1028	-0.2024	0.2022	0.0000	0.0859	-0.1691	0.1690
BT	0.1458	0.1235	-0.0974	0.3887	-0.4093	0.1181	-0.6419	-0.1769	-0.3399	0.0987	-0.5343	-0.1457
CV	-0.0149	0.1185	-0.2481	0.2182	-0.0258	0.1133	-0.2489	0.1971	-0.0256	0.0947	-0.2120	0.1607
te300	0.2312	0.1914	-0.1456	0.6078	0.1166	0.1831	-0.2438	0.4767	0.2373	0.1530	-0.0639	0.5383
Temx	-0.1206	0.1475	-0.4111	0.1696	-0.0966	0.1411	-0.3744	0.1810	-0.1598	0.1179	-0.3920	0.0722
tf	0.0684	0.1122	-0.1524	0.2891	0.0327	0.1073	-0.1785	0.2437	0.0801	0.0897	-0.0964	0.2565
ti	0.1858	0.1731	-0.1551	0.5264	0.0319	0.1656	-0.2941	0.3576	0.3015	0.1384	0.0291	0.5737
W	-0.0651	0.1222	-0.3056	0.1752	-0.3239	0.1168	-0.5540	-0.0941	-0.0963	0.0962	-0.2855	0.0929
Bpo	0.2940	0.1710	-0.0425	0.6302	0.2506	0.1678	-0.0798	0.5807	0.0234	0.1345	-0.2414	0.2881
	t60 - HF = 70 kW m^{-2}				HT - HF = 70 kW m^{-2}				Tmx - HF = 70 kW m^{-2}			
Fixed effects	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q
(Intercept)	0.0000	0.1039	-0.2044	0.2042	0.0000	0.0898	-0.1768	0.1767	0.0000	0.0942	-0.1854	0.1852
BT	-0.0735	0.1120	-0.2940	0.1467	-0.4421	0.0969	-0.6328	-0.2516	-0.4260	0.1016	-0.6259	-0.2263
CV	-0.0346	0.1181	-0.2671	0.1977	-0.0744	0.1022	-0.2755	0.1265	0.0656	0.1071	-0.1452	0.2763
te300	-0.1052	0.1235	-0.3483	0.1376	0.0231	0.1068	-0.1871	0.2332	-0.0702	0.1120	-0.2906	0.1501
Temx	-0.1166	0.1072	-0.3275	0.0942	-0.2981	0.0927	-0.4806	-0.1158	-0.1449	0.0972	-0.3361	0.0463
tf	0.2316	0.1203	-0.0053	0.4682	-0.1633	0.1041	-0.3681	0.0414	-0.0506	0.1091	-0.2654	0.1640
ti	0.0203	0.1083	-0.1929	0.2333	0.1175	0.0937	-0.0669	0.3017	-0.0105	0.0982	-0.2038	0.1827
W	0.1540	0.1216	-0.0855	0.3932	0.0145	0.1053	-0.1928	0.2216	-0.0568	0.1054	-0.2642	0.1504
Bpo	0.0362	0.1228	-0.2056	0.2777	0.1271	0.1063	-0.0822	0.3363	0.3149	0.1064	0.1056	0.5241

Supplementary material 2

Table S2. Fixed effects. Mean, standard deviation (SD) and 95% credible interval for field data in spring burnings and autumn burnings.

	t60 – Spring burning				HT - Spring burning				Tmx - Spring burning			
Fixed effects:	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q
(Intercept)	-0.0044	0.0892	-0.1799	0.1710	0.0000	0.0945	-0.1859	0.1858	-0.0027	0.0873	-0.1745	0.1690
BT	0.0859	0.1120	-0.1345	0.3062	-0.0246	0.1192	-0.2592	0.2098	-0.0480	0.1097	-0.2638	0.1676
CV	-0.1285	0.1126	-0.35	0.0928	-0.1244	0.1203	-0.3610	0.1120	-0.0576	0.1102	-0.2745	0.1590
te300	0.3375	0.0961	0.1485	0.5264	0.0140	0.1028	-0.1882	0.2160	0.4263	0.0940	0.2412	0.6111
Temx	0.1992	0.0974	0.0076	0.3906	0.3016	0.1038	0.0973	0.5056	0.1412	0.0953	-0.0463	0.3286
	t60 – Autumn burning				**HT - Autumn burning**				**Tmx - Autumn burning**			
Fixed effects:	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q	Mean	SD	0.025q	0.975q
(Intercept)	0.0004	0.1202	-0.2363	0.2368	0.0000	0.1176	-0.2316	0.2314	0.0003	0.1175	-0.2311	0.2316
BT	0.0293	0.1284	-0.2236	0.2819	0.0395	0.1272	-0.2109	0.2897	-0.2063	0.1256	-0.4536	0.0409
CV	0.0233	0.1295	-0.2317	0.2781	0.0299	0.1257	-0.2175	0.2771	0.0240	0.1267	-0.2255	0.2732
te300	0.1707	0.2309	-0.284	0.6249	0.1588	0.2302	-0.2945	0.6117	-0.2061	0.2258	-0.6508	0.2383
Temx	-0.2459	0.2317	-0.7021	0.2100	-0.2267	0.2310	-0.6815	0.2278	0.1496	0.2266	-0.2967	0.5955

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Chapter 4

Tree growth response to low-intensity prescribed burning in *Pinus nigra* stands: effects of burn season and fire severity

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Tree growth response to low-intensity prescribed burning in *Pinus nigra* stands: effects of burn season and fire severity.

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Abstract

Prescribed burning must not only reduce wildfire hazard but also minimize the negative fire effects on tree and even maximize the resistance and resilience of stand. However, fire effects on tree growth, particularly in European pines, are poorly understood. The study of the short-term post-burn growth response of trees in a mixed stand of *Pinus nigra* and *Pinus pinaster* and in a pure stand of *P. nigra* in the Cuenca Mountains (Spain) will enable to determine the disturbance on tree growth of prescribed burning treatments conducted in two different seasons. An experimental factorial design, with three treatments (no burning, spring burning and autumn burning) was established. Dendrochronological methods and mixed modelling were used to investigate whether tree growth responses are influenced by stand and tree characteristics, fire season and fire severity variables. The findings revealed that prescribed burning scarcely affected tree growth, although overall, a slight reduction was observed at short-term. The type of stand (mixed or pure) was not critical for tree growth. The individual tree characteristics were significant factors in all scenarios studied, being even more significant than the type of stand or plot traits. The inclusion of post-burn severity variables for the first time in tree growth models showed that maximum scorch height determined a main part of the variability of tree growth. The time during which temperature was above 60 °C in the cambium region was a significant factor after spring burnings and was not significant after autumn burning, what reinforced the importance of the insulating capacity of the thick bark in *Pinus nigra*. Litterfall one year after prescribed burning was not a significant factor in any of the models. Overall, the findings confirm the characteristic resistance of *Pinus nigra* to surface fires and favors the potential application of prescribed burning programmes for this species in the Mediterranean Basin.

Keywords: forest ecology; disturbance; fire ecology; forest management; forest fires.

Introduction

Large wildfires are a common occurrence in Mediterranean forests. However, within the framework of global climate change, interactions between climate and fire may accentuate the effects, or at least, alter the stress on ecosystems (Moritz and Stephens, 2008; Seidl et al., 2017). Prescribed burning (PB) is an active forest management tool that can reduce the intensity and severity of forest fires, when it strategically applied in both time and space (Fernandes and Botelho, 2003; Agee and Skinner, 2005; Piqué and Domènech, 2018). Beyond fire prevention, PB may also be beneficial for the vitality of ecosystems, recovering the distribution of the most balanced vegetation layers and occasionally improving biodiversity (Fernandes et al., 2013; Ferreira et al., 2015; Shakesby et al., 2015). Nonetheless, PB activity in southern Europe remains local in scope and the area treated is quite modest (Fernandes et al., 2016), although in recent years an effort is being made to include PB in forest management plans. In addition, forest managers often do not have access to accurate and research-supported information about the potential effects of prescribed burning on different forest ecosystems. This may lead to the application of unsuitable prescribed fire regimes that can cause significant damage or mortality to tree species and indirectly alter biotic and abiotic processes (Kerns and Day, 2018).

The responses of plant function to fire activity are complex and can vary widely (Bär et al., 2019). Thus, fire-surviving trees can be compromised in their physiological functionality, show reduced growth and be more likely to succumb to delayed death (e.g. Nesmith et al., 2015; Maringer et al., 2016; Thompson et al., 2017). Conversely, the injured trees may also benefit in the short- and mid-term from reduced competition (Battipaglia et al., 2014; Valor et al., 2015; Valor, 2018). However, few studies have modelled individual growth responses as a function of tree and stand characteristics including fire intensity (e.g. residence time above a lethal temperature) and severity variables (e.g. scorch height or total amount of litterfall after fire treatment) (Valor et al., 2015; Valor, 2018). In the Cuenca Mountains (Central Spain), prescribed burning is usually carried out in the early season (spring) and late season (early autumn). Although burn season can be easily controlled in PB treatments, little is known about how burn season affects post-fire tree growth (Valor, 2018). Some studies have shown that early season burning at the beginning of the annual growth period is more likely to cause heat damage (Hough, 1968; Garrison, 1972). Conversely, late season burning is likely to be of greater intensity because the fuels are drier (Skinner and Chang, 1996), which may exacerbate tree mortality (Thies et al., 2005).

Other specific stand and individual tree characteristics are widely recognized sources of variability in relation to tree growth (e.g. density, age, phenotypic plasticity, genetic variability and interactions with site factors) (Fritts, 1976; Rossi et al., 2008; Lucas-Borja and Vacchiano, 2018) and should be considered in tree growth studies. The present study involved a mixed stand of *Pinus nigra* Arn. ssp *salzmannii* (Spanish black pine) and *Pinus pinaster* Ait. (maritime pine) and a pure stand of *P. nigra*. The Cuenca Mountains are representative of areas where the *P. nigra*-*P. pinaster* ecotone generates stable stands with ecosystem services of high ecological value. This species richness has previously been associated with stand-level stability in the face of disturbance (DeClerck et al., 2006; Lloret et al., 2007; Klos et al., 2009), which may be explained by aggregate properties of species. Thus, exploration of the resilience of pure and mixed stands to perturbations such as prescribed burning is essential to establish recommendations for management and fire prevention strategies in this area. In addition, these species have been recognized to be adapted to surface fires with different characteristics: thick bark, high crown base height, self-pruning strategy and open structure in the case of *Pinus nigra* (e.g. Fernandes et al., 2008; Fulé et al., 2008; Pausas et al., 2009); or serotine cones and thick bark, large buds shielded by scales and by long needles in the case of *Pinus pinaster* (e.g. Tapias et al., 2001; Fernandes and Rigolot, 2007; Fernandes et al., 2008).

