Universitat Autònoma de Barcelona

The effects of prescribed burning on the vigour of Mediterranean pine species

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PhD Thesis

Teresa Valor Ivars

to be eligible for the Doctor degree

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PhD in Terrestrial Ecology
Consorci Centre de Ciència i Tecnologia Forestal de Catalunya (CTFC)
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With the approval of the supervisors

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Que nada nos limite. Que nada nos defina. Que nada nos sujete.
Que la libertad sea nuestra propia sustancia. Hay que vivir y ser libre.
Por que alguien dijo y estoy de acuerdo, que el tiempo cura, el dolor pasa,
que la decepción no mata y que la vida,
la vida siempre continua.

Simone de Beauvouir

Als meus pares

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Abstract

Prescribed burning is the planned use of fire to meet clear management objectives under suitable environmental conditions. It is usually executed to reduce fire hazard, but also to manage range and conserve biodiversity. Prescribed burning applied under a forest canopy can reduce crown fire hazard; however, underburning might affect the primary and secondary metabolism of trees. Planning underburning to reduced fire hazard, while minimizing the negative effects and maximizing the positive effects on trees, requires understanding how specific components of the fire regime, such as fire intensity, severity and season, affect tree performance. The goal of this doctoral thesis is to understand the influence of prescribed burning regime factors and related fire impacts on the primary and secondary metabolisms of three pine species with contrasting fire tolerances: *Pinus nigra* ssp. *salzmannii* (Dunal) Franco, *P. sylvestris* L. and *P. halepensis* Mill., using a combination of dendrochronological, isotope and terpene quantification techniques.

Post-burning growth variations depended on the time since burning, the pine species, tree resistance, fire severity and tree performance before burning. In the year of burning, growth was reduced in *P. halepensis* and unaffected in *P. nigra* and *P. sylvestris* (Chapter 2). However, as time passed, growth increased in *P. nigra*, recovered in *P. halepensis* and decreased in *P. sylvestris*. *P. nigra* had a lower probability of dying than *P. sylvestris* (Chapter 3). Burning season emerged as an important factor for explaining initial post-burning pine mortality, since for a certain level of crown injury the probability of a pine dying was higher in spring than in fall. In contrast, delayed pine mortality was higher in fall than in spring burns probably due to the longer combustion times recorded during the fall burns at the base of the trunk. A relevant release of tree competition increased growth through a positive effect on the latewood of *P. nigra* and *P. sylvestris* as stem injury decreased (Chapter 4). Moreover, we showed that burning just after a dry year did not reduce the growth resilience of pines in comparison with unburned pines. In *P. halepensis* a relevant competition release, especially in pines with lower crown volume scorched, resulted in higher growth rates as time since burning increased. This growth response coincided with a dry year and was associated with higher stomatal conductance, suggesting that water availability was enhanced after burning. Burning also affected the secondary metabolism of pines, and specifically the amount and type of terpene production depending on the pine species and fire severity (Chapter 6). Thus, as crown injury increased, needle terpene concentration 24h post-burning also augmented (Chapter 6). However, a remarkable decrease occurred at one year post-burning. This reduction was more pronounced in pines benefited by the increase in resource availability after burning, suggesting that pines were allocating assimilates to growth rather than to defence.

From a fuel management point of view, this thesis provides valuable information that can be used to better plan prescribed burning in Mediterranean *Pinus* forests, in terms of required fire intensity, severity and burning season, offering a new window of opportunity for the use of prescribed burning as a forest management tool.

Resum

La crema prescrita consisteix en l'ús planificat del foc en condicions ambientals adequades per tal d'aconseguir objectius de gestió prèviament definits. De manera general la crema s'executa amb l'objectiu de reduir el risc d'incendis tot i que també s'utilitza per a la gestió de pastures i la conservació de la biodiversitat. L'aplicació d'una crema sota dosser arbori d'una banda pot reduir el risc d'incendi de capçades, però de l'altra banda també pot afectar el metabolisme primari i secundari de l'arbre. Tanmateix, les cremes prescrites haurien de minimitzar els efectes negatius del foc als arbres i maximitzar els positius. Per assolir dits efectes, és important comprendre de quina manera els components específics del règim de foc, com són la intensitat del foc, severitat i estació de crema, afecten el funcionament de l'arbre. L'objectiu d'aquesta tesi doctoral és comprendre la influència que els factors del règim de cremes prescrites tenen en el metabolisme primari i secundari de tres espècies de pi amb tolerància al foc contrastada: *Pinus nigra* ssp. *salzmannii* (Dunal) Franco, *P. sylvestris* L. i *P. halepensis* Mill., utilitzant una combinació de tècniques dendrocronològiques, isotòpiques i de quantificació terpènica.

Les variacions de creixement post-crema van dependre del temps transcorregut des de la crema, l'espècie de pi, la resistència de l'arbre, la severitat del foc i del creixement de l'arbre abans de la crema. Al llarg de l'any de la crema, el creixement es va reduir en *P. halepensis* i no es va veure afectat en *P. nigra* i *P. sylvestris* (Capítol 2). No obstant això, amb el pas del temps, el creixement es va incrementar en *P. nigra*, es va recuperar en *P. halepensis* i es va reduir en *P. sylvestris*. La probabilitat de morir va ser menor en *P. nigra* que en *P. sylvestris* (Capítol 3). L'estació de crema va emergir com un factor important per explicar la mortalitat inicial dels pins: per a un cert nivell de capçada socarrimada la probabilitat que un pi mori és més alta a la primavera que a la tardor. En contrast, la mortalitat tardana del pi va ser més alta a la tardor que a les cremes de primavera probablement a causa dels majors temps de combustió registrats a la base dels troncs durant les cremes de tardor.

Una reducció rellevant de la competència arbrada va augmentar significativament el creixement mitjançant un efecte positiu en la fusta de tardor de *P. nigra* i *P. sylvestris*, a mesura que el dany causat pel foc a la tija va disminuir (Capítol 4). A més, vam

demostrar que una crema prescrita executada just després d'un any sec no redueix la capacitat de resiliència dels pins en comparació amb la dels pins no cremats. En *P. halepensis* una reducció significativa de la competència va donar com a resultat majors creixements amb el pas del temps, especialment en pins amb un menor volum de capçada socarrimada. Aquest augment va coincidir amb un any sec i es va associar amb una major conductància estomàtica, el que suggereix que la disponibilitat d'aigua va millorar després de la crema. La crema també va afectar al metabolisme secundari dels pins, i específicament a la quantitat i al tipus de terpens segons l'espècie de pi i la severitat del foc (Capítol 6). A mesura que el volum de capçada socarrimada va augmentar, la concentració de terpens a l'acícula dels pins 24 h post-crema també ho va fer (Capítol 6). No obstant això, 1 any després de la crema es va produir una notable disminució. Aquesta reducció va ser més pronunciada en pins beneficiats per l'augment de la disponibilitat de recursos després de la crema, el que suggereix que els pins estaven assignant als productes assimilats al creixement en lloc de a mecanismes de defensa.

Des del punt de vista de la gestió de combustible, aquesta tesi proporciona informació valuosa per ajudar en la millora de la planificació de les cremes prescrites als boscos de pi mediterranis, en termes d'intensitat de foc requerida, severitat i estació de crema, oferint una nova finestra d'oportunitat per a l'ús de la crema prescrita com a eina de gestió forestal.

Resumen

La quema prescrita consiste en el uso planificado del fuego en condiciones ambientales adecuadas con el fin de conseguir objetivos de gestión previamente definidos. Generalmente, la quema se ejecuta con el objetivo de reducir el riesgo de incendios, aunque también se utiliza para la gestión de pastizales y la conservación de la biodiversidad. La quema prescrita aplicada bajo dosel arbóreo puede reducir el riesgo de incendio de copas, sin embargo, también puede afectar al metabolismo primario y secundario del árbol. Por este motivo, las quemas precritas deberían minimizar los efectos negativos del fuego en los árboles y maximizar los positivos. Para ello, es necesario comprender de qué manera los componentes específicos del régimen de fuego, como son la intensidad del fuego, severidad y estación de quema, afectan al funcionamiento del árbol. El objetivo de esta tesis doctoral es comprender la influencia de los factores del régimen de quemas prescritas en el metabolismo primario y secundario de tres especies de pino con tolerancia al fuego contrastada: *Pinus nigra* ssp. *salzmannii* (Dunal) Franco, *P. sylvestris* L. y *P. halepensis* Mill., utilizando una combinación de técnicas dendrocronológicas, isotópicas y de cuantificación terpénica.

Las variaciones de crecimiento post-quema dependieron del tiempo transcurrido desde la quema, la especie de pino, la resistencia del árbol, la severidad del fuego y del crecimiento del árbol antes de la quema. En el año de la quema, el crecimiento se redujo en *P. halepensis* y no se vio afectado en *P. nigra* y *P. sylvestris* (Capítulo 2). Sin embargo, con el paso del tiempo, el crecimiento se incrementó en *P. nigra*, se recuperó en *P. halepensis* y se redujo en *P. sylvestris.* La probabilidad de morir fue menor en *P. nigra* que en *P. sylvestris* (Capítulo 3). La estación de quema emergió como un factor importante para explicar la mortalidad inicial de los pinos, ya que, para un cierto nivel de copa chamuscada, la probabilidad de que un pino muera es mayor en primavera que en otoño. En contraste, la mortalidad tardía del pino fue más alta en otoño que en las quemas de primavera, probablemente, debido a los mayores tiempos de combustión registrados en la base de los árboles durante las quemas de otoño.

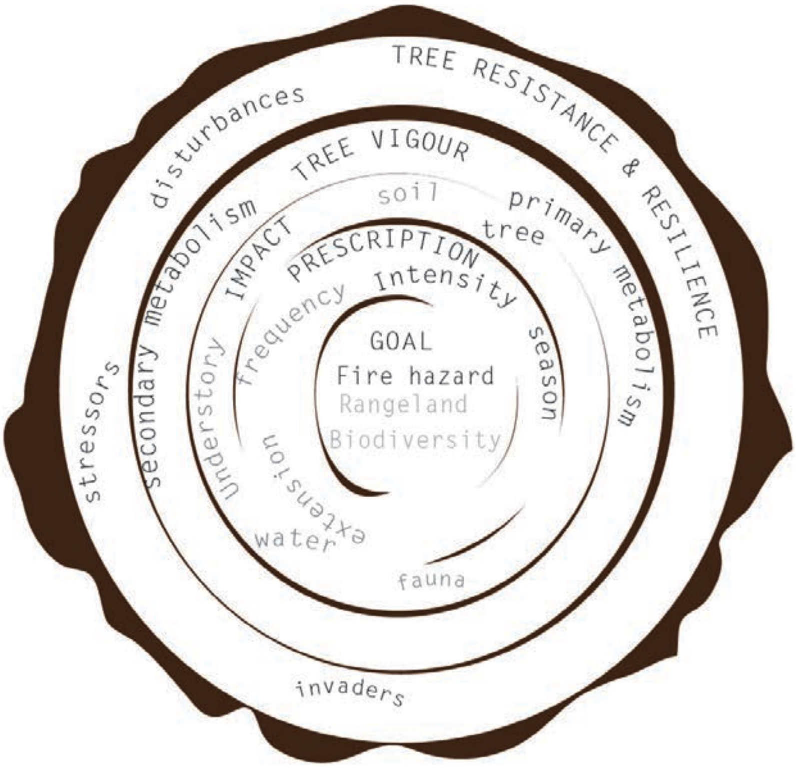
Una reducción relevante de la competencia arbolada aumentó significativamente el crecimiento de los pinos, especialmente de la madera tardía de *P. nigra* y *P. sylvestris*, a medida que el daño causado por el fuego al tallo disminuyó (Capítulo 4). Además,

demostramos que una quema prescrita ejecutada justo después de un año seco no redujo la capacidad de resiliencia de los pinos en comparación con pinos no quemados. En *P. halepensis* una reducción significativa de la competencia resultó en mayores crecimientos con el paso del tiempo, especialmente en pinos con un menor volumen de copa chamuscada. Este aumento coincidió con un año seco y se asoció con una mayor conductancia estomática, lo que sugiere que la disponibilidad de agua mejoró después de la quema. La quema, también afectó al metabolismo secundario de los pinos, y específicamente a la cantidad y al tipo de terpenos según la especie de pino y la severidad del fuego (Capítulo 6). A medida que el chamuscado de copa aumentó la concentración de terpenos en la acícula de los pinos 24 h post-quema también lo hizo (Capítulo 6). Sin embargo, 1 año post-quema se produjo una notable disminución. Esta reducción fue más pronunciada en pinos beneficiados por el aumento en la disponibilidad de recursos después de la quema, lo que sugiere que los pinos estaban asignando los productos asimilados al crecimiento en lugar de a mecanismos de defensa.

Desde el punto de vista de la gestión de combustible, esta tesis proporciona información valiosa para ayudar a mejorar la planificación de las quemas prescritas en los bosques de pino mediterráneos, en términos de intensidad de fuego requerida, severidad y estación de quema, ofreciendo una nueva ventana de oportunidad para el uso de la quema prescrita como herramienta de gestión forestal.

Chapter 1

General introduction



1.1 Prescribed burning

In Mediterranean landscapes fire regimes have shifted from fuel-limited to more drought-driven (Pausas and Fernández-Muñoz 2012). In the second half of the last century many people left the countryside for urban areas and thus land was abandoned and traditional land uses decreased, mainly extensive livestock and multipurpose forestry (for timber, woodfuel and charcoal). In many cases, this has led to a progressive build-up of fuel across the landscapes. In addition, contemporary fire suppression strategies contribute to the increase in fuel load and continuity (Piñol et al. 2005). Moreover, the expansion of the urban-rural interface and the rise in recreational uses increase the likelihood of fire ignition (Radeloff et al. 2005; Syphard et al. 2007). Consequently, the number of catastrophic fires has increased in the last decades in southern Europe (Gonzalez and Pukkala 2007). All of this has prompted the idea of establishing a less harmful fire regime by allowing the spread of low-intensity unplanned fires and applying prescribed burns extensively as a cost-efficient way of reducing fuel load and continuity (Piñol et al. 2007; Regos et al. 2014).

Prescribed burning (PB) is the planned use of fire to meet clear management objectives under suitable environmental conditions (Wade et al. 1989). In Europe, PB is usually carried out to reduce the fire hazard, but also to manage the range and conserve biodiversity (Montiel and Kraus 2010) (Figure 1.1). The Mediterranean basin has a long history of traditional burning, and fire was also part of the system prior to human settlement (see the revision by Pausas and Keeley 2009). However, PB is used marginally in Mediterranean countries like Portugal, Spain and France and its use is still questioned in Europe (Montiel and Kraus 2010). Obstacles range from technical (i.e. risk-related concerns, shortage of experienced professionals and organizations), social (i.e. negative public perception of fire), operational (i.e. predominance of private lands over public, land treatable by PB is restricted by the extent of the rural-urban interface), economical (i.e. low investment in forest and fuel management) to legal limitations (i.e. in Greece the use of fire is prohibited) (Fernandes et al. 2013).

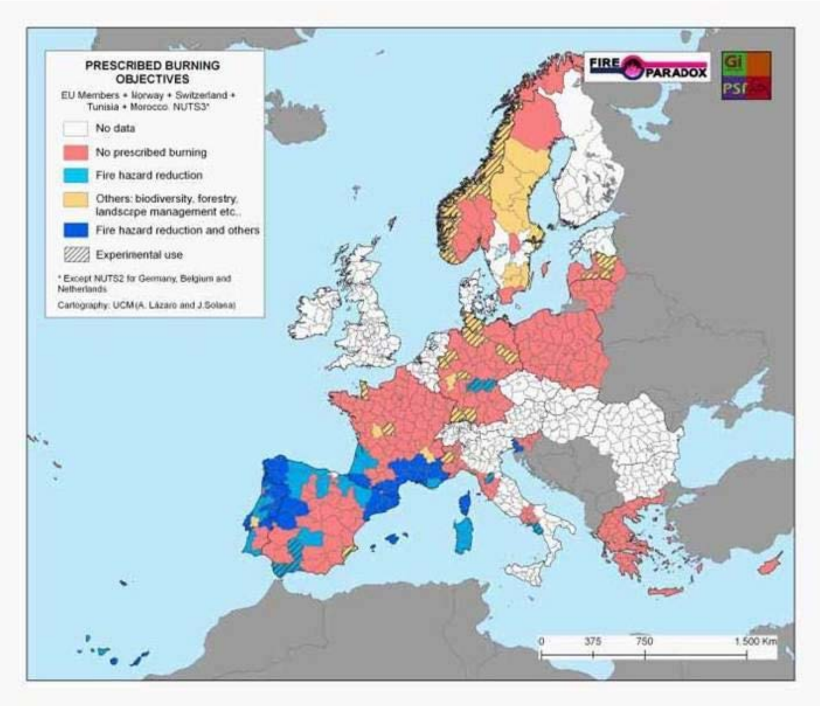


Figure 1.1: Map of main prescribed burning objectives in Europe (Lazaro and Montiel 2010).

Although fuel is less important than weather as a driving force of wildfire behaviour under extreme fire danger conditions (Bessie and Johnson 1995; Bradstock et al. 1998), PB aims to reduce the fire hazard by decreasing fuel loads (Pyne et al. 1996) and disrupting the horizontal continuity (Agee and Skinner 2005). When PB is carried out under the forest canopy, the vertical continuity is disrupted and the fire hazard is reduced more effectively because the possibility of crown fire propagation is decreased (Stephens et al. 2012; Kobziar et al. 2015; Casals et al. 2016; Fernandes et al. 2018) (Figure 1.2).

Figure 1.2: *P. nigra* and *P. sylvestris* stand before (left) and after the execution of prescribed burning (right).

The main goal of PB carried out under the forest canopy is to reduce fuel load under a threshold level without impacting ecosystem functions, as well as increase the longevity of the fuel treatment. The ability of plants to resprout after the destruction of all their aboveground biomass is a wide spread phenomenon in different ecosystems, including the Mediterranean and temperate forests (e.g. Bond and Midgley 2001; Lloret et al. 2005). Therefore, the understory can re-grow quickly, which potentially reduces the benefit-cost ratio of the PB treatment. Disparities in the recovery time of the understory fuel complex after PB depends on diverse ecological and management factors, such as plant composition and burning season; however, fire severity clearly determines the resprouting vigour of the woody understory, at least in Submediterranean pine forests under dense canopies (Casals and Ríos 2018). Higher intensity burns can impact tree vigour depending on the intrinsic characteristics of the species and the performance and physiological status of the individual species. In the context of the present thesis, by understanding the effects of the factors of the PB regime on the vigour of trees, we can achieve the goal of reducing understory fuel while minimizing potentially undesired impacts on the forest overstory.

1.1.1 From single burns to prescribed burning regime

The implementation of landscape-scale fuel treatments to prevent the spread of high intensity fires across landscapes should be the norm; however, in southern Europe, PB is usually applied as single burns and rarely as part of PB plans (Fernandes et al. 2013). In Catalonia, the study area, PB is generally carried out in strategic areas at the landscape level to support the firefighting actions.

To achieve the management objective using PB, a window prescription needs to be adopted that determines burning behaviour and the associated impacts on the different ecosystem components. The window prescription consists in selecting the ignition pattern to be applied, the thresholds of weather-fuel related variables, the season of the burn, and the extension and frequency of the treatment. The intensity, season, extension, frequency and severity define a wildfire regime. Thus, adopting the wildfire regime concept to PB planning can be useful for better understanding how these features impact not only the fuel dynamics but also the tree functioning. Especially, this concept can be applied to PB management to improve the design of PB regimes that not only reduce fuel hazard but also minimize the negative fire effects on tree performance and even could maximize tree growth resilience and resistance. This information would contribute to establishing connections between burning conditions, fire behaviour characteristics and fire impacts to developed window prescriptions that fulfil multiple management objectives (Fernandes 2018).

1.2 Window prescription

1.2.1 Ignition pattern and weather-fuel conditions

Fire intensity represents the energy released during various phases of the fire. Broadly, fire intensity is a function of spread rate and the amount of available fuel for combustion (Byram 1959); however, there are different metrics to capture different aspects of fire intensity (i.e. reaction intensity, fire line intensity, temperature, combustion time, radiant energy) (Keeley 2009). Within the range of intensities allowed during a PB, firing techniques can be applied to control the intensity of the fire, from the least intense fires (backing fires) to the most intense (heading fires) or intermediate ones (flanking fires or Point source fires). To apply PB safely and minimize the impacts on the ecosystem, weather and fuel related variables must be within threshold limits. The range of wind speeds at eye level should be between 2 and 5 km/h and the preferred relative humidity must be between 30 and 55 %. The rainfall in the previous days, water content of the soil, fine fuel and duff are other variables that must be taken into account to avoid undesired impacts.

1.2.2 Burning season

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 post-fire tree mortality and the growth of surviving trees (see Knapp et al. 2009 for review). PB is generally performed in the Mediterranean basin either at the beginning of autumn, just after the first rains but before the rainy period, or at the end of winter and early spring, before the onset of spring rains (Figure 1.3).

Figure 1.3: Prescribed burning behaviour in Mediterranean (Colladetes) and Submediterranean pine forests (Lloreda and Miravé) performed in different seasons.

Spring burns occur in the period of vegetation activity, whereas autumn burns are executed during the dormant season. The varying effects of burning season on tree functioning have generally been attributed to the phenological status of the tree, the tree's carbohydrate storage during the burning season and differences between the intensity of the burns (Harrington 1993; Thies et al. 2005). However, most of the studies have shown little or no effect of burning season on different ecological variables (see Knapp et al. 2009 for review). The review in Fernandes et al. (2008) on the fire resistance of European pines highlighted that most studies have been conducted during the trees' dormant season.

1.2.3 Frequency and Extension

Research into PB frequency is especially important for understanding fuel dynamics and determining the timing between treatments to keep surface fuel loads at a desirable level (Stephens et al. 2012). In North America and Australia, there is a considerable amount of research on the effects of repeated burning on several ecological variables (e.g. Bottero et al. 2017; Collins et al. 2014b; Covington and Sackett 1986; Guinto et al. 1999; Peterson et al. 1994). In contrast, in Europe, little is known about the cumulative effects of the repeated application of PB on fuel dynamics probably because research has been focused on single events (Fernandes 2018). In Europe, PB is typically small, rarely exceeding 200 ha, even if the study of the spatial scale is relevant for preventing the spread of wildfires (Fernandes 2015). However, the effects of PB frequency and extension have not been explored in this thesis.

1.3 Fire impacts

Fire severity describes the effects of burning on the ecosystem and different metrics can be used to measure the amount of organic material lost above and below ground (Keeley 2009). The impacts of PB depends on the window prescription adopted and the specific component of the ecosystem affected (i.e. soil, water, trees, understory, fauna). In the case of vegetation, fire severity depends on, among others, the plant composition, plant structure, physiological status and plant water content of the stand.

PB can impact a single tree by affecting its photosynthetic capacity or its meristematic (i.e. cambium, buds) and vascular tissues (i.e. phloem and xylem) in stems and roots. Crown damage can be measured by different methods, such as percentage of crown volume scorch, crown height scorch or a visual estimation of the volume scorched (Peterson 1985). Stem injury can be estimated by proxies of the potential damage to the cambium and xylem, such as bole scorched height (Regelbrugge and Conard 1993), char depth and the percentage of the trunk charred (Peterson and Arbaugh 1986), as well as the number of basal trunk quadrants with dead cambium (Hood et al. 2008; Ryan 1982). Root injury is rarely reported in PB studies because of the difficulties involved in assessing root distribution (Swezy and Agee 1991) and because the soil supposedly protects the roots from heat. However, stands with a thick duff layer may contain superficial roots that are susceptible to being killed during smouldering fires (Varner et al. 2009). A tree can die due to these crown, stem or root impacts. Tree mortality is, then, one of the most evident metrics of fire severity. However, tree vigour and increased risk of pathogen attack should also be considered when the effect of fire severity on trees is assessed.

1.4 Fire resistance of Mediterranean *Pinus* species

Tree damage due to fire depends on fire characteristics, namely temperature and combustion time, and the capacity of each tree to protect sensitive tissues (Dickinson and Johnson 2004, Michaletz and Johnson 2007). This capacity to protect sensitive tissues, and therefore survive, depends on the species and size of the tree (Catry et al. 2010).

Although smaller trees can be expected to show differences in fire resistance among species, the fire intensities used in PB are generally insufficient to affect the status of larger trees, and thus fire resistance differences between species are not important (Fernandes 2018).

Depending on the coevolution of wildfire regimes and species response, we can define five general plant strategies to cope with fire: avoiders, invaders, endurers, evaders and resisters (Rowe 1983). In this thesis, the pine species studied are *Pinus nigra* ssp. *salzmannii* (Dunal) Franco; *P. sylvestris* L. and *P. halepensis* Mill.. The Mediterranean *P. halepensis* is a clear example of the evader strategy with serotiny, early flowering and successful post-fire recruitment (Tapias et al. 2004). Fire resistant species are adapted to recurrent and fast spreading surface fires that periodically clear the lower stratum of the forest of dead and live fuels (Fonda 2001). The aim of these species is to survive surface fires by putting the maximum possible distance between their sensitive tissues and the fire. Submediterranean pine species such as *P. sylvestris* and *Pinus nigra* ssp. *salzmannii* are fire-resisters. The best fitness for these species with late flowering and absence of serotinous cones corresponds to thick-barked individuals capable of surviving surface fires (Tapias et al. 2004). In terms of fire resistance, Fernandes et al. 2008 ranked *P. sylvestris* as the most vulnerable in terms of cambium and crown vulnerabilities, while *P. halepensis* has the lowest cambium vulnerability. *P. nigra* is supposed to be less vulnerable to fire tissue damage due to its pyro-resistant traits (e.g. thicker bark, higher crown base height) (Figure 1.4). The fire resistance ranking provided by Fernandes et al. 2008 reflects the vulnerability to death, but whether this ranking is also true for the growth response and defence of surviving pines is not clear.

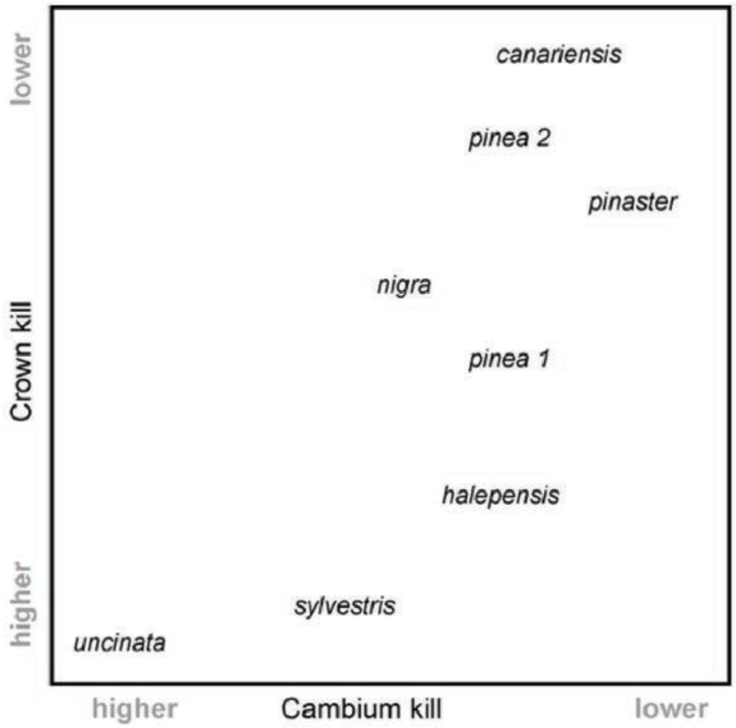


Figure 1.4: Fire resistance rating of European pine species, based on morphological traits and lethal heating experiments (Fernandes et al. 2008).

1.5 From binary to a continuous quantification of tree vigour after burning

Metrics of tree vigour after PB range from the simple distinction between "alive" and "dead" trees, to variations in tree growth and tree defence production to detect the continuous effects of tissue injury on the vitality of surviving trees (Figure 1.5). For example, necrosis of meristematic tissues due to fire occurs when the temperature exceeds the threshold of about 60ºC (Dickinson and Johnson 2004). The time above this threshold, the tissue injured and the tree resistance will determine whether the damage inflicted by fire will affect the primary and secondary metabolism of individuals and ultimately cause their death. Crown, stem and root fire injuries generally occur together, influencing the whole tree functioning. However, the mechanisms driving the vigour of underburned trees could be different for each type of tissue affected.

Figure 1.5: Gradient of crown (upper) and stem (bottom) fire impact on the vigour of pines.

The crown damage, caused by heat convection, has an important impact on photosynthetic rate (Wallin et al. 2003) and stomatal conductance of leaves (Cernusak et al. 2006). However, the impact on buds seems more critical than needle consumption (Peterson and Ryan 1986). Partial defoliation can improve the water balance and increase the photosynthetic efficiency of the remaining foliage; however, when defoliation is severe the uptake of carbon decreases, and negatively impacts the growth (Lilieholm and Hu 1987; Ryan 1993; Waldrop and Van Lear 1984). Severe defoliation and generalized bud damage can cause tree death (see Fernandes et al. 2008 for review of crown volume scorched thresholds in Mediterranean pine species) (Figure 1.5).

Stem injury due to fire results from heat released by radiation, conduction and convection. Depending on the bark thickness and combustion time near the trunk, fire can lead to a lasting reduction in xylem conductivity via wall deformation or cavitation. This results in a permanent reduction in hydraulic safety and an increase in embolism vulnerability (Bär et al. 2018; Michaletz et al. 2012). There is recent evidence for a species-specific response to fire-induced hydraulic impairments (Bär et la. 2018). Depending on tree size and species, stem injuries after fire can have little or no effect (Ducrey et al. 1996; Jimenez et al. 2012), reduce tree growth (Seifert et al. 2017; Sudachkova et al. 2016) or cause tree death, especially in small individuals (Fernandes et al. 2012) (Figure 1.5).

Extended smouldering increases the transfer of heat into the soil, which can result in root or basal meristem fire injury (Wyant et al. 1983). The replacement of dead fine roots requires photosynthetic carbohydrates, which are generally used at the expense of tree growth (Varner et al. 2009). This can also result in the death of trees (Ryan and Frandsen 1991).

1.5.1 Initial and delayed post-burning tree mortality

There is abundant information on the fire severity thresholds of trees, especially, for conifers of America (see Woolley et al. 2012); however, there is less information for European pines (Fernandes et al. 2008 for review).

Tree mortality caused by planned or unplanned fires is usually predicted using tree resistance variables (i.e. DBH, bark thickness) and fire severity indicators (see Woolley et al. 2012 for review) that describe the damage of foliage, stems and roots (Dickinson and Johnson 2004, Michaletz and Johnson 2007). To assess post-fire tree mortality it is necessary to carry out a minimum of 3 years of post-fire monitoring to account for initial and delayed mortality. In conifers, initial mortality can be observed within a month after PB by the absence of green foliage in the crowns if the buds have been killed (Peterson and Ryan 1986). In contrast, delayed mortality usually occurs in girdled trees, in which death is evident after two or three years post-burning (Hood 2010), or in severely fire-damaged trees, in which factors such as drought, insects or wind episodes contribute to a gradual process of tree weakening and finally cause the death of the tree (Ryan and Amman 1996). However, few studies have split the temporal framework of tree mortality. This could reveal differences in the mechanisms behind initial and delayed mortality. Furthermore, fire severity, tree size, and burning season could interact to explain differences in tree mortality rate over time. However, the effects of burning season on tree mortality have been neglected and the results, when they exist, are conflicting (e.g. see Knapp et al. 2009 for review): some studies did not detect any effect (Schwilk et al. 2006); while others found higher mortality after spring burns (Glitzenstein et al. 1995; Harrington 1987; Harrington 1993) or after autumn burns (Perrakis and Agee 2006; Thies et al. 2005).

1.5.2 Post-burning tree growth: effect of competition release

After a fire, tree growth can be positively influenced by the increase in soil nutrient availability (Certini 2005), the reduction in the competition for the resources due to the removal of the understory (Battipaglia et al. 2014, Battipaglia et al. 2016; Bottero et al. 2017), and, less often, by fire-induced tree thinning (Mutch and Swetnam 1995). The final effects of this competition release on tree growth will depend on the degree of the physiological dysfunctions caused by the fire. The studies in which fire led to a release from tree competition obtained a wide range of results (see Chambers et al. 1986 for review), from positive (Alfaro-Sánchez et al. 2015; Mutch and Swetnam 1995; Valor et al. 2013) to neutral (Morris and Mowat 1958; Sutherland 1989) or negative effects (Wooldridge and Weaver 1965).

Tree growth response to burning is usually diagnosed by comparing the post-burning and preburning growths of the same tree or the growth of a target tree against other nearby unstressed trees (Dobbertin 2005). The traditional approach compares the mean tree growth. There are only a few studies that model individual growth responses as a function of tree attributes and the direct and indirect effects of fire. The contrasting results reported previously can be explained by differences in the degree of fire damage sustained by surviving trees, the temporal scale analysed, the pre-fire stand density, thinning intensity and the spatial scale used to compute the competition release (stand vs. tree level). Furthermore, the interaction between the fire damage of individual tress and the release from tree competition might be key to understanding the diverse results. Tree attributes (e.g. species, size, age), specific characterises of PB (e.g. intensity, season) and the meteorological conditions before, during and after PB are sources of variation that also need to be controlled in order to understand post-burning tree growth.

1.5.2.1 Impact of prescribed burning and drought on tree growth

In the context of climate change, it is important to consider the interaction between drought and fire. The increase in the frequency and intensity of warmer and drier periods might result in lower tree resistance to stressors such as pathogens, drought or fires. The review by Kane et al. (2017) evidences the importance of the sequence of disturbance interaction (drought and fire) on tree mortality: a severe drought can reduce tree vigour enough to promote a synergistic effect on tree mortality during a subsequent fire; in contrast, if fire precedes drought, the evidence suggests that the interaction has less effect. However, few studies have analysed the effects of PB-drought interactions on the growth of surviving trees.

There is some evidence that the effects of PB on tree growth are more visible in drought years (Skov et al. 2004) because the indirect effects of fire could increase the drought resilience capacity of trees as a consequence of increased resource availabilities (i.e. soil, water and nutrients). This effect would depend on the level of damage to each individual because physiological changes caused by the fire could constrain tree functioning even more during a drought event. No differences in tree growth between burned and unburned trees under subsequent drought events have been described (Bottero et al. 2017; Lloret et al. 2011). This could be due to the low release from tree competition after PB (Bottero et al. 2017) or a lack of fire severity measures at the individual level (Lloret et al. 2011). Other studies have found reduced drought resilience of burned pines (Collins et al. 2014b; Slack et al. 2016). At the spatial scale, Alfaro-Sánchez et al. (2018) suggest that the effects of drought are cancelled out in burned sites with high water availability but not in water-limited sites. Reversely, an intense drought before PB could exacerbate the fire severity experienced by each individual (Kane et al. 2017) resulting in additive or synergic effects on tree growth. However, as time since burning increases the indirect effects of fire could reverse this situation. Furthermore, fire-damaged trees are more vulnerable to insects, especially bark beetles, and infections by root fungi; therefore, understanding the defence system of pines after PB is crucial (Wallin et al. 2003).

1.5.3 Secondary metabolism and fire

Secondary metabolites are so named because they have no function in growth. They play a defence role against biotic threats or a protection function in relation to abiotic stressors. They can be classified as constitutive or induced; direct or indirect; and localized or systemic (Howe and Jander 2008). Plants usually have a permanent level of constitutive defences but under stress conditions they can induce new defences by increasing the concentration of some compounds, or by inducing the *de novo* compounds (Karban and Myers 1989). Repelling substances are released that attack the pathogen directly or indirectly by attracting its antagonist. Furthermore, when the attack occurs, the plant tissue affected reacts near the injury. However, systemic reactions have also been observed in other parts of the plants.

Terpenes are the most numerous group of secondary metabolites with more than 25,000 different products and are formed though the union of isoprene units (5-carbon atoms). Depending on the number of isoprene units, terpenes are classified as Monoterpenes (10C), Sesquiterpene (15C), Diterpenes (20C), Triterpenes (25C) and Tetraterpenes (40C). In *Pinus* species, oleoresin is a mixture of terpenes including monoterpenes (volatile metabolites), sesquiterpenes (metabolites with intermediate volatility) and diterpenes (semi-volatile compounds), which are stored in resin ducts of woody and needle tissues (Phillips and Croteau 1999). After mechanical damage or insect attack, monoterpenes serve as a solvent to transport non-volatile diterpenes to the injured tissue where monoterpenes evaporate and the wound seals by oxidation and polymerization of diterpenes in the trunk (Croteau and Johnson 1985) or needles (Pasqua et al. 2002).

Biotic factors can alter the production of terpene in plants as well as abiotic factors such as water stress, light, and temperature (Loreto and Schnitzler 2010). Moreover, the plant's physiological status (Sampedro et al. 2011) and genetics (Pausas et al. 2016) constrain the quantity and composition of terpene produced against a stressor as well as the nature and severity of the stress, and the species affected. Therefore, the direct and indirect effect of PB can alter the production of terpenes, and thus change needle flammability (Ormeno et al. 2009) and susceptibility to insects (Hood et al. 2015) (Figure 1.6).

Figure 1.6: Bark beetle attacking *P. sylvestris* one year after burning. Author: Pere Casals.

1.5.3.1 Effect of temperature and crown fire injury on terpene production

Direct effects of fire such as rising temperatures or heat induced needle damage can alter terpene production. As the temperature increases terpenes are released (Alessio et al. 2004) but if the temperature does not surpass the optimal temperature of enzymes, terpene production can be stimulated, which gives the needles thermoprotection against heat (Loreto and Schnitzler 2010). Usually the studies that determine the effects of fire on secondary metabolites focus on the effects of fire wounding on the wood resin flow and composition (Cannac et al. 2009; Lombardero et al. 2006; Powell and Raffa 2011), but little attention has been paid to the effect of fire on needle terpenes. The few studies that have analysed needle terpene content (Lavoir et al. 2013) were based on data from relatively low intensity fires with little effect on tree vigour and terpene concentration. However, there is evidence that crown fire injury can affect the production of tannins, phenols and pigments of needles (Alonso et al. 2002).

1.5.3.2 Effect of resource availability on terpene production

Indirect effects of fire can affect terpene concentrations by increasing resource availability (Ormeño et al. 2008). The 'growth differentiation balance hypothesis' (GDBH) (Herms and Mattson 1992; Stamp 2003) predicts that under poor water and nutrient availabilities, growth is more limited than photosynthesis. Since carbon assimilation is maintained, the excess of carbohydrates favours the synthesis of carbon-based secondary metabolites. On the contrary, when resource availability is high, the plant growth is not expected to be limited and plants allocate a greater proportion of assimilates to growth rather than to defence traits (Herms and Mattson 1992; Stamp 2003). Accordingly, if trees are damaged, a short-term response following PB should be an increased demand by the plant for chemical defence. However, as time since burning increases, the higher resource availability resulting from PB could favour carbon allocation to growth rather than chemical defences (Feeney et al. 1998). Indeed, time-course terpene responses of the direct and indirect effects of PB could differ between tree species depending on their fire resistance strategies.

1.6 Objectives

The goal of this doctoral thesis is to understand the influence of prescribed burning regime factors on the primary and secondary metabolisms of three pine species with contrasting fire tolerances: *Pinus nigra* ssp. *salzmannii* (Dunal) Franco; *P. sylvestris* L. and *P. halepensis* Mill.. In this thesis, the PBs used varied in terms of burning intensity and season, which resulted, at the tree level, in a wide range of fire severities. This allowed us to analyse how certain features of the burning regime affect the growth and defence reactions of surviving trees and which ones lead to tree death. Answering this question is not only important from the fuel and forest management points of view, but also from an ecophysiological perspective. To achieve this goal we applied a combination of dendrochronological, isotope and terpene quantification techniques. The specific objectives of this thesis are:

1. to characterize the post-burning growth response and mortality of *P. nigra*, *P. sylvestris* and *P. halepensis* (Chapters 2, 3, 4, 5);
2. to determine the effect of burning season on initial and delayed post-burning mortality of *P. nigra* and *P. sylvestris* (Chapter 3);

3. to assess the resilience capacity of *P. nigra* and *P. sylvestris* suffering two consecutives stressful events (drought and PB) (Chapter 4);
4. to determine the mechanisms underlying the post-fire physiological responses of dominant *P. halepensis* (Chapter 5);
5. to evaluate the effects of fire severity and fire-induced physiological tree changes on mono-, sesqui- and diterpene storage in *P. nigra*, *P. sylvestris* and *P. halepensis* (Chapter 6).

1.7 Structure of the doctoral thesis

The thesis is organized in chapters, written in the format of scientific articles, with the aim of publishing them in international, peer-reviewed, scientific journals. The current introduction provides the conceptual framework of the thesis (Figure 1.7). The first study (Chapter 2) examines the effects of low intensity PBs on the short- and mid-term growth response of *P. nigra* ssp. *salzmannii*, *P. sylvestris* and *P. halepensis*, based on tree resistance and individual fire severity as influencing variables. In Chapter 3, the mortality over time of *P. nigra* ssp. *salzmannii* and *P. sylvestris* after burning is assessed considering fire severity and burning season. In Chapter 4, the growth resilience of *P. nigra* and *P. sylvestris* suffering two consecutives stressful events (drought and PB) is modelled as a function of burning season and release from tree competition. In Chapter 5, we determine the effect of crown injury and release from tree competition on the growth and physiological response of *P. halepensis* (Objective 4). Then, in Chapter 6, we assess the temporal production of terpene compounds after burning in *P. nigra* ssp. *salzmannii*, *P. sylvestris* and *P. halepensis* with different levels of crown injury and fire-induced physiological tree changes (Objective 5). Finally, Chapter 7 includes the discussion of the main results of the thesis and the thesis Conclusions are listed in Chapter 8.

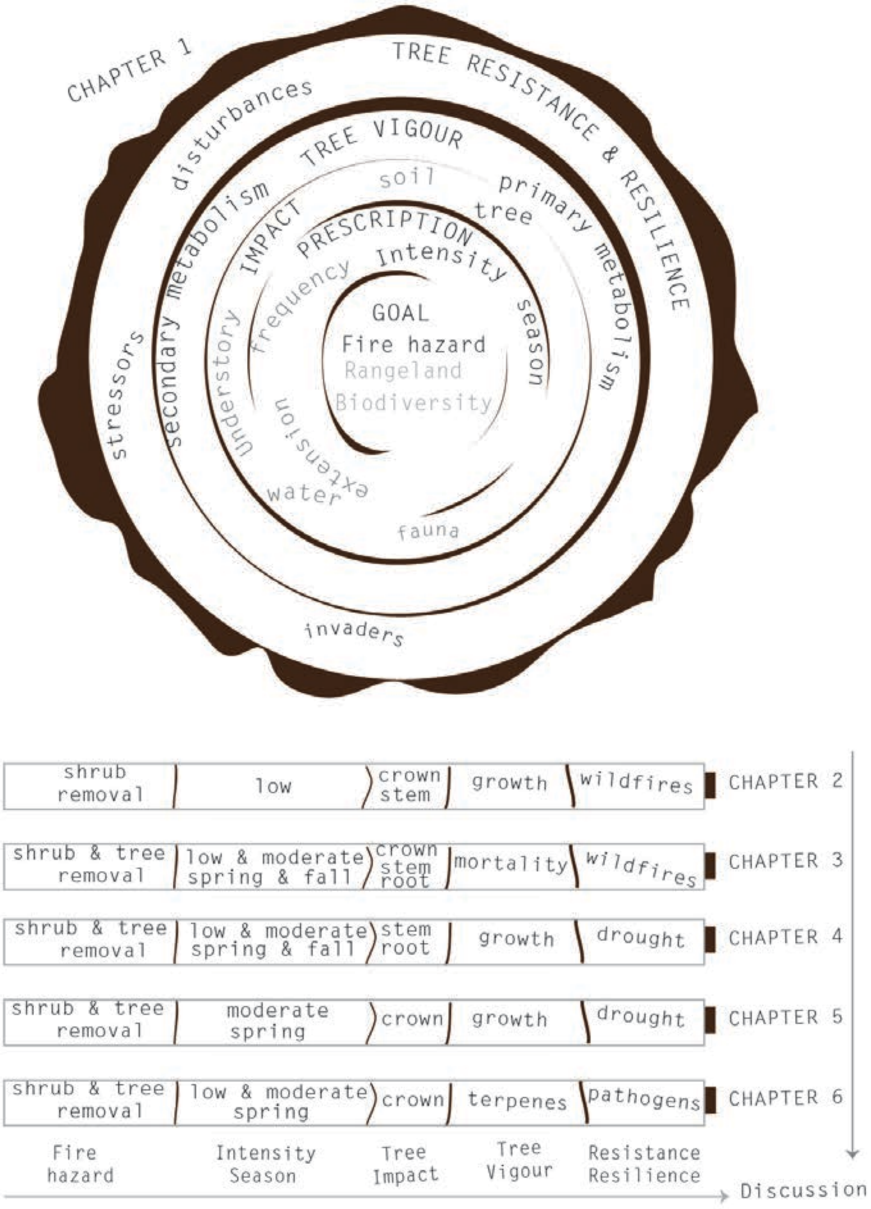


Figure 1.7: Conceptual framework of the thesis represented as a trunk section. The inner rings influence the outer rings, starting from PB goals to tree resistance and resilience. Within each ring the topic addressed is written in capital letters and the features of the topic are in small letters and in bold when studied in the thesis. A wood core represents each chapter. In each core/chapter, the specific features analysed in the thesis are shown in each ring.

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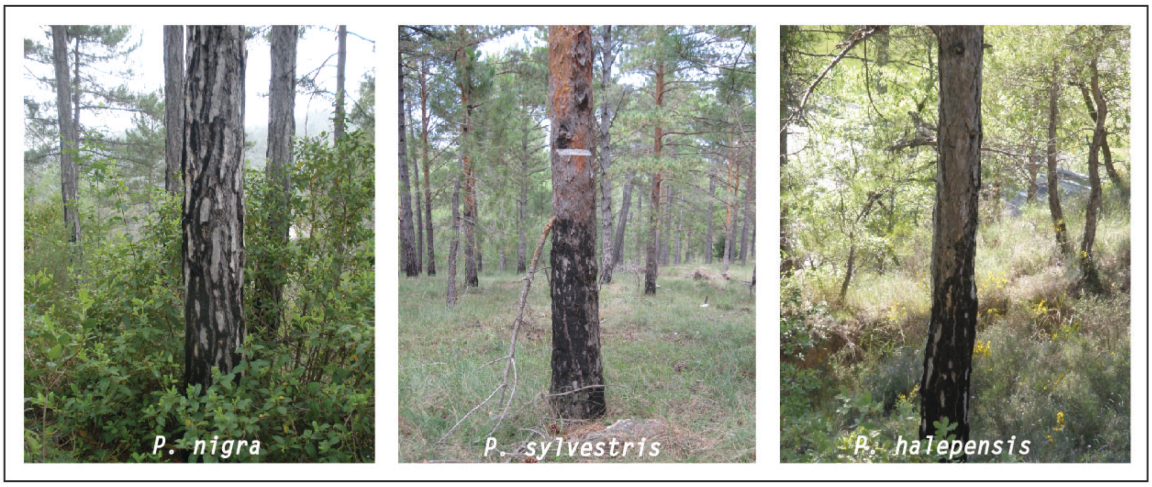
Chapter 2

Assessing the impact of prescribed burning on the growth of European pines



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Abstract

Prescribed burning to reduce surface fuel loads could help preserve pine stands by increasing forest fire-resistance, but its effects on tree growth, especially growth of European pines, are poorly understood. Characterizing the short and mid-term effects of prescribed burning on *Pinus* growth could provide valuable input to inform fire and forest manager decision-making. Here we use dendrochronology and mixed modelling to investigate whether prescribed burning has differential effects on the mid-term growth of dominant and suppressed *Pinus* trees under different levels of fire severity via an approach comparing observed post-burning growth against inferred tree growth without burning. Results showed reduced growth of *P. halepensis* and suppressed *P. sylvestris* at year of prescribed burning. Mid-term post-burning growths were good for dominant *P. halepensis* and *P. nigra salzmannii* trees subjected to higher fire severities, whereas suppressed *P. nigra nigra*, *P. sylvestris* and *P. halepensis* grew less than expected without burning. Although prescribed burning tended towards negatively affect the mid-term growth of *P. sylvestris* and *P. nigra nigra*, trees with higher pre-burn growth rates showed better post-burn recovery. The effect of fire severity on growth was positive for *P. nigra salzmannii* but negative for *P. nigra nigra.* These findings show that as time since burning elapses, growth recovery may depend on fire-tolerance of the pine species, degree of fire severity, tree characteristics and tree performance prior to prescribed burn. Understanding and balancing these factors in *Pinus* forests should help better plan prescribed burning, both in terms of desired fire intensity and required burning intervals, without altering tree vitality.

Keywords: Prescribed fire; Dendrochronology; Tree growth; *Pinus halepensis*; *Pinus nigra*; *Pinus sylvestris.*

2.1 Introduction

High-intensity fires threaten important ecological and social functions of *Pinus* species and their production value (Pausas et al. 2008). The negative impact of forest fires on the forest and its associated functions is expected to increase in the future, as model predictions point to increasing forest fire frequency, intensity and severity due to land-use and climate change (Flannigan et al. 2009).

Landscape-level fuel treatments strategically allocated in time and space can be combined with forest management efforts to reduce the extent and severity of forest fires, depending on vegetation type and historical fire regime (Agee et al. 2000). Fuel management in Europe traditionally relies on mechanical tools, but 10,000 ha yr^{-1} of forest is currently being managed by prescribed burning (PB) in which planned fires are set and used by fire experts under mild weather conditions to meet a defined management objective (Fernandes et al. 2013). PB is widely recognized in North America, South Africa and Australia, but it is still questioned in Europe although used marginally in Mediterranean countries like Portugal, Spain and France. *Pinus*-dominated stands cover approximately 64,9807 ha in Catalonia (NE Spain) without considering *Pinus uncinata* (Gracia et al. 2004), but only approximately 0.01 % of the forest area was treated annually with PB (GRAF 2011) whereas wildfires accounted annually for 0.8 % of the total burned forest over the period 1999-2011 (Gonzalez et al. 2007). The increase in number of large catastrophic fires in past decades (Gonzalez and Pukkala 2007) in Southern Europe has prompted the idea of establishing a less harmful fire regime, where the controlled spread of low-intensity unplanned fires is to be allowed and PB extensively applied as a cost-efficient way to reduce fuel continuities (Pinol et al. 2007; Regos et al. 2014). However, the requisite changes to the social, economic and legal restrictions limit the deployment of this new fire management policy for PB (Fernandes et al. 2013) and especially for unplanned fires (Regos et al. 2014). Besides, research of its potential effects on forest ecosystems and their accompanying services is still required, adding uncertainty.

PB plans can lead into conflicts between protection, conservation and production goals, as PB may be beneficial for some ecosystem functions but indirectly affect others in an undesirable way (Driscoll et al. 2010). Post-PB tree vitality is a key aspect that warrants closer attention. PB may be perceived as aggravatory factor to the gradual process of tree death, both directly due to physical fire damage to the tree, and indirectly as a contributory factor exacerbating the effects of drought episodes and other stressors (Allen et al. 2010). In contrast, tree vitality can be partially affected, unaffected or even improved with time since burn, depending, among others, on the species, fire severity and tree size (Peterson et al. 1991; Valor et al. 2013). It is difficult to obtain a metric of tree vigour, as there are no direct measures to reflect it, but indicators such as crown transparency or tree growth, among others, are feasible proxies of tree vitality (Dobbertin 2005). For instance, some dendroecological studies use tree growth as an indirect measure of tree vitality after a perturbation such as drought (Martinez-Vilalta et al. 2011; Linares et al. 2014) or defoliation (Linares et al. 2014) and in some cases even to predict tree mortality (Bigler and Bugmann 2004). Tree vitality is diagnosed by comparing the stressed growth of a tree to a reference growth of the same tree (e.g. growth prior to the stress or modelled without stress) or the growth of other unstressed trees nearby (Dobbertin 2005).

Postfire growth is essentially regulated by tree attributes (e.g. species, size, competition, age) and fire regimes (e.g. pattern, size, intensity, severity, season and recurrence) (Keyser et al. 2010). There is a consensus that specific characteristics of affected trees are major sources of variability in tree growth response to fire, yet few studies have actually characterized these variations. Reductions in tree growth result from altered photosynthetic processes due to the physical damage to tree tissues caused by the fire (Chambers et al. 1986), whereas increases in tree growth can be observed in unaffected trees or in trees healing over time since fire due to increased light and soil nutrient availability or reduced tree competition (Certini 2005).

