Trees

Short-term effects of prescribed fire and soil mulching with fern on natural regeneration of Quercus Frainetto L.

--Manuscript Draft--

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Abstract:	To avoid the negative impacts of wildfire, prescribed fire is applied in several environments, often with post-fire soil mulching, to control wildfire hazard and erosion in burned areas. However, uncertainties remain about impacts of these forest management techniques on post-fire regeneration, especially for some forest species, such as oak, which is predominant in Mediterranean fire-prone areas. This study evaluates the effects of prescribed fire and post-fire soil mulching with fern on initial recruitment of an oak forest of Southern Italy. Acorn emergence and seedling survival as well as some important plant and root biometric characteristics (height, diameter, and dry weight) have been monitored in plots burned by prescribed fire with or without post-fire treatment with fern. The acorn rain among the experimental conditions was not statistically different, whereas the prescribed fire significantly increased acorn emergence compared to the unburned area. About 30% of acorns germinated, of which more than 70% survived. Soil mulching with fern did not significantly increase acorn emergence (35%) and plant survival (85%) compared to the burned and untreated sites, presumably due to the shadowing effect of the cut fern, which reduces the light availability for juvenile plants. However, this post-fire treatment significantly

	enhanced plant height (+44%) and root mass (+30%) but not its diameter (+6%) and root length (+1%). These contrasting effects in part support the initial hypothesis that soil mulching may be synergistic with the prescribed fire. Overall, the knowledge of the beneficial influences of prescribed fire and post-fire treatments on oak recruitment, thanks to the fire-tolerance character of this forest species, is useful to develop sustainable management plans for the delicate forest ecosystems of the semi-arid Mediterranean environment.
Response to Reviewers:	AUTHORS' REPLIES TO THE COMMENTS OF THE COMMUNICATING EDITOR AND REVIEWER #2 Comment CE: Thank you for revising the MS. I have read the revised MS and your responses, and found that the MS is well revised. However, as Reviewer 2 pointed out, there is a need for further minor correction. The comment is fair and reasonable. Following the comments shown below, please reconsider your analysis. Reply Dear Communicating Editor, we are glad that You have appreciated our revision work. Thank You again for the new suggestions, which we have addressed in the revised MS. Below, You will find the replies to the new considerations by the Second Reviewer. Kind regards. Reviewer #2: Comment Dear Author, The manuscript has undergone significant improvements after the revisions but there are still some points on which I invite you to think about: Reply Dear Prof./Dr., thanks a lot for the new review round and the related considerations, which we appreciate very much. As done before, we have addressed these suggestions in the revised MS. Below, the relevant replies. Kind regards. Comment - Section Methods-study site: the species composition of the forest stand remains untold, I deduce it is a pure oak wood, it would be better to make it explicit (e.g. row 146 and Table 1 within the Tree characteristics). Reply We have specified that the natural forest is a pure stand in the main text and heading of Table 1. See the marked changes in the revised MS. Comment - Experimental design & so on: I appreciated your clarifications in response to the reviewer's criticism and the studies you indicated to support your analytical choices. However, I am still perplexed how in the Results (e.g. between lines 246 and 249) the mean values for each group are reported in an inappropriate but formally correct

	manner which are essentially the mean of three counts/measures. Strictly speaking, it is also possible to compute a mean value between two measurements and also apply the analysis of variance between n + 1 samples (with n = the number of groups, so in your case the minimum number of samples required is 4); it's not advisable to have so few, but it's possible to do ANOVA in that situation and still have the theory work when the assumptions hold (data distributions for normality). But are the Results consistent with reality? Are the results of the tests significant? In this regard, in the Figures you could indicate this situation using scatter plots rather than histograms (since you have 3 points for each group). In my opinion, if you want to carry out your analysis scheme as proposed, I recommend that you state the weaknesses of the statistical analysis inherent in the low number of samples available (albeit conditioned by the situation you described in response to the reviewer). The biggest concerns at really small sample sizes would be very low power and higher-than-usual sensitivity to the normality assumption at very low sample size. You can estimate the effect of size on your results. When you obtain ANOVA results, see the sum of square results. Calculate the sum of squares between groups/ total sum of the square. According to Cohen's (1988, pp.284-7), if the value 0.01 the effect is small, 0.06; the effect is medium, and 0.14; the effect is large. Cohen, J., 1988.Statistical power analysis for the behavioral sciences (No. 300.72 C6). Reply Many thanks again for this valuable observation. Accordingly, we have stated the limitation of our study, due to the small number of plots in regeneration surveys (lines 271-277 of the revised MS with tracked changes). Moreover, we have replaced the histogram of Figure 2 with three scatter plots (one for the acorn rain, another for the acorn emergence, and the last for the seedling survival) with points related to the plot surveys and their mean and standard deviation, as You have suggested. The other figures do not need changes, since the observations are much more than three. Also in this case, please see the revised MS with marked changes.

Short-term effects of prescribed fire and soil mulching with fern on natural regeneration of *Quercus Frainetto* L.

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Abstract

Key message

In Mediterranean oak stands prescribed burning increases acorn emergence and plant survival, while post-fire soil mulching with fern does not significantly enhance the initial recruitment of plants.

To avoid the negative impacts of wildfire, prescribed fire is applied in several environments, often with post-fire soil mulching, to control wildfire hazard and erosion in burned areas. However, uncertainties remain about impacts of these forest management techniques on post-fire regeneration, especially for some forest species, such as oak, which is predominant in Mediterranean fire-prone areas. This study evaluates the effects of prescribed fire and post-fire soil mulching with fern on initial recruitment of an oak forest of Southern Italy. Acorn emergence and seedling survival as well as some important plant and root biometric characteristics (height, diameter, and dry weight) have been monitored in plots burned by prescribed fire with or without post-fire treatment with fern. The acorn rain among the experimental conditions was not statistically different, whereas the prescribed fire significantly increased acorn emergence compared to the unburned area. About 30% of acorns germinated, of which more than 70% survived. Soil mulching with fern did not significantly increase acorn emergence (35%) and plant survival (85%) compared to the burned and untreated sites,

presumably due to the shadowing effect of the cut fern, which reduces the light availability for juvenile plants. However, this post-fire treatment significantly enhanced plant height (+44%) and root mass (+30%) but not its diameter (+6%) and root length (+1%). These contrasting effects in part support the initial hypothesis that soil mulching may be synergistic with the prescribed fire. Overall, the knowledge of the beneficial influences of prescribed fire and post-fire treatments on oak recruitment, thanks to the fire-tolerance character of this forest species, is useful to develop sustainable management plans for the delicate forest ecosystems of the semi-arid Mediterranean environment.

Keywords: initial recruitment; oak stands; burning; acorn rain; acorn emergence, seedling survival.

1. Introduction

A detailed understanding of processes driving the dynamics of forest ecosystems is essential to develop sustainable management plans. This understanding must include the process of natural regeneration, since the initial recruitment of forest species is fundamental to achieve stand persistence under the pressure of future climate change (Lucas-Borja et al. 2012b), which forecasts reduced lower precipitation and higher temperatures (Collins et al. 2013). Acorn emergence and seedling survival stages, which determine the future forest structure, have been recognised as the most limiting stages for natural regeneration (Calama et al. 2017). In early stages of forest trees, abiotic factors, such as soil moisture and temperature, and sunlight, primarily control seedling establishment and growth (Lucas-Borja et al. 2011). Some biotic factors, such as canopy cover, acorn and seedling predation, and/or sapling herbivore, may also influence these stages (Lucas-Borja et al. 2012b).

Among these factors, fire may favour or hinder early seedling recruitment, since several forest species depend on the impact of fire to germinate (Keeley and Pausas 2019). However, fire, when at high severity, such as wildfires, may pose pervasive effects on human goods and assets (Wittenberg and Pereira 2021). To avoid the negative impacts of wildfire, prescribed fires - the planned use of low-intensity fire to achieve very different goals given certain weather, fuel and topographic conditions (Fernandes et al.

2013; Alcañiz et al. 2018) - has been suggested and applied in several environments (Klimas et al. 2020), for management of fuel accumulation and future fire hazard in forests that are prone to the wildfire risk (Fernandes and Botelho 2003). On an ecological approach, prescribed fire is thought to change the structure and composition of the forest (Romeo et al. 2020), increasing the heterogeneity of the landscape, and can be purposely used for restoration of fire-associated ecosystems (Ryan et al. 2013), promoting or regenerating fire-adapted species (Van Lear and Waldrop 1991). Its controlled and rational use can improve forest habitats (Fontaine and Kennedy 2012), facilitating the emergence and growth of understory vegetation (Neary et al. 1999; Hunt et al. 2014), discouraging establishment of some plant species, while favouring some others (Galford 1989; Barnes and Van Lear 1998). Post-fire recovery is generally accomplished by direct regeneration, i.e., the recovery of a plant community composed of the same pool of species that existed before the fire (Romeo et al. 2020).

Despite these beneficial effects of prescribed fires, uncertainties remain about its ecological impacts (Fuentes et al. 2018). The forest species may perform differently after prescribed fires (Lucas-Borja et al. 2016), and even the results of relevant research on the fire effect on tree regeneration are often contrasting. For some forest species, such as oak, which is predominant in Mediterranean fire-prone areas and has adapted to fire (Curt et al. 2009), studies of fire effects on regeneration have been shown varying results (Petersson et al. 2020). Oak woodlands rely on the ability of this species to survive fire, resprout efficiently after fire and regenerate from seeds (Pausas 2006; Arthur et al. 2012). However, failure of oak regeneration from acorns is frequent in Mediterranean countries (Maltez-Mouro et al. 2007; Curt et al. 2009). Oak is fire-adapted and moderately shade-intolerant, and therefore prescribed fire can be used as a management tool to decrease competition and increase light levels, thus promoting oak regeneration in some ecosystems (Brose et al. 2013; Izbicki et al. 2020). Prescribed fire also eliminates excess fire-sensitive vegetation and reduces litter, favouring the emergence of acorns (Blankenship and Arthur 2006). Many studies have shown that fire alone or in combination with other treatments, such as partial canopy removal, can improve the establishment and growth of regenerating oak trees (Hutchinson et al. 2005). However, several oak species have exhibited unsuccessful regeneration in recent years, and it is difficult to identify the reasons, due to a multitude of biotic and abiotic factors influencing this ecological processes (Pausas et al. 2004; Curt et al. 2009; Royse et al. 2010). In this context, the role of how prescribed burning affects initial seedling recruitment is still not understood and more research is needed with particular focus for Mediterranean mountainous areas, where the fire hazard is high and the post-fire recruitment of vegetation is limited by drought and some adverse soil characteristics (Shakesby 2011; Moody et al. 2013).

In spite of these beneficial effects on forest ecology, prescribed fire shows negative impacts on burned ecosystems, especially on soil hydrology. More specifically, removing litter and understory vegetation and modifying some important hydrological properties of soil (such as reducing the hydraulic conductivity of soil and inducing water repellency (Zavala et al. 2014; Lucas-Borja et al. 2018; Plaza-Álvarez et al. 2018, 2019), prescribed fire can increase the runoff and erosion rates, also by some order of magnitude (Vega et al. 2005; González-Pelayo et al. 2010; Cawson et al. 2012). Soil mulching with vegetal material (commonly pruning residues or straw) may mitigate runoff and erosion in burned areas (Lucas-Borja 2021; Zema 2021), since this post-fire management technique protects soil from raindrop impacts and reduces the velocity of overland flow (Patil Shirish et al. 2013; Prosdocimi et al. 2016; Zituni et al. 2019). This mulch action may also increase moisture and reduce temperature of burned soils, which are key factors in initial seedling recruitment of forest species (Calama et al. 2017). However, post-fire mulching can also have negative effects, especially regarding the mulching material. For instance, the residues of straw can be displaced by wind in some areas and may contain seeds, chemicals and parasites, which can be the sources of non-native vegetation and plant diseases. On this regard, forest residues (e.g. wood strands, chips or shreds) or dead plants may be preferable to straw, because these substrates do not carry non-native seeds or chemical residues, and are more resistant to wind displacement (Robichaud et al. 2020). In Mediterranean forest floor, fern - *Pteridium aquilinum* (L.) Kuhn - is widely available, and this avoids transport from other locations. On an ecological approach, fern stands act as an ecological filter that influences tree regeneration and favours emergence of late-successional species (Ssali et al. 2019).

