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Microsite manipulation in lowland oak forest restoration results in indirect effects on acorn predation

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Abstract

Taking advantage of facilitation mechanisms to counteract harsh environmental conditions may prove to be a successful strategy in the restoration of degraded man-made ecosystems. Seed sowing is often a viable and less expensive technique to restore forest cover in environments subjected to heavy anthropic disturbance. In this context, nurse plants and shield objects can improve microsite conditions, enhancing the emergence and growth of seedlings. However, their
presence can also create preferred microhabitats for seed predators, and as a result, may alter their movement decisions and foraging behavior. In this study, we investigated whether nurse shrubs and artificial shade structures, with and without previous soil amelioration, could facilitate acorn emergence. We also tested their effects on the predation of acorns by small mammals, within the framework of a larger project aimed at restoring a *Quercus robur* L. forest. The research was conducted in a former roadbuilder’s yard located along the Mediterranean Corridor, in Northern Italy. The yard had been in use during the widening of a highway that runs parallel to a stretch of the Corridor. The area was restored in 2014. Mechanical preparation of the soil included two treatments, with and without the addition of compost and zeolite. Acorns were then sown in plots in different microsites, i.e., 1) close to a shrub (*Cytisus scoparius* L., Scotch broom), 2) to the north and 3) south of a shield object (a 30 cm x 30 cm wood particle board), and 4) without any protection element (control). We recorded acorn emergence and predation during the first growing season. At the end of the season, overall acorn emergence was observed to be low, and, surprisingly, was higher in the non-amended soil treatment. The amended soil had a higher temperature and a lower water volumetric content in the summer (JJA). No evidence of direct facilitation on emergence by shield objects or Scotch broom was found, but indirect effects were detected. Acorn predation was generally high (67.45%), but was negatively affected by the presence of *C. scoparius*. The distance of the plot from the rainwater drainage ditch surrounding the study site also influenced winter predation; a larger number of acorns were removed by rodents at longer distances from the drainage ditch. In order to restore degraded
sites to lowland oak forests through direct seeding, it is necessary to deal with complex interactions between direct and indirect facilitation mechanisms, competition and predation. Predation was particularly important because of the high predation pressure on acorns.

**Key words:** *Quercus robur*; acorn; nurse shrub; artificial shield object; facilitation; seed predation.

1. Introduction

The ecological restoration of degraded man-made ecosystems usually requires plant establishment and survival in harsh or stressful environments, while limiting restoration costs and further interventions. Seed sowing is often a viable and less expensive alternative to planting nursery-grown seedlings (Madsen and Löf, 2005), with seeds being easier to transport and manage than bareroot and container seedlings. Direct seeding also allows the plant root system to develop naturally, thereby avoiding the root malformations that are generally associated with containers (Stanturf et al., 2000; Dey et al., 2008). Instead, the use of container stock can reduce the stress linked to nutrient or water limitation, the risk of poor survival, and number of operations necessary for site preparation and management, e.g. litter removal, mechanical scarification, repeated mowing to control competing ground vegetation (Smith et al., 1997; Dey et al., 2008). Moreover, after germination, seedlings can show slower development rates than planted trees (Nyland 2007).
The early stages of seedling establishment are critical for the regeneration of plant populations. Several mortality factors, including predation, competition and abiotic stress can hamper seed regeneration (Nathan and Muller-Landau, 2000; Castro et al., 2004; Schurr et al., 2004; Davis et al., 2005; Riginos et al., 2005; Kipfer et al., 2009). In addition, excess light, high temperatures and summer drought, which are accentuated in degraded areas without forest cover, can severely limit the survival and growth of seedlings (Castro, 2006; Gómez-Aparicio et al., 2006). Site conditions and mechanisms that operate at a small scale may in particular limit the emergence and performance of seedlings (Collins and Good, 1987).

Taking advantage of intra- or interspecific facilitation mechanisms and the sheltering effects of abiotic elements in natural environments has proven to produce beneficial effects on the survival, initial growth and fitness of the neighbouring seedlings, particularly in climatically stressing sites (Callaway, 2007; Brooker et al., 2008; Marzano et al., 2013). Increasing evidence has pointed out the potential benefits of the application of this nursing effect to improve the success of restoration projects in degraded environments (Padilla and Pugnaire, 2006; Torroba-Balmori et al., 2015).