There is far less information on postfire tree growth in European species than species studied in North America and Australia. The few European studies on this subject have focused on growth responses in fire-resistant species (Agee 1998) after low-severity forest fires in *Pinus nigra salzmannii* (Valor et al. 2013), *Pinus sylvestris* (Beghin et al. 2011; Blanck et al. 2013) and the fire-resistant fire-endurer (Fernandes et al. 2008) *Pinus canariensis* (Rozas et al. 2011), with rare studies on tree growth response to PB in *Pinus pinaster* (McCormick 1976; Botelho et al. 1998). There has been little focus on fire-evader species (Agee 1998) like *P. halepensis*, whether after forest fires (Battipaglia et al. 2014a) or after PB (Battipaglia et al. 2014b). Here, we studied the effects of PB as a surface fuel hazard reduction strategy on the short-and mid-term growth of pine species with contrasted tolerance to fire. Our aims were: (1) to ascertain whether there are consistent variations in post-PB growth (release or suppression) in *Pinus halepensis* Mill., *Pinus nigra* ssp. *salzmannii*, *Pinus nigra* ssp. *nigra* and *Pinus sylvestris* L. subjected to PB and on sites with contrasting climatic conditions, and (2) to characterize individual tree growth response to PB over time since burning, based on crown status and fire severity as influencing variables. We hypothesize that low-intensity PB should be neutral or even improve tree vigour in more fire-resistant species like *P. nigra* and *P. sylvestris* but weaken tree vigour in the fire-evader species *P. halepensis*, but that these responses may change over time post-PB. The studied pine species are not only ecologically important in the region but also in terms of economic value as they represent annual cuttings of 350.000 m^2, i.e. over 60% of the region's total harvested timber (Idescat 2014) and subtain the yield of non-wood forest products as relevant in the region as mushrooms (Bonet et al. 2014). Determining the duration and intensity of PB impact on growth and the subsequent recovery patterns (if any) should help refine PB timing and intensity.

2.2 Methods

2.2.1 Study sites

Study sites were obtained from the PB database developed by the Forest Actions Support Group of the Catalan Government (GRAF). From the database, we selected study sites treated with a PB at least 4 years prior to starting the study, (including year of PB) and dominated (>50% of basal area) by one of the main pines in the region (*Pinus halepensis* Mill., *Pinus nigra*, including two subspecies *Pinus nigra* ssp. *salzmannii* and *Pinus nigra* ssp. *nigra*, and *Pinus sylvestris* L.). A total of 14 study sites, distributed across the region of Catalonia (NE Spain), were selected for tree-ring sampling: 4 dominated by *P. halepensis*, 4 by *P. nigra* ssp. *salzmannii*, 3 by *P. nigra* ssp. *nigra* and 3 by *P. sylvestris* (Table 2.1 and Figure 2.1).

Table 2.1: Description of the PB sites.

Pine species	Study sites	Burn year	Burnt surface (ha)	Tree density (tree/ha)	Slope (%)	Aspect	Elev. (m.a.s.l)
P. halepensis	*Ph1*	2003	0.8	1089.4	24.0	E	340
	Ph2	2005	6.0	520.0	9.9	NW	261
	Ph3	2006	0.4	1645.6	22.0	NW	400
	Ph4	2007	5.8	980.8	20.0	NE	210
P. nigra salzmannii	*Pns1*	2003	2.2	836.1	34.6	N	670
	Pns2	2003	3.7	753.2	35.9	N	492
	Pns3	2003	0.9	1652.8	30.0	NE	737
	Pns4	2003	1.8	808.1	30.0	NE	730
P. nigra nigra	*Pnn1*	2002	9.5	830.7	27.7	NE	1049
	Pnn2	2003	3.4	1323.2	24.9	W	902
	Pnn3	2003	2.6	1031.5	10.0	NW	878
P. sylvestris	*Ps1*	2002	9.5	1425.5	30.5	N	1049
	Ps2	2002	5.2	256.1	10.0	N	1010
	Ps3	2003	0.9	708.4	30.0	NE	737

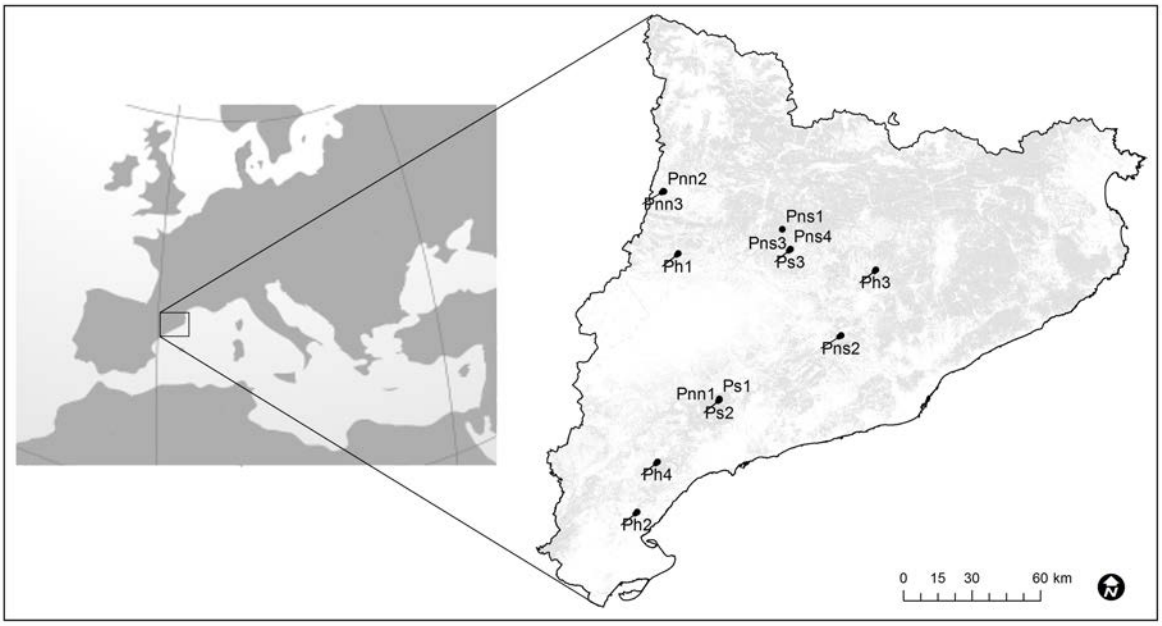

Figure 2.1: Site distribution across Catalonia (NE Spain). Ph =*P. halepensis* sites, Pns =*P. nigra salzmannii* sites, Pnn = *P. nigra nigra* sites, Ps = *P. sylvestris* sites.

P. halepensis study sites tend to be in areas of dry Mediterranean climate whereas *P. nigra salzmannii*, *P. sylvestris* and *P. nigra nigra* study sites fell into temperate cold sub-Mediterranean climate. Based on Spanish Meteorological Agency (AEMET) data, over 1986–2010 (Figure 2.2), annual mean temperature and mean precipitation on the selected study sites were 14.3ºC and 491.4 mm for *P. halepensis*, 12.9ºC and 631 mm for *P. nigra salzmannii*, 11.8ºC and 589.3 mm for *P. nigra nigra*, and 11.1ºC and 534.9 mm for *P. sylvestris*.

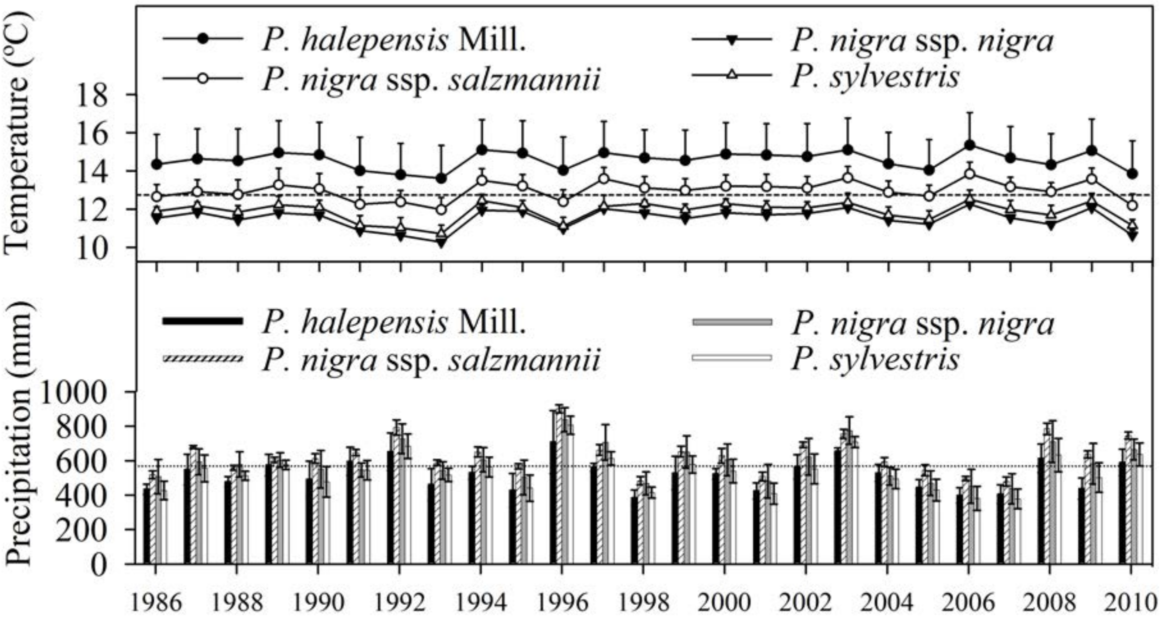

Figure 2.2: Evolution in mean annual temperature and cumulative precipitation on the study sites, grouped by pine species. The standard deviation of the climatic variables among sites dominated by the same pine species is shown.

2.2.2 Tree selection and tree-ring measurement

2.2.2.1 Tree selection

The criteria for cored-tree sampling aimed to select a minimum of 8 trees from each combination of crown status (dominant or suppressed) and fire severity class (high or low) at each study site. However, the target of 8 trees per combination was not always reached due to an absence of candidate trees in some of the grouping categories (Table A1).

Dominant trees were defined as trees whose crown width above the main canopy layer of the stand, intercepting direct sunlight, and among the larger of the stand in terms of stem diameter (Kraft 1884). Suppressed trees were defined as trees whose crown remains entirely or partially below the main canopy, receiving none or little direct sunlight, and among the smaller of the stand in terms of tree diameter (Kraft 1884). The classification of the trees on these two crown status categories was implemented to facilitate the analysis, requiring from the assumption that codominant trees were considered as a dominant and intermediate trees fell into the suppressed category. In order to define the fire severity classes (FSEV), the tree's bole char height (BCH) was measured as an indirect measure of cambium damage (Regelbrugge and Conard 1993; Kobziar et al. 2006; Keeley 2009). The degree of fire severity at the tree level can vary within a single PB depending mainly on micro-site fuel load and continuity, as meteorological conditions are rather constant. Trees were grouped into the high-severity class when BCH >1 m and the low-severity class when BCH < 0.5 m. Therefore, both crown status (CS) and severity classes (FSEV) were defined as categorical variables with two levels of Dominant/Suppressed and High/Low, respectively. We assumed that CS at burn year was the same as in 2012 when we collected the data, because PB did not reduce tree density and no management operations were executed after the PB. Diameter at breast height (DBH) and total height (Ht) were measured in each tree (Table 2.2). In addition, the 5 trees closest to the target tree were measured for BCH, and their average BCH, including the measured target tree BCH, was calculated ($\mathrm{BCH}_{\mathrm{local}}$) and used as a proxy of the intensity of the fire once it reached our target tree (Table 2.2).

Table 2.2: Means and standard deviation of diameter at breast height (DBH), total height (Ht), bole char height (BCH) and local fire severity ($\text{BCH}_{\text{local}}$) at sampling year (2012) and burn-year diameter at breast height (DBH_{b}) and age (AGE_{b}), for each crown status–fire severity combination within each pine species.

Pine species	Crown status–Severity	Sample size (n)	DBH (cm)	DBH_{b} (cm)	Ht (m)	BCH (m)	$\text{BCH}_{\text{local}}$ (m)	AGE_{b}
P. halepensis								
	Dominant-High	25	22.5±4.8	12.5±3.3	12.4±4.2	1.2±0.1	0.8±0.06	32.7±10.4
	Dominant-Low	30	19.7±3.6	12.6±2.5	12.8±5.8	0.3±0.1	0.4±0.04	36.6±20.0
	Suppressed-High	16	17.7±4.0	9.0±1.9	10.8±1.9	1.2±0.2	0.9±0.09	35.5±17.5
	Suppressed-Low	17	14.3±2.7	8.6±2.0	10.3±2.6	0.3±0.1	0.4±0.04	34.7±16.8
P. nigra salzmannii								
	Dominant-High	20	24.9±5.8	13.6±6.3	12.9±2.4	1.2±0.2	0.8±0.12	55.9±14.1
	Dominant-Low	20	20.1±5.0	10.4±3.0	12.9±2.1	0.3±0.1	0.4±0.06	51.6±18.2
	Suppressed-High	20	15.9±4.3	11.8±4.6	9.2±1.4	1.2±0.2	0.9±0.06	52.7±18.0
	Suppressed-Low	20	13.3±2.7	9.6±2.4	9.4±1.8	0.3±0.1	0.5±0.03	55.8±15.2
P. nigra nigra								
	Dominant-High	21	21.6±3.7	13.1±2.2	11.3±1.3	1.2±0.2	0.9±0.09	31.2±10.4
	Dominant-Low	20	21.1±5.4	12.7±3.5	10.6±1.4	0.2±0.1	0.4±0.06	32.7±10.7
	Suppressed-High	21	15.6±4.1	9.8±3.1	10.3±1.9	1.2±0.1	1.0±0.11	31.2±10.6
	Suppressed-Low	18	14.2±3.8	9.2±2.5	9.1±1.6	0.3±0.1	0.4±0.05	32.4±11.1
P. sylvestris								
	Dominant-High	20	23.9±3.7	16.3±3.5	12.7±1.9	1.2±0.2	0.7±0.11	46.2±10.4
	Dominant-Low	20	20.7±4.6	15.1±3.7	12.5±1.6	0.3±0.1	0.5±0.03	44.3±11.6
	Suppressed-High	20	15.9±3.8	12.0±4.0	10.0±1.9	1.2±0.3	0.9±0.39	42.3±11.4
	Suppressed-Low	20	15.4±4.7	9.8±3.7	10.2±1.8	0.3±0.1	0.7±0.34	40.1±13.2

2.2.2.2 Tree-ring measurement

A total of 328 trees (88 *P. halepensis*; 80 *P. nigra salzmannii*; 80 *P. nigra nigra*; 80 *P. sylvestris*) were ultimately selected and cored to the pith during May and June 2012. Trees were cored with an increment borer at 1.30 m and as far away as possible from fire scars (Mutch and Swetnam 1995) or in our case the most scorched side of the stem, as scars were seldom visible. All extracted cores were prepared following standard dendrochronological techniques (Stokes and Smiley 1968). Tree-rings were measured from year 2011 until pith, as late wood in spring 2012 was not still defined (Rinn 1996), using a Lintab 3 measuring system coupled to TSAP tree-ring software (Frank Rinn, Heidelberg, Germany), with a precision of 0.001 mm. All cores were dated and visually cross-dated to detect the presence of false and incomplete rings. Within each of the crown status and fire severity combinations, cross-dating was validated using COFECHA software (Holmes 1983), which calculates cross-correlations amongst individual series of tree growths. For each cored tree, the $\mathrm{DBH_b}$ and $\mathrm{AGE_b}$ were estimated by dendrochronological methods (Table 2.2). Mean interseries correlation (Rbar) was calculated to assess agreement between individual series in each combination (Table A1). The majority of the trees showed Rbar values above 0.3, except for some suppressed trees that still were kept for growth modelling as the correlation was positive (Martín-Benito et al. 2007; Martínez-Vilalta et al. 2011).

2.2.3 Climatic data

Monthly precipitation (P) and temperature (T) from 1985 to 2010 were downscaled for each study site from climatic records and projections elaborated by the Spanish Meteorological Agency for each study site (AEMET) (Ninyerola et al. 2000; Ninyerola et al. 2007). Monthly potential evapotranspiration (PET) was estimated using the Thornthwaite (1948) method. Cumulative values (1985-2010) of P and PET were calculated for three time windows ranging from June (jun), August (aug) and September (sep) of the year before the formation of the tree-ring (*t-1*) to September of the year of ring formation (t). For each time window, two classes of drought indices (DRI) were calculated: difference between P and PET (*P-PET*), and P to PET ratio (P/PET). In total, six drought indices were calculated for each study site: $P\text{-}PET_{\mathrm{jun(t-1)\text{-}sep(t)}}$, $P\text{-}PET_{\mathrm{aug(t-1)\text{-}sep(t)}}$, $P\text{-}PET_{\mathrm{sep(t-1)\text{-}sep(t)}}$, $P/PET_{\mathrm{jun(t-1)\text{-}sep(t)}}$, $P/PET_{\mathrm{aug(t-1)\text{-}sep(t)}}$ and $P/PET_{\mathrm{sep(t-1)\text{-}sep(t)}}$. The time intervals and their associated drought indexes were selected based on previous knowledge on the influence of climate on the radial growth of the studied pines (Gutiérrez 1989; Martín-Benito et al. 2007; Sanchez-Salguero et al. 2012; Herrero et al. 2013).

2.2.4 Data analysis and growth modelling

The yearly radial increments were converted into yearly basal area increments (BAI) as initial predictor variable to quantify growth variation due to PB, using the equation:

$$(1)\ \ BAI = \pi \cdot (r_t^2 - r_t^2)$$

where r is the tree radius and t is the year of tree-ring formation.

The approach for assessing growth variation due to PB encompassed two phases and was based on comparing observed BAI (BAI_{obs}) against inferred BAI (BAI_{inf}). First, pre-burning BAI was modelled using tree characteristics and climate conditions as predictors and BAI_{obs} prior to burning as predicted variable (from 1985 till one year prior to PB). The selected models were used to estimate BAI_{inf}, thus assuming that no PB ever happened. Afterwards, the BAI_{inf} was compared against the post-burning BAI_{obs} so as to assess the PB effect on annual variations in BAI. Therefore, if on a given year PB had a positive effect on growth, the BAI_{obs} of that year was supposed to be greater than the corresponding BAI_{inf}. On the other hand, if PB had a negative effect on tree growth, the BAI_{obs} was supposed to be smaller than the BAI_{inf}. Second, the standardized difference between BAI_{obs} and BAI_{inf} was used as a predicted variable (BAI_{dif}) to model the impact of PB on growth during the first four years after PB, using tree and fire severity variables.

2.2.4.1 Inferring growth under the no-burning scenario

For each pine species, a linear mixed model was fitted for predicting BAI under a non-burning scenario using the best-fit drought index (DRI) (see section Climatic data) and breast height diameter at burnt year (DBH_{b}) as independent variables:

$$(2)\ \ ln(BAI_{k(jy)} + 1) = \beta_0 + \beta_1 \cdot f(DRI_{ky}) + \beta_2 \cdot DBH_b + \gamma_{0k} + \alpha_{0k(j)} + \epsilon_{k(jy)}$$

where β is the overall intercept; β_1 and β_2 are the parameters adjusting the fixed effects, k is the index of the study site, *k(j)* is the index for tree nested in the study site, y is the index for year of measurement, γ_{0k} and $\alpha_{0k(j)}$ are the random effects associated with study site and tree, respectively, and $\epsilon_{k(jy)}$ is the error term. Temporal autocorrelation of the error term was accounted for using an AR-(1) correlation structure. Models with the lowest Akaike Information Criterion (AIC) (Burnham and Anderson 2002) were selected and used, as mentioned, to infer the hypothetical growth of the no-burning scenario (BAI_{inf}), after applying a Snowdon correction factor (1991) to avoid bias due to log-transformation of the predicted variable. Annual BAI_{inf} was inferred until 2010, as growth in 2011 was excluded as 2011 climate data was unavailable. For each pine, the observed mean growth BAI_{obs} and mean BAI_{inf} was plotted against time-since-burning (TSB), which refers to number of years since application of the PB.

2.2.4.2 Modelling the impact of prescribed burning on tree growth

For each pine species, a linear mixed model was developed to describe short and mid-term impact of PB on growth. The predicted variable was the BAI_{dif} calculated as the difference between each of the first four years post-burning BAI_{obs} (including the year of PB) and the corresponding BAI_{inf}, divided by the square root of the pre-burning BAI_{obs} time series variance, in order to remove the effect of growth variability from the actual growth rate (Martínez-Vilalta et al. 2011). Models considered the following as potential fixed factors: time-since-burning (TSB), crown status (CS), fire severity class (FSEV) and local fire severity (BCH_{local}). Second interactions of FSEV with the other fixed factors except BCH_{local} were also included. All fixed factors and interaction terms were incorporated, and only the significant effects were retained. An AR(1) correlation structure was included to consider the temporal autocorrelation of the error terms. The non-independence of tree-ring growth data, as each tree growth responds to consecutive post-burning years, was resolved by adding a random intercept, which is a baseline for the multiple growth responses on the same individual tree. In order to characterize each tree baseline response to BAI_{dif}, random intercepts of each pine species model were regressed against age at burnt year (AGE_b) and the mean of the 5-year pre-burning BAI_{obs} (BAI_{obs5}). These two variables were considered potential factors explaining the random intercepts of the PB impact models of each pine. The residuals of all models developed presented no pattern, and highly correlated explanatory variables in the two phases of modelling were avoided. All analyses were performed using R software (v. 3.0.1, R Foundation for Statistical Computing) using the nlme package for linear mixed-effects modelling.

2.3 Results

2.3.1 Inferred growth under the no-burning scenario

For all pines, the best-fitted linear mixed models indicated that pre-burning BAI was significantly influenced by tree size at burning year and by the same drought month interval (Table 2.3).

The results showed that for all tree species, larger trees showed more growth than smaller ones, especially during years with no water restriction. The fixed part of each pre-burning BAI model accounted for 0.47 of total variance in *P. halepensis* Mill., 0.11 in *P. nigra salzmannii*, 0.40 in *P. nigra nigra* and 0.40 in *P. sylvestris*.

Table 2.3: Best-fitted linear mixed models of the pre-burning basal area increment (BAI) from 1986 to the year before of the prescribed burning for each pine species.

Pine species	Best pre-burning BAI fitted model	R^2	AIC_{null} $-AIC_{best}$	Snowdon correction
P. halepensis	$-0.285+0.129 \cdot DBH_b+0.001 \cdot P\text{-}PET_{jun(t\text{-}1)\text{-}sep(t)}$	0.64	494.6	1.11
P. nigra salzmannii	$0.552+0.027 \cdot DBH_b+0.001 \cdot P\text{-}PET_{jun(t\text{-}1)\text{-}sep(t)}$	0.64	178.3	1.08
P. nigra nigra	$-0.431+0.122 \cdot DBH_b+0.398 \cdot P/PET_{jun(t\text{-}1)\text{-}sep(t)}$	0.57	171.0	1.03
P. sylvestris	$-0.482+0.109 \cdot DBH_b+0.197 \cdot P/PET_{jun(t\text{-}1)\text{-}sep(t)}$	0.55	133.1	1.08

DBH_b, diameter at breast height at burnt year.

$P\text{-}PET_{jun(t\text{-}1)\text{-}sep(t)}$, drought index calculated as the difference between precipitation and potential evapotranspiration from June before tree-ring formation to September of the year of tree-ring formatio.

$P/PET_{jun(t\text{-}1)\text{-}sep(t)}$, drought index calculated as the ratio between precipitation and potential evapotranspiration from June before tree-ring formation to September of the year of tree-ring formation.

Plotting the mean BAI_{inf} obtained using the fixed part of the models (Table 2.3) against the corresponding post-burning mean BAI_{obs} revealed that both followed a similar evolution within each pine (Figure 2.3).

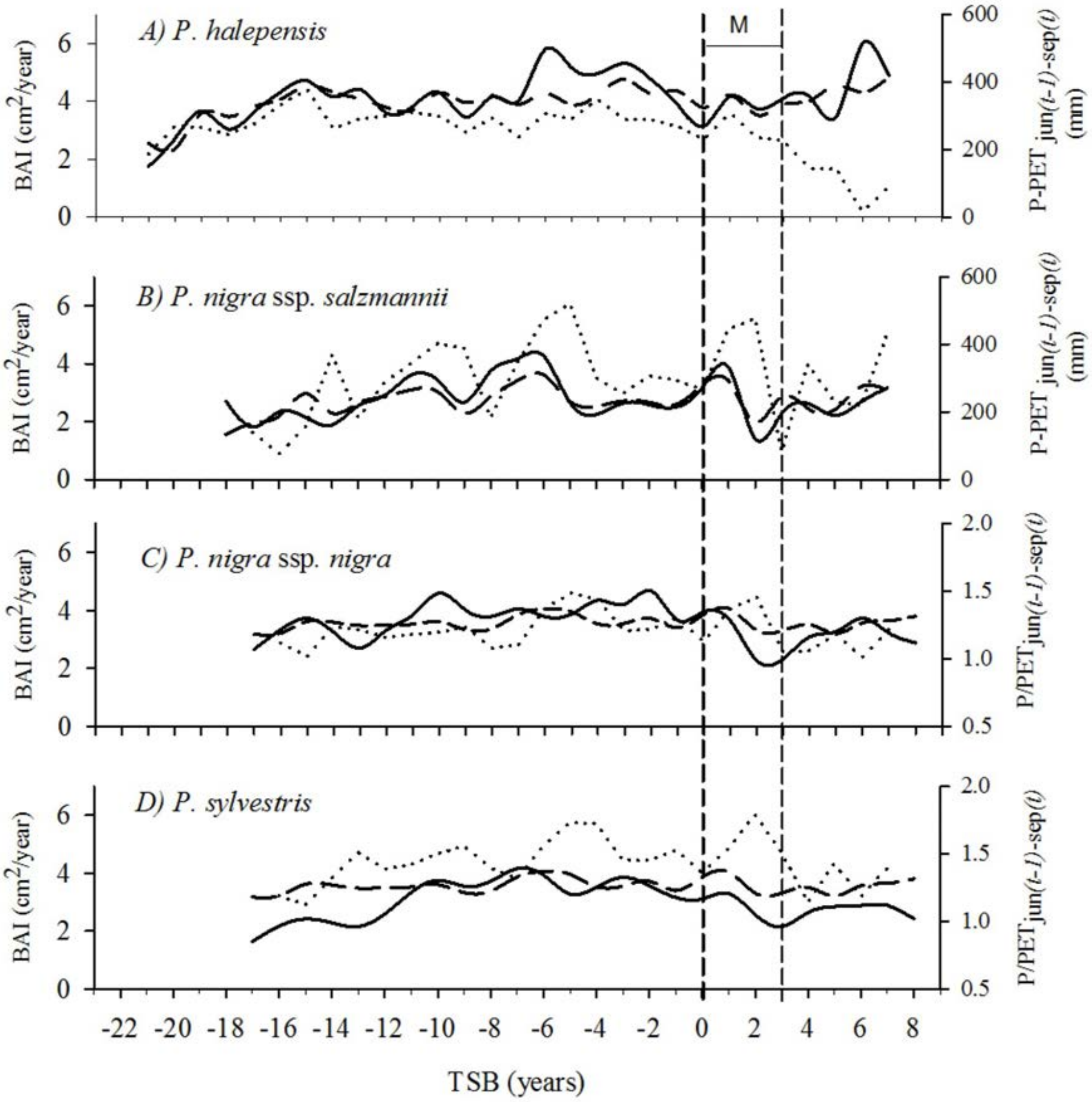

Figure 2.3: Comparison of time-course trends of mean observed basal area increment (BAI_{obs}) (solid lines), inferred basal area increment (BAI_{inf}) in the case of no PB application (dashed lines), and the influencing drought indices (dotted lines) against time-since-burning (TSB) for each pine specie. PB year (dashed vertical line) and *M*, modelling period of the PB impact on growth are also shown.

However, important variation appeared in specific years. For instance, at the year of PB, the BAI_{obs} was significantly lower than BAI_{inf} for *P. halepensis* and *P. sylvestris* (Figure 2.3, A and D) yet almost identical in the two *P. nigra* subsp. (Figure 2.3, B and C). The first year following the PB, there was a strong increase in BAI_{obs} compared to BAI_{inf} in *P. nigra salzmannii* (Figure 2.3, B). Lastly, the second year following the PB, there was a significant drop in BAI_{obs} compared to BAI_{inf} in *P. nigra nigra* which did not recover until year five after the fire, whereas *P. sylvestris* grew less than the predicted BAI_{inf} during the entire post-burning period (Figure 2.3, C and D).

2.3.2 Modelled impact of prescribed burning on tree growth

For all pines, growth variation was found to be significantly affected by TSB, with different signs depending on the year, and also by CS (except for *P. nigra nigra*), with dominant trees showing higher postburning growth (Table 2.4).

Severity of fire around the target tree, $\mathrm{BCH}_{\mathrm{local}}$, had a significant effect on the $\mathrm{BAI}_{\mathrm{dif}}$ of *P. nigra* species: positive in *P. nigra salzmannii* and negative in *P. nigra nigra* (Table 2.4). None of the interactions was significant, and there were no differences between FSEV classes.

Table 2.4: Summary of the models characterizing the impact of prescribed burning on growth variation (BAI_{dif}) in pine species.

Fixed effect	*P. halepensis*			*P. nigra salzmannii*			*P. nigra nigra*			*P. sylvestris*		
	Estimate	SE	p-value	Estimate	SE	p-value	Estimate	SE	p-value	Estimate	SE	p-value
Intercept	-1.035	0.208	<0.001	-1.185	0.237	<0.001	0.572	0.253	<0.05	-0.551	0.188	<0.01
TSB=1	0.374	0.117	<0.01	0.332	0.123	<0.01	-0.549	0.153	<0.001	-0.209	0.108	0.055
TSB=2	0.567	0.128	<0.001	-0.132	0.133	0.324	-0.840	0.159	<0.001	-0.394	0.104	<0.001
TSB=3	0.422	0.130	<0.01	-0.335	0.135	<0.05	-0.811	0.160	<0.001	-0.427	0.104	<0.001
CS=Dominant	0.693	0.246	<0.01	0.749	0.169	<0.001	0.087	0.221	0.694	0.608	0.250	<0.05
BCH_{local}	0.364	0.489	0.550	0.639	0.262	<0.05	-0.838	0.279	<0.01	-0.083	0.268	0.757

TSB, time since burning; CS, crown status; BCH_{local}, local fire severity.

Model $R^2 = 0.71$ for *P. halepensis* (AIC=1040.5), $R^2 = 0.52$ for *P. nigra salzmannii* (AIC=897.7), $R^2 = 0.56$ for *P. nigra nigra* (AIC=1031.1) and $R^2 = 0.79$ for *P. sylvestris* (AIC=871.4)

Fixed Model $R^2 = 0.08$ for *P. halepensis*, $R^2 = 0.17$ for *P. nigra salzmannii*, $R^2 = 0.11$ for *P. nigra nigra* and $R^2 = 0.07$ for *P. sylvestris*

Evaluations of the short-term (TSB=0) impact of PB on BAI_{dif} of *Pinus* species showed a negative BAI_{dif} for *P. halepensis*, *P. nigra nigra* with high BCH_{local} and suppressed *P. sylvestris* (Table 2.4). In contrast, PB was found to have a positive impact on BAI_{dif} in dominant *P. sylvestris* trees and all *P. nigra salzmannii* trees regardless of crown status, but in this case when BCH_{local} was high or extremely high (Table 2.4).

As time since PB elapsed (from TSB=1 to TSB=3), the impact of PB on the BAI_{dif} of dominant *P. halepensis* trees was found to be positive across the whole period, in contrast to suppressed trees that failed to show positive BAI_{dif} in any TSB year (Table 2.4). In the case of dominant *P. nigra salzmannii*, BAI_{dif} was also positive and increased in magnitude with increasing BCH_{local}, whereas suppressed *P. nigra salzmannii* showed negative BAI_{dif} values unless BCH_{local} was particularly high (Table 2.4). In the case of *P. nigra nigra* and *P. sylvestris* trees, PB had a negative impact on BAI_{dif} across the whole 3-year post PB period, regardless of tree status or fire severity (Table 2.4).

Finally, evaluation of the impact of random effects (AGE_b and BAI_{obs5}) on growth variation revealed that an increase in trees age had a negative effect on BAI_{dif} in *P. nigra nigra* and *P. sylvestris*, whereas an increase in pre-burning BAI_{obs5} had a positive effect on post-burn growth recovery in *P. nigra nigra* and *P. sylvestris* (Figure 2.4) but no effect on the other two species.

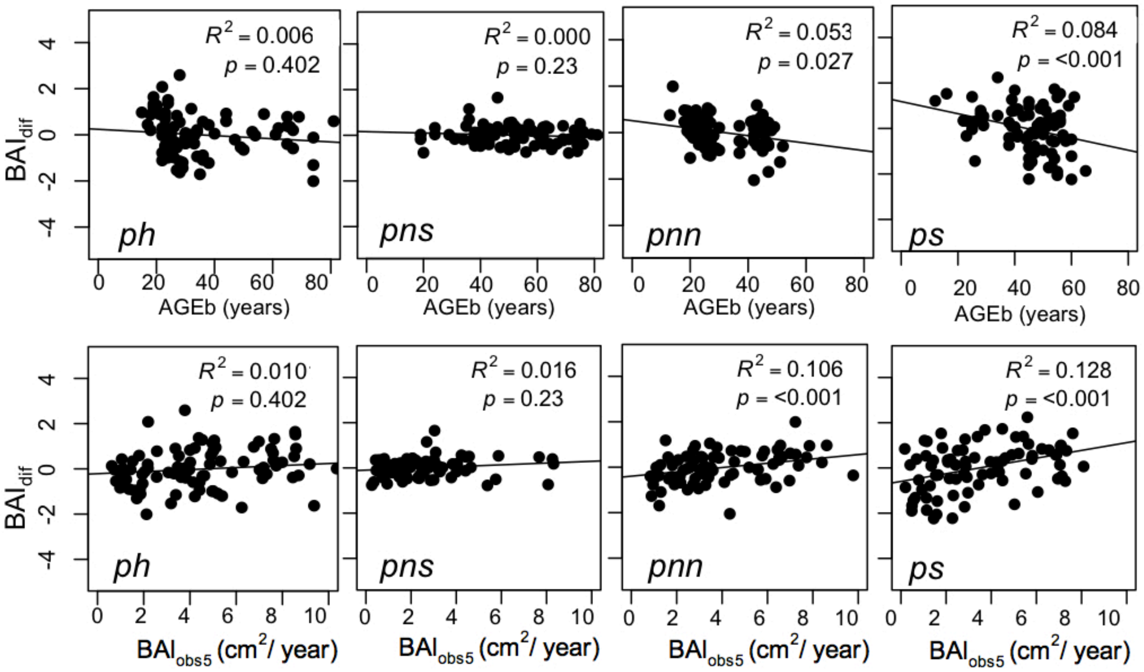

Figure 2.4: Adjusted R^2 of tree age at burnt year (AGEb) and mean of the 5-year pre-burning BAI (BAI_{obs5}) on growth variation (BAI_{dif}) for all pine species. The regression lines were estimated by fitting a linear model. *Ph* = *P. halepensis*, *Pns* =*P. nigra salzmannii*, *Pnn* = *P. nigra nigra*, and *Ps* = *P. sylvestris*.

2.4 Discussion

This study was designed to assess the impact of PB on the growth of European pines. The information generated here provides a framework to establish species-specific PB recommendations on timing and intensity designed to reduce the potentially negative impacts of PB on tree vitality.

Our results show that when the impact of PB on tree growth is assessed at the year of the PB its effect varies among pines (Figure 2.3). Immediately after the PB, growth variations can be interpreted in the context of the ecological fire adaptations of the studied *Pinus* species. Especially those related with potential differences in bark thickness that may confer distinctive degrees of fire tissue protection among them particularly when young (Fernandes et al. 2012). Firstly, growths remained unaffected in fire resister species such as *P. nigra salzmannii* (Figure 2.3, B), probably, due to its pyro-resistant morphological traits conferring fire tissues protection (e.g. thicker bark) (Fule et al. 2008) and to a lesser extend in *P. nigra nigra* (Figure 2.3, C). Although there is some evidence that *P. halepensis*, when adult, can withstand surface fires (Fournier et al. 2013), generally it is assume that as a fire evader specie its traits are less favourable to cope with low-intensity fires (e.g. thinner bark) (Keeley 2012) explaining probably the observed reduction in growth immediately after the fire (Figure 2.3, A). Lastly, the growth reduction suffered by the moderately fire resistant *P. sylvestris* may be related to cambium damages caused by the fire due to its thinner bark when compared with *P. nigra* (Figure 2.3, D) (Tapias et al. 2004; Fernandes et al. 2008).

The findings of the study also highlight the importance of modelling tree growth response to PB as a function of tree and fire attributes to gain insight into individual tree responses (Table 2.4), as our results point to variations in the above-mentioned trends not just at the year of PB but also as time increases. For example, as expected, crown status plays a major role in defining the immediate impact of PB on tree growth (Sutherland et al. 1991), with dominant trees suffering less growth reduction immediately after PB than suppressed trees. The reasons for this impact is presumably that smaller trees are more exposed to cambial injuries due to their thinner bark, especially at higher stem heights (Fernandes et al. 2012). The fact that *P. nigra nigra* was used for reforestation purposes, with homogeneous tree development, may be the cause of the absence of differences between crown status. Bark thickness, together with the temperature and the residence time of the fire beneath the tree, determine ultimately the degree of cambium damage (Bova and Dickinson 2005). However, the fire severity indicator, BCH, used to create FSEV classes was not found to have a significant effect on post-burning growth. The lack of a measurable impact of fire severity on growth could be due to the relatively small variability between the defined FSEV classes (Table 2.2). With this regard the short-term reduction in growth observed in dominant *P. halepensis* may be explained by longer duration of burning at the base of the trunk due to their shorter needles present in the litter (Fonda 2001) but also for its greater crown vulnerability to fire as it has the lowest canopy base height among Mediterranean pine species (Mitsopoulos and Dimitrakopoulos 2007). In this sense the reduced growth in suppressed trees could be also be due to the combining effects of fire damages, not only on the cambium, but, also, on the crown due to their lower canopy base heights, which probably allows flames to scorch a larger portion of the tree crown foliage, leading to higher rates of transpiration and inefficient photosynthesis bringing about a reduction in stem growth (Keyser et al. 2010). The amount of canopy scorched, if any, would have been an appropriate indicator for defining fire severity at the crown level (Fernandes et al. 2008), but the retrospective nature of our study impeded the measurement of crown scorch. Indeed, we did not find a significant interaction between FSEV and CS that could explain, partially, potential fire crown damages and its effects on growth. However, when we looked at local fire intensity on our target trees, as expressed by $\text{BCH}_{\text{local}}$, we found a positive effect of $\text{BCH}_{\text{local}}$ on the growth of *P. nigra salzmannii*, in agreement with a previous study by Valor et al. (2013). A possible explanation for this increased growth could be that the average BCH on a small area can provide an approximation of flame length and fire intensity, with higher intensities translating to higher soil organic matter mineralization and therefore higher post-fire soil fertility (Pausas et al. 2003), although the effect of duff moisture may disrupt this relationship. This potential effect of enhanced nutrient availability has been observed to last from 1 month to 5 years depending on type of nutrient, burnt tree species, soil properties, burn intensity, and pathway of leaching processes (Certini 2005). Conversely, the effect of $\text{BCH}_{\text{local}}$ on growth was significantly negative on *P. nigra nigra*, highlighting the possibility of developing different adaptive characteristics depending on species origin and provenances. Thus, *P. nigra nigra*, a sp. whose origin has being traced to central Europe (Kreyling et al. 2012), may be more proximately adapted to cope with perturbations such as snow or wind (e.g. open crown) rather than fire. In the case of *P. halepensis* and *P. sylvestris*, $\text{BCH}_{\text{local}}$ had no significant effect on tree growth, which means there may be a trade-off between the effects driven by fire damage on cambium tissues and partially on the crowns, on these more fire-sensitive species, and the presence of fire-induced fertilization and removal of understory in the years following PB (Wyant et al. 1983). In contrast to *P. halepensis* and *P. nigra salzmannii*, which showed a tendency towards increasing post-burn growth, *P. sylvestris* and *P. nigra nigra* showed an intensified decrease in growth, similar to results reported on *P. sylvestris* during the first 5 years following fire (Beghin et al. 2011). Although these reductions in growth may be related to fire causing tissue damage, this growth variation might also be explained by the effect of cumulative drought events on post-PB growth (e.g. 2005, 2006 and 2007 (Figure 2.2) that were not captured in the pre-burn models, resulting in an overestimation of BAI_{inf} in *P. sylvestris* and *P. nigra nigra* which are particularly sensitive to drought events in this region. Nonetheless, it is also remarkable that in these species a better pre-PB growth performance resulted in a faster post-PB recovery (Figure 2.4). Additionally, the age of trees was found to have a negative or none-significant effect on growth, depending on the tree species. However, it has to be mentioned that as age most probably correlates to the dbh of the trees, a variable used to estimate the BAI_{inf}, and subsequently the BAI_{dif}, interpreting the impact of age on the variation of post-fire growth is difficult.

The models presented could be improved by considering fire intensity (e.g. residence time above a lethal temperature) or additional fire severity variables (e.g. crown scorch, duff consumed), which can be only obtained during or immediately after PB, but again, the retrospective nature of the study made this impossible to study. It should also be note that the climatic dataset used, which is combination of records and projections using spatial interpolation tools could not reflect exactly the actual climate of the study sites. Note that on specific years, we used a lower number of tree-ring series to calculate mean BAI, as BAI was referenced to TSB and not to actual year growth, thus explaining the BAI_{obs} departures observed in *P. halepensis* from year 5 to year 8 (Figure 2.3, A). Future research should look closer at the short-term mechanisms and physical and biological processes liable to shape the mid-term effects of PB on tree vitality, and also explore the effects of recurrent PB on growth.

2.5 Conclusion

This study brings valuable information for fire and forest management decision-makers. It shows that the impact of PB on tree growth varies as time increases, and that this response is modified by the tree and fire characteristics of the pines. Our findings show that for all pines analysed, immediate post-PB growth was reduced in *P. halepensis* and *P. sylvestris* but remained unaffected in *P. nigra* spp. As time since burning increased, *P. halepensis* growth recovered and *P. nigra salzmannii* growth increased whereas *P. sylvestris* and *P. nigra nigra* growth decreased continuously. As a rule, dominant trees grew better than suppressed trees, indicating that larger trees have greater protection against fire probably due to its thicker bark and greater crown base height, even if suppressed trees grew less per se than dominant trees. Interestingly, higher fire intensities around the target trees appeared to enhance the growth of *P. nigra salzmannii* and to a lesser extent *P. halepensis*, probably due to fire-induced soil fertilization. Indeed, dominant *P. nigra salzamannii* and *P. halepensis* doubled diameter in 7-10 years after the PB (Table 2.2), being such increments observed in the region particularly at fertile sites (Trasobares et al. 2004a; Trasobares et al. 2004b). From the presented results, it can also be stated that low intensity fires, as the ones promoted through PB, have a small impact on the short term growth of pine forests, and its use as a silvicultural tool has to be framed on the context of fuel reduction activities. However, it has to be mentioned that as fire intensity increases and post-fire time elapses significant and even positive increments on tree growth can be expected (Valor et al. 2013) if an adequate balance between competition reduction and the tissue damage caused to remnant trees is accomplished, offering a new window of opportunity for the use of PB as a forest management tool.

In summary, growth recovery may depend on the species morphological traits (e.g. tree size, crown status, bark thickness), the severity and intensity of the PB, tree performance prior to PB, the effects of tree healing, and increased light and soil nutrient availability postfire. Understanding and balancing these factors in *Pinus* forests should help forest and fire managers better plan PB operations, both in terms of desired fire intensity and required burning intervals.

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2.7 Appendix

Table A1: Means standard deviation of diameter at breast height (DBH), total height (Ht), bole char height (BCH) and local fire severity ($\text{BCH}_{\text{local}}$) at sampling year (2012) and burn-year diameter at breast height (DBH_{b}) and age (AGE_{b}), for each crown status–fire severity combination within each pine species.

Pine species	Study site	Total trees (n)	Crown Status- Fire Severity							
			Dominant- High		Suppressed-High		Dominant-Low		Suppressed-Low	
			Time-span (years)	Rbar[a] (trees)	Time-span (years)	Rbar (trees)	Time-span (years)	Rbar (trees)	Time-span (years)	Rbar (trees)
P. halepensis	Ph1	22	1971-2011 (41)	0.731 (5)	1980-2011 (32)	0.470 (5)	1976-2011 (36)	0.601 (6)	1980-2011 (31)	0.500 (6)
	Ph2	26	1968-2011 (44)	0.548 (7)	1974-2011 (38)	0.348 (6)	1970-2011 (42)	0.676 (8)	1978-2011 (34)	0.726 (5)
	Ph3	14	1976-2011 (36)	0.708 (5)	NA	NA	1983-2011 (29)	0.852 (8)	1989-2011 (23)	—[c] (1)
	Ph4	26	1946-2011 (66)	0.559 (8)	1934-2011 (78)	0.181 (5)	1934-2011 (78)	0.422 (8)	1927-2011 (85)	0.296 (5)
P. nigra salzmannii	Pns1	27	1913-2011 (99)	0.424 (8)	1931-2011 (81)	0.364 (5)	1907-2011 (105)	0.378 (6)	1925-2011 (87)	0.433 (8)
	Pns2	15	1912-2011 (100)	0.442 (7)	1928-2011 (84)	0.302 (5)	1930-2011 (82)	—c (1)	1964-2011 (48)	0.346 (2)
	Pns3	21	1928-2011 (84)	0.474 (5)	1931-2011 (81)	0.235 (5)	1942-2011 (70)	0.617 (5)	1958-2011 (54)	0.432 (6)
	Pns4	17	NA[b]	NA	1946-2011 (66)	0.323 (5)	1935-2011 (77)	0.573 (8)	1940-2011 (72)	0.262 (4)
P. nigra nigra	Pnn1	32	1951-2011 (61)	0.489 (8)	1955-2011 (57)	0.444 (8)	1954-2011 (58)	0.525 (8)	1952-2011 (60)	0.430 (8)
	Pnn2	28	1974-2011 (38)	0.775 (8)	1978-2011 (34)	0.783 (8)	1976-2011 (36)	0.746 (7)	1978-2011 (34)	0.726 (5)
	Pnn3	20	1977-2011 (35)	0.439 (5)	1976-2011 (36)	0.434 (5)	1974-2011 (38)	0.685 (5)	1975-2011 (37)	0.713 (5)
P. sylvestris	Ps1	31	1944-2011 (68)	0.440 (8)	1943-2011 (69)	0.106 (7)	1939-2011 (73)	0.301 (8)	1949-2011 (63)	0.086 (8)
	Ps2	24	1950-2011 (62)	0.389 (6)	1957-2011 (55)	0.247 (8)	1950-2011 (62)	0.344 (4)	1954-2011 (58)	0.342 (6)
	Ps3	25	1956-2011 (56)	0.466 (6)	1967-2011 (45)	0.471 (5)	1964-2011 (48)	0.619 (8)	1963-2011 (49)	0.629 (6)

[a] Rbar, mean interseries autocorrelation.

[b] NA, not available due to an absence of candidate trees for sampling in the burnt site in a specific crown status–fire severity combination.

[c] Rbar cannot be computed given the sample size.

Chapter 3

The effects of burning season and severity on the mortality over time of *Pinus nigra* spp. *salzmannii* (Dunal) Franco and *P. sylvestris* L.

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Abstract

Understanding tree mortality after fires can be used to improve planning of prescribed burning and post-burning salvage logging. This study aims to assess the effects of burning season, fire severity and tree size on the evolution of post-burning mortality of pine species with different fire resistance traits. In 2013, two paired plots (1 ha each) were burned, one in spring and the other in fall, in two mixed-stands of *P. sylvestris* and *P. nigra,* and the fire severity and tree size variables were measured. Mortality was assessed individually in a total of 933 pines during the following 3 years post-burning. We modeled the initial (1 year post-burning) and delayed (3 years post-burning) tree mortality using Firth logistic regression and Conditional Inference trees. The probability of dying after burning was lower in *P. nigra* than in *P. sylvestris* confirming that *P. nigra* has higher fire-resistance compared to the moderate fire-resistance of *P. sylvestris.* Initial mortality was slightly higher for pines burned in spring than for those burned in fall; however, after 3 years the proportion of pines that had died after the fall burns was higher than that after the spring burns. Nevertheless, for the two periods modeled, the likelihood of a pine dying was significantly higher in spring than in fall given a certain level of crown scorch. This suggests that pines with damaged crowns were more likely to die when burning occurred during periods of active growth. However, the higher fire severities at the base of the trees that occurred during the fall burns compared to the spring burns are probably the cause of the higher rates of delayed tree mortality. Higher likelihood of mortality in trees burned in fall, with higher percentages of white ashes in the stem base suggest basal stem girdling as a cause of delayed pine mortality. We can conclude that crown damage is more detrimental when burning occurs during the growth season, while longer combustion times during fall burns may have caused the higher delayed pine mortality in comparison with spring burns.

Keywords: delayed mortality; dormant season; forest management; fire ecology; prescribed fire; prescribed burning; post-fire mortality models; tree phenology.

3.1 Introduction

Prescribed burning (PB) is the planned use of fire under mild weather conditions to meet defined management objectives (Wade and Lunsford 1989). PB is used, among other management objectives, to reduce surface fuel load to lessen wildfire intensity and difficult the transition of surface fire to the crown fuel layer. For certain forest structures eliminating the ladder fuel is advisable in order to significantly reduce the fire hazard and tree mortality (Gonzalez-Olabarria et al. 2007). These treatments require more intense fires and careful PB planning to achieve the desired goals while limiting the negative impact on the remaining overstory. PB, like unplanned fires, can cause tree death by damaging sensitive tissues such as foliage, stems and roots (Dickinson and Johnson 2004, Michaletz and Johnson 2007). The impact of burning on the overstory is normally predicted based on a combination of the size of the trees and fire severity indicators (see Woolley et al. 2012 for review). However, the effect of the burning season on tree mortality has generally been neglected or the results of analyses have been conflicting (e.g. see Knapp et al. 2009 for review), either showing no effect (Schwilk et al. 2006) or higher mortality after spring (Glitzenstein et al. 1995; Harrington 1987; Harrington 1993) or fall burns (Perrakis and Agee 2006; Ryan et al. 1988; Thies et al. 2005; Thies et al. 2006). According to Ryan et al. (1988), these conflicting results are due to difficulties in separating the effect of fire behavior from burning season.

Post-fire tree mortality models require a minimum of 3 years of post-fire monitoring to account for initial and delayed mortality. After this time, mortality generally returns to baseline rates (Wooley et al. 2012). In conifers, initial mortality can be observed within a month after PB by the absence of green foliage in the crowns if buds are killed (Peterson and Ryan 1986). In contrast, delayed mortality usually occurs in girdled trees, in which death is evident after two or three years post-burning (Hood 2010), or in severely fire-damaged trees, in which factors such as drought, insects or wind episodes contribute to a gradual process of tree death (Ryan and Amman 1996). Most studies use data for the longest post-burning period available to model tree mortality, with only a few studies (Catry et al. 2010) splitting the temporal framework, which could reveal differences in the mechanisms behind initial and delayed mortality. Consequently, we hypothesized that fire severity, tree size, and the phenological state of the trees when PB occurred could explain the different tree mortalities over time.

The review in Fernandes et al. (2008) on the fire resistance of European pines highlighted the need for long-term studies on post-fire tree mortality, as most studies only included 1 or 2 years of post-fire data. In addition, the need for experimental fires of higher intensities and different burning seasons was also evident, as most studies have been conducted during the trees' dormant season. Here we studied the effects of low to moderate intensity spring and fall PB on the mortality of *Pinus nigra* spp. *salzmannii* Dunal (Franco) and *P. sylvestris* L., 1 and 3 years after burning. *P. nigra* is considered more fire resistant than *P. sylvestris* due to its thicker bark, thicker needles and higher canopy base height, although there is considerably more information about post-fire tree mortality for *P. sylvestris* than for *P. nigra* (see Fernandes et al. 2008 for review). Our specific objectives were (i) to compare the degree of fire resistance of *P. nigra* and *P. sylvestris*; (ii) to analyze the effect of burning season on tree mortality; and (iii) to determine whether crown, stem and basal stem injury indicators explain differences between initial and delayed post-burning tree mortality. We expected that the interaction between burning season, fire severity and tree variables (if any) could vary according to the time scale analyzed.

3.2 Methods

3.2.1 Study site, experimental design and prescribed burns

The study was performed in two localities in the foothills of the Pyrenees, Miravé and Lloreda, in the NE Iberian Peninsula (Table 3.1), separated by a distance of ca. 20 km. We selected one mixed *P. sylvestris* and *P. nigra* stand in each locality, Mirave and Lloreda. Within each stand, we selected two paired sites (1 ha each) to perform PB either in spring or fall. One plot (30 x 30m), per each of the four sites, was set up to monitor the effects of PB on pine mortality.

Table 3.1: Characteristics of PB sites.

Locality	Burn season –PB site	Lat. (ºE)	Long. (ºN)	Altitude (m.a.s.l.)	Slope (%)	Aspect	Rainfall (mm)	T (ºC)
Lloreda								
	Spring	1.5706	42.0569	715	30	N	731.6	11.7
	Fall	1.5771	42.062	826	25	N	766.3	11.1
Miravé								
	Spring	1.4494	41.9515	723	25	NE	677.3	11.5
	Fall	1.4496	41.9508	723	25	NE	677.3	11.5

The climate is sub-Mediterranean with milder summers and colder winters than the typical Mediterranean climate. At both sites, soils developed from calcareous colluviums, classified as Calcaric inceptisols (FAO 2006), were between 0.5 m and 1 m deep, basic (pH 6.8-7.5) and with a fine texture.

The forest overstory is dominated by *Pinus nigra* ssp. *salzmanii* (Dunal) Franco and *P. sylvestris* L. and broadleaf trees (i.e. *Quercus pubescens*, *Acer monspessulanum*, *Sorbus aria*) are also common. The understory is composed mainly of *Buxus sempervirens* and deciduous species (e.g. *Lonicera etrusca, Viburnum lantana*, *Amelanchier ovalis*). Other evergreen shrubs were also common in the understory (e.g. *Juniperus oxycedrus*, *Q. coccifera*).

