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

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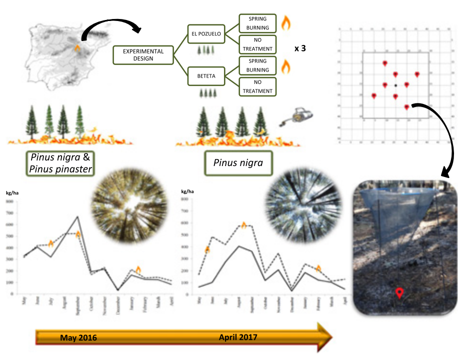
HIGHLIGHTS

- Short-term effects of low-intensity prescribed burns on litterfall biomass were studied.
- There is an increase of needle fall 3–4 months immediately after burning.
- One year after burning, there are no differences on litterfall biomass between burned and unburned plots (control).
- Burning could reduce production of inflorescences.
- Low intensity prescribed burning did not produce changes in LAI (Leaf Area Index).

GRAPHICAL ABSTRACT



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ABSTRACT

Fire severity, defined as the magnitude of fire effects in an ecosystem, is a key factor to consider in planning management strategies for protecting forests against fire. Although prescribed burning has been used as a fuel reduction tool in forest ecosystems, it is quite limited in the Mediterranean region. Furthermore, little is known about how tree crowns are affected by prescribed underburning aimed at reducing fire severity in conifer stands. As part of an ongoing study to assess the effects of prescribed burning on the tree canopy, litterfall is currently being monitored in a network of experimental plots located in mixed (*Pinus nigra* and *Pinus pinaster*) and pure (*P. nigra*) conifer stands in the Cuenca Mountains (Castilla La Mancha, Spain). A total of 12 study plots (30 m × 30 m) were established in a completely randomized experimental design to determine the effect of burning, with 2 treatments: no burning (control) and burning (i.e. with three replicate plots for each treatment and site). Burning was conducted in May 2016. In each plot, 8 litterfall collectors were installed at regular intervals, according to international protocols (ICP Forests), and all biomass falling into the collectors is being monitored monthly. The specific objective of this study is to assess how prescribed burning affects the rate of generation of foliar and non-foliar litterfall biomass due to the fire. In addition, the Leaf Area Index was estimated before burning and one year later to verify possible changes in the structure of the stands. This information could be used to help minimize the negative impacts of prescribed underburning on litterfall. To our knowledge, this study represents the first attempt to evaluate the effect of prescribed burning on litterfall biomass in Europe.

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1. Introduction

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

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

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

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

Pure stands of *Pinus nigra* Arn. ssp. salzmannii (Spanish black pine) and mixed stands of *Pinus pinaster* Ait. (Maritime pine) and *Pinus nigra* Arn. ssp. salzmannii have been chosen. Spanish black pine is one of the most widely distributed species in Central and Eastern Spain and Southeast France. The convention for Conservation of European Wildlife and Natural Habitats (European Union, 1996) has classified stands of this species as "habitat of European interest" (Lucas-Borja et al., 2016). Spanish black pine has disappeared from some regions, mainly as a result of wildfires or interspecific competition (mostly with *Pinus pinaster*) (Barbero et al., 1998). Spanish black pine is a Mediterranean tree species that is resistant to low intensity surface fires due to the thick bark characteristic of the species (Fulé et al., 2008; Touchan et al., 2012; Pausas, 2015). However, recurrent large wildfires are threatening the permanence of *P. nigra* forests in some Mediterranean areas as a result of the almost total lack of capacity of this non-serotinous pine to regenerate after fire (Espelta et al., 2003; Ordóñez et al., 2005; Fernandes et al., 2008; Christopoulou et al., 2013; Lucas-Borja et al., 2017). In addition, the species releases its seeds early in the year, at the beginning of spring, and therefore no seed bank is maintained after summer wildfires (Ordóñez et al., 2005). According to Lucas-Borja et al. (2016), little is known about the impact of prescribed burning on the natural regeneration of Spanish black pine. On the other hand, maritime pine is a conifer from the Western Mediterranean basin that covers >1,200,000 ha of land in Spain as a dominant species, under different elevation, climate and soil conditions, resulting in a high level of genetic variation (Alía et al., 1996). Important reforestation projects carried out during the 20th century, motivated by different factors, led to the wide expansion of this species. Fire is an important threat to maritime pine, but also plays a crucial role in the perpetuation of natural stands (Fernandes and Rigolot, 2007; Cruz and Fernandes, 2008). Some provenances of this species have a thick bark (that allows survival of the trees after low-intensity fire) and also produce serotinous cones (Fernandes and Rigolot, 2007). The provenance under study (from the Cuenca Mountains) does not produce serotinous cones (Alía et al., 1996). In Spain, the total area occupied by maritime pine that was burned between 1974 and 2010 was 674,055 ha, representing 31% of the total burned forest area. The area of Spanish black pine burned in the same period represents 4% of the total (Vázquez De La Cueva, 2016).