Nurse plants improve the emergence, survival and growth of seedlings (Castro et al., 2004; Gómez-Aparicio et al., 2004; Gómez-Aparicio et al., 2006; Torroba-Balmori et al., 2015) providing a favourable microhabitat in their surroundings. They offer shade, buffering against high radiation and temperatures, and can increase soil moisture and nutrient content (Callaway, 1995; Rey Benayas, 2005; Legras et al., 2010; Leiva et al., 2015).
In highly degraded systems, and in particular at the grassland stage, nurse objects, such as woody debris or artificial shade-structures can emulate the beneficial effects of nurse plants by reproducing the shaded environments that can be found under their canopies (Gómez-Aparicio et al., 2005; Rey Benayas et al., 2005; Badano et al., 2011).

A key point that influences the recruitment process success is then the interaction among environmental factors that affect regeneration and the behavior of seed dispersal and predation agents (Crawley and Long, 1995; Sunyer et al., 2015). This interaction can actually alter the probability of seed survival and seedling establishment, with the spatial pattern of seed removal usually resulting from predator preferences for certain microhabitats (Clark et al., 1999; Jordano and Schupp, 2000; Pérez-Ramos and Marañón, 2008). Thus, even though nurse plants and shield objects could improve microhabitat conditions and promote seed germination and growth, they could also influence animal behavior, driving seed and seedling predator movements. Their presence, which in particular affects the foraging behavior of animals that usually feed on seeds, could result in increasing predation.

It is well known that wild ungulates and granivorous rodents in Europe can predate a large number of seeds and browse saplings, thus reducing the success of direct seeding in restoration projects (Birkedal et al., 2009; Jinks et al., 2012; van Ginkel et al., 2013). The seed predation rate is strongly affected by habitat complexity, which in turn can influence the abundance of ungulates and rodents. For instance, wild ungulates tend to avoid habitats with complex structure that could hamper their movements, while small mammals prefer to forage in habitats
with shrubs and rocks, where they perceive a minor predation risk (Gómez et al., 2003; Orrock et al., 2004; Fedriani and Manzaneda, 2005; Pérez-Ramos et al., 2008; Leverkus et al., 2013). The presence of coarse woody debris in forests usually intensifies the removal of seeds by rodents, and the presence of shrubs and shelters lengthens the time that the rodent spends handling and choosing viable seeds (Perea et al., 2011; van Ginkel et al., 2013).

In the context of a large lowland oak forest restoration project, we have investigated whether the presence of shelter elements can improve acorn emergence and seedling survival of pedunculate oak (*Quercus robur* L.) or whether it can support the movement decisions and foraging behavior of small rodents, by analyzing the main factors that affect spatio-temporal variations in seed predation.

Since acorn germination in *Quercus* species is conditioned by soil moisture (Pérez-Ramos et al., 2013) and can present delayed emergence (González-Rodríguez et al., 2012), using shrubs (Gómez-Aparicio et al., 2004) and artificial shield objects on planting sites could ameliorate microclimatic conditions, and thus enhance emergence rates (Smit et al., 2008). The large size of oak cotyledons can increase the odds of both regeneration success and seed predation. The latter, which is mainly by small mammals (Gómez et al., 2003; Smit et al., 2008), is usually more intense in the post-dispersal phase and is one of the main factors that endangers oak recruitment. The regeneration performance of oak is related to several variables (Annighöfer et al. 2015). Acorn predation before germination largely determines the success of regeneration
(Harmer, 1994), with competition, pests, water supply, light availability and browsing being other variables leading to recruitment failure (Nilsson et al., 1996). We have hypothesized that improving the physical and chemical conditions of the soil and facilitating seed germination with nurse shrubs and shield objects could influence oak establishment but also the removal of acorns by small mammals. Our main aims were thus to 1) assess the impact of soil amelioration on acorn emergence and predation rate; 2) evaluate the role of enhanced seeding microsites (close to nurse shrubs and shield objects) on the regeneration performance and acorn predation; 3) determine whether the relative position of sowing sites can modify the predation pattern.