PB was carried out in late spring (5 and 12 June, 2013) and fall (17 October and 8 November, 2013) by the Forest Actions Support Group (GRAF) of the Autonomous

Catalan Government (*Generalitat de Catalunya*), with the objective of reducing surface and ladder fuels. All PBs were carried out following a strip headfire ignition pattern under similar meteorological conditions. During burning, combustion time (minutes) above 120 ºC and the maximum temperature at the base of the trunk, placed at the surface of the soil litter, were measured in 18 trees with K-thermocouples (4 mm) connected to dataloggers (Testo 175), packed with a fireproof blanket and buried in the soil at a depth of ca. 20 cm. Temperatures were recorded every 10 s. Trees for temperature monitoring were spatially distributed by dividing each of the 30 x 30 plots into 4 subplots (15x15m) and randomly selecting a number of 4-5 pines within each subplot to gain a representative spatial characterization of the temperature in the plots.

3.2.2 Data collection

Before PB, in each plot, all pines with a diameter larger than 2.5 cm at a height of 1.30 m (DBH) were tagged with a metal number. The potential fire resistance of the pines was characterized by measuring the following tree variables: DBH, bark thickness (BT), total height (Ht) and canopy base height (CBH). BT at 0.20, 0.50 and 1.30 m height and the corresponding diameter were measured on the uphill and downhill sides of 10 trees per species and plot using a standard bark gauge following Fernandes et al. (2012). Then, allometric equations were generated that related BT to diameter at different heights, and between DBH and diameters at 0.20 and 0.50 m height. These equations were used later to estimate diameters at 0.20 and 0.50 m (Figure A1) and BT (Figure A2) for the rest of the pines. Since resistance to cambium injury is expected to increase with the square of the stem diameter (Ryan 1998), we also calculated the square of the BT and diameter at different heights.

Several indicators were used to characterize the degree of crown injury. Two weeks after PB, crown volume scorched (CVS) was visually estimated to the nearest 5 % as the change in needle color resulting from transferred heat via convection. Foliage combustion was rare and not measured. We calculated the squared CVS as there is evidence of its non-linear influence on tree survival (Peterson and Ryan 1986). In addition, we measured maximum crown scorch height (CSH) (Ryan and Reinhardt 1988) and calculated crown length scorched (CLS) (Wyant et al. 1986) as the difference between pre-burning crown length (crown length= Ht-CBH) and post-burning crown length scorched (crown length scorched= CSH-CBH). Stem injury was characterized by bole scorch height (BSH) and bole scorch proportion (BSP) (Wyant et al. 1986; Rigolot 2004). We measured the maximum (BSH_{max}) and minimum (BSH_{min}) bole scorch height and computed the mean (BSH_{mean}). BSP was calculated as the bole scorch height expressed as a percentage of tree height for maximum, minimum and mean values. Straight after PB, for each tree we visually estimated the proportion of white and black ashes and unburned duff layer in a 1 m radius from the tree center. Black ash deposition indicates low intensity and short-residence time and may result in little or no damage to the basal stem or root collar, while white ashes indicate smoldering and potential damage to the basal stem and root collar (McCandliss 2002).

Pine mortality was monitored one month after PB and every 6 months during the 3 following years. A tree was considered alive when any green foliage was present in the crown, otherwise it was considered dead.

3.2.3 Data analysis

In order to describe dead trees, we used a sparse partial least squares discriminant analysis (sPLS-DA) to select the variables that best separated the time of death (1, 2 or 3 years post-burning). The sPLS-DA is a supervised technique that takes the time since death of each individual pine into account and tries to reduce the dimensions while maximizing the separation between times.

The probability of initial (1 year) or delayed (3 years) post-burning pine mortality was modeled using the Firth logistic regression and conditional inference trees. Firth's penalized likelihood approach is a method for dealing with separability (i.e. death of only small trees), rare events, small sample sizes, and bias in the parameter estimates (Firth 1993). Conditional inference trees estimate a regression relationship by binary recursive partitioning in a conditional inference framework that is based on maximally selected rank statistics (Hothorn et al. 2006). We used them here to help interpret the results.

Firstly, for 1 and 3 post-burning years, the most influential tree and fire severity variables for predicting tree mortality were selected using a random forest permutation-based score (Table 3.2). All variables mentioned in *Data collection* were included. Selected variables were classified into indicators of tree size, direct measurements of crown damage and proxies of cambium injury to account for potential damage to the base of the tree and to the stem (Table 3.2).

Table 3.2: Tree size variables and injury indicators. M1 and M3 are the variables selected by the random forest permutation-based score to predict 1- and 3-year post-burning mortality respectively.

Tree size	Stem injury	Crown injury	Basal stem injury
DBH (cm)	BSH_{mean} (cm)^{M3}	CVS (%)$^{M1-M3}$	white ashes (%)^{M3}
DBH^2(cm^2)	BSH_{max} (cm)	CVS^2 (%)	black ashes (%)
D_{50} (cm) $^{M1-M3}$	BSH_{min} (cm)	CLS (m)	unburned (%)
D^2_{50}(cm^2)	BSP_{mean} (cm)^{M3}	CSH (m)^{M1}	
D_{20} (cm)	BSH_{max} (cm)^{M1}		
D^2_{20}(cm^2)	BSH_{min} (cm)		
BT_{130} (cm)			
BT^2_{130}(cm)			
BT_{50} (cm)			
BT^2_{50}(cm^2)			
BT_{20} (cm)			
BT^2_{20}(cm^2)			
Ht (m)			
CBH (m)			

D, diameter at 20, 50 cm height. D^2, squared diameter at 130, 50, 20 cm height. BT, bark thickness at 130, 50 and 20 cm height. BT^2, squared bark thickness at 130, 50 and 20 cm height. Ht, total height. CBH, canopy base height. BSH, bole scorch height (mean, max and min). BSP, bole scorch proportion (mean, max and min). White/black ashes, percentage of white or black ashes in 1 m radius from tree center. Unburned, percentage of unburned surface in 1 m radius from tree center. CVS, crown volume scorched. CVS^2, squared crown volume scorched. CLS, crown length scorched. CSH, crown scorch height.

Secondly, a set of candidate models including separately crown, stem and basal stem indicators was developed by incorporating one of the indicators selected by the random forest permutation in each model to avoid highly correlated variables (Table A1, Table A2, Figure A3). Tree size variables were included in the stem models as they protect from cambium injury. All models included species, burning season and second interactions. Per each type of modeled damage, we selected the best model based on corrected Akaike criterion (AICc). Then, the indicators used in the selected models were used to construct a model that combined crown, stem and basal stem injury indicators.

Finally, selected models (crown, stem, basal stem and combined fire damage models) were run using a penalized Firth logistic regression. The best model, regardless of the type of damage, was selected based on the corrected Akaike criterion (AICc). Model performance was assessed by the area under the receiver operating characteristic curve (AUC), and by agreement rates between the observed and predicted tree status.

For each post-burning date, a Ctree model was developed using the variables of the best model fitted using the Firth logistic regression.

In addition, for each species, linear mixed models (LMM) considering plot as a random factor were used to analyze potential differences in the independent variables selected by the random forest permutation tests between tree status and burning season and their interaction.

All the analyses were performed using the R software (R Development Core Team, 2014) using “VSURF”, “logistf”, “MuMin”, “visreg”, “party” (Horton et al. 2008), “nlme” (Pinheiro et al. 2013) and “mixOmics” packages of R.

3.3 Results

3.3.1 Fire behavior

The meteorological conditions during PB were similar, although forest floor moisture was much lower in fall burns than in spring burns (Table 3.3). Fire behavior was comparable among sites and fire seasons, except in the Lloreda fall burn (Table 3.3) where the combustion time above 120 ºC at the soil surface was longer than the other plots. The forest characteristics were similar across plots except for the understory phytovolume, which was lower in the Lloreda spring burn, and the basal area of the stands, which was higher in the Lloreda fall burn (Table 3.3).

Table 3.3: Characteristics of PB and forest experimental units.

	Meteorological conditions			PB characteristics						Forest characteristics				
Burn Season	Air Temp. (ºC)	Rel. Humid. (%)	Wind Speed (km.h^{-1})	Burn Surf. (ha)	Tmax (ºC)	CT120 (min.)	FFMC (%)	BSH$_{max}$ (cm)	CSH (m)	UP (m^3 ha^{-1})	DBH (cm)	Ht (m)	Density (trees ha^{-1})	BA (m^2 ha^{-1})
Mirave														
Spring	25.8±2.1	54.9±4.4	17.6±3.6	0.9	409 (60-718)	9 (0-18)a	48.3±15.4a	76.0±61.8a	2.6±3.0b	1637	10.7±6.0a	7.9±3.4a	2722	32.3
Fall	21.4±1.5	55.3±5.2	2.0±1.7	1.2	310 (60-747)	9 (0-36)a	22.9±3.7b	47.0±35.0b	3.4±3.9a	1805	10.7±5.9a	8.0±3.4a	2744	32.6
Lloreda														
Spring	21.8±1.4	60.4±4.9	6.8±5.9	1.1	418 (107-816)	8 (0-19)a	47.8±11.1a	71.0±48.1a	3.0±3.2a	949	11.9±3.4a	8.5±1.9a	2611	31.5
Fall	15.2±0.9	63.0±4.6	5.9±2.7	1.6	492 (222-729)	95 (5-408)b	19.2±5.6b	170.3±122.6c	0.3±1.3b	1693	15.0±5.0b	11.5±2.4b	2277	44.9

Different small letters within a column indicate statistically significant differences between sites ($P < 0.05$).

General lineal model was applied for the following variables CT120 (combustion time (minutes) above 120 ºC), FFMC (forest floor moisture content), BSH$_{max}$ (maximum bole scorch height), CSH (crown scorch height), DBH and Ht with plot as a fixed factor.

Wind speed was measured outside the forest.

Mean and range between parenthesis of maximum temperatures (Tmax) and combustion time above 120º (CT120) at the base of 18 trees per site.

Forest parameters (mean ±std) were estimated for trees with DBH   2.5 cm; Ht, total height; BA, basal area.

UP, understory phytovolume. Understory phytovolume was calculated using the crown maximum and normal diameter and height of each understory woody plant in a 10 x 10 m2 plot by approximating the volume of each woody to plant to a cylinder.

3.3.2 Pine mortality between tree status and burning season

In the two species, dead trees had significantly lower diameters and higher CVS than the surviving trees (Table 3.4). The pines that died after spring burns had higher CVS than those that died after fall burns (Table 3.4). We also observed differences in stem injury indicators between living and dead *P. nigra*, particularly in spring (Table 3.4). No significant differences were found in the proportion of white ashes between tree species, status or burning season (Table 3.4).

Table 3.4: Tree size variables and fire injury indicators selected by the random forest permutation test for each pine species grouped by season and tree status (mean and standard error between brackets).

Species	Burn Season	Tree status	D_{50} (cm)	CVS (%)	CSH (m)	BSH_{mean} (cm)	BSP_{mean} (%)	BSP_{max} (%)	WA (%)
P. nigra	Spring	Alive (n=408)	13.8 (0.2)a	15.9 (1.1)a	2.8 (0.1)	51.9 (1.6)a	6.4 (0.2)a	9.1 (0.3)a	63.1(1.1)
		Dead (n=34)	6.4 (0.4)b	67.9 (6.6)b	2.9 (0.3)	80.8(19.8)b	16.5 (3.5)b	19.1 (3.5)b	68.5 (3.3)
	Fall	Alive (n=324)	16.0 (0.4)a	19.9 (1.9)a	2.1 (0.2)	74.2 (3.8)a	7.4 (0.3)a	11.7 (0.5)a	55.6 (1.5)
		Dead (n=40)	8.1 (0.5)b	50.3 (7.4)c	2.4 (0.4)	50.5 (6.1)c	9.2 (1.0)c	13.0 (1.4)c	51.4 (4.4)
P. sylvestris	Spring	Alive (n=35)	15.2 (1.1)a	8.7 (3.0)a	2.2 (0.5)	39.4 (3.4)	5.2 (0.6)	8.0 (0.9)	58.4 (3.9)
		Dead (n=4)	4.7 (0.2)b	48.7(26.8)b	2.4 (1.4)	33.0 (11.4)	6.6 (2.5)	8.9 (3.7)	62.7 (11.7)
	Fall	Alive (n=72)	14.6 (0.6)a	6.4 (2.1)a	1.1 (0.3)	35.9 (3.8)	3.4 (0.4)	5.4 (0.7)	52.9 (2.7)
		Dead (n=16)	9.3 (1.0)b	27.5 (9.9)c	2.2 (0.7)	33.1 (6.9)	5.1 (1.4)	6.5 (1.4)	39.4 (4.4)

For each species, different small letters within a column indicate statistically significant differences between tree status and burning season ($P < 0.05$).
D_{50}, diameter at 50 cm height.
CVS, crown volume scorched.
CSH, crown scorch height.
BSH, bole scorch height (mean).
BSP, bole scorch proportion (mean and max).
WA, percentage of white ashes in 1 m radius from the center of the tree.

Pine mortality decreased as the diameter class increased: 30 % of the trees within the 5 cm diameter class died, whereas trees in the 10 and 15 cm diameter classes showed mortality rates of 6 % and 1.8 % respectively. During the first six months, mortality was higher after spring burns than after the fall ones, regardless of the species. After one year, tree mortality was slightly higher in trees burned during spring than those burned in fall (15.9 % vs. 15.4 %), and was similar between seasons in *P. nigra* (Figure 3.1a) and higher in *P. sylvestris* after spring burnings (Figure 3.1b). Dead trees had higher CVS (Figure 3.1, c and d). Two years post-burning mortality was higher in trees burned in fall than in spring (Figure 3.1, a and b). At this time, however, dead trees were characterized by lower CVS and higher D^2_{20} with respect to pines that died at 1-year post-burning (Figure 3.1, c and d). After 3 years, mortality was higher in fall burns, particularly in *P. sylvestris* with ca. 20 % of trees dying (Figure 3.1, a and b). The few trees that died during the third year were larger in size and had lower CVS compared to the 1-year post-burning dead trees (Figure 3.1, c and d). They also had a higher percentage of ashes nearby (either white or black).

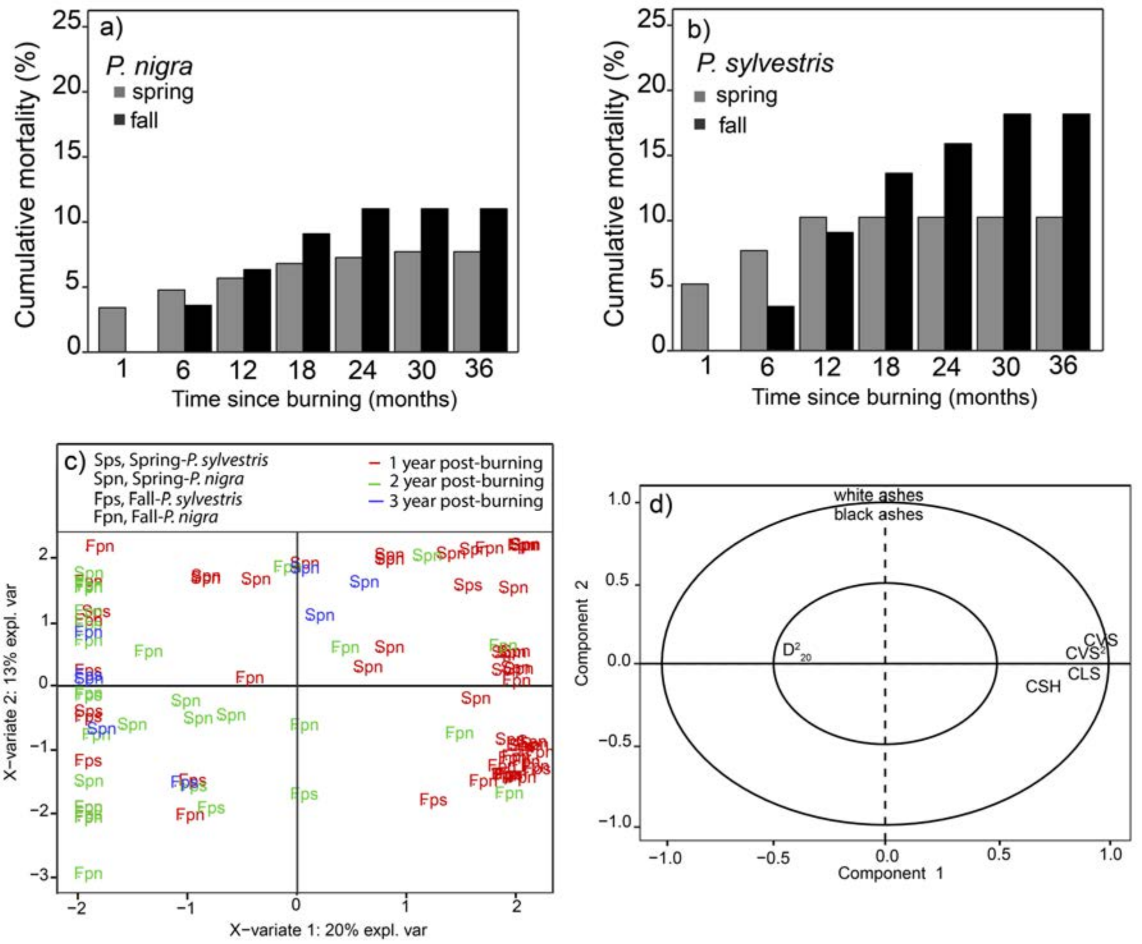

Figure 3.1: Time cumulative-mortality for *P. nigra* (a) and *P. sylvestris* (b) after spring and fall burns. Scores of sPLS-DA for dead trees after 1 (red), 2 (green) and 3 (blue) years post-burning for *P. nigra* (pn) and *P. sylvestris* (ps), after spring (S) and fall (F) burns (c). Correlation Circle plot for the first two sPLS-DA dimensions with the 5 variables selected (d); CVS, crown volume scorched; CLS, crown length scorched; CSH, crown scorch height.

3.3.3 Post-burning tree mortality modeling

3.3.3.1 Model selection

From all crown, stem, basal stem and combined damage models developed using the variables selected by the random forest permutation test (Table 3.2), those that combined crown and stem injury indicators explained the initial tree mortality best (1 year post-burning) (Table 3.5; see Tables A1 and A2 for all the models developed). In contrast, those models that combined crown and basal stem injury indicators explained delayed tree mortality better (3 years post-burning) (Table 3.5).

Table 3.5: Firth logistic regression models ranked by the AICc representing combined, stem, crown and basal stem damage following 1 and 3 years post-burning. The best model for each post-burning time is marked in bold. x, represents the interaction between two terms of the models; ↑, positive relationship with tree mortality; ↓, negative relationship with tree mortality. For species and season the arrows show the effect for *P. sylvestris* to *P. nigra* and for spring to fall respectively.

Time since PB	Model	AICc	Significant parameters
1 year	Combined	217	↑Season +↑sp+↑BSP_{max}+↓ D_{50}+↑CVS+↑CVS x Season+↓D_{50} x Season
	Stem	282	↑Season +↑BSP_{max}+↓D_{50}+↓D_{50}xSeason
	Crown	282	↑Season+↑sp+↑CVS+↑Season x CVS
3 year	Combined	364	↑Season+↑sp+ ↑WA+↓D_{50}+↑CVS+↑CVS x Season
	Stem	394	↑Season+↑sp+↑BSP_{min} +↓D_{50}+↓D_{50}x Season
	Basal stem	402	↑Season+↑sp+↑WA+↓D_{50}+↓D_{50}x Season
	Crown	481	↑Season+↑sp+↑CVS+↑Season x CVS

sp, species

D_{50}, diameter at 50 cm height.

CVS, crown volume scorched.

BSP_{max}, bole scorch proportion (maximum).

BSP_{min}, bole scorch proportion (minimum).

WA, percentage of white ashes in 1 m radius from the center of the tree.

3.3.3.2 Modeled 1 year post-burning tree mortality

At one year post-burning the moderately fire-resistant *P. sylvestris* showed significantly higher mortality than the fire-resistant *P. nigra* (Table 3.6, Figure 3.2, a1).

Table 3.6: Firth logistic regression coefficients for the probability of tree mortality 1 year post-burning and 3 years post-burning. x, represents the interaction between two terms of the models.

Time since burning modeled	AUC	Parameters	Estimate±std. Error
1 year post-burning	0.93	Intercept	-1.740 ± 0.678*
		BSP_{max}	0.032 ± 0.015*
		D_{50}	-0.190 ± 0.05**
		CVS	0.019 ± 0.005***
		Species (*P. sylvestris*)	1.283 ± 0.459**
		Season (Spring)	0.479 ± 1.230
		Season x CVS	0.023 ± 0.009**
		Season x D_{50}	-0.282 ± 0.138*
3 years post-burning	0.9	Intercept	0.219 ± 0.518*
		White ashes	0.016 ± 0.005**
		D_{50}	-0.314 ± 0.040***
		CVS	0.009 ± 0.004*
		Species (*P. sylvestris*)	0.967 ± 0.343**
		Season (Spring)	-1.831 ± 0.425***
		Season x CVS	0.019 ± 0.006**

BSP_{max}, bole scorch proportion (maximum).

D_{50}, diameter at 50 cm height.

CVS, crown volume scorched.

WA, percentage of white ashes in 1 m radius from the center of the tree.

For species and season the coefficients show the change from *P. sylvestris* to *P. nigra* and from spring to fall respectively. The levels of significance are represented as *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

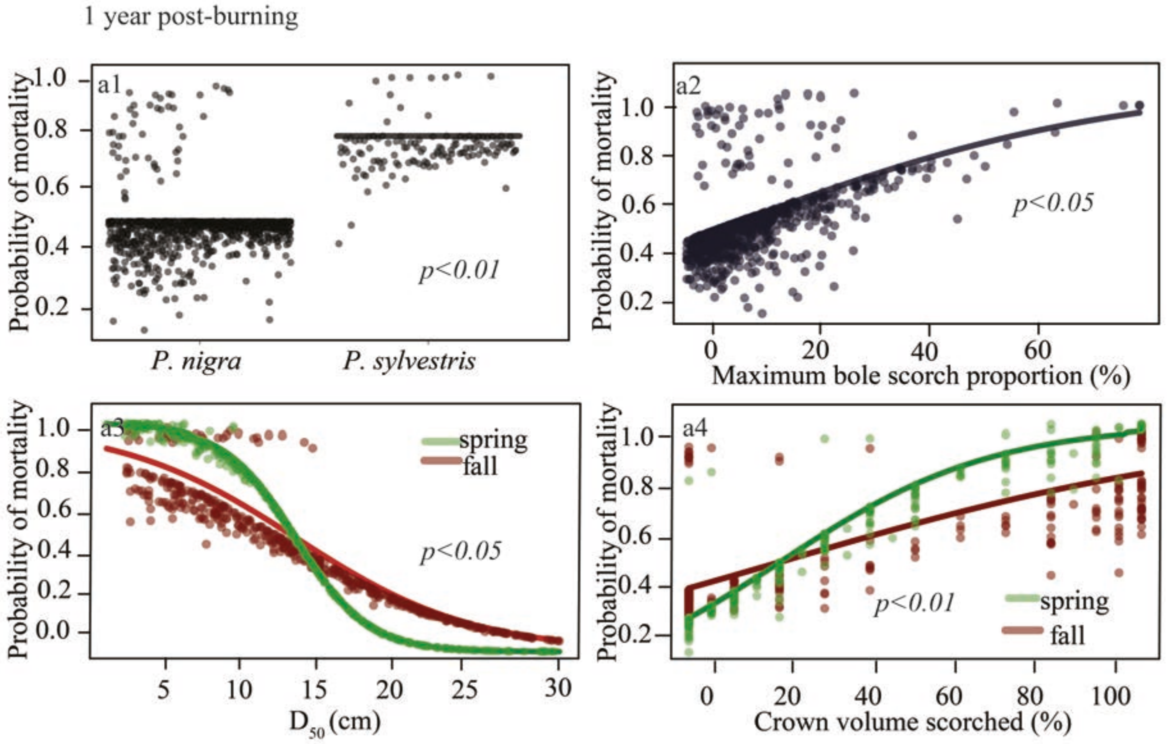

Figure 3.2: Partial regression residuals of the significant parameters for the Firth logistic regression model explaining the tree mortality 1 year post-burning. D_{50}, diameter at 50 cm height.

The maximum bole scorch proportion was positively associated with pine mortality 1 year after burning (Table 3.6, Figure 3.2, a2). The probability of tree mortality increased with increasing CVS and decreasing D_{50} but both variables interacted with burning season (Table 3.6). Thus, trees showed a higher probability of dying in spring than in fall, as the proportion of the crown scorched increased and D_{50} decreased (Figure 3.2, a3 and a4). The conditional inference tree model showed similar results: when CVS was greater than 95 % tree mortality in spring was generalized, while in fall the probability was halved (Figure 3.3).

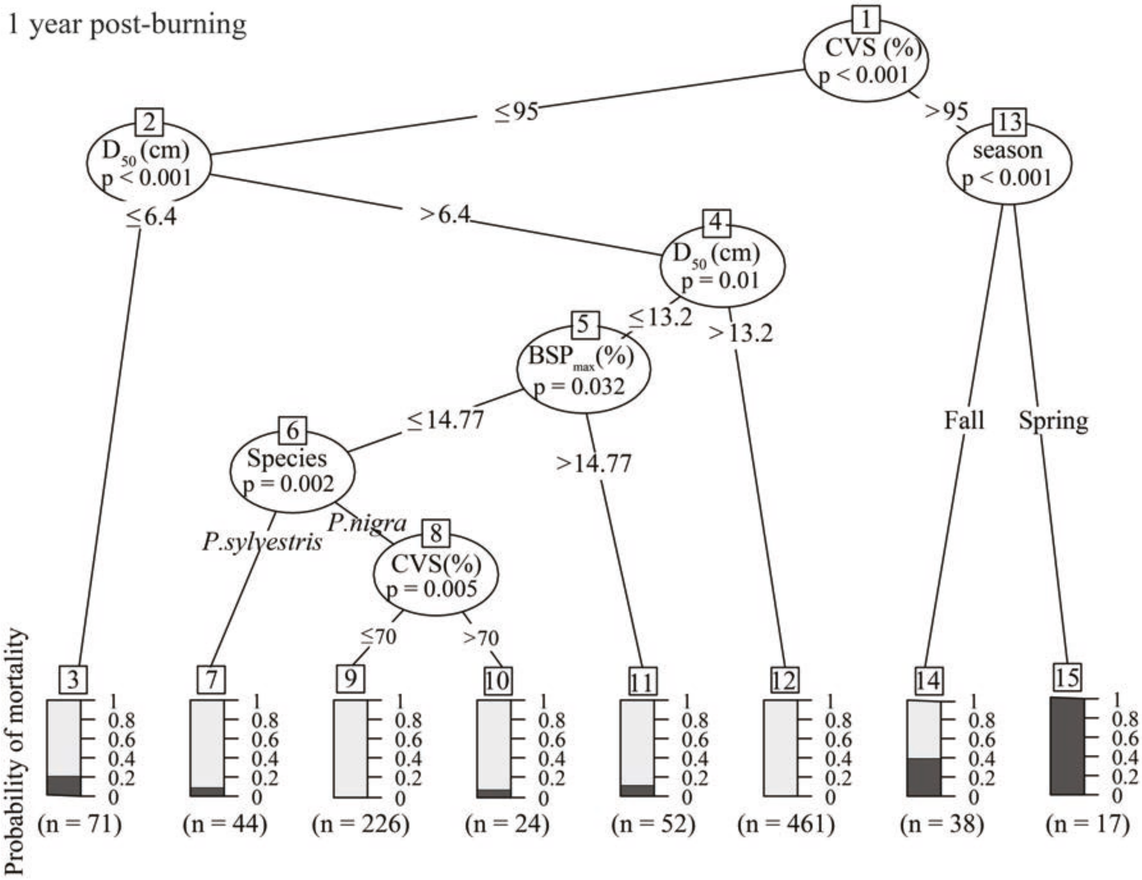

Figure 3.3: Conditional inference tree for 1 year post-burning tree mortality. BSP_{max}, maximum bole scorch proportion (D_{50}, diameter at 50 cm height; CVS, crown volume scorched. The numbers inside the squares identify the nodes and the order of variable/partition entrances, starting with 1 in the root node. The predicted probability of mortality for each node is specified by the black proportion of 0-1 bars in the lower part of the figure.

When CVS was below 95 %, smaller trees (D_{50}<6.4 cm) had a 20 % chance of dying, while when D_{50} >13.2 cm, all trees survived (Figure 3.3). Pines with intermediate D_{50} (6.4 cm-13.2 cm) had a very low probability of dying but this increased slightly when the maximum bole scorch proportion increased (Figure 3.3).

Both models had similar accuracy (ca. 0.96, Table A3) and showed the same significant parameters, but the logistic regression model had a higher rate of true positives (TPR=0.41) than the Ctree model (TPR=0.28). The precision of the logistic regression indicated that the model correctly predicted a tree as dead 78 % of the times when it was in fact dead.

3.3.3.3 Modeled 3 years post-burning tree mortality

At three years post-burning, the interaction between burning season and CVS was still significant as well as the species (Table 3.6), showing the same effect on the probability of tree mortality than when initial mortality was modeled (Figure 3.4, a1 and a4). In contrast, the interaction between burning season and D$_{50}$ was not significant. Thus, tree mortality was higher as D$_{50}$ decreased (Table 3.6, Figure 3.4, a3) and those trees with higher percentages of white ashes at the stem base had a higher probability of mortality (Table 3.6, Figure 3.4, a2).

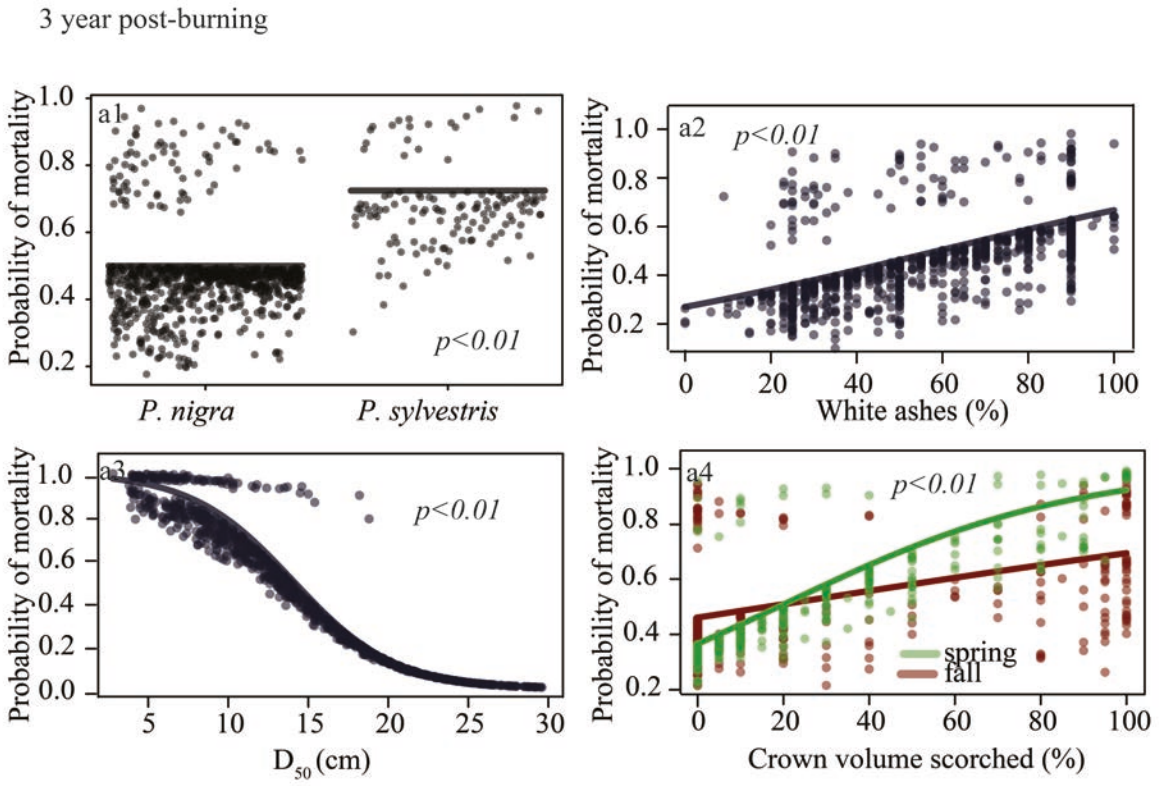
3 year post-burning

Figure 3.4: Partial regression residuals of the significant parameters for the Firth logistic regression model for tree mortality 3 years post-burning.

In the conditional tree model the first variable entered was D$_{50}$; thus, smaller trees (D$_{50}$<7.5cm) had a 0.8 probability of dying when CVS was almost complete and the probability was reduced to 0.3 when it was lower than 95 % (Figure 3.5).

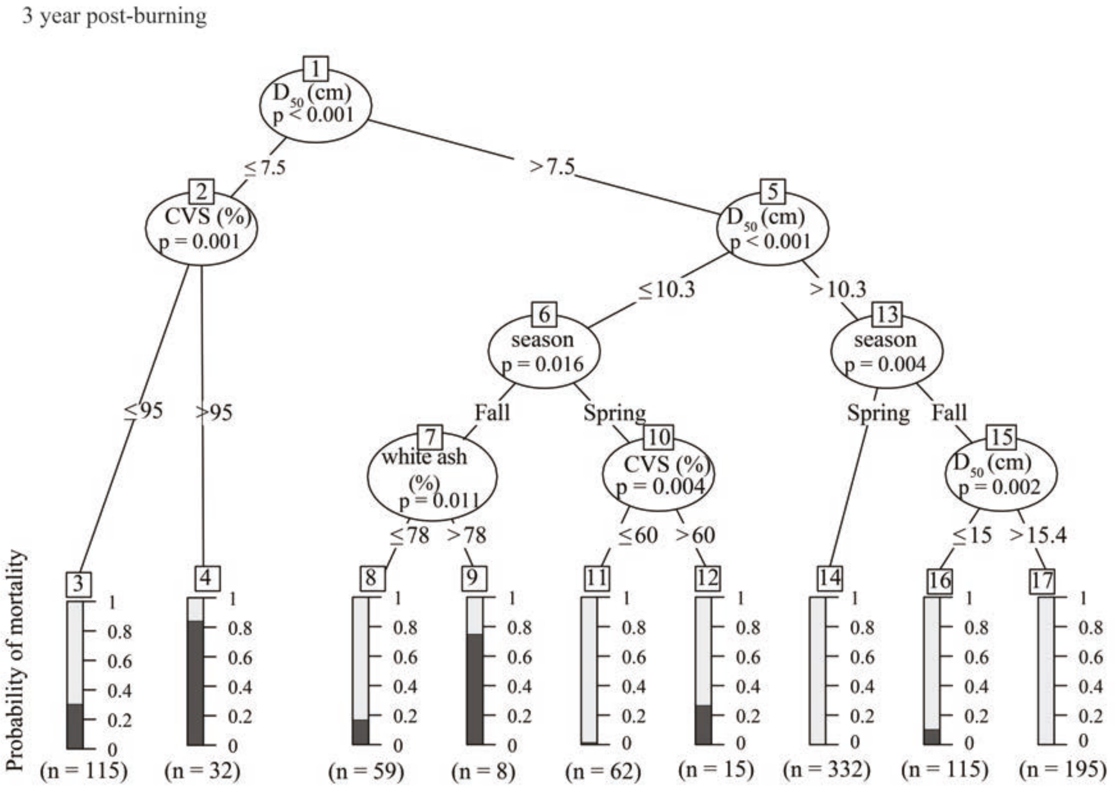

Figure 3.5: Conditional inference tree for 3 years post-burning tree mortality. D_{50}, diameter at 50 cm height; CVS, crown volume scorched. The numbers inside the squares identify the nodes and the order of the variable/partition entrances, starting with 1 in the root node. The predicted probability of mortality for each node is specified by the black proportion of 0-1 bars in the lower part of the figure.

For bigger trees (D_{50} >10.3cm), the probability of dying was generally null, while in intermediate sized pines (7.5 cm>D_{50}<10.3cm) mortality was determined by the burning season, white ashes and CVS (Figure 3.5). In trees burned in spring the likelihood of tree death was controlled by CVS (0.3 when CVS>60 %) (Figure 3.5). In contrast, in trees burned in fall the percentage of white ashes defined the probability of tree mortality (0.8 when white ashes >78 %) (Figure 3.5). Finally, species was not entered as a significant parameter in the conditional inference tree model.

The performance of the logistic and conditional inference models was similar (Table A3) but the conditional tree model had a lower rate of false positives (0.8 % *vs.* 2 %). However, these models did not share the same significant parameters because Species was not a significant variable in the Ctree model.

3.4 Discussion

Prescribed burning planning as a forest management option requires acknowledging the expected impact of the fire on the surface fuels and trees. Identifying the interactions between fire severities, species traits, tree size and phenological state can be the difference between the success and failure of the burning operation.

Initial tree mortality was similar (*P. nigra*) or higher (*P. sylvestris*) after spring burns than after fall burns, whereas delayed mortality was greater after fall burns for the two pine species. *P. nigra*, considered a fire-resistant species (Fernandes et al. 2008), showed a lower probability of mortality than the moderately fire-resistant *P. sylvestris*. Thus, *P. sylvestris* seems more vulnerable, even though it had lower values for crown and stem injury indicators than *P. nigra*, suggesting that the tissues are less protected from heat. In the case of *P. sylvestris,* we acknowledge that the sample size is relatively small but we partially corrected for this by using Firth logistic regression as it provides adjusted *p*-values and unbiased parameter estimates.

3.4.1 Initial tree mortality

Initial tree mortality was slightly higher after spring than after fall burns. Higher levels of crown injury in trees dying after spring burns compared to fall burns may have caused this trend. The probability of tree mortality increases strongly when fire damaged the upper foliage of the tree (CVS >80 %), as observed in most studies (see Fernandes et al. 2008 and Wooley et al. 2013 for review). The results of our study revealed that for a given CVS, trees are more vulnerable to dying during periods of high physiological activity than during the dormant season. On one hand, it can be expected that a larger number of buds will survive after dormant season burns than after spring burns (Ryan 1990) as buds are protected by old needles, producing new foliage in the next season. This explains the fact that even with 95 % CVS, the probability to death was reduced by half in fall compared to spring burns. On the other hand, foliage injury during periods of high physiological activity seems to be more harmful than during dormant seasons. The contrasting effect of burning season on tree mortality may be explained not only by differences in the tree physiological activity but also by carbohydrate storage during the burning season as discussed by Harrigtong (1993). After spring burns, the lower amount of non-soluble carbohydrates (Oberhuber et al. 2011) and the loss of photosynthetic capacity in scorched trees (Wallin et al. 2003) could lead to a reduction in the photosynthesis-to-respiration ratio, and thus the growth and chemical defense demand in severely damaged trees is not fulfilled, therefore increasing the probability of mortality (Harrigton et al. 1993). Indeed, in the same spring sites, we previously found that trees with a greater proportion of the crown scorched invested more in chemical defense than undamaged trees 1 year post-burning (Valor et al. 2017). This potentially increases the chances of carbon starvation by increasing carbon loss. Conversely, scorched trees burned in fall, without damage in the roots, can continue with belowground growth and metabolism activities as carbon reserves are expected to be highest during dormant seasons (Ursino et al. 1968). Carbon reserves in trees burned in fall could be used to favor foliage production in the next season, thus restoring photosynthesis capacity. Field observations suggested that this was the case in our study areas as re-foliage was only observed in pines burned in fall.

In our study, increasing the maximum bole scorch proportion was associated with stem death, as previously reported (Regelbrugge and Conard 1993; Rigolot 2004; Thies 2006). A higher maximum bole scorch proportion probably reflects higher fire intensities in the stem, as demonstrated by the weak correlation between bole scorch proportion and CVS. This weak correlation reflects the effect of plume inclination caused mainly by the steep slope and probably by wind.

Stem injury not only increased with increasing bole scorch proportion but also with decreasing tree size (D_{50}) as generally reported (Woolley et al. 2013). Considering BSH_{max} (Table 3.2), the fact that D_{50} instead of D_{130} or D_{20} predicted tree mortality better may indicate a higher susceptibility to cambium injury at this stem height than at the stem base, where bark is thicker, or at breast height, where combustion time is lower. In fact, in smaller *P. nigra* trees this effect could be amplified as bark thickness decreased with height in the stem (Fernandes et al. 2012), and it could be even higher in *P. sylvestris* given the characteristic thinner bark on the upper trunk. Moreover, the expected detrimental effect of heat on the cambium of trees with thinner barks (Michaletz and Johnson 2007) could be further enhanced during active cambial growth, as indicated by the higher probability of dying for small trees after spring burns. Although the burning season and D_{50} interaction could reflect the effect of CVS instead of tree size, the weak correlation between CVS and D_{50} does not support this.

3.4.2 Delayed tree mortality

Contrary to initial tree mortality, pines had a higher likelihood of dying 3 years after fall burnings than after spring ones. Delayed tree mortality occurring after a fall burn has been previously reported in some studies (Perrakis and Agee 2006; Ryan et al. 1988; Thies et al. 2005; Thies et al. 2006). However, other studies have found higher mortality 4 and 10 years after spring burns (Ryan 1998; Swezy and Agee 1991) than after fall burns. This lack of consistent results may be explained by differences in the type of variables used in the models, time since burning modeled, fire intensities, species studied and heat release history (i.e. differences in fuel consumption and fuel complex structure). More importantly, the lack of replication in terms of fire behavior and burn conditions in our study as well as among studies and among seasons of burning treatments within the same study adds further difficulties for assessing the significance of the results.

The possibility that burning season and fire severity variables could interact, in terms of their effect on tree mortality, was not explored by the studies mentioned above, making it difficult to compare our results with previous research on this topic. Only the study by Harrington (1987) assessed the probability of tree mortality between burning seasons for a certain level of crown volume scorched. He reported a higher probability of tree mortality after spring burns than after fall burns for the same level of damage in *P. ponderosa* 5 years post-burning, which is in accordance with our findings regarding not only initial but also delayed tree mortality.

Three years after PB, smaller trees, expressed by $D_{50,}$ had a higher probability of dying than bigger trees, as indicated in most post-fire tree modeling studies (see Fernandes et al. 2008 and Wolley et al. 2012 for review). However, the discriminant analysis showed that delayed tree mortality occurred in trees with lower CVS and higher D^2_{20} compared to those that died during the first year. Tree mortality after several years, as indicated by Hood (2010), occurs mainly in girdled trees because the xylem is intact and the tree can continue to transport water to support the crown; however, the products of photosynthesis cannot be transported down to the roots and they eventually suffer from carbohydrate starvation. This supports the hypothesis that delayed tree mortality was mainly due to injuries in the basal stem and/or in the root collar, even though trees were bigger than those that died during the first year, and therefore more resistant.

Basal stem girdling or root collar damage, expressed by white ashes instead of stem injury indicators, best explained delayed tree mortality. Moreover, the Ctree model showed that the percentage of white ashes significantly increased the probability of tree mortality but only when trees were burned in fall. Thus, higher levels of delayed tree mortality in fall burns may be explained by higher fire severities at the base of the trees rather than an effect of tree phenology because in fall burns the combustion time and forest floor moisture content was higher than in spring burns, previously observed in several studies (Perrakis and Agee 2006; Schwilk et al. 2006; Thies et al. 2005). We acknowledge that white ashes could indicate not only the effect of fire severity in the basal stem or root collar but also in the stem, as both variables were positively correlated.

Tree size, crown, stem and basal stem injury indicators, did not interact with each other, which could illustrate the relative contribution of these variables to tree death (Fernandes et al. 2008). This suggests that the interaction between burning season and CVS contributed much more strongly to mortality than the interaction between tree size, crown, stem and basal stem injury indicators.

Delayed tree mortality after PB has been associated with bark beetle attacks, especially when fire has affected a significant amount of the crown (Lombardero 2006).

However, we did not observe any sign of borer insects in the pines monitored in this study, although some *P. sylvestris* outside our plot but in one of the study sites showed signs of a bark beetle attack.

3.5 Management implications

Fuel management programs need to adjust the wildfire hazard reduction goals with an adequate PB regime to achieve the desired objective without negatively affecting the ecosystem. Previous research in the region showed that low intensity PB reduces the modeled intensity of wildfires after 8 years of prescribed burns (Casals et al. 2016) and they have a lower impact on the vitality of surviving trees (Valor et al. 2015). Although the results of the study are clear, the potential application of the presented knowledge in PB planning may require for verification from future studies. In this study, as a management recommendation we can suggest that spring burns, with flame lengths that are high enough to affect the crown of the smaller trees, would be advisable when the management goal seeks dominated trees thinning. This would likely concentrate tree mortality in the first year. If the management objective is to keep tree mortality minimal, spring burns with little impact on tree crowns could be recommended. For forests stands with a large fuel ladder, fall burns, which would allow higher crown damage without being detrimental to the tree, would be advisable as long as the forest floor moisture content is high to prevent delayed tree mortality.

According to our study, delayed mortality seems to be related more to the fire severity than the phenological status when burning occurs. Higher delayed mortality as a consequence of basal stem girdling seems to be the result of the higher combustion time recorded in fall burns. The mortality of larger trees was delayed and concentrated in fall burns probably due to the lower forest floor moisture content and longer combustion times registered. This suggests that managers should pay attention to forest floor moisture content when the objective of PB is to keep larger trees alive.

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3.7 Appendix

Table A1: Competing stem, crown, basal stem and combined damage models for the probability of tree mortality 1 year post-burning using the variables selected by the random forest permutation-based score. X reflects the interaction term.

Competing models	**AIC$_c$**
Stem models	
tree status $\sim$ season+BSP$_{min}$+D$_{50}$+sp+BLS$_{min}$ x sp+BSP$_{min}$ x season+D$_{50}$ x BSP$_{min}$ +D$_{50}$ x season+sp x D$_{50}$+season x sp	263.4
tree status $\sim$ season+BLS$_{max}$+D$_{50}$+sp+BSP$_{max}$ x sp+BSP$_{max}$ x season+D$_{50}$ x BSP$_{max}$ +D$_{50}$ x season+sp x D$_{50}$+season x sp	261.9
Crown models	
tree status $\sim$ season+CSH+sp+season x CSH+CSH x sp+sp x season	427.1
tree status $\sim$ season+CVS^2+sp+season x CVS^2 + CVS^2 x sp+sp x season	296.2
tree status $\sim$ season+CVS+sp+season x CVS+CVS x sp+sp x season	275.3
Basal stem models	
None	
Combined models	
tree status $\sim$ season+BSP$_{max}$+D$_{50}$+sp+CVS+CVS x D$_{50}$+CVS x BSP$_{max}$+ season x CVS+CVS x sp+sp x season+BSP$_{max}$ x sp+BSP$_{max}$ x season+D$_{50}$ x BSP$_{max}$+D$_{50}$ x season+sp x D$_{50}$+season x sp	165.3

Table A2: Competing stem, crown, basal stem and combined damage models for the probability of tree mortality 3 year post-burning using the variables selected by random forest permutation-based score. X reflects the interaction term.

Competing models	AICc
Stem models	
tree status $\sim$ season+BSP$_{mean}$+D$_{50}$+sp+BSP$_{mean}$ x sp+ BSP$_{mean}$ x season+ D$_{50}$ x BSP$_{mean}$ +D$_{50}$ x season+sp x D$_{50}$+season x sp	376.5
tree status $\sim$ season+BSP$_{min}$+D$_{50}$+sp+season+BSP$_{min}$ x sp+ season+BSP$_{min}$ x season+D$_{50}$ x season+BSP$_{min}$ + D$_{50}$ x season+sp x D$_{50}$+season x sp	376.7
Crown models	
tree status $\sim$ season+CVS+sp+season x CVS+CVS x sp+sp x season	475.6
tree status $\sim$ season+CVS+sp+season x CVS	481.7
Basal stem models	
tree status $\sim$ season+white ashes+D50+sp+white ashes x sp+ white ashes x season+D$_{50}$ x white ashes+D$_{50}$ x season+sp x D$_{50}$+season x sp	373.0
Combined models	
tree status $\sim$ season+ season+BSP$_{mean}$ +white ashes+CVS+D$_{50}$+ sp+season x CVS+CVS x sp+white ashes x CVS+sp x season+white ashes x season+D$_{50}$ x white ashes+ D$_{50}$ x season+sp x D$_{50}$+season x sp+ season+BSP$_{mean}$ x sp+ season+BSP$_{mean}$ x season+D$_{50}$ x season+BSP$_{mean}$	315.0

Table A3: Measures of model performances for 1 and 3 years post-burning tree mortality.

	1 year post-burning		3 years post-burning	
	Firth LR	Ctree	Firth LR	Ctree
Accuracy	0.96	0.95	0.93	0.93
Error rate	0.04	0.04	0.08	0.07
TPR	0.41	0.28	0.34	0.34
FPR	0.008	0	0.02	0.008
Specificity	0.99	1	0.97	0.97
Precision	0.78	1	0.63	0.63
Prevalence	0.06	0.06	0.10	0.10

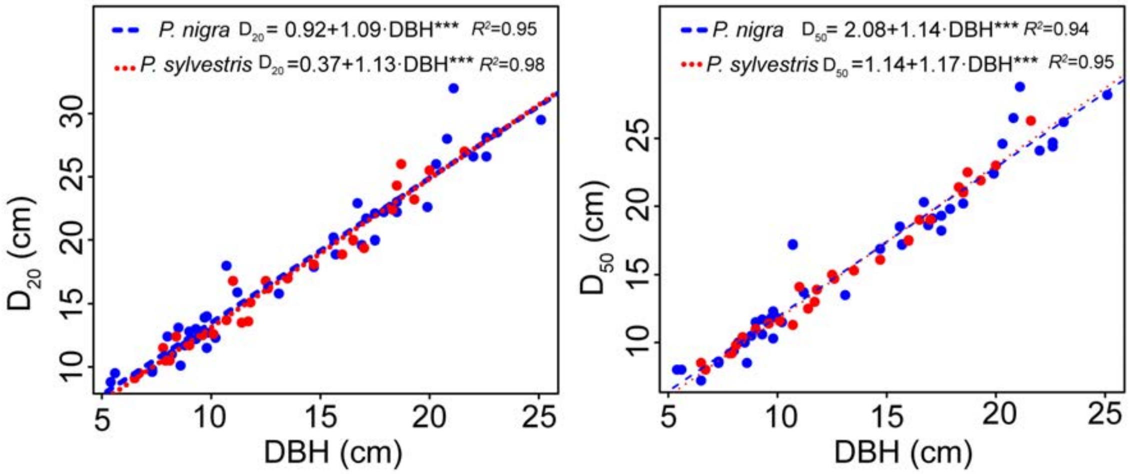


Figure A1: Relationships between diameter at 20 cm and 50 cm (D_{20} and D_{50}, cm) heights and diameter at breast height (DBH, cm) for *P. nigra* (n=40) and *P. sylvestris* (n=40).

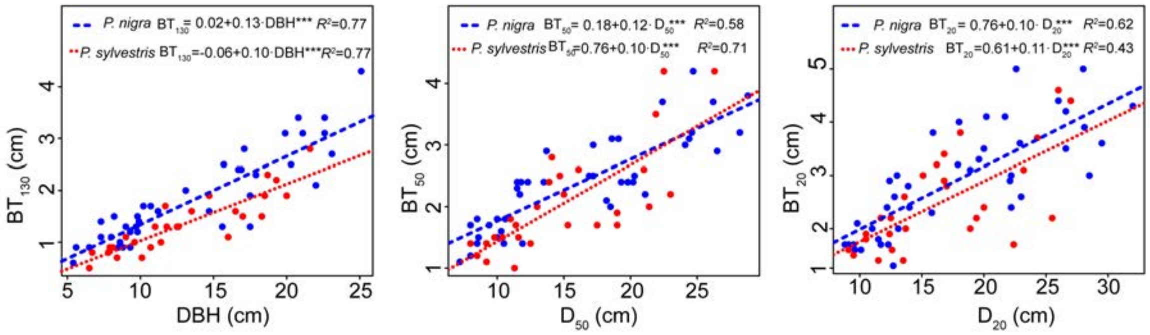


Figure A2: Relationships between bark thickness (BT) (cm) and diameter at breast height (DBH, cm) at different stem heights (130 cm, 50 cm and 20 cm) for *P. nigra* (n=40) and *P. sylvestris* (n=40).