However, to the best authors' knowledge, very few evaluations about the effect of fern on early recruitment of oak species in burned soil are available in literature. It results that the question of how prescribed fire and post-fire mulching affects the early seedling recruitment of an important forest species, as oak, is still not understood in

Mediterranean forest ecosystems. To fill these gaps, this study evaluates the effects of prescribed fire and post-fire soil mulching with fern on initial recruitment of an oak forest of Southern Italy. More specifically, acorn emergence and seedling survival as well as some important plant and root biometric characteristics (height, diameter, and dry weight) have been monitored in plots burned by prescribed fire with or without post-fire treatment with fern. Since oak is a forest species that is adapted to fire, we hypothesised that prescribed burning enhances the initial recruitment of plants, and this effect may be synergistic with mulching application.

2. Material and Methods

2.1. Study site

The study was carried out in a natural and pure stand of oak (*Quercus frainetto* Ten.) in the locality of “Rungia”, geographical coordinates 588635 E and 4216172 N, municipality of Samo, Calabria, Southern Italy). The climate of the area is semi-arid (“Csa” class, “Hot-summer Mediterranean” according to Koppen (Kottek et al. 2006) with mild and wet winters, and warm and dry summers. The mean annual precipitation and temperature are 1100 mm and 17.4 °C, respectively.

3The oak stand under investigation is between 900 and 950 m above sea level and is exposed to North-East (Figure 1). The stand is an adult forest (about 150 years old) with a monoplane structure and a large contribution of natural mulching from the fall of the leaves and fern. No management actions have been accomplished in this forest stands in the last century. Table 1 reports the main dimensional characteristics of the tree layer and the composition of the herbaceous layer. No shrub species are found in the understory vegetation.

The soil of the experimental site, with a mean slope of 19.1 ± 1.65%, are loamy sand with contents of silt, clay and sand of 11.5 ± 1.89%, 9.2 ± 0.58% and 79.1± 1.59%, respectively.

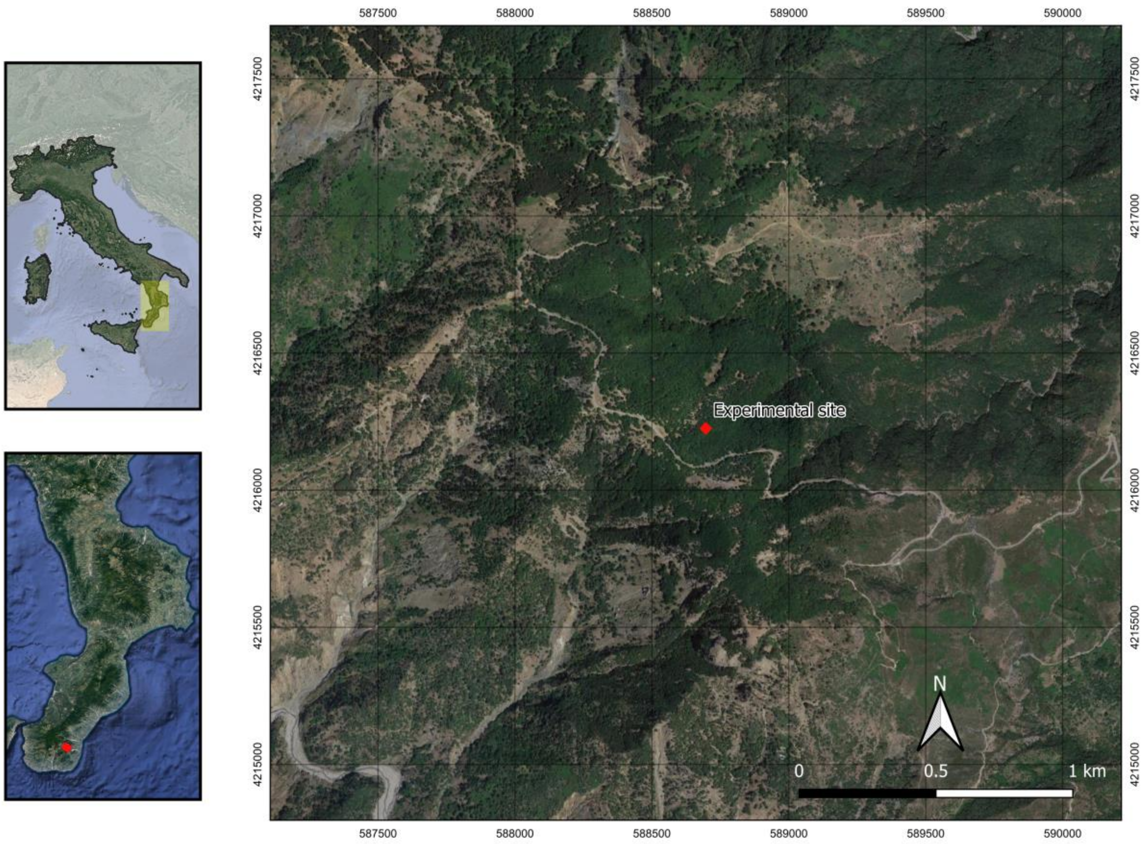


Figure 1 - Location of the experimental site (Samo, Calabria, Southern Italy).

Table 1 - Main characteristics of the pure oak forest in the experimental site (Rungia, municipality of Samo, Calabria, Southern Italy).

Characteristics		**Value**
Tree	density (n./ha)	225 ± 44.7
	diameter at the breast height (cm)	40.7 ± 8.9
	height (m)	18.2 ± 1.9
	basal area (m^2/ha)	31.1 ± 3.6
Litter	height (cm)	12.2 ± 3.9
Main herbaceous species		*Cyclamen hederifolium, Bellis perennis* L.

2.2. Experimental design

The prescribed fire was carried out in an area (about 250 m^2) of the oak forest in early June 2019 with the support of the Forest Regional Agency (Calabria Verde) and the surveillance of the National Corp of Firefighters. Wind was practically absent and air humidity was between 50 and 60%. The mean and maximum temperatures of soils, which were measured at a depth of 2.5 cm by a thermocouple connected to a datalogger, were 21 and 26.9 °C.

The burn severity of soils after the prescribed fire was evaluated according to the classification by (Parson et al. 2010). Accordingly, one day after prescribed fire, ground surface of all sites was visually checked, observing ash colour and fine roots. Following (Parson et al. 2010), the burn soil severity of all sites were low, that is, with small change from pre-fire status, black ground surface with recognizable fine fuels remaining on surface, and fine roots unchanged.

In the burned area, three small portions (about 9 m^2) was mulched with a cover (more or less 3 to 5 cm of thickness) of fern residues, cut from an adjacent zone, and distributed over ground at a dose of 500 g/m^2 of fresh weight (equivalent to 200 g/m^2 of straw dry matter, usually applied in burned and mulched areas after fire (Vega et al. 2014; Lucas-Borja et al. 2018).

A third area, which was not burned and was located less than 10 m from the burned areas, in each site was selected to be considered as “control”.

Immediately after the prescribed fire and soil mulching, a total of nine small plots (three series of plots, area of 3 m^2, each series consisting of three replicated plots) were selected and delimited using 0.3-m high metallic sheets inserted up to 0.2 m below the ground surface. The plots were at a reciprocal distance between 1.5 and 2 m. Three plots were set up in the unburned soils (considered as “control”), while six plots were located in the burned area, of which three in the area without treatment (Figure 2a) and three in the mulched area (Figure 2b).

Overall, the experimental design consisted of three soil conditions (unburned, burned and untreated, and burned and mulched) × three replicated plots, for a total of nine plots.

(a) (b)

(c)

(d)

Figure 2 - Photos of the experimental plots (a, burned and untreated soils; b, burned and mulched soils), acorn emergence (c) and measurement of seedling height and diameter (d) in oak forest under different soil conditions (Samo, Calabria, Southern Italy).

2.3. Vegetation sampling and analysis

Acorn rain was estimated in November 2019 (five months after the prescribed fire application and soil treatment with fern mulch) in each plot of the three soil conditions.

Seed fall was calculated at each experimental treatment as a sum of acorns found at each plot. The plots were protected with wire netting (1 × 1 cm mesh size) to avoid acorn predation.

Acorn emergence (Figure 2c) was measured one year (June 2020) after the prescribed fire application and soil treatment with fern mulch, while seedling survival was surveyed at the end of the experiment in June 2021 (24 months after fire and mulching).

Moreover, the total length of the main stem and root-collar diameter of all surviving seedlings was measured in June of 2020 and 2021 (Figure 2d). Then, in June 2021, the soil supporting seedling growth was gently excavated and the aerial part of the plant was separated from the root system. On the latter, the root length and dry weight (at a temperature of 60 °C for 48 hours) were determined on samples of ten seedlings per plot.

2.4. Statistical analyses

Previously, Cochran's C test, a one-sided upper limit variance outlier test, was aèèòoed to the BMP values, in order to detect possible outliers.

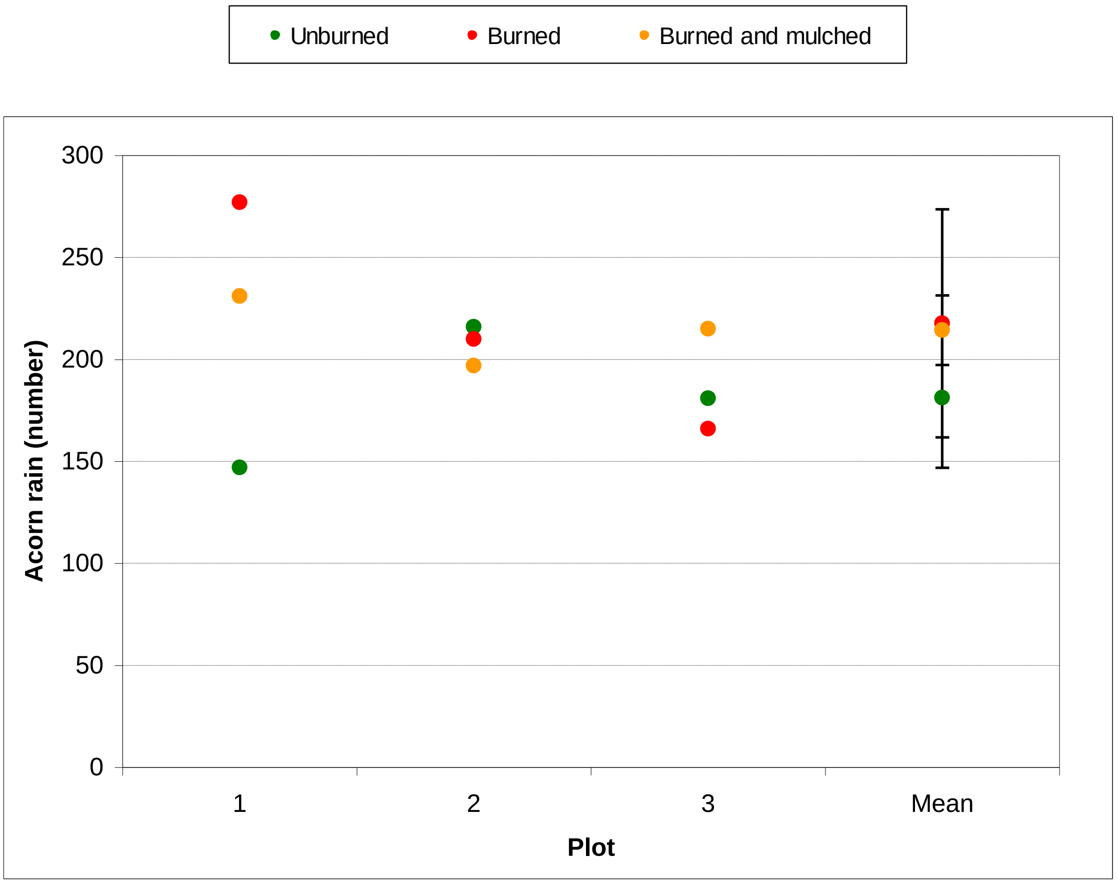
Then, two-way ANOVA was applied to evaluate the statistical significance of the differences in the BMP (considered as dependent variables) among the number of replicates and test duration (independent factors).