2. Materials and methods

2.1 Study site.

The experiment was conducted in a degraded area in northwestern Italy (45°11'38.60" N, 7°50'38.04" E, ca. 190 m a.s.l.). The site is located along the Rail Freight Corridor 6 (Mediterranean Corridor) (European Commission, 2018), linking the southwestern Mediterranean region of Spain and the Hungarian border with Ukraine. The area, which is bordered by a highway, was formerly a roadbuilder’s yard. In November 2014, after the road works ended, the area was restored through both seed sowing and seedling planting (2 year old nursery plants), followed by hand sowing with a grassland species mixture. The tree and shrub species used in the experiment were those that usually grow in the lowland oak-hornbeam Mesophytic deciduous forest, i.e., the natural late seral forest
ecosystem of the Po Plain, which is dominated by *Q. robur* and *Carpinus betulus* L.

No trees were present when the experiment was started. The soil texture was sandy loam. The pH ranged from 7.41 to 8.08 and soil organic matter content ranged from 1.5% to 3%. The cation exchange capacity (CEC) was lower than 10 meq/100 g.

The climate is temperate, with an annual mean temperature of 11.4 °C and average annual precipitation of 806.2 mm (ARPA Piemonte-Verolengo meteorological station 10 km from the study site, period 1988-2010). The rainfall is not homogeneously distributed during the year, with spring and autumn being the wettest seasons.

A rainwater drainage ditch surrounds the area. At the beginning of the experiment, the area was fenced off against the introduced eastern cottontail (*Sylvilagus floridanus*) and wild ungulates to avoid browsing damage to seedlings and seed predation.

The experimental design (Fig. 1) included two soil treatments (non-amended and amended). In November 2014, the treatments were applied in rows after a mechanical site preparation. Using a tractor the rows were ripped (to a 70 cm depth) and ploughed (to a 40 cm depth). The distance between rows was approximately 2.5 m. Furrows were then formed along the southern edge of the rows. Half of the rows were just ripped and ploughed (non-amended). Since we wanted to improve the organic matter content up to 3% and raise the CEC to 15 meq/100g, the other rows were ripped, ploughed and amended using 4.2 Mg/ha of compost and 43 Mg/ha of zeolite, with a diameter of 3-8 mm. The compost
had a pH of 8.7, 24% w/w of organic carbon content on a dry matter basis, and a 2.19% total N content. Zeolitite was chosen as it is a slow release fertilizer (Allen and Ming, 1995; Campisi et al., 2016). It has a very good cation exchange capacity and retains water and nutrients thanks to its high adsorption ability. In the sampling design, amended rows alternated with non-amended ones (Fig. 1). Rectangular plots (4 m x 5 m) were established, 1.5 m apart from each other, within each row. Six plots per treatment were used for the direct seeding of *Q. robur* acorns, while the remaining 60 plots per treatment were used for seedling planting.

**Fig. 1.** Sampling design. Plots were established in rows after mechanical site preparation. Rows amended with compost and zeolitite (fully colored in grey) alternated with the non-amended ones (no color; grey border). Among the amended plots, the black ones are those used for the direct seeding of *Q. robur*
acorns. Among the non-amended plots, those with a black border were used for the direct seeding of *Q. robur* acorns. The rest of the amended and non-amended plots were used for seedling planting (data not reported). A rainwater drainage ditch (black dashed line) surrounds the study site, which is also bordered by a highway.

2.2 Acorn facilitation and predation experiment

In order to assess the role of shield and nurse elements in the oak emergence, and their influence on rodent predation, we performed a sowing experiment, the set-up of which included four different treatments within each of the 12 main-plots that were sown (6 x 2 soil treatments). In each soil treatment, acorns were sown 1) close to a *Cytisus scoparius* L. (Scotch broom) shrub (B); 2) close to the northern (N) and 3) southern side (S) of a shield object; 4) without protection elements in the surrounding area (control) (C).

Scotch broom is a native nitrogen-fixing species, which grows in dense clumps, and has been found to facilitate *Q. robur* recruitment (Muhamed et al., 2015). The nurse shrubs were 2 year old nursery Scotch brooms, with a mean height of 72.5 cm (± 10.8) at planting time in November 2014. Any dead Scotch brooms were replaced during the experiment. The artificial shield objects consisted of wood particle boards (30 cm x 30 cm), erected vertically in an east-west direction.