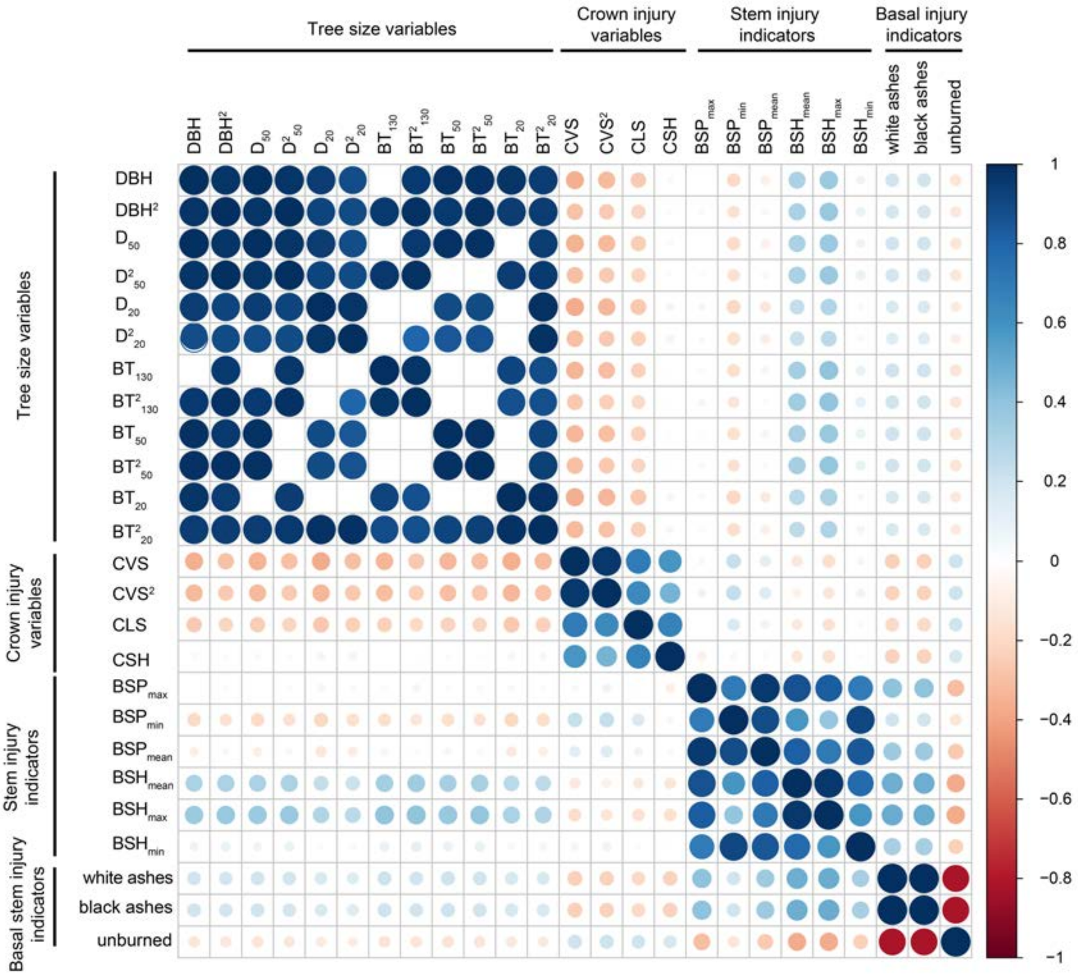
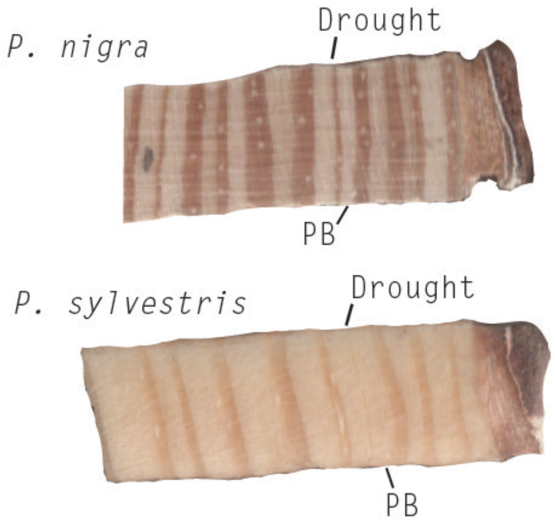


Figure A3: Correlation matrix between tree size and crown fire damage variables, as well as stem and basal stem injury indicators.

Chapter 4

The effect of prescribed burning on the drought resilience of ***Pinus nigra*** **spp.** ***salzmannii*** **(Dunal) Franco and** ***P. sylvestris*** **L.**



In preparation
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Abstract

Applying prescribed burning (PB) after a dry year can be controversial as fire might exacerbate the negative effects of drought on tree growth; however, PB can also increase resource availability and enhance drought resilience and, even, resistance to further drought events. In two mixed-stands of *Pinus nigra* spp. *salzmannii* (Dunal) Franco and *P. sylvestris* L., we assessed the effects of PBs executed one year after a dry year (2012), on total tree ring (TTR), earlywood (EW) and latewood (LW), resilience, considering pines' resistances to drought and PB by comparing pines burned during the spring, the fall season and control pines. We investigate possible variations in growth resilience over time caused by burning season and release from tree competition as well as on LW/EW. No differences were found in the resilience capacity of the less resistant individuals, independently of whether the pines were burnt or not. By contrast, resistant pines burned during the fall had higher TTR and LW resilience indices than control and pines burned during the spring. Particularly, TTR and LW resilience increased significantly in resistant individuals with high pre-burning competition index that experienced a significant amount of release from tree competition and were less damage by fire in terms of bole scorch height. *P. nigra* showed higher resilience than *P. sylvestris* but lower resistance suggesting a trade-off between resistance and resilience in terms of tree reserves. Another plausible explanation is related to differences in the climate-growth relationship of the species to the same dry year and/or in fire sensitivity. In fact, *P. sylvestris* showed high values of LW δ^{13}C at the year of fall PB suggesting lower stomata conductance due to the fire stress. By the second year of fall PB, TTR resilience between both species was similar suggesting that *P. sylvestris* recover from both stresses, as partially indicated by the more negative values of δ^{13}C. Likewise, post-burning LW/EW increased after fall PB suggesting that PB can increase pine's resistance to upcoming drought events. Overall moderate PB after a dry year did not exacerbate the vitality of drought-weakened pines increasing the resilience of LW in resistant individuals when a significant amount of release from tree competition occurred. PB may be a valuable management tool to overcome the immediate effects of a dry year and increase drought resistance especially in *P. nigra*.

Keywords: drought; resistance; recovery; prescribed fire; carbon isotope; tree rings; fire ecology.

4.1 Introduction

The comparison of tree growth before, during and after a drought event can be used as a proxy of tree's resilience and resistance (Lloret et al. 2011). Successive drought events can deplete individual tree's reserves diminishing tree vitality and, ultimately, causing tree death (Galiano et al. 2011). If drought weakened trees are further stressed by natural or management perturbations, such as prescribed burning (PB), the resilience capacity of trees can be constrained as fire injured trees required available carbohydrate storage to replenish killed or injured tissues (Varner et al. 2009). In contrast, PB can have positive effects on tree growth by means of increasing resource availability due to the removal of understory and small trees (Alfaro-Sánchez et al. 2015; Battipaglia et al. 2014) and the release of nutrients after the fire (Certini 2005). Although some studies have analysed the effect on tree-growth of PB affected by subsequent drought events (Bottero et al. 2017; Collins et al. 2014; Lloret et al. 2011), few studies have studied the reverse situation: PB after a drought event. In particular, how the application of PB after a dry year influences tree resilience, where we define resilience as the capacity to return to pre-disturbance level in terms of function and growth (Holling 1973, Walker et al. 2004). Furthermore, as resilience *per se* does not consider the impact inflated by the disturbance, the inclusion of resistance, which is the reversal of the reduction in ecological performance during disturbance (Kaufman 1982), in the analysis of tree growth resilience could avoid underestimations of resilience (Lloret et al. 2011).

To understand in detail individual's resilience capacity to overlapping disturbances requires knowing their relative intensities (Bansal et al. 2013) and the stochastic extrinsic factors associated with them (e.g. release from tree competition induced by fire or drought) (Lloret et al. 2011). Resistance to drought and PB together with the fire severity experienced by each individual (e.g. crown, root and stem injury, death of neighbour trees), specific characteristics of PB (e.g. intensity, season), tree attributes (e.g. species, size, tree reserves status) and time since burning should be major sources of variability revealing trees resilience after drought and PB. Yet, few studies have actually characterized these variations.

The direct and indirect impacts of drought and PB on tree growth could differently influence the earlywood and latewood resilience of pines. Trees that survived to drought events generally have a higher proportion of latewood compared to those that succumb to drought (Martinez-Meier et al. 2008). Latewood tracheid's are of smaller-diameter and with thicker cell walls than earlywood and, therefore, a higher proportion of latewood respect to earlywood increases the resistance to future embolism of upcoming drought events. However, fire stem injury could lead to a lasting reduction of xylem conductivity via conduit wall deformation or cavitation resulting in a permanent reduction of hydraulic safety and the increase of embolism vulnerability (Michaletz et al. 2012; Bär et al. 2018). We expect that burning season, the most controllable component of the PB, can influence further pine's resistance to cavitation because burning in spring or fall could differently impact the development of the next seasonal growth likely producing changes in the proportion of latewood and earlywood in a tree ring.

The boreo-alpine *P. sylvestris* L. is less tolerant to drought that the sub-Mediterranean *P. nigra* spp. *salzmannii* (Dunal) Franco (Martinez-Vilalta and Piñol 2002). Additionally, *P. nigra* is more fire resistant than *P. sylvestris* due to its thicker bark, thicker needles and higher canopy base height (see Fernandes et al. 2008 for review), which is also supported by lower mortality of *P. nigra* after PB in comparison to *P. sylvestris* (Valor et al. 2017). For both species, with iso-hydric behaviour, combining tree growth data with wood isotope C information can help to understand more specifically the physiological response of each species to the combined effect of fire and drought. The δ^{13}C is a good indicator of plant intrinsic water-use efficiency (WUE$_i$) (Farquhar et al. 1989), and analysed at the seasonal scale can provide specific information on the physiological response of trees to the environmental conditions created by PB. Whereas earlywood is usually formed from carbohydrates assimilated in the previous year (Kagawa et al. 2006; Kress et al. 2009; Eilmann et al. 2010), latewood carbon isotope values are clearly related to current environmental conditions and can contribute to explain the physiological mechanism behind tree resilience after drought and PB.

This study takes advantage of moderate-intensity PBs, enough to induce around 10-15 % of tree mortality 2 years post-burning (Valor et al. 2017), executed in spring and fall of 2013, one year after 2012, considered one of the driest year recorded in the region (Sánchez-Costa et al. 2015). We studied the effects of drought and spring or fall PB on total tree ring, earlywood and latewood resilience capacity of *P. nigra* spp. *salzmannii* and *P. sylvestris*, using dendrochronological and stable isotope analysis by selecting pines of similar size and without any apparent damage to the crown but in which stem or basal stem injury was plausible. The goal of this study was to answer the following questions:

1. Is the resistance of *P. nigra* and *P. sylvestris* to two consecutives stressful events (drought and PB) different in comparison to pines experiencing only drought?
2. Is the resilience capacity of *P. nigra* and *P. sylvestris* suffering two consecutives stressful events (drought and PB) different in comparison to pines experiencing only drought? How much is resilience influenced by the degree of resistance to PB and drought?
3. Is resilience to drought and PB individual or species specific? How much is resilience influenced by the degree of resistance to PB and drought, PB characteristics (such as burning season) and release from tree competition induced by PB?

4. Does burning season affect the proportion of latewood to earlywood in *P. nigra* and *P. sylvestris*?

Understanding to what extend PB and the season of burning may exacerbate, cancel out or reverse the effects of a drought event on the resilience capacity of trees may contribute to the prescription of this management technique. This is an important issue given the predicted increase in drought frequency and intensity under climate change.

4.2 Methods

4.2.1 Study site, experimental design and prescribed burns

The study was performed in two localities in the foothills of the Pyrenees, Miravé and Lloreda, in the NE Iberian Peninsula (Table 4.1). We selected one mixed *P. sylvestris* and *P. nigra* stand in each locality, Mirave and Lloreda. Within each stand, we selected two paired sites (1 ha each) to perform PB either in spring or fall. One plot (30 x 30 m), per each of the four sites, was set up to monitor the effects of PB on tree growth and pine mortality.

Table 4.1: Characteristics of PB sites.

Locality	Burn season	Long. (ºE)	Latitude (ºN)	Altitude (m.a.s.l.)	Slope (%)	Aspect	Rainfall (mm)	T (ºC)
Lloreda								
	Spring	1.5706	42.0569	715	30	N	731.6	11.7
	Fall	1.5771	42.0620	826	25	N	766.3	11.1
Miravé								
	Spring	1.4494	41.9515	723	25	NE	677.3	11.5
	Fall	1.4496	41.9508	723	25	NE	677.3	11.5

The mean annual values of climatic variables were estimated using a georeferenced model (Ninyerola et al. 2000).

The climate is sub-Mediterranean with milder summers and colder winters than the typical Mediterranean climate. At both sites, soils developed from calcareous colluviums, classified as Calcaric inceptisols (FAO 2006), were between 0.5 m and 1 m deep, basic (pH 6.8-7.5) and with a fine texture.

The forest overstory is dominated by *Pinus nigra* ssp. *salzmannii* (Dunal) Franco and *P. sylvestris* L. and broadleaf trees (i.e. *Quercus pubescens* Willd., *Acer monspessulanum* L., *Sorbus aria* (L.) Crantz) are also common. The understory is composed mainly of *Buxus sempervirens* and deciduous species (e.g. *Lonicera etrusca* Santi, *Viburnum lantana* L., *Amelanchier ovalis* Medik.). Other evergreen shrubs were also common in the understory (e.g. *Juniperus oxycedrus* L., *Q. coccifera* L.).

PB was carried out in late spring (5 June at Lloreda and 12 June at Miravé, 2013) and fall (17 October at Miravé and 8 November at Lloreda, 2013) by the Forest Actions Support Group (GRAF) of the Autonomous Catalan Government (*Generalitat de Catalunya*), with the objective of reducing surface and ladder fuels. All PBs were carried out following a strip headfire ignition pattern under similar meteorological conditions. During burning, combustion time (minutes) above 120 ºC and the maximum temperature at the base of the trunk, placed at the surface of the soil litter, were measured in 18 trees with K-thermocouples (4 mm) connected to dataloggers (Testo 175), packed with a fireproof blanket and buried in the soil at a depth of ca. 20 cm. Temperatures were recorded every 10 s. Trees for temperature monitoring were spatially distributed by dividing each of the 30 x 30 plots into 4 subplots (15x15m) and randomly selecting a number of 4-5 pines within each subplot to gain a representative spatial characterization of the temperature in the plots.

Straight after PB, for each tree we visually estimated the proportion of white ashes in 1 m radius from the tree center. White ashes indicate smoldering and potential damage to the basal stem and root collar (McCandliss 2002). Stem injury was characterized by bole scorch height (BSH) (Wyant et al. 1986; Rigolot 2004). We measured the maximum (BSH_{max}) and minimum (BSH_{min}) bole scorch height. One week after PB, the crown volume scorched was visually estimated to the nearest 5 % as the change in needle colour resulting from transferred heat via convection. In each plot, tree mortality was monitored 1 month after PB and every 6 months during the 2 following years. A tree was considered alive when any green foliage was present in the crown otherwise it was considered dead. Details on tree mortality over time are reported in Valor et al. 2017.

4.2.2 Tree selection and tree competition index

The resilience capacity of pines affected by a drought and managed by PB was analysed by randomly selecting at each plot 15 trees per species and burning season, with similar DBH and without any damage on the crown (Table 4.2). In *P. sylvestris* the target of 15 trees was not always reached due to an absence of candidate trees, even though we select some trees outside from the monitoring plot but within the site. Additionally, we select 15 control trees per species growing in an adjacent site left unburned.

Table 4.2: Tree, fire and tree competition characteristics (mean ± SD) grouped by locality, species and plot.

Locality	Species	Plot	n (trees)	Age (years)	dbh (cm)	C_{12}	BSH_{max} (cm)	BSH_{min} (cm)	WA (%)	RCI_{15}
Mirave										
	P. nigra	Control	15	58±13	16.3±1.3					
		Spring PB	15	65±13	15.9±1.6	3.7±3.5	112.7±64.7	46.0±38.3	75.4±17.4	0.11±0.20
		Fall PB	15	64±13	15.2±1.3	3.6±1.6	52.2±36.6	14.4±12.6	33.8±13.0	0.11±0.13
	P. sylvestris	Control	15	55±7	20.3±2.0					
		Spring PB	15	46±11	20.3±3.8	2.6±1.5	39.0±21.9	19.5±14.5	60±21.9	0.12±0.17
		Fall PB	15[1]	53±6	18.2±2.4	0.8±0.3	57.7±4.24	14.4±6.3	49±22.6	0.02±0.04
Lloreda										
	P. nigra	Control	15	54±7	17.4±1.3					
		Spring PB	15	49±12	14.1±2.2	4.1±2.8	114.7±71.0	34.7±25.0	71.5±14.1	0
		Fall PB	14	56±10	15.5±0.7	3.2±1.7	161.5±113.3	30.3±22.8	78.9±15.6	0.02±0.03
	P. sylvestris	Control	15	65±5	18.4±2.4					
		Spring PB	12[2]	51±13	15.5±3.0	2.8±2.9	62.2±37.5	23.6±16.9	58.2±23.6	0
		Fall PB	15	59±6	17.5±3.1	2.3±1.1	102.9±75.5	29.0±23.6	70.9±19.0	0.14±0.13

[1] The sample size for the severity variables is 7.

[2] The sample size for the severity variables is 10.

CI_{12}, pre-burning Hegyi competition index.

RCI_{15}, relative competition index in 2015.

BSH_{max}, maximum bole scorch height (cm).

BSH_{min}, minimum bole scorch height (cm).

WA, white ashes in 1 m radius from tree centre (%).

For each burned tree, we computed the Hegyi's competition index (CI) (Hegyi 1974) before burning (CI_{12}) and 2 years post-burning (CI_{15}) (Table 4.2). The subindices referring to the year of measurement (2012 and 2015). The Hegyi's index is a distance dependent tree competition index, and it was calculated using the *pairwaise* function of the Siplab package for each year as follows:

$$CI_i = \sum_{j=1}^{n} \frac{DBH_j/DBH_i}{dist_{ij}}$$

where CI_i is the competition index for the subject tree *i*, DBH the diameter at breast height, $dist_{ij}$ the distance between the subject tree *i* and the competitor tree *j*, and n is the number of competitors in the neighborhood zone. A search radius of 3.5 m defined the neighborhood zone. Following Lorimer (1983), the radius was defined by multiplying the mean crown radius of overstory, 1, by 3.5. Then, to quantify the release from tree competition experienced by each tree, we compute a relative competition index (RCI_{15}) as the difference between pre (CI_{12}) and post-burning competition (CI_{15}) indices relative to pre-burning levels (4.2).

4.2.3 Tree ring measurements

A total of 176 pines were cored to the pith during November and December 2016. Two cores per tree were extracted with an increment borer of 4.5 mm diameter at 50 cm height above ground, with an angle of 120º between them. Cores were prepared following standard dendrochronological techniques (Stokes and Smiley 1968). The total tree-ring (TTR), earlywood (EW) and latewood (LW) widths of each annual tree ring were measured from year 2015 until pith, as latewood in early fall 2016 was not still defined in all cores. We used a Lintab 3 measuring system coupled to TSAP tree-ring software (Frank Rinn, Heidelberg, Germany), with a precision of 0.001 mm. Cores of each locality, species and plot were dated and visually cross-dated by comparing characteristic narrow rings presents in all trees to detect the presence of false and missing rings. Cross-dating was validated in the 12 chronologies developed, resulting from the combination of locality, species and plot, using COFECHA software (Holmes 1983), which calculates cross-correlations amongst individual series of tree growths.

Tree-ring series of pines were detrended with a cubic smoothing spline with a 50 % frequency response cut-off of 10 years to remove long-term growth trends embedded in the raw tree-ring series that were thought to be induced by non-climatic influences, such as aging and competition between trees (Fritts 1976), using the Dendrochronology Program Library (dplR) package in R, Version 2.10.1. The standard chronology was calculated as the biweight robust mean value of each detrended series. On the other hand, individual series were subjected to autoregressive modelling and also averaged using a bi-weight robust mean to obtain the residual chronology (Cook 1985). The residual chronology was used to analyse climate-growth relationships because by removing the autoregressive component stronger more reliable correlations can be found with climate. We used the standard chronology to calculate common dendrochronological statistical measures, and so understand how much growth for any given year has been strongly influenced by growth in preceding year.

TTR, EW and LW were converted into basal area increments (BAI) using the INBAI function of the Dplr package. The use of BAI was preferred over raw ring tree widths because it is less dependent on tree age and avoids the need for detrending, which could obscure the effects of reduction in tree competition on tree growth, as it removes the low frequency variability (Biondi 1999).

We studied tree responses to one individual dry year that occurred in 2012, just one year before the application of PB. To ascertain that the 2012 event was a year with significant growth reduction in both species we used the package Pointres (Figure A1). For each tree and year, we calculate the relative growth change (in percentage) (RGC) of a specific year compared to the 3 preceding years in each species. A threshold of 25% was established, below which the relative change in growth in a specific tree and a year was considered a negative pointer year or a positive pointer year when the RGC was above 60%. For each year, when more than 50% of all trees within the control chronologies exceed the 25% or 60% threshold, the year was considered a negative or positive pointer year, respectively.

4.2.4 Resistance and resilience indices calculations

The resistance and resilience indices were calculated following Lloret et al. (2011) as follows:

Resistance = BAI_{St} / BAI_{preSt}

Resilience = BAI_{postSt} / BAI_{preSt}

where BAI_{St} is the average BAI during the stress period (drought 2012 and PB 2013), BAI_{preSt} is the average BAI of the three years preceding the stress period (2009 to 2011) and BAI_{postSt} is the average BAI of the two years after the stress period (2014 and 2015); resilience was also calculated separately for year 2014 and 2015. Resilience was calculated for TTR, EW and LW while TTR was used for the resistance index.

To understand the effect of burning season on drought resistance to future stressful events we used the LW/EW ratio as an estimate for wood density (Bodig and Jayne 1993). For each tree, we subtracted the mean pre-burning LW/EW (2006-2012) to the LW/EW for each of the 2 following years post-burning (dif LW/EW). Positive values of dif LW/EW indicated higher LW/EW than before burning and negative values lower LW/EW than before burning.

4.2.5 Climatic data

Monthly precipitation (P) and temperature (T) from 1975 to 2015 were obtained for both sites using climatic records and projections elaborated by the Spanish Meteorological Agency (AEMET) (see Ninyerola et al. 2000 for the spatial resolution of the original climatic data and the approach applied to downscale the climatic records). Monthly potential evapotranspiration (*PET*) was estimated using the Thornthwaite (1948) method. For each month, the difference between P and PET (*P-PET*) was calculated as a measure of water availability.

4.2.6 Carbon isotopes

Once the chronologies per each site, specie and burning season were developed, we selected the 5 trees with the highest inter-series correlation and of similar age. In these selected trees, one additional core was extracted using a borer of 7 mm diameter with a drill at 50 cm height. From each sampled core, we selected the section corresponding to the period 2011-15. EW and LW were divided and separated for each year. The EW and LW of the 5 trees were pooled per each combination of year, site, specie and burning season. To estimate the standard deviation of the samples, the EW and LW of 2011 were not pooled. Pooled and individual samples were ground with a milling chamber.

The carbon stable isotope composition was measured at the IRMS Lab of University of Campania "L. Vanvitelli" by continuous-flow isotope ratio mass spectrometry (Delta V Advantage, Thermo Scientific, Bremen, Germany) using 0.06 mg of dry matter for ^{13}C measurements. Late- and early-wood was processed since recent studies showed that the use of wood isotope values, instead of cellulose, is justified for ecophysiological and dendrochronological studies that investigate the response of trees to environmental changes recorded within the sapwood, i.e. in a relatively short-term period (Weight et al. 2015, Riechelmann et al. 2016, Valor et al 2018). The δ^{13}C series were corrected for the Suess Effect (decrease in δ^{13}C of atmospheric CO_2 since the beginning of industrialization) resulting from the emission of fossil carbon dioxide, which is depleted in ^{13}C (Francey et al. 1999, McCarroll and Loader 2004). The corrected series were used for all statistical analyses. Isotopic compositions are expressed in delta notation (‰) relative to accepted reference standards Vienna PeeDee Belemnite for carbon isotope values. The standard deviation for the repeated analysis of an internal standard (commercial cellulose) was lower than 0.2 ‰ for C.

4.2.7 Data analysis

The following parameters were calculated to assess the goodness of the developed chronologies: Mean inter-series correlation (Rbar), to assess agreement between individual series in each fire severity class; Expressed Population Signal (EPS), to understand to what extent the chronologies, which are based on a limited number of trees, were representative of the 'hypothetical' true chronologies (Wigley et al. 1984). For each species, Pearson correlations were calculated between control residual chronologies (TRW, EW and LW) and *P-PET* of monthly data, and data of two and three consecutive months using the package TreeClim.

First, we tested for differences in resistance to drought and PB between control and burned pines using a general linear mixed model (GLMM) including species and burning treatment (burned and control trees) as fixed factors and the interaction between them. Additionally, in burned pines, we modelled resistance as a function of BSH_{min} and burning season (spring and fall) (First goal question).

Second, we used a general mixed model for each resilience variable studied (TTR, EW and LW) to understand if resilience differs between pines suffering one stress (drought), that is control pines, and those that have experience two consecutive stressful events (drought and PB), such as the pines burned during the spring and fall after the dry year (Second goal question). The models include pine species, burning treatment (spring, fall and control) and the resistance index, as well as the two and three-way interactions between them. Third, using only burned pines we analysed how fire severity at the individual level affects the resilience of burned pines using a GLMM for each resilience variable studied and burning season (TTR, EW and LW for the whole post stress period and separately for 2014 and 2015) (Third goal question). The models include pine species, the resistance index, BSH_{min} as a proxy of stem or basal stem injury, RCI_{15} as the release from tree competition experienced by each individual and CI_{12} as the pre-burning competition index. The models include all two-way interactions and a three-way interaction between BSH_{min}, RCI_{15} and species. We used BSH_{min} instead of BSH_{max} or the proportion of white ashes because the model with BSH_{min} showed a better fit than the ones using the other fire severity indicators. Model selection was based on a robust form of Akaike's information criterion (AICc) (Burnham and Anderson, 2002), a model selection index favouring both model fit and model simplicity (Burnham and Anderson, 2002). Maximum likelihood was used for computing the AICc, while restricted maximum likelihood was used to estimate model coefficients.

To understand how burning season can influence tree ring density (Fourth goal question), we used a GLMM to assess the effects of burning season on the dif LW/EW at each of the 2 following years post-burning considering the pre-burning LW/EW, burning season and pine species as independent variables. All three way-interactions were included. Last, to detect time variations in the δ^{13}C LW, the mean and error standard of δ^{13}C LW for each species, site and burning season was plotted over time. For each species, we use a general lineal model to analyse the relationship between the δ^{13}C EW and δ^{13}C LW of the current year and between the δ^{13}C EW of the current year and the δ^{13}C LW of the previous years in the pre-burning years (2011-2012-2013) and the post-burning years (2014-2015). We include 2013 as pre-burning years because as spring burns occurred late in the season, we consider that PB has not impacted the EW of pines burned in spring.

All mixed models developed included a random factor (locality). Residuals of the mixed models developed presented no pattern and highly correlated explanatory variables were avoided. All analyses were conducted with the software R (v. 3.2.1, the R Foundation for Statistical Computing) using the package nlme for linear mixed-effects modelling and the package AICcmodavg for model selection. The model variances explained by fixed effects (marginal R^2) and by both fixed and random effects (conditional R^2) are provided (Nakagawa and Schielzeth 2013).

4.3 Results

4.3.1 Tree-ring chronologies and climate

Trees presented similar age within and between chronologies (Table 4.2 and Table A1). In all chronologies, EPS showed high values (> 0.85) indicating that constructed chronologies from detrended individuals were representative of the whole population of trees (Table A1). The mean BAI for each chronology revealed a similar growth pattern during the whole period (Figure 4.1).

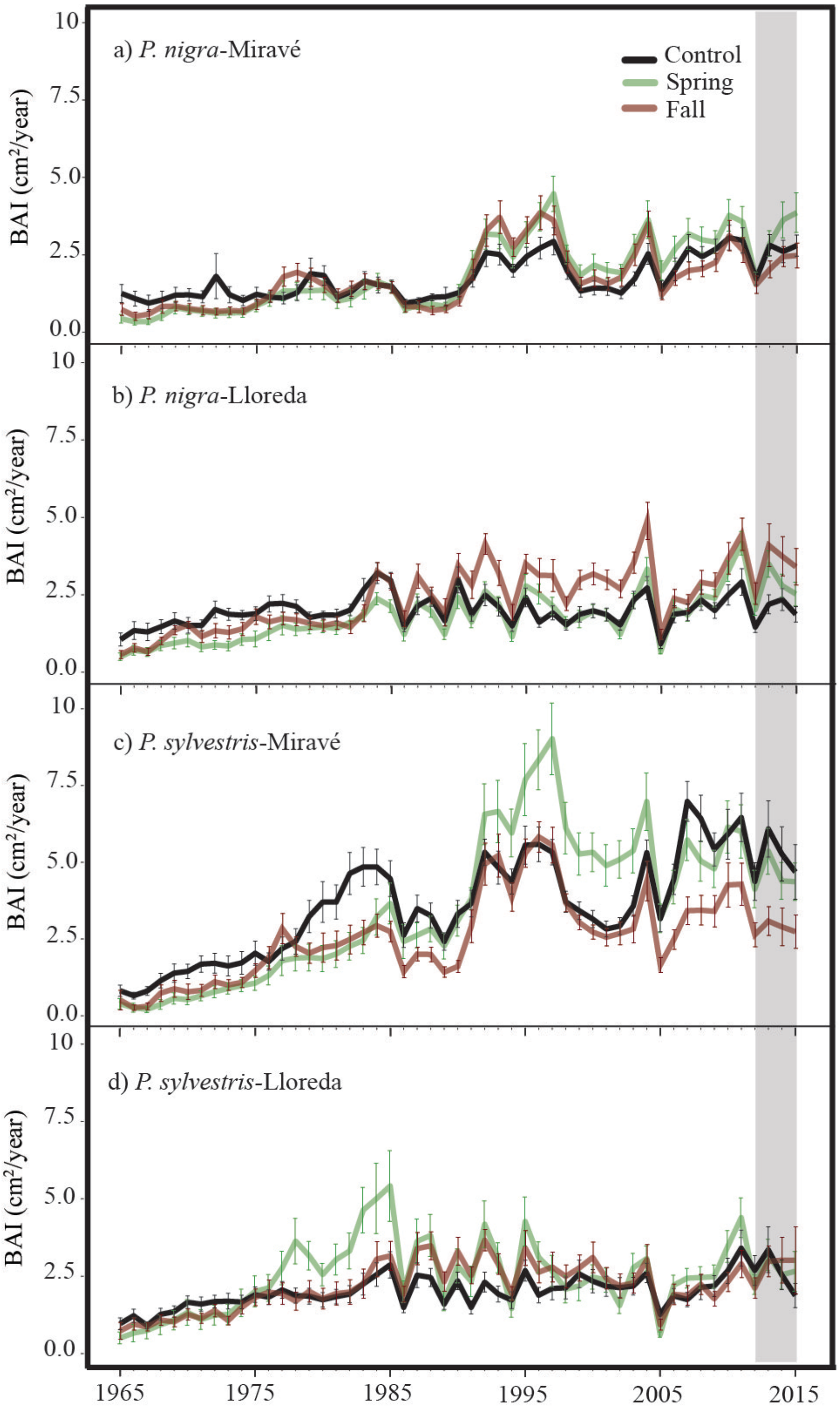

Figure 4.1: Mean basal area increment (BAI) with error standard for the whole-time span for *P. nigra* (a and b) and for *P. sylvestris* (c and d) at Miravé and Lloreda localities for control (black line), spring (green) and fall (brown) plots. Grey shading underlines the year of PB (2013) and the post-burning period 2014-2015.

The strongest correlations between growth and P-PET were found when 3 consecutive months were considered in the analysis rather than with monthly P-PET or when two consecutive months of *P-PET* were used (correlations not shown for 1 and 2 consecutive months).

In *P. sylvestris*, TTR was positively correlated with accumulated *P-PET* of previous November and December and current January and, also, with current summer *P-PET* (June, July and August) (Figure A2, a). Specifically, EW showed higher positive correlations with *P-PET* of November-December-January (Figure A2, c) and LW with the accumulate *P-PET* of summer months (Figure A2, e). In the 2012 dry year accumulated *P-PET* in previous November-December and current January, as well as in the summer months, was the lowest respect to the historical values (Figure A3).

In *P. nigra*, TTR was significantly correlated with accumulated *P-PET* of April-May-June and current fall (September, October and November) (Figure A2, b). EW was highly correlated with *P-PET* in April-May-June and LW with accumulated *P-PET* in the fall months (Figure A2, d and f). In the 2012 dry year, accumulated *P-PET* in April-May-June and fall were lower compared to the historical values (Figure A3).

For years 2012 and 2013, in *P. nigra,* the δ^{13}C of EW was related to LW δ^{13}C of the current year ($P < 0.001$, $R^2 = 0.90$) (Figure 4.2, a) but not to the previous δ^{13}C of LW (Figure 4.2, b); in contrast, in *P. sylvestris* the δ^{13}C of the current EW was directly correlated to the δ^{13}C of LW of the previous season ($P < 0.05$, $R^2 = 0.48$) (Figure 4.2, b) but not to the δ^{13}C of LW of the current year (Figure 6.2, a). For 2014 and 2015, the δ^{13}C of EW did not significantly correlated with the current or previous δ^{13}C of LW (data not shown).

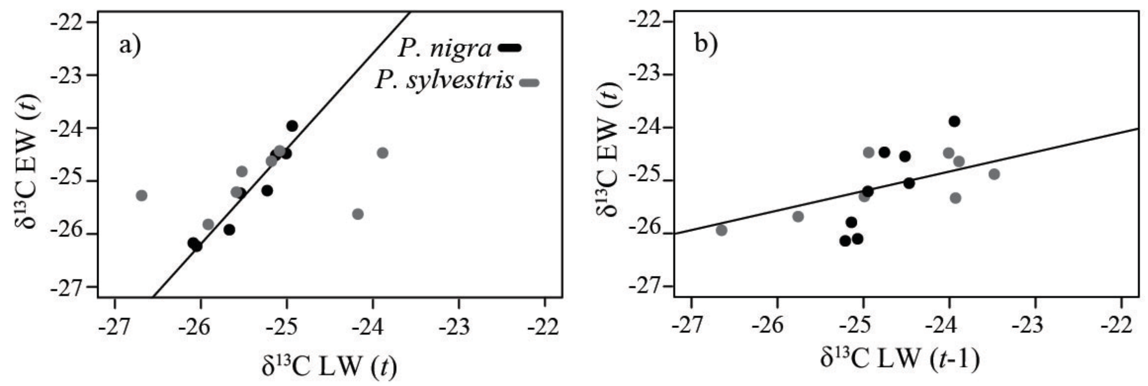

Figure 4.2: δ^{13}C values in current earlywood (EW (t)) versus current latewood (LW (t)) (a) and in current earlywood (EW (t)) versus previous latewood (LW (t-1)) (b) for *P. nigra* and *P. sylvestris* for pre-burning years 2012 and 2013. The regression for *P. sylvestris* in b was $y = -15.98x + 0.36$, $R^2 = 0.43$, $n = 8$, $P < 0.05$ and for *P. nigra* in a was $y = 20.60x + 1.80$, $R^2 = 0.90$, $n = 8$, $P < 0.001$.

4.3.2 Resistance to drought and PB of burned pines in comparison with control pines

Resistance was higher in *P. sylvestris* than in *P. nigra* (Table 4.3) and in control pines compared to burned pines (Table 4.3). Species per treatment interaction was not significant. Considering only burned pines, the resistance of pines burned in fall was lower than that of those burned during spring as BSH_{min} increased (Table 4.3).

Table 4.3: Summary of the mixed model (Estimate± std. Error) describing TTR resistance to the stress events (drought and prescribed burning) for burned and control pines and in burned pines as a function of fire severity and season.

	Parameters	Resistance
Burned and control pines		
	Intercept	0.80±0.06***
	Species (*P. sylvestris*)	0.13±0.03***
	Treatment (Burning)	-0.07±0.04*
	R^2Conditional	0.08
	R^2 Marginal	0.17
Burned pines		
	Intercept	0.85 ±0.10***
	Species (*P. sylvestris*)	0.14±0.05**
	Burn season (spring)	-0.14±0.07*
	BSH_{min}	-0.004±0.001**
	Season (spring) X BSH_{min}	0.004±0.002*
	R^2Conditional	0.10
	R^2 Marginal	0.29

*** Significant at *P* <0.001; ** significant at *P* <0.01; * significant at *P* <0.05.
BSH_{min}, minimum bole scorched height.
The coefficients show the change from burned pines to control pines for the treatment factor and from spring to fall for the burn season factor.

4.3.3 Resilience to drought and PB of pines burned in the fall and spring in comparison with control pines as a function of resistance

P. nigra showed higher TTR, EW and LW drought resilience than *P. sylvestris* independently of burning treatment (Table 4.4). For TTR and LW, there was a significant burning season by resistance interaction: as resistance increased the resilience of pines burned in the fall season was significantly higher than that of control or pines burned during the spring (Table 4.4 and for TTR resilience Figure 4.3, a). For EW, resistance had a positive effect on resilience regardless of burning treatment (Table 4.4).

Table 4.4: Summary of the mixed models (Estimate ± std. error) describing total tree ring, earlywood and latewood resilience from drought and PB as a function of resistance, species and burning season.

Parameters	Post-stress resilience		
	TTR	Earlywood	Latewood
Intercept	0.43±0.14**	0.17±0.10*	0.57±0.18**
Resistance	0.59±0.15***	0.88±0.11***	0.51±0.20
Species (*P. sylvestris*)	-0.24±0.04***	-0.19±0.04***	-0.27±0.05***
Season (Spring)	0.00±0.20		0.12±0.27
Season (Fall)	-0.40±0.19		-0.49±0.26†
Resistance X Season (Spring)	0.05±0.24		-0.03±0.32
Resistance X Season (Fall)	0.60±0.23**		0.81±0.30**
R^2 Conditional	0.33	0.29	0.26
R^2 Marginal	0.38	0.34	0.27

*** Significant at $P < 0.001$; ** significant at $P < 0.01$; * significant at $P < 0.05$; † significant at $0.05 < P < 0$.

For species, the coefficient shows the change from *P. sylvestris* to *P. nigra*; for season from spring or fall PB to control pines.

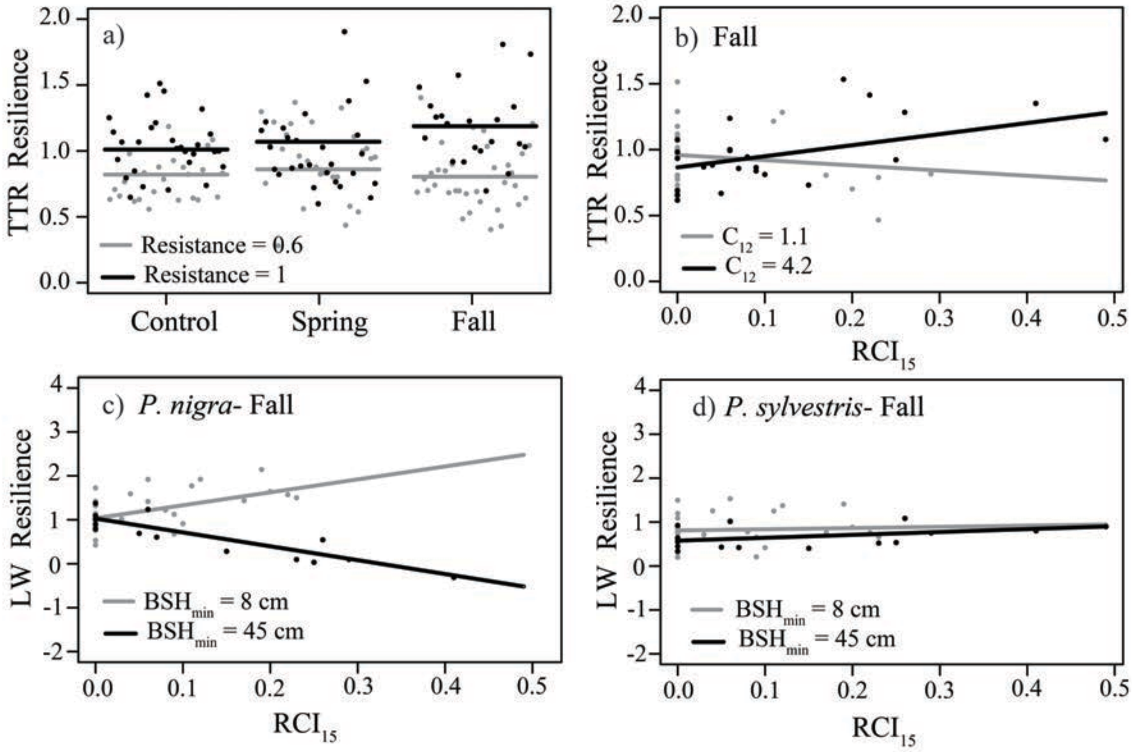

Figure 4.3: Partial regression residuals of the TTR resilience model showing (a) the interaction in Table 4.4 between resistance and treatment; and the interactions in Table 4.5 (b) for pines burned in the fall season between the release from tree competition (RCI_{15}) and the pre-burning competition index (CI_{12}); and for the LW resilience model in pines burned during the fall season between RCI_{15} and minimum bole scorch height (BSH_{min}) for *P. nigra* (c) and for *P. sylvestris* (d) to explain the interaction between species, RCI_{15} and BSH_{min}.

4.3.4 Resilience of pines burned in the fall as a function of resistance and fire severity

For pines burned during the fall season, except for LW 2014, there was a significant interaction between RCI_{15} and CI_{12} indicating that the positive effect of RCI_{15} on the resilience of TTR and LW was of higher magnitude as CI_{12} increased (Table 4.5 and Figure 4.3b for TTR resilience). In agreement with the model including the control pines (Table 4.4), the resistance had a positive effect on the TTR and LW resilience of both species (Table 4.5). The TTR resilience was higher in *P. nigra* than in *P. sylvestris* with the exemption of 2015, when the species factor was no significant (Table 4.5). For LW resilience, the differences between species depended on the level of RCI_{15} and BSH_{min} as indicated by a three-way interaction between species, RCI_{15} and BSH_{min} (Table 4.5): this interaction implied that as RCI_{15} increased and BSH_{min} decreased the LW resilience capacity of *P. nigra* significantly increased compared to *P. sylvestris* (Figure 4.3c and 4.3d, for *P. nigra* and *P. sylvestris* respectively).

We did not explore the effects of fire severity on spring burns since no differences were detected in the TTR and LW between pines burned in spring and control pines (Table 4.4), but the models can be found in Table A3.

Table 4.5: Summary of the mixed models (Estimate ± std. error) describing total tree ring (TTR) and latewood (LW) resilience of pines burned during the fall for the whole period post-stress (2014-2015) and at two (2014) and three (2015) years post-drought.

Parameters	TTR			LW		
	2014+2015	2014	2015	2014+2015	2014	2015
Intercept	0.25±0.16	0.12±0.17	0.14±0.19	0.09±0.27	-0.20±0.37	0.31±0.26
Resistance	1.07±0.18***	1.26±0.20***	0.92±0.20***	1.47±0.29***	1.76±0.39***	1.10±0.27***
Species (*P. sylvestris*)	-0.20±0.09*	-0.28±0.10*		-0.17±0.22	-0.04±0.30	-0.23±0.21
RCI_{15}	-0.83±0.67	-0.47±0.75	-0.23±0.95	1.60±1.73	4.26±2.22	0.36±1.65
CI_{12}	-0.03±0.02	-0.05±0.03	-0.001±0.02	-0.06±0.04		
BSH_{min}	-0.003±0.001*		-0.002±0.002		-0.002±0.005	-0.002±0.004
Sp. (*Ps*) X RCI_{15}				-4.07±2.18 †	-5.24±3.04 †	-2.34±2.07
Sp. (*Ps*) X BSH_{min}				0.006±0.006	-0.007±0.009	-0.003±0.006
RCI_{15}X CI_{12}	0.39±0.16**	0.38±0.18*	0.56±0.18**	1.02±0.42**		1.14±0.40***
RCI_{15} X BSH_{min}			-0.04±0.02*	-0.16±0.05**	-0.10±0.05*	-0.14±0.05*
Sp. (*Ps*) X RCI_{15} X BSH_{min}				0.17±0.06**	0.15±0.07*	0.12±0.06*
R^2 Conditional	0.52	0.50	0.45	0.46	0.37	0.41
R^2 Marginal	0.52	0.50	0.45	0.46	0.37	0.41

*** Significant at P <0.001; ** significant at P <0.01; * significant at P <0.05; † significant at $0.05<P<0.1$.
For species, the coefficient shows the change from *P. sylvestris* to *P. nigra*.
RCI_{15}, relative competition indec in 2015.
CI_{12}, pre-burning Hegyi competition index.
BSH_{min}, minimum bole scorch height (cm).
Ps, *P. sylvestris*.

4.3.5 Impact of burning season on the post-burning LW/EW of pines

The years before burning the LW/EW of *P. sylvestris* was significantly lower than that of *P. nigra* (P<0.05), without differences between control and PB sites (P>0.05).

At 1 and 2 years post-burning, there was a significant interaction between pre-burning LW/EW, burning season and species to explain the difLW/EW (Table 4.6). After fall burns the difLW/EW significantly increased in *P. nigra* individuals with low pre-burning LW/EW compared to control pines with low pre-burning LW/EW (Figure 4.4, a) and in *P. sylvestris* in those with high pre-burning LW/EW (Figure 4.4, b).

Table 4.6: Anova table of the linear mixed-effects model results (F value) of the difference in LW/EW (LW/EW post-burning minus pre-burning LW/EW).

Parameters	1 year post-burning	2 year post-burning
Intercept	111.35***	7.86**
pre-burning LW/EW	9.8**	3.24†
Species	32.15***	13.40***
Season	8.86***	0.11
pre-burning LW/EWX Species	6.60*	2.60
pre-burning LW/EW X Season	2.53†	1.63
Species X Season	1.36	0.56
pre-burning LW/EW X Season X Species	3.76*	4.58*

*** Significant at P <0.001; ** significant at P <0.01; * significant at P <0.05; † significant at 0.05<P<0.1.

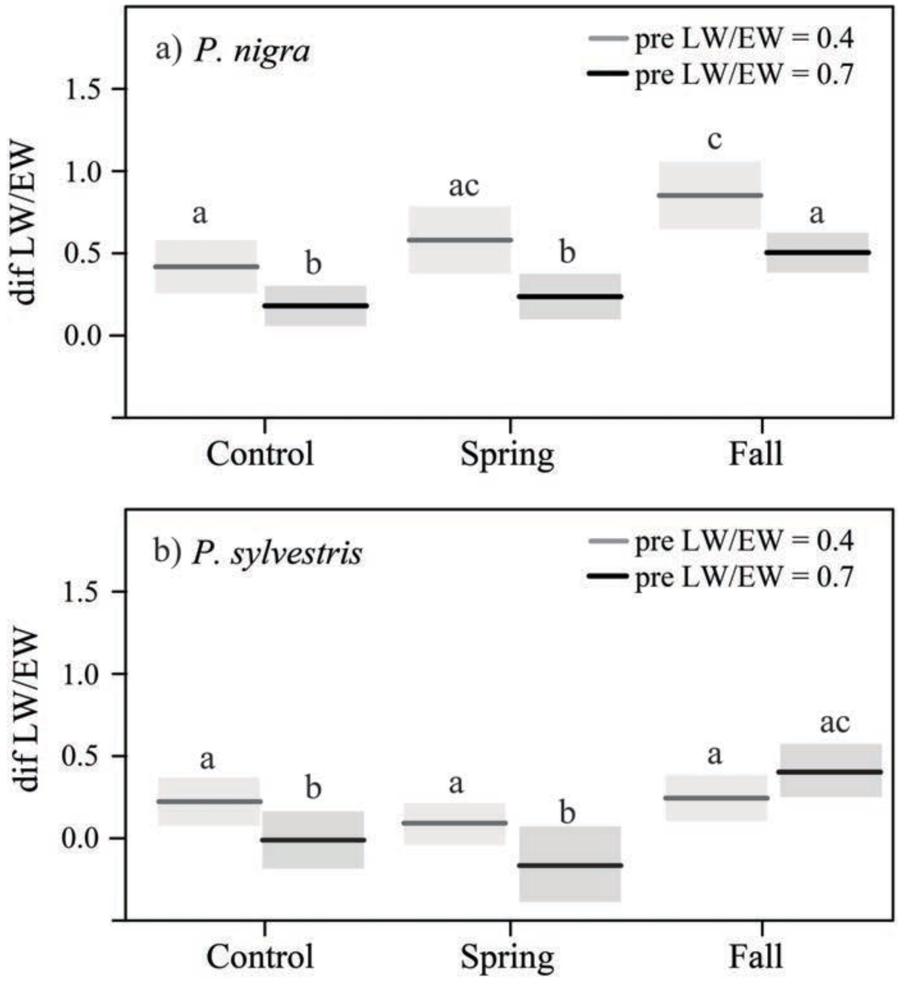

Figure 4.4: Prediction line and confidence intervals of the mixed model describing the dif LW/EW at 1 year post-burning (the LW/EW at 1 year post-burning minus mean pre-burning LW/EW) showing the interaction between burning season and pre-burning LW/EW for *P. nigra* (a) and *P. sylvestris* (b) to explain the interaction between burning season, species and pre-burning LW/EW. For each species, different letters indicate statistical significant differences ($P < 0.05$) among burning seasons and categories of pre-burning LW/EW using the LMM of Table A3 at 1 year post-burning followed by Tukey post-hoc test.

4.3.6 Carbon isotope ratio in *P. nigra* and *P. sylvestris* latewood

Before burning the δ^{13}C of LW of each pine species was similar between stands, except for *P. sylvestris* in the Mirave site (Figure 4.5). After burning, in the Lloreda site, the year of PB (2013), the δ^{13}C of LW of *P. sylvestris* was higher in fall burns than in spring burns (Figure 4.5, a and b). After 2 years of PB, *P. sylvestris* burned in fall had lower LW δ^{13}C than in spring burns at both sites (Figure 4.5, a and b).

The δ^{13}C of LW of *P. nigra* tend to be lower the year of burning and the years after in comparison with pre-burning years, without clear differences between burning seasons.

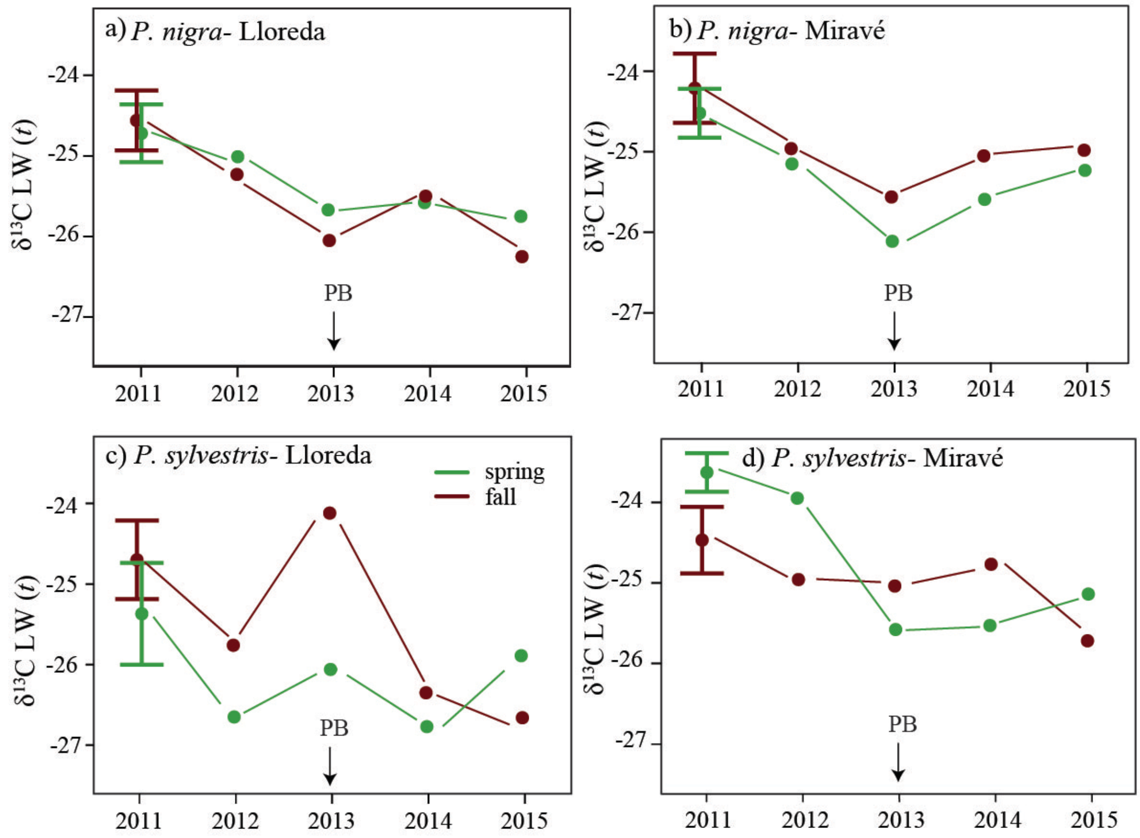

Figure 4.5: δ^{13}C pool values in current latewood (LW) for *P. nigra* burned in fall and spring at Lloreda (a) and Mirave (b) sites and for *P. sylvestris* burned in fall and spring at Lloreda (c) and Mirave (d). The standard deviation is showed for the 2011 year (n=5).

4.4 Discussion

A PB performed the year after a dry year did not reduce the growth resilience of pines in comparison with pines growing in control (unburned) stands. Moreover, burning in the fall after the dry year seems to increase the growth resilience of pines compared with those burned in spring or left unburned. However, the individual response varied between pine species being positively influenced by drought and PB resistance and the degree of release from tree competition experienced, but, negatively by the damage caused by the fire.