Several authors have reported higher stability of mixed species stands than of monocultures (e.g. Schütz et al., 2006; Felton et al., 2010). However, others argue that damage to mixed stands will only be reduced in the same proportion of the stable species (Lupke and Spellmann, 1997). Exploration of the resilience of pure and mixed stands to perturbations such as prescribed burning is therefore essential to establish recommendations for management and fire prevention strategies for these stands.

To our knowledge no previous studies have evaluated the effect of prescribed burning on litterfall biomass. This study is a first attempt to compare litterfall patterns in pure stands of *P. nigra* and mixed stands of *P. nigra* and *P. pinaster* in the short term, after prescribed burning, and one year after the disturbance. The hypothesis proposed in this study is that low-intensity prescribed burning in pure stands of *Pinus nigra* and mixed stands of *Pinus nigra* and *Pinus pinaster* does not affect crown trees, to corroborate this hypothesis the specific aims of this study were as follows: (1) to analyze the effect of prescribed burning on the quantity, patterns and fractions of the litterfall in pure *P. nigra* and mixed *P. nigra* and *P. pinaster* stands in the Cuenca Mountains; (2). to compare changes in LAI one year after treatment.

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

2. Materials and methods

2.1. Study site

As mentioned the study is part of a wider study being carried out in the community of Castilla La Mancha (Central-Eastern Spain). Two sites in the Northwestern zone of the Cuenca Mountains (Central System), El Pozuelo (40° 33′ 36″ N/002° 15′ 56″ W) and Beteta (40° 33′ 06″ N/002° 06′ 32″ W), were chosen for study. The average altitude is 1015 and 1294 m a.s.l. in El Pozuelo and Beteta respectively. Both areas have slopes between 3 and 10%. Both stands are natural; the Beteta stand was managed in 1971, but the El Pozuelo stand has not been managed.

Spanish black pine forests in the Cuenca Mountains have traditionally been managed using the shelterwood method, with a shelter-phase of 20–25 years and a rotation period of 100–125 years (Tiscar Oliver et al., 2011). Controlled grazing and hunting-based management are also carried out in the stands.

The soils in the Cuenca Mountains are calcareous: shallow and rendzina soils predominate in the most steep areas and calcimorphic brown soils in the flat areas and troughs (Lucas-Borja et al., 2017). In both study areas the soil is classified in the order Inceptisol according to the Soil Taxonomy classification (USDA, 1987) used in the National Soil Atlas of Spain (1: 200,000).

The climate is classified as humid Mediterranean (Allué, 1990). The mean annual temperature is 10.7 °C (the warmest month is July with an average of 19.8 °C and the coldest month, December with 2.0 °C), and the mean annual precipitation is 537 mm (56 mm in summer months).

The area of El Pozuelo is included in the public utility forest CU217 and is characterized by being a mixed stand of *P. nigra* and *P. pinaster*. The mean tree density is 627 trees/ha, with *P. nigra* comprising 89% and *P. pinaster* 11% of the trees (Table 1). The mean tree height is 12.2 m with the first live branch appearing at a mean height of 6.4 m. The mean breast diameter is 19.8 m. The understory vegetation is mainly dominated by *Cistus laurifolius* L., *Genista scorpius* Sibth. & Sm. ex Boiss, *Prunus spinosa* L., *Arrhenatherum bulbosum* and *Bupleurum rigidum* L. Beteta is included within in public utility forest CU179 and is a pure stand of *P. nigra*, with *Genista scorpius* Sibth. & Sm. ex Boiss. and *Rosa canina* L. occurring as the main understory species. The mean density of trees is 1286 trees/ha, of mean height 13.2 m, and first live branch appearing at a mean height of 8.2 m. The mean breast diameter is 18.8 m. Both zones have an irregular distribution of pine regenerated under canopy, with density ranging from 78 to 11,611 seedlings/ha. The mean shrub cover in all plots is in a range of 5–20%, and the fuel model is TU1 (El Pozuelo and Beteta) according to the classification of Scott and Burgan (2005).