We established 10 sowing sites (replicates) for each treatment. Three acorns were planted in each site, 5 cm apart, one per hole; they were buried vertically at a depth of 3 cm and covered with soil. The acorns were sown at the minimum possible distance from the wooden boards and *C. scoparius* (2-5 cm). This
resulted in a total of 10 replicates x 3 acorns x 4 treatments x 6 plots x 2 soil treatments (= 1440 buried acorns). Before sowing, the seeds were floated in water to exclude any non-viable ones (Gómez et al., 2003). A single batch of acorns was used (Bosco della Mesola, 44°50'20.66” N, 12°15'08.70” E, ca. 0 m a.s.l.). Sowing was performed twice, in winter (December 2014) and spring (March 2015), thus allowing us to test for seasonality in predation. The sowing site position was recorded in order to investigate whether the distance from potential preferential paths (e.g. the drainage ditch and furrows), where rodents would be protected from possible predators, affected their foraging behavior and thus the seed removal rate.

2.3 Data Collection

Seedling emergence was recorded 3 times during the experiment, in May, July and September 2015. It was calculated in a cumulative way.

The meteorological data that might have influenced emergence (air temperature, precipitation) were obtained from the ARPA Piemonte-Verolengo meteorological station (45°10'54.86” N, 8°1'55.46” E; 163 m a.s.l.). The study year (2015) had a particularly hot and dry summer. Mean summer temperature (JJA) was 1.9 °C warmer than the 1989-2014 period (23.1 °C vs. 21.2 °C), and precipitation amounted to 168.4 mm (mean summer precipitation 1991-2014 was 196.8 mm). The soil temperature and moisture were collected using Lascar EasyLog EL-USB-2 data loggers (n=16) and Spectrum WatchDog 1400 Micro Stations with Spectrum WaterScout SM 100 soil moisture sensors (n=16), respectively. The soil sensors were buried at a depth of 10 cm in each sowing manipulation for the
two soil conditions (amended and non-amended). Data were collected from the beginning of June to the end of August 2015.

The final fate of the acorns was recorded twice: early spring (March), and late summer (September). By means of a hunting camera, rodents were identified as the main acorn predators. Removed and gnawed acorns were considered as predated.

We confirmed the removal of acorns in each survey by digging all the sowing points. During the first survey, acorns were recorded as predated or intact. During the second survey, acorns were recorded as seedlings (epicotyl visible aboveground), ungerminable (empty or unhealthy endosperm/cotyledon), or predated (removed or gnawed seeds) (Birkedal et al., 2010).

2.4 Data analysis

We firstly analyzed the role of soil treatments on acorn emergence and predation rate by means of the $\chi^2$ test. As expected, soil amelioration had no effect on predation rates, and was thus not considered in subsequent predation analyses ($\chi^2; p>0.05$). We then tested the effect of the different treatments on acorn predation by fitting generalized linear models (GLM) in which the presence or absence of shrubs (B), the location south (S) or north (N) of the board, distance from the surrounding drainage ditch (DIST), and the period (TIME) were included, along with all their possible interactions, as explanatory variables. The DIST parameter was computed in a GIS (ArcMap 10.1) environment: eight 5.5 m wide buffers were created and each plot was assigned to a buffer in relation to its
distance from the ditch. The control plots were included in the analysis with absence values (0) for all treatments (i.e. no shrubs, no boards).

In order to define the influence of different factors on the rate of emergence we ran generalized linear mixed models (GLMM) considering soil amelioration (A), presence of shrubs (B), location south (S) or north (N) of the board as fixed parameters, along with their possible interactions, and plot location defined by distance from the drainage ditch (DIST) as a random factor. GLMM analyses were implemented in the R software environment (2.15.2; R Development Core Team, 2012) using the lme4 package (Bates et al., 2015). The GLM analyses were run using STATGRAPHICS centurion XVII (Statpoint Inc., USA, 2014).

Since the predation and emergence data showed a binary response, a binomial error distribution with a logit-link function was adopted in both analyses. Model simplification was accomplished by computing the Akaike information criterion (AIC). Starting from the full model, the minimal adequate GLMM or GLM was obtained by sequentially removing any non-significant model terms until no further reduction in AIC was observed.