PB mostly impacted growth resilience through the effect on LW while EW resilience was not clearly affected by fire. Moreover, the release in tree competition had a higher positive effect on the LW resilience of pines with high pre-burning competition index suggesting that PB benefit the LW performance of the more stressed individuals.

In pines with improved condition due to increased environmental resources caused by PB-induced thinning, a long-lasting differentiation of latewood tracheid's in summer and fall months could have occurred. Our findings are similar to those of Linares et al. 2009, who found that the greatest difference between unthinned and intensively thinned treatments in *Abies pinsapo* corresponded to the number of latewood tracheid's. Others studies have attributed crown scorch or releases from tree competition as possible causes of latewood increases after fire (Alfaro-Sánchez et al. 2018; De Micco et al. 2013).

Pines burned during the spring had similar resilience than control pines while pines burned in the fall increased the resilience capacity. Two years post-burning tree mortality was greater in fall burns than in the spring burns (Valor et al. 2017) and, therefore, the release from tree competition in fall burns may have had a greater positive effect on TTR and LW resilience of pines than in those burned in the spring burns. This occurred, even though, resistance was lower in pines burned during the fall. It is likely that the prolonged heating in fall burns, as indicated by the higher combustion times recorded (Valor et al. 2017), caused higher basal stem girdling and killed small pine roots (Varner et al. 2009) reducing the resistance of some individuals in the short-term, inducing the death of some trees but enhancing the resilience of the most resistant pines. In fact, resistant individuals burned in the fall after the year of drought showed higher resilience than resistant individuals burned in the spring. Surprisingly, the impact of PB on the less resistant individuals in spite of being additive was irrelevant as it was similar between pines burned in the spring, fall and control pines. We speculate that dissection of finer root in the less resistant individuals during drought could explain why the impact of PB was nearly negligible in the less resistant individuals and similar to that of control pines.

P. sylvestris had higher resistance index than *P. nigra,* indicating an unexpectedly higher sensitivity of *P. nigra* to drought and PB. Contrary to Martin-Benito et al. (2013), we found that the growth of *P. nigra* was stronger correlated with the *P-PET* of the current year, whereas in *P. sylvestris* the effect of previous-year climate was more important. Likewise, the relationship between δ^{13}C in earlywood and current or previous δ^{13}C latewood suggests a tight coupling of wood formation and currently produced photosynthetic in *P. nigra* while wood formation of *P. sylvestris* seems that relied more on stored carbohydrates from the previous year. Alternatively, if resistance and resilience depended on the amount of stored reserves (Galiano et al. 2011; Lloret et al. 2011), then the differences observed between species could reflect a trade-off and, therefore, higher resistance in *P. sylvestris* resulted, later, in lower resilience while lower resistance in *P. nigra* resulted in higher resilience.

Furthermore, the lower resilience of *P. sylvestris* compared to *P. nigra* could also be related with the higher sensitivity of *P. sylvestris* to fire than *P. nigra* (Valor et. al 2017). For the same level of damage, the effect of release from tree competition was greater on the LW resilience of *P. nigra* than for *P. sylvestris* suggesting a higher fire sensitivity of *P. sylvestris*. In fact, in *P. sylvestris*, the higher δ^{13}C in the LW of the year of PB in fall burns suggests a lower stomata conductance due to the fire stress likely resulting in a reduction of resilience. Indeed, Bär et al. 2018 showed that *P. sylvestris* is especially vulnerable to the structural changes in the xylem caused by the fire. However, at second year after PB we found no difference between *P. sylvestris* and *P. nigra* in TTR resilience suggesting that *P. sylvestris* may have recovered. This was supported by the more negative values of δ^{13}C in the LW of *P. sylvestris* suggesting an improvement of water availability induced by the higher release from tree competition observed in fall burns.

Post-burning LW/EW was higher in pines burned in fall compared to pines burned in spring and control pines. This may be due to the use of available carbohydrates to replenish dead tissues after fall burns, which were more severe than spring burns, rather than for earlywood cells (Cown 1977) and/or to the higher reduction in tree competition after the fall burns that resulted in an increase in LW. Recently, Alfaro et al. (2018) found higher latewood proportion in burned *P. halepensis* compared to unburned one in sites with high water availability but not in those water-limited. This increase in LW/EW compared to pre-burning levels, may confer a higher resistance to embolism and provide a minimal hydraulic conductivity at lower water potentials (Mayr and Cochard 2003) as can act as a water storage (Domec and Gartner 2002).

4.5 Conclusion

A PB performed the year after a dry year did not reduce the growth resilience of pines in comparison with pines in control (unburned) stands. Moreover, burning in the fall after the dry year seems to increase the growth resilience of pines in comparison with pines burned in spring or unburned. Resistant *P. nigra* and to a lesser extend *P. sylvestris* individuals burned during the fall increased TTR and LW resilience, especially when pines experienced a release from tree competition. The higher resilience of *P. nigra* compared to *P. sylvestris* may be explained by the higher resistance of *P. sylvestris*, suggesting a trade-off between resistance and resilience in terms of tree reserves, by the specific climate-growth relationship of the species to the same dry year and/or by the higher sensitivity of *P. sylvestris* to fire compared to *P. nigra*. However, by the second year after PB *P. sylvestris* showed similar TTR resilience than *P. nigra*. Our results suggest that the increase in resource availability induced by PB increases the production of dense latewood resulting in higher LW/EW and increasing pines resistance to cavitation.

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4.7 Appendix

Table A1: Dendrochronological characteristics of the ring-width chronologies of *P. halepensis* for control, scorched and unscorched trees. SNR, signal-to-noise ratio; RBAR, mean interseries correlation; EPS, expressed population signal; S.I., series intercorrelation; MS, mean sensitivity. Statistics calculated for the maximum interval period.

Locality	Species	Plots	Trees (radii)	Age (years)	Max. Interval	RBAR	SNR	EPS
Mirave								
	P. nigra	Spring	15 (30)	58±13	1949-2015	0.51	15.6	0.94
		Fall	15 (30)	65±13	1931-2015	0.46	12.8	0.92
		Control	15 (30)	64±13	1946-2015	0.30	6.6	0.86
	P. sylvestris	Spring	15 (30)	55±7	1961-2015	0.45	12.4	0.92
		Fall	15 (30)	46±11	1954-2015	0.46	13.1	0.92
Lloreda		Control	15 (30)	53±6	1947-2015	0.40	9.8	0.90
	P. nigra	Spring	15 (30)	54±7	1945-2015	0.52	16.7	0.94
		Fall	14 (28)	49±12	1947-2015	0.50	14.1	0.93
		Control	15 (30)	56±10	1956-2015	0.36	7.8	0.88
	P. sylvestris	Spring	12 (24)	65±5	1949-2015	0.60	18.6	0.94
		Fall	15 (30)	51±13	1947-2015	0.40	9.4	0.90
		Control	15 (30)	59±6	1946-2015	0.42	11.0	0.91

Table A2: Summary of the mixed models (Estimate ± std. Error) describing total tree ring (TTR) and latewood (LW) resilience of pines burned during the spring season for the whole period post-stress (2014-2015) and at two (2014) and three (2015) years post-drought.

Parameters	TTR			LW		
	2014+2015	2014	2015	2014+2015	2014	2015
Intercept	0.81±0.33	0.60±0.31†	0.93±0.09***	0.70±0.20	0.73±0.20***	
Resistance	0.01±0.43	0.30±0.41		0.46±0.25†	0.43±0.09†	
Species (*P. sylvestris*)	-0.17±0.08*	-0.18±0.08*	-0.19±0.10†	-0.27±0.09***	-0.32±0.09***	
RCI$_{15}$	0.48±0.26†					
CI$_{12}$	-0.21±0.05	-0.19±0.05**				
BSH$_{min}$	0.016±0.006*					
Resistance X BSH$_{min}$	-0.02±0.01*	-0.02±0.01*				
Resistance X CI$_{12}$	0.29±0.07***	0.27±0.07				
R^2 Conditional	0.39	0.44	0.05	0.15	0.20	0.03
R^2 Marginal	0.42	0.48	0.10	0.16	0.21	0.03

*** Significant at P <0.001; ** significant at P <0.01; * significant at P <0.05; † significant at 0.05<P<0.
For species, the coefficient shows the change from *P. sylvestris* to *P. nigra*
RCI$_{15}$, relative competition index in 2015
CI$_{12}$, pre-burning Hegyi competition index
BSH$_{min}$, minimum bole scorch height (cm).

Table A3: Summary of the mixed models describing the dif LW/EW for the post-burning years.

Parameters	Estimate ± std. Error	
	1 year post-burning	2 year post-burning
(Intercept)	0.77±0.16***	0.62±0.18***
pre-burning LW/EW	-0.88±0.24***	-0.56±0.24**
Species (*P. sylvestris*)	-0.21±0.24	-0.24±0.42
Season (spring)	0.34±0.32	-0.08±0.32
Season (fall)	0.51±0.28	0.20±0.28
pre LW/EW X Species (*Ps*)	0.01±0.41	0.15±0.42
pre LW/EW X season (spr)	-0.40±0.51	0.25±0.53
pre LW/EW X season (fall)	-0.29±0.41	-0.24±0.42
Species (*Ps*) X season (spr)	-0.46±0.40	0.28±0.41
Species (*Ps*) X season (fall)	-1.07±0.37**	-0.74±0.38*
pre LW/EW X Species (*Ps*) X season (spr)	0.40±0.72	-0.82±0.73
pre LW/EW X Species (*Ps*) X season (fall)	1.70±0.62**	1.29±0.63*
R^2 Conditional	0.34	0.12
R^2 Marginal	0.34	0.23

*** Significant at *P* <0.001; ** t, *P* <0.01; * ,*P* <0.05.

For species, the coefficient shows the change from *P. sylvestris* to *P. nigra* for season from spring or fall to control.

Ps, *P.sylvestris.*

pre LW/EW, preburning latewood to earlywood ratio.

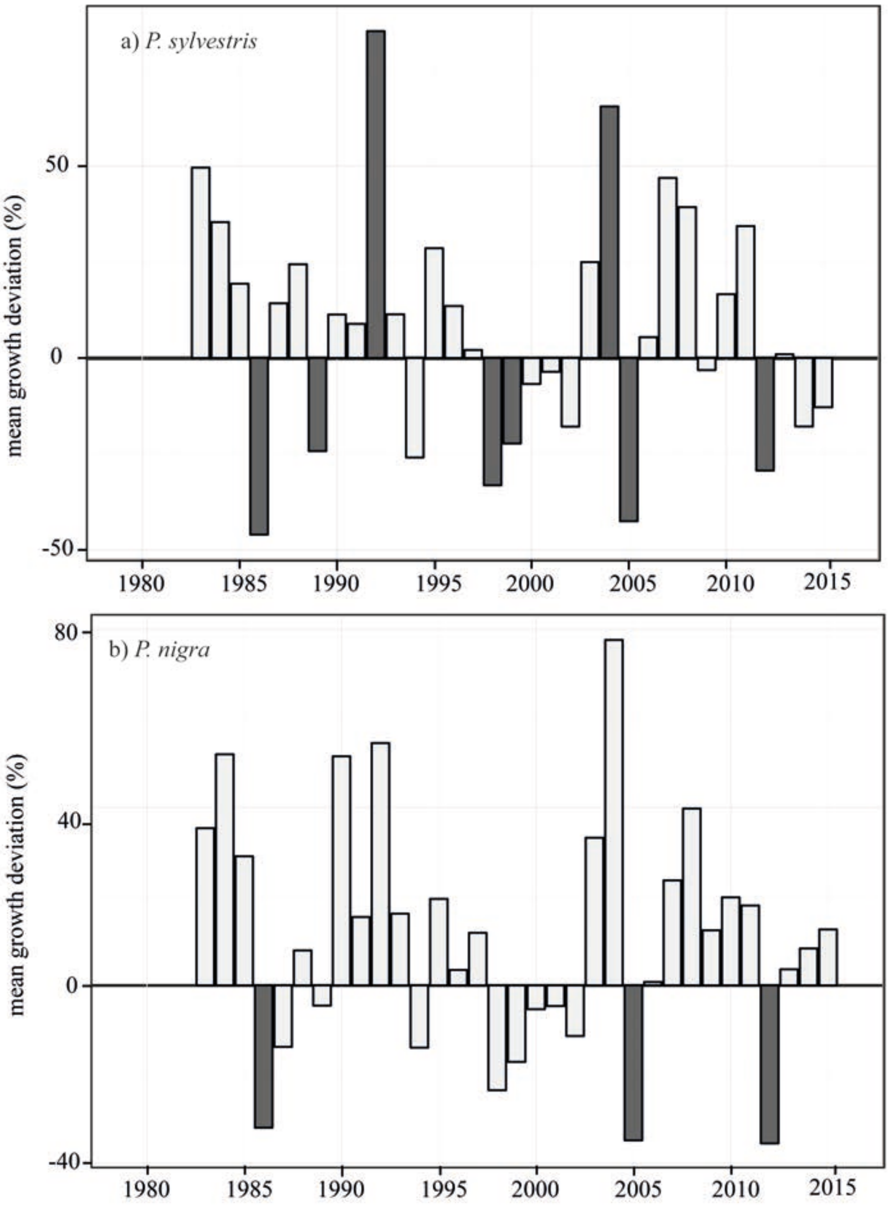


Figure A1: Mean growth deviation for the period 1983-2015 for *P sylvestris* (a) and *P. nigra* (b). In grey years identified as positive or negative pointer years are highlighted.

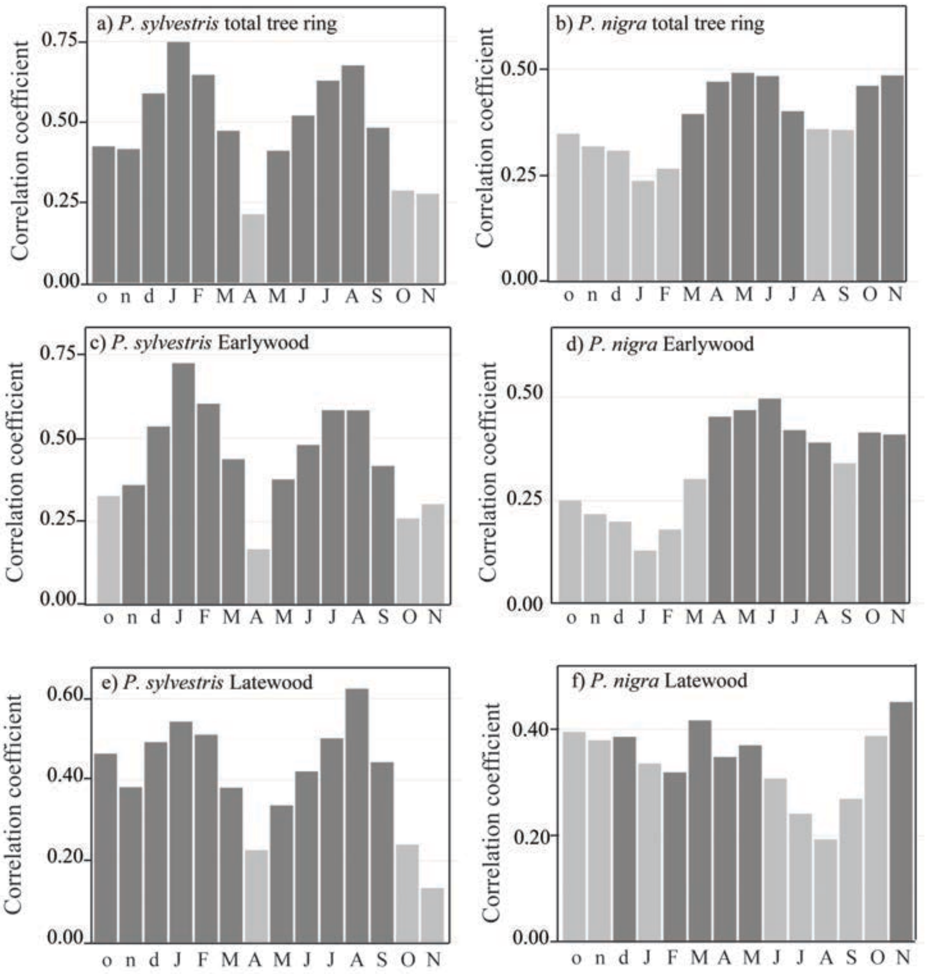


Figure A2: Correlation between the TRW index of the residual chronologies of control pines and *P-PET* of three consecutive months in *P. sylvestris* for total tree ring (a), earlywood (c) and latewood (d) and in *P. nigra* for total tree ring (b), earlywood (d) and latewood (f). Months with small letters denotes months of the year before tree-ring formation.The correlation showed for a given month includes the two preceding months (e.g. correlation showed in August represents the correlation of June, July and August). Significant coefficients are in dark grey.

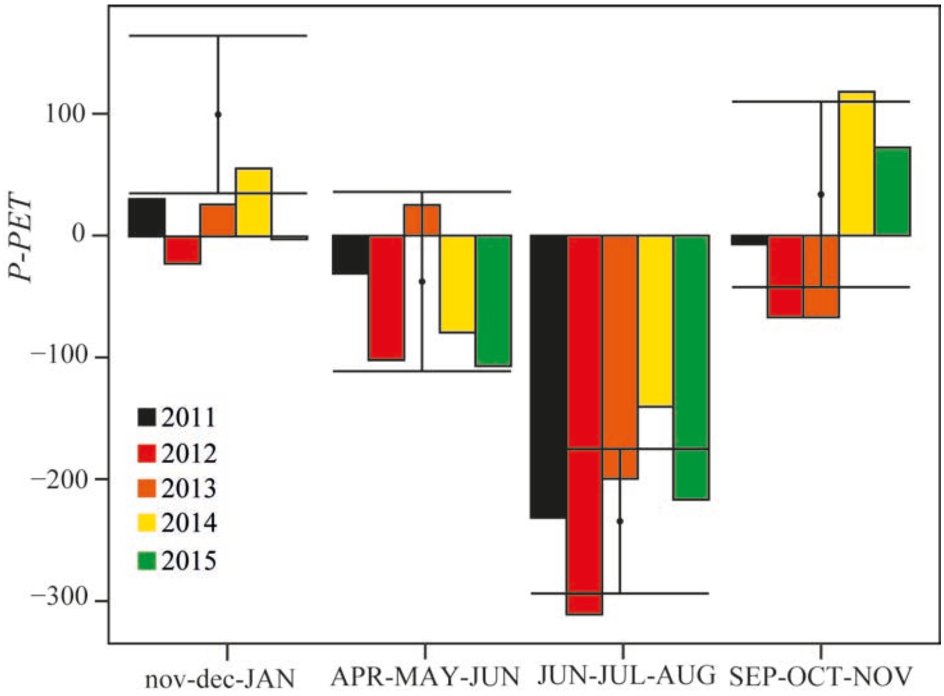
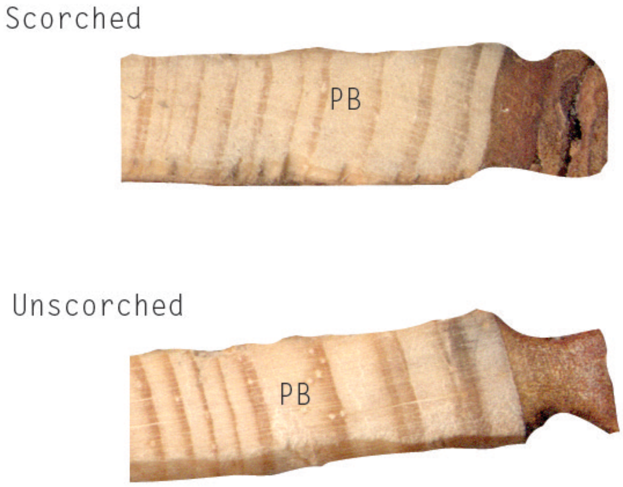


Figure A3: Precipitation minus Potential Evapotranspiration (*P-PET*) for the temporal windows that significantly correlated with the growth of *P. nigra* and *P. sylvestris* (See Figure A2) during the period 2011-2015. The mean and standard deviation of *P-PET* over the entire period (1975-2015) is showed as a reference of historical values. Months with small letters denotes months of the year before tree-ring formation.

Chapter 5

Disentangling the effects of crown scorch and competition release on the physiological and growth response of ***Pinus halepensis*** Mill. using δ^{13}C and δ^{18}O isotopes



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Abstract

Prescribed burning (PB) can decrease the likelihood of crown fires by increasing canopy base height via canopy scorching and sometimes by reducing tree density through fire-induced tree mortality, especially in fire-prone stands. However, little is known about the effect of moderate PB on tree functioning, especially in Mediterranean species such as *Pinus halepensis* Mill. In this study we combined dendrochronology and isotope analysis to understand the physiological effects of PB that determine the short-term post-burning growth response of crown-scorched and unscorched *P. halepensis* with different levels of competition release. PB was carried out in spring 2013. Scorched and unscorched pines showed higher post-burning growth rates than before PB as well as control pines. In the first year post-burning, unscorched pines had similar growth rates and δ^{18}O-δ^{13}C values to the control pines, which indicates that PB only had a minor impact on tree functioning. In contrast, scorched pines showed a significant reduction in growth and wood δ^{13}C, but had similar δ^{18}O as the unscorched and control (no-PB managed) pines. This suggests that the pines' response mechanism to scorch was to reduce their photosynthetic capacity. At two years post-burning (2015), the growth of scorched pines was similar to control pines. Moreover, the radial growth of pines increased significantly when PB resulted in a relevant competition release and crown volume scorch was low. At two years post-burning (2015), a smaller change in δ^{13}C in scorched trees compared to 2014 was found and no changes in δ^{13}C in unscorched pines; however, lower δ^{18}O was found in scorched and unscorched pines compared to the controls, which suggests an increase in stomatal conductance probably due to improved water availability through fire induced thinning. The increase in stomatal conductance in scorched and unscorched pines coincided with an extreme drought year (2015), which indicates that the control pines adopted a more conservative water-use efficiency. No effects of burning on needle N content or δ^{15}N were detected. As a management conclusion, our study suggests that moderate PB can be implemented to disrupt the vertical continuity of fuels in crown fire prone landscapes, enhancing water availability during drought episodes with only minor effects on tree functioning.

Keywords: Aleppo pine; prescribed burning; scorched pines; stable isotopes; photosynthetic activity; stomatal conductance; tree-ring; water use efficiency.

5.1 Introduction

Prescribed burning (PB) is the planned use of fire under specific and predetermined fuel and weather conditions to manipulate the environment and achieve a desired outcome (Wade et al. 1989), including reducing fuel hazard (Fernandes and Botelho 2003). PB can benefit the productivity of trees by nutrient deposition after fire (Certini 2005) and reducing plant competition by removing the understory (Battipaglia et al. 2014b). However, in crown fire prone ecosystems, a higher burning intensity could be useful for decreasing the likelihood of crown fires by increasing canopy base height through crown scorching and inducing the death of small trees. In this case, although in general PB aims to maintain forest productivity without negatively impacting standing trees, the growth and performance of remaining trees may be reduced. High intensity PB may negatively impact tree growth through foliage, root and stem injuries, leading to changes in their photosynthetic capacity and tree water relations (Chambers et al. 1986, Ryan 1993). However, higher intensity PB can improve the growth of standing trees by reducing the competition with small and medium trees (Py et al. 2006). Although the scientific community has studied the impact of these contrasted factors on tree growth, little is known about the interaction among them and this has become a priority for planning successful PB.

Crown fire injury can have important impacts on photosynthetic rate (Wallin et al. 2003), stomatal conductance (Cernusak et al. 2006), transpiration (Clinton et al. 2011), xylem pressure potential (Ryan 1993), sap flow rate (Cernusak et al. 2006), phloem transport (Alexou and Dimitrakopoulos 2014) and chemical defenses (Alonso et al. 2002). While partial defoliation can improve tree water relations and increase the photosynthetic efficiency of the remaining foliage, severe defoliation may result in a decrease in carbon uptake, and hence, a reduction in growth (Waldrop and Van Lear 1984, Lilieholm and Hu 1987, Ryan 1993). This may potentially lead to carbon starvation and ultimately tree death (Galiano et al. 2011). Moreover, the functioning of scorched trees facing future stressful events, such as drought, could be constrained by the physiological changes caused by the fire.

Studies using reconstructed or direct measures of fire thinning intensity have shown either positive (Mutch 1995, Py et al. 2006, Valor et al. 2013, Alfaro-Sánchez et al. 2015), negative (Wooldridge and Weaver 1965) or no effect (Morris and Mowat. 1958, Sutherland 1989) on tree growth (see Landsberg 1994 for review). These discrepancies may be attributed to differences in the reduction of tree competition in the different studies, the scale used to compute the competition release (stand vs. tree level) and the degree of fire damage sustained by surviving trees. Fire induced thinning can be expected to have a positive effect on growth and also impact intrinsic water use efficiency (WUE_i) in surviving trees when water availability is a limiting factor, similarly to mechanical thinning (McDowell et al. 2003, Martín-Benito et al. 2010, Giuggiola et al. 2016). Furthermore, fire can enhance nutrient availability through ash deposition and stimulation of post-fire mineralization, leading to an increase in N concentrations in plant tissues (Carter and Foster 2004), which potentially enhances

WUE_i and tree growth (Guerrieri et al. 2011). Nonetheless, increases in WUE_i do not always lead to an enhancement in tree growth because other environmental factors or alternative carbon sinks may limit growth (Peñuelas et al. 2008). WUE_i is the ratio between photosynthetic rates (*A*) and stomatal conductance (*gs*), which is the amount of water that is lost by the leaf for each CO_2 molecule assimilated by photosynthesis (Farquhar et al 1989). The dual-isotope approach (Roden and Farquhar 2012), which combines the analysis of $\delta^{13}C$ and $\delta^{18}O$, can be used to differentiate between the effects of the photosynthesis rate (*A*) and stomatal conductance (*gs*) on WUE_i, especially in drought sensitive species and after fire events (Battipaglia et al. 2014a, Battipaglia et al. 2016). The $\delta^{13}C$ is a good indicator of plant intrinsic WUE_i (Farquhar et al. 1989), which is given by the ratio of net photosynthetic rate (*A*) and stomatal conductance (*gs*). The $\delta^{18}O$ of plant organic material is related, for a similar water source, to the ratio of atmospheric (ea) to leaf intercellular (ei) water vapor pressure (ea/ei), which is strongly affected by changes in gs (Barbour 2007). As plant $\delta^{18}O$ is related to *gs* but unaffected by *A* (Barbour 2007) simultaneous estimations of $\delta^{13}C$ and $\delta^{18}O$ can isolate the impacts of *A* and *gs* on $\delta^{13}C$ (Scheidegger et al. 2000; Moreno-Gutiérrez et al. 2012).

In the Mediterranean basin, *P. halepensis* is the pine with the lowest canopy base height (Mitsopoulos and Dimitrakopoulos 2007) and forms highly flammable stands that are particularly prone to crown fires (Tapias et al. 2004). Thus, moderate-intensity burns in *P. halepensis,* scorching the crown of larger trees to increase the canopy base height, could be advisable for reducing the risk of fire crowning, as long as crown injury does not lead to irreversible damage to tree functioning. In recent years, research on post-fire *P. halepensis* growth has indicated that the overall effect of low-intensity fires is small (Fournier et al. 2013, Battipaglia et al. 2014b, Alfaro-Sánchez et al. 2015, Valor et al. 2015). This study analyses the effects of a moderate-intensity PB executed in 2013. The intensity of the fire affected the crown of dominant trees and induced tree mortality. We combined dendrochronological and stable isotope teiques to determine the mechanisms underlying the short-term physiological and tree growth responses of dominant *P. halepensis* in relation to certain levels of crown-fire damage and competition release. In addition, taking advantage of the extreme drought that occurred in the year 2015, we explored the drought sensitivity of scorched and unscorched trees compared to control trees. We hypothesized that scorching would improve the water relations of *P. halepensis* due to foliage loss, but in turn the loss in the total leaf area of the tree would reduce tree growth. In unscorched pines, we expected an increase in post-burning growth caused by fire-induced fertilization and thinning leading to a reduction in the competition for soil resources (i.e. water and nutrients). In contrast, the effects of fire induced thinning and fertilization on the growth of scorched pines may be more complex as fire injury may cancel out the positive effects of PB.

5.2 Methods

5.2.1 Study site

The study was carried out at a 2-ha site situated in the El Perelló locality, in the southern part of Catalonia (Spain). The climate is typically Mediterranean with a mean annual rainfall of 610 mm and mean temperature of 15.5 ºC. Based on Spanish Meteorological Agency (AEMET) data, over 1975–2015, the warmest and coldest months were August (mean T = 24.5 ºC) and January (mean T = 8.2 ºC), respectively. The driest and wettest months were July (P = 15.8 mm) and October (P = 62.6 mm), respectively. The site is situated on a flat terrain, soils have developed from limestones (0.4-0.5 m depth) and are classified as Leptic Regosol (FAO 2006). The forest is an even-aged forest of *P. halepensis* planted during the decades of the 1950s and 60s. The average diameter at breast height (DBH) of the trees was 20.6±2.7 cm. Site tree density (DBH>2.5 cm) before burning was 727 tree ha^{-1} and the basal area was 23 m^2 ha^{-1}. The understory is dominated by *Pistacia lentiscus* L. and *Quercus coccifera* L., which occupies about 604 m^3 ha^{-1}.

5.2.2 Prescribed burning and fire severity measurements

Before PB, two plots (30 x 30 m) were set up to monitor fire behavior, tree mortality and the growth of surviving trees. In addition, an unburned plot, adjacent to the burned site, was established as a control. In each plot, trees were identified with a metal tag. For each tree, we measured the distance and angle to the center of the plot and the DBH.

PB was conducted in spring on 13 May 2013, by the Forest Actions Support Group (GRAF) of the Autonomous Government of Catalonia (Generalitat de Catalunya) using a strip headfire ignition pattern. PB was of moderate intensity with the aim of increasing canopy base height of dominant trees and reducing tree density. The average meteorological conditions during the PB were: 19.5 ºC air temperature; 58% relative humidity; 3.2 km h^{-1} wind speed; and 7.5% mineral soil water content. The combustion time (minutes) above 60 ºC and the maximum temperature at the base of the stem, placed at the surface of the soil litter, was measured in a total of 18 trees with K-thermocouples (4 mm diameter) connected to dataloggers (Testo 175) packed with a fireproof blanket and buried in the soil. Temperatures were recorded every 10 s. The mean combustion time above 60 ºC ranged from 2 minutes to 241 minutes, while maximum temperatures ranged from 65 ºC to 750 ºC. Between 70-80% of the surface fuel load was consumed (Fuentes et al. 2018). One week after PB, the crown volume scorched was visually estimated to the nearest 5% as the change in needle color resulting from heat transferred via convection. In each plot, tree mortality was monitored 1 month after PB and every 6 months during the 2 following years in a total of 135 pines. A tree was considered alive when there was green foliage in the crown, otherwise it was considered dead. One year post-burning 10% of pines had died while at 2 years post-burning the accumulated mortality was 20%.

5.2.3 Tree selection and tree competition index

The effect of crown injury on tree growth was analyzed by selecting trees with different degrees of crown volume scorched (CVS). Trees were grouped into two scorch classes: 'Scorched' (30%> CVS <100%) and 'Unscorched' (CVS<30%) (Table 5.1).

Table 5.1: Tree, fire and tree competition characteristics (mean ± SD, and range in parenthesis) of the control and the two crown-severity classes.

Tree and fire characteristics	Control (n=15)	Unscorched (n=19)	Scorched (n=18)
DBH (cm)	23.1±2.5	21.3±2.7	23.6 ± 2.3
Ht (m)	9.1±1.5	9.2±1.1	9.3±1.7
CVS (%)		11.3±1.1(0-20)	56.6±4.0(30-95)
CI_{12}	1.0±0.1(0.5-1.8)	1.8±0.3(0.2-4.9)	1.0±0.1(0.3-2.6)
CI_{13}		1.8±1.4(0.2-4.9)	0.9±0.5(0-2.6)
CI_{14}		1.6±1.2(0.2-4.0)	0.8±0.5(0-2.6)
CI_{15}		1.6±1.2(0.2-3.9)	0.8±0.4(0-1.9)
RCI_{15}		0.1±0.03(0-0.5)	0.2±0.06(0-1.0)

Ht, total height (m).
CVS, crown volume scorched (%).
CI, Hegyi competition index before burning in 2012 (CI_{12}), just after the PB 2013 (CI_{13}), 1 year post-burning 2014 (CI_{14}) and 2 years post-burning 2015 (CI_{15}).
RCI_{15} , relative competition index in 2015 with respect to pre-burning CI_{12}.

Besides the crown-severity classes, there was also a control class composed by trees from the control plot. For each class, we randomly selected 15 trees with a similar DBH and without any apparent damage on the stem (Table 5.1). For each burned and control tree, we computed the Hegyi's competition index (CI) (Hegyi 1974) before burning (CI_{12}). In addition, for those trees belonging to the crown-severity classes we computed the CI in the year of PB (CI_{13}) and during the post-burning years (CI_{14} and CI_{15}) (Table 5.1); the sub-indices refer to the year of measurement (2012, 2013, 2014, and 2015). Hegyi's index is a distance dependent tree competition index, and it was calculated using the *pairwaise* function of the Siplab package for each year as follows:

$$CI_i = \sum_{j=1}^{n} \frac{DBH_j/DBH_i}{dist_{ij}}$$

where CIi is the competition index for the subject tree i, DBH the diameter at breast height, dist$_{ij}$ the distance between the subject tree i and the competitor tree j, and n is the number of competitors in the neighborhood zone. A search radius of 5 m was used to define the neighborhood zone. The selected radius was defined by multiplying the mean crown radius of the overstory (1.4 m) by 3.5 (Lorimer 1983). Then, to quantify the competition release of each tree, we computed a relative competition index (RCI$_{15}$) as the difference between the pre-(CI$_{12}$) and post-burning competition (CI$_{15}$) indices relative to pre-burning levels (Table 5.1).

5.2.4 Tree ring measurements

A total of 52 trees (15 Control; 19 Unscorched; 18 Scorched) were cored to the pith during October and November 2016. Two cores per tree were extracted with an increment borer of 4.5 mm diameter at 50 cm height above the ground, with an angle of 120º between them. Cores were prepared following standard dendrochronological techniques (Stokes and Smiley 1968). All cores were visually cross-dated by comparing characteristic narrow rings present in all trees to detect any false or missing rings. Tree-rings were measured from year 2015 until pith (when present), as late wood in early fall 2016 was still not defined, using a Lintab 3 measuring system coupled to TSAP tree-ring software (Frank Rinn, Heidelberg, Germany), with a precision of 0.001 mm. Within each class, cross-dating was validated using the COFECHA software (Holmes 1983), which calculates cross-correlations amongst individual series of tree growth.

Tree-ring series were detrended with a cubic smoothing spline with a 50% frequency response cut-off of 10 years to remove long-term growth trends embedded in the raw tree-ring series that were thought to be induced by non-climatic influences, such as aging and competition between trees (Fritts 1976), using the Dendrochronology Program Library (dplR) package in R, Version 2.10.1. The standard chronology was calculated as the bi-weight robust mean value of each detrended series. In addition, individual series were subjected to autoregressive modelling and averaged using a bi-weight robust mean to obtain the residual chronology (Cook 1985). The residual chronology was used to analyze climate-growth relationships because by removing the autoregressive component, stronger and more reliable correlations with climate can be found. We used the standard chronology to calculate common dendrochronological statistical measures, and thus determine how much growth for any given year had been strongly influenced by growth in the preceding year.

To understand the effect of crown-fire severity and reduced tree competition on tree growth, ring widths were converted into annual basal area increments (BAI) using the INBAI function of the Dplr package. We used BAI because it is less dependent than ring width on tree age and makes detrending unnecessary (Biondi 1999).

The period of interest was set to 2011-2015 to include data before and after the PB. For each core, we estimated the mean annual BAI of the tree during the five years prior to the studied period ($BAI_{2006-2010}$) and the relative BAI (BAI_{rel}). BAI_{rel} was calculated for each tree and year analyzed by dividing the annual BAI of the studied years (BAI_{2011}, BAI_{2012}... up to BAI_{2015}) by $BAI_{2006-2010}$. BAI_{rel} is a unitless variable, as it is the result of normalizing post-fire growth by pre-burn growth rates, so that each tree serves as its own control (Reinhardt and Ryan 1988). For burned years, this means that when the BAI_{rel}value was less than 1, the tree decreased its growth compared to its pre-burn growth rates; and when BAI_{rel} was higher than 1, the tree increased its growth.

5.2.5 Carbon and oxygen isotopes

Once the control and the crown-severity chronologies had been developed, we selected the 5 trees with the highest inter-series correlation with a similar age and which had different degrees of competition release for taking annual isotope measurements (Table A1). In selected trees, one core was extracted using a borer of 7 mm diameter with a drill at 0.50 m height. From each sampled core, we selected the section corresponding to the period 2011-15. Samples were annually divided and ground with a milling chamber. The carbon and oxygen stable isotope compositions were measured at the IRMS Lab of the University of Campania "L. Vanvitelli" by continuous-flow isotope ratio mass spectrometry (Delta V Advantage, Thermo Scientific, Bremen, Germany) using 0.06 mg and 0.3 mg for ^{13}C and ^{18}O determinations, respectively. Cellulose extraction from tree rings is recommended for isotope analyses for determining climate signals (Ferrio and Voltas 2005, Battipaglia et al. 2008). In our case, whole wood was processed since recent studies have shown that the use of whole-wood isotope values is justified for ecophysiological and dendrochronological studies that analyze the response of trees to environmental changes recorded within the sapwood, i.e. in a relatively short-term period (Weight et al. 2015, Riechelmann et al. 2016). The $\delta^{13}C$ series were corrected for the Suess Effect (decrease in $\delta^{13}C$ of atmospheric CO_2 since the beginning of industrialization) resulting from the emission of fossil carbon dioxide, which is depleted in ^{13}C (Francey et al. 1999, McCarroll and Loader 2004). The corrected series were used for all statistical analyses. Isotopic compositions are expressed in delta notation (‰) relative to accepted reference standards: Vienna PeeDee Belemnite for carbon and

Vienna Standard Mean Ocean Water for oxygen. The standard deviation for the repeated analysis of an internal standard (commercial cellulose) was lower than 0.1 (‰) for C and lower than 0.5(‰) for O.

5.2.6 Needles N isotope ratio and concentration

We selected 5 trees with similar crown volume scorched and DBH as those selected for wood isotope measurements for needle N isotope ratio and concentration determinations. The analysis was carried out on 1-year-old unscorched needles sampled from the top of the south-facing crown before burning (spring 2013) and 1 year post-burning (spring 2014). Needles were oven-dried at 60 ºC for 48 h, ground and analyzed at the Stable Isotope Facility of the University of California at Davis (USA) using an ANCA interfaced to a 20–20 Europa ®isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK).

5.2.7 Climatic data

Monthly precipitation (*P*) and temperature (*T*) from 1975 to 2015 were obtained for the study site from climatic records and projections elaborated by the Spanish Meteorological Agency (AEMET) (see Ninyerola et al. 2000 for the spatial resolution of the original climatic data and the approach applied to downscale the climatic records). Monthly potential evapotranspiration (*PET*) was estimated using the Thornthwaite (1948) method. The difference between *P* and *PET* (*P-PET*) was calculated for each month.

5.2.8 Data analysis

The following parameters were calculated to assess the goodness of the developed chronologies: Mean inter-series correlation (Rbar), to assess agreement between individual series in each fire severity class; Expressed Population Signal (EPS), to determine to what extent the chronologies, which are based on a limited number of trees, were representative of the 'hypothetical' true chronologies (Wigley et al. 1984); and Mean sensitivity (MS), to determine the degree to which tree ring width changes from year to year and how it is influenced by high-frequency climatic variation. In addition, Pearson correlations were calculated between the control residual chronology and monthly *P-PET* using the packages Bootres and Treeclim. The *P-PET* of the months that significantly correlate with tree growth were plotted for the studied period (2011-2015). For comparison, the mean and standard deviations of *P-PET* over the entire period (1975-2015) were used as references of historical values.

The effect of crown scorch on tree growth was analyzed using a general linear model and on carbon and oxygen isotopes using the dual δ^{13}C-δ^{18}O approach (Scheidegger et al. 2000). Although the interpretation of the double model δ^{13}C-δ^{18}O is not straightforward (Gessler et al. 2014), it can still be informative when it is applied in strongly water-limited ecosystems (Moreno-Gutiérrez et al. 2012, Gessler et al. 2014, Altieri et al. 2015, Battipaglia et al. 2016). Firstly, differences in BAI$_{rel}$, among control and crown-severity classes were analyzed for each year of the studied period (2011-2015) using a general linear model with crown-severity class as a fixed factor. A Tukey post-hoc test was used for multiple comparisons. In addition, Pearson correlation coefficients were calculated between BAI$_{rel}$, δ^{13}C and δ^{18}O for the period studied and the post-burning period. Secondly, combinations of δ^{13}C-δ^{18}O values as well as BAI$_{rel}$ for the scorched and unscorched classes were evaluated as the difference from the control (crown severity class-control) for the pre-burning period (2011 and 2012), the year of PB and the following 2 years post-burning (Figure A1). The conceptual model of Scheidegger et al. 2000 was used to differentiate whether changes in WUE$_i$ were the result of changes in photosynthetic capacity (A) and/or stomatal conductance (gs) by plotting how δ^{13}C-δ^{18}O isotopes co-vary. Following Roden and Siegwolf (2012), we consider that A represents an integrated or average A rather than maximum photosynthetic potential as indicated in Scheidegger et al. (2000). As humidity has strong effects on δ^{18}O enrichment (Roden and Siegwolf 2012), we selected pines of the same site and observed that humidity was similar between the years studied (mean relative humidity 65.8 ± 2.6% of the years studied).

The effect of release from tree competition on post-burning tree growth, including the year of PB, was analyzed using a linear mixed model (LMM). The model included year as a fixed factor and pre-burning competition index (CI$_{12}$), the relative competition index (RCI$_{15}$) and the crown volume scorched as continuous (independent) variables. A three-way interaction was included between year, crown volume scorched and the relative competition index. Tree was added as a random factor and an AR(1) correlation structure was included to account for the non-independent data of tree growth. Control trees were excluded from the analysis because they did not have competition release or crown scorched. Finally, to analyze the effect of tree competition on δ^{13}C and δ^{18}O, we modelled pre-burning δ^{13}C and δ^{18}O as a function of the pre-burning competition index (CI$_{12}$), regardless of the crown severity class. Then, post-burning δ^{13}C and δ^{18}O was modelled as a function of the post-burning competition index (CI$_{13}$ CI$_{14}$ CI$_{15}$) for scorched, unscorched and control trees. Time was included as a random factor.

Residuals of the mixed models developed did not show any pattern (Figure A2) and highly correlated explanatory variables were avoided. The variance explained for the fixed effects was obtained by comparing the final model with the null model (containing only the random structure). All analyses were conducted with the software R (v. 3.2.1, the R Foundation for Statistical Computing) using the package nlme for linear mixed-effects modelling. The model variances explained by fixed effects (marginal R^2) and by both fixed and random effects (conditional R^2) were provided (Nakagawa and Schielzeth 2013).

5.3 Results

5.3.1 Tree-ring chronologies and climate

EPS showed high values (>0.85) in all chronologies, indicating that chronologies constructed from detrended individuals were representative of the whole population of trees (Table 5.2).

Table 5.2: Dendrochronological characteristics of the ring-width chronologies of *P. halepensis* for control, scorched and unscorched trees. SNR, signal-to-noise ratio; RBAR, mean inter-series correlation; EPS, expressed population signal; MS, mean sensitivity; AC, autocorrelation. Statistics were calculated for the maximum interval period.

	Control	Unscorched	Scorched
Trees (radii)	15 (30)	19 (38)	18 (36)
Max. Interval	1963-2015	1955-2015	1960-2015
Age (years)	49±1	47±2	48±1
RBAR	0.52	0.50	0.47
SNR	15.0	14.7	14.0
EPS	0.93	0.93	0.93
MS	0.37	0.36	0.35
AC	0.59	0.54	0.57

The mean TRW index for each chronology revealed a similar growth pattern during the entire period (Figure 5.1) as indicated by the significant correlation between them (Unscorched Vs. Control, 0.91, $P<0.001$; Scorched vs. Control, 0.85, $P<0.001$; Scorched vs. Unscorched, 0.90, $P<0.01$).

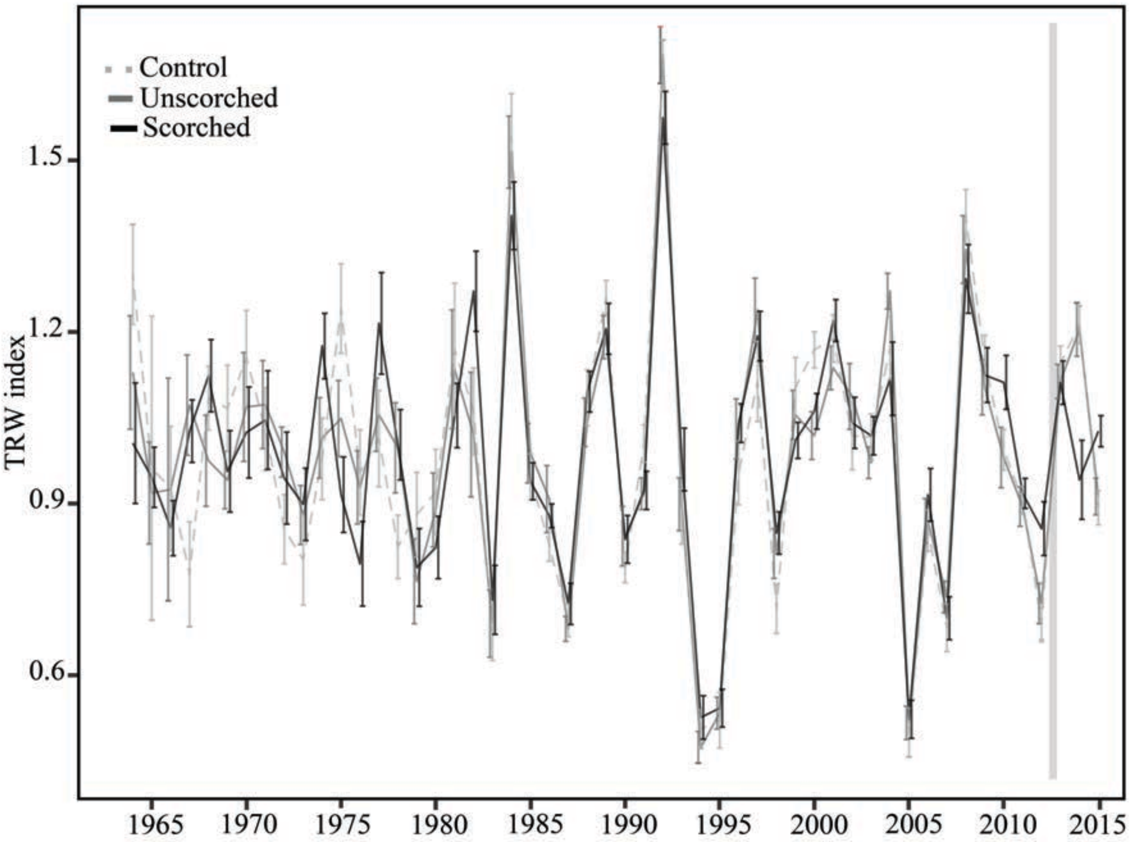

Figure 5.1: Mean tree-ring width (TRW) index with standard error for the entire time span (a) and for the 2003-2015 period (b) for control, unscorched and scorched trees. Grey shading indicates the year of prescribed burning (2013).

Mean sensitivity was similar among chronologies showing high year-to-year variability in tree ring growth related to inter-annual changes in weather conditions (Table 5.2). We found positive correlations between the residual control chronology and the *P-PET* index of May (0.53, P<0.001) and February (0.31, P<0.05) (Figure A3). During the studied period, May 2015 and February 2011 had the lowest *P-PET* of the entire period 1975-2015, while February 2013 showed the highest *P-PET* value (Figure A4). Moreover, May 2012 and 2011, the years preceding the PB, were among the driest of the entire period (Figure A4).

5.3.2 Effects of crown injury on the time evolution of BAI$_{rel}$

The years before PB (2011-2012), no significant differences were observed in BAI$_{rel}$ among the crown-severity and control classes (Figure 5.2). Pines continued to grow as before in the pre-studied period (2006-2010), as BAI$_{rel}$ was *ca.* 1.0 (Figure 5.2).

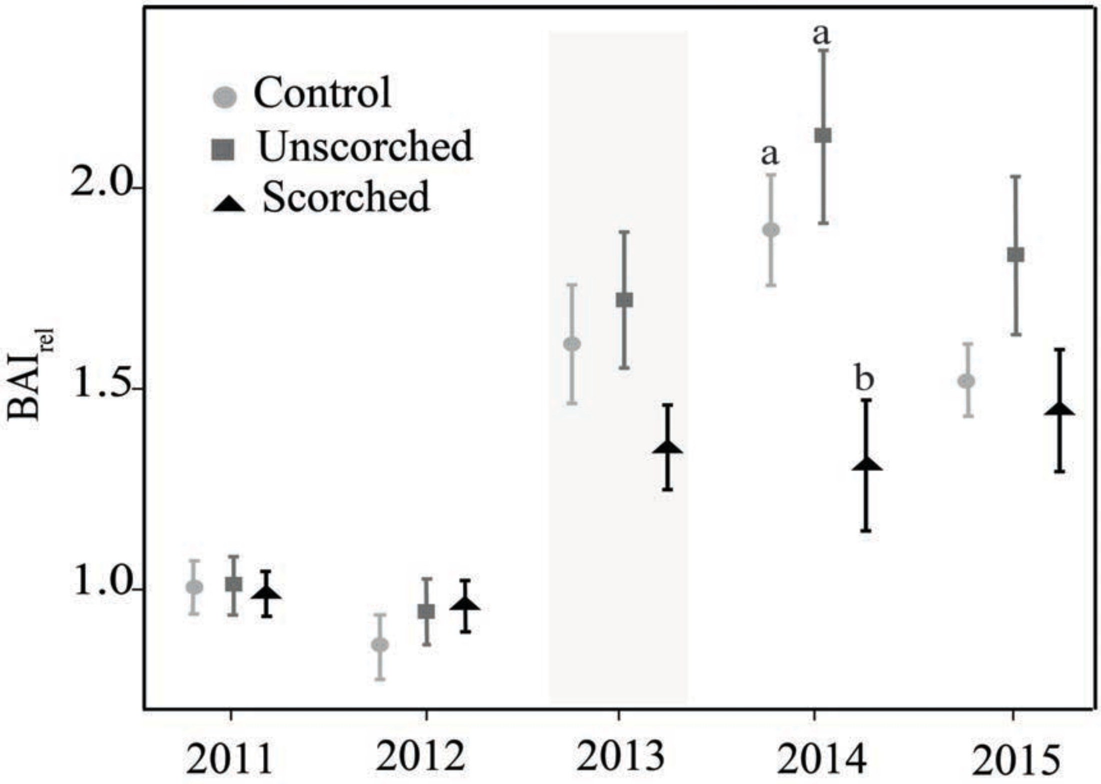

Figure 5.2: Relative basal area increment (BAI_{rel}), BAI of the year divided by the mean BAI of the period 2006-2010 for control (n=15), scorched (n=18) and unscorched (n=19) trees, 2 years before, during (2013) and 2 years after the prescribed burning (Mean ± SE). Grey shading indicates the year of prescribed burning (2013). Significant differences ($P<0.05$) among crown-severity and control classes are indicated by different letters.

Following PB (2013-2015), pines showed higher growth rates ($BAI_{rel}>1.0$), regardless of the crown-severity class, than the pre-burning period (2011-2012) or the pre-studied period (2006-2010) (Figure 5.2). However, 1 year post-burning (2014), this increase was lower in scorched pines, as the BAI_{rel} was significantly lower than that of the control and unscorched pines (Figure 5.2).

Considering the entire period studied (2011-2015), only control trees showed significant correlations between BAI_{rel} and $\delta^{13}C$ (r = 0.6, $P<0.001$, n=25) and between $\delta^{13}C$ and $\delta^{18}O$ (r = 0.36, $P<0.07$, n=25) (Table A2). In contrast, for unscorched and scorched trees, no significant correlations were found between any parameter and period (Table A2).

5.3.3 Changes in δ^{13}C-δ^{18}O relationships of scorched and unscorched pines with respect to the controls

In unscorched pines, in the year of PB and 1 year post-burning, the association between δ^{13}C and δ^{18}O values, expressed in terms of their difference from the control, was similar to the pre-burning years (Figure 5.3, a). In contrast, in scorched pines, the association δ^{13}C-δ^{18}O was clearly different between the post- and pre-burning years, including the year of PB (Figure 5.3, b). Thus, in the year of PB (2013), scorched pines had a slight increase in δ^{18}O accompanied by a decrease in δ^{13}C. This coincided with a decrease in BAI_{rel} (Figure 5.3, d). Similarly to 2013 in the first year post-burning (2014), there was a decrease in δ^{13}C and BAI_{rel} but of higher magnitude with little change of δ^{18}O (Figure 5.3, b and d).