For both tests, to satisfy the assumptions of the statistical tests (equality of variance and normal distribution), the data were subjected to normality test or were square root-transformed whenever necessary.

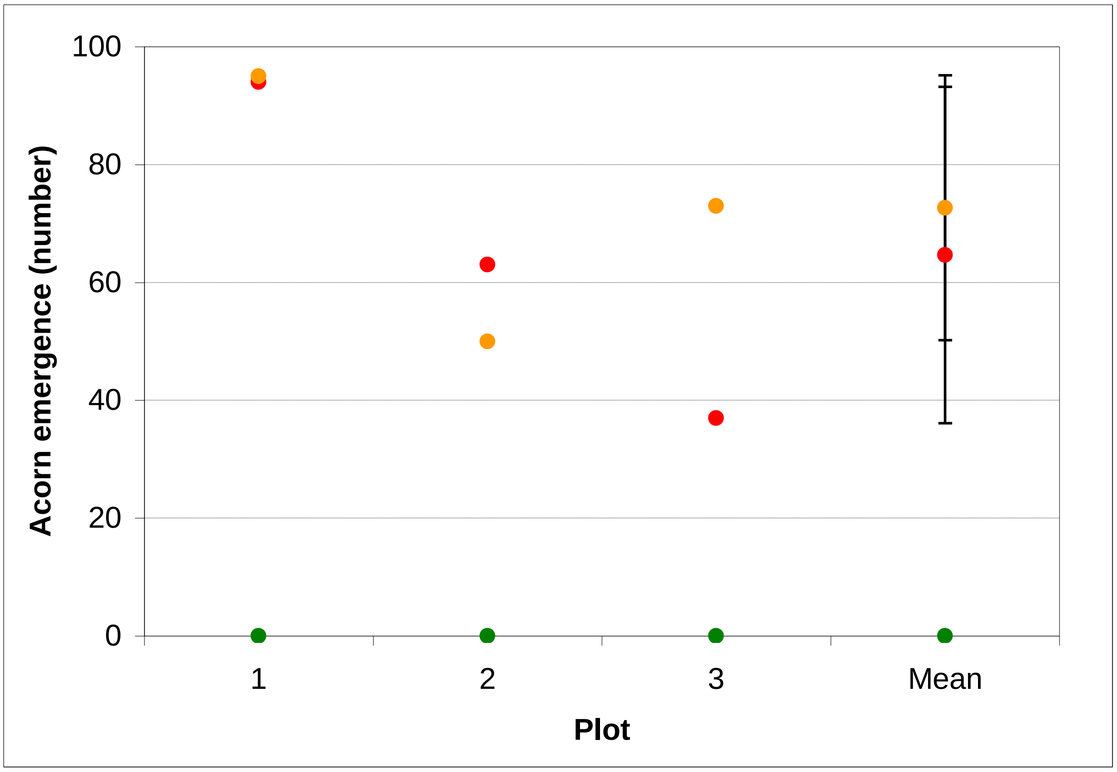
All the statistical tests were carried out by with the XLSTAT software (release 2019).

3. Results

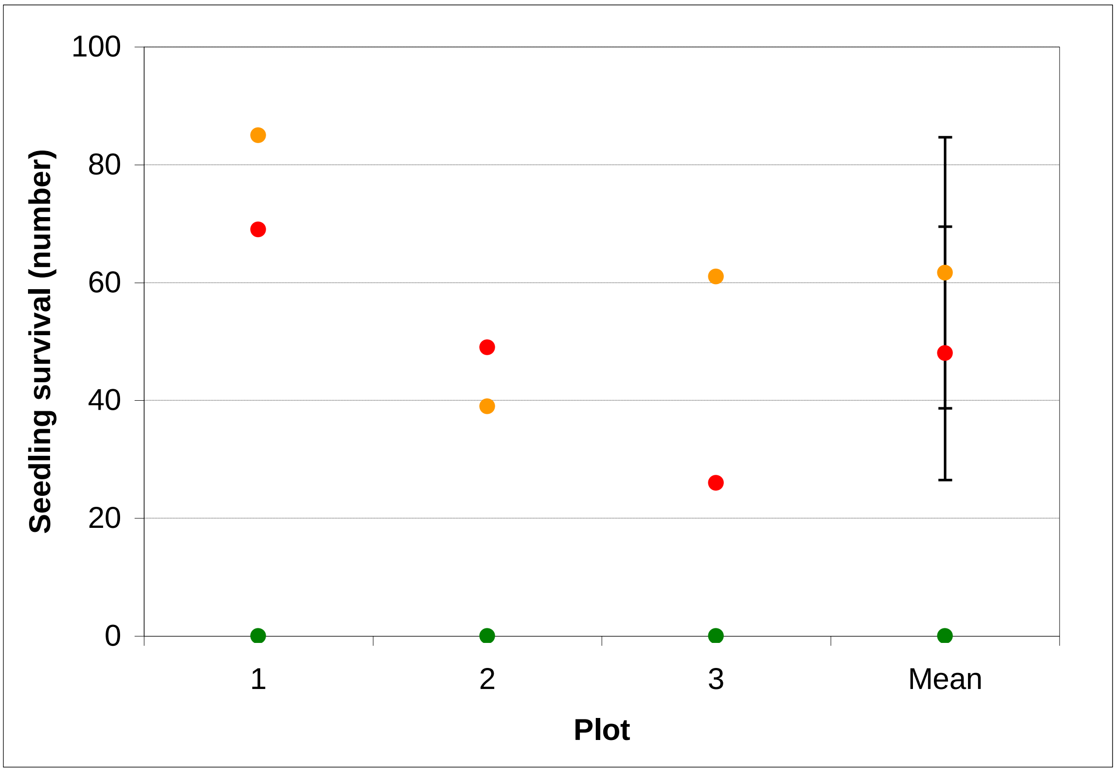
Compared to the unburned sites, where acorn rain was on average 181 ± 35, in burned, and burned and mulched plots 218 ± 56 and 214 ± 17 acorns were counted, respectively (Figure 3), although the differences among the different soil conditions were not significant ($p = 0.497$, $n = 3$, after the pairwise comparison by Tukey's test).


Unburned
Burned
Burned and mulched
300
250
200
150
100
50
0
Acorn rain (number)
1
2
3
Mean
Plot

(a)


100
80
60
40
20
0
Acorn emergence (number)
1
2
3
Mean
Plot

(b)



(c)

Figure 3 - Acorn rain (a) and emergence (b), and seedling survival (c) counted in nine plots (three per each soil condition, unburned, burned, and burned and mulched) of oak forest under different soil conditions (Samo, Calabria, Southern Italy). The vertical bar expresses the standard deviation of data in the three plots.

While no acorn emergence was noticed in unburned sites, 65 ± 29 acorns germinated on burned soils and 73 ± 23 on burned and mulched plots (Figure 3). Only the difference between unburned, and burned soils (mulched or not) was significant ($p < 0.01$, $n = 3$, after the pairwise comparison by Tukey's test).

Of the emerged acorns, 48 ± 22 and 62 ± 23 seedlings survived on burned, and burned and mulched soils (Figure 3), but the difference between this soil conditions was not significant ($p = 0.068$, $n = 3$, after the pairwise comparison by Tukey's test).

It should be highlighted that the values of oak regeneration may be affected by a bias, due to the low number of samples (three plots per soil condition), and the effect of this small size is large. This effect, which was measured by the ratio between the sum of squares between groups and the total sum of the squares (given by ANOVA), was large, according to the reference values proposed by (Cohen 2013), since this ratio is higher than 0.21 for the three variables analyzed (acorn rain and emergence, and seedling survival).

At both survey dates (June 2020 and 2021), plant height was higher in burned and mulched plots (92.8 ± 31.2 mm, June 2020, and 110.5 ± 40.3 mm, June 2021) compared to the burned and untreated soils (64.6 ± 35.3 mm, June 2020, and 85.3 ± 41.9 mm, June 2021) (Figure 4). According the 2-way ANOVA, both soil condition and survey date were significant factors ($p < 0.0001$ and 0.01, respectively, $n = 3$, after the pairwise comparison by Tukey's test) on plant height, but not its interaction ($p = 0.630$).

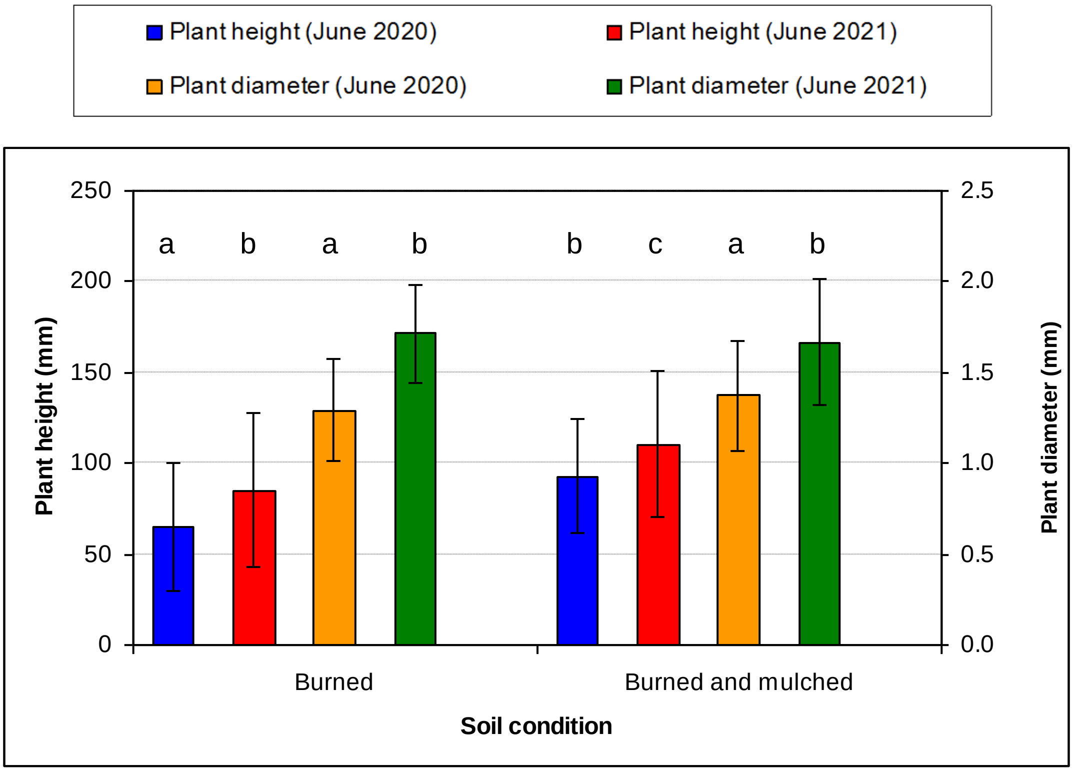


Figure 4 - Plant height and diameter (mean ± standard deviation) surveyed in oak forest under different soil conditions at two survey dates (Samo, Calabria, Southern Italy). Different letters indicate significant differences between the soil conditions after the pairwise comparison by Tukey's test ($p < 0.05$, $n = 3$).