Multi-comparison tests were run using Kyplot 2.0 (KyensLab Inc.) for the parameters that appeared to significantly affect acorn predation or emergence.

We tested the relationship between distance from the drainage ditch (DIST) and predation rate for each plot using a linear regression model. A similar analysis was conducted considering the distance of sowing sites from the furrows on the southern side of the rows.

3. Results
The mean summer temperature in the topsoil (0-10 cm) was 28.0 °C, while the mean summer volumetric water content was 6.1%. A significantly higher daily mean temperature of the soil was observed in the amended sowing sites located close to shrubs (B) and north of the boards (N), compared to the non-amended ones (t-paired test; p<0.01), but no difference was found between treatments for both amended and non-amended sites (Table 1; Tukey test, Pairwise multiple comparisons; p>0.01). Both daily maximum temperature and temperature range showed higher values in amended sites than in non-amended ones (t-paired test; p<0.01), except for control sites. No significant differences were detected for daily maximum temperature between sowing treatments for both amended and non-amended sites. The soil in amended sites on the southern side of the board (S) showed the largest mean daily temperature range (compared to C and B; Tukey test, Pairwise multiple comparison; p<0.01), while the daily range was significantly wider in S non-amended sites than in N ones (Tukey test, Pairwise multiple comparisons; p<0.01). The mean volumetric water content in amended and non-amended sites was significantly different for each sowing treatment (t-paired test; p<0.01). No differences were found in amended sites, while N showed a significantly higher water content in non-amended ones (Table 1; Tukey test, Pairwise multiple comparisons; p<0.01).

Table 1. Mean soil temperatures (daily mean, maximum and range) and volumetric water content (± standard deviation) for the summer months (JJA) in amended and non-amended sowing sites. Different letters indicate significant differences between treatments according to a Tukey post-hoc test (p<0.01). The
treatments are C, control; N, acorns sown north of a shield object (a wood particle board); S, acorns sown south of a shield object (a wood particle board); B, acorns sown close to Scotch broom (Cytisus scoparius L.).

<table>
<thead>
<tr>
<th></th>
<th>amended soil</th>
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<th></th>
<th>non-amended soil</th>
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<tr>
<td></td>
<td>JJA</td>
<td>C</td>
<td>N</td>
<td>S</td>
<td>B</td>
<td>C</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>Soil T mean</td>
<td>28.26 ± 4.34</td>
<td>27.85 ± 4.28</td>
<td>28.19 ± 4.46</td>
<td>28.16 ± 4.34</td>
<td>27.89 ± 4.50</td>
<td>27.21 ± 4.22</td>
<td>28.30 ± 4.79</td>
<td>27.87 ± 4.26</td>
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<tr>
<td>°C</td>
<td></td>
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<tr>
<td>Soil T max</td>
<td>33.21 ± 5.85</td>
<td>33.28 ± 5.94</td>
<td>34.29 ± 6.45</td>
<td>33.40 ± 6.11</td>
<td>33.05 ± 6.43</td>
<td>31.87 ± 5.79</td>
<td>33.80 ± 6.98</td>
<td>32.87 ± 5.84</td>
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<tr>
<td>°C</td>
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<td></td>
</tr>
<tr>
<td>Water Content</td>
<td>6.17 ± 5.10</td>
<td>4.37 ± 3.86</td>
<td>4.40 ± 3.80</td>
<td>4.73 ± 4.05</td>
<td>5.12 ± 3.84</td>
<td>11.83 ± 8.20</td>
<td>4.82 ± 3.33</td>
<td>7.00 ± 5.99</td>
</tr>
<tr>
<td>(%)</td>
<td></td>
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In September, acorn emergence was 7.98% (based on the total sown amount). No significant difference in emergence rates was observed for the two soil treatments ($\chi^2$ test; $p>0.05$).

Predation rate was high for both periods (42.09% in March; 67.45% in September). No significant soil treatment effects were observed on the acorn predation rate for either period ($\chi^2$ test; $p>0.05$). A significant difference between sowing treatments was instead found in both amended and non-amended sites ($\chi^2$ test; $p<0.01$). In March no significant difference was found in the number of predated acorns between treatments. In contrast, predation was significantly
lower in September (Steel-Dwass multiple comparisons test; p<0.05) in B (55.56%) than in C (77.50%) or S (70.05%) (Fig. 2).