2.2. Experimental design

2.2.1. Plot establishment and collection of pre-burn data

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

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

2.2.2. Burning

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

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

2.2.3. Litterfall

The litterfall collection system was designed in accordance with the recommendations outlined in the Manual of the United Nations Economic Commission for Europe under the project entitled "International Co-operative Program on Assessment and Monitoring of Air Pollution Effects on Forests " (ICP Forests) (Ukonmaanaho et al., 2016), to guarantee the quality and quantity of the sample. The system consisting of 8 collectors per replicate plot was installed immediately after the prescribed burning. The spatial distribution is systematic and covers the entire working area in the 30 m × 30 m plot, to guarantee the representativeness of the sample (Fig. 1a). The catchment area of the collectors is always horizontal, to correct for the effect of the slope in collecting material. The catchment area is 0.38 m^2. The bag is 0.75 m deep, to prevent the sample from being lost as a result of wind action. The collectors were placed at a height of 1.2 m from the ground to

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

Z	PN	PT	Dt	DPn	DPp	Pn	Pp	Ht	H1lb	D30	D60	D130	G	Bmt
			Trees/ha	Trees/ha	Trees/ha	%	%	m	m	cm	cm	cm	m^2/ha	cm
El Pozuelo	P2C P5C P9C	Control	563	526	37	93	7	11.50	5.09	20.84	19.69	18.66	22,54	1.43
			(74.0)	(89.8)	(17.0)	(4.2)	(4.2)	(3.2)	(1.9)	(4.2)	(3.8)	(3.7)	(7.5)	(0.5)
El Pozuelo	P3B P4B P6B	Burned	881	770	111	89	11	12.25	7.06	22.59	21.27	19.75	33.74	1.92
			(227.0)	(148.0)	(88.9)	(7.8)	(7.8)	(0.8)	(0.6)	(1.2)	(1.0)	(1.0)	(9.7)	(0.7)
Beteta	B1C B3C B9C	Control	1456	1456	0	100	0	10.07	6.05	16.30	15.10	13.84	25.46	1.29
			(507.1)	(507.1)	(0.0)	(0.0)	(0.0)	(3.2)	(3.3)	(1.8)	(1.7)	(1.6)	(2.1)	(0.4)
Beteta	B2B B5B B6B	Burned	1215	1215	0	100	0	12.70	7.53	21.45	19.98	18.18	39.14	1.85
			(208.6)	(208.6)	(0.0)	(0.0)	(0.0)	(0.8)	(1.3)	(2.1)	(1.9)	(2.0)	(6.7)	(0.7)

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

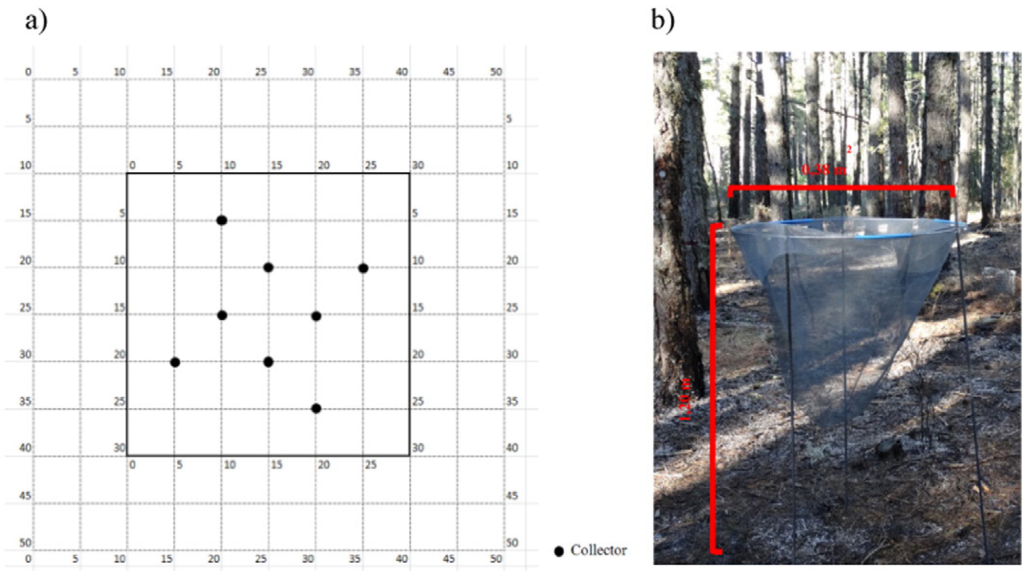

Fig. 1. (a) Example of plot design (distances in m) and standard distribution of collectors in the sampling plots. (b) Collector installed in El Pozuelo.