In June 2020, seedling diameter was lower in the burned and untreated plots (1.29 ± 0.28 mm) compared to the burned and mulched soils (1.38 ± 0.30 mm). In contrast, in

June 2021, the latter soils showed seedlings with lower diameter (1.67 ± 0.35 mm vs. 1.71 ± 0.27 mm of burned and untreated plots) (Figure 4). For seedling characteristic, both the date and its interaction with the soil condition determined significant differences in plant diameter ($p < 0.0001$ and 0.01, respectively, $n = 3$, after the pairwise comparison by Tukey's test). In contrast, soil condition did not statistically influence the seedling diameter ($p = 0.485$).

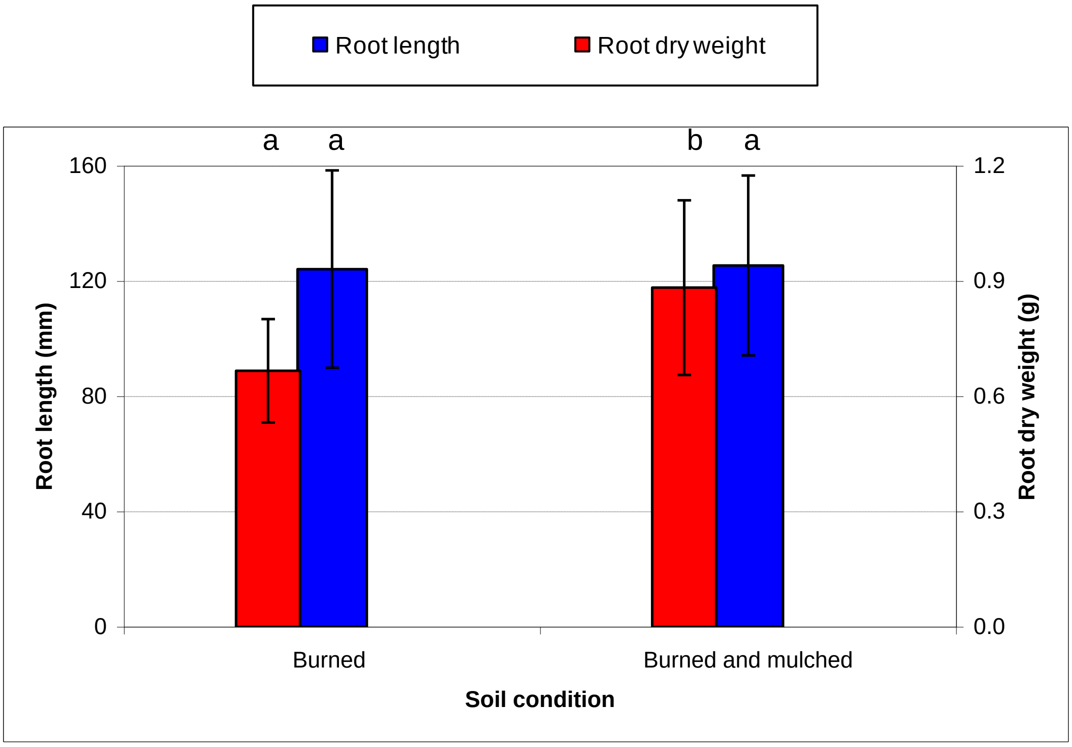


Figure 5 - Root length and dry weight (mean ± standard deviation) of oak seedlings under different soil conditions at two survey dates (Samo, Calabria, Southern Italy). Different letters indicate significant differences between the two soil conditions after the pairwise comparison by Tukey's test ($p < 0.05$, $n = 3$).

Root length (125 ± 34.3 mm and 126 ± 34.0 mm, respectively) was practically the same in burned, and burned and mulched soils. In contrast, the latter soils showed a higher root dry weight (0.88 ± 0.23 g) compared to the burned and untreated plots (0.67 ± 0.14 g) (Figure 5). These differences were significant for root dry weight ($p < 0.001$, $n = 3$, after the pairwise comparison by Tukey's test), but not for root length ($p = 0.905$).

4. Discussions

Since seedling recruitment is ultimately limited by the quantity of viable acorn fall, an evaluation of seedling patterns is necessary to understand the dynamics of recruitment (Lucas-Borja and Vacchiano 2018). Oak acorns are dispersed across a wide variety of sites covering a range of abiotic and biotic conditions. Given that a similar forest structure of oak forest among all plots, we assumed that acorns fall was similar at all plots. Furthermore, since parent rock material, topography, climate and forest structure were similar among selected plots, differences with respect to seedling emergence, survival and initial growth can be associated with the influence of fire and mulch.

While none of the fallen acorns sprouted in the unburned area, about 30% of acorns germinated in the burned areas, of which more than 70% survived. Higher regeneration in burned areas is in agreement with other studies dealing with oak recruitment, which reported higher survival rates (Royse et al. 2010; Brose et al. 2013; Petersson et al. 2020), the latter in combination with other treatments (e.g., tree canopy openness or ungulate exclosure) in burned oak stands, while (Hutchinson et al. 2005) found reduced sapling density in areas with burn treatments. Many reasons may explain the greater early recruitment of oak in the burned sites. First, the reduction in tree density and canopy cover increases light availability on the forest floor, which is very important for regeneration of oak species, which are considered light demanding for survival and growth once energy reserves of the cotyledons are exhausted (Annighöfer et al. 2015; Petersson et al. 2020). Second, the lower competition with herbaceous vegetation presumably enhances vegetation growth in this semi-arid environment, having a limited water availability (Garcia-Fayos et al. 2020; Petersson et al. 2020); in contrast, the presence of herb layer found in unburned plots could have delayed or impeded acorn emergence development (Caccia and Ballaré 1998). Third, low-intensity fire creates more favourable seedbed conditions to oak establishment, promoting the contact with mineral soil and thus favouring acorn emergence (Facelli and Pickett 1991; Hutchinson et al. 2005). Fourth, the increases in organic carbon, total nitrogen and available phosphorous measured in the same experimental site immediately after the prescribed fire compared to the unburned sites, followed by significant decreases one year after fire, when the plant regeneration was higher (Carra et al. 2021). A higher availability of organic matter and nitrogen contents of burned soils, which have been well documented after prescribed fires (Úbeda et al. 2005; Alcañiz et al. 2018, 2020; Hueso-González et al. 2018), may have supported the recruitment of oak seedlings detected in this study. These changes in soil properties also improve microbial diversity, which may increase soil fertility (Nannipieri et al. 2003; Lucas-Borja et al. 2012a). The latter effect together with the low damage to canopy acorn bank and the low impact on forest floor conditions after prescribed fires might generate better conditions for initial seedling recruitment. In line with this, (Madrigal et al. 2010) have also shown a positive influence of the soil organic layer remaining after fire on seedling recruitment, which has been related to a higher water content of soils with higher organic matter. However, since several studies have indicated that oak regeneration may be more effective after a single prescribed fire when combined with other treatments (Ssali et al. 2019; Petersson et al. 2020; Izbicki et al. 2020), the opening of tree canopy by mechanized operations and replicated applications of prescribed fire can be suggested, in order to remove midstory competition, increasing understory light, and increasing oak seedling growth and density (Green et al. 2010; Brose et al. 2013; Izbicki et al. 2020).

Mulching determined increases - although not being significant - in these percentages (about 35% of germinated acorns, and 85% of survived seedlings) that can be quantified in 14% and 12% for emergence and survival, respectively. (Lucas- Borja et al. 2021) have demonstrated that seedling survival but not emergence rates is largely controlled by post-fire treatments enhancing the establishment of vegetation cover, due to the beneficial effect of shrubs on the recruitment of seedlings located under their canopies (Emborg 1998; Heydari et al. 2017). Moreover, the mulch cover spread over the ground of burned soils acts as a barrier that reduces drought by lowering solar radiation and soil temperature and increasing its water content (Castro et al. 2003; Lucas- Borja et al. 2021). Also (Ssali et al. 2019) reported higher seedling survival on soils of tropical forests subjected to post-fire treatment with fern.

Many studies have stated that growth of oak seedlings in burned areas is higher compared to fire-excluded sites, since larger diameters (Royse et al. 2010), faster biomass growth (Wang et al. 2005), and greater heights (Petersson et al. 2020) were observed in oak seedlings after prescribed fires.

In spite of the non-significant difference in the initial recruitment of oak seedlings between mulched and untreated areas, this post-fire treatment enhanced plant growth at both survey dates, as shown by the significantly greater height (+44% one year after the treatment) compared to the plants grown on burned and untreated plots. In contrast, the plant diameter was not significantly influenced by the treatment (only +6% after one year). These enhancements of the plant morphology lost weight over time (+30% in height two years after the treatment) and even became negative (-3% in diameter) over time. Higher growth in soils burned and mulched with fern was reported also by (Ssali et al. 2019).

Regarding the root system, mulching did not significantly influence the length (only +1% in mulched areas compared to the burned and untreated plots), but determined a significant increase (by more than 30%) of the root mass.

The faster growth of seedlings in mulched areas compared to the untreated sites may be due to the higher water content, since it is well known that mulching enhances soil moisture which is a key point in natural regeneration process, especially in forest ecosystems of the semi-arid environments with limited water availability (Prosdocimi et al. 2016; Lucas-Borja et al. 2018). (Bautista et al. 2009) theorized that the main advantage of mulch application is the immediate increase in vegetal cover of soil, which result in an effective protection during the first rain events after fire. Moreover, the mulch material can be fast incorporated into the soil, thus increasing the its content of organic matter and nutrients (Bombino et al. 2019).

5. Conclusions

This study has demonstrated that the application of prescribed burning to an oak stand of the Mediterranean environment play significant effects on plant regeneration compared to the unburned sites. In relation to emergence, in burned areas, about 30% of acorns emerged, of which more than 70% survived. This may be due to the higher light availability on the forest floor, lower competition with herbaceous vegetation, more favourable seedbed conditions to oak establishment, and increases in soil contents of organic carbon, total nitrogen and available phosphorous. This result confirms the first working hypothesis that prescribed burning enhances the initial recruitment of plants, since oak is a forest species that is adapted to fire.

Soil mulching with fern applied as anti-erosive post-fire treatment did not significantly increase acorn emergence and plant survival compared to the burned and untreated sites. However, this post-fire treatment significantly enhanced plant height and root mass but not its diameter and root length. With the same root length, higher root biomass with more soil water availability led to greater height growth in the measured seedlings. These contrasting effects, which may be attributed to the higher water content of mulched area of this water-limited in forest ecosystem of the semi-arid environments, in part support the initial hypothesis that soil mulching may be synergistic with the prescribed fire.

Overall, the knowledge of the beneficial influences of prescribed fire and post-fire treatments on oak recruitment, thanks to the fire-tolerance character of *Quercus frainetto* L., is useful to develop sustainable management plans for the delicate forest ecosystems of the semi-arid Mediterranean environment. More research is needed in order to validate the outcomes of this study on different forest species and to evaluate the effects of prescribed fire and mulching with other forest operations.