**Fig. 2.** Predation rate of *Q. robur* acorns for the two surveys (March and September). Different letters indicate significant differences between treatments according to a Steel-Dwass multiple comparisons test (p<0.05). The treatments are C, control; N, acorns sown north of a shield object (a wood particle board); S, acorns sown south of a shield object (a wood particle board); B, acorns sown close to Scotch broom (*Cytisus scoparius* L.).
GLM showed that different factors affected predation rate (Table 2). The final model (F=6.27; p<0.001) showed that only the shrub presence, among the different treatments, significantly affected predation rate, and that distance from the drainage ditch was the most important variable. The period was only important when combined with distance from the drainage ditch and with treatments (B, N, S). This result was confirmed by the linear regression analysis (Fig.3). In March, there was a significant increase in predation rate as distance from the drainage ditch increased (R² = 0.696, p = 0.001). No significant effect was found in September (R² = 0.017, p = 0.689), the predation rate being high and almost constant (always >50%) along the distance gradient (Fig.3).

**Table 2.** Summary of the GLM for acorn predation. B, acorns sown close to Scotch broom (*Cytisus scoparius* L.); N, acorns sown north of a shield object (a wood particle board); S, acorns sown south of a shield object (a wood particle board); DIST, distance from drainage ditch; TIME period of the survey.

<table>
<thead>
<tr>
<th>Source</th>
<th>Estimate± S.E.</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.064±0.021</td>
<td>1, 69</td>
<td>9.63</td>
<td>0.003</td>
</tr>
<tr>
<td>N</td>
<td>0.021±0.021</td>
<td>1, 69</td>
<td>1.06</td>
<td>0.301</td>
</tr>
<tr>
<td>DIST</td>
<td>-0.182±0.038</td>
<td>5, 69</td>
<td>8.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TIME</td>
<td>-0.051±0.033</td>
<td>1, 69</td>
<td>2.29</td>
<td>0.135</td>
</tr>
<tr>
<td>B x TIME</td>
<td>-0.041±0.024</td>
<td>1, 69</td>
<td>2.76</td>
<td>0.101</td>
</tr>
<tr>
<td>N x TIME</td>
<td>-0.031±0.024</td>
<td>1, 69</td>
<td>1.58</td>
<td>0.213</td>
</tr>
<tr>
<td>S x TIME</td>
<td>-0.042±0.024</td>
<td>1, 69</td>
<td>2.96</td>
<td>0.090</td>
</tr>
</tbody>
</table>
Fig. 3. *Q. robur* predation rate in relation to plot distance from the drainage ditch. 
$R^2=0.696$, $p=0.001$ in March (M, solid line). $R^2=0.017$, $p=0.689$ in September (S, dashed line).

Overall net acorn emergence was computed at the end of the season considering only non-predated acorns. Just 24.3% of non-predated acorns germinated. A higher emergence rate was found in non-amended sites ($\chi^2$ test; $p<0.05$), but there were no significant differences between sowing treatments ($\chi^2$ test; $p>0.05$).

GLMM confirmed that soil treatment was the only factor that affected emergence (Table 3).
The distance between different sowing points in the plot and furrows had no significant effect on the predation rate in either March or September (p>0.05). Since there was a high predation rate and low germination success, which greatly reduced the number of emerged seedlings, the growth rate and survival data were not considered in the subsequent analyses.

Table 3. Model parameter estimates derived from the GLMM models for acorn emergence at the end of the growing season (net of predation). B, acorns sown close to Scotch broom (*Cytisus scoparius* L.); S, acorns sown south of a shield object (a wood particle board); SOIL, acorns sown in amended soil.