enable adequate drainage by gravity and to prevent capture of biomass from shrub strata. They were anchored firmly to the ground to provide greater resistance to the inclement conditions (snow, wind or rain) or other types of disturbance (cattle, wildlife, etc.). The fiberglass mesh (pore size, 2 mm) (Fig. 1b) was firmly attached to the structure. Fiberglass provides resistance against external weather conditions and also ensures drainage while preventing loss of smaller elements, such as needles. The material was collected monthly to prevent decomposition of the biomass or the chemical leachate. This frequency of collection also ensures the easy identification of, for example, the fine elements of the flower and terminal shoots, which are rapidly compressed. Samples were taken to the laboratory on the same day and oven-dried at 65 °C to constant weight (i.e. for at least 48 h). The samples from each plot were then combined and the fractions separated. For the purposes of this study, these fractions were established: needles, branches of diameter < 2 cm, bark, cones, seeds, inflorescences, lichens, leaves of other species and miscellaneous.

2.2.4. Leaf Area Index (LAI)

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

2.3. Statistical analysis

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

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

All above analyses were carried out with STATISTICA 10.0 (Statsoft Inc., Tulsa, USA).

3. Results

3.1. Burning

The mean daily air temperature during prescribed burning was 21.5 °C in El Pozuelo and 20.4 °C in Beteta; the relative humidity was 48% in El Pozuelo and 33% in Beteta. In relation to fire behavior, rate of spread in El Pozuelo was 0.65 ± 0.10 m/min and 0.76 ± 0.09 m/min in Beteta. The flame height was 53.7 ± 15.6 cm and 43.7 ± 7.8 cm and the flame length 62.0 ± 18.0 cm and 50.4 ± 9.0 in El Pozuelo and Beteta, respectively. The characteristics of the prescribed burnings in each plot are shown in Table 2. The maximum flame temperature reached during burning was high (between 559 and 787 °C), but only small proportion of monitored trees (6–13%) were affected by cambial heating. It was generated a moderate maximum scorched height in the leeward side

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

Z	PN	PT	T	RH	WS	FLc	TmM litter	TmM soil	HmMS	TMxB	TMxC	t > 60 °C	Trees T > 60 °C
–	–	–	°C	%	m/s	%	°C	°C	cm	°C	°C	s	%
El Pozuelo	P3B	Burned	20.17	53.12	0.17	59.31	157.25	37.80	32	559.5	151.5	322	6.66
El Pozuelo	P4B	Burned	21.97	47.47	0.81	61.19	261.87	31.10	66	668.0	43.0	0	0.00
El Pozuelo	P6B	Burned	22.42	42.61	1.32	75.21	372.13	37.20	113	787.0	67.0	112	13.33
Beteta	B2B	Burned	18.80	34.77	0.79	77.22	394.30	42.00	151	688.5	81.5	174	6.66
Beteta	B5B	Burned	20.65	33.10	0.71	65.16	384.57	40.30	150	754.5	82.0	554	6.66
Beteta	B6B	Burned	21.69	30.15	0.82	62.23	303.96	30.00	178	605.5	61.5	217	6.66

Note = Z: zone; PN: plot number; PT: plot type; T: temperature air; RH: relative humidity; WS: mean wind speed; FLc: percentage of fuel load consumed; TmM Litter: maximum mean temperature in litter horizon; TmM Soil: maximum mean temperature in soil; HmMS: mean maximum scorch height; TMxB: maximum bark temperature; TMxc: maximum cambium temperature; t > 60°: time that the temperature in the cambium was higher than 60 °C; Trees T > 60 °C: percentage of trees in which the temperature in the bark was higher than 60 °C.

of trunks (70 cm in El Pozuelo and 160 cm in Beteta), but in most cases lower than the height of the first living branch (Table 1). Crown damage caused by the effects of fire, was observed in different proportions of trees in each plot generating a moderate scorched crown range of 0–13% in El Pozuelo and 1–17% in Beteta however, no trees were completely scorched, except seedlings under canopy.