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Author contributions

Conceptualization, B.G.C., G.B., M.E.L.B. and D.A.Z.; methodology, B.G.C., A.L., P.A.P.A., M.E.L.B. and D.A.Z.; validation, G.B., M.E.L.B. and D.A.Z.; formal analysis, G.B., M.E.L.B. and D.A.Z.; investigation, B.G.C., A.L., and P.A.P.A.; data curation, B.G.C., A.L., P.A.P.A., and D.A.Z.; writing - original draft preparation, B.G.C., and D.A.Z.; writing - review and editing, B.G.C., G.B., M.E.L.B and D.A.Z.; supervision, M.E.L.B and D.A.Z.; project administration, D.A.Z.; funding acquisition, D.A.Z. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest statement

The authors declare that they have no conflict of interest.

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Short-term effects of prescribed fire and soil mulching with fern on natural regeneration of *Quercus Frainetto* L.

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Abstract

Key message

In Mediterranean oak stands prescribed burning increases acorn emergence and plant survival, while post-fire soil mulching with fern does not significantly enhance the initial recruitment of plants.

To avoid the negative impacts of wildfire, prescribed fire is applied in several environments, often with post-fire soil mulching, to control wildfire hazard and erosion in burned areas. However, uncertainties remain about impacts of these forest management techniques on post-fire regeneration, especially for some forest species, such as oak, which is predominant in Mediterranean fire-prone areas. This study evaluates the effects of prescribed fire and post-fire soil mulching with fern on initial recruitment of an oak forest of Southern Italy. Acorn emergence and seedling survival as well as some important plant and root biometric characteristics (height, diameter, and dry weight) have been monitored in plots burned by prescribed fire with or without post-fire treatment with fern. The acorn rain among the experimental conditions was not statistically different, whereas the prescribed fire significantly increased acorn emergence compared to the unburned area. About 30% of acorns germinated, of which more than 70% survived. Soil mulching with fern did not significantly increase acorn emergence (35%) and plant survival (85%) compared to the burned and untreated sites,

presumably due to the shadowing effect of the cut fern, which reduces the light availability for juvenile plants. However, this post-fire treatment significantly enhanced plant height (+44%) and root mass (+30%) but not its diameter (+6%) and root length (+1%). These contrasting effects in part support the initial hypothesis that soil mulching may be synergistic with the prescribed fire. Overall, the knowledge of the beneficial influences of prescribed fire and post-fire treatments on oak recruitment, thanks to the fire-tolerance character of this forest species, is useful to develop sustainable management plans for the delicate forest ecosystems of the semi-arid Mediterranean environment.

Keywords: initial recruitment; oak stands; burning; acorn rain; acorn emergence, seedling survival.

1. Introduction

A detailed understanding of processes driving the dynamics of forest ecosystems is essential to develop sustainable management plans. This understanding must include the process of natural regeneration, since the initial recruitment of forest species is fundamental to achieve stand persistence under the pressure of future climate change (Lucas-Borja et al. 2012b), which forecasts reduced lower precipitation and higher temperatures (Collins et al. 2013). Acorn emergence and seedling survival stages, which determine the future forest structure, have been recognised as the most limiting stages for natural regeneration (Calama et al. 2017). In early stages of forest trees, abiotic factors, such as soil moisture and temperature, and sunlight, primarily control seedling establishment and growth (Lucas-Borja et al. 2011). Some biotic factors, such as canopy cover, acorn and seedling predation, and/or sapling herbivore, may also influence these stages (Lucas-Borja et al. 2012b).

Among these factors, fire may favour or hinder early seedling recruitment, since several forest species depend on the impact of fire to germinate (Keeley and Pausas 2019). However, fire, when at high severity, such as wildfires, may pose pervasive effects on human goods and assets (Wittenberg and Pereira 2021). To avoid the negative impacts of wildfire, prescribed fires - the planned use of low-intensity fire to achieve very different goals given certain weather, fuel and topographic conditions (Fernandes et al.

2013; Alcañiz et al. 2018) - has been suggested and applied in several environments (Klimas et al. 2020), for management of fuel accumulation and future fire hazard in forests that are prone to the wildfire risk (Fernandes and Botelho 2003). On an ecological approach, prescribed fire is thought to change the structure and composition of the forest (Romeo et al. 2020), increasing the heterogeneity of the landscape, and can be purposely used for restoration of fire-associated ecosystems (Ryan et al. 2013), promoting or regenerating fire-adapted species (Van Lear and Waldrop 1991). Its controlled and rational use can improve forest habitats (Fontaine and Kennedy 2012), facilitating the emergence and growth of understory vegetation (Neary et al. 1999; Hunt et al. 2014), discouraging establishment of some plant species, while favouring some others (Galford 1989; Barnes and Van Lear 1998). Post-fire recovery is generally accomplished by direct regeneration, i.e., the recovery of a plant community composed of the same pool of species that existed before the fire (Romeo et al. 2020).

Despite these beneficial effects of prescribed fires, uncertainties remain about its ecological impacts (Fuentes et al. 2018). The forest species may perform differently after prescribed fires (Lucas-Borja et al. 2016), and even the results of relevant research on the fire effect on tree regeneration are often contrasting. For some forest species, such as oak, which is predominant in Mediterranean fire-prone areas and has adapted to fire (Curt et al. 2009), studies of fire effects on regeneration have been shown varying results (Petersson et al. 2020). Oak woodlands rely on the ability of this species to survive fire, resprout efficiently after fire and regenerate from seeds (Pausas 2006; Arthur et al. 2012). However, failure of oak regeneration from acorns is frequent in Mediterranean countries (Maltez-Mouro et al. 2007; Curt et al. 2009). Oak is fire-adapted and moderately shade-intolerant, and therefore prescribed fire can be used as a management tool to decrease competition and increase light levels, thus promoting oak regeneration in some ecosystems (Brose et al. 2013; Izbicki et al. 2020). Prescribed fire also eliminates excess fire-sensitive vegetation and reduces litter, favouring the emergence of acorns (Blankenship and Arthur 2006). Many studies have shown that fire alone or in combination with other treatments, such as partial canopy removal, can improve the establishment and growth of regenerating oak trees (Hutchinson et al. 2005). However, several oak species have exhibited unsuccessful regeneration in recent years, and it is difficult to identify the reasons, due to a multitude of biotic and abiotic factors influencing this ecological processes (Pausas et al. 2004; Curt et al. 2009; Royse et al. 2010). In this context, the role of how prescribed burning affects initial seedling recruitment is still not understood and more research is needed with particular focus for Mediterranean mountainous areas, where the fire hazard is high and the post-fire recruitment of vegetation is limited by drought and some adverse soil characteristics (Shakesby 2011; Moody et al. 2013).

In spite of these beneficial effects on forest ecology, prescribed fire shows negative impacts on burned ecosystems, especially on soil hydrology. More specifically, removing litter and understory vegetation and modifying some important hydrological properties of soil (such as reducing the hydraulic conductivity of soil and inducing water repellency (Zavala et al. 2014; Lucas-Borja et al. 2018; Plaza-Álvarez et al. 2018, 2019), prescribed fire can increase the runoff and erosion rates, also by some order of magnitude (Vega et al. 2005; González-Pelayo et al. 2010; Cawson et al. 2012). Soil mulching with vegetal material (commonly pruning residues or straw) may mitigate runoff and erosion in burned areas (Lucas-Borja 2021; Zema 2021), since this post-fire management technique protects soil from raindrop impacts and reduces the velocity of overland flow (Patil Shirish et al. 2013; Prosdocimi et al. 2016; Zituni et al. 2019). This mulch action may also increase moisture and reduce temperature of burned soils, which are key factors in initial seedling recruitment of forest species (Calama et al. 2017). However, post-fire mulching can also have negative effects, especially regarding the mulching material. For instance, the residues of straw can be displaced by wind in some areas and may contain seeds, chemicals and parasites, which can be the sources of non-native vegetation and plant diseases. On this regard, forest residues (e.g. wood strands, chips or shreds) or dead plants may be preferable to straw, because these substrates do not carry non-native seeds or chemical residues, and are more resistant to wind displacement (Robichaud et al. 2020). In Mediterranean forest floor, fern - *Pteridium aquilinum* (L.) Kuhn - is widely available, and this avoids transport from other locations. On an ecological approach, fern stands act as an ecological filter that influences tree regeneration and favours emergence of late-successional species (Ssali et al. 2019).

However, to the best authors' knowledge, very few evaluations about the effect of fern on early recruitment of oak species in burned soil are available in literature. It results that the question of how prescribed fire and post-fire mulching affects the early seedling recruitment of an important forest species, as oak, is still not understood in

Mediterranean forest ecosystems. To fill these gaps, this study evaluates the effects of prescribed fire and post-fire soil mulching with fern on initial recruitment of an oak forest of Southern Italy. More specifically, acorn emergence and seedling survival as well as some important plant and root biometric characteristics (height, diameter, and dry weight) have been monitored in plots burned by prescribed fire with or without post-fire treatment with fern. Since oak is a forest species that is adapted to fire, we hypothesised that prescribed burning enhances the initial recruitment of plants, and this effect may be synergistic with mulching application.

2. Material and Methods

2.1. Study site

The study was carried out in a natural and pure stand of oak (*Quercus frainetto* Ten.) in the locality of "Rungia", geographical coordinates 588635 E and 4216172 N, municipality of Samo, Calabria, Southern Italy). The climate of the area is semi-arid ("Csa" class, "Hot-summer Mediterranean" according to Koppen (Kottek et al. 2006) with mild and wet winters, and warm and dry summers. The mean annual precipitation and temperature are 1100 mm and 17.4 °C, respectively.

3The oak stand under investigation is between 900 and 950 m above sea level and is exposed to North-East (Figure 1). The stand is an adult forest (about 150 years old) with a monoplane structure and a large contribution of natural mulching from the fall of the leaves and fern. No management actions have been accomplished in this forest stands in the last century. Table 1 reports the main dimensional characteristics of the tree layer and the composition of the herbaceous layer. No shrub species are found in the understory vegetation.

The soil of the experimental site, with a mean slope of 19.1 ± 1.65%, are loamy sand with contents of silt, clay and sand of 11.5 ± 1.89%, 9.2 ± 0.58% and 79.1± 1.59%, respectively.