Moreover, in order to achieve the management objectives of PB, the burn window must be applied to reach the desired outcomes regarding fire behaviour and the associated impacts. Maximum scorch height and time of exposure to a critical temperature of 60 °C in the cambium area (e.g. Bauer et al., 2010) were used in the present study as proxies for damage to the cambium and severity of burning at tree level (Madrigal et al., 2019; Espinosa et al., 2020a). As far as we are aware, this is the first time that these variables have been included together in tree growth models. Inclusion of these variables may improve the results in relation to predicting the effect of burn intensity and duration of high temperatures on living tissues below the bark (damage or severity of fire in cambium and phloem), which have been considered the most important stress factors affecting trees (Rötzer et al., 2012).

In addition, the time of exposure to a critical temperature of 60 °C in the cambium area has been proposed for improving growth models (Valor et al., 2015). At the same time, tree growth is closely related to the amount of leaf-fall (Vanninen and Mäkelä, 2000). Tree growth often declines after fire (Poljanšek et al., 2015), because burning affects photosynthesis by increasing leaf-fall and thus reducing the photosynthetic efficiency of the remaining leaves (Wyant et al., 1983) and also by altering transpiration patterns and water use efficiency (Chambers et al., 1986). However, some positive effects on tree growth after PB have been described in relation to crown damage (Villarrubia and Chambers, 1978). Variables commonly used to indicate crown injury criterion include the level of crown scorched and crown scorch height (e.g. Botelho et al., 1998); However, nothing is known about the effects on tree growth of an increase in litterfall biomass after PB. In a recent study in the same experimental plots, we found that even low-intensity PB (with low values of crown scorched and crown scorch height) can increase the litterfall relative to that in unburned plots in the short-term (Espinosa et al., 2020b). Thus, litterfall biomass after PB was included for the first time in the model as a predictor of burn severity at stand level, relating crown damage and tree growth.

In this study, we examined how PB conducted as a surface fuel hazard reduction treatment in two different seasons (spring and autumn) affected the short-term growth of two types of *Pinus nigra* stands (mixed and pure). In the context of a more comprehensive study of the introduction of prescribed fire as management tool in Spanish forest stands (Madrigal et al., 2018), we hypothesize that low-intensity PB will not have an important effect on tree growth in the short-term and that the fire effects will be more notable in pure stands owing to the greater resilience of mixed stands to perturbations (DeClerck et al., 2016).

The study aims were as follows: (i) to ascertain any consistent variations in post-PB growth of *P. nigra*; (ii) to examine the potential effects of burning season; (iii) to analyze the interactions between species mixture and burning on tree growth, and (iv) to explore the effect of fire severity during burning at tree and stand level.

Materials and methods

Study sites

Two areas in the Cuenca Mountains (Iberian System, Central Eastern Spain) separated by a straight-line distance of 14 km were selected for the study (Fig. 1): a mixed stand of *Pinus nigra* (89±11%) and *Pinus pinaster* (11±11%) in El Pozuelo and a pure stand of *P. nigra* in Beteta. The main characteristics of both stands are shown in Table 1.

According to the data recorded at the Cañizares weather station (940 m asl), and provided by the State Meteorological Agency of the Spanish Government (AEMET, 2018), the mean annual temperature was 11.3 °C and the average precipitation, 747 mm in the last 46 years.

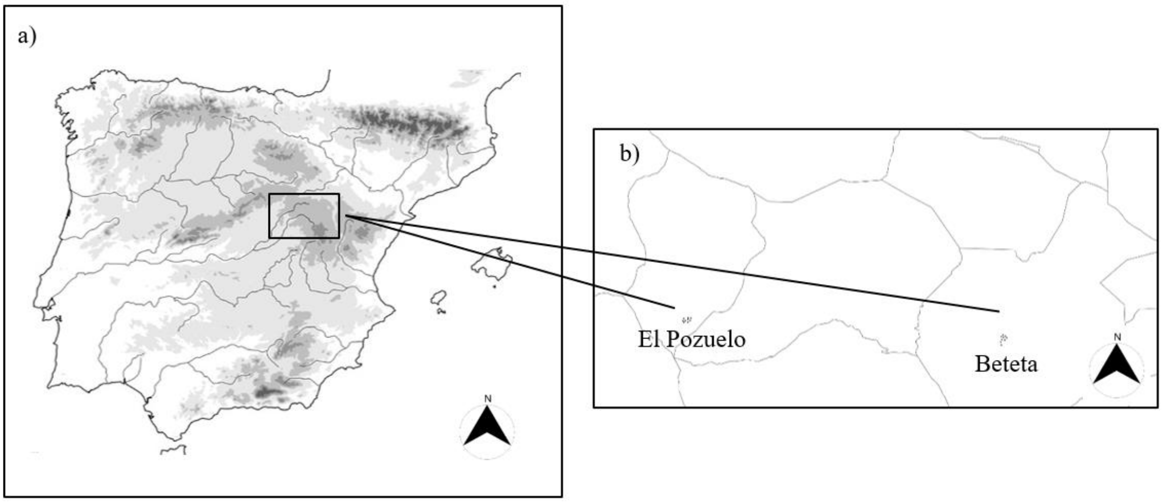


Fig. 1. Locations of the study plots in (a) the Iberian Peninsula (1:1.250.000); (b) El Pozuelo (mixed *P. nigra*-*P. pinaster* stand) and Beteta (pure *P. nigra* stand) (1:50.000).

Table 1. Main characteristics of mixed and pure stands (mean and standard deviation) (Espinosa et al., 2020c)

--	Mixed stand	Pure stand
Coordinates - Longitude	40º 33' 36'' N	40º 33' 06'' N
Coordinates - Latitude	002º 15' 56'' W	002º 06' 32'' W
Main species	*Pinus nigra* (89±11%) *Pinus pinaster* (11±11%)	*Pinus nigra* (100%)
pH of topsoil *	7.3 (clay texture)	6.9 (loamy-sand texture)
Elevation	1016±5 m asl	1232±7 m asl
Slope	3-8%	3-10%
Stand density	627±238 trees ha^{-1}	1286±339 trees ha^{-1}
Stand basal area	25.4±9.7 m^2 ha^{-1}	36.6±10.7 m^2 ha^{-1}
Dominant tree height	18.6±0.8 m	17.0±1.6 m
Tree height	12.2±2.0 m	13.2±2.7 m
Diameter at breast height (DBH)	19.8±2.6 cm	18.8±4.1 cm
Bark thickness	1.7±0.3 cm	1.7±0.4 cm

(*) Data from Plaza-Álvarez et al. (2017).

Experimental Design

Within the study site, a total of 18 plots (n = 9 in the mixed stand and n = 9 in the pure stand), each measuring 50 m × 50 m, were selected following a block design. For data collection, subplots of 30 m × 30 m were established in order to prevent the edge effect. Three treatments (no burning, spring burning and autumn burning) were applied to each type of stand, with 3 replicates per block (for further details, see Espinosa et al., 2018). In order to obtain data on post-fire growth, 15 *P. nigra* trees in each plot were selected for study (n = 135 in the mixed stand and n = 135 in the pure stand). The trees were chosen to represent all of the diametric classes present in each particular plot.

Burning and fire severity at tree level

Spring burning was conducted in May 2016, before the main growth season; while autumn burning was carried out in November 2016, after the main growth season. The strip ignition technique was applied at a distance of 1-2 m downhill facing a headwind. This technique is the most widely used in the study area to produce low- medium-intensity fire (Vega, 2001). During the burning, the temperature and relative humidity (Geonica; STH-5031) and wind speed (Casella; 178031C-3) were recorded every 10 minutes at a meteorological station located adjacent to the study plots. In order to estimate burn severity at tree level during the burning, the temperature in the cambial region (inner bark) of the 15 selected *P. nigra* trees in each plot was monitored at a height of 0.6 m (Graves et al., 2014) with type K 1 mm diameter inconel-sheathed thermocouples (response time, 0.3 s). The thermocouples were connected to data loggers (DT-USB TCDirect®), which recorded the data with a frequency of 1 second. Maximum scorch height (hypsometer VERTEX IV) was recorded to evaluate fire severity in the trunk (Martinson and Omi, 2013; Espinosa et al., 2019). The main parameters measured during prescribed burning in the selected trees are shown in Table 2.

Table 2. Main parameters measured during prescribed burning in mixed and pure stands of *P. nigra* (Espinosa et al., 2020a).

S	PT	T	RH	WS	RS	FLI*	FH	FL*
--	--	ºC	%	m s^{-1}	m min^{-1}	kW m^{-1}	cm	cm
Mixed stand	Spring burning	21.5 (1.2)	47.7 (5.3)	0.8 (0.6)	0.65 (0.21)	20.0 (8.8)	53 (15)	30 (6)
Mixed stand	Autumn burning	11.9 (0.4)	67.0 (1.3)	0.3 (0.3)	0.59 (0.31)	11.2 (6.6)	17 (10)	23 (6)
Pure stand	Spring burning	20.4 (1.5)	32.7 (2.3)	0.8 (0.1)	0.76 (0.24)	32.6 (13.3)	43 (8)	38 (8)
Pure stand	Autumn burning	12.0 (0.9)	43.5 (0.8)	0.1 (0.1)	0.72 (0.22)	13.8 (10.7)	26 (13)	25 (9)

S: type of stand; PT: plot treatment; T: air temperature; RH: relative humidity; WS: wind speed; RS: fire rate of spread; FLI: fire-line intensity; FH: flame height; FL: flame length. Standard deviation in brackets. (*) Data from Byram (1959).

Tree-ring width analysis

In November 2018 (30 months after spring burning and 24 months after autumn burning), one core was extracted from each selected *P. nigra* tree at breast height (n = 135 in each stand), perpendicular to the terrain slope, with an increment borer (0.5 cm inner diameter) (Fritts, 1976). All cores were prepared following standard dendrochronological techniques (Stokes and Smiley, 1968). The cores were mounted on grooved boards and sanded until the tree rings were clearly visible.

Tree ring widths were measured using a tree ring measuring stage with a precision of 0.01 mm (Lintab, Rinntech) and recorded in a computer with TSAP software (Rinn, 2003). Our study species at our study site form clearly defined rings boundaries with abrupt transitions between late wood and early wood (Martin-Benito et al., 2017). All cores were dated and visually cross-dated to detect the presence of false and incomplete rings. COFECHA software was used to check cross-dating and validate measurement quality (Holmes, 1983) (Table 3). The ring widths were converted into annual basal area increments (BAI) by using the equation BAI = p (r^2_t - $r^2_{(t-1)}$), where “r” is the tree radius and “t” is the year of tree-ring formation. BAI was used as a proxy for tree growth because it is less dependent on age and thus prevents the need for detrending (Biondi, 1999), which could also remove low frequency variability. In addition, the BAI was standardized and shown in box plots. The standardization was performed using the mean tree growth value of the immediately five preceding years unaffected by the burning treatment (2010-2015).

Table 3. Main dendrochronology statistics.