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Estimate± S.E.</th>
<th>Z-value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-1.45±0.25</td>
<td>-5.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S</td>
<td>0.47±0.29</td>
<td>1.61</td>
<td>0.107</td>
</tr>
<tr>
<td>B</td>
<td>0.39±0.31</td>
<td>1.23</td>
<td>0.218</td>
</tr>
<tr>
<td>SOIL</td>
<td>-0.83±0.35</td>
<td>-2.35</td>
<td>0.019</td>
</tr>
<tr>
<td>B x SOIL</td>
<td>0.61±0.44</td>
<td>1.39</td>
<td>0.165</td>
</tr>
</tbody>
</table>

4. Discussion

Different biotic and abiotic factors were found to affect the regeneration performance in our manipulation experiment.
Contrary to our expectations, *Q. robur* emergence was low in all treatments. However, the short-term results of the restoration activities were to a great extent influenced by rodent predation.

The summer of the study year (2015) was hotter and dryer than average (see also Orth et al., 2016), and this probably reduced the soil moisture, whose influence on acorn germination in *Quercus* species is well known (Löf and Birkedal, 2009; Pérez-Ramos et al., 2013). A rainfall deficit, coupled with high air temperatures, affected the microclimatic conditions in the top soil, and this led to an extremely low water content and high temperatures, far from optimal values for acorn germination and seedling growth.

The stressful environment determined by the higher temperatures and lower precipitations in the summer months was also attested to by the high mortality rate of planted seedlings, all of which presented symptoms of drought stress and increased transplant shock (data not shown).

Summer drought, the frequency and length of which are predicted to increase in Central Europe due to climate change (Orth et al., 2016; Trnka et al., 2016), can exacerbate the harsh conditions faced by seedlings, particularly in areas undergoing restoration activities. In these areas, its negative effects could potentially be mitigated through microsite amelioration provided by shading elements (Castro et al., 2002; Man and Greenway, 2011). Nurse shrubs and natural or artificial shield objects can affect microsites anisotropically (Lingua et al., 2008; Marzano et al., 2013), thus making the relative location of the seedling crucial. In our manipulation experiment, soil conditions on the two sides of the wooden board differed. Higher maximum temperatures, which lead to an increase
in the daily range, are commonly found on the sun-exposed side, as a result of both a lack of shade and an increase in reflected solar radiation.

Artificial shield objects have proved important in enhancing microsite conditions in harsh environments, protecting plants from wind, snow, and/or excessive heat (Helgerson, 1990; Carlsson and Callaghan, 1991). Being non-living elements, artificial objects can sometimes induce a net positive effect since they provide benefits to seedlings without competing for resources (e.g. water, nutrients), in a similar way to in-situ produced deadwood and woody debris (Castro et al., 2011; Marzano et al. 2013).

In our treatments, the amended soil reached the highest daily mean and maximum temperatures, as well as the widest temperature range, since organic matter probably affected solar radiation adsorption and reradiation (Helgerson, 1990). The sites located on the southern side of the boards, which were those that reached the highest diurnal temperatures during summer, had the widest temperature range for both soil treatments. On non-amended sites, the temperature on the northern side of the boards was always lower (even if not significantly different from the southern side). However, it should be underlined that the sensors were buried in the soil at a depth of 10 cm, and this probably led to a buffering of the signal at ground level, where the effect of the nurse objects might be higher.

The soil water content did not differ among the amended sites. Even though zeolitite could improve water retention (Xiubin and Zhanbin, 2001; Polat et al., 2004), the amount applied in the area was calculated only considering soil CEC
amelioration and may have been insufficient to affect the soil water holding capacity.

Conversely, in non-amended sites the board significantly enhanced the water content in the soil shaded from the midday sun's rays, thus suggesting that in harsh environments even small artificial objects are able to reduce an excess of irradiance and thus influence soil moisture (Egerton et al., 2000), as well as the suitability of a regeneration site.

Fabaceae shrubs have been found to facilitate tree regeneration by providing enhanced microsite conditions, but this facilitation probably acts more at the seedling survival stage than at the emergence level (Burrows et al., 2014). In addition, newly emerged oak seedlings depend to a great extent on cotyledon reserves, and are thus probably less influenced by the potential nursing effects of neighboring shrubs (Pérez-Ramos et al., 2010).