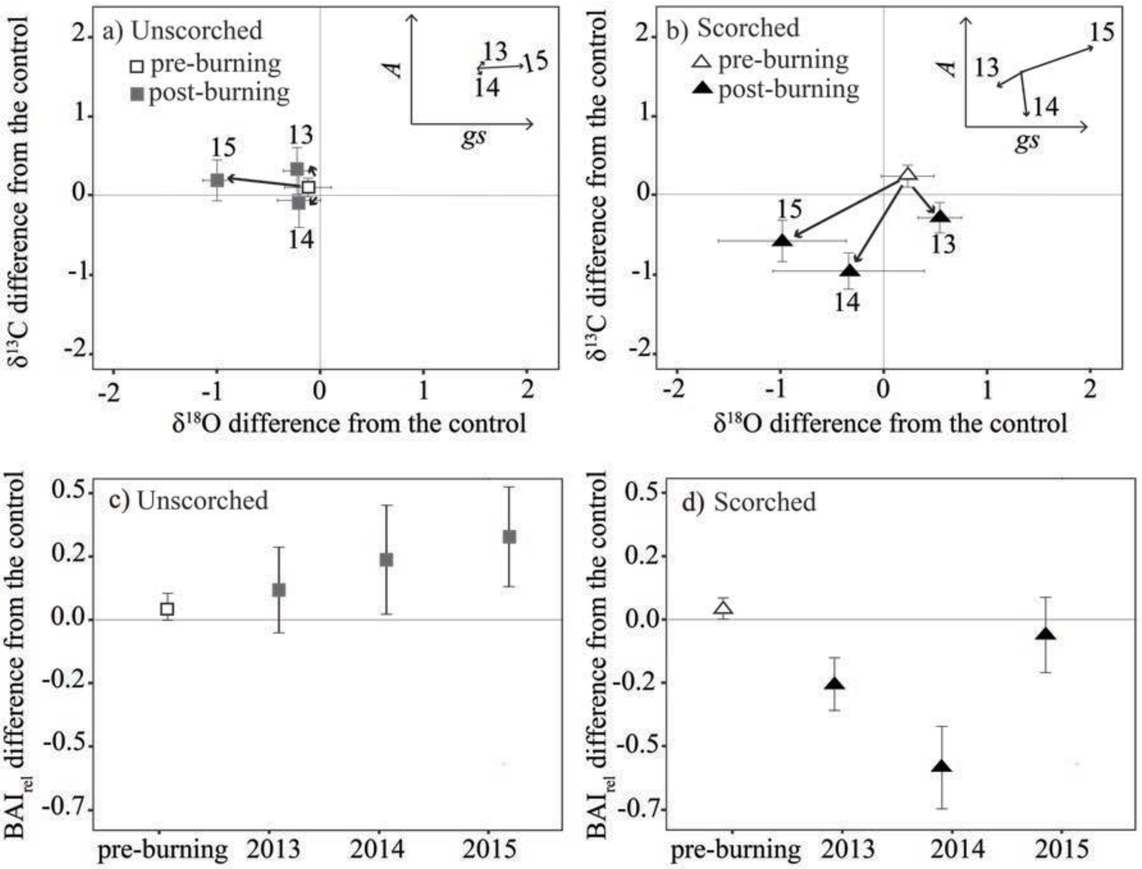

Figure 5.3: Combination of δ^{13}C and δ^{18}O values (mean± SE) for unscorched (a) and scorched (b) crown-fire severity classes, evaluated as the difference from the control (crown-fire severity class-control), for the year of PB 2013 (13), and the post-burning year 2014 (14) and 2015 (15) and pre-burning periods. The insets indicate the inferred changes in the photosynthesis rates (*A*) vs. stomatal conductance (*gs*) based on the conceptual model (Scheidegger et al. 2000). Relative basal area increment (BAI_{rel}), BAI of the year divided by the mean BAI of the period 2006-2011 of unscorched (c) and scorched (d) classes, expressed as the difference from the control (crown fire severity-control) for pre-burning, the year of PB 2013 (13) and post-burning years 2014 (14) and 2015 (15).

In 2015, two years post-burning, we observed a tendency for δ^{18}O to decrease in both scorched and unscorched trees (Figure 5.3, a and b), with a smaller change in δ^{13}C in scorched trees compared to 2014 (Figure 5.3, b). In this year (2015), the BAI$_{rel}$ of scorched trees was similar to that of control trees (Figure 5.3, d), while the highest differences were observed between unscorched and control trees (Figure 5.3, c).

5.3.4 Changes in the first year post-burning needle N concentration and δ^{15}N in scorched and unscorched pines with respect to pre-burning

No significant differences in N concentration or δ^{15}N in one-year old needles of scorched and unscorched pines were observed between pre-burning and 1 year post-burning (Table 5.3).

Table 5.3: Needle δ^{15}N and N concentration for unscorched and scorched trees.

	Unscorched (n=5)	Scorched (n=5)
Needle δ^{15}N (‰)		
Pre-burning	-2.8±0.7	-3.1±0.6
1 year post-burning	-3.1±0.6	-3.3±0.6
Needle N content ($\mathrm{mgg}_{DM}{}^{-1}$)		
Pre-burning	15.3±2.0	14.6±1.3
1 year post-burning	14.9±2.1	14.7±0.8

5.3.5 Effect of tree-to-tree competition on post-burning growth, δ^{13}C and δ^{18}O

Post-burning BAI$_{rel}$, including the year of PB, was affected by a three-way interaction involving year, crown volume scorched (CVS) and the relative competition index (RCI$_{15}$) (Table 5.4). Specifically, 1 year post-burning growth was similar to the year of PB regardless of the RCI$_{15}$. In contrast, 2 years post-burning, BAI$_{rel}$ increased with respect to the year of PB as RCI$_{15}$ increased and CVS decreased (Figure 5.4, a and b). In addition, independently of the post-burning year, trees with a higher competition index before burning (CI$_{12}$) showed higher post-burning growth (Table 5.4).

Table 5.4: Summary of the mixed model characterizing the impact of crown volume scorch (CVS) and reduction in tree competition induced by prescribed burning on the post-burning relative basal area increment. *P*-values lower than 0.05 are indicated in bold.

Parameters	Value	SE	*P*-value
Intercept	1.232	0.303	0.000
CVS (%)	0.003	0.006	0.621
year (2014)	0.355	0.176	0.048
year (2015)	-0.023	0.144	0.871
CI_{12}	0.267	0.101	**0.012**
RCI_{15}	-1.107	1.290	0.397
CVS: RCI_{15}	0.001	0.022	0.943
CVS: year (2014)	-0.004	0.004	0.361
CVS: year (2015)	0.003	0.004	0.383
RCI_{15}: year (2014)	0.780	0.924	0.401
RCI_{15}: year (2015)	1.501	0.755	0.051
CSV: year (2014): RCI_{15}	-0.018	0.016	0.269
CSV: year (2015): RCI_{15}	-0.030	0.013	**0.023**
Marginal R^2= 0.27			
Conditional R^2= 0.85			

CVS, crown volume scorched (%).

For year, the coefficient shows the change from 2015 and 2014 to the PB year, 2013.

CI_{12}, pre-burning competition index.

RCI_{15}, relative competition index.

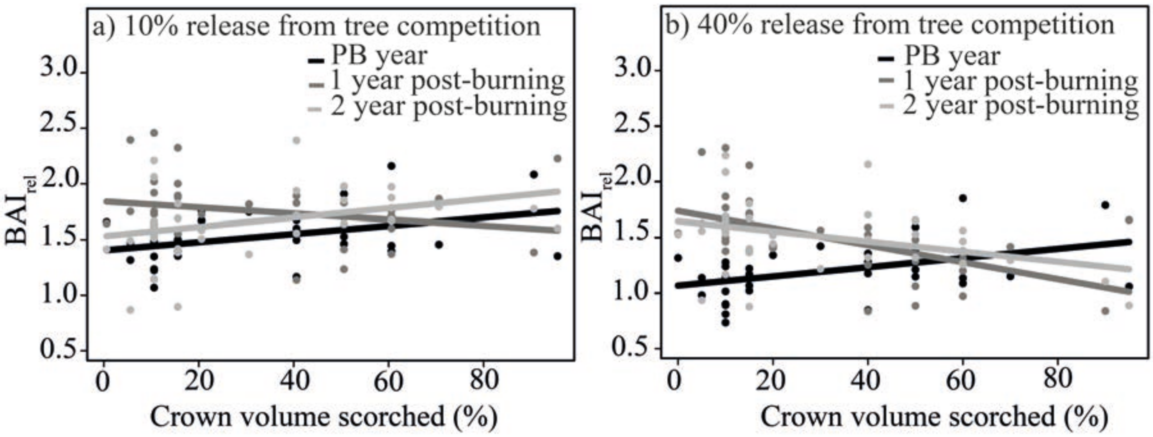

Figure 5.4: Partial regression residuals of the relative basal area increment (BAI_{rel}) for the year of PB and 1 and 2 years post-burning against crown volume scorched for 10% (a) and 40% (b) competition release.

Pre-burning $\delta^{13}C$ and $\delta^{18}O$ values were not influenced by the pre-burning competition index (Table 5.5). In contrast, in scorched and unscorched trees, the post-burning competition indices had a positive effect on the post-burning δ^{18}O but not on δ^{13}C (Table 5.5). However, in control pines, the competition indices of years 2013, 2014 and 2015, which correspond to the post-burning period, had a marginal positive effect on δ^{13}C but not on δ^{18}O (Table 5.5).

Table 5.5: Effect of competition index on pre-burning δ^{13}C and δ^{18}O considering all pines and on post-burning δ^{13}C and δ^{18}O of unscorched, control and scorched pines. The models in which the competition index was significant are in bold. Marg., marginal; Cond.,conditional.

Period-variable response	Intercept	Competition Index	Sample size	Marg. R^2	Cond. R^2
Pre-burning					
δ^{18}O	29.50±0.2***	0.08±0.14	30		
δ^{13}C	-25.93±0.12***	0.05±0.07	30		
Post-burning					
Control δ^{18}O	29.76±1.08***	0.40±0.93	15		
Unscorched δ^{18}O	**29.43±0.21*** **	**0.15±0.06***	**15**	**0.17**	**0.54**
Scorched δ^{18}O	**27.96±0.64*** **	**0.15±0.72** **	**12**[1]	**0.28**	**0.56**
Control δ^{13}C	**-26.08±0.30*** **	**0.50±0.23** ·	**15**	**0.18**	**0.43**
Unscorched δ^{13}C	-25.42±0.33***	0.01±0.14	15		
Scorched δ^{13}C	-26.71±0.55***	0.87±0.61	12		

*** Significant at P <0.001; ** significant at P <0.01; * significant at P <0.05; · significant at 0.05<P<0.1

[1] One scorched pine was excluded from the analysis as the CVS and competition index were extreme values within the scorched pines. The inclusion of this pine resulted in a non-significant effect of the competition index when the δ^{18}O was modelled.

5.4 Discussion

Post-burning growth of *P. halepensis* was affected by the levels of crown fire injury and the degree of competition release undergone by each individual tree. In control pines, the significant correlation between δ^{13}C and δ^{18}O indicates a high WUE$_i$ via stomatal regulation (Scheidegger et al. 2000). In contrast, the lack of correlation between scorched and unscorched pines suggests that different physiological mechanisms were operating in the short term after burning. Moreover, the presumable increase in soil N availability after burning was not detected 1 year post-burning in pine needles. The post burning growth rates of both scorched and unscorched pines, as well as a control pines, were higher than during the pre-burning years (2011 and 2012). Pre-burning years were characterized by very dry springs suggesting that the effect of fire-induced defoliation on tree growth was minor compared to the effects of two consecutive dry years. Nonetheless, we found post-burning differences in BAI_{rel} and isotopes between scorched, unscorched and control pines that are following discussed.

5.4.1 Effect of PB-caused defoliation on tree growth and physiology

Defoliation caused by crown scorching resulted in a moderate reduction in BAI_{rel} during the year of PB and a significant sharp decrease 1 year post-burning compared to the control and, especially, unscorched trees. The moderate reduction, rather than severe, in BAI_{rel} in the year of PB can be explained by the fact that PB was carried out in May, when the earlywood formation of *P. halepensis* has been completed (De Micco et al. 2016) and only the latewood formation could have been affected. Similarly, in the year of PB, scorched pines did not suffer major changes in WUE_i, as the δ^{13}C was similar to the control trees. However, assuming that there were no changes in water source and ambient humidity before and after PB, the conceptual model of Scheidegger et al. (2000) suggests that the slight decrease in BAI_{rel} could result from a reduction in the photosynthetic capacity and stomatal conductance (inserts in Figure 5.4). In contrast, 1 year post-burning, the significant sharp reduction in the BAI_{rel} of scorched pines was accompanied by a decrease in δ^{13}C compared to control and unscorched pines, suggesting lower WUE_i. A reduction in WUE_i could be a consequence of a decrease in the photosynthetic capacity of the surviving needles or an increase in stomatal conductance. No change in the δ^{18}O suggests that the decrease in WUE_i was related to a reduction in photosynthetic capacity (Scheidegger et al. 2000), presumably due to the needle scorch. Recently, several papers have shown that, under severe drought events, plants show a decrease in WUE_i associated with a growth reduction, due to the reduction in leaf mass during tree decline (Colangelo et al. 2017, Petrucco et al. 2017, Timofeeva et al. 2017). In contrast, a considerable increase in WUE_i was linked with a reduction in *P. halepensis* growth after wildfire, which was explained by a reduction in stomata conductance (Battipaglia et al. 2014a). Beghin et al. (2011), without measuring δ^{18}O, related the reduction in *P. sylvestris* growth to the wood damage caused by the fire and suggested that the decrease in δ^{13}C after wildfire resulted in weaker competition for water among the surviving trees. This diversity of results may be explained by differences in the sites and in the species studied, the different time resolutions and, especially, the lack of direct measures of fire severity at the individual level.

Despite the reduction in photosynthetic capacity compared to control trees, scorched pines still grew after PB, as indicated by the higher $\mathrm{BAI}_{rel}$with respect to pre-burning years. This may be partially explained by the wide range of crown volume scorched (CVS between 30% and 95%) of the scorched class used in our study. Considering that a reduction in photosynthetic capacity was observed in pines with 60% CVS (that is, the pines used for the isotope analysis), scorched pines with less than 60% CVS may have maintained carbon assimilation rates like those before PB because surviving top-needles are more efficient in terms of photosynthesis (Meir et al. 2002). Other potential factors, such as higher needle N, to explain increased photosynthetic efficiency, were discarded as no differences were observed between pre- and 1 year post-burning one-year-old needle N concentration of scorched pines. Similarly, the absence of visible re-greening foliage in our study excluded the possibility of needle regrowth as a factor for explaining the variation in post-burning BAI_{rel} (Wiley et al. 2017).

Contrary to scorched pines, PB had minor effects on the growth and physiological status of unscorched pines the year of PB and 1 year post-burning, as BAI_{rel} was only slightly higher than that of control pines and no changes in δ^{13}C, δ^{18}O and δ^{13}C-δ^{18}O were observed. This agrees with previous studies that report little or no variation in the post-fire growth of undamaged pines (Valor et al. 2015, Battipaglia et al. 2016). An increase in the growth of unscorched pines has been linked to higher wood N concentrations and δ^{15}N (Battipaglia et al. 2014b). However, we did not find variations in N concentration and δ^{15}N of unscorched 1-year-old needles with respect to pre-burning levels. Thus, the moderate BAI_{rel}increase observed 1 year post-burning seems to be related more to a reduction in competition due to fire-induced tree mortality and understory removal, rather than a potential fertilization effect. Moreover, the higher pre-burning competition index of unscorched pines compared to control and scorched pines could have obscured the expected higher BAI_{rel} of unscorched pines with respect to control pines. Indeed, this slight increase seems to be more linked to the effect of immediate understory removal rather than the thinning effect because the degree of thinning intensity did not influence 1 year post-burning growth, probably because tree mortality was higher at 2 years post-burning. Moreover, the favorable climatic conditions recorded in 2014 may have obscured the effects of fire thinning, as the effect of competition release on the growth of standing trees seems to be more noticeable under drought conditions (Skov et al. 2004), as discussed in the next section.

5.4.2 Effect of reduced competition induced by PB on tree growth and physiology

Two years after PB, a higher competition release clearly translated into enhanced BAI_{rel} levels as the crown volume scorched decreased, indicated by the analysis of individual pines. This finding highlights the importance of modelling the growth response of pines to PB as a function of tree and fire severity variables to gain insights into individual pine responses, as the mean BAI_{rel} of each crown-fire severity class suggested that scorched, unscorched and control trees at 2 years post-burning grew to similar degrees. According to Scheidegger et al. (2000) the reduction in δ^{18}O observed in scorched and unscorched pines indicates an increase in stomatal conductance compared to control trees (Figure 5.4, insets). Moreover, the positive relationship between δ^{18}O and tree competition indexes found in scorched and unscorched pines, but not in control pines, suggests higher stomatal conductance because pines had a higher competition release. Reduced canopy interception and transpiration due to fire-induced thinning probably resulted in an enhancement of water availability, stomatal conductance and hence tree growth, emulating similar processes that occur under mechanical thinning (Giuggiola et al. 2016). Higher photosynthetic rates after mechanical thinning have been related to an increase in light availability (Martín-Benito et al. 2010); however, in our study, light was not a limiting factor as the stand was rather open and trees were dominant.

The δ^{18}O-derived increase in stomatal conductance in pines managed by PB in the driest year of the entire series (2015) highlights the role of burning in reducing competition when resources, especially water, are limiting (Skov et al. 2004, Keyser et al. 2010). Thus, our results suggest that moderate-intensity PB can help to overcome episodes of intense drought and compensate for the negative effects of future drought episodes.

5.5 Conclusion

Isotope and dendrochronological analysis suggested that the physiological responses of scorched and unscorched pines differed 1 year post-burning but became similar by the second post-burning year. Still, pines managed by PB grew more than during the pre-burning years regardless fire damage. One year after burning, the sharp decrease in both BAI_{rel} and δ^{13}C and that there were no changes in δ^{18}O suggest that there was a reduction in the photosynthetic capacity due to crown scorch. Two years post-burning, the growth of pines increased as tree-to-tree competition was reduced, especially in pines with lower crown volume scorched. The increase in BAI_{rel} of unscorched and scorched pines 2 years post-burning was associated with an increase in stomatal conductance due to lower resource competition resulting from fire induced thinning. This coincided with the driest year in the climatic data series, which suggests that burning could contribute to mitigating the effects of drought episodes on surviving trees. Further research should focus on the accumulative effects on tree growth of fire-caused defoliation occurring simultaneously with a drought event.

As a management conclusion, our study indicates that moderate PB but with enough intensity to partially affect the crown of the dominant *P. halepensis* could be used to disrupt the vertical continuity without irreversible damage to the functioning of scorched trees. As time since burning increases, PB may even enhance the water availability of the surviving trees. This information may help managers in adjusting the window prescription and ignition pattern when the management objective is to reduce crown fire hazard.

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5.7 Appendix

Table A1: Characteristics of the trees selected for isotope measurements for control, scorched and unscorched trees.

Tree and fire characteristics	Scorched (n=5)	Unscorched (n=5)	Control (n=5)
Age	49±6	50±7	49±2
Rbar	0.73±0.12	0.73±0.04	0.77±0.02
CVS (%)	62.0±4.5(60-70)	10.0±7.0 (0-20)	
CI_{12}	0.9±0.3(0.4-1.3)	2.0±1.6 (0.2-3.5)	1.0±0.4(0.5-1.8)
CI_{13}	0.7±0.3 (0.4-1.2)	1.8±1.4 (0.2-3.2)	
CI_{14}	0.6±0.4 (0-1)	1.7±1.3 (0.1-2.9)	
CI_{15}	0.5±0.3 (0-0.8)	1.7±1.3 (0.2-2.7)	
RCI_{15}	0.4±0.3 (0.1-1.0)	0.2±0.2 (0-0.5)	

CVS, crown volume scorched (%).

CI, Hegyi competition index in 2012, 2013, 2014, 2015.

RCI_{15}, relative competition index in 2015 respect to CI_{12}.

Table A2: Correlation coefficients between growth and isotopic variables for different periods and crown-fire severity classes.

Periode	Sample class	Variables		Correlation coefficent	P value	Sample size
All						
	C-S-U	**δ^{13}C**	**δ^{18}O**	**0.22**	**0.05**	75
	C-S-U	δ^{13}C	BAI_{rel}	0.17	0.13	75
	Control	**δ^{13}C**	**δ^{18}O**	**0.36**	**0.07**	25
	Control	**δ^{13}C**	**BAI_{rel}**	**0.60**	**0.001**	25
	Scorched	δ^{13}C	δ^{18}O	0.17	0.39	25
	Scorched	δ^{13}C	BAI_{rel}	0.07	0.71	25
	Unscorched	δ^{13}C	δ^{18}O	0.29	0.14	25
	Unscorched	δ^{13}C	BAI_{rel}	-0.17	0.40	25
Pre-burning						
	C-S-U	δ^{13}C	δ^{18}O	0.14	0.44	30
	C-S-U	δ^{13}C	BAI_{rel}	0.10	0.58	30
Post-burning						
	S-U	δ^{13}C	δ^{18}O	0.12	0.52	30
	S-U	δ^{13}C	BAI_{rel}	-0.01	0.93	30
	Scorched	δ^{13}C	δ^{18}O	0.20	0.46	15
	Scorched	δ^{13}C	BAI_{rel}	0.34	0.21	15
	Unscorched	δ^{13}C	δ^{18}O	0.36	0.18	15
	Unscorched	δ^{13}C	BAI_{rel}	-0.43	0.10	15

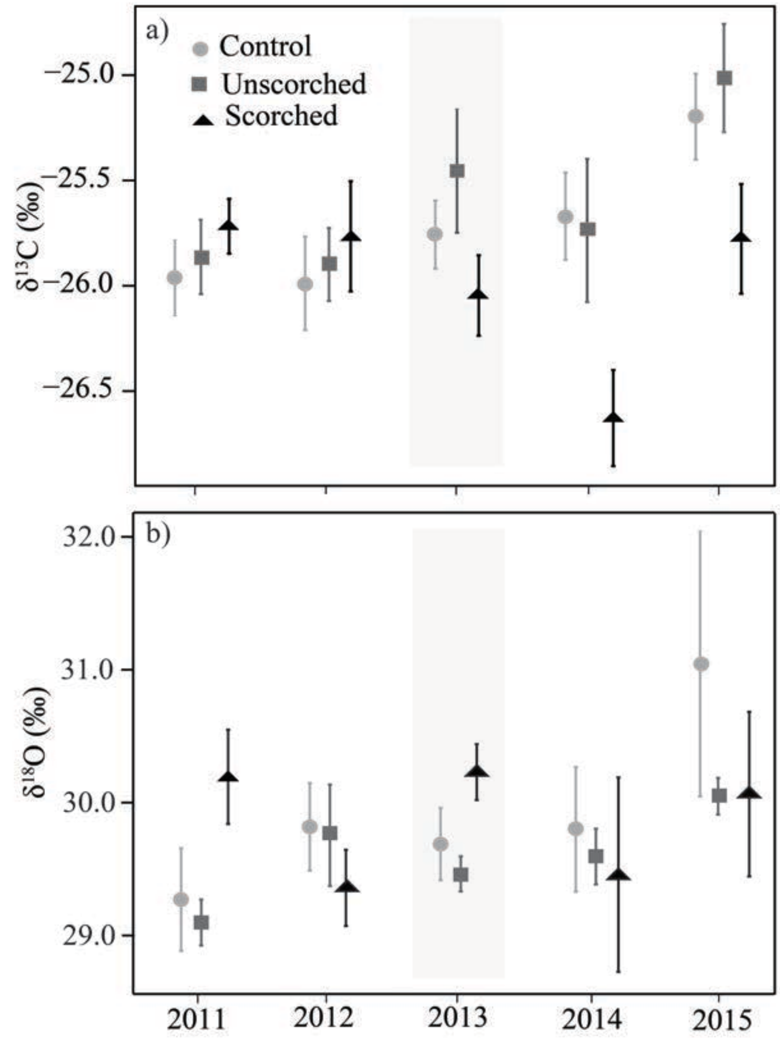


Figure A1: δ^{13}C (‰) (a) and δ^{18}O (‰) (b) for control (n=5), scorched (n=5), and unscorched (n=5) trees, 2 years pre-burning, the year of PB (2013) and 2 post-burning (Mean ± SE). Grey shading indicates the year of prescribed burning (2013).

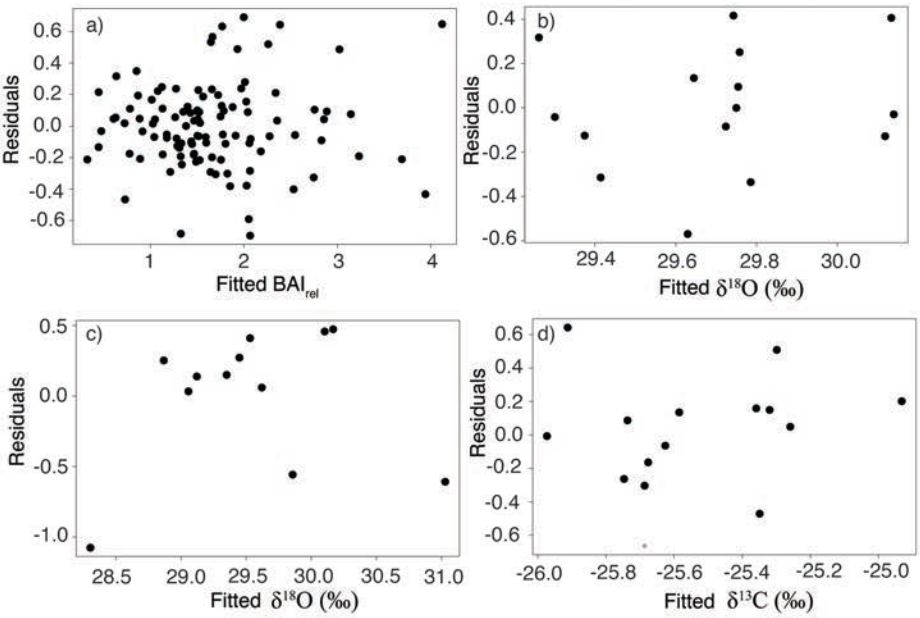


Figure A2: Residuals of the linear mixed model analyzing the effect of crown volume scorch, relative competition index and time on post-burning BAI$_{rel}$ (a), the effect of tree competition index on the post-burning δ^{18}O of unscorched pines (b) and scorched pines (c) and the effect of the tree competition index on the post-burning δ^{13}C of control pines (d).

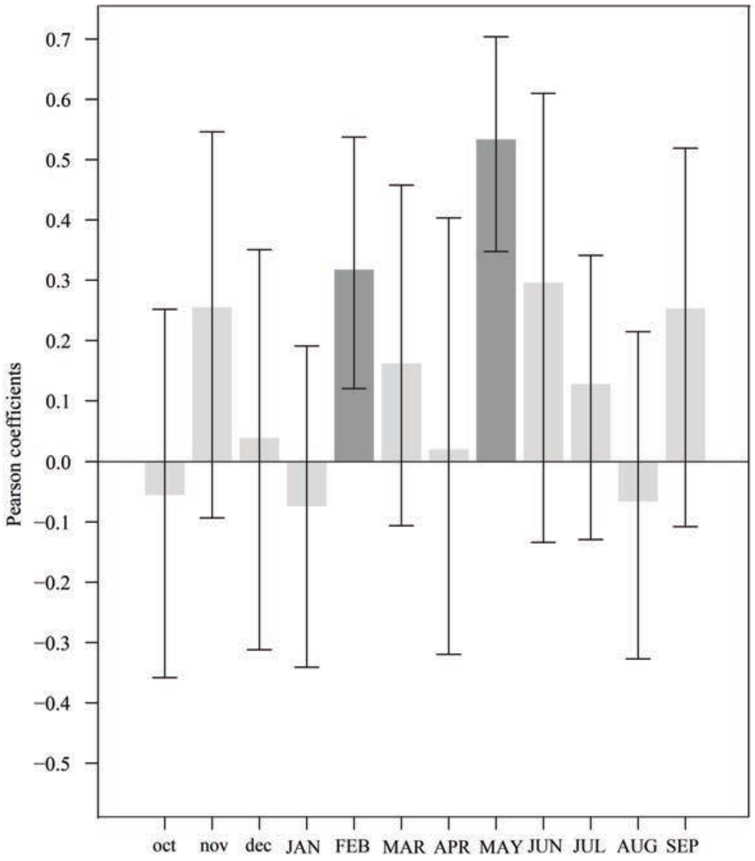


Figure A3: Correlation between the TRW index of the residual chronology of control pines and monthly *P-PET*. Months with small letters denote months of the year before tree-ring formation. Bars in dark grey represent significant coefficients.

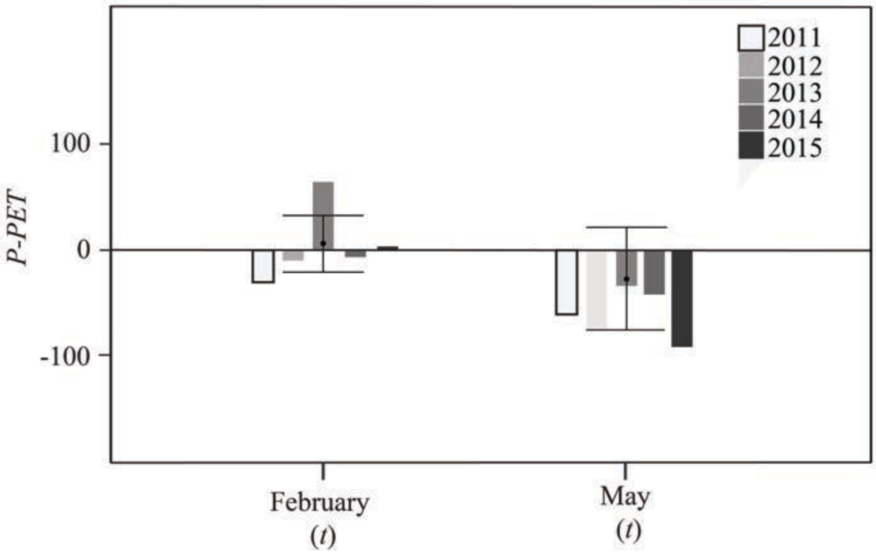
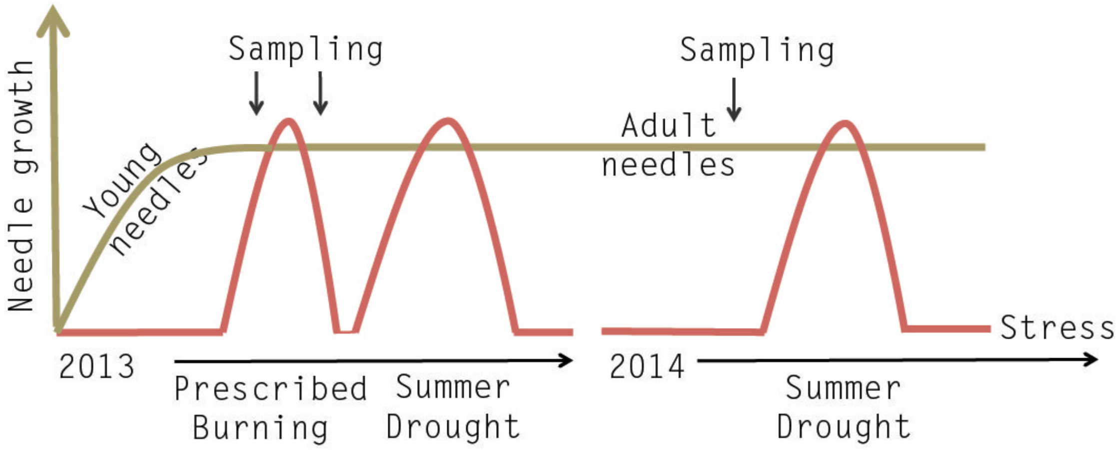


Figure A4: Precipitation minus potential evapotranspiration (*P-PET*) for May and February (the *P-PET* of these months significantly correlated with the residual tree chronology) during the studied period (2011-2015). The mean and standard deviation of P-PET over the entire period (1975-2015) for May and February is showed as a reference of historical values.

Chapter 6

Temporal effects of prescribed burning on terpene production in Mediterranean pines



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Abstract

Prescribed burning is used to reduce fuel hazard but underburning can damage standing trees. The effect of burning on needle terpene storage, a proxy for secondary metabolism, in fire-damaged pines is poorly understood despite the protection terpenes confer against biotic and abiotic stressors. We investigated variation in needle terpene storage after burning in three Mediterranean pine species featuring different adaptations to fire regimes. In two pure-stands of *Pinus halepensis* and two mixed-stands of *P. sylvestris* and *P. nigra,* we compared 24 h and 1 year post-burning concentrations with pre-burning concentrations in 20 trees per species, and evaluated the relative contribution of tree fire severity and physiological condition (δ^{13}C and N concentration) on temporal terpene dynamics (for mono- sesqui- and diterpenes). Twenty-four hours post-burning, monoterpene concentrations were slightly higher in *P. halepensis* than at pre-burning, while values were similar in *P. sylvestris*. Differently, in the more fire-resistant *P. nigra* monoterpene concentrations were lower at 24 h, compared to pre-burning. One year post-burning, concentrations were always lower compared to pre- or 24 h post-burning, regardless of the terpene group. Mono- and sesquiterpene variations were negatively related to pre-burning δ^{13}C, while diterpene variations were associated with fire-induced changes in needle δ^{13}C and N concentration. At both post-burning times, mono- and diterpene concentrations increased significantly with crown scorch volume in all species. Differences in post-burning terpene contents as a function of the pine species' sensitivity to fire suggest that terpenic metabolites could have adaptive importance in fire-prone ecosystems in terms of flammability or defence against biotic agents post-burning. One year post-burning, our results suggest that in a context of fire-induced resource availability, pines likely prioritize primary rather than secondary metabolism. Overall, this study contributes to the assessment of the direct and indirect effects of fire on pine terpene storage, providing valuable information about their vulnerability to biotic and abiotic stressors throughout time.

Keywords: secondary metabolism; plant volatiles; prescribed fire; *Pinus nigra*; *Pinus halepensis*; *Pinus sylvestris*; fire ecology; conifers.

6.1 Introduction

Prescribed burning (PB) is the planned use of fire under mild weather conditions to meet defined management objectives (Wade et al. 1989). PB is executed mostly for fire risk reduction, but also for forest management, restoring habitats or improving grazing. Generally, prescribed burns are low intensity fires, but certain management objectives require a higher burning intensity to effectively achieve specific goals, such as significantly removing understory or slash. In this case, PB can partially damage trees and affect their vitality in the short-term. Some studies have analysed the effects of PB on post-burning growth (Battipaglia et al. 2014, Valor et al. 2015) and tree vitality (see Woolley et al. 2012 for review). Less attention has been dedicated to understanding the effect of PB on secondary metabolites produced by pines (Lavoir et al. 2013), despite the protection they confer against biotic and abiotic stressors, and their potential to increase plant flammability (Ormeno et al. 2009, Loreto and Schnitzler 2010).

The quantity and composition of terpenes produced against a stressor can be constrained by the plant's physiological status (Sampedro et al. 2011) and genetics (Pausas et al. 2016), but also by the nature and severity of the stress, and the species affected. The main secondary metabolites biosynthesized in conifers are terpenes and phenols (Langenheim 2003). In *Pinus* species, oleoresin is a mixture of terpenes including monoterpenes (volatile metabolites), sesquiterpenes (metabolites with intermediate volatility) and diterpenes (semi-volatile compounds), which are stored in resin ducts of woody and needle tissues (Phillips and Croteau 1999). Upon stress, plants follow a constitutive or induced strategy to defend themselves from a stressor. Although most *Pinus* spp. favour the production of constitutive terpenes under stress conditions, they can also synthesize new induced defences (Phillips and Croteau 1999). The induction timing may be different depending on the chemical groups of terpenes, type of stress, and the species or tissue attacked (Lewinsohn et al. 1991, Achotegui-Castells et al. 2015).

Direct effects of fire such as rising temperatures or heat-induced needle damage can alter terpene production. Increases in air and leaf temperature trigger the emission of volatile terpenes (Alessio et al. 2004) but their synthesis can also be stimulated if the optimal temperature of enzymes is not exceeded (Loreto and Schnitzler 2010). Benefits of such stimulation include thermoprotection against heat, since terpene volatiles neutralize the oxidation pressure encountered by chloroplasts under thermal stress (Vickers et al. 2009). As the emission of volatile terpenes in several Mediterranean pines cease 24 h after fire (Alessio et al. 2004) or wounding (Pasqua et al. 2002), we hypothesized that the accumulation of monoterpenes will be higher 24 h post-burning, than before PB.

Indirect effects of fire can affect terpene concentrations by means of increasing resource availability (Certini 2005). In turn, terpene variations induced by fire could change needle flammability (Ormeno et al. 2009) and susceptibility to insects (Hood et al. 2015). The "growth differentiation balance hypothesis" (GDBH) (Herms and Mattson 1992, Stamp 2003) predicts that under poor water and nutrient availabilities, growth is more limited than photosynthesis. Since carbon assimilation is maintained, the excess of carbohydrates favours the synthesis of carbon-based secondary metabolites. On the contrary, when resource availability is high, the growth of plants is not expected to be limited and plants allocate a greater proportion of assimilates to growth rather than to defence traits (Herms and Mattson 1992, Stamp 2003). Accordingly, a short-term response following PB should be an increasing demand on the plant for chemical defence if trees are damaged, but with time, if trees heal, increased fertilization and reduced water competition induced by PB (Feeney et al. 1998) could favour carbon allocation to growth rather than chemical defences.

Time-course terpene responses of the direct and indirect effects of PB could differ between tree species depending on their fire resistance strategies. In this study, we used pines with contrasting tolerance to surface fires: *Pinus halepensis*, a fire sensitive species, *P. sylvestris*, moderately fire-resistant and the fire-resister *P. nigra,* which is supposed to be less vulnerable to fire tissue damage due to its pyro-resistant traits (e.g. thicker bark, higher crown base height) (Fernandes et al. 2008). In agreement with these strategies, we previously found that radial growth was reduced the year of PB in the most fire sensitive species and unaffected in *P. nigra*, while 1 year post-burning, growth augmented in *P. nigra* and *P. halepensis*, and reduced in *P. sylvestris* (Valor et al. 2015). In consequence, we hypothesized that 1 year post-burning, the concentration of terpenes would be, as a whole, lower than before PB, if fire induces a decrease in nutrient and water competition; this reduction would be lower on damaged trees and in pines defined as having lower fire resistance (e.g. *P. halepensis* and *P. sylvestris*).

The objectives of this study were to evaluate the effects of relatively high-intensity PB (enough to remove understory and ladder fuels) on mono-, sesqui- and diterpene storage in *Pinus* spp., comparing 24 h and 1 year post-burning concentrations with pre-burning concentrations. We modelled the relative change of terpene concentrations at two sampling times: (1) 24 h post-burning, as a function of fire severity and pre-burning physiological condition, and (2) 1 year post-burning, as a function of fire severity and PB-induced changes in pine physiological conditions. Additionally, we aimed to identify the most representative terpenes of each sampling time since burning.

6.2 Methods

6.2.1 Study sites

The study was established in three sites situated in the NE Iberian Peninsula (Catalonia, Spain): two plots in mixed-stands of *P. nigra* ssp. *salzmanni* (Dunal) Franco and *P. sylvestris* L. at Miravé and Lloreda localities, situated in the foothills of the Pyrenees; and two other plots in a pure-stand of *P. halepensis* Mill. at El Perelló locality, in the Southern part of Catalonia. The *P. halepensis* stand is located in areas of dry Mediterranean climate while the mixed-stands of *P. nigra* and *P. sylvestris* are situated in temperate cold sub-Mediterranean climate with milder summers and colder winters (Table 6.1). In the sub-Mediterranean sites, soils are developed from calcareous colluviums (0.5-1 m deep) and thus classified as Calcaric cambisols (FAO 2006); in the Mediterranean site, they are developed from limestones (0.4-0.5 m deep) and classified as Leptic Regosol (FAO 2006). The understory is dominated by *Buxus sempervirens* L. and *Viburnum lantana* L., in the *P. nigra* and *P. sylvestris* mixed-stands, and by *Pistacia lentiscus* L. and *Quercus coccifera* L. in the *P. halepensis* stand.

Table 6.1: Topographical and climate characteristics of the study localities.

Study sites			Topography			Climate[1]	
Localities	Lat. (º)	Long. (º)	Aspect	Slope (%)	Elevation (m.a.s.l)	Annual Rainfall (mm)	Mean annual Temperature (ºC)
Lloreda	1.5706	42.0569	N	30	715	731.6	11.7
Miravé	1.4494	41.9515	NE	25	723	677.3	11.5
El Perelló	0.6816	40.9068	NW	10	244	609.9	15.5

[1] Climate variables, annual rainfall and annual mean temperature, were estimated using a georeferenced model (Ninyerola et al. 2000).

6.2.2 Experimental design: tree selection and prescribed burning

A total of four plots (30x30 m) were set up: one in each of the mixed-stand of *P. nigra* and *P. sylvestris*, and two in the pure *P. halepensis* stand. Each plot was burnt in spring 2013 (Table 6.2). PB were conducted by the Forest Actions Support Group (GRAF) of the Autonomous Government (Generalitat de Catalunya) using a strip headfire ignition pattern. PB aimed to decrease fuel hazard by reducing surface and ladder fuel loads. Between 90-100 % of the surface fuel load was consumed in all plots. Needle terpene concentration, fire features and tree physiological condition were studied in 9/10 dominant or co-dominant pines per species in each plot. Each tree was sampled on three occasions for analysing terpene concentration: 24h before PB (pre-burning), 24 h and one year after PB (24 h post-burning and 1 year post-burning, respectively). δ^{13}C and N concentrations of 1-year-old needles were also analysed as a proxy of physiological condition in pre-burning and 1 year post-burning samples.

Table 6.2: Characteristics of prescribed burnings executed in 2013 and forest experimental units (mean ± std.).

	Meteorological conditions				PB characteristics					[3] Forest characteristics				
Exp. Unit	Burn date (day/ month)	Air Temp. (ºC)	Rel. Hum. (%)	Wind Speed[1] (km.h^{-1})	Burn Surf. (ha)	Tmax[2] (ºC)	RT60[2] (min.)	SWC (%)	BSH (cm)	Sp.	Phyto-vol. (m^3 ha^{-1})	DBH (cm)	Density (trees ha^{-1})	BA (m^2 ha^{-1})
Perelló1	13/05	19.5±1.6	49.2±2.9	2.5±1.0	1.0	65-750	2-89	7.5±0.5	84.2±57.1	*Ph*	597	20.6±3.5	533	18.4
Perelló2	13/05	19.9±1.6	66.7±7.7	4.0±0.5	0.7	127-561	7-241	7.5±0.5	50.6±30.5	*Ph*	611	18.5±5.9	922	27.6
Miravé	12/06	25.8±2.1	54.9±4.4	17.6±3.6	0.9	60-718	6-28	30.3±12.0	71.2±53.3	*Pn/Ps*	1637	14.1±5.1	1711	30.3
Lloreda	5/06	21.8±1.4	60.4±4.9	6.75±5.9	1.1	107-834	7-30	34.3±10.3	65.1±48.2	*Pn/Ps*	949	12.4±3.1	2411	30.9

Exp. unit., experimental unit; Air. Temp., air temperature; Rel. Hum., relative humidity; Burn surf., burn surface; SWC, soil water content; BSH, bole scorch heigh. *Ph*, *Pinus halepensis*. *Pn-Ps*, *P. nigra* and *P. sylvestris*.

[1] Wind speed was measured outside the forest.

[2] Range of maximum temperatures (Tmax) and residence time above 60º (RT60) in 10 trees in each of the Perelló experimental units and in 20 trees in Miravé and Lloreda.

[3] Phytovol., phytovolume calculated using the cover and height of the understory shrubs; diameter at breast height (DBH), density and basal area of trees with DBH $\geq$ 7.5 cm.

Before PB, selected trees were identified with a metal tag. Their diameter at breast height (DBH), total height and height lo live crown base were measured. During fires, the fire residence time (minutes) above 60 ºC and the maximum temperature at the base of the trunk were measured for the selected trees with K-thermocouples (4 mm) connected to dataloggers (Testo 175), packed with a fireproof blanket and buried into the soil. Temperatures were recorded every 10 s. The maximum temperatures registered at the soil surface occurred in the *P. nigra* and *P. sylvestris* plots, while the highest residence time above 60 ºC was recorded in the *P. halepensis* plots (Table 6.2). One week after PB, the crown volume scorched was visually estimated to the nearest 5 % as an indicator of fire severity. Foliage scorch was defined as a change in needle colour resulting from direct foliage ignition or indirect heating (Catry et al. 2010).

6.2.3 Needle sampling

In each plot, we cut an unscorched branch from the top of the south-facing crown in the 9/10 trees selected per species for each sampling time studied: pre-burning, 24 h and 1 year post-burning. Five twigs with unscorched healthy needles were cut immediately, covered with aluminium paper and stored in a portable refrigerator at 4 ºC until being stored at -20 ºC in the laboratory for terpene analysis. The time period between the field and the laboratory did not exceed 2 hours. Additionally, about five twigs were transported to the laboratory, dried at 60 ºC and stored in tins before δ^{13}C and N concentration analysis.

6.2.4 Needle terpene concentration

In the studied pine species, needles reached up to 3 years old. Before terpene extraction, we collected the 1-year-old needles from each twig to control for the effect of age for each sampling time. Needles were cut in small parts (approx. 5 mm) and placed in well-filled, tightly closed amber glass vials to avoid exposure to light and oxygen (Guenther 1949, in Farhat et al. 2001). The extraction method consisted in dissolving 1 g of cut 1-year-old unscorched green needles in 5 ml of organic solvent (cyclohexane + dichloromethane, 1:9), containing a constant amount of undecane, a volatile internal standard to quantify terpene concentrations which was not naturally stored in the needles. Extraction occurred for 20 min, under constant shaking at room temperature, similar to extractions shown in Ormeno et al. (2007). The extract was stored at -20 ºC and then analysed within the following three weeks. Analyses were performed on a gas chromatograph (GS-Agilent 7890B) coupled to a mass selective detector (MSD 5977A). Compound separation was achieved on an HP-5MS capillary column with helium as the carrier gas. After sample injection (1μL), the start temperature (40 ªC for 5 min) was ramped up to 245 ªC at a rate of 3 °Cmin^{-1}, and then until 300 ºC a rate of 7 °Cmin^{-1}. Terpene identifications were based on the comparison of terpene retention times and mass spectra with those obtained from authentic reference samples (Sigma- Aldrich®) when available, or from databases (NIST2008, Adams 2007) when samples were unavailable. Also, we calculated the Kovats retention index and compared it with bibliographical data. Terpenes were quantified based on the internal standard undecane (36.6 ng μL^{-1} of injected solution). Thus, based on calibrations of terpene standards of high purity (97-99%), also prepared using undecane as internal standard, chromatographic peak areas of an extracted terpene where converted into terpene masses based on the relative response factor of each calibrated terpene. Results were expressed on a needle dry mass (DM) basis. The identified terpenes were grouped in mono-, sesqui- and diterpenes. At each post-burning time, we calculated the relative change of terpene concentration as the difference between the pre- and post-burning concentration of each terpene group expressed as percentage.

6.2.5 Tree physiological condition: δ^{13}C and N analysis

δ^{13}C and N analysis were carried out on 1-year-old unscorched needles in pre-burning and 1 year post-burning samples. For δ^{13}C and N, needles were oven-dried at 60 ºC for 48 h, ground and analysed at the Stable Isotope Facility of the University of California at Davis (USA) using an ANCA interfaced to a 20-20 Europa ®isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

6.2.6 Climatic data before and during sampling years

Monthly precipitation (P) and temperature (T) from March 2012 to August 2014 were downloaded from the 3 nearest meteorological stations to the sub-Mediterranean and the Mediterranean plots. Monthly potential evapotranspiration (PET) was estimated using the Thornthwaite (1948) method. For each sampling year (t), 2013 and 2014, accumulated values of P and PET of different periods were calculated for each meteorological station. Seven periods of accumulated climate data were compiled: annual, from June before the sampling year (t-1) to May of the sampling year (t); spring, summer, fall and winter before the sampling year (t-1); spring and summer of the sampling year (t). For each period, we calculated the difference between P and PET (P-PET) for each meteorological station and sampling year.

6.2.7 Data analysis

Linear mixed models (LMM), considering plot as a random factor, were used to:

1. Analyse potential differences in pre-burning tree physiological condition and fire parameters among pine species.

2. Test for differences in total terpene and terpene group concentrations (expressed in a needle mass basis and as the percentage of the terpene group from the total) between times since burning for each pine species.

3. Model 24 h and 1 year impact of PB on the relative concentration change of mono-, sesqui- and diterpenes respect to pre-burning concentration. The 24 h and 1 year post-burning models considered pine species as a fixed factor, needle δ^{13}C and N concentration pre-burning, and the proportion of crown scorched and fire residence time above 60 ºC as covariables. In addition, in the 1 year post-burning model, δ^{13}C and N concentration changes were also included (1 year post-burning minus pre-burning levels of δ^{13}C and N concentration). Second interactions of pine species with each co-variable were included.

Terpene concentration were log transformed to accomplish normal distribution requirement. When the relative concentration change of terpenes was modelled, 100 was summed as a constant before taking the logarithm. Therefore, log-transformed data higher than 2 indicate higher terpene concentrations than pre-burning, while values lower than 2 mean lower terpene concentrations. Residuals presented no pattern and highly correlated explanatory variables were avoided. The variance explained for the fixed effects was obtained by comparing the final model with the null model (containing only the random structure). A Tukey post-hoc test was used for multiple comparisons when needed.

For each pine species, terpene profiles were evaluated using a principal component analysis (PCA) to show potential qualitative and quantitative variation in needle terpene within and between plots and time since burning. Terpene concentrations were centered and the variance-covariance matrix used to understand how terpene profiles varied. Moreover, for each pine species, we used a multilevel sparse partial least squares discriminant analysis (sPLS-DA) to select the terpenes that best separated each time since burning in terms of their concentration. The sPLS-DA is a supervised technique that takes the class of the sample into account, in this case time since burning, and tries to reduce the dimension while maximizing the separation between classes. To conduct the analysis, we selected those compounds that were present in at least 75% of the sampled trees, resulting in a total of 48, 37 and 35 compounds in *P. halepensis*, *P. nigra* and *P. sylvestris,* respectively. We used the multilevel approach to account for the repeated measures on each tree to highlight the PB effects within trees separately from the biological variation between trees. The classification error rate was estimated with leave-one-out cross validation with respect to the number of selected terpenes on each dimension. Lastly, differences in *P-PET* between sampling years were tested by a Student's t-test for the Mediterranean and Sub-Mediterranean plots. All analyses were conducted with the software R (v. 3.2.1, the R Foundation for Statistical Computing) using the package *nlme* for linear mixed-effects modelling and the package mixOmics for the sPLS-DA analysis. The model variances explained by fixed effects (marginal R^2) and by both fixed and random effects (conditional R^2) are provided (Nakagawa and Schielzeth 2013).

6.3 Results

6.3.1 Tree, fire and climate characteristics

The proportion of crown scorched was significantly higher in *P. halepensis* than in the other species despite that the three pine species presented similar height to live crown base (Table 6.3). By contrast, no differences in fire residence time above 60 ºC were encountered among species (Table 6.3). Needle δ^{13}C decreased significantly 1 year post-burning in the three species while N concentration was similar (Table 6.3).

Table 6.3: Studied pine trees and fire characteristics (mean ± std.) before and after prescribed burnings grouped by species.

Tree and fire characteristics	*P. halepensis*	*P. nigra*	*P. sylvestris*
n (trees)	20	19	19[1]
DBH (cm)	20.0±6.9a	13.6±5.5b	12.7±5.3b
Total height (m)	9.1±2.4a	8.3±2.4a	8.6±1.9a
Height to live crown base (m)	5.2±1.0a	4.8±1.3a	6.6±13.2b
Crown scorched (%)	44.0±32.1a	6.6±13.2b	5.5±9.5b
Fire residence time >60ºC(min)	38.2±54.1a	16.6±6.9a	15.2±6.4a
Needle δ^{13}C ($^{0}/_{00}$)			
pre-burning	-25.8±0.5Aa	-26.6±1.0 Ab	-26.5±0.6Ab
1 year post-burning	-27.6±0.9Ba	-28.5±1.4Ba	-28.0±0.8Ba
Needle N content (mg $g_{DM}{}^{-1}$)			
pre-burning	14.8±1.9Aa	10.1±0.8Ab	12.3±1.6Ac
1 year post-burning	14.9±3.2Aa	9.1±2.7Ab	11.0±3.1Aa

[1] Sample size is 18 for 1 year post-burning data because of death of one tree.

Different small letters within a row indicate statistical significant differences ($P < 0.05$) among pine species using LME (where fixed factor=species, random factor=plot) followed by Tukey post-hoc test. Different capital letters within a column indicate statistical significant differences ($P < 0.05$) between pre-burning and 1 year post-burning for each pine species using LMM (where fixed factor =time since burning, random factor=plot) followed by Tukey post-hoc test.

This decrease in δ^{13}C contrasted with the drier conditions found 1 year post-burning (*P-PET*= 200 mm and = 135 mm in Mediterranean and Sub-Mediterranean plots, respectively) in comparison with pre-burning (*P-PET*= 481 mm and =290 mm in Mediterranean and Sub-Mediterranean plots, respectively (Supplementary Figure A1).