--	Both stands	Mixed stand	Pure stand
Series intercorrelation	0.439	0.542	0.431
Average mean sensitivity	0.337	0.374	0.308

Litterfall: fire severity at stand level

After the prescribed burning, 8 litterfall collectors (0.38 m^2) were installed in each plot (total n = 144) to evaluate fire severity at stand level (crown damage). In order to ensure the representativeness of the samples, the collectors were distributed so that they covered the entire working area. The litterfall collection system was designed in accordance with the recommendations and parameters outlined in the Manual of the United Nations Economic Commission for Europe (UNECE), under the project entitled "International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests" (ICP Forests, Level II Plots) (Ukonmaanaho et al., 2016) to guarantee the quality and quantity of the sample (for more details, see Espinosa et al., 2018). On each collection day, the samples were transported to the laboratory and oven-dried at 65 °C to constant weight. Litterfall biomass was collected during the year immediately after spring burning and autumn burning in mixed and pure stands and used to characterize fire severity at stand level (Table 4).

Statistical analysis

Three models were constructed in order to analyse the tree growth results: model SB for spring burned plots; model AB, for autumn-burned plots; and model NB for non-burned plots. For each treatment, a linear mixed model (Eq. 1) was used to describe short-term impact of PB on tree growth. BAI was selected as the target variable. A collinearity analysis was performed, tree height (H) which had a strong collinearity with DBH was excluded. Likewise, nested effect between random variables were considered. According to the collinearity and significance the best fit model considered the following potential predictors (fixed factors): tree height (H), percentage of live crown height (Hc), diameter at breast height (DBH), maximum scorch height (SMx), time during which the temperature in the cambium area was higher than 60 °C (t60) and time during which the temperature in the bark surface was higher than 300 °C (t300). Possible differences in composition and characteristics of stand (S), variability between plots (P) and total amount of litterfall collected per plot one year after PB (L) were resolved by adding a random effect.

[Eq 1]

$$ln\left(BAI_{k(jy)}+1\right)=\beta_0+\beta_1\cdot Hc+\beta_2\cdot DBH+\beta_3\cdot SMx+\beta_4\cdot t60+\beta_5\cdot t300+\gamma_{0k(j)}+\alpha_{0k(j)}+L\alpha_{k(jy)}+\varepsilon_{k(jy)}$$

where β_0 is the overall intercept; β_i (i =1,…,5) are the parameters adjusting the fixed effects; k is the study site index; $k(j)$ is the tree index nested in the study site; y is the month of measurement index; $\gamma_{0k(j)}$, $\alpha_{0k(j)}$ represents the random effects associated with study plots and stand respectively, $L\alpha_{k(jy)}$ is the interaction between stand and litterfall; and $\varepsilon_{k(jy)}$ is the error term. No pattern was observed in the residuals of any models.

All statistical tests were performed using R software (v. 3.0.1, R Foundation for Statistical Computing), specifically with the *nlme* and *lme4* packages for linear mixed-effects modelling. A significance level of 95% was established for detecting differences between treatments.

Table 4. Main variables used to model the impact of prescribed burning on tree growth.

S	PT	H	Hc	DBH	SMx	t60	t300	L16	L17
--	--	m	%	cm	cm	s	s	kg ha^{-1}	kg ha^{-1}
Mixed stand	No burning	12.2 (5.0)	50.3 (16.2)	19.5 (10.3)	--	--	--	3171 (649)	3532 (585)
Mixed stand	Spring burning	11.9 (5.0)	48.0 (15.2)	18.1 (10.0)	54 (50)	31 (82)	10 (26)	3257 (599)	--
Mixed stand	Autumn burning	12.2 (5.2)	50.1 (15.8)	19.4 (10.4)	46 (45)	16 (59)	6 (20)	--	2732 (325)
Pure stand	No burning	13.1 (4.9)	39.4 (14.4)	19.6 (10.1)	--	--	--	1989 (519)	2393 (739)
Pure stand	Spring burning	13.9 (4.4)	37.9 (15.1)	19.5 (9.4)	167 (201)	23 (93)	44 (167)	3482 (129)	--
Pure stand	Autumn burning	14.5 (5.3)	36.2 (12.5)	21.7 (11.3)	132 (169)	16 (81)	33 (149)	--	3629 (527)

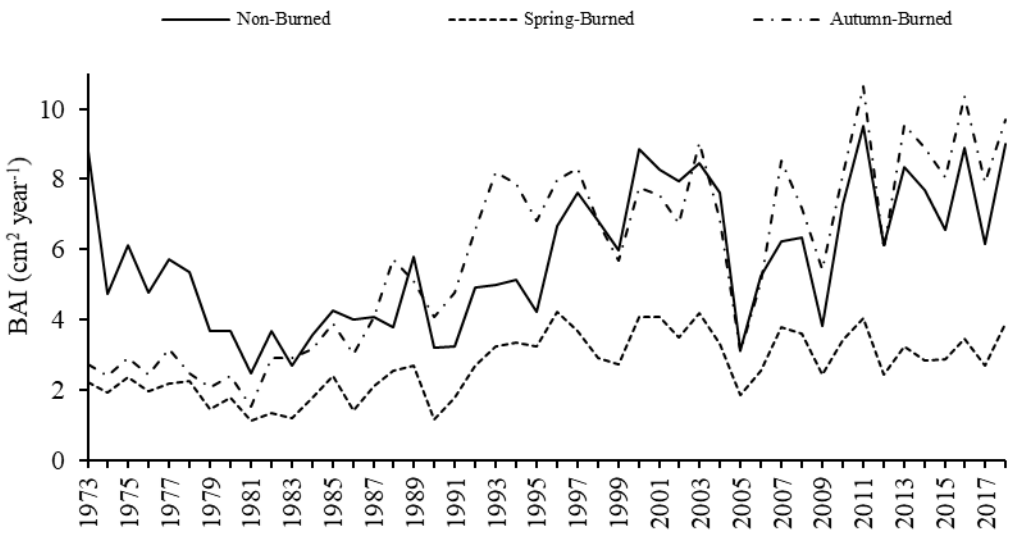
S: type of stand; PT: plot treatment; H: tree height; Hc: percentage of live crown height; DBH: diameter at breast height; SMx: maximum scorch height; t60: time during which the temperature in the cambium area was higher than 60 °C; t300: time during which the temperature in the bark surface was higher than 300 °C; L16: litterfall biomass from May 2016 to April 2017; L17: litterfall biomass from November 2016 to October 2017. Standard deviation in brackets.

Results

The annual trends in mean basal area increment (BAI) for the three treatments in the mixed and pure stand were similar (Fig. 2). However, tree growth was lower in the spring burned plots in the mixed stand than in the unburned and autumn burned plots (Fig. 2a). Hence, the BAI was standardized and shown in box plots per type of stand (mixed or pure) for comparison of tree growth (Fig. 3 and Fig. 4). In both stands, tree growth was lower after spring burning (Fig. 3 and Fig. 4). Conversely, in the mixed stand, tree growth increased in the following year after autumn burning (Fig. 3), but not in the pure stand (Fig. 4).

The type of stand (mixed or pure) did not have influence on tree growth (Table 5). The tree variables, percentage of live crown height and diameter at breast height, were significant factors in the three models (although percentage of live crown height in Model NB was weakly significant; $p < 0.1$) (Table 5). Regarding fire severity variables at tree and stand level (maximum scorch height, time during which temperature in the cambium area was higher than 60 °C, time during which temperature in the bark surface was higher than 300 °C and litterfall biomass), maximum scorch height was a significant factor (Model SB and AB). A higher scorch height is related to a greater fire severity, which may negatively influence on tree growth. Time during which temperature in the bark surface was higher than 300 °C was only significant in Model SB, as well as t60 which was significant in the same model. In this sense, the spring burning appeared to have a greater effect on tree growth than autumn burning. The values of the variables related with the residence time above lethal temperatures did not have an impact on the autumn burning due to the low-intensity of fire. Litterfall was not a significant factor in any of the models.

a) Mixed stand



b) Pure stand

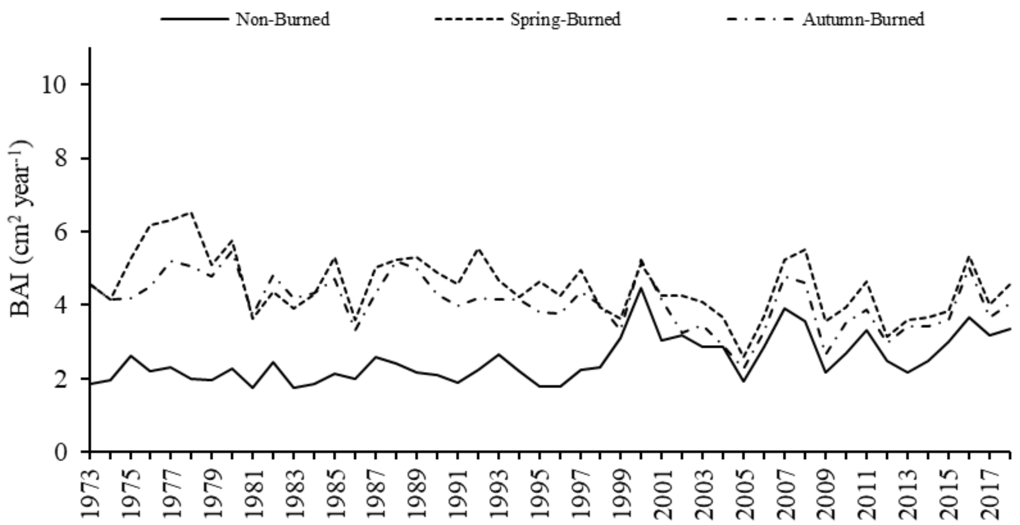


Fig. 2. Comparison of annual trends in mean basal area increment (BAI) in (a) the mixed stand and in (b) the pure stand. Unburned plots (solid line), spring-burned plots (dashed line) and autumn-burned plots (dotted line). Spring burning was conducted in May 2016, autumn burning was carried out in November 2016.