3.2. Litterfall

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

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

3.3. Litterfall main fractions

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

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

In El Pozuelo, two months immediately after the burning (July) the amount of needles was significantly higher (little significance) in the burned than in the control plots ($F = 6.97$, $p = 0.0576$) and from September onwards the differences were not significant (Fig. 4a). This highlights the main short-term effect of burning on the needle fraction, which was masked in the analysis of total litterfall (Fig. 3a). In Beteta, the maximum was reached in August and in the burned remains in September. The amount of needles was always higher in the burned plots

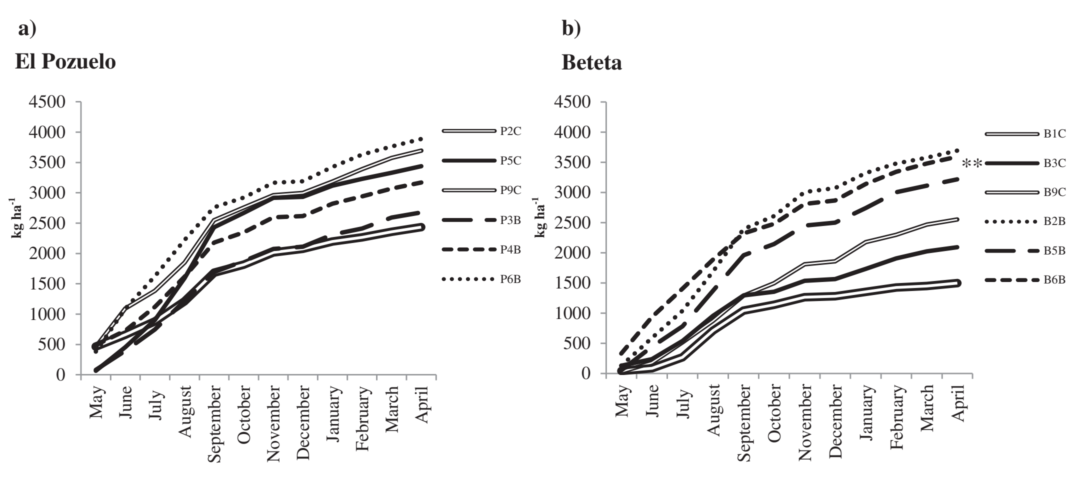

Fig. 2. Total litterfall accumulated per plot (control and burned) during the period May 2016–April 2017 in (a) El Pozuelo and (b) Beteta. Control in solid line and burned in dashed line. Statistical significance of difference between treatments (control vs burned) is indicated as **$p < 0.05$.

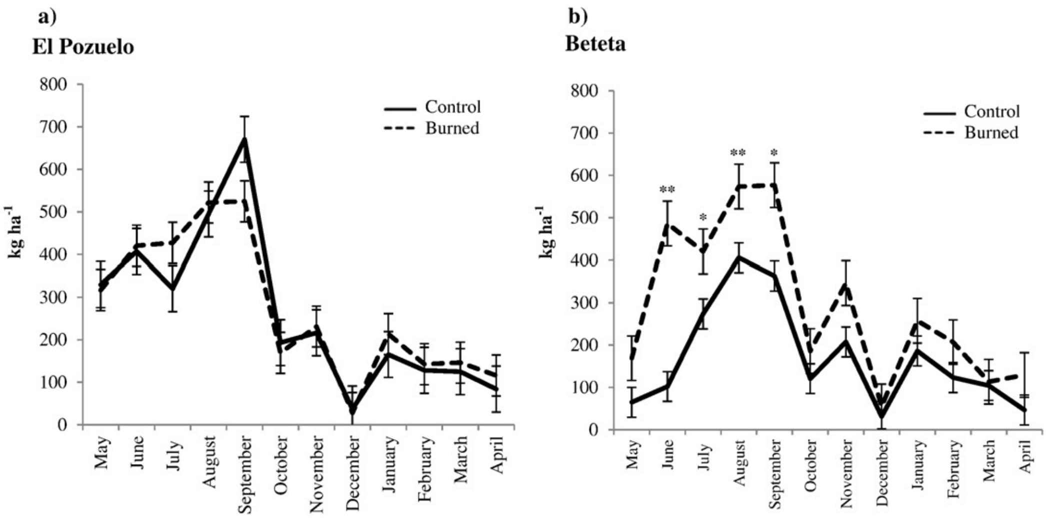

Fig. 3. Variation in mean litterfall in all control and burned plots, collected during the period May 2016–April 2017 in (a) El Pozuelo and (b) Beteta. Control in solid line and burned in dashed line. Litterfall accumulation is expressed as the mean ± standard error, n = 6. Statistical significance of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