6.3.2 Terpene concentration variation across time since burning for each pine species

A total of 56, 59 and 49 terpenes were identified and quantified in *P. halepensis*, *P. nigra* and *P. sylvestris,* respectively (Supplementary Table A1). Pre-burning, *P. nigra* showed the highest terpene concentration (65.6±7.1 mg $g_{DM}{}^{-1}$) followed by *P. halepensis* and *P. sylvestris* (41.2±5.8 mg $g_{DM}{}^{-1}$ and 21.4±2.6 mg $g_{DM}{}^{-1}$, respectively). Before PB, more than 45 % of total terpene concentration was represented by diterpenes in *P. halepensis* while sesquiterpenes represented about 59 % in *P. nigra* and monoterpenes represented 83 % in *P. sylvestris* (Supplementary Table S2). Considering all sampling times, the diterpene thunbergol in *P. halepensis*, the sesquiterpene β-caryophyllene in *P. nigra* and the monoterpene α-pinene in *P. sylvestris* were the major compounds found, representing an average of 22 %, 22 % and 40 % of the total terpene concentration, respectively (Supplementary Figure A2). Terpene concentration and composition strongly varied within plots in all species, with no clear differences in terpene composition among plots (Supplementary Figure A3a, A4a, A5a). The variation in terpene concentrations was high within pre- and 24 h post-burning samples, while variation for 1 year post-burning concentrations was much lower (Supplementary Figure A3b, A4b, A5b). In all species, the quantity of dominant compounds in pre- and 24 h post-burning samples were clearly different from those of 1 year post-burning samples (Supplementary Figure A3b, A4b, A5b). For instance, the quantity of α-pinene was higher in pre- and 24 h post-burning times in all pine species in opposition with 1 year post-burning samples. Limonene was characteristic 24 h post-burning in the needles of *P. halepensis* and *P. nigra* while the quantity of camphene and myrcene was higher in pre- and 24 h post-burning needles samples of *P. sylvestris.*

Differences in total terpene concentration between pre- and 24 h post-burning were only detected in *P. nigra*, which decreased ca. 39 % (Figure 6.1, a). When analysing terpene groups, the 24 h post-burning needle concentration of both mono- and sesquiterpenes were, in comparison with pre-burning, slightly higher in *P. halepensis*, lower in *P. nigra* and similar in *P. sylvestris* (Figure 6.1, b and c). No differences were detected in the diterpene concentration between pre- and 24 h post-burning times (Figure 6.1, d).

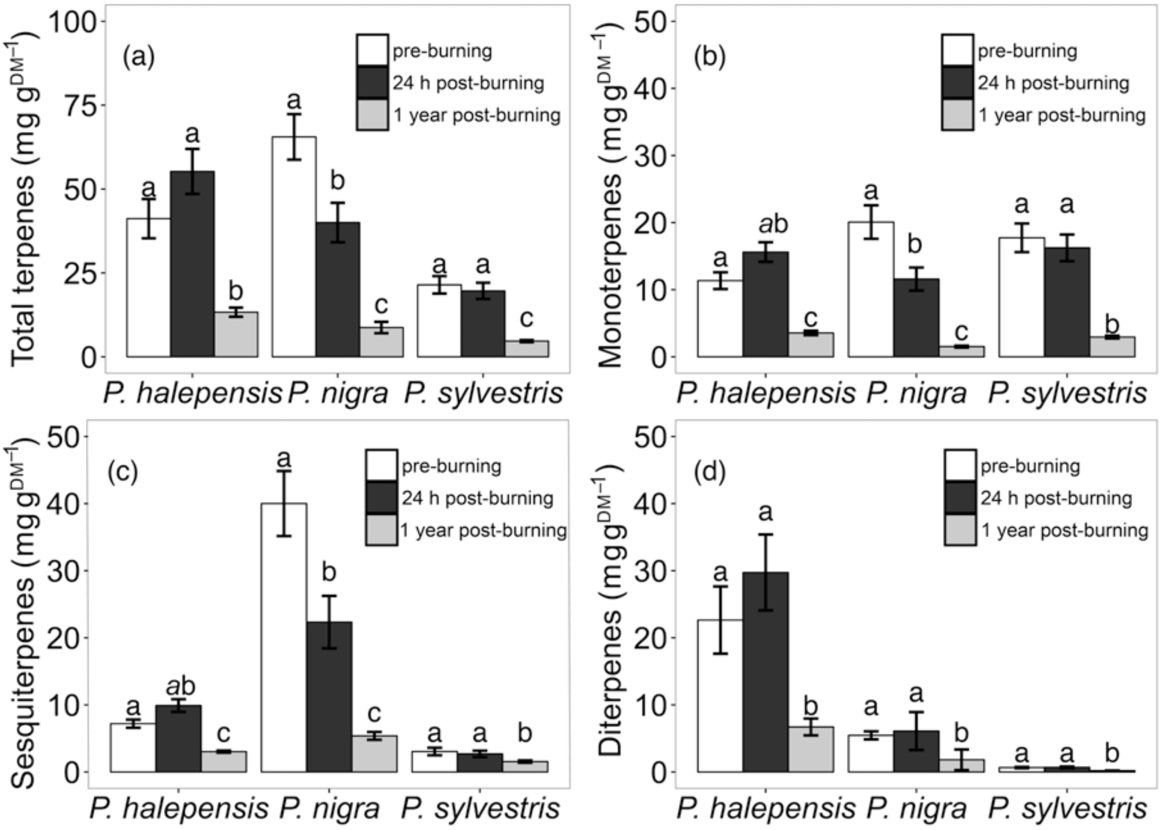


Figure 6.1: Concentration (mean ± SE) of total terpene (a), monoterpenes (b), sesquiterpenes (c) and diterpenes (d) across time since burning (TSB) for each pine species (*P. halepensis*,n=20; *P. sylvestris,* n=19; *P. nigra,* n=19 and n=18 in 1 year-post-burning). Differences in the concentration between TSB within each pine species were tested using LMM considering plot as a random factor. Within each pine species, different letters indicate differences between TSB using a Tukey post-hoc, where regular letters indicate significant differences at $P<0.05$; italic letters represent a marginal significant difference ($0.05<P<0.1$)

One year after burning, total terpene concentration was lower compared to the levels observed pre- and 24 h post-burning in the three species (Figure 6.1, a). In *P. halepensis* this reduction was similar for each terpene group while, in the two Sub-Mediterranean species, it was mostly due to a decrease in the proportion of monoterpenes (Supplementary Data, Table A2). In contrast, an increase in the relative contribution of the sesquiterpene group to the total terpenes was found 1 year post-burning in both Sub-Mediterranean species.

6.3.3 Relative change of terpene concentration 24 h post-burning

The relative changes of mono- and diterpene concentrations 24 h post-burning were directly related to the proportion of crown scorched (Table 6.4).

Table 6.4: Summary of the models characterizing the impact of prescribed burning and tree vitality on the 24 h post-burning relative concentration change of mono-, sesqui- and di-terpenes, calculated as the standardized difference between 24 h post-burning and pre-burning concentration expressed as percentage (logarithmically transformed). Only the significant interaction terms are shown. Bold characters indicate significant effects ($P < 0.05$).

	24 h post-burning relative concentration change								
	Monoterpenes			Sesquiterpenes			Diterpenes		
	Est.	SE	*P*	Est.	SE	*P*	Est.	SE	*P*
Intercept	**1.146**	**0.203**	**0.00**	**2.780**	**0.633**	**0.00**	**1.585**	**0.074**	**0.00**
CS^1	**0.009**	**0.003**	**0.00**	0.001	0.001	0.48	**0.027**	**0.005**	**0.00**
RT60^2	0.000	0.000	0.29	**0.002**	**0.001**	**0.04**	0.001	0.001	0.33
δ^{13}C	-0.035	0.030	0.25	0.000	0.040	0.99	-0.011	0.054	0.83
HLCB^3	**0.041**	**0.020**	**0.04**	0.008	0.026	0.73	-0.052	0.035	0.14
Foliar N	**0.033**	**0.016**	**0.03**	**-0.112**	**0.062**	**0.07**	0.019	0.026	0.47
Sp.=*P. halepensis*	0.228	0.128	0.21	-1.358	0.735	0.20	**0.476**	**0.132**	**0.07**
Sp.=*P. sylvestris*	**0.209**	**0.069**	**0.00**	**-1.604**	**0.688**	**0.02**	**0.395**	**0.106**	**0.00**
CS x *Ph*	**-0.007**	**0.003**	**0.02**				**-0.025**	**0.005**	**0.00**
CS x *Ps*	-0.005	0.005	0.32				**-0.024**	**0.009**	**0.00**
Foliar N x *Ph*				**0.155**	**0.067**	**0.02**			
Foliar N x *Ps*				**0.174**	**0.065**	**0.01**			
AIC	24.48			32.81			67.39		
R^2 (fixed)	0.54			0.46			0.44		
R^2 (random+fixed)	0.59			0.57			0.44		

[1] CS, crown scorched (%).

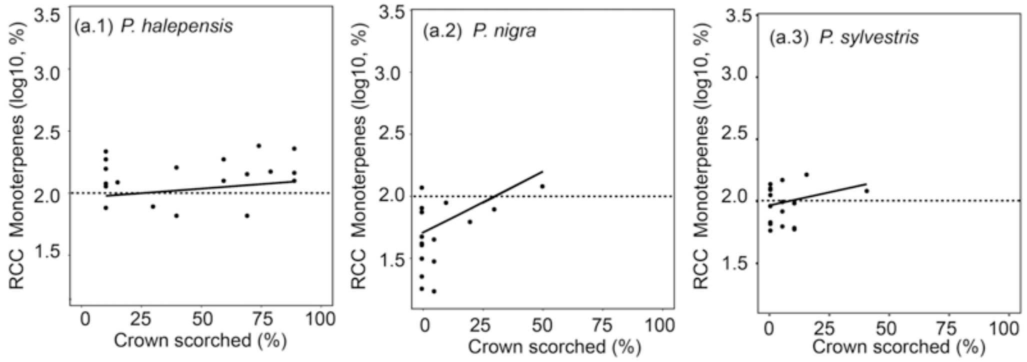
[2] RT60, fire residence time above 60 ºC(min).

[3] HLCB, height to live crown base (m).

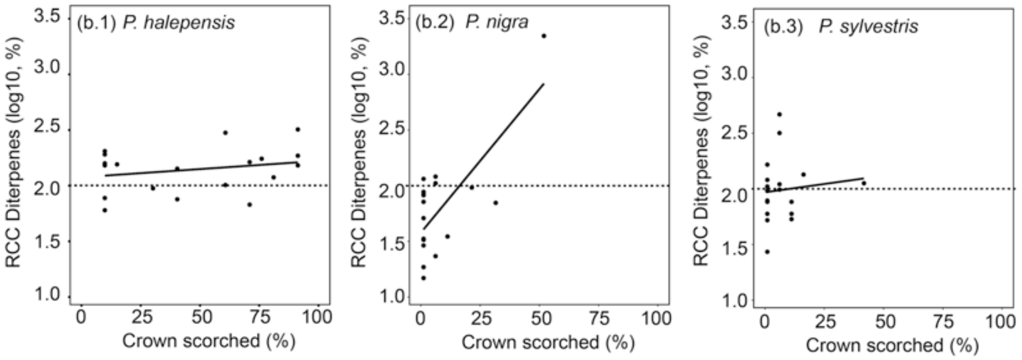
However, crown scorch volume interacted with pine species to explain the relative changes in mono- and diterpene concentrations (Table 6.4). Thus, in both *P. halepensis* and *P. sylvestris*, the 24 h post-burning concentration of monoterpenes was higher than pre-burning and increased with crown scorched (Figure 6.2, a.1 and a.3); only individual pines with low proportion of the crown scorched (<15-20 %) showed similar or lower concentration than pre-burning. In contrast, the relative change of monoterpene concentration in *P. nigra* was generally lower than pre-burning, at least in the range of crown scorch measured (0-50 %) (Figure 6.2, a.2). The relationship between the relative concentration change in diterpenes and crown scorch followed a similar trend as in monoterpenes for *P. halepensis* and *P. sylvestris* (Figure 6.2, b.1 and b.2), while in *P. nigra*, the ratio of change in crown scorch was higher and shifted from lower to higher concentrations than pre-burning in the middle of the measured crown scorch range (Figure 6.2, b.3).

24 h post-burning

(a) Relative content change (RCC) in Monoterpenes vs. Crown scorched



(b) Relative content change (RCC) in Diterpenes vs. Crown scorched



(c) Relative content change (RCC) in Sesquiterpenes vs. needle N

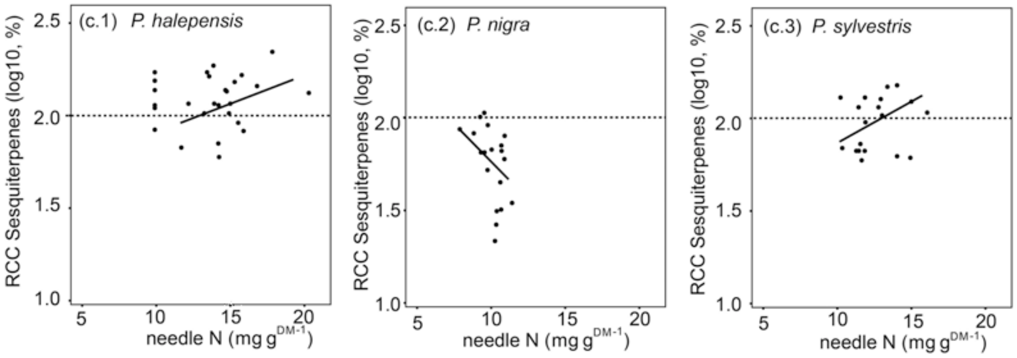


Figure 6.2: Measured and predicted (line) relative concentration change (log-transformed) using 24 h post-burning models (see Table 6.4) of monoterpenes and diterpenes against crown scorched (a and b) and for sesquiterpenes against needle N (c). Before the log-transformation, 100 was summed. The dashed line indicates no changes between pre- and post-burning terpene concentrations: higher values indicate a higher terpene concentration than those of pre-burning while the opposite is indicated by lower values.

The relative concentration change of monoterpene was also directly related with the needle N concentration and the height to live crown base (Table 6.4). In the case of sesquiterpenes, needle N concentration interacted with pine species (Table 6.4, Figure 6.2). Thus, the relative concentration change of sesquiterpenes 24 h post-burning was higher in *P. halepensis* and *P. sylvestris*, and augmented as needle N concentration increased (Figure 6.2, c.1 and c.3), whereas it was always lower in *P. nigra*, decreasing inversely with increasing needle N concentration (Figure 6.2, c.2). Finally, fire residence time above 60 ºC directly affected the relative change of sesquiterpene concentration in all species (Table 6.4).

6.3.4 Relative change of terpene concentration 1 year post-burning

One year after PB, the relative change of mono- and sesquiterpene concentrations were always lower than pre-burning and inversely related with δ^{13}C of pre-burning needles (Table 6.5, Figure Figure 6.3, a.1). The 1 year post-burning relative change concentration of diterpenes were also lower than pre-burning, but variations were associated with changes in δ^{13}C or N concentration of needles (Figure 6.3, a.2 and a.3).

Table 6.5: Summary of the models characterizing the impact of prescribed burning and tree vitality on the 1 year post-burning relative concentration change of mono-, sesqui- and di-terpenes, calculated as the standardized difference between 1 year post-burning and pre-burning content expressed as percentage (logarithmic transformed). Only the significant interaction terms are shown (P<0.05). Bold characters indicate significant effects.

	1 year post-burning relative concentration change								
	Monoterpenes			Sesquiterpenes			Diterpenes		
	Est.	SE	*P*	Est.	SE	*P*	Est.	SE	*P*
Intercept	**-1.879**	**0.812**	**0.05**	**-2.699**	**0.907**	**0.00**	**0.812**	**0.107**	**0.00**
CS^1	**0.002**	**0.001**	**0.07**	**0.002**	**0.001**	**0.06**	**0.035**	**0.005**	**0.00**
RT60^2	-0.000	0.000	0.50	-0.000	0.000	0.53	-0.000	0.001	0.80
δ^{13}C	**-0.103**	**0.030**	**0.00**	**-0.144**	**0.034**	**0.00**	-0.065	0.063	0.30
Foliar N	0.019	0.017	0.26	0.012	0.019	0.53	0.001	0.029	0.96
HLCB^3	-0.003	0.020	0.86	0.001	0.023	0.94	-0.058	0.035	0.11
Sp=*P. halepensis*	**0.603**	**0.073**	**0.01**	**0.611**	**0.067**	**0.01**	**0.696**	**0.150**	**0.04**
Sp=*P. sylvestris*	**0.351**	**0.056**	**0.00**	**0.647**	**0.063**	**0.00**	**0.847**	**0.110**	**0.00**
Change δ^{13}C^4	-0.029	0.030	0.33				**0.101**	**0.041**	**0.01**
Change N^5	0.009	0.013	0.47				**-0.062**	**0.021**	**0.00**
CS x *Ph*							**-0.031**	**0.006**	**0.00**
CS x *Ps*							**-0.029**	**0.009**	**0.00**
AIC	0.87			-0.30			81.79		
R^2 (fixed)	0.70			0.69			0.67		
R^2 (random+fixed)	0.70			0.69			0.67		

[1] CS,percentage of crown scorched.[2] RT60, fire residence time above 60ºC (min). [3] HLCB, height to live crown base (m). [4] Change δ^{13}C, change in δ^{13}C (difference between 1 year post-burning and pre-burning δ^{13}C). [5] Change N, change in foliar N content (difference between 1 year post-burning and pre-burning N content).

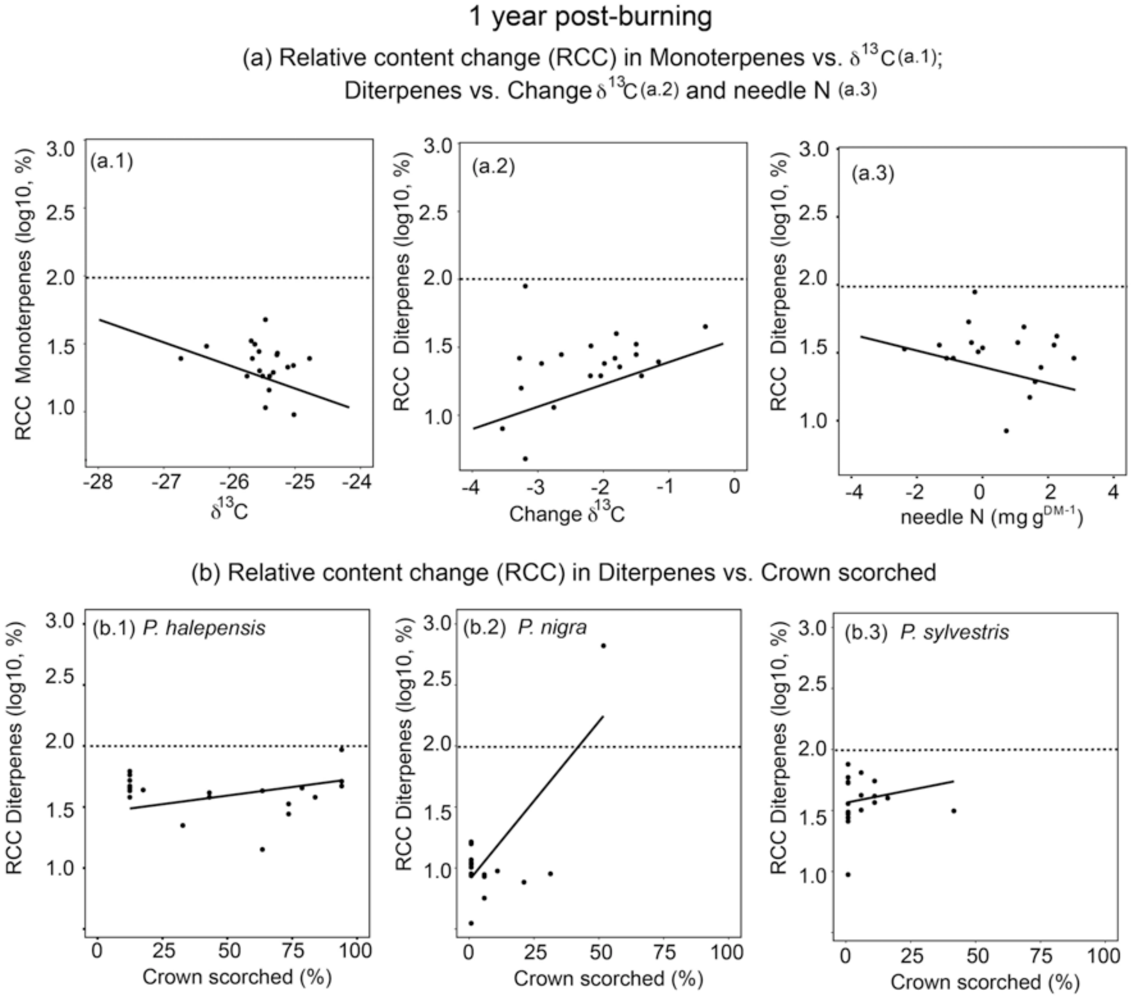


Figure 6.3: Measured and predicted (line) relative concentration change (log-transformed) using 1 year post-burning models (see Table 6.5) for monoterpenes against δ^{13}C (a.1), for diterpenes against change in δ^{13}C (a.2), change in needles N (a.3), and the interaction between species and crown scorch (b). Before the log-transformation, 100 was summed. The dashed line indicates no changes between pre- and post-burning terpene concentrations: higher values indicate a higher terpene concentration than those of pre-burning while the opposite is indicated by lower values.

Similar to 24 h post-burning, the proportion of crown scorched had a direct effect on the relative concentration change of all terpene groups, although marginally significant in mono- and sesquiterpene models (Table 6.5). This variable interacted with pine species in the case of diterpenes (Figure 6.3, b) and showed that as crown scorch increased, the relative concentration change in *P. nigra* was more acute than in the other species (Table 6.5, Figure 6.3, b.2).

6.3.5 Discriminant terpenes across time since burning for each pine species

The multilevel sPLS-DA in *P. halepensis* led to optimal selection of six and one terpenes on the first two dimensions with a classification error rate of 0.26 and 0.06, respectively, reflecting a clear separation between times since burning (Figure 6.4). Among compounds, terpinen-4-ol separated pre-burning (Cluster 2) from both post-burning times; whereas E-β-ocimene and α-thujene discriminated the 24 h post-burning sampling time from the others (Cluster 1). Four sesquiterpenes characterized the 1 year post-burning needle samples (Cluster 3).

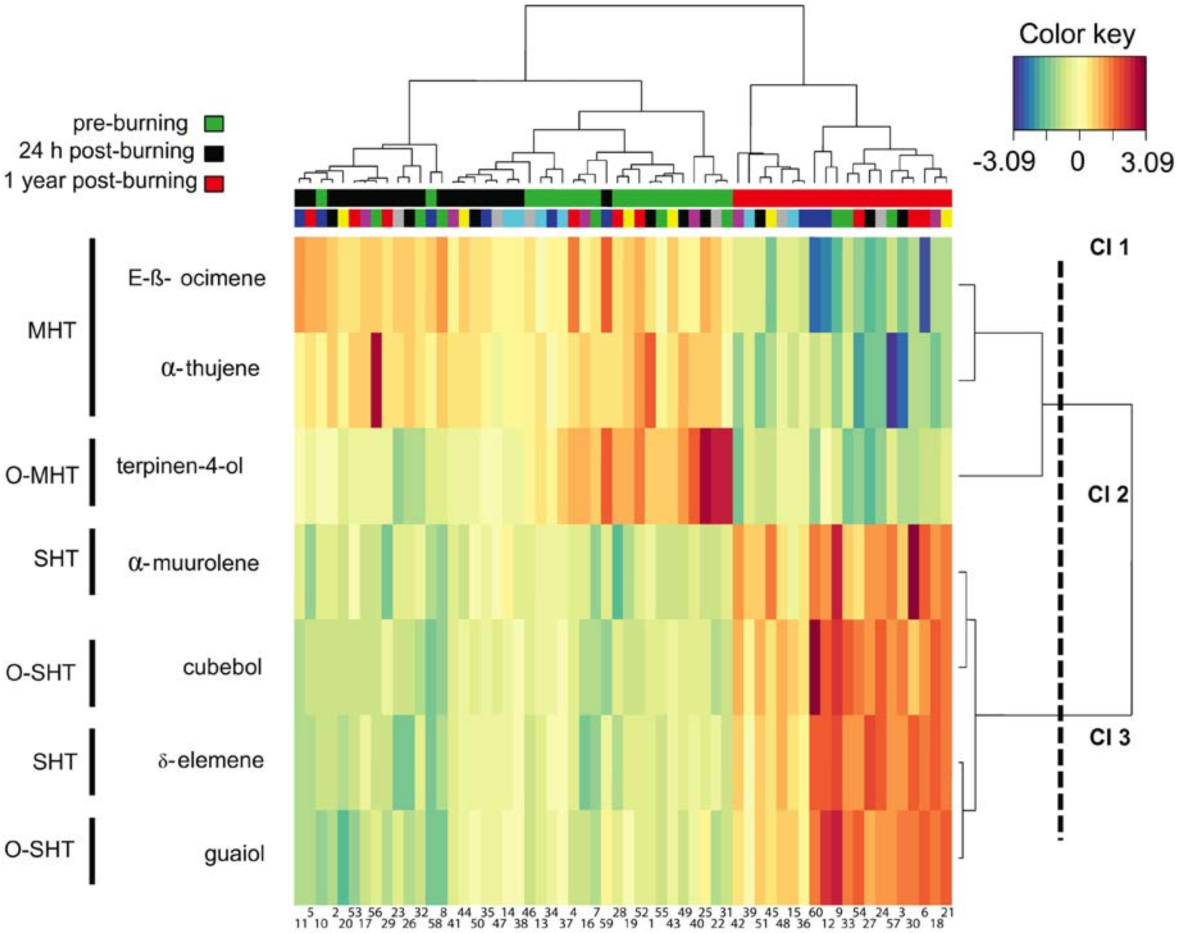


Figure 6.4: Hierarchical clustering for *P. halepensis* of the seven terpenes selected with multilevel sPLS-DA using terpene content. Samples are represented in columns and terpenes in rows. MHT, monoterpene hydrocarbon; SHT, sesquiterpene hydrocarbon; O, oxygenated compounds; der, derivative compounds.

In *P. nigra*, we chose three dimensions and the corresponding terpenes selected for each were four, one and one (Figure 6.5). The classification error rates were 0.35, 0.33 and 0.18, respectively, for the first three dimensions. Two clusters were differentiated:

pre-burning was discriminated, mainly, by three sesquiterpenes (Cluster 1) and bornyl acetate and β-springene represented, post-burning samplings (Cluster 2) (Figure 6.5).

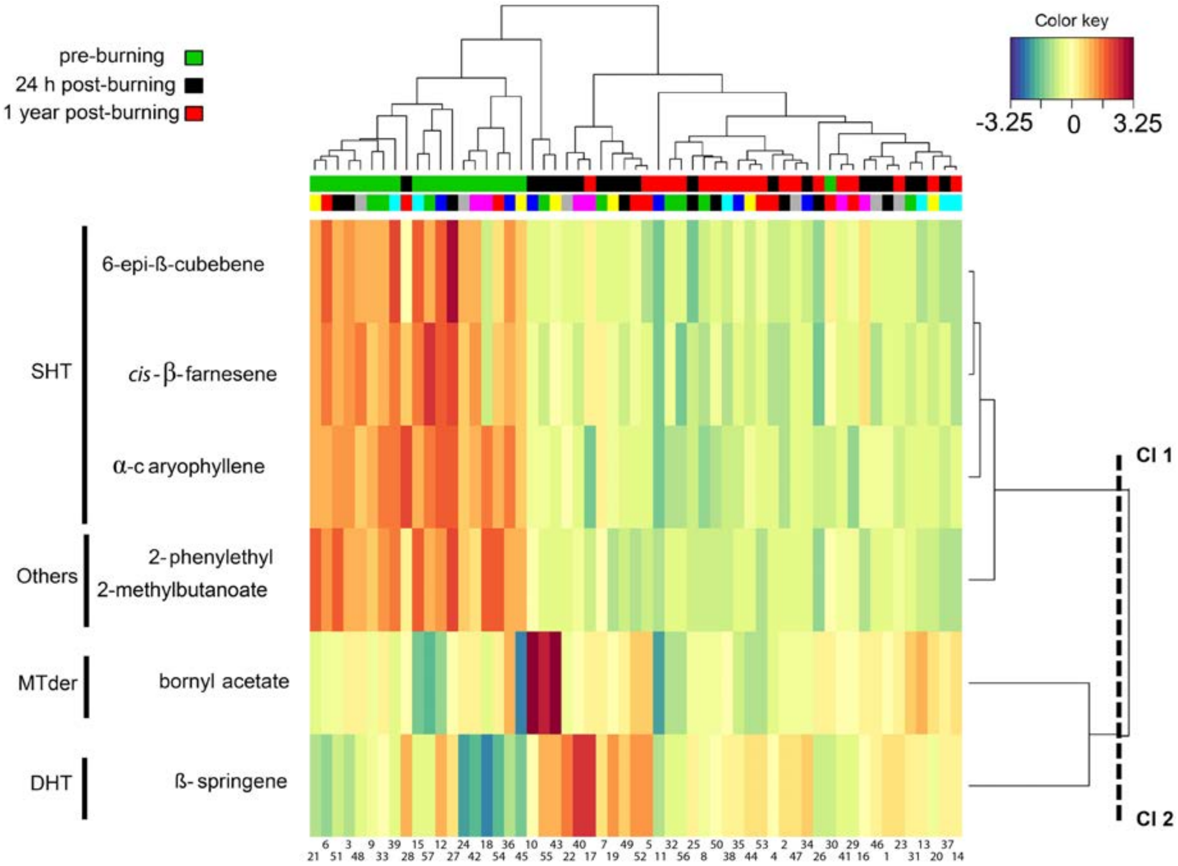


Figure 6.5: Hierarchical clustering for *P. nigra* of the six terpenes selected with multilevel sPLS-DA using terpene content. Samples are represented in columns and terpenes in rows. MT, monoterpene hydrocarbon;SHT, sesquiterpene hydrocarbon; DHT, diterpene hydrocarbon; der, derivative compounds; others, compounds other than terpenes.

Finally, two dimensions were selected for *P. sylvestris* (Figure 6.6) with 11 terpenes on each component. The classification error rates were 0.66 and 0.33. As in *P. nigra*, two clusters were distinguished: sesquiterpenes characterized the pre-burning sampling time, whereas both post-burning times were characterized mainly by mono- and sesquiterpenes (Figure 6.6).

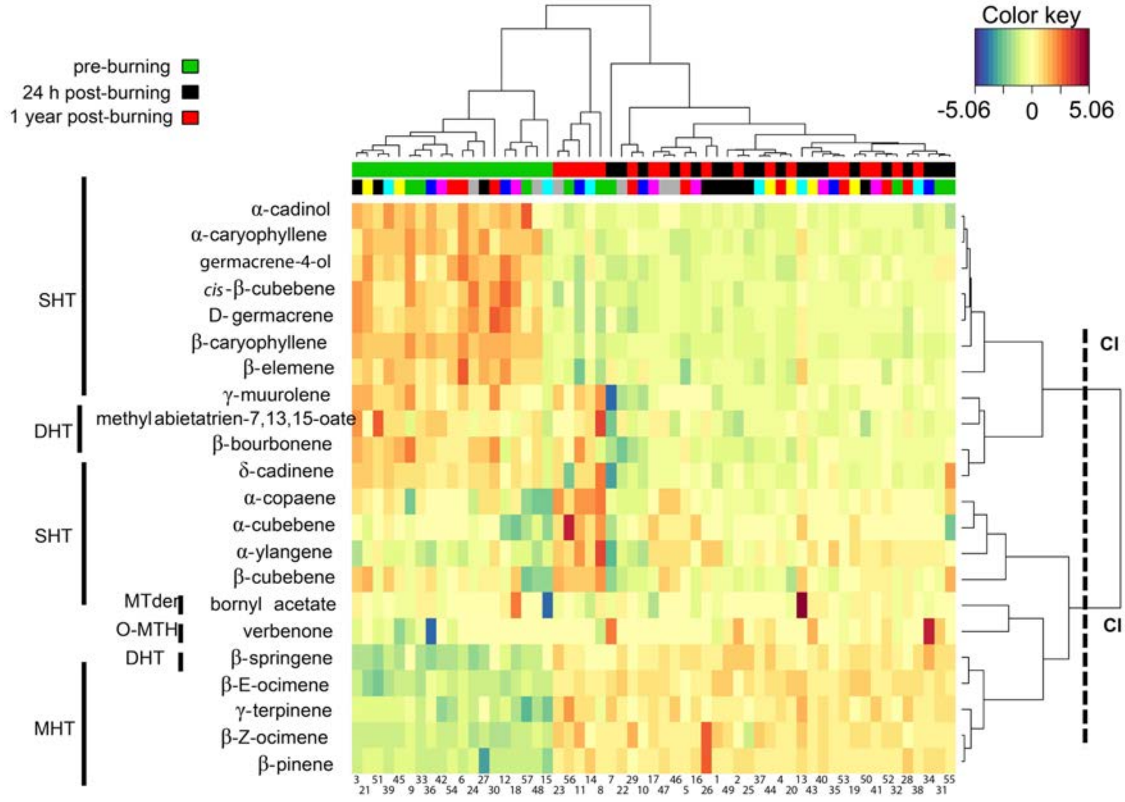


Figure 6.6: Hierarchical clustering for *P. sylvestris* of the 22 terpenes selected with multilevel sPLS-DA using terpene content. Samples are represented in columns and terpenes in rows. MHT, monoterpene hydrocarbon; SHT, sesquiterpene hydrocarbon; DHT, diterpene hydrocarbon; O, oxygenated compounds; MTder, derivative monoterpenes.

6.4 Discussion

6.4.1 PB effects on terpene concentration

Pinus nigra is a species considered to be resistant to medium-low fire intensities, *P. sylvestris* a moderately fire-resistant species and *P. halepensis* a fire-sensitive species (Agee 1998, Fernandes et al. 2008). While concentration of the semi-volatile diterpenes was not affected 24 h post-burning, concentration of mono- and sesquiterpenes seems to decrease in *P. nigra,* was sustained in *P. sylvestris* and tended to increase in *P. halepensis.* Although massive needle terpene emissions have been reported at ambient temperatures often reached during PB (Alessio et al. 2004, Loreto and Schnitzler 2010, Zhao et al. 2012), various explanations may justify the different terpene contents observed 24 h post-burning between species. For instance, terpenes stored in needle resin ducts are likely to encounter different resistance to volatilisation due to differences in the specific characteristics of the epistomatal chambers which are, respectively, unsealed, sealed and buried in needles of *P. nigra, P. sylvestris* and *P. halepensis* (Hanover and Reicosky 1971, Boddi et al. 2002, Kim et al. 2011). These differences in needle morphology may contribute to explaining the reduction of terpenes observed 24 h post-burning in *P. nigra.* Another reason for variable terpene contents may be different respiration sensitivity between species. As the consumption of assimilates increases relative to the photosynthetic production at high temperatures (Farrar and Williams 1991), this could bring about a decrease in the weight of carbohydrates and, thus, an apparent increase in needle terpene concentrations. If the respiration sensitivity to increasing temperature is higher in *P. halepensis* than in the other two species, this may explain the slight increase in terpene concentration in this species 24 h post-burning. Alternatively, the increase in monoterpene concentration in unscorched needles of *P. halepensis* 24 h post-burning may partly reflect systemic induced resistance, triggered by burning needles from lower parts of the canopy, although no data was found in literature to support this hypothesis. Finally, although we carefully selected only 1-year-old unscorched needles and from the same part of the crown, we cannot fully exclude that terpene variation between pre-and post-burning are reflecting differences in light availability between the sampled needles.

Terpene dynamics within the species were modulated by fire severity. Thus, relative concentration changes of mono- and diterpenes increased with the proportion of crown scorched 24 h post-burning. This trend was evident 1 year post-burning, suggesting that the damaged pines were still investing in chemical defences. According with the "growth differentiation balance hypothesis" (GDBH) (Herms and Mattson 1992, Stamp 2003) and the reduction in radial growth detected in *P. halepensis* and *P. sylvestris* (Valor et al. 2015), we hypothesised that the increase in monoterpenes by *P. halepensis* and, to a lesser extent, in *P. sylvestris,* may constrain primary metabolism. Although the rate of increase in diterpenes post-burning was greater in *P. nigra* than in the other two species, *P. nigra* required a greater proportion of scorched crown in order to achieve higher concentrations than those observed pre-burning. Therefore, trees with a greater proportion of scorched crown could be investing in secondary metabolism rather than primary metabolism, although this potential trade-off on carbon investment deserves further research.

6.4.2 Tree physiological conditions and terpene concentration

Needle N concentration was positively associated with the relative concentration change of monoterpenes in the three species and of sesquiterpenes in the case of *P. halepensis* and *P. sylvestris.* As resin canal ducts are limited by N (Björkman et al. 1998), these positive relationships may be explained by an increase in the number and size of the ducts in needles with higher N content. In contrast, we did not detect any effect of pre-burning water status, as estimated by δ^{13}C, for 24 h post-burning terpene concentration change in individual pines.

According to our study, tree-to-tree variation in terpene concentration is known to be naturally high, even over short spatial distances, or when plants grow in the same soil in the same geographic area (Kännaste et al. 2013, Ormeño 2008). Our study reveals, however, that this variation is reduced 1 year post-burning within and between plots. One year post-burning, the terpene concentration was lower than pre-burning, while an increase could be expected given the drier meteorological conditions during the year after burning (Loreto and Schnitzler 2010). In contrast, lower needle δ^{13}C values, compared to pre-burning, suggest a decrease in water competition 1 year post-burning, an increase in the photosynthetic rate or stomatal conductance (Battipaglia et al. 2014), or an improvement in water conditions in the remaining needles of highly scorched trees (Wallin et al. 2003). A lower terpene concentration 1 year after burning differs from other studies (Cannac et al. 2009, Lavoir et al. 2013) comparing burned versus unburned plots. These studies concluded that needle terpene concentration return to normal values one year after fire. They suggested that short-term increases in nutrient availability had minor effects on terpene concentration. The discrepancies with our investigation may be explained by the higher burning intensity in our study which impacted water availability as indicated by δ^{13}C values. In agreement with the growth differentiation balance hypothesis (Herms and Mattson 1992, Stamp 2003), our results showed that the relative concentration change of diterpenes was lower in trees which had an improvement in their physiological condition 1 year post-burning, as suggested by needle δ^{13}C change and changes in needle N concentration. Despite that no relationships were found between mono- or sesquiterpenes regarding the change in δ^{13}C or N, the direct relationship between the relative terpene concentration change and the pre-burning δ^{13}C suggested that the decrease in both terpene groups occurred in pines that were more stressed pre-burning.

6.4.3 Ecological implications

The ecological functions of many mono-, sesqui- and diterpene compounds are still not well understood, although in recent years significant achievements have been made via genetic engineering (Cheng et al. 2007, Loreto and Schnitzler 2010). Likewise, research on terpenes and flammability is generally scarce, though there are some studies that have shown a correlation between both variables (Owens et al. 1998, Alessio et al. 2004, Ormeno et al. 2009). The reduction in terpene concentration 24 h post-burning in the fire-resister *P. nigra* could imply a reduction of needle flammability with respect to pre-burning, strengthened by a reduction in the highly flammable α-caryophyllene (also known as α-humulene) and the increase in bornyl acetate, which is inversely related to flammability (Owens et al. 1998). By contrast, increases of monoand sesquiterpene concentrations in *P. halepensis* may involve greater flammability, which would favour fire reaching the canopy to effectively open the serotinous cones. Specifically, the sPLS-DA showed E-β-ocimene, which is correlated with flammability (Page et al. 2012), as representative of 24 h post-burning samples. In *P. sylvestris*, the poor terpene discrimination in relation to time since burning limits the interpretation of any compound in terms of flammability.

Fire-damaged trees are more vulnerable to insects, especially bark beetles, and infections by root fungus, which contribute to trees susceptibility to beetle attack (Sullivan et al. 2003, Parker et al. 2006). The accumulation of high amounts of monoterpenes 24 h post-burning in the lower fire resistant species (*P. halepensis* and *P. sylvestris*) when fire partially scorches the crowns, might accomplish several functions, such as; effective transport of diterpenes to the affected tissues (Phillips and Croteau 1999), better protection of the photosynthetic apparatus (Vickers et al. 2009) or ensuring the needs for chemical defence against pathogens (Phillips and Croteau 1999). According with this last function, E-β-ocimene and α-thujene with antifungal activity (Bajpai et al. 2007, Deba et al. 2008) appear to correctly classify 24 h post-burning needle samples of *P. halepensis.* Although the discriminant analysis in *P. sylvestris* showed poor classification power, the presence of E-β-ocimene and γ-terpinene also suggests that trees possess a higher resistance to fungus compared to pre-burning (Espinosa-García et al. 1993). In the case of the fire-resistant *P. nigra,* the pre-burning concentration of monoterpenes may be sufficient to cope with biotic stresses related with medium intensity fires. Nonetheless, bornyl acetate seems to represent 24 h post-burning samples conferring resistance to defoliators immediately after fire (Zou and Cates 1995). The high accumulation of diterpenes 24 h post-burning in *P. nigra* as the proportion of the scorched crown increases in respect to the other species, and possibly indicates a better chemical protection against xylophagus insects (LaFever et al. 1994). In *P. nigra* and *P. sylvestris,* the fact that the percentage of sesquiterpenes augmented significantly 1 year post-burning respect to pre-burning, together with the increase in the relative concentration change as crown scorch augmented, might indicate the importance of sesquiterpenes as indirect defences to a wide range of biotic stressors (Phillips and Croteau 1999; Schnee et al. 2006) and, as reported in Lavoir et al. (2013), were representative in repeatedly burned plots. Similarly, our classification found the sesquiterpenes guaiol, α-muurolene and δ-elemene as being characteristic in 1 year post-burning *P. halepensis* needle samples. These compounds might have defensive roles in defoliated trees against insects (Wallis et al. 2008; Liu et al. 2013).

After fire, bark beetles pose a significant threat to trees, especially when a significant amount of the crown has been scorched (Lombardero 2006). Several volatile terpenes such us α-pinene, camphene, and myrcene can be released during PB and facilitate the attack of bark beetles (Coyne and Lott 1976). 24 h post-burning *P. sylvestris* tended to present higher amounts of these terpene compounds, suggesting higher susceptibility to bark beetle attack respect to the other species. Finally, limonene, which is highly toxic for several types of beetles (Raffa et al. 2005), was present in higher amounts in *P. nigra* and *P. halepensis,* suggesting a higher resistance to bark beetle attack for both species 24 h post-burning.

6.5 Conclusion

The concentration of mono- and sesquiterpenes 24 h post-burning was similar to the pre-burning ones in the more fire sensitive species (*P. halepensis* and *P. sylvestris*) and lower in the fire-resistant *P. nigra* species. Terpene dynamics were modulated within the species by fire severity, as indicated by the direct relation between the proportion of scorched crown and the concentration of terpenes 24 h post-burning. As discussed, a combination of morphological and physiological mechanisms may be operating during and short-term after PB, but no clear conclusions may be stated. However, differences in terpene contents as a function of the pine species sensitivity to fire suggest that terpenic metabolites could have adaptive importance in fire-prone ecosystems, in terms of flammability and defence against biotic agents short-term after fire. In agreement with the growth differentiation balance hypothesis (Herms and Mattson 1992, Stamp 2003) trees may be allocating assimilates to growth rather than to defence, as suggested by the remarkable decrease in terpene concentration and the negative relation between terpene concentration and the change in needle δ^{13}C. This decrease in terpene concentration, in turn, could imply a higher susceptibility to fire-related pathogens and insects.

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6.7 Appendix

Table A1: Terpene concentration (Mean ± SE) expressed in μg g^{DM-1} in needles of *P. halepensis*, *P. nigra* and *P. sylvestris* at each time since burning.

						P. halepensis						*P. nigra*						*P. sylvestris*					
						pre-burning		24 h post-burning		1 yr post-burning		pre-burning		24 h post-burning		1 yr post-burning		pre-burning		24 h post-burning		1 yr post-burning	
Nature	Compounds	KI	Ex.KI	Ex.RT	CAS Num.	Mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
MTH	tricyclene	921	918	11.17	508-32-7	-	-	-	-	-	-	-	-	-	-	-	-	481	83.5	443.1	77.6	132.1	13.1
MTH	α-thujene	924	925	11.55	2867-05-2	133.2	19.8	183.9	31.8	7.9	4.5	-	-	-	-	-	-	-	-	-	-	-	-
MTH	α-pinene*	932	928	11.71	80-56-8	2233.7	185.7	3061.2	255.6	753.4	47	9752.1	995.6	6397.6	879.2	999.2	104	8366.2	874.6	7755.7	755.3	1743.8	127.7
MTH	camphene*	946	941	12.35	79-92-5	-	-	-	-	-	-	163.8	45.7	29.4	17.2	<LOD	<LOD	2967.8	450.3	2711.7	404.5	614.7	48.9
MTH	sabinene	969	971	13.85	3387-41-5	1849.9	239.8	2884.2	333.9	1083	117.3	-	-	-	-	-	-	-	-	-	-	-	-
MTH	β-pinene*	974	972	13.91	127-91-3	-	-	-	-	-	-	2744.7	987.1	1550	665.2	141.5	43.4	1155.6	137.5	1063	129.8	115.5	10.9
MTH	β-myrcene*	988	992	14.92	123-35-3	2888.8	548.1	3714.3	550.8	732	124.3	2239.7	530.8	937.9	158.4	182.1	35.6	691.4	87.6	618.4	82.6	123	9.6
MTH	α-phellandrene*	1002	1002	15.42	99-83-2	-	-	-	-	-	-	280.6	68.8	179.9	15.4	<LOD	<LOD	-	-	-	-	-	-
MTH	δ -3-carene*	1008	1008	15.72	13466-78-9	867.9	208.6	1222.4	316.9	184.7	45.2	3204.9	-	1099.8	-	196.5	-	<LOD	<LOD	<LOD	<LOD	10.6	2.4
MTH	α-terpinene*	1014	1015	16.05	99-86-5	53.9	7.7	66.4	8.6	29.2	4.2	17.5	10.8	14.8	7.9	<LOD	<LOD	7.2	3.6	3.7	2.3	<LOD	<LOD
MTH	*p*-cymene*	1020	1023	16.45	99-87-6	10.4	5	5.2	0	<LOD	<LOD	-	-	-	-	-	-	5.9	2.1	4.6	2.8	<LOD	<LOD
MTH	limonene*	1024	1028	16.69	138-86-3	382.8	36.3	530.7	40.7	124.8	11.2	1954.3	274.9	1056.3	209.4	139.1	19.6	384.7	67.4	318.9	55.6	59.6	5.6
MTH	Z-β-ocimene	1032	1044	17.52	29714-87-2	9.5	1.2	15.2	1.4	<LOD	<LOD	13.4	7.9	6.6	2.0	<LOD	<LOD	19.6	2.5	19.2	3.1	<LOD	<LOD
MTH	E-β-ocimene	1044	1052	17.88	13877-91-3	854.6	83.8	1182	71.7	32.5	4.3	2494.1	397.2	1206.6	226.6	44.7	7.3	3326	525.5	3002.2	502.8	127.6	16.4
MTH	γ-terpinene*	1054	1059	18.25	99-85-4	199.8	22.1	228.5	21.7	87.8	7.8	23.9	6.6	7.7	2.5	<LOD	<LOD	27.3	4.9	23.2	4.6	<LOD	<LOD
MTH	terpinolene*	1086	1088	19.70	586-2-9	1771	221.5	2493.2	288.4	519	61.5	53.6	17.1	22.9	5.5	<LOD	<LOD	210.4	71.3	189.5	69.2	15.5	6.1
O-MTH	linalool	1095	1106	20.60	78-70-6	73.7	22.1	<LOD	<LOD	<LOD	<LOD	232.8	98.2	75.6	33.1	<LOD	<LOD	-	-	-	-	-	-
O-MTH	α-campholenal	1122	1132	21.90	91819-58-8	-	-	-	-	-	-	-	-	-	-	-	-	46.9	27	33.8	7.7	<LOD	<LOD
MTH	neo-allo-ocimene	1140	1137	22.17	3016-19-1	-	-	-	-	-	-	21.8	4.1	9.6	5.1	<LOD	<LOD	15.3	2.2	13.1	2.8	<LOD	<LOD
MTH	unkown 1	T^1		22.40		-	-	-	-	-	-	-	-	-	-	-	-	178.6	73.6	100.4	47.3	<LOD	<LOD
MTH	camphor*	1141	1145	22.60	76-22-2	-	-	-	-	-	-	566.8	-	-	-	-	-	<LOD	<LOD	23.6	-	<LOD	<LOD
O-MTH	pinocarvone	1160	1165	23.59	34-41-3	-	-	-	-	-	-	67.8	13	31.6	11.2	<LOD	<LOD	19.9	2.8	17.8	2.9	<LOD	<LOD
O-MTH	terpinen-4-ol	1174	1178	24.27	562-74-3	96	17	34.6	4.8	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
O-MTH	myrtenal*	1195	1197	25.24	23727-16-4	16.9	2.3	14.4	1.7	<LOD	<LOD	-	-	-	-	-	-	11.9	5.2	14.3	4.5	<LOD	<LOD
O-MTH	verbenone*	1204	1206	25.64	80-57-9	-	-	-	-	-	-	-	-	-	-	-	-	24.2	11.1	11.6	4.2	<LOD	<LOD
MTH	unknown 2	T^1		26.40		-	-	-	-	-	-	-	-	-	-	-	-	16.9	8.1	13.8	6.3	<LOD	<LOD
MT der	thymyl methyl ether	1235	1238	27.16	1076-56-8	-	-	-	-	-	-	89.7	36.2	39.5	11.4	2.6	1.3	-	-	-	-	-	-

						P. halepensis						*P. nigra*						*P. sylvestris*					
						pre-burning		24 h post-burning		1 yr post-burning		pre-burning		24 h post-burning		1 yr post-burning		pre-burning		24 h post-burning		1 yr post-burning	
Nature	Compounds	KI	Ex.KI	Ex.RT	CAS Num.	Mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
MT der	linalyl acetate*	1254	1257	28.07	115-95-7	-	-	-	-	-	-	698.4	329.7	496.3	167.9	9.1	6	-	-	-	-	-	-
MT der	bornyl acetate*	1287	1283	29.29	76-49-3 (125-12-2)	4.9	1.6	6.4	2.2	2.9	0.9	48.6	13.5	15.2	4	3.1	0.8	51.1	28.6	38.7	24.4	7.5	3.3
O-MTH	trans-verbenyl acetate	1291	1296	29.90	1203-21-0	-	-	-	-	-	-	69.8	42.5	51.4	24.8	<LOD	<LOD	-	-	-	-	-	-
SQTH	δ-elemene	1335	1335	31.54	20307-84-0	32.3	3.3	43.5	5.5	27.6	2.3	-	-	-	-	-	-	-	-	-	-	-	-
SQTH	1,5,5-Trimethyl-6-methylene-cyclohexene	1338	1335	31.55	514-95-4	-	-	-	-	-	-	152	27.1	108	25.1	32.9	5.4	-	-	-	-	-	-
SQTH	α-cubebene	1345	1348	32.10	31141-66-9	39.6	3.2	54.3	6.3	12.6	1	407.7	76.1	242.7	53	22.9	7.1	37.2	12.4	45.9	13.7	5.4	0.8
SQTH	α-ylangene	1373	1370	33.03	15917-91-6	<LOD	<LOD	<LOD	<LOD	2.1	1.2	107.5	21.5	80.2	21.3	8.4	2.4	5.8	0.7	8.1	2.1	0.8	0.4
SQTH	α-copaene	1374	1374	33.22	13567-62-9	143.5	14.5	196.3	21.8	46.4	4.6	321.4	42.9	180.1	40.4	23.9	4.7	27.2	4.9	28.3	5.3	6	0.8
SQTH	β-bourbonene	1387	1383	33.60	5208-59-3	-	-	-	-	-	-	172	29.3	102.4	23.7	13.5	1.6	32.5	5.5	40.2	12.6	13.1	1.8
SQTH	β-cubebene	1387	1389	33.85	23526-21-8	27.9	3	37.3	4.1	10.1	1.4	232.5	38.3	156.9	30.8	20.7	4.8	20.8	3.7	21.7	4.3	5.2	0.7
SQTH	β-elemene	1389	1392	33.96	33880-83-0	22.9	5	33.1	5.7	14.4	1.7	221.6	43.4	130.7	28.1	37.2	5.9	70.9	16.1	51.7	11.4	28.9	4.5
SQTH	β-caryophyllene*	1417	1417	34.99	17627-40-6	5395.8	445.4	7247.3	690.4	1703.2	97	13990.2	1272.1	7971.6	1384.1	1912.6	213.2	1059.1	134.8	954.9	106.5	454	40.8
SQTH	β-copaene	1430	1432	35.63	18252-44-3	-	-	-	-	-	-	1409.7	205.9	746.4	103.6	259.2	33.7	141.5	28.8	141.3	39.1	58.2	8.6
SQTH	6-epi-B-cubebene	T[1]		35.84		14	2.1	21.4	3.7	11.6	0.9	353.5	56.1	173.1	35.5	107.6	14	27	5.4	20.2	3.8	23.4	4
SQTH	α-caryophyllene	1452	1451	36.40	6753-98-6	775.7	75.1	1161.4	130	476.1	26.7	2506.2	267.7	1326	249.7	425.7	41.8	149.5	22.1	137.2	16.2	99.8	8.4
SQTH	E-β-farnesene	1454	1458	36.72	18794-84-8	16.2	5.2	37.4	8.5	15.4	1.4	367	226.4	298.6	146.6	<LOD	<LOD	-	-	-	-	-	-
SQTH	*cis*-β-farnesene	1457	1457	36.87	28973-97-9	6	1.9	11.3	2.9	11.5	0.8	202.4	39.6	98.7	22.2	44.6	6.3	16.4	3.9	13.3	2.5	10.5	2
SQTH	γ-muurolene	1478	1476	37.44	30021-74-0	8.2	1.6	24.8	5.2	7.3	0.6	2144.2	606.1	1114.8	316.3	101.2	38.8	50.1	7.8	71.3	19.8	24.3	3.9
SQTH	D-germacrene	1484	1480	37.60	105453-16-5	63.5	17.3	111.4	26.4	19.8	1.8	12274.1	2008.5	6358.6	1252.2	1369.6	170.3	628.6	156.5	525.5	150.8	316.8	47.4
others	2-Phenylethyl 2-methylbutanoate	1486	1489	37.74		-	-	-	-	-	-	217.8	57.1	103.5	35.2	104.7	12.1	-	-	-	-	-	-
SQTH	β-selinene	1489	1491	38.08	17066-67-0	50.9	8	89.9	10.4	90.6	11.1	511.1	192.5	1003	684.3	<LOD	<LOD	-	-	-	-	-	-
others	2-phenylethyl 3-methylbutanoate	1490	1493	38.16	140-26-1	677.2	62.5	1012.5	83	345	37.6	2010.9	639.7	1252.4	331.5	<LOD	<LOD	-	-	-	-	-	-
SQTH	bicyclogermacrene	1500	1494	38.21	24703-35-3	-	-	-	-	-	-	-	-	-	-	-	-	94.4	18.7	86.7	17	57	8.9
SQTH	α-muurolene	1500	1500	38.44	31983-22-9	56.7	13.3	105.6	20.3	82.3	7.2	600.6	146.5	276	74.3	<LOD	<LOD	32.1	10.3	29.4	6.4	4.5	2.5
SQTH	δ-amorphene	1511	1503	38.58	189165-79-5	-	-	-	-	-	-	184.9	50.8	105.3	34.7	165	65.9	8.1	3.5	14.3	8.2	4.8	2.8