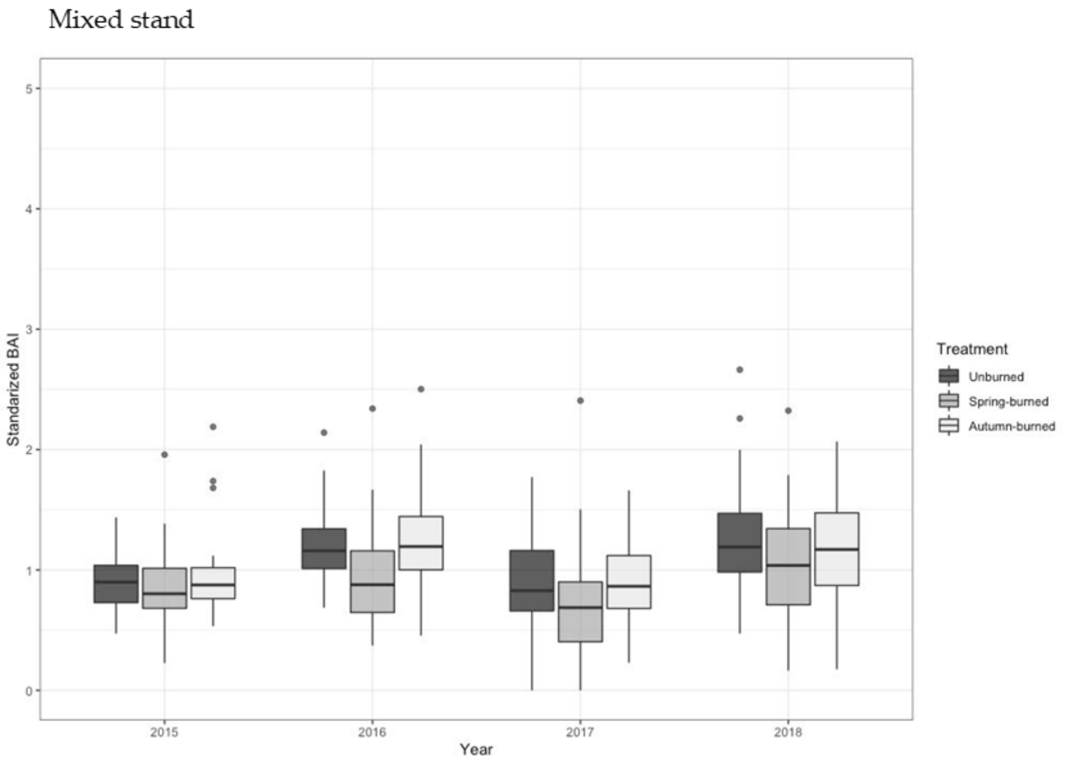


Fig. 3. Box plot showing the differences in standardized BAI in the mixed stand. Spring burning was conducted in May 2016, autumn burning was carried out in November 2016.

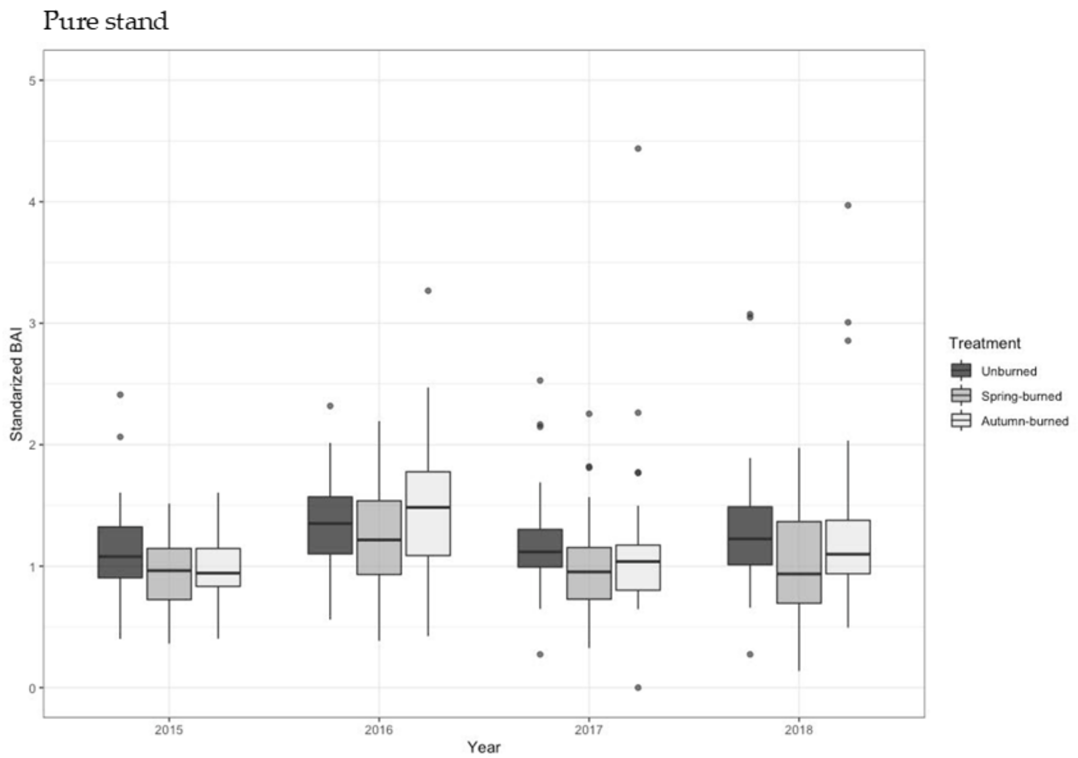


Fig. 4. Box plot showing the differences in standardized BAI in the pure stand. Spring burning was conducted in May 2016, autumn burning was carried out in November 2016.

Table 5. Summary of statistical results.

Fixed effects	Model SB				Model AB				Model NB			
	Estimate	SE	*t* value	*p* value	Estimate	SE	*t* value	*p* value	Estimate	SE	*t* value	*p* value
Intercept	1.364×10^{1}	6.925×10^{-2}	19.696	<0.001	1.297×10^{1}	9.274×10^{-2}	13.988	0.0026	1.488×10^{1}	1.397×10^{-1}	10.653	0.0087
Hc	**6.541×10^{-2}**	**9.378×10^{-3}**	**6.974**	**<0.001**	**5.233×10^{-2}**	**1.221×10^{-2}**	**4.285**	**<0.001**	1.855×10^{-2}	1.068×10^{-2}	1.738	0.0824
DBH	**6.448×10^{-1}**	**9.864×10^{-3}**	**65.367**	**<0.001**	**6.619×10^{-1}**	**8.516×10^{-3}**	**77.728**	**<0.001**	**5.093×10^{-1}**	**1.037×10^{-2}**	**49.138**	**<0.001**
SMx	**-3.762×10^{-2}**	**8.500×10^{-3}**	**-4.426**	**<0.001**	**-2.291×10^{-1}**	**3.092×10^{-2}**	**-7.411**	**<0.001**	--	--	--	--
t60	**-1.264×10^{-2}**	**5.560×10^{-3}**	**-2.273**	**0.0231**	-1.790×10^{-1}	1.175×10^{-1}	-1.524	0.1277	--	--	--	--
t300	**-2.796×10^{-2}**	**6.159×10^{-3}**	**-4.540**	**<0.001**	-1.039×10^{-1}	7.387×10^{-2}	-1.406	0.1598	--	--	--	--

Random effects	Model SB			Model AB			Model NB		
	Variance	SE	Pr (>Chisq)	Variance	SE	Pr (>Chisq)	Variance	SE	Pr (>Chisq)
S	0.0000	0.0000	1.0000	0.0030	0.0554	0.8590	0.0287	0.1696	0.2553
P	0.0177	0.1330	1.0000	0.0190	0.1379	1.0000	0.0013	0.0364	1.0000
L	0.0105	0.1028	1.0000	0.0188	0.1373	1.0000	0.0287	0.1696	1.0000

Hc: percentage of live crown height; DBH: diameter at breast height; SMx: maximum scorch height; t60: time during which the temperature in the cambium area was higher than 60 °C; t300: time during which the temperature in the bark surface was higher than 300 °C; S: type of stand; P: plot; L: accumulated litterfall during the year following the burning treatment; SE: standard error. Values shown in bold indicate significant effects ($p < 0.05$).

Discussion

The study findings highlight the importance of modelling tree growth response to PB as a function stand and tree characteristics, fire season and fire severity variables to gain some insight into individual tree responses. As far as we know, this the first time that variables associated with fire severity, such as time of exposure to a critical temperature in the bark surface and cambium area and the effect of litterfall after PB, have been included in a model of tree growth response to prescribed burning. These variables play a key role in determining the stress level in trees after fire and they may be essential to enable estimation of post-burn growth and mortality rate (Varner et al., 2009).

The findings showed that prescribed burning at short-term scarcely affected tree growth. Similar findings have been reported by other authors (e.g. Sala et al., 2005; Valor et al., 2015; Bottero et al., 2017). Nevertheless, in the following year after autumn burning in the mixed stand, a slight increase in tree growth was observed relative to the unburned plots (Fig. 4). One possible explanation for this is that fire acts as a mineralizing agent releasing nutrients instantaneously, in contrast to slower natural decomposition processes (Debano et al., 1998; DeLuca and Zouhar, 2000; Alfaro-Sánchez et al., 2018). However, such nutrient pulses are usually temporary and the values return to or fall below pre-treatment values within 1-2 years (Sala et al., 2005), as in the 3rd year of the study. In spring-burned plots in the mixed stand, and in spring and autumn-burned plots in the pure stand, no increase in tree growth was observed after burning. The trend in reduction of tree growth has generally been described as short-term (1-3 years), with growth rates returning to approximately pre-fire levels thereafter (Seifert et al., 2017), as it happened in the mixed stand. Reduced growth of *Pinus ponderosa*, *P. contorta* and *P. palustris* has been observed 1 and 2 years after burning (by Sutherland et al., 1991; Peterson et al., 1991; Ford et al., 2010, respectively). However, other longer growth recovery rates have also been reported, such as the slight decline in tree growth observed 6 years after fire in *Pinus ponderosa* and *Pinus sylvestris* (Busse et al., 2000; Blanck et al., 2013).

Although several authors have reported that mixed stands are more stable than pure stands (e.g. Felton et al., 2010), with a lower response after disturbance and faster recovery (Loreau, 2001; Jactel et al., 2009); and even, other studies involving soil properties and litterfall carried out in the same experimental plots (Plaza-Álvarez et al., 2017; Espinosa et al., 2020b) have revealed the same positive effect on mixed stands after prescribed burning, the present findings did not allow confirmation of this point in relation to tree growth.

Although there is abundant literature relating growth responses to climate and to potential decline in vitality in Mediterranean forests (e.g. Linares et al., 2009; de Luis et al., 2013), the relationships may depend on many stand- and tree-level factors (Martin-Benito et al., 2011; Lucas-Borja and Vacchiano, 2018). Our study findings showed that the tree variables included in statistical analysis (percentage of live crown height and DBH) are significant factors in all three models (although percentage of live crown height showed less significance in Model non-burned). Therefore, regardless of the type of stand (mixed or pure), the particular characteristics of each tree may explain the variability in growth after fire. This may be partly explained by the low percentage of *Pinus pinaster* in the mixed stand or the low-intensity of burning. In this regard, some researchers proposed that factors affecting tree growth rate before fire continue to affect post-fire growth (e.g. Sutherland et al., 1991), although others have suggested the opposite (Busse et al., 2000).

Maximum scorch height was a significant variable that negatively influenced tree growth after spring and autumn burning. It is therefore possible that higher scorch heights may imply greater added stress on trees and should therefore be avoided. Indeed, fire-damaged trees require stored carbohydrates to replenish tissues, depleting carbohydrate reserves, often compromising tree growth (Varner et al., 2009). Despite these results, maximum scorch height proved to be a random variable regarding litterfall in the same experimental plots, although it was probably due to maximum scorch height was reached below the mean height of the first live branch (Espinosa et al., 2020b).