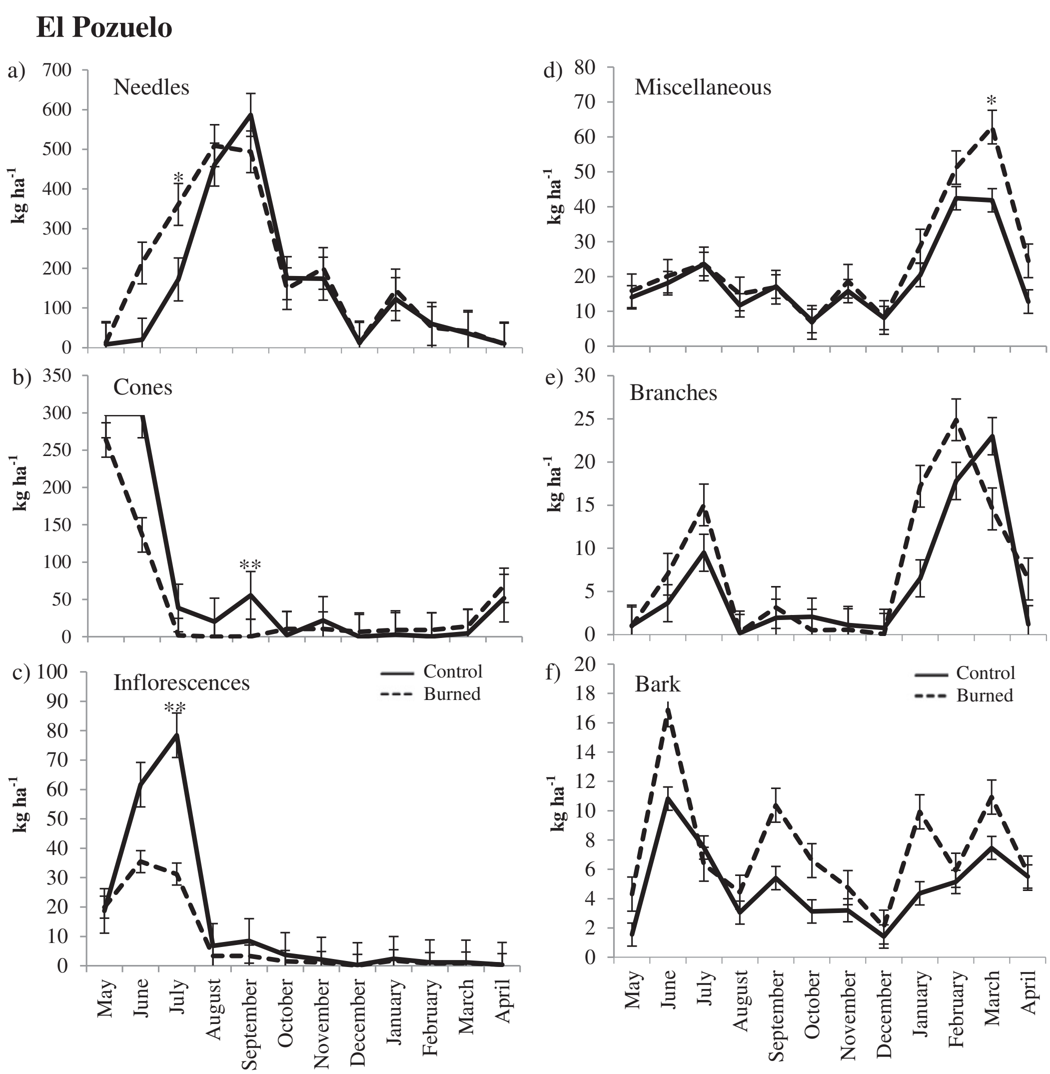

Fig. 4. Changes in the main litterfall fractions (a) needles, (b) cones, (c) inflorescences, (d) miscellaneous, (e) branches and (f) bark, collected during the period May 2016–March 2017 in El Pozuelo. Control in solid line and burned in dashed line. Litterfall production is expressed as the mean ± standard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

than in control plots confirming the significant differences in accumulate (Fig. 2b) and total litterfall (Fig. 3b) but only significant differences were detected from July (F = 149.17, p = 0.0002) to September (F = 5.37; p = 0.0532) and during winter (January, F = 25.35; p = 0.0073).