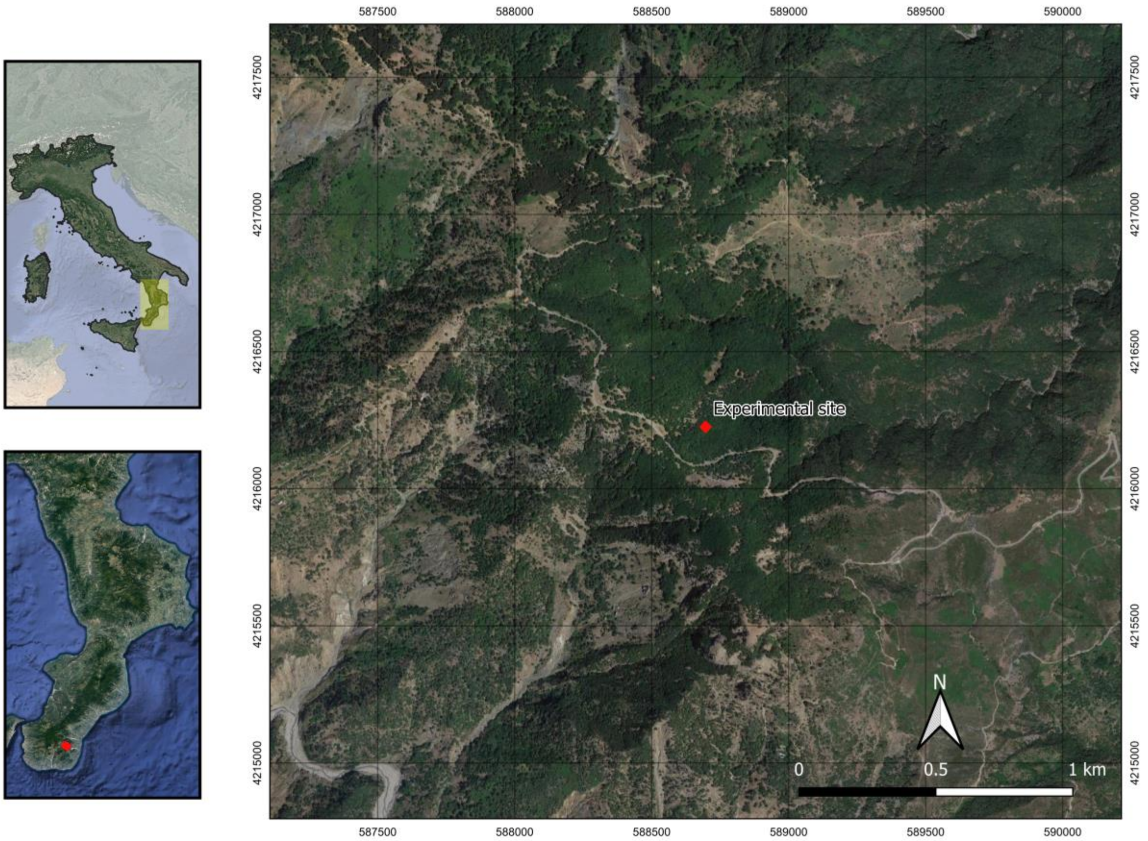


Figure 1 - Location of the experimental site (Samo, Calabria, Southern Italy).

Table 1 - Main characteristics of the pure oak forest ~~stand~~ in the experimental site (Rungia, municipality of Samo, Calabria, Southern Italy).

~~Oak forest c~~Characteristics		Value
Tree	density (n./ha)	225 ± 44.7
	diameter at the breast height (cm)	40.7 ± 8.9
	height (m)	18.2 ± 1.9
	basal area (m^2/ha)	31.1 ± 3.6
Litter	height (cm)	12.2 ± 3.9
Main herbaceous species		*Cyclamen hederifolium, Bellis perennis* L.

2.2. Experimental design

The prescribed fire was carried out in an area (about 250 m^2) of the oak forest in early June 2019 with the support of the Forest Regional Agency (Calabria Verde) and the surveillance of the National Corp of Firefighters. Wind was practically absent and air humidity was between 50 and 60%. The mean and maximum temperatures of soils, which were measured at a depth of 2.5 cm by a thermocouple connected to a datalogger, were 21 and 26.9 °C.

The burn severity of soils after the prescribed fire was evaluated according to the classification by (Parson et al. 2010). Accordingly, one day after prescribed fire, ground surface of all sites was visually checked, observing ash colour and fine roots. Following (Parson et al. 2010), the burn soil severity of all sites were low, that is, with small change from pre-fire status, black ground surface with recognizable fine fuels remaining on surface, and fine roots unchanged.

In the burned area, three small portions (about 9 m^2) was mulched with a cover (more or less 3 to 5 cm of thickness) of fern residues, cut from an adjacent zone, and distributed over ground at a dose of 500 g/m^2 of fresh weight (equivalent to 200 g/m^2 of straw dry matter, usually applied in burned and mulched areas after fire (Vega et al. 2014; Lucas-Borja et al. 2018).

A third area, which was not burned and was located less than 10 m from the burned areas, in each site was selected to be considered as “control”.

Immediately after the prescribed fire and soil mulching, a total of nine small plots (three series of plots, area of 3 m^2, each series consisting of three replicated plots) were selected and delimited using 0.3-m high metallic sheets inserted up to 0.2 m below the ground surface. The plots were at a reciprocal distance between 1.5 and 2 m. Three plots were set up in the unburned soils (considered as “control”), while six plots were located in the burned area, of which three in the area without treatment (Figure 2a) and three in the mulched area (Figure 2b).

Overall, the experimental design consisted of three soil conditions (unburned, burned and untreated, and burned and mulched) × three replicated plots, for a total of nine plots.

(a) (b)

(c)

(d)

Figure 2 - Photos of the experimental plots (a, burned and untreated soils; b, burned and mulched soils), acorn emergence (c) and measurement of seedling height and diameter (d) in oak forest under different soil conditions (Samo, Calabria, Southern Italy).

2.3. Vegetation sampling and analysis

Acorn rain was estimated in November 2019 (five months after the prescribed fire application and soil treatment with fern mulch) in each plot of the three soil conditions.

Seed fall was calculated at each experimental treatment as a sum of acorns found at each plot. The plots were protected with wire netting (1 × 1 cm mesh size) to avoid acorn predation.

Acorn emergence (Figure 2c) was measured one year (June 2020) after the prescribed fire application and soil treatment with fern mulch, while seedling survival was surveyed at the end of the experiment in June 2021 (24 months after fire and mulching).

Moreover, the total length of the main stem and root-collar diameter of all surviving seedlings was measured in June of 2020 and 2021 (Figure 2d). Then, in June 2021, the soil supporting seedling growth was gently excavated and the aerial part of the plant was separated from the root system. On the latter, the root length and dry weight (at a temperature of 60 °C for 48 hours) were determined on samples of ten seedlings per plot.

2.4. Statistical analyses

Previously, Cochran's C test, a one-sided upper limit variance outlier test, was aèèòoed to the BMP values, in order to detect possible outliers.

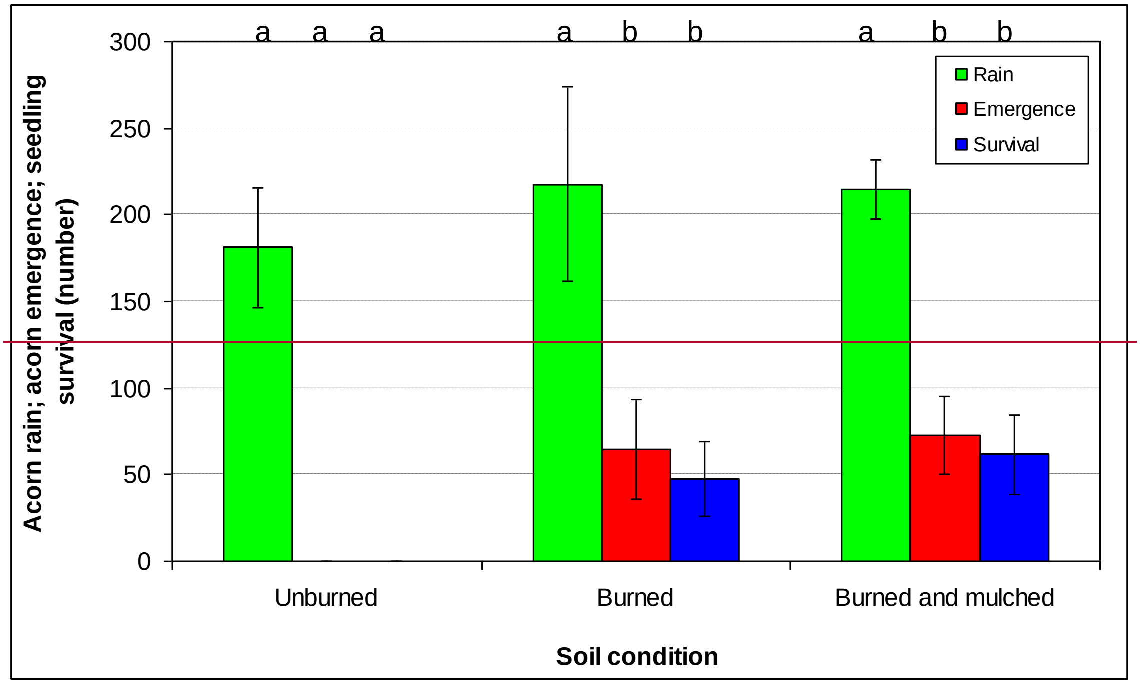
Then, two-way ANOVA was applied to evaluate the statistical significance of the differences in the BMP (considered as dependent variables) among the number of replicates and test duration (independent factors).

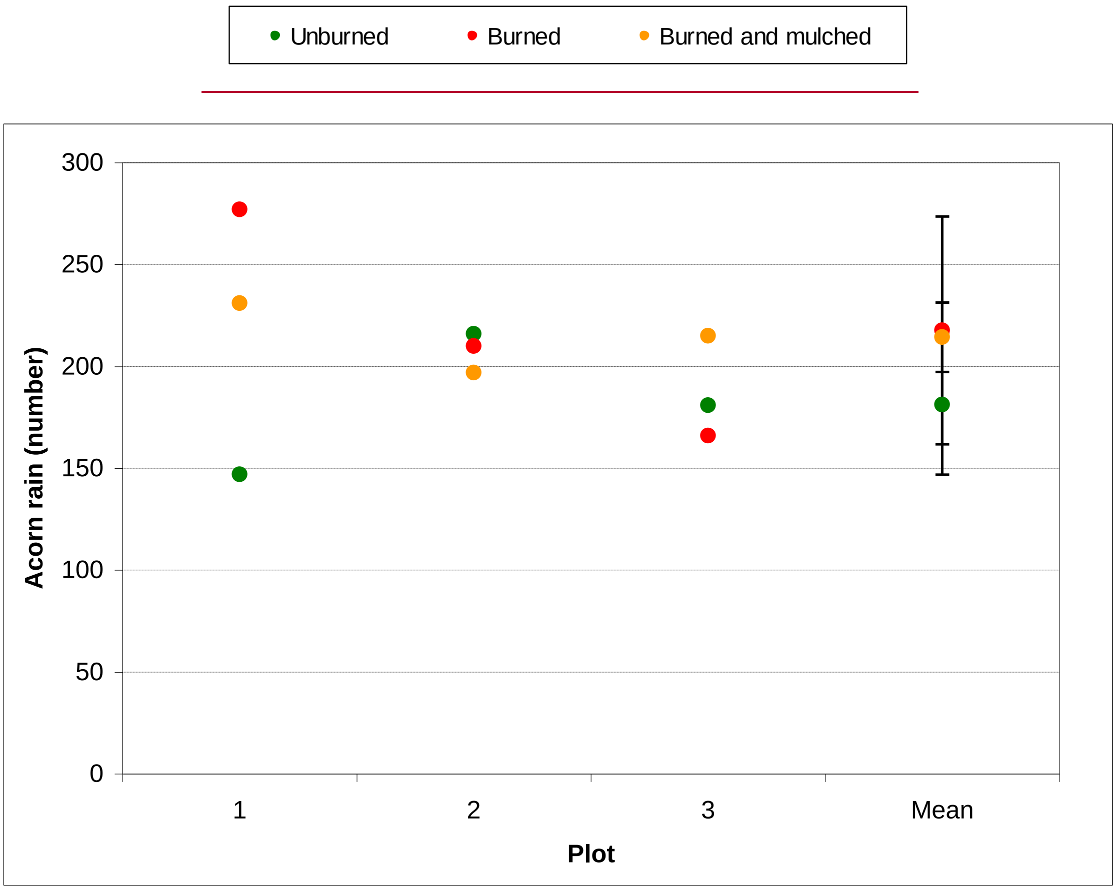
For both tests, to satisfy the assumptions of the statistical tests (equality of variance and normal distribution), the data were subjected to normality test or were square root-transformed whenever necessary.