Time during which the temperature remained above 300 °C in bark surface had a significant response regarding tree growth only during spring PB; in addition, time during which the temperature remained above 60 °C in inner bark (cambium area) was also significant during spring PB. Although, as mentioned, maximum scorch was a significant variable after spring and autumn burning treatments, it seemed to have a slightly greater effect after spring PB. Overall, spring burning has been described to be more disruptive to trees because carbohydrate reserves are at their lowest levels at the beginning of the annual growth period (Hough, 1968; Garrison, 1972). Furthermore, a higher level of damage to fine roots (abundant during this period) has been pointed out (Swezy and Agee, 1991; Harrington, 1993). Hence, PB carried out in autumn seemed to have less impact on tree growth, however disturbance in other tree and stand processes (e.g. impact on litterfall) should be also considered in the prescriptions plans (Espinosa et al., 2020b). In this regard, fuels have been described as typically drier later in the year (Skinner and Chang, 1996), which correspond with the regular autumn burning season, what may imply higher mortality rates (Thies et al., 2005). The relationship between tree growth and t60 may be because the insulating capacity of bark completely protects the cambium at low-intensity PB (Espinosa et al., 2020a). *Pinus nigra* is known to possess thick bark (especially in the lowest part of stem), as a result of the adaptation to frequent surface fires, which protects the cambium against overheating by fire (e.g. Fulé et al., 2008; Odhiambo et al., 2014; Wesolowski et al., 2014). In addition, bole scorch height does not necessarily imply an effect on the cambium if it does not affect the entire bole circumference (Seifert et al., 2017), which in turn disturbs the supply of water and nutrients to the leaves and translocation of photosynthates to the roots (Rozas et al., 2011). Furthermore, Bottero et al. (2017) also suggested that the minimal presence of fire scars associated with heat damage to the cambium and other live tissues does not significantly affect tree growth. However, this reflected the importance of including variables associated with the flame residence time in the tree growth models.

The total litterfall biomass collected one year after prescribed burning was not a significant variable in the tree growth response. The low-intensity of the prescribed burning and the high height of the first live branch may explain the weak significance of this variable. Nevertheless, litterfall was found to be beneficial to tree growth, in some cases. In this regard, PB improves tree efficiency by eliminating the unproductive lower branches

(Villarrubia and Chambers, 1978) and less efficient needles from the lower part of the crown (Wyant et al., 1983), which may enhance tree growth (Certini, 2005; Retzlaff et al., 2018). Or by contrast, fire disturbance in the crown is typically related to the reduction in tree growth (Busse et al., 2000), which represents photosynthetically active tissue and a source of energy (e.g. De Micco et al., 2013; Poljanšek et al., 2015). Indeed, some authors maintain that allocation of resources prioritises foliage and buds (Gordon and Larson, 1968; Waring and Schlesinger, 1985; Oliver and Larson, 1996), so that an increase in litterfall may mobilize resources in these parts (mainly to guarantee photosynthesis) at the expense of tree growth. However, the findings obtained did not allow confirming the significance of this variable on tree growth, at least in the short-term.

Conclusions

The findings suggest that PB (spring and autumn) is a potentially valuable management tool for reducing fire hazard, increasing the resilience in pure and mixed *P. nigra* stands with scarcely effect on short-term tree growth. Although a slight increase in tree growth was observed after autumn burning in the mixed stand after fire, the overall trend was for a reduction in growth. A longer-term study may be necessary to establish the recovery rate of the stands, particularly in pure stand. Differences between mixed and pure stands were not critical to tree growth, what do not allow confirmation of the initial hypothesis about the weaker effect of perturbation in the mixed stand. Individual tree characteristics proved to be more important for growth than the type of stand, treatment or fire severity in low-intensity PB. The differences between burning seasons were notable. The inclusion of post-burn severity variables for the first time in tree growth models showed that maximum scorch height influences tree growth. This is an easily measurable variable that must be considered in burning prescriptions. Recognized surface fire adaptations of *Pinus nigra* such as the insulating capacity of the thick bark and the high crown height completely protected the tree; however, special attention should be given to variables related to exposition to a critical temperature in outer and inner bark, particularly after spring burning. Although autumn prescribed burning seemed to have less impact on tree growth, disturbance in other stand dynamics should be also considered. The litterfall biomass one year after prescribed burning was not a significant variable for tree growth. These findings could be used to improve burn prescriptions and evaluation of PB in *P. nigra* stands.

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Chapter 5

General discussion and management implications

Doctoral thesis, ***Prescribed burning to reduce fire severity: effects on pine forests in the Iberian System*, 2021**

Juncal Espinosa Prieto

The findings of this doctoral thesis confirmed the proposed hypothesis that low-intensity PB, at tree and stand level, causes scarce damage in mixed and pure *Pinus nigra* stands in the Iberian System. The findings can be used by managers to improve prescriptions and to evaluate fire severity and fire impacts after prescribed burning.

Effect of low-intensity prescribed burning in two seasons on litterfall biomass and nutrient content

The accumulated litterfall biomass during the study period was within the range of amounts of natural litterfall in Spanish conifer stands (Subchapter 2.1 and 2.2); this suggests that the PB treatment did not significantly alter the amount of litterfall accumulated. The annual patterns of biomass cycles were also not significantly altered, with maximum peaks occurring in summer months (Subchapter 2.1 and 2.2). The results obtained from the analysis of the LAI one year after the spring burning treatment confirm the scarce impact of PB at stand level (Subchapter 2.1), although long-term monitoring and data from autumn PB were required to confirm this finding. In addition, the treatments had a scarce impact on carbon and macronutrient contents (Subchapter 2.2).

The influence on litterfall of stand composition, burn season and longevity of prescribed burning

Subchapter 2.2 reported no significant differences in the total amount of litterfall biomass collected in the two types of stand (which may be due to all treatments being considered together in the statistical analysis), although the contrasting results following spring and autumn burns may be explained by the composition of stands. However, in Subchapter 2.3, significant differences appeared by using a Bayesian approach, an in-depth analysis of several variables involved in the litterfall process. One year after the spring PB treatment in the mixed stand, the difference between litterfall biomass in burned and unburned plots was 3%, while in the pure stand the corresponding difference was 75% (Subchapter 2.1). By the end of the study (Subchapter 2.2), litterfall was more abundant in unburned plots than in burned plots in the mixed stand (which may be explained by the fact that fire hastened the needle cycle) and although the differences decreased over time, they remained visible (17%) in the pure stand. Similarly, after autumn PB, no effect was observed in the mixed stand; however, there were significant differences in the pure stand, particularly in the maximum litterfall season. Overall, a higher level of resistance and resilience and faster recovery after disturbance are expected in mixed stands (e.g. Loreau et al., 2001; Jactel et al., 2009; Felton et al., 2010). The contrasting results obtained following PB treatments can be partly explained by the different species composition in the two stands.

Along with the type of stand, the Bayesian approach revealed a positive response of litterfall due to season (Subchapter 2.3). The effect of spring PB was more marked than autumn PB, at least during the first year (Subchapter 2.1) and particularly in the pure stand. In this context, the spring PB preceded the maximum litterfall season, coinciding with the months of physiological drought in the Mediterranean basin (Subchapter 2.3). However, the recovery rate was slower after autumn PB, although further information about the effect of fire disturbance of soils is required. In addition, a delayed effect of perturbation was observed in the pure stand after autumn PB, with significant differences mainly observed in the period between November and January (Subchapter 2.2). A delayed effect has also reported by Blanco et al. (2006) and is probably related to the subsequent impact of meteorological events, particularly snowfall and storms (Subchapter 2.3).

The immediate impact of treatment and the subsequent recovery of fuel load and structure are considered critical for evaluating the treatment effectiveness. The PB carried out in this study had an immediate effect (3-4 months after PB) (Subchapter 2.1), which then decreased over time (30-24 months) (Subchapter 2.2). If PB has a severe and immediate impact, it may cause an increase in fallen biomass, thus leading to a higher risk of future fire and a short-lived treatment efficacy.

The findings obtained have important management implications. Thus, PB must be carefully planned by taking into account high mean maximum temperatures and severe dry conditions. In addition, specific meteorological events (snowfalls and storms during winter-autumn) may increase the abundance of litterfall biomass. Overall, low-intensity PB had a short-term impact on litterfall biomass in mixed and pure stands, and the effect decreased over time. Assessing the equilibrium between litter consumption during burning and the litterfall after burning can help managers to improve prescriptions with the aim of reducing fuel accumulation and increasing the duration of the effect of the treatment (time required for recovery of fuel load to pre-burn state). A moderate fuel consumption and a low rate of litterfall in the experimental plots were observed, thus the effects of the treatment lasted around 4 years (although this must be confirmed). This duration of treatment effect was also suggested by Espinosa et al. (2019) for a wide range of *Pinus pinaster* stands. The treatment effectiveness has important implications regarding spatio-temporal PB planning, and this is the first scientific reference on this topic in relation to forest stand management in Europe.

Influence of low-intensity prescribed burning on litterfall fractions

The needle fraction followed a similar trend to that observed for total litterfall biomass. This was expected as needles comprise the most abundant fraction, accounting for 58% and 57% of litterfall in mixed and pure stands respectively (on average for the overall study period) (Subchapter 2.2). Therefore, PB appears to have had a limited effect on the needle fraction.

Fluctuations in cone production were observed during the years considered. A peak was almost always distinguished after the pine flowering season. In the pure stand, there were more cones in the burned plots than in the unburned plots, whereas the opposite was found in the mixed stand (Subchapter 2.1 and 2.2). A marked seasonality was also observed in the inflorescences, with variation in peaks between May and August. Following spring burning, a greater number of inflorescences were collected in unburned plots than in burned plots in both stands (Subchapter 2.1 and 2.2). Longer-term study of this effect is required because, if fructification is similar among plots, a decrease in inflorescence would not be a good indicator of fructification potential, probably because PB does not affect the female flowers mainly found in the apical fast-growing shoots at the top of the tree. However, a larger number of cones in the unburned plots would presumably indicate that

PB significantly affects the regeneration potential in *Pinus nigra* stands. Although it is difficult to determine the scope of fire effects on the cone and inflorescence fractions, given the particular fructification characteristics of each species, as well as other external factors (Del Cerro Barja et al., 2009), the findings are nonetheless important for forest managers, who would be able to plan PB in non-masting years or initial seedling recruitment to ensure periodic fructification and natural regeneration, thus contributing to the persistence of black pine forests.