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

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

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

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

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

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

3.4. LAI

In El Pozuelo, the LAI decreased in all plots (control and burned), except P6B. The most important decrease occurred in plot P3B (decrease of 0.4 points), as also observed in one of the control plots (P9C, 0.38). In

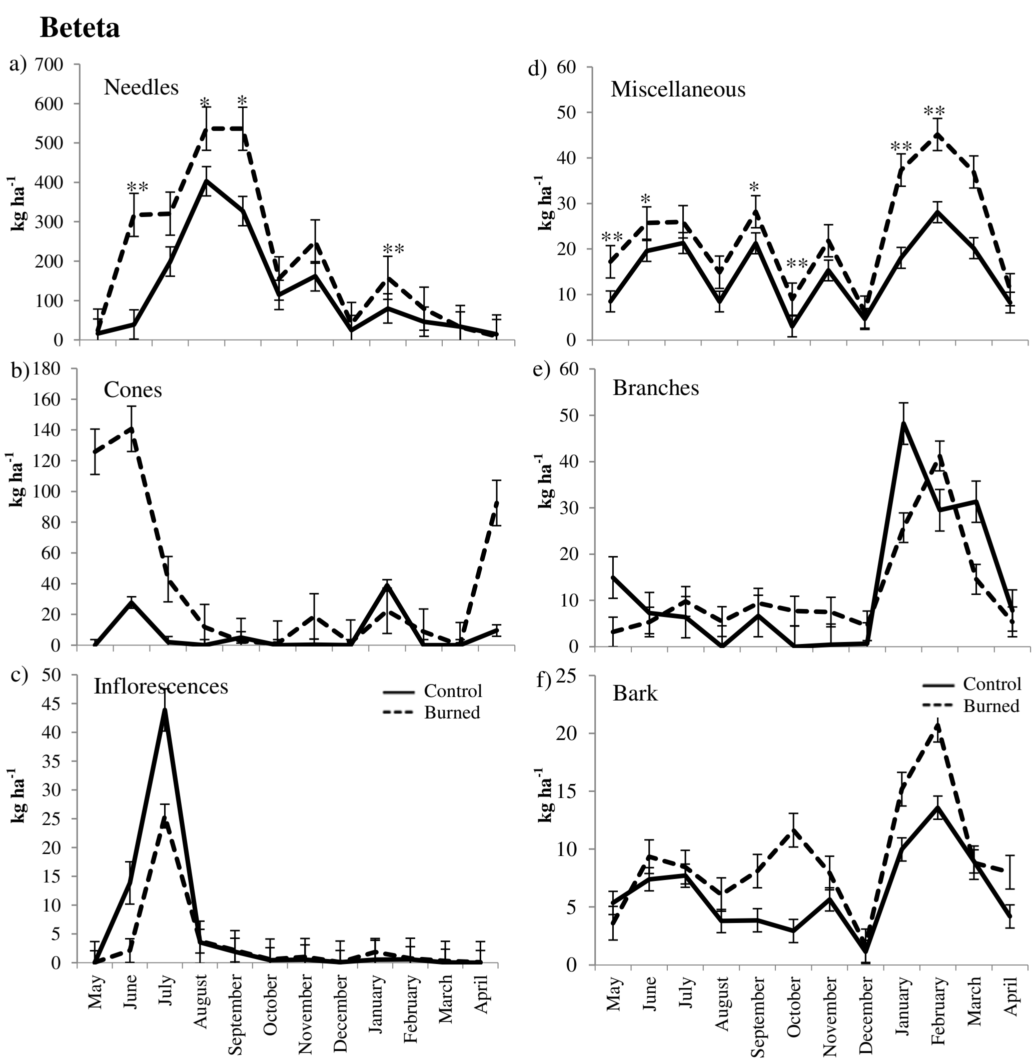

Fig. 5. Variation in the main litterfall fractions (a) needles, (b) cones, (c) inflorescences, (d) miscellaneous, (e) branches and (f) bark, collected during the period May 2016–March 2017 in in Beteta. Control in solid line and burned in dashed line. Litterfall production is expressed as the mean ±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

Beteta, the LAI only increased in two burned plots (B2B and B6B). Nevertheless, there were no significant differences in LAI one year after burning in any of the plots (Table 3) (Wilcoxon paired test $p = 0.2393$).