All the statistical tests were carried out by with the XLSTAT software (release 2019).

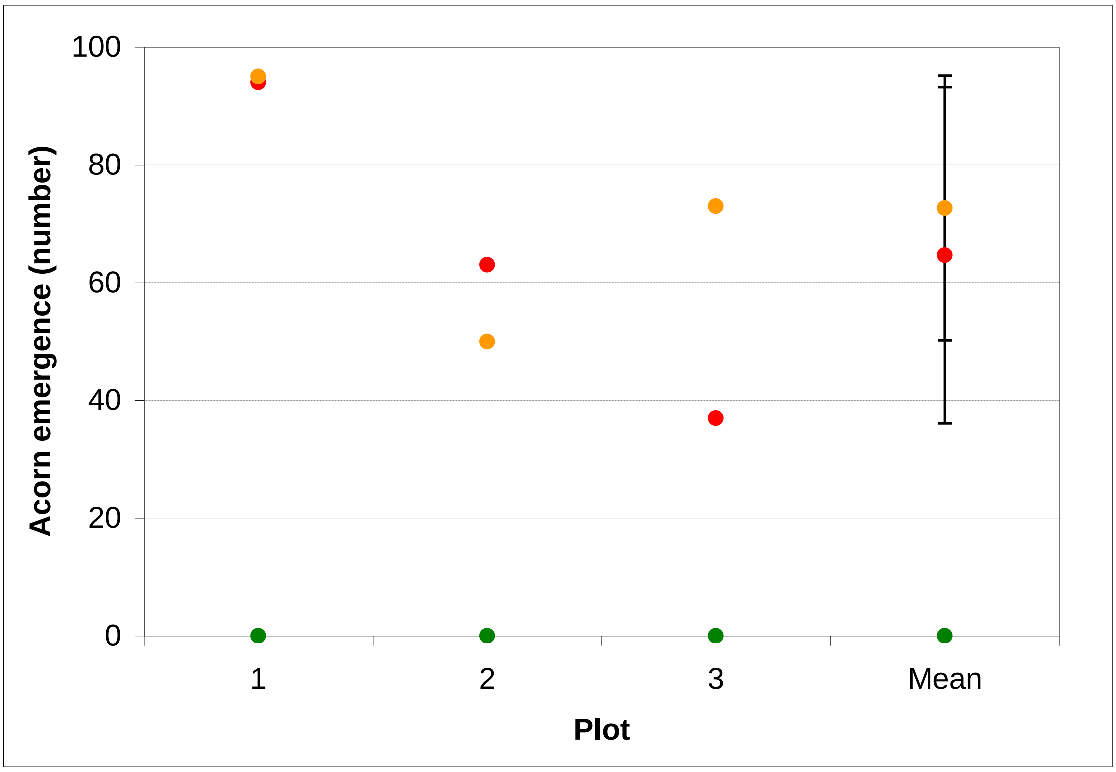
3. Results

Compared to the unburned sites, where acorn rain was on average 181 ± 35, in burned, and burned and mulched plots 218 ± 56 and 214 ± 17 acorns were counted, respectively (Figure 3), although the differences among the different soil conditions were not significant ($p = 0.497$, $n = 3$, after the pairwise comparison by Tukey’s test).

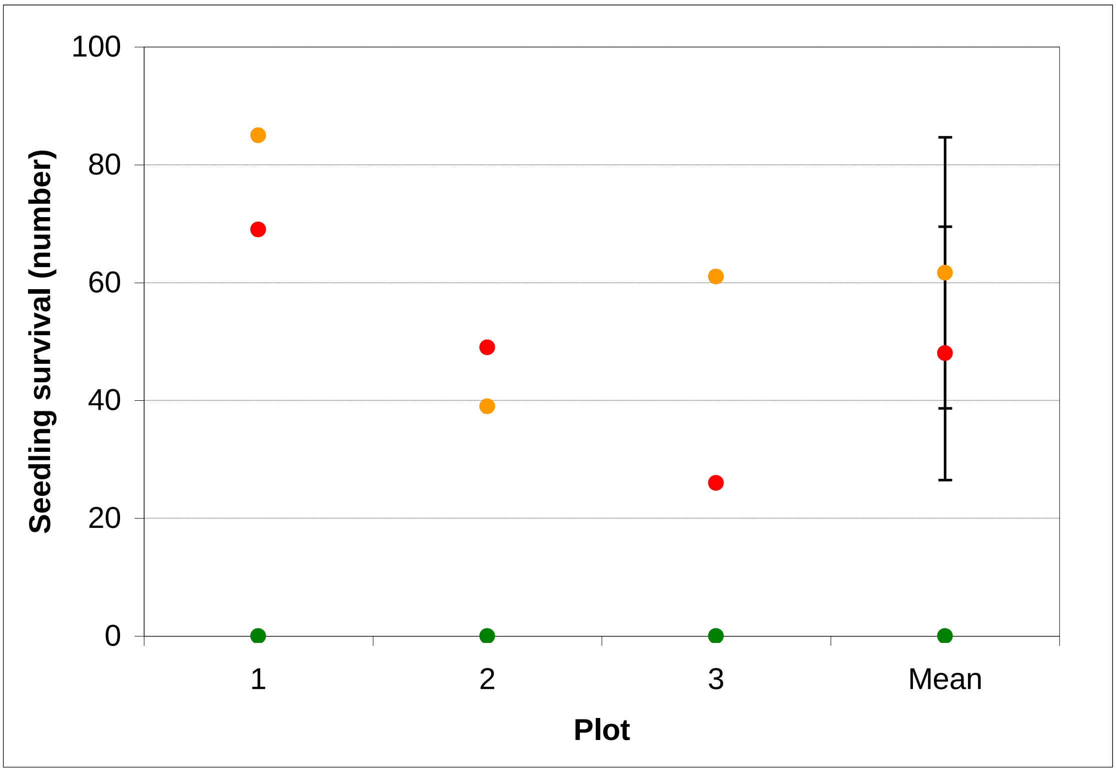

a a a a b b a b b
Rain
Emergence
Survival
Acorn rain; acorn emergence; seedling survival (number)
300
250
200
150
100
50
0
Unburned
Burned
Burned and mulched
Soil condition


Unburned
Burned
Burned and mulched
Acorn rain (number)
300
250
200
150
100
50
0
1
2
3
Mean
Plot

(a)


Acorn emergence (number)
100
80
60
40
20
0
1
2
3
Mean
Plot

(b)


Seedling survival (number)
100
80
60
40
20
0
1
2
3
Mean
Plot

(c)

Figure 3 - Acorn rain (a) and emergence (b), and seedling survival (c) ~~(mean ± standard deviation)~~ counted in nine plots (three per each soil condition, unburned, burned, and burned and mulched) of oak forest under different soil conditions (Samo, Calabria, Southern Italy). The vertical bar expresses the standard deviation of data in the three plots.

~~Different letters indicate significant differences among the soil conditions after the pairwise comparison by Tukey's test ($p < 0.05$, $n = 3$).~~

While no acorn emergence was noticed in unburned sites, 65 ± 29 acorns germinated on burned soils and 73 ± 23 on burned and mulched plots (Figure 3). Only the difference between unburned, and burned soils (mulched or not) was significant ($p < 0.01$, $n = 3$, after the pairwise comparison by Tukey's test).

Of the emerged acorns, 48 ± 22 and 62 ± 23 seedlings survived on burned, and burned and mulched soils (Figure 3), but the difference between this soil conditions was not significant ($p = 0.068$, $n = 3$, after the pairwise comparison by Tukey's test).

It should be highlighted that the values of oak regeneration may be affected by a bias, due to the low number of samples (three plots per soil condition), and the effect of this small size is large. This effect, which was measured by the ratio between the sum of squares between groups and the total sum of the squares (given by ANOVA), was large, according to the reference values proposed by (Cohen 2013), since this ratio is higher than 0.21 for the three variables analyzed (acorn rain and emergence, and seedling survival).

At both survey dates (June 2020 and 2021), plant height was higher in burned and mulched plots (92.8 ± 31.2 mm, June 2020, and 110.5 ± 40.3 mm, June 2021) compared to the burned and untreated soils (64.6 ± 35.3 mm, June 2020, and 85.3 ± 41.9 mm, June 2021) (Figure 4). According the 2-way ANOVA, both soil condition and survey date were significant factors ($p < 0.0001$ and 0.01, respectively, $n = 3$, after the pairwise comparison by Tukey's test) on plant height, but not its interaction ($p = 0.630$).

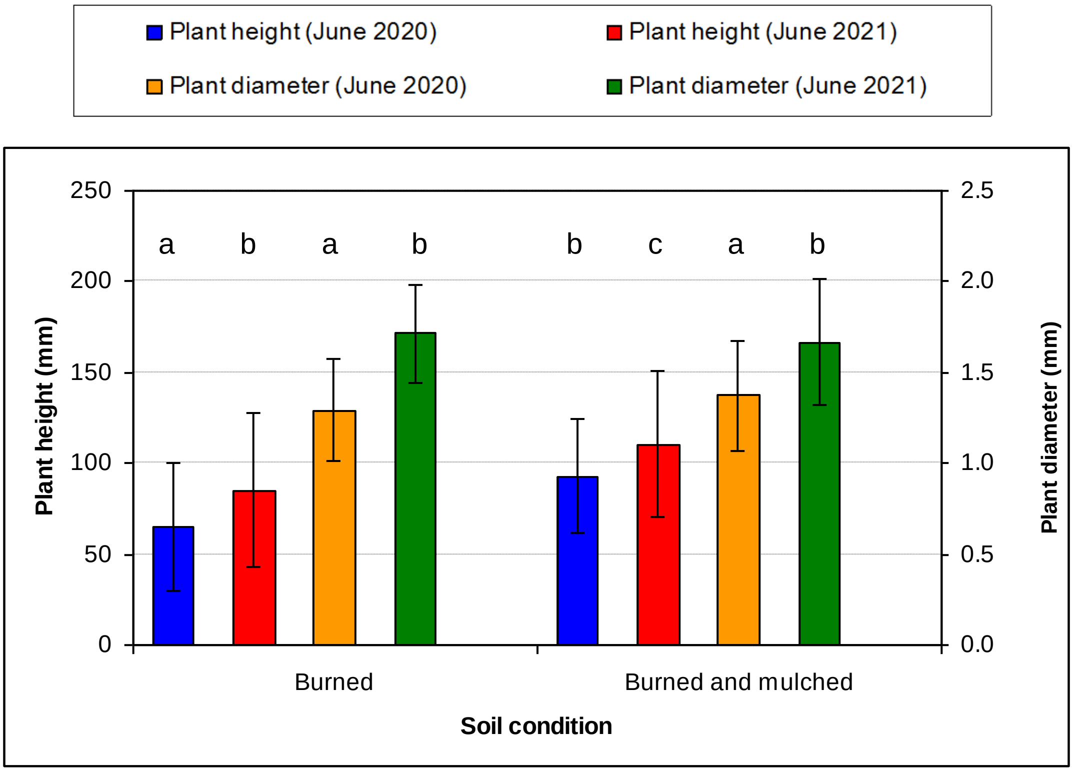


Figure 4 - Plant height and diameter (mean ± standard deviation) surveyed in oak forest under different soil conditions at two survey dates (Samo, Calabria, Southern Italy). Different letters indicate significant differences between the soil conditions after the pairwise comparison by Tukey’s test ($p < 0.05$, $n = 3$).