						P. halepensis						*P. nigra*						*P. sylvestris*					
						pre-burning		24 h post-burning		1 yr post-burning		pre-burning		24 h post-burning		1 yr post-burning		pre-burning		24 h post-burning		1 yr post-burning	
Nature	Compounds	KI	Ex.KI	Ex.RT	CAS Num.	Mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
SQTH	γ-cadinene	1513	1516	39.05	39029-41-9	-	-	-	-	-	-	1558.6	234.7	921.6	187	129.1	23.8	-	-	-	-	-	-
O-SQT	cubebol	1514	1518	39.14	23445-02-5	57.7	5.9	71.9	9	145.9	12.9	-	-	-	-	-	-	145.3	36.6	147.6	28.9	65	8.1
SQTH	δ-cadinene	1522	1526	39.45	60305-17-1	48.0	6.4	81	10.9	32.5	2.2	2057.3	383.4	1231.5	276.5	182.2	49.2	158.8	51.1	161.7	40.4	58.8	10.4
SQTH	α-cadinene	1537	1542	40.08	82468-90-4	-	-	-	-	-	-	420.5	134.4	209.3	112.7	<LOD	<LOD	-	-	-	-	-	-
SQTH	unkown 3	T^1		40.53		49.2	7.7	67.2	8.2	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
O-SQT	elemol	1557	1558	40.69	639-99-6	165.2	17.5	190.6	31.3	98	9.9	-	-	-	-	-	-	-	-	-	-	-	-
O-SQT	germacrene-4-ol	1574	1578	41.49	72120-50-4	<LOD	<LOD	<LOD	<LOD	12.3	2.1	676.1	142	447.7	127.3	111.4	17.4	347.1	150.4	212.5	64.4	120.9	40.2
O-SQT	caryophyllene oxide	1582	1582	41.64	1139-30-6	71.2	8.1	101.8	16.4	105.3	16.8	360.3	76.1	325.4	105.2	84.4	20.9	79	52.1	27	17.5	99.8	36.1
O-SQT	guaiol	1600	1609	42.65	489-86-1	163.6	18.4	223.3	33.2	110.8	10.5	-	-	-	-	16	-	-	-	-	-	-	-
O-SQT	τ-cadinol	1638	1643	43.90	65700-78-9	-	-	-	-	-	-	195.6	49.2	96.3	35.5	53.9	11.3	<LOD	<LOD	<LOD	<LOD	17.8	4.6
O-SQT	α-cadinol	1652	1654	44.29	481-34-5	-	-	-	-	-	-	220.7	37.7	123.4	34.3	384.5	94.8	21.3	12.8	20.1	9.3	169.8	30
DTH	isopimara-9(11),15-diene	1905	1900	53.01	39702-28-8	-	-	-	-	-	-	48.1	6.6	29.2	6.5	<LOD	<LOD	-	-	-	-	-	-
DTH	β-springene	T^1	1922	53.71	70901-63-2	134.3	11.6	194.3	18.9	39.7	3.5	298.1	37.6	134.4	30	5.9	3.3	29.1	4.6	23.9	3	<LOD	<LOD
DTH	cembrene	1937	1932	54.04	57345-20-7	1961.8	616.6	3182.4	813.4	788.8	198.2	672.1	584	460	381.3	<LOD	<LOD	-	-	-	-	-	-
DTH	(3Z)-cembrene A	1965	1965	55.15	73246-00-1	492.4	126	697.9	164.9	110.1	30	-	-	-	-	-	-	-	-	-	-	-	-
O-DT	13-epi-manool oxide	2009	2008	56.54	77171-57-4	-	-	-	-	-	-	2522.9	428.7	4906.9	3343.8	1974.4	1954.2	-	-	-	-	-	-
DTH	abieta 8,12 diene	2022	2020	56.90	122712-77-0	86.7	10.7	119.9	23.8	31.6	4.5	-	-	-	-	-	-	-	-	-	-	-	-
O-DT	thunbergol	2047	2055	57.94	63568-83-2	13240.2	4191.8	15652.4	4661.4	3812.2	1078.5	229.2	54.2	167.6	52.3	35	9.6	-	-	-	-	-	-
DTH	abietatriene	2055	2059	58.08	19407-28-4	24.1	12.1	50.6	0.7	<LOD	<LOD	-	-	-	-	-	-	-	-	-	-	-	-
DTH	abietadiene	2087	2084	58.81	35241-40-8	128.4	24.9	188	59.7	56.8	9.8	174.7	45.4	124.6	26.2	11.7	3.3	32	5	41.6	10	6.5	3.4
DT der	abieta-(8(14),13(15)-diene	2153	2148	60.79	57119-12-7	98.9	10.5	134.4	17.8	44.5	6.5	-	-	-	-	-	-	-	-	-	-	-	-
DT der	sandaracopimarinal	2184	2179	61.73	3855-14-9	33.8	4.4	38.5	6.4	18.6	3.9	874.8	193.6	535.3	164.9	109.6	29.2	-	-	-	-	-	-
DT der	methyl sandaracopimarate	T^1	2249	63.73	1686-54-0	187	19.8	213.3	22.5	52.8	7.2	566	81.6	377.2	91.8	43.6	8	-	-	-	-	-	-
O-DT	dehydroabietal	2274	2273	64.39	13601-88-2	977.9	374.4	1279	453	318.3	132.5	-	-	-	-	-	-	-	-	-	-	-	-
DT der	methyl isopimarate	2297	2298	65.09	1686-62-0	87.2	10.8	412.8	312.8	152.1	90.4	-	-	-	-	-	-	-	-	-	-	-	-
DT der	methyl levopimarate	2306	2311	65.45	3513-69-7	3526.2	442.7	4646.4	534.1	890.3	156.7	-	-	-	-	-	-	-	-	-	-	-	-

						P. halepensis						*P. nigra*						*P. sylvestris*					
						pre-burning		24 h post-burning		1 yr post-burning		pre-burning		24 h post-burning		1 yr post-burning		pre-burning		24 h post-burning		1 yr post-burning	
Nature	Compounds	KI	Ex.KI	Ex.RT	CAS Num.	mean	se	Mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se	mean	se
DT der	methyl dehydroabietate	2341	2339	66.20	1235-74-1	774.1	122.8	919.6	145.2	223.3	31.5	435.6	93.6	294.5	70.9	18.8	2.4	176.2	72.2	204.2	78.6	36.2	13
DT der	methyl abietate	2385	2388	67.52	127-25-3	241.6	33.3	304.1	45.3	82.3	12.3	-	-	-	-	-	-	-	-	-	-	-	-
DT der	methyl neoabietate	2443	2440	68.90	3310-97-2	1521.3	259	2855.4	1006.8	479.7	75.7	778.7	319.4	367.4	100.9	66.6	21.8	169.4	34.2	162.6	37.7	54.5	11.5
DT der	methyl abietatrien-7,13,15-oate	T[1]	2498	70.39	54850-32-7	452.9	63.6	548.6	74.5	204.7	30	669.2	328.1	237.5	76.3	37	14.6	287.4	41.9	296.9	43.1	101.3	15.4

T[1], tentatively identified

LOD, under 0.2 limit of detection

MTH: monoterpene hydrocarbon; SQTH: sesquiterpene hydrocarbon; DTH: diterpene hydrocarbon; O: oxygenated compounds; der: derivative compounds; others: other than terpenes; KI: Kovats retention index; Ex.KI: experimental Kovats retention index, Exp. RI: experimental retention index; CAS number: chemical abstract service number, *: available commercial standards

Table A2: Total terpene content, monoterpene, sesquiterpene and diterpene (mean ± se) expressed as percentage respect to total terpene content across time since burning for each pine species.

	n (trees)	Terpene content (mg g^{DM-1})	Monoterpenes (%)	Sesquiterpenes (%)	Diterpenes (%)
P. halepensis					
pre-burning	20	41.2±5.8a	30.4±2.5a	22.9±3.2a	45.6±4.0a
24 h post-burning	20	55.2±6.7a	31.3±2.7a	21.2±2.6a	45.7±3.8a
1 year post-burning	20	13.3±1.3b	28.4±2.5a	27.0±2.9a	42.2±3.8a
P. nigra					
pre-burning	19	65.6±7.1a	31.1±2.2a	59.4±2.1a	8.9±0.9a
24 h post-burning	19	40.0±5.8a	31.8±2.7a	54.4±3.7a	13.0±4.0a
1 year post-burning	19	8.7±1.7b	21.4±1.9b	70.7±3.7b	7.8±4.0a
P. sylvestris					
pre-burning	19	21.4±2.6a	83.2±1.4a	13.2±1.4a	3.3±0.6a
24 h post-burning	19	19.6±2.4a	83.0±1.4a	12.9±1.2a	3.7±0.6a
1 year post-burning	18	4.7±0.3b	62.8±1.8b	32.2±1.9b	4.5±0.9a

Differences between time since burning (TSB) for each pine species were tested using LMM (linear mixed model) considering TSB as fixed and plot as a random factor. Different letters in the TSB column indicate significant differences ($P<0.05$) in total terpene content and groups of terpenes for each pine species using a Tukey post-hoc test.

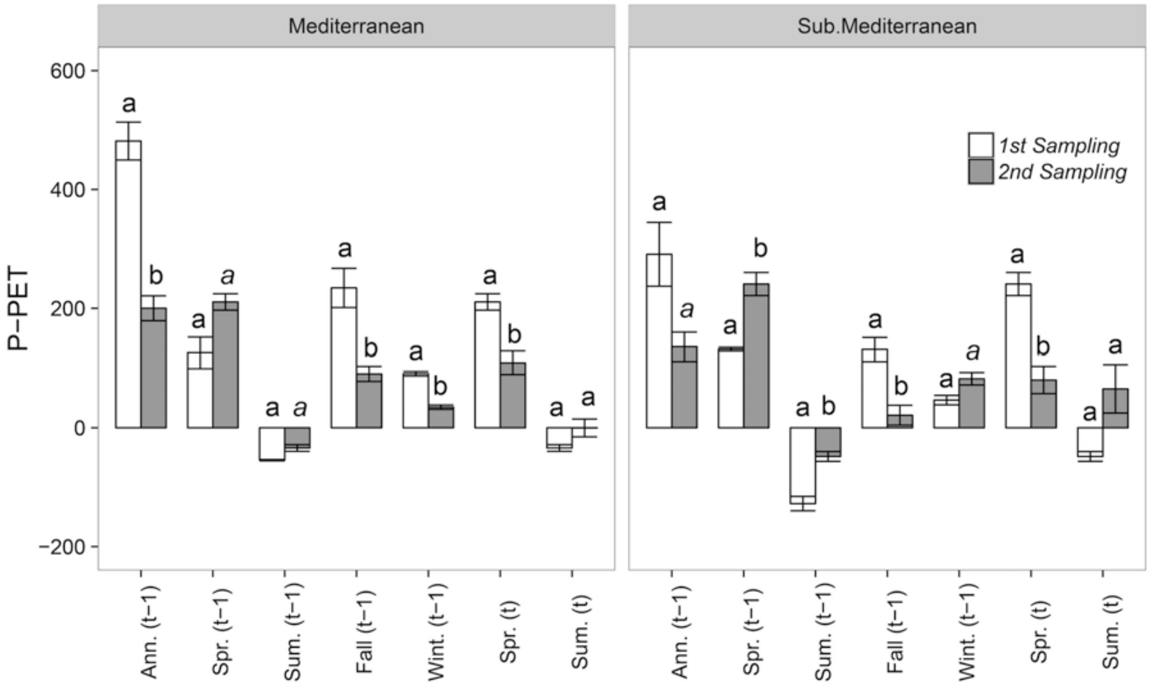


Figure A1: Drought index (*P-PET*) (mean ± se) for each annual or seasonal period before (*t-1*) and during (*t*) the first (pre-burning and 24 h post-burning sampling times) and second sampling (1 year post-burning samples) at the *P. halepensis* stand (Mediterranean region) and at the *P. nigra* and *P. sylvestris* stands (Sub-Mediterranean). For each period, different letters indicate statistically significant differences ($P<0.05$) between sampling times using a Student's t-test; italic letters represent a marginal significant difference ($0.05<P<0.01$). Periods in relation to sampling times are classified as the following: Ann., from June before the sampling year (t-1) to May of the sampling year (t); Spr., spring; Sum., summer; Wint.,Winter.

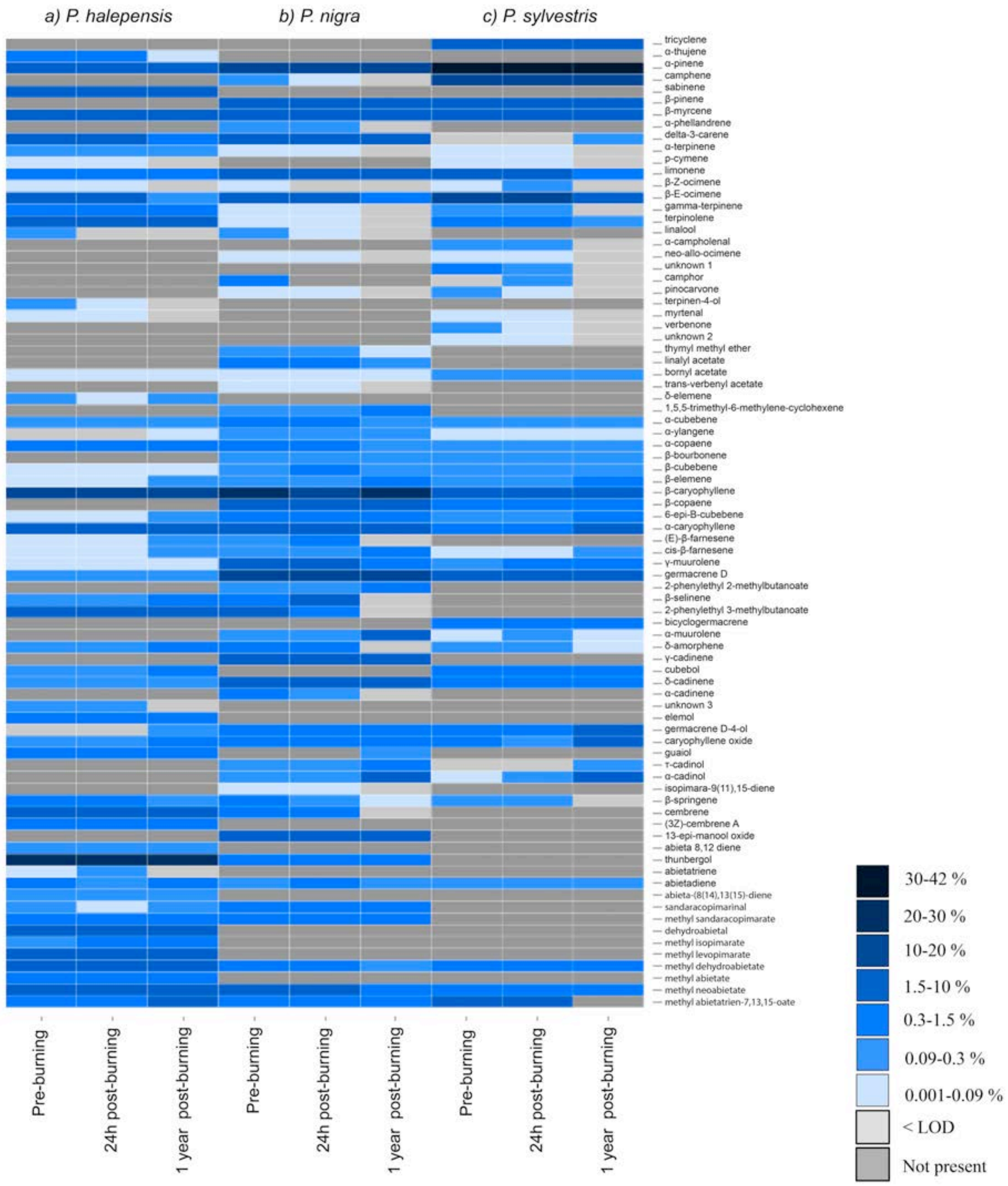


Figure A2: Mean terpene compound percentage respect to total terpene content in needles of *P. halepensis*, *P. nigra* and *P. sylvestris* at each time since burning.

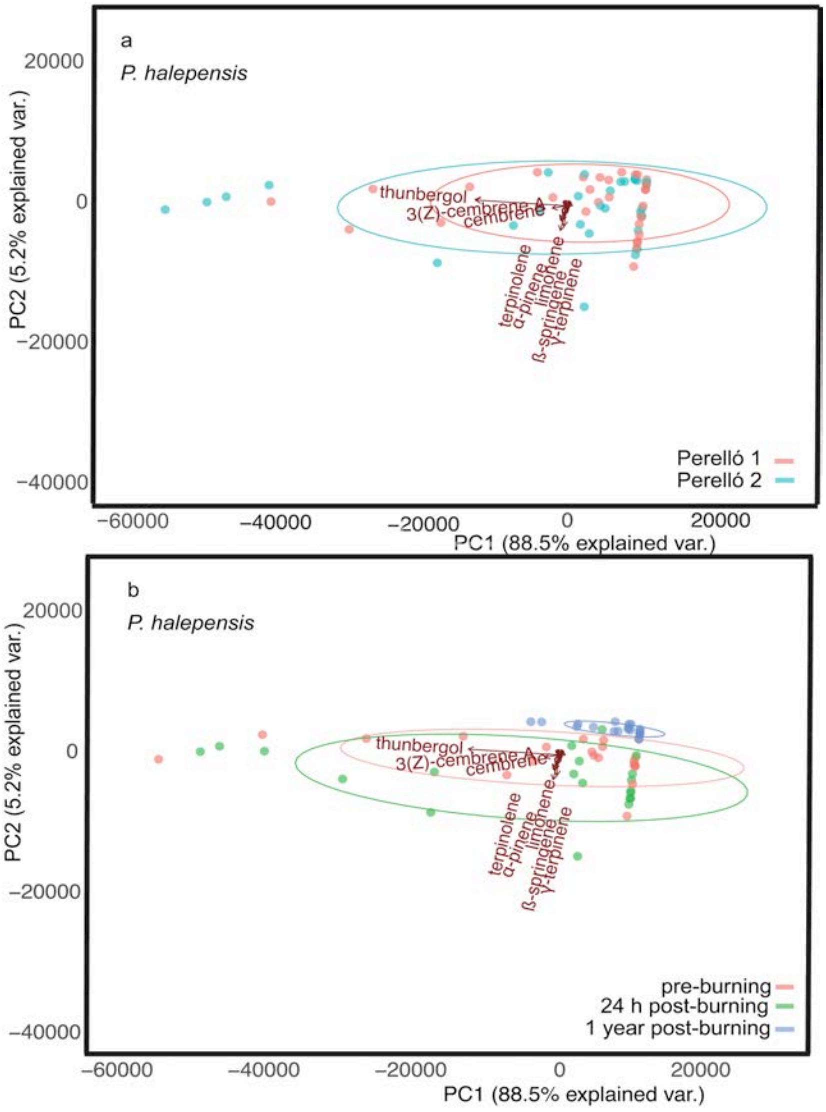


Figure A3: Biplot of trees and terpenes compounds of PCA analysis based on terpene content (μg g^{DM-1}) in the needles of *P. halepensis*. (a), each symbol represents a sample of a tree of sites of Colladetes 1 (pink) and Colladetes 2 (blue), while in (b) each symbol represents the sampling times of a tree pre-burning (pink), 24 h post-burning (green) and 1 year post-burning (blue). Only the 8 terpene compounds responsible for contributing the most to the formation of the axes are shown.

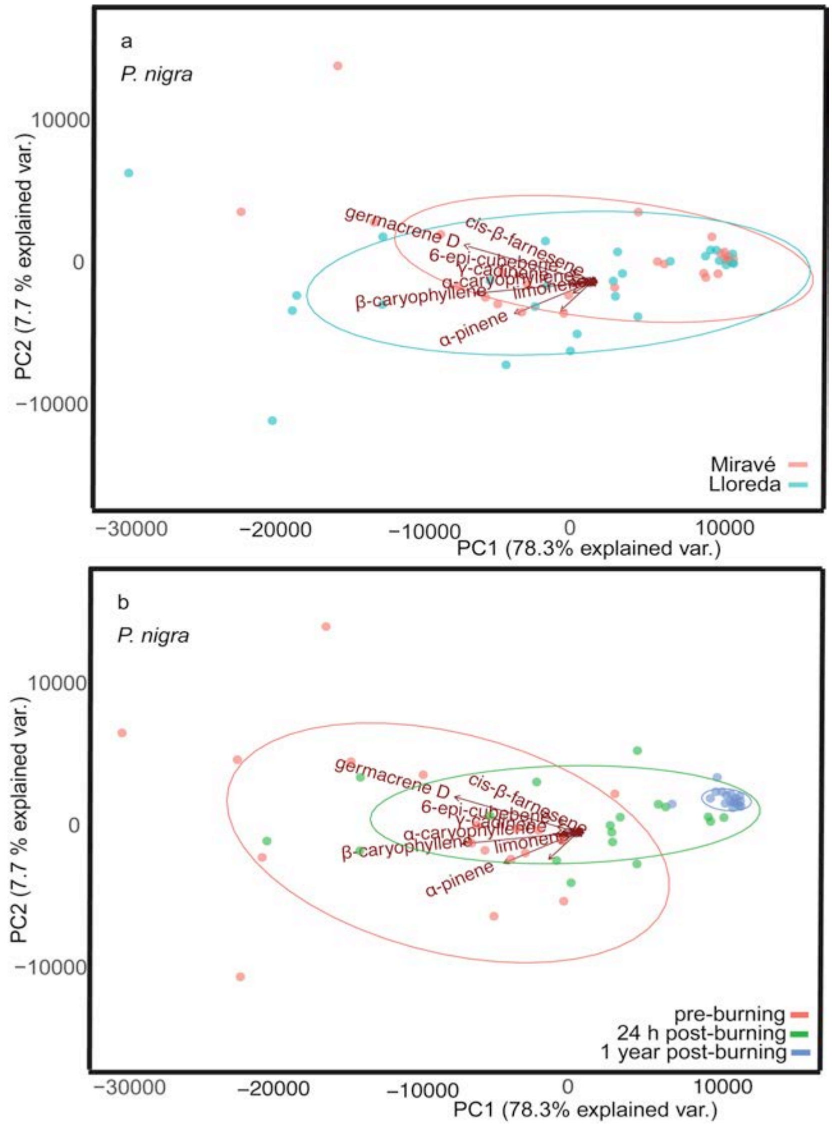


Figure A4: Biplot of trees and terpenes compounds of PCA analysis based on terpene content (μg g^{DM-1}) in the needles of *P. nigra*. (a), each symbol represents a sample of a tree of sites of Miravé (pink) and Lloreda (blue), while in (b) each symbol represents the sampling times of a tree of pre-burning (pink), 24 h post-burning (green) and 1 year post-burning (blue) times. Only the 8 terpene compounds responsible for contributing the most to the formation of the axes are shown.

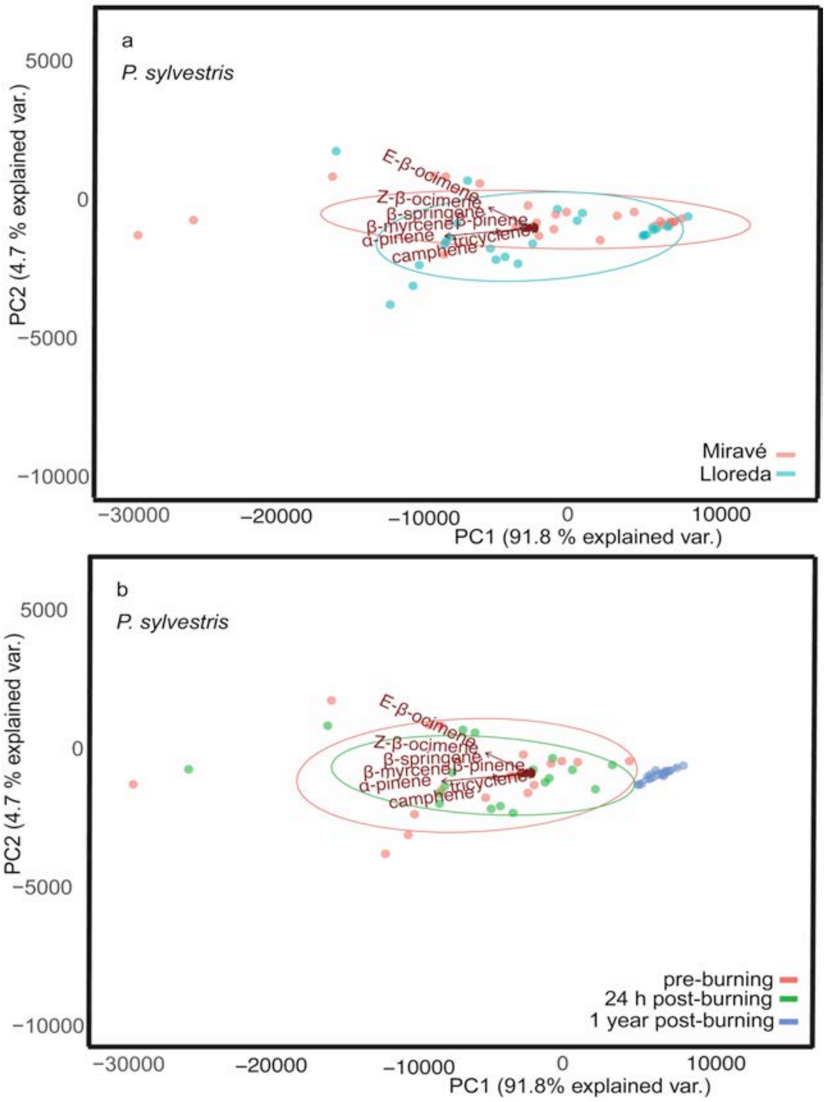
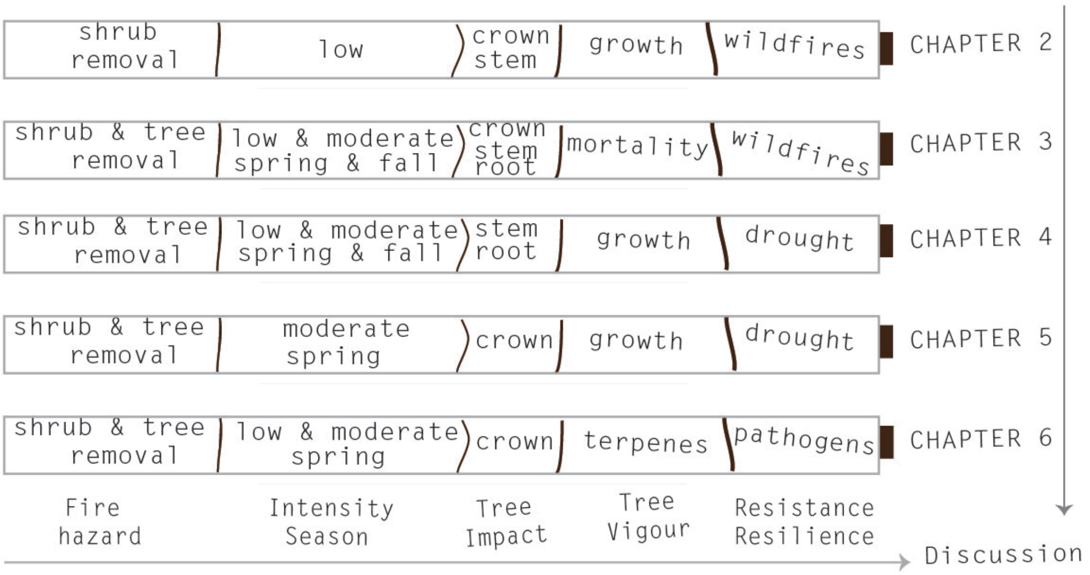


Figure A5: Biplot of trees and terpenes compounds of PCA analysis based on terpene content (μg g^{DM-1}) in the needles of *P. sylvestris*. (a), each symbol represents a sample of a tree of sites of Miravé (pink) and Lloreda (blue), while in (b) each symbol represents the sampling times of a tree of pre-burning (pink), 24 h post-burning (green) and 1 year post-burning (blue) times. Only the 8 terpene compounds responsible for contributing the most to the formation of the axes are shown.

Chapter 7

General discussion



Fire hazard	Intensity Season	Tree Impact	Tree Vigour	Resistance Resilience	
shrub removal	low	crown stem	growth	wildfires	CHAPTER 2
shrub & tree removal	low & moderate spring & fall	crown stem root	mortality	wildfires	CHAPTER 3
shrub & tree removal	low & moderate spring & fall	stem root	growth	drought	CHAPTER 4
shrub & tree removal	moderate spring	crown	growth	drought	CHAPTER 5
shrub & tree removal	low & moderate spring	crown	terpenes	pathogens	CHAPTER 6

This doctoral thesis contributes to increasing the understanding of the role played by different window prescriptions on the tree vigour of Mediterranean pines, providing valuable information about their growth response and vulnerability to biotic and abiotic stressors over time. Applying the fire regime concept to PB management contributes to the understanding of the effects on tree functioning of specific management characteristics, such as burning season and fire intensity and related fire impacts. The thesis shows that the damage inflicted by fire affects the primary and secondary metabolism of pine individuals depending on the pine species type, tree characteristics, individual performance before burning and meteorological conditions before and after burning. All these features affect the primary and secondary metabolisms of individuals and can sometimes cause their death. This knowledge improves the PB design to achieve the management objectives and minimize negative impacts on tree vigour.

7.1 The role of tree resistance on pine mortality and growth after burning

Tree size plays a major role in defining the immediate impact of PB on tree vigour. Initial tree mortality due to stem injury increases with increasing bole scorch proportion but also with decreasing tree size, as generally reported (Woolley et al. 2012) (Chapter 3). Similarly, crown status, hence tree resistance, largely determines the immediate impact of low intensity PBs on tree growth (Sutherland et al. 1991), with dominant trees having less growth reduction immediately after PB than suppressed trees (Chapter 2). These results suggest that smaller trees are more exposed to cambial injuries due to their thinner bark, especially at higher stem heights (Fernandes et al. 2012). In fact, the diameter at 50 cm height predicts tree mortality better than diameter at 20 or 130 cm height, indicating a higher susceptibility to cambium injury at this stem height than either at the tree base, where bark is thicker, or at breast height, where combustion time is lower. In suppressed pines, a reduction in tree growth could be due to the combined effects of fire damages, not only to the cambium, but also to the crown due to their lower crown base heights. A low crown base height could imply a larger portion of crown foliage scorched, which would lead to higher rates of transpiration and inefficient photosynthesis.

As time since burning increases, dominant trees grow better than suppressed trees. This is probably because they have greater protection against fire due to their thicker bark and greater crown base height (Chapter 2). Three years after PB, small trees still had a higher probability of dying than big trees (Chapter 3). However, delayed tree mortality occurred in trees with less crown volume scorched and a larger diameter at 20 cm height compared to those that died during the first year (Chapter 3). Tree mortality after several years occurred mainly in girdled trees (Hood, 2010). In these trees, fire does not usually affect the xylem and the tree can continue to transport water to support the crown; however, the products of photosynthesis cannot be transported down to the roots and the trees eventually suffer from carbohydrate starvation. This supports the hypothesis that delayed tree mortality is mainly due to injuries in the basal stem and/or in the root collar, even though trees were bigger than those that died during the first year, and therefore more resistant.

7.2 Season and fire severity: effect on mortality and growth

The probability of tree mortality increased strongly when fire damaged the upper foliage of the tree (crown volume scorched $> 80\%$), as observed in most studies (see Fernandes et al. (2008) and Woolley et al. 2012 for review). In our study, initial tree mortality was slightly higher after spring than after fall burns (Chapter 3). Our results revealed that for a given crown volume scorched, trees are more vulnerable to dying during periods of high physiological activity (i.e. spring) than during the dormant season (i.e. fall). This can be expected because a larger number of buds will survive during dormant season burns because old needles protect buds, which are going to produce new foliage in the next season (Ryan 1990). This explains the fact that even with 95% of the crown volume scorched, the probability to death was reduced by half after fall burns compared to spring burns. The contrasting effect of burning season on tree mortality can be explained not only by differences in the tree physiological activity but also by carbohydrate storage during the burning season as discussed by Harrington (1993). Scorched trees burned during the fall season, without damage in the roots, can continue with belowground growth and metabolism activities. This is because carbon reserves are expected to be highest during dormant seasons (Ursino et al. 1968), favouring foliage production in the next season, and thus restoring photosynthesis capacity. Field observations suggested that this was the case in our study areas as re-foliage was only observed in pines burned in fall. After spring burns, the lower amount of non-structural carbohydrates (Oberhuber et al. 2011) and the loss of photosynthetic capacity in scorched trees (Chapter 5) could lead to a reduction in the photosynthesis-to-respiration ratio and, therefore, if the growth and chemical defence demand is not fulfilled, the probability of mortality increases (Harrington 1993). Indeed, at the same spring sites, we found that trees with a greater proportion of the crown scorched invested more in chemical defence than undamaged trees (Chapter 6). The effect of burning season on initial tree growth response was the opposite to the pattern found for tree mortality: growth reduction at the year of PB occurred in unscorched pines burned during the fall season (Chapter 4). Rather than an effect of tree phenology, it is likely that the prolonged heating in fall burns caused higher basal stem girdling and killed small pine roots, and consequently, reduced growth in the year of PB.

After 3 years post-burning, pines had a higher likelihood of dying after fall burns than after the spring ones, in contrast to initial tree mortality. Delayed tree mortality occurring after a fall burn has been previously reported in some studies (Perrakis and Agee 2006; Ryan 1998; Thies et al. 2005). However, other studies have found higher mortality 4 and 10 years after spring burns (Ryan 1998; Swezy and Agee 1991) than after fall burns. Basal stem girdling or root collar damage, expressed by white ashes instead of stem injury indicators, best explained delayed tree mortality. Thus, higher levels of delayed tree mortality in fall burns may be explained by higher fire severities at the base of the trees rather than an effect of tree phenology because in fall burns the combustion time and forest floor moisture content was higher than in spring burns, as previously observed in several studies (Perrakis and Agee 2006; Schwilk et al. 2006; Thies et al. 2005). In contrast to the initial growth response, the growth of surviving pines was higher after fall burns than after spring burns (Chapter 4). We attributed this response to the higher release of competition due to the higher mortality found after fall burns compared to spring burns. Further, the negative impact suffered at the year of PB after fall burns did not constrain tree functioning and pines were able to benefit from the release in tree competition.

7.3 Fire severity as a driver of changes in the growth and terpene production of pines species

In the short term after PB, growth remained unaffected in *P. nigra* ssp. *salzmannii*, whereas the moderately fire-resistant *P. sylvestris* suffered growth reductions after low intensity burns (Chapter 2). The growth reduction in *P. sylvestris* was likely related to cambium damage due to its thinner bark, especially higher up in the trunk, compared with *P. nigra* (Fernandes et al. 2008; Tapias et al. 2004). In the case of *P. halepensis*, we observed a reduction in growth immediately after PB probably because the traits of this fire evader species are less favourable for coping with low-intensity fires (Keeley 2012). This reduction was associated with its greater crown vulnerability to fire as it has the lowest canopy base height among Mediterranean pine species (Mitsopoulos and Dimitrakopoulos 2007); however, the retrospective nature of the study meant we could not measure crown scorch and this remained speculative (Chapter 2).

When PB impacted the crowns of the pines, we found that scorched dominant *P. halepensis* significantly decreased growth 1 year post-burning. This was accompanied by a decrease in δ^{13}C compared to control and unscorched pines, suggesting lower

WUE$_i$ (Chapter 5). A reduction in WUE$_i$ could be a consequence of a decrease in the photosynthetic capacity of the surviving needles or an increase in stomatal conductance. No change in the δ^{18}O suggests that the decrease in WUE$_i$ was related to a reduction in photosynthetic capacity (Scheidegger et al. 2000), presumably due to the needle scorch. In unscorched *P. sylvestris*, we found higher δ^{13}C in the year of the fall PB with respect to pre-burning levels, which suggests a lower stomata conductance due to the fire stress. However, in *P. nigra* the δ^{13}C values were similar to those before burning (Chapter 4). After fall burns, growth was reduced as the minimum bole scorch height increased, suggesting a dysfunction of xylem tissue, which may have limited xylem water flow. This probably reduced stomata conductance and carbon assimilation rates (higher δ^{13}C). Bär et al. (2018) showed that *P. sylvestris* is especially vulnerable to the structural changes in the xylem caused by the fire. Thus, *P. sylvestris* seems more vulnerable, even though it had lower values for crown and stem injury indicators than *P. nigra*, suggesting that the tissues are less protected from heat (Chapter 3).

In the short term after burning, the growth response patterns observed could also be driven by a different investment in chemical defence in the pine species, depending on the fire damage experienced and their degree of fire resistance. Terpene dynamics within the species were modulated by fire severity (Chapter 6). Thus, relative concentration changes of mono- and di-terpenes increased with the proportion of crown scorched 24 h post-burning, and this was still evident 1-year post-burning. The accumulation of high amounts of monoterpenes 24 h post-burning in the lower fire-resistant species (*P. halepensis* and *P. sylvestris*) when fire partially scorches the crowns, might accomplish several functions, such as effective transport of diterpenes to the affected tissues (Phillips and Croteau 1999), better protection of the photosynthetic apparatus (Vickers et al. 2009) or ensuring the needs for chemical defence against pathogens (Phillips and Croteau 1999). This would partially explain the reduction in growth observed in scorched *P. halepensis* and in stem injured *P. sylvestris*. In the case of the fire-resistant *P. nigra*, which did not reduce growth, the pre-burning concentration of monoterpenes may be sufficient to cope with biotic stresses related to medium intensity fires with less need for investing in chemical defence. Therefore, pines with a greater proportion of scorched crown could be investing in secondary metabolism rather than primary metabolism because scorched pines reduced growth. This potential trade-off in carbon investment deserves further research. After fire, bark beetles pose a significant threat to trees, especially when a significant amount of the crown has been scorched (Lombardero et al. 2006). Several volatile terpenes such as α-pinene, camphene and myrcene can be released during fire and facilitate the attack of bark beetles (Coyne and Lott 1976). 24-h post-burning *P. sylvestris* tended to show higher amounts of these terpene compounds, suggesting higher susceptibility to bark beetle attack with respect to the other species. Limonene, which is highly toxic for several types of beetle (Raffa et al. 2005), was present in higher amounts in *P. nigra* and *P. halepensis*, suggesting a higher resistance to bark beetle attack for both species 24 h post-burning. This may explain why outside our plot but in one of the study sites we found some *P. sylvestris* with evident signs of bark beetle attack.

Mid-term post-burning growths were good for dominant *P. nigra* ssp. *salzmannii* and neutral for *P. halepensis*, whereas dominant *P. sylvestris* grew less than expected without burning after low intensity burns. In Chapter 2, BCH_{local} was used as an approximation of flame length and fire intensity, with higher intensities translating to higher soil organic matter mineralization and therefore higher post-fire soil fertility. BCH_{local} had a significant positive impact on *P. nigra* growth, a non-significant negative effect on *P. sylvestris* and a positive effect on *P. halepensis*. This suggests that the effects due to fire damage were more important for *P. sylvestris* than the presence of fire-induced fertilization and removal of understory in the years following PB, while, in *P. halepensis* a trade-off between these two processes could have occurred. It is important to note that we found little evidence of fire-induced fertilization (Chapter 5) as we did not find variations in N concentration and δ^{15}N of unscorched 1-year-old needles with respect to pre-burning levels (Chapter 5). This suggests that short-term growth responses are driven by fire damage and the effect of immediate understory removal rather than the fertilization effect.

The reduction of tree competition following fire-induced mortality gained importance as the main driver of a positive effect of fire on tree growth, especially as fire damage decreased (Chapter 4 and Chapter 5). For the same level of fire stem injury, the positive effect of release from tree competition was greater for *P. nigra* than for *P. sylvestris*, suggesting that *P. sylvestris* has a higher fire sensitivity (Chapter 4). However, at second year after PB no differences in growth between the two species were found, suggesting that *P. sylvestris* made a fast recovery probably due to higher water availability caused by fire thinning and supported by the more negative values of δ^{13}C. This result partially contradicts the results observed in Chapter 2, where *P. sylvestris* showed a tendency to reduce growth during the following years after burns. This could suggest that in the case of *P. sylvestris*, PB can impact its growth positively as long as the fire significantly reduces tree-to-tree competition. However, the damage caused by the fire to sensitive tissues may prevail in burns that do not change stand density, which constrains the pine's functioning. Furthermore, in *P. nigra* and *P. sylvestris* the release in tree competition had a higher positive effect on the latewood of pines with a high pre-burning competition index. This suggests that in pines with an improved condition due to increased environmental resources caused by PB-induced thinning, a long-lasting differentiation of latewood tracheids in summer and fall months could have occurred. In addition, in the case of *P. halepensis*, a higher competition release clearly translated into enhanced BAI_{rel} levels as the crown volume scorched decreased. According to Scheidegger et al. (2000) the reduction in δ^{18}O observed in scorched and unscorched pines indicates an increase in stomatal conductance compared to control trees. Reduced canopy interception and transpiration due to fire-induced thinning probably resulted in an enhancement of water availability, stomatal conductance and therefore tree growth, emulating similar processes that occur under mechanical thinning (Giuggiola et al. 2016).

The terpene concentration was lower 1-year post-burning than pre-burning, although an increase could be expected given the drier meteorological conditions during the year after burning (Loreto and Schnitzler 2010). A lower terpene concentration 1-year after burning differs from other studies (Cannac et al. 2009; Lavoir et al. 2013) that compared burned versus unburned plots. These studies concluded that the needle terpene concentration returns to normal values 1 year after the fire. They suggested that short-term increases in nutrient availability had minor effects on the terpene concentration. The discrepancies with our investigation may be explained by the higher burning intensity in our study. In agreement with the Growth Differentiation Balance Hypothesis (GDBH) (Herms and Mattson 1992; Stamp 2003), our results showed that the relative concentration change of diterpenes was lower in trees that had an improvement in their physiological condition 1 year post-burning, as suggested by changes in the needle δ^{13}C and N concentrations. Lower δ^{13}C, compared with pre-burning, suggests a decrease in water competition 1-year post-burning, an increase in the photosynthetic rate or stomatal conductance (Battipaglia et al. 2014), or an improvement in water conditions in the remaining needles of highly scorched trees (Wallin et al. 2003). However, we found that in scorched pines, lower δ^{13}C reflects a reduction in photosynthetic capacity that leads to growth reductions (Chapter 5), and therefore the previous interpretation needs to be considered with caution. However, it is possible that scorched pines with less than 60% of the crown volume scorched had lower δ^{13}C but maintained or increased carbon assimilation rates like those before PB, as the reduction in photosynthesis capacity occurred in pines with 60% of the crown volume scorched. Moreover, it needs also to be take it into account that the change in δ^{13}C and crown volume scorched did not interact to explain 1-year post-burning terpene concentration. In agreement with the GDBH (Herms and Mattson 1992, Stamp 2003) trees may be allocating assimilates to growth rather than to defence, as suggested by the remarkable decrease in terpene concentration and the negative relationship between terpene concentration and the change in needle δ^{13}C and N concentration. This decrease in terpene concentration, in turn, could imply a higher susceptibility to fire-related pathogens and insects.

7.4 The effects of burning and drought on tree growth

When burning precedes drought, the effects of drought on tree growth will be reduced, whereas a severe drought does not promote a synergistic effect on tree growth during a subsequent burning. In *P. halepensis*, the δ^{18}O-derived increase in stomatal conductance in pines managed by PB coincided with the driest year of the entire series (2015), highlighting the role of burning in reducing competition when resources, especially water, are limiting (Keyser et al. 2010; Skov et al. 2004). Thus, a moderate-intensity PB can help to overcome episodes of intense drought and compensate for the negative effects of future drought episodes as long as PB results in a relevant reduction in tree competition. In fact, the lack of a significant reduction in tree competition after low intensity burns could explain why PB did not cancel out the plausible negative effects of cumulative drought events (e.g. 2005, 2006 and 2007) on post-PB growth in *P. sylvestris* (Chapter 2). Furthermore, post-burning latewood to earlywood ratio (LW/EW) of *P. nigra* and *P. sylvestris* was higher in pines burned in fall compared to pines burned in spring and control pines. This may be due to the use of available carbohydrates to replenish dead tissues after fall burns, which were more severe than spring burns, rather than to the development of earlywood cells (Cown 1977) and/or to the higher reduction in tree competition after the fall burns that resulted in an increase in LW. This increase in LW/EW compared to pre-burning levels may confer a higher resistance to embolism and provide minimal hydraulic conductivity at lower water potentials (Mayr and Cochard 2003), which can act as water storage (Domec and Gartner 2002) in future upcoming stresful events. Recently, Alfaro-Sánchez et al. (2018) found a higher latewood proportion in burned *P. halepensis* compared to unburned ones at sites with high water availability but not in those that were water-limited. A PB performed the year after a dry year did not reduce the growth (i.e. growth resilience) after two consecutive stressful events (drought and burning) in comparison with pines growing in control stands. Surprisingly, the impact of PB on individuals with low resistance to drought (i.e. growth resistance), in spite of being additive, was irrelevant. We speculate that desiccation of finer roots in the less resistant individuals during a drought could explain why the impact of PB was nearly negligible in the less resistant individuals and similar to that of control pines. Moreover, burning in the fall after the dry year seems to increase the growth resilience of pines compared with those burned in spring or left unburned as a consequence of the higher release of competition due to the higher mortality found after fall burns compared to spring burns (Chapter 3).

To sum up, the main results discussed are summarized in the following Figure 7.1.

Figure 7.1: Effect of the species factor, fire injury and competition release on pine's survial, growth and terpene production. In *P. halepensis*, mortality/survival was not modelled. The sign of the effect is shown with a black arrow for each species and burning season. For each temporal scale, the total effect of fire severity variables and the species factor on tree vigour variables is computed by the sum of the number of black arrows. The net effect at the temporal scale is also highlighted in red, when the effect is negative, and in green when positive.

	Spring			Fall	
	Pinus halepensis	*Pinus sylvestris*	*Pinus nigra*	*Pinus sylvestris*	*Pinus nigra*
Survival					
Overall effect		↓(x8)	↓(x6)	↓(x8)	↓(x6)
Initial		↓(x5)	↓(x4)	↓(x4)	↓↓↓
Species		↓↓	↓	↓↓	↓
Crown injury		↓↓	↓↓	↓	↓
Stem/Basal injury		↓	↓	↓	↓
Delayed		↓(x3)	↓↓	↓(x4)	↓↓↓
Species		↓↓	↓	↓↓	↓
Crown injury		↓	↓	○	○
Stem/Basal injury		○	○	↓↓	↓↓
Growth					
Overall effect	↑↑	○	○	↑	↑ (x4)
Short-term	↓↓	○	○	↓	○
Crown injury	↓↓				
Stem/Basal injury		○	○	↓	○
Mid-term	↑ (x4)	○	○	↑↑	↑ (x4)
Crown injury	○				
Stem/Basal injury		○	○	○	○
Competition release	↑ (x4)	○	○	↑↑	↑ (x4)
Terpene production					
24 h post-burning	↑ (x4)	↑↑	↑		
Species	↑↑	○	↓↓		
Crown injury	↑↑	↑↑	↑		
1 year post-burning	↓(x4)	↓(x4)	↓(x4)		
Species	↓↓↓	↓↓↓	↓↓↓		
Crown injury	↑	↑	↑		
Competition release	↓↓	↓↓	↓↓		

↓, negative effect

↑, positive effect

○, no effect

7.5 References

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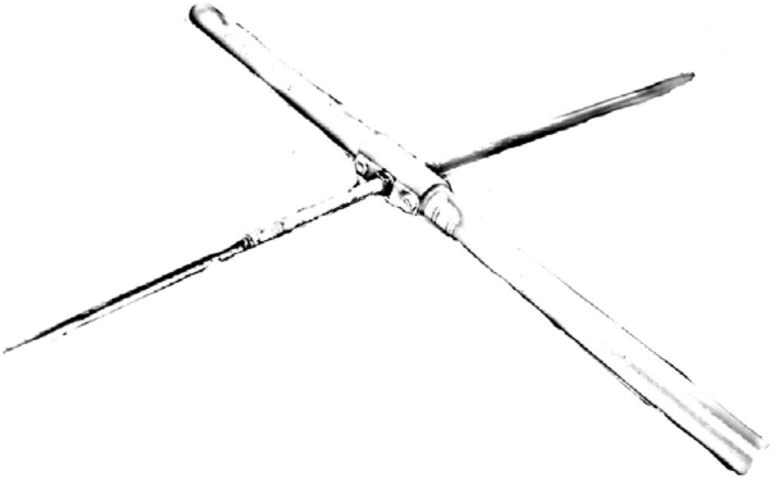
Chapter 8

Conclusions



1. Tree size largely determines the impact of burning on tree growth and mortality. Smaller trees are more susceptible to being damaged by burning than bigger trees. However, as time since burning increases, delayed tree mortality occurs in bigger trees compared to initial tree mortality.

2. Burning season contributes to explaining initial pine mortality because the probability of a tree dying is greater after spring than after autumn burns, for a certain level of crown volume scorched.

3. The effect of burning on tree mortality depends on the pine species: *P. nigra* has a lower probability of dying than *P. sylvestris*. This confirms the higher fire-resistance of the former species compared to the moderate fire-resistance of *P. sylvestris*.

4. Post-burning tree growth variations were related to differences in the fire-resistance of the pine species, the degree of fire severity, tree characteristics and tree performance prior to fire.

5. An increase in tree growth after burning can be expected if the balance between competition reduction and tissue damage is positive. In the fire-resistant *P. nigra*, prescribed burning had little or no effect on the short-term growth response. As time passed, this species was clearly benefited from the competition release induced by burning. In the moderately fire-resistant *P. sylvestris*, the growth immediately after the less severe burns was not affected but growth can be reduced when PB results in stem and basal stem injuries. Stem injury does not constrain mid-term growth as long as burning results in a relevant reduction in tree-to-tree competition. The fire evader *P. halepensis*, reduces growth in the short term after burning when a significant proportion of the crown is scorched due to a reduction in the photosynthetic capacity. As time passes, growth can be augmented through an increase in stomatal conductance, as the release in competition increases and crown damage diminishes.

6. A prescribed burn performed the year after a dry year does not seem to reduce the growth resilience of pines in comparison with pines in unburned stands. If burning induces a significant release in competition after a dry year, the resilience capacity of pines might even increase in comparison with unburned pines. The increase in production of dense latewood may, in addition, favour the resistance to cavitation of pines managed with prescribed burnings. A prescribed burn performed before a dry year can contribute to mitigating the effects of drought episodes on surviving pines.

7. The concentration of mono- and sesqui-terpenes 24 h post-burning was similar to the pre-burning concentrations in the more fire-sensitive species (*P. halepensis* and *P. sylvestris*) and lower in the fire-resistant *P. nigra* species. This suggests that terpenic metabolites could have adaptive importance in fire-prone ecosystems, in terms of flammability and defence against biotic agents in the short term.

8. In all pine species, the concentration of mono- and di-terpenes augmented as crown injury increased 24 h and 1 year post-burning, even though terpene concentrations 1 year post-burning were much lower than 24 h post-burning. This suggests that as time since burning passes, damaged pines continue to invest in chemical defences.

9. The remarkable decrease in total terpene concentration 1 year post-burning and the negative relationship between terpene concentration and indicators of resource availability suggest that pines may be allocating assimilate to growth rather than to defence. This decrease in terpene concentration, in turn, could imply a higher susceptibility to fire-related pathogens and insects.