4. Discussion

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

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

Similarities between control and burned plots were found in relation to the temporal pattern of litterfall (Fig. 3). Although annual litterfall patterns may vary slightly from year to year (Cañellas et al., 1996; Pausas, 1997), is accepted a maximum in the summer months in conifer stands. Maximum amounts were observed in September in El Pozuelo and in August in Beteta. Blanco et al. (2006) reported peaks in two stands of *P. sylvestris* in the Western Pyrenees in September and October. Peaks have also been described for *P. sylvestris* in September–October (Guerrero et al., 1998) and in August–October in *P. pinea* and *P. sylvestris* (Hernández et al., 1992). Other peaks were observed in November and January in both areas (June–July by Blanco et al., 2006). A winter peak in litterfall in conifer stands was also reported by Roig et al. (2005). Litterfall was minimal in December in both areas.

Litterfall accumulation appears to be related to the annual climatic characteristics. The litterfall data (Fig. 3) were related to the temperature, precipitation and wind data from a meteorological station located in Mariana, Cuenca (40° 09′ 09″ N/002° 08′ 29″ W), close to the study area (Annex 1). Litterfall seems to be maximal in the months with physiological drought. A relationship between dry conditions during the growth period and the timing of needle fall (main fraction) has been reported for coniferous forests by different authors (Hennessey et al., 1992; Pausas, 1997). The gradual leaf-fall may also depend on the duration of light period, (Wareing and Thompson, 1975). The Mediterranean area is characterized by an irregular distribution of rainfall in the leaf-growing season, which results in some degree of variability in the litterfall (Roig et al., 2005). Precipitation and wind tend to increase in November in the zone, which may accelerate the shedding of needles, branches and other biomass elements, thus leading to an increase in the amount of litterfall collected (Table 4). The similar patterns obtained for control and burned plots for both locations (Fig. 3) suggest that ecophysiological tree processes have not been altered by prescribed burning (Battipaglia et al., 2016; Jiménez et al., 2017).

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

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

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

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

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

Table 3
Results of LAI measurement.

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

Note = LAI values and crown cover before and one year after the prescribed burning. Z: zone; PT: plot type; S LAI: standard deviation of LAI.

two plot types occurred in the months of maximum collection (February and March).

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

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

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

In El Pozuelo, the amount of needles in the total litterfall was 44 and 43% (control and burned plots), and in Beteta it was 66 and 65%. There is no difference in percentage between untreated and treated plots, although in Beteta, the total amount of litterfall was greater in burned than in control plots. In El Pozuelo, there was scarcely any difference in the annual mean and the proportions of the fractions are still maintained. Differences were observed in the two zones possibly due to the different stand characteristics. Blanco et al. (2006) reported that the needle fraction was 50–60% of the litterfall, similar to values reported by Gallardo et al. (1995). Several authors have proposed a mean needle fall value estimate on a global basis of about 70% (Meentemeyer et al., 1982; Albrektson, 1988).

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

In the El Pozuelo and Beteta stands the LAI values were between 1.8 and 3.0. There were no significant differences before and 1-year after the burning in the plots, and there were also no significant differences between control and burned plots. Some authors (Montes et al., 2007) have used the same method to studied LAI in *P. sylvestris* stands in the Central Mountain Range of Spain and have reported values ranging from 4.30 to 6.78 for this species.. The absence of changes in this parameter 1 year after burning assess that physiological processes and biomass cycles were not altered by PB at stand level. Long-term monitoring is required to confirm this finding.

5. Conclusions

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

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Appendix A. Annex 1

Table 4
Mean litterfall collected between May 2016 and March 2017 in the burned and control plots in El Pozuelo and Beteta in relation to data recorded at the Mariana weather station (40° 09′ 09″ N/002° 08′ 29″ W).

	Unit	Zone	Plot type	May	June	July	August	September	October	November	December	January	February	March
Rainfall	mm	–	–	52.91	10.19	6.96	5.39	13.87	48.21	65	6.5	15.07	38.07	35.4
Mean temperature	°C	–	–	11.9	18.2	22.5	21.3	16.2	11.6	5.4	2.8	1.0	4.9	7.0
Wind	m/s	–	–	0.88	0.75	0.66	0.67	0.59	0.52	0.66	0.48	0.95	1.06	1
Mean litterfall	kg ha^{-1}	El Pozuelo	Control	330	407	320	495	670	193	216	37	165	128	125
Mean litterfall	kg ha^{-1}	El Pozuelo	Burned	317	420	427	522	525	169	231	28	213	143	146
Mean litterfall	kg ha^{-1}	Beteta	Control	65	102	273	405	363	121	208	31	186	123	105
Mean litterfall	kg ha^{-1}	Beteta	Burned	169	486	420	574	577	186	346	55	258	207	113

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