In June 2020, seedling diameter was lower in the burned and untreated plots (1.29 ± 0.28 mm) compared to the burned and mulched soils (1.38 ± 0.30 mm). In contrast, in June 2021, the latter soils showed seedlings with lower diameter (1.67 ± 0.35 mm vs. 1.71 ± 0.27 mm of burned and untreated plots) (Figure 4). For seedling characteristic, both the date and its interaction with the soil condition determined significant differences in plant diameter ($p < 0.0001$ and 0.01, respectively, $n = 3$, after the pairwise comparison by Tukey’s test). In contrast, soil condition did not statistically influence the seedling diameter ($p = 0.485$).

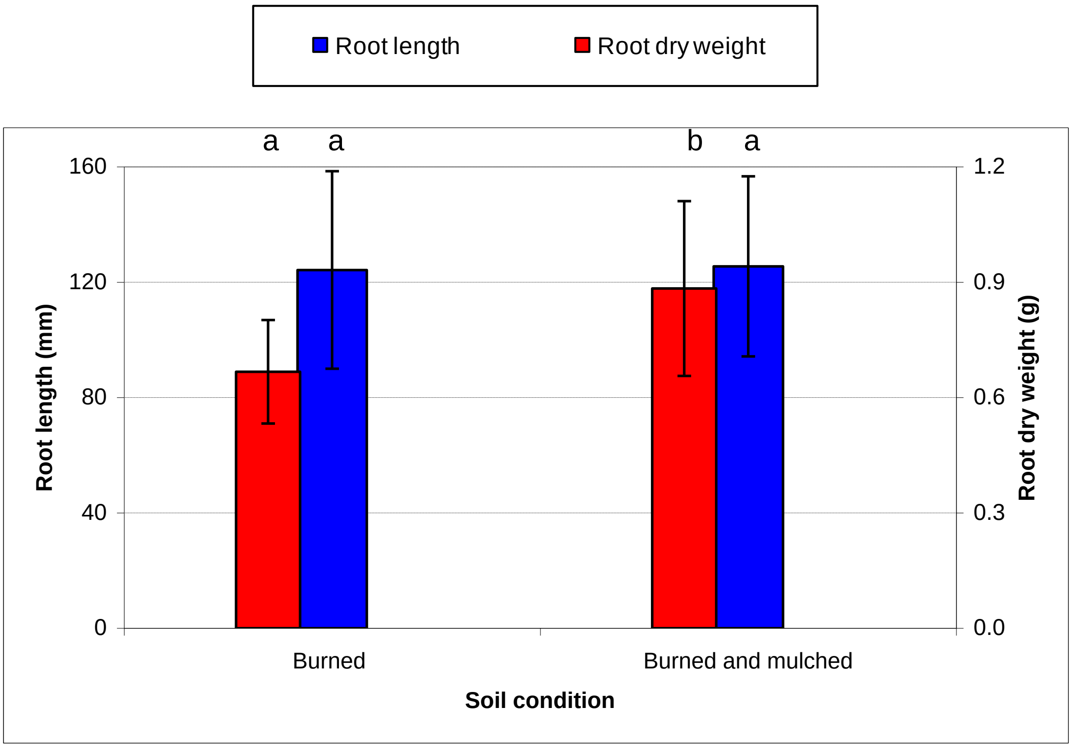


Figure 5 - Root length and dry weight (mean ± standard deviation) of oak seedlings under different soil conditions at two survey dates (Samo, Calabria, Southern Italy). Different letters indicate significant differences between the two soil conditions after the pairwise comparison by Tukey's test ($p < 0.05$, $n = 3$).

Root length (125 ± 34.3 mm and 126 ± 34.0 mm, respectively) was practically the same in burned, and burned and mulched soils. In contrast, the latter soils showed a higher root dry weight (0.88 ± 0.23 g) compared to the burned and untreated plots (0.67 ± 0.14 g) (Figure 5). These differences were significant for root dry weight ($p < 0.001$, $n = 3$, after the pairwise comparison by Tukey's test), but not for root length ($p = 0.905$).

4. Discussions

Since seedling recruitment is ultimately limited by the quantity of viable acorn fall, an evaluation of seedling patterns is necessary to understand the dynamics of recruitment (Lucas-Borja and Vacchiano 2018). Oak acorns are dispersed across a wide variety of sites covering a range of abiotic and biotic conditions. Given that a similar forest structure of oak forest among all plots, we assumed that acorns fall was similar at all plots. Furthermore, since parent rock material, topography, climate and forest structure were similar among selected plots, differences with respect to seedling emergence, survival and initial growth can be associated with the influence of fire and mulch.

While none of the fallen acorns sprouted in the unburned area, about 30% of acorns germinated in the burned areas, of which more than 70% survived. Higher regeneration in burned areas is in agreement with other studies dealing with oak recruitment, which reported higher survival rates (Royse et al. 2010; Brose et al. 2013; Petersson et al. 2020), the latter in combination with other treatments (e.g., tree canopy openness or ungulate exclosure) in burned oak stands, while (Hutchinson et al. 2005) found reduced sapling density in areas with burn treatments. Many reasons may explain the greater early recruitment of oak in the burned sites. First, the reduction in tree density and canopy cover increases light availability on the forest floor, which is very important for regeneration of oak species, which are considered light demanding for survival and growth once energy reserves of the cotyledons are exhausted (Annighöfer et al. 2015; Petersson et al. 2020). Second, the lower competition with herbaceous vegetation presumably enhances vegetation growth in this semi-arid environment, having a limited water availability (Garcia-Fayos et al. 2020; Petersson et al. 2020); in contrast, the presence of herb layer found in unburned plots could have delayed or impeded acorn emergence development (Caccia and Ballaré 1998). Third, low-intensity fire creates more favourable seedbed conditions to oak establishment, promoting the contact with mineral soil and thus favouring acorn emergence (Facelli and Pickett 1991; Hutchinson et al. 2005). Fourth, the increases in organic carbon, total nitrogen and available phosphorous measured in the same experimental site immediately after the prescribed fire compared to the unburned sites, followed by significant decreases one year after fire, when the plant regeneration was higher (Carra et al. 2021). A higher availability of organic matter and nitrogen contents of burned soils, which have been well documented after prescribed fires (Úbeda et al. 2005; Alcañiz et al. 2018, 2020; Hueso-González et al. 2018), may have supported the recruitment of oak seedlings detected in this study. These changes in soil properties also improve microbial diversity, which may increase soil fertility (Nannipieri et al. 2003; Lucas-Borja et al. 2012a). The latter effect together with the low damage to canopy acorn bank and the low impact on forest floor conditions after prescribed fires might generate better conditions for initial seedling recruitment. In line with this, (Madrigal et al. 2010) have also shown a positive influence of the soil organic layer remaining after fire on seedling recruitment, which has been related to a higher water content of soils with higher organic matter. However, since several studies have indicated that oak regeneration may be more effective after a single prescribed fire when combined with other treatments (Ssali et al. 2019; Petersson et al. 2020; Izbicki et al. 2020), the opening of tree canopy by mechanized operations and replicated applications of prescribed fire can be suggested, in order to remove midstory competition, increasing understory light, and increasing oak seedling growth and density (Green et al. 2010; Brose et al. 2013; Izbicki et al. 2020).

Mulching determined increases - although not being significant - in these percentages (about 35% of germinated acorns, and 85% of survived seedlings) that can be quantified in 14% and 12% for emergence and survival, respectively. (Lucas- Borja et al. 2021) have demonstrated that seedling survival but not emergence rates is largely controlled by post-fire treatments enhancing the establishment of vegetation cover, due to the beneficial effect of shrubs on the recruitment of seedlings located under their canopies (Emborg 1998; Heydari et al. 2017). Moreover, the mulch cover spread over the ground of burned soils acts as a barrier that reduces drought by lowering solar radiation and soil temperature and increasing its water content (Castro et al. 2003; Lucas- Borja et al. 2021). Also (Ssali et al. 2019) reported higher seedling survival on soils of tropical forests subjected to post-fire treatment with fern.

Many studies have stated that growth of oak seedlings in burned areas is higher compared to fire-excluded sites, since larger diameters (Royse et al. 2010), faster biomass growth (Wang et al. 2005), and greater heights (Petersson et al. 2020) were observed in oak seedlings after prescribed fires.

In spite of the non-significant difference in the initial recruitment of oak seedlings between mulched and untreated areas, this post-fire treatment enhanced plant growth at both survey dates, as shown by the significantly greater height (+44% one year after the treatment) compared to the plants grown on burned and untreated plots. In contrast, the plant diameter was not significantly influenced by the treatment (only +6% after one year). These enhancements of the plant morphology lost weight over time (+30% in height two years after the treatment) and even became negative (-3% in diameter) over time. Higher growth in soils burned and mulched with fern was reported also by (Ssali et al. 2019).

Regarding the root system, mulching did not significantly influence the length (only +1% in mulched areas compared to the burned and untreated plots), but determined a significant increase (by more than 30%) of the root mass.

The faster growth of seedlings in mulched areas compared to the untreated sites may be due to the higher water content, since it is well known that mulching enhances soil moisture which is a key point in natural regeneration process, especially in forest ecosystems of the semi-arid environments with limited water availability (Prosdocimi et al. 2016; Lucas-Borja et al. 2018). (Bautista et al. 2009) theorized that the main advantage of mulch application is the immediate increase in vegetal cover of soil, which result in an effective protection during the first rain events after fire. Moreover, the mulch material can be fast incorporated into the soil, thus increasing the its content of organic matter and nutrients (Bombino et al. 2019).

5. Conclusions

This study has demonstrated that the application of prescribed burning to an oak stand of the Mediterranean environment play significant effects on plant regeneration compared to the unburned sites. In relation to emergence, in burned areas, about 30% of acorns emerged, of which more than 70% survived. This may be due to the higher light availability on the forest floor, lower competition with herbaceous vegetation, more favourable seedbed conditions to oak establishment, and increases in soil contents of organic carbon, total nitrogen and available phosphorous. This result confirms the first working hypothesis that prescribed burning enhances the initial recruitment of plants, since oak is a forest species that is adapted to fire.

Soil mulching with fern applied as anti-erosive post-fire treatment did not significantly increase acorn emergence and plant survival compared to the burned and untreated sites. However, this post-fire treatment significantly enhanced plant height and root mass but not its diameter and root length. With the same root length, higher root biomass with more soil water availability led to greater height growth in the measured seedlings. These contrasting effects, which may be attributed to the higher water content of mulched area of this water-limited in forest ecosystem of the semi-arid environments, in part support the initial hypothesis that soil mulching may be synergistic with the prescribed fire.

Overall, the knowledge of the beneficial influences of prescribed fire and post-fire treatments on oak recruitment, thanks to the fire-tolerance character of *Quercus frainetto* L., is useful to develop sustainable management plans for the delicate forest ecosystems of the semi-arid Mediterranean environment. More research is needed in order to validate the outcomes of this study on different forest species and to evaluate the effects of prescribed fire and mulching with other forest operations.

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Author contributions

Conceptualization, B.G.C., G.B., M.E.L.B. and D.A.Z.; methodology, B.G.C., A.L., P.A.P.A., M.E.L.B. and D.A.Z.; validation, G.B., M.E.L.B. and D.A.Z.; formal analysis, G.B., M.E.L.B. and D.A.Z.; investigation, B.G.C., A.L., and P.A.P.A.; data curation, B.G.C., A.L., P.A.P.A., and D.A.Z.; writing - original draft preparation, B.G.C., and D.A.Z.; writing - review and editing, B.G.C., G.B., M.E.L.B and D.A.Z.; supervision, M.E.L.B and D.A.Z.; project administration, D.A.Z.; funding acquisition, D.A.Z. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest statement

The authors declare that they have no conflict of interest.

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