The branch fraction showed high inter- and intra-annual variability in both stands (Subchapter 2.1 and 2.2), with many peaks mainly due to meteorological events (e.g. snowfall, storms, high winds or drier conditions). Indeed, autumn PB was suggested to increase the number of branches that fell as a result of a combination of thermal damage and snowy or stormy days (Subchapter 2.3). However, the low-intensity PB carried out in this study and the fire-prone strategies described in *Pinus nigra* and *Pinus pinaster* stands (e.g. high crown base height and self-pruning ability) did not enable confirmation of the existence of this aggregate effect (Subchapter 2.3). From a management perspective, moderate high-intensity PB should be avoided in later autumn to prevent the accumulation of branches around the tree, which may imply a longer residence of the fire, overheating the tree stem and often affecting the cambium area (Subchapter 2.3 and Chapter 3).

Some inter-annual variability in bark was also observed, although no clear trend was apparent. As with branches, meteorological events can influence bark litter production. Although not significant, bark biomass was higher in burned plots from both stands during the first year, suggesting a direct effect of scorching on the tree trunk, leading to detachment of the outer bark. Significant differences in this fraction between zones may be due the particular characteristics of bark in maritime pine, which is laminated and the outer layer is exfoliated during combustion (Fernandes and Rigolot, 2007). However, this cannot be confirmed given the irregularity of the bark patterns.

Several peaks were observed in the miscellaneous fractions. Some winter peaks can be attributed to the leaves of other deciduous species, mainly *Quercus* spp., although these are scarce in the study area. Peaks in February and May–July may correspond to seeds, but the amount of seed is very small. Miscellaneous peaks may also be associated with heavy rain and storms (Navarro et al., 2013).

Nutrient changes in litterfall after prescribed burning

The differences between stands in carbon (C) and macronutrient concentrations in litterfall (nitrogen, N; phosphorus, P; potassium, K; calcium, Ca; and magnesium, Mg) reflected the particular characteristics at each site (Subchapter 2.2). Burning treatments had little effect on the C concentrations in the different fractions in both types of stand. A slight overall increase in N, P and K was recorded, being this nutrient critical to the tree recovery.

The N foliar values are consistent with the N values in old needles (ICP Forests). In addition, neither transport of photosynthate to the crown nor nutrient and water storage were expected to be disrupted (Subchapter 2.3 and Chapter 3), because prescribed burning scarcely affected photosynthetic production in the upper part of the crown. Scorch height was generally lower than the height of the first live branch and the highest scorch marks on the stems were due to the presence of lichens, which burned briefly. Therefore, the random effect of these two variables (scorch height and height of the first live branch) on litterfall reported in Subchapter 2.3 is not surprising. An increase in all fractions, at least in the short-term, was detected in P concentrations (particularly in the mixed stand). Similarly, a short-term increase in K after spring PB was detected in all fractions in the mixed stand and in the needle fractions in the pure stand. Calcium is the most abundant element in litterfall nutrient content, as the leaves have the greatest need for Ca. Calcium is abundant in the soils in the study area and was not expected to be a limiting element. Magnesium content exhibited no clear trend, with reductions in some fractions probably due to the competition with other cations.

The role of bark traits in fire protection: bark thickness and coefficient of variation of bark thickness

The laboratory test using a mass loss colorimeter device (MLC) in a vertical configuration confirmed the influence of bark thickness in preventing cambium thermal damage for all response variables (time that the cambium is above 60 °C, t60; heating rate in cambium area, HT; and maximum absolute temperature in cambium, Tmx) and for the four heat flux rates considered (20, 25, 50 and 75 kW m^{-2}) (Chapter 3). A threshold of 17 mm of bark thickness appears to be a critical point at which the probability of cambium death decreases. This value is very similar to the corresponding values reported by other authors for other species (e.g. Wesolowski et al., 2014; Madrigal et al., 2019). The present findings showed that at low heat flux (below 20 kW m^{-2}, conditions that can be reached in a low-intensity PB), bark provided complete protection, and other variables, such as the flame residence time, may be more critical. In this context, a threshold of 25 kW m^{-2} is proposed as representative of a low- moderate-intensity fire.

Forest managers will be able to assess the proportion of dominated trees (bark thickness less than 17 cm) that will have a greater probability of suffering from effects that will reduce their survival or even lead to their death. Bark thickness profile models (e.g. Madrigal et al., 2019) will also enable more accurate assessment of the scorch height above which mortality will increase due to cambium damage. In addition, a value of 25 kW m^{-2} may be a qualitative reference point, after which the bark does not guarantee total protection of the cambium area.

The coefficient of variation in bark thickness (used as proxy for bark roughness) seemed to have a slightly positive effect in protecting the cambium, at least in two response variables in the laboratory test: time during which the temperature of the sample was higher than 60 °C in the cambium area (t60) and heating rate in the cambium area (HT) (significant at low heat flux). Although some authors (e.g. Bauer et al., 2010; Odhiambo et al., 2014) have suggested that bark roughness protects against overheating of the cambium, others (e.g. Frejaville et al., 2013) did not observe any significant influence. In any case, rougher bark has been identified as characteristic of fire-prone species (Graves et al., 2014).

Despite the findings of the laboratory test, no significant results were observed in the burning conducted in field in regard to the fire protection characteristic of bark thickness and coefficient of variation of bark thickness. The lack of significance made sense because temperatures higher than 60 °C were only reached in a small proportion of trees (less than 18%). In low-intensity fires, the bark presumably fully protects the cambium, particularly in species with thick bark, such as *Pinus nigra*. This means that only local conditions at tree level can generate local burn intensities that can potentially cause damage to the cambium; thus, managers should prevent excess accumulation of branches (for example). These results are very similar to those reported in Subchapter 2.3, in which a Bayesian model was also used to assess the protection of minimum bark thickness. However, more data on moderate- high-intensity fires are needed to clarify the effects of these variables.

The importance of fire intensity in cambium damage

In order to characterize fire intensity, the time during which the temperature of the samples was higher than 300 °C in bark surface (te300) and the maximum absolute temperature in bark surface (Temx) were recorded. These variables did not seem to be good indicators of cambium damage in laboratory test. As the thermocouples were located on the outer bark (23 mm from radiation source), and radiation fluxes ranged from 20 to 70 kW m^{-2}, it is reasonable to expect temperatures above 300 °C to be reached. Similarly, temperature peaks may not affect the cambium if they are not prolonged. However, these two variables significantly affected t60 and Tmx and t60 and HT respectively in field prescribed burning. The variables indicate overheating of individual trees which did not occur in laboratory test, as the trials are more homogeneous. This shows the importance of conducting field studies to check laboratory findings.

Sustainability, ignitability and consumability as predictor variables of cambium damage

Flame residence time (tf) higher than 5 min may be critical to cambium damage. In this context, a significant relationship between tf and t60 was observed at 20 kW m^{-2} and although not significant for HT and Tmx, it was notable. As already mentioned, bark provided complete protection from low-intensity fire, and flame residence time may be more critical in terms of cambium damage. In addition, the findings showed that a longer time to ignition (which implied a longer exposure time in laboratory test) may yield higher values of t60 and HT. In this regard, even at high flux rate, the level of cambium damage (temperatures above 60 °C) was highest after 300 s (maximum exposure time), during the combustion phase. The intensity of the radiant source and exposure time are proposed as a critical combination for establishing cambium damage (especially in thin bark) exacerbated by bark flammability.

These findings may help forest managers to establish quantitative limits of bark resistance in *Pinus nigra* stands (5 min of exposure to more than 20-25 kW m^{-2}) and provide additional information to support PB planning by using available fire behaviour models. In addition, the burning technique selected should favour rapid advancement of the fire front, avoiding long residence times of fire but at the same time ensuring that the burning goals are achieved. Smouldering after the passage of fire may increase the temperature of cambium (bark flammability significantly affects the potential damage to the cambium), and this effect should therefore be considered in burn prescriptions.

Two variables were selected in order to measure the consumability: percentage of weight (W) and bark consumed (Bpo). Weight did not seem to be clearly related to t60, HT or Tmx. However, a greater reduction in bark surface negatively affected the cambium. The percentage weight loss is probably related to water loss and volatilization of gases. However, reduction in bark surface is related to loss of bark thickness and thus less protection of the cambium. Estimation of bark consumed could therefore be used as a proxy for cambium damage. Although detailed analysis with additional data is necessary to develop an operational tool for evaluating the impact of PB, the preliminary data appear favourable.

Short-term response of tree growth after two seasons of prescribed burning in *Pinus nigra* stands

The results obtained highlight the importance of modelling the tree growth response to PB as a function of tree and stand characteristics, burn season and fire severity variables to gain some insight into individual tree responses (Chapter 4).

The findings showed that in the short-term, PB scarcely affected tree growth, although a slight overall reduction was observed. The reduced tree growth has been described as a short-term trend (1-3 years), with growth rates returning to approximately pre-fire levels thereafter (Seifert et al., 2017). Thus, longer-term trials may be necessary to establish the recovery rate of the stands.

The influence on tree growth of tree characteristics

The tree variables included in the statistical analysis (percentage of live crown height and DBH) proved to be significant factors in all models tested. Therefore, regardless of the type of stand (mixed or pure), the particular characteristics of each tree may explain the variability in growth after fire. This may be partly explained by the low percentage of *Pinus pinaster* in the mixed stand (mean, 11%) and the low-intensity characteristic of burning.

The influence of stand composition on tree growth

The differences between mixed and pure stands did not explain the variability in tree growth after PB. In this respect, it is widely recognized that mixed stands are more stable than pure stands (e.g. Felton et al., 2010); reduced effects were even detected in mixed stands after PB regarding total litterfall (Subchapter 2.3) and soil properties (Plaza-Álvarez et al., 2017) in the same experimental plots. However, the present findings did not enable confirmation of this point in relation to tree growth.

The influence on tree growth of burn season and fire severity variables

Burn season influenced tree growth, as observed in the litterfall studies (Subchapter 2.1, 2.2 and 2.3). Time during which the temperature remained above 60 °C in inner bark (t60) and time during which the temperature remained above 300 °C in bark surface (t300) had significant effects only after spring PB. In addition, although maximum scorch height was significant after spring and autumn PB, it seemed to have a greater effect after spring PB. Overall, spring burning has been reported to be more disruptive to trees because carbohydrate reserves are at their lowest levels at the beginning of the annual growth period (Hough, 1968; Garrison, 1972). Furthermore, the abundance of fine roots during this period may imply a higher level of damage (Swezy and Agee, 1991; Harrington, 1993). Hence, the effects of autumn PB may be less disruptive; however, disturbance of other tree and stand processes (e.g. impact on litterfall) should be also considered in the burning prescriptions (Subchapter 2.3). Indeed, fuels have been described as typically drier later in the regular autumn burning season (Skinner and Chang, 1996), which may imply higher mortality rates (Thies et al., 2005).

Maximum scorch height had a significant negative influence on tree growth after spring and autumn burning. Higher scorch heights may therefore imply greater added stress on trees and should be avoided. Scorch height is easy to measure in the field and could help forest managers in PB planning and post-burning assessment. Nonetheless, maximum scorch height had random effects on litterfall in the same experimental plots, although this was probably because the maximum scorch height was reached below the mean height of the first live branch (Subchapter 2.3).