In our sowing trial, the facilitation provided by the shrubs was not direct in the first year, as a result of microsite enhancement, but was instead indirect, thus protecting the acorns from predation. The presence of shrubs is usually associated with improved microclimate and soil properties (Pugnaire et al., 2004; Smit et al., 2008), as well as the protection of seedlings against herbivory (Smit et al., 2006; Callaway, 2007). On the contrary, the risk of seed predation by rodents is generally first increased by the sheltered conditions created under the shrub (Callaway, 2007), potentially leading to a short-term disruption of the net facilitation effect (Bruno et al., 2003). This complex interaction has still not been exhaustively explored (Chaneton et al., 2010).
The crown architecture of our *C. scoparius* transplants was probably not developed enough to create a fully sheltered environment for rodents; moreover, since rodents detect seeds through odorants, i.e., volatile molecules from the seed surface, it is possible that Scotch broom releases substances that mask the smell of acorns buried in their proximity (Vander Wall, 2003). Scotch broom is known to be an allelopathic shrub that produces a set of different alkaloid compounds (Gresser et al., 1996; Grove et al., 2012). Further analyses should be conducted to investigate whether these shrubs can effectively alter the feeding behavior of rodents, as has already been shown for other plants (Curtis et al., 2002; Fischer et al., 2013; Hansen et al., 2016).

Seed predation was high in the area in both winter and summer. The presence of the drainage ditch, furrows, shield objects and shrubs could provide protected sites and safe pathways for rodents, thus hiding them from predators. Seed predation rates are usually significantly higher in more complex habitats (Gómez et al., 2003; Madsen and Löff, 2005). Protected by vegetation, rodents can spend more time in finding and removing acorns (Orrock et al., 2004, Pérez-Ramos et al., 2008; Pérez-Ramos and Marañón, 2008; van Ginkel et al., 2013). We therefore expected the acorn predation rate to be higher under shrubs or close to wooden boards since these shelter elements can provide safer sites for foraging, potentially increasing rodent activity (Perea et al., 2011). We also expected a higher predation rate close to the furrows and drainage ditch surrounding the area.

Summer acorn predation was instead lower under Scotch broom, thus highlighting that the influence of nurse shrubs on predator feeding habits could
result from the interplay of several factors, not merely related to their cover and the resulting sheltering effect. Finding shrub species that are able to both enhance microsite conditions and limit acorn predation would be of great value for forest restoration activities.

In winter (Dec-Mar), the distance from the drainage ditch played a key role in determining the predation rate, but it was not inversely related as had been hypothesized. In fact, the observed predation rate increased with distance. A possible explanation for this is the presence of the fence along the highway (and the drainage ditch) enclosing the experimental area. Fence poles provided the only perching sites for rodent predators, given that there was no tall vegetation on the site. The predation pressure of raptors on small mammal populations may depend on the availability of perch sites (Wolff et al., 1999; Meunier et al., 2000). Car and street lights could also have contributed to increasing the perception of danger in the bordering area (Perea et al., 2011). The rodents thus perceived a higher predation risk near the edge of the experimental site rather than in its central part, and modified their feeding behavior accordingly (Diaz et al., 2005).

This spatial relationship disappeared in late spring and summer, when tall herbaceous vegetation covered the entire area homogeneously, thus making rodent movements safer over the whole site.

Restoring degraded sites to a lowland oak forest through direct seeding requires dealing with the intensive predation pressure that acorns are usually subjected to. With so many animals relying on acorns as a main part of their diet, restoration projects that wish to apply seed sowing must adopt adequate strategies for the direct or indirect protection of acorns (Madsen and Löf, 2005; Leverkus et al.,
2015; Torroba-Balmori et al., 2015). Seed protection should not greatly impinge on the total cost of the restoration effort. Cost-effective restoration in highly disturbed areas (e.g. after road, highway or railway construction) is currently a challenge for ecologists and practitioners (Cuperus et al., 1999; Corbin and Holl, 2012; Löf et al., 2012; Meli et al., 2014; Stark et al., 2015). Restoring degraded areas, particularly where disturbed site conditions might be made worse by a harsh environment and climate conditions, may require more complex approaches, thus making it necessary to understand the various interactions between direct and indirect facilitation mechanisms, competition, predation and herbivory.

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Authors’ contributions: S.M.: conception and design of the research, data collection; data analysis; paper writing; E.L. and R.M.: conception and design of the research, data analysis; paper writing. F.M.: conception and design of the research, data collection; paper revision. M.F., R.M. and A.N.: paper revision.
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