The total litterfall biomass collected one year after prescribed burning did not significantly affect the tree growth response (Chapter 4). As already mentioned, the low-intensity of the prescribed burning and adaptation of *Pinus nigra* to stressful fire conditions may limit the associated impact of PB (Subchapter 2.3 and Chapter 3). PB may improve tree efficiency by eliminating the unproductive lower branches (Villarrubia and Chambers, 1978) and less efficient needles from the lower part of the crown (Certini, 2005; Retzlaff et al., 2018). By contrast, PB may reduce tree growth as a consequence of crown disturbance (Busse et al., 2000), which represents photosynthetically active tissue and a source of energy (e.g. De Micco et al., 2013; Poljanšek et al., 2015); although no significant short-term effect was observed in the present study.

The proposed models could help managers to predict the effect of PB on tree growth. In the studies developed in this doctoral research, alterations in growth were minimal, confirming the resilience of stands in the Iberian System to under-canopy PB. However, the sensitivity of the model to variable t60 (and even variable t300) suggests that cambium heat may be a key variable regarding the potential decrease in growth, at least in the short term after PB. Therefore, this effect should be considered if the main objective of the stand is timber production.

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Chapter 6

General conclusions

Doctoral thesis, ***Prescribed burning to reduce fire severity: effects on pine forests in the Iberian System,*** **2021**

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1. Low-intensity prescribed burning, in both spring and autumn, had a limited medium-term effect (which decreased steadily over time) on litterfall dynamics (in terms of amount and nutrient content) in a mixed *Pinus nigra–Pinus pinaster* stand and a pure *Pinus nigra* stand.

2. The variability in litterfall was affected by stand composition and characteristics. Along with the type of stand, the season in which the prescribed burning was carried out also affected litterfall biomass dynamics. The effect of spring prescribed burning was more marked than autumn prescribed burning in the pure stand, at least in the short-term. However, the rate of recovery was faster after prescribed burning in spring. Nonetheless, prescribed burning in spring must be carefully planned under high mean maximum temperatures and severe dry conditions. A delayed effect of perturbation was observed in the pure stand after autumn prescribed burning, owing to the subsequent impact of snowfalls and storms. Indeed, meteorological variables were found to be more significant than variables associated with stand characteristics or with low-intensity prescribed burning.

3. Considering that needles constitute an important nutrient sink, prescribed burning did not appear to disturb the nutrient return to the forest soil. Inflorescences decreased significantly after the spring burning treatment in both stands, suggesting that reproductive organs may be affected by heat. Branches, bark and miscellaneous fractions were irregularly distributed throughout the year, mainly in relation to meteorological events. In particular, snowfall and storms during autumn-winter may cause peaks in the abundance in the branch fraction. Significant differences in bark fraction between stands may be due to the bark characteristics of *Pinus pinaster*.

4. Although there were some differences in nutrient concentrations between sites and fractions, the similar trend in nutrient content of plots treated and not treated by prescribed burning indicated the scarce impact of fire on nutrient cycling. The values obtained for nitrogen were similar to those in old needles, and thus no great damage is expected in the crown, particularly in the short-term, as confirmed by analysis of the Leaf Area Index. The burning treatment had little effect on the carbon concentration. A slight overall increase in nitrogen, phosphorus and potassium was detected after burning. All of these nutrients are key to stand recovery following disturbance. Calcium was not found to be a limiting factor due to its abundance in the study soils. Magnesium content exhibited no clear trend.

5. The study findings confirmed the influence of bark thickness in preventing cambium damage during the passage of fire in *Pinus nigra* stands, as only certain conditions at tree level will generate burn intensities that can potentially cause cambium damage. In this regard, a minimum bark thickness of 17 mm was identified as the critical threshold at which the probability of cambium death decreases. In addition, the coefficient of variation of bark thickness seemed to have slightly positive effect on protecting the cambium.

6. A threshold of 25 kW m^{-2} may be considered a reference value for improving fire prescriptions regarding mortality rates after wildfires.

7. Temperature peaks in bark surface during low-intensity prescribed burning may not affect the internal cambium if not accompanied by prolonged exposure to high temperatures.

8. The flame tends to become extinguished shortly after the end of radiant heat exposure (300 s), even at high heat fluxes. However, bark continued burning sustained by the heat without flame (smouldering).

9. The study demonstrated for the first time that bark flammability (duration of flame and bark surface burned) significantly affects the potential level of damage to the cambium.

10. The findings showed that low-intensity prescribed burning had scarce effect on short-term tree growth. Individual tree characteristics proved to be more important to growth than the type of stand. The differences between treatment seasons were notable. Although autumn prescribed burning seemed to have less impact on tree growth, disturbance of other stand dynamics should also be considered (e.g. litterfall). The inclusion of post-burn severity variables for the first time in tree growth models showed that maximum scorch height influences tree growth. Special attention should be given to variables related to exposition to a critical temperature in outer and inner bark, particularly after spring burning. Litterfall biomass one year after prescribed burning was not a significant variable.

In conclusion, and in view of the results obtained, low-intensity prescribed burning (spring and autumn) is a potentially valuable management tool for reducing fire hazard, increasing the resilience in pure and mixed *Pinus nigra* stands in the Iberian System.

1. La quema prescrita de baja intensidad, tanto la ejecutada en primavera como en otoño, tiene un efecto limitado a medio plazo (que disminuye progresivamente con el paso del tiempo) sobre la dinámica de desfronde (en términos de cantidad y contenido de nutrientes) en masa mixtas de *Pinus nigra* y *Pinus pinaster* y puras de *Pinus nigra*.

2. La variabilidad en el desfronde se ve afectada por la composición y características de la masa. Junto con el tipo de masa, la época en que se realiza la quema prescrita también influye en la dinámica de desfronde. El efecto de la quema prescrita de primavera fue más acentuado que el de la quema prescrita de otoño en la masa pura, al menos a corto plazo. Aunque la tasa de recuperación fue más rápida después de la quema de primavera. La quema prescrita en primavera debe planificarse cuidadosamente, particularmente cuando las temperaturas máximas son altas y las condiciones de sequía severas. Además, se observa un efecto retardado de la perturbación en la masa pura después de la quema prescrita de otoño, que se relaciona con el impacto posterior al fuego de nevadas y tormentas. De hecho, las variables meteorológicas resultaron ser más significativas que las asociadas a las características de la propia masa o que las características de la quema prescrita de baja intensidad.

3. Teniendo en cuenta que las acículas constituyen un importante sumidero de nutrientes, la quema prescrita, no parece perturbar el retorno de nutrientes al suelo. La fracción de inflorescencias se redujo de manera significativa después de la quema prescrita de primavera en las dos masas, lo que sugiere que los órganos reproductores podrían verse afectados por el calor. Las fracciones de ramas, corteza y miscelánea se distribuyeron irregularmente a lo largo del año, principalmente afectadas por fenómenos meteorológicos. En particular, las nevadas y tormentas durante el otoño-invierno pueden causar picos en la fracción de ramas. Las diferencias significativas en la fracción de corteza entre las dos masas pueden deberse a las propias características de la corteza de *Pinus pinaster*.

4. Aunque hubo diferencias en la concentración de nutrientes entre las dos masas y entre fracciones, el contenido de nutrientes tuvo una tendencia similar en las parcelas tratadas y no tratadas con quema prescrita, lo que sugiere que el impacto del fuego en el ciclo de nutrientes es escaso. El contenido de nitrógeno de las acículas recolectadas en el desfronde se correspondía con valores propios de acículas maduras, por lo que no se esperan daños graves en la copa de los árboles; este dato también se confirmó a través del análisis del Índice de Área Foliar que se realizó durante el primer año de estudio. La quema prescrita también tuvo un efecto escaso sobre la concentración de carbono. En general, se detectó un ligero aumento de nitrógeno, fósforo y potasio, siendo estos nutrientes clave para la recuperación del árbol después de una perturbación. Dado que el calcio es un elemento abundante en los suelos de estudio, no fue un elemento limitante. El contenido de magnesio no mostró una tendencia clara.

5. El estudio confirmó el papel del espesor de corteza en la protección del cambium frente al fuego en masas de *Pinus nigra*; únicamente condiciones puntuales a nivel árbol pueden generar intensidades que potencialmente podrían dañar el cámbium. En este sentido, un espesor de corteza de más 17 mm puede considerarse el umbral crítico a

partir del cual la mortalidad del árbol por daño cambial disminuye. Además, el coeficiente de variación del espesor de corteza pareció tener un efecto ligeramente positivo sobre la protección del cambium.

6. Un valor de flujo de calor de 25 kW m^{-2} puede ser un dato de referencia para mejorar las prescripciones con respecto a las tasas de mortalidad después de incendios forestales.

7. Los picos de temperatura puntuales alcanzados en la corteza del árbol durante la quema prescrita de baja intensidad pueden no afectar al cambium si no existe, además, un tiempo prolongado de exposición a alta temperatura.

8. La llama tiende a extinguirse poco tiempo después de cesar la exposición al foco radiante (300 s), incluso trabajando con flujos de calor elevados. Sin embargo, la corteza continúa ardiendo (sin llama) sostenida por el propio calor (rescoldo).

9. Por primera vez se observó que la inflamabilidad de la corteza (duración de la llama y porcentaje de corteza quemada) afecta significativamente al daño cambial.

10. A corto plazo, los resultados muestran que la quema prescrita de baja intensidad tiene un efecto escaso en el crecimiento. Las características propias del árbol demostraron ser más decisivas para el crecimiento que el tipo de masa. La estación en la que se ejecuta la quema prescrita influye en el crecimiento. Aunque la quema prescrita de otoño parece tener menos impacto en el crecimiento de los árboles, también debe considerarse la alteración en otros procesos (por ejemplo, el desfronde). La inclusión de variables de severidad post-quema por primera vez en modelos de crecimiento mostró que la altura máxima de chamuscado influye en el crecimiento del árbol. Se debe prestar especial atención a las variables relacionadas con la exposición a temperaturas crítica en la corteza y el cambium, particularmente después de la quema de primavera. El desfronde, un año después de la quema prescrita, no fue una variable significativa para el crecimiento.

Para concluir, y a la vista de los resultados obtenidos, se puede afirmar que la quema prescrita de baja intensidad (ejecutada en primavera y otoño) es una herramienta de manejo forestal potencialmente valiosa para reducir el riesgo de incendio, aumentando la resiliencia en masas puras y mixtas de *Pinus nigra* del sistema Ibérico.

Chapter 7

Future research perspectives

Doctoral thesis, ***Prescribed burning to reduce fire severity: effects on pine forests in the Iberian System,*** **2021**

Juncal Espinosa Prieto

The research reported in this doctoral thesis addressed the effect of low-intensity PB treatment on two *Pinus nigra* stands in the Iberian System. Overall, the effect on litterfall biomass, cambium area and tree growth were very limited, and the results obtained will allow more effective prescriptions to be designed. However, during the study, some questions emerged which may open the door to new areas of research; in addition, some aspects related to the methodology could be improved.

The experimental design used in El Pozuelo and Beteta included a network of permanent plots where the litterfall has continued to be collected monthly. Therefore, data series of more than 4 years are already available. It would be interesting to establish whether the litterfall dynamics have completely stabilized after the fire-induced disturbance, returning the stand to the initial pre-burning state. This point could not be entirely clarified 30 months after spring PB and 24 months from autumn PB.

The ForeStereo® device was used to obtain data on the Leaf Area Index before and one year after spring burning. The ForeStereo® device is easy to use and does not require a permanent infrastructure in the plot. The speed at which it produces data in the field and its post-processing in laboratory allow data to be obtained about crown disturbance. The use of litterfall models based on ForeStereo® (or other remote sensing methods) may represent a powerful method of improving the evaluation of PB plots in the short-, medium- and long-term at a low cost and with high accuracy.

Differences in cone and inflorescence production were observed in unburned and burned plots. Given the particular fructification of some conifers (masting), it would be useful to confirm the potential effect of burning on flowering production in order to consider this in burning plans incorporated in other forest management and silviculture plans (e.g. thinning and regeneration treatments).

The carbon and macronutrient contents of the different litterfall fractions after prescribed burning were studied. It would be useful to complete the analysis of the nutrient cycle by studying the balance between the carbon and macronutrient provided via litterfall and those that are lost in the soil by fire. This could confirm the vulnerability of the ecosystem to possible repeat burning treatment. In addition, although data on micronutrient content are available, these data have not been published and could be considered for a more complete research.

Meteorological variables seemed to have a great impact on litterfall after prescribed burning; these variables were even more important than other tree and stand characteristics or fire severity variables. The meteorological data used in the study were provided by the general network of weather stations from State Meteorological Agency of Spanish Government (AEMET). Given the importance of this data, the installation of mobile meteorological stations in burned plots during and after the burning could provide information about the real scope (at local scale) of the influence of meteorological events such as snowfall, storms and frost.

The mass loss calorimeter was used for the first time in a vertical configuration with *Pinus nigra* samples. As *Pinus nigra* is adapted to tolerate surface fire, the study of other conifer species may provide useful information about cambium resistance to fire and the importance of bark in fire protection, indicating its importance as an evolutionary trait. It would also be interesting to redefine the protocols with the mass loss calorimeter, particularly the maximum exposure times. The samples were exposed to radiant heat until extinction of the flame or for up to 5 min (maximum exposure time). This seems to be a critical factor that does not always occur in field burns.

The study demonstrated the relationship between flammability and cambium damage which leads the way to new studies relating ecological and combustion properties of tree bark and the potential influence of these properties in protecting living tissues after fire of different levels of intensity and severity. In addition, the importance of the proportion of bark consumed (Bpo) was suggested as a proxy for cambium damage; however, detailed analysis of additional data is necessary before the measure can be used operationally.

Post-fire tree growth was studied. Only microcores were extracted, and the use of complete growth cores would probably have added extra information about the past growth and the tree age. In addition, a long-term post-fire study would reveal any delayed effect of fire.

Another issue raised during the study was the possible effect of burning in inducing the secondary defense mechanisms (increase in the number of resin canals due to the effect of stress during burning) and the efficiency of water use. This would open the door to examining possible interactions between PB and susceptibility/resistance to biotic (e.g. pests or diseases) and abiotic factors (e.g. climate change).

High-intensity PB was carried out within the same experimental design. A new line of research could compare the effects of different types of burning, in order to establish the tolerance (vulnerability) of the ecosystems under study (fire-prone habitats) to prescribed fire. The study of interactions between trees, soil properties and soil biome (especially mycorrhizal fungi species) after different levels of fire severity in prescribed burns has important ecological and management implications. At the same time, other conifer stands should be tested to extend the findings obtained.

Finally, the experimental burning was conducted as a result of a close collaboration between Forestry Services from Castilla-La Mancha and the INIA (Forest Research Centre). Continuing this management-research alliance will contribute to improving the design, study and implementation of PB, in combination with other treatments (e.g. thinning) or in place of these.

Appendix I

Journals articles included as academic merit to present the doctoral thesis

Doctoral thesis, ***Prescribed burning to reduce fire severity: effects on pine forests in the Iberian System,*** **2021**

Juncal Espinosa Prieto

Chapter 2. Subchapter 2.1

Title:

Short-term effects of prescribed burning on litterfall biomass in mixed stands of *Pinus nigra* and *Pinus pinaster* and pure stands of *Pinus nigra* in the Cuenca Mountains (Central-Eastern Spain).

Authors:

Espinosa, J.[1,2], Madrigal, J.[1,2], De la Cruz, A.C.[1], Guijarro, M.[1,2], Jiménez, E.[3], Hernando, C.[1,2]

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Corresponding author: Espinosa, J.

Journal: Science of the Total Environment (STOTEN).

Editorial: Elsevier.

Date of publication: March 2018 (online, November 2017).

ISSN: 0048-9697.

DOI: https://doi.org/10.1016/j.scitotenv.2017.08.291.

Journal impact factor: 5.589.

Journal ranking: 27 de 242 (Q1) in Environmental Sciences.

Indexation database: Journal Citation Reports (JCR).

Contribution of the authors:

Juncal Espinosa, designed the experiment, carried out the field and laboratory work, ran the data analysis, discussed the results and wrote the manuscript. **Javier Madrigal**, designed the experiment, carried out the field work, supported the statistical analysis and corrected the manuscript. **Ana Carmen de la Cruz**, designed the experiment, carried out the field and laboratory work and corrected the manuscript. **Mercedes Guijarro**, designed the experiment, carried out the field work and corrected the manuscript. **Enrique Jiménez**, provided soil data. **Carmen Hernando**, designed the experiment, carried out the field work, corrected the manuscript and coordinated the research project.

Chapter 2. Subchapter 2.2

Title:

The effect of low-intensity prescribed burns in two seasons on litterfall biomass and nutrient content.

Authors:

Espinosa, J.[1,2], Madrigal, J.[1,2], Pando, V.[2,3], De la Cruz, A.C.[1], Guijarro, M.[1,2], Hernando, C.[1,2]

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Corresponding author: Espinosa, J.

Journal: International Journal of Wildland Fire (IJWF).

Editorial: Csiro Publishing.

Date of publication: November 2020 (online, August 2020).

ISSN: 1029-1041.

DOI: https://doi.org/10.1071/WF19132.

Journal impact factor: 2.627.

Journal ranking: 9 of 68 (Q1) in Forestry.

Indexation database: Journal Citation Reports (JCR).

Contribution of the authors:

Juncal Espinosa, designed the experiment, carried out the field and laboratory work, ran the data analysis, discussed the results and wrote the manuscript. **Javier Madrigal**, designed the experiment, carried out the field work and corrected the manuscript. **Valentín Pando**, supported the statistical analysis. **Ana Carmen de la Cruz**, designed the experiment, carried out the field and laboratory work and corrected the manuscript. **Mercedes Guijarro**, designed the experiment, carried out the field work and corrected the manuscript. **Carmen Hernando**, designed the experiment, carried out the field work, corrected the manuscript and coordinated the research project.

Chapter 2. Subchapter 2.3

Title:

Use of Bayesian modeling to determine the effects of meteorological conditions, prescribed burn season, and tree characteristics on litterfall of *Pinus nigra* and *Pinus pinaster* stands.

Authors:

Espinosa, J.[1,2], Rodríguez de Rivera, O.[3], Madrigal, J.[1,2], Guijarro, M.[1,2], Hernando, C.[1,2]

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Corresponding author: Espinosa, J.

Journal: Forests.

Editorial: MDPI.

Date of publication: September 2020 (online, September 2020).

ISSN: 1999-4907.

DOI: https://doi.org/10.3390/f11091006.

Journal impact factor: 2.221.

Journal ranking: 17 of 68 (Q1) in Forestry.

Indexation database: Journal Citation Reports (JCR).

Contribution of the authors:

Juncal Espinosa, designed the experiment, carried out the field and laboratory work, discussed the results and wrote the manuscript. **Óscar Rodríguez de Rivera**, supported the statistical analysis and corrected the manuscript. **Javier Madrigal**, designed the experiment, carried out the field work and corrected the manuscript. **Mercedes Guijarro**, designed the experiment, carried out the field work and corrected the manuscript. **Carmen Hernando**, designed the experiment, carried out the field work, corrected the manuscript and coordinated the research project.

Chapter 3

Title:

Predicting potential cambium damage and fire resistance in *Pinus nigra* Arn. ssp. s*alzmannii*.

Authors:

Espinosa, J.[1,2], Rodríguez de Rivera, O.[3], Madrigal, J.[1,2], Guijarro, M.[1,2], Hernando, C.[1,2]

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Corresponding author: Espinosa, J.

Journal: Forest Ecology and Management (FORECO).

Editorial: Elsevier.

Date of publication: October 2020 (online, July 2020).

ISSN: 0378-1127.

DOI: https://doi.org/10.1016/j.foreco.2020.118372.

Journal impact factor: 3.17.

Journal ranking: 5 of 68 (Q1) in Forestry.

Indexation database: Journal Citation Reports (JCR).

Contribution of the authors:

Juncal Espinosa, designed the experiment, carried out the field and laboratory work, discussed the results and wrote the manuscript. **Óscar Rodríguez de Rivera**, supported the statistical analysis and corrected the manuscript. **Javier Madrigal**, designed the experiment, carried out the field work and corrected the manuscript. **Mercedes Guijarro**, designed the experiment, carried out the field work and corrected the manuscript. **Carmen Hernando**, designed the experiment, carried out the field work, corrected the manuscript and coordinated the research project.

Chapter 4

Title:

Tree growth response to low-intensity prescribed burning in *Pinus nigra* stands: effects of burn season and fire severity.

Authors:

Espinosa, J.[1,2], Martin-Benito, D.[3], Rodríguez de Rivera, O.[4], Hernando, C.[1,2], Guijarro, M.[1,2], Madrigal, J.[1,2,5]

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Corresponding author: Espinosa, J.

Journal: in the review phase in European Journal of Forest Research.

Contribution of the authors:

Juncal Espinosa, designed the experiment, carried out the field and laboratory work, ran the data analysis, discussed the results and wrote the manuscript. **Dario Martin-Benito**, carried out the laboratory work and corrected the manuscript. **Óscar Rodríguez de Rivera**, supported the statistical analysis and corrected the manuscript. **Carmen Hernando**, designed the experiment, carried out the field work and corrected the manuscript. **Mercedes Guijarro**, designed the experiment, carried out the field work and corrected the manuscript. **Javier Madrigal**, designed the experiment, carried out the field work, corrected the manuscript and coordinated the research project.