Lack of local adaptation to the establishment conditions limits assisted migration to adapt drought-prone *Pinus nigra* populations to climate change

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ABSTRACT

The intentional movement of tree genotypes, or assisted migration, is an operational approach proposed to adapt forest management to climate change. Assisted migration assumes that tree populations are locally adapted to environmental conditions, but this fact should be tested specifically. In this study, both a reciprocal transplant and a common garden experiment were carried out to test local adaptation to variable environmental conditions of seedling emergence and survival of the tree species *Pinus nigra*. Reciprocal sowing experiments involved the cross-sowing of four seed origins among four destination habitats, located in high- and low-altitude sites within central and peripheral populations of *Pinus nigra*. Evidence of local adaptation was taken to be a significant destination x origin interaction. Then, the possibility of local adaptation was further evaluated under the "local versus foreign" and the "home versus away" criteria. Results showed negative evidence of local adaptation to the emergence and survival environment faced by *Pinus nigra* seedlings, which would reduce the utility of assisted migration as an effective tool to adapt forests to ongoing climate change.

1. Introduction

Climate envelope models predict the movement of tree geographical ranges toward higher elevations and latitudes as temperature and evapotranspiration increase due to ongoing climate change (Araújo and Rahbek, 2006; Ruiz-Labourdette et al., 2012; García-Valdés et al., 2013). Other climate changes have occurred in the past, forcing the migration of tree species to new areas as they tracked the climate to which they were adapted (Davis and Shaw, 2001; Hampe and Petit, 2005; Iverson and McKenzie, 2013). However, a similar natural migration in response to current climate change is unlikely to occur for at least two reasons: expected change will be more rapid than trees are able to disperse to spread their domains, and natural migration would be almost impossible because of forest fragmentation and existing barriers to seed dispersal (Aitken et al., 2008; Valladares et al., 2014). Consequently, it has been argued that the persistence of forests will need to rely on the presence of sufficient genetic variation within tree populations, the maintenance of habitat connectivity and the implementation of some management strategies such as 'assisted migration' (Loss et al., 2011; Schwartz et al., 2012).

Assisted migration (also referred to as assisted colonization, assisted translocation and managed relocation in scientific literature) has been defined as the movement of species and populations to facilitate natural range expansion in direct management response to climate change (Vitt et al., 2010). Here, we will emphasize the intentional movement of tree populations (genotypes) so that propagates from the warmer range areas could be moved upward or northward in order to catch up with their expected future ranges (Millar et al., 2007; Pedlar et al., 2012; Williams and Dumroese, 2013).

Although the proposal derives from logical reasoning, it comes with various issues that have raised concerns among forest managers and ecologists (Loss et al., 2011; Williams and Dumroese, 2013; Schwartz et al., 2012). For instance, it has been highlighted that projections made by climate envelope models may be unsuitable to guide long-term forest planning under a climate change scenario, because the effect of climate on tree distribution is frequently mediated through topographic variation, soil properties and inter-specific interactions (Lo et al., 2010; Liancourt et al., 2013; Tíscar et al., 2017). There is also a risk of causing genetic introgression through the hybridization of the relocated and the native genotypes (Hufford and Mazer, 2003; Ricciardi and Simberloff, 2009).

Genotypes differences along species distribution ranges are mostly

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due to natural selection acting under the specific environmental conditions at each site. In this sense, assisted migration assumes that natural tree populations are adapted to their local environmental conditions and, particularly, to the climatic ones. However, local adaptation seems to be less common in plant populations than usually thought (Leimu and Fischer, 2008) and, when present, its scale and relative strength might vary (Galloway and Fenster, 2000; Garrido et al., 2012). For this reason, studies on local adaptation should be undertaken before considering assisted migration as a suitable management strategy to adapt forests to climate change.

Local adaptation can be seen as an outcome of the natural selection process whereby the frequency of the traits that enhance fitness increases within a population. It is typically assessed by means of reciprocal transplant experiments in which the average relative fitness of different demes is measured across a set of habitats, and the deme x habitat interaction statistically tested (Kawecki and Ebert, 2004). Experiments of reciprocal transplant can be carried out in the field, in a common garden or combining both methodologies (Pahl et al., 2013).

In this study, both a reciprocal transplant and a common garden experiment were performed in order to test for local adaptation in Spanish black pine (*Pinus nigra* Arn. subsp *salzmanii* (Dunal) Franco, *Pinus nigra* hereafter). Although fecundity is more advisable for fitness evaluation (Kawecki and Ebert, 2004), its use was inadequate here because *Pinus nigra* takes several years to produce fruiting cones (Alía et al., 2009). Alternatively, we used seedling emergence and early seedling survival as estimates of fitness. These plant traits are typically important in the recruitment dynamic of tree species and are particularly relevant for Mediterranean forests, where summer drought imposes an extremely high seedling mortality during the first years after emergence (Tíscar and Linares, 2011; Matías et al., 2012; Vizcaíno-Palomar et al., 2014).

Opportunities for local adaptation may be enhanced at the latitudinal and altitudinal limits of species' geographical ranges, since populations on the distribution edges might become isolated from the central ones due to any barrier that limits gene flow (García-Ramos and Kirkpatrick, 1997; Herrera and Bazaga, 2008; Bastida et al., 2015). This would be the case of Spanish black pine, which distribution is fragmented over the eastern mountains of Spain with areas isolated from each other by unsuitable lowland habitat and, within each area, by altitudinal gradients that may involve the variation of biotic and abiotic factors. We thus selected low- and high-altitude demes originating from central and peripheral localities for the experimental design. Aridity increases north to south along the *Pinus nigra* natural range (Tíscar and Linares, 2014). Taking all this into account, the study hypothesis was that, if local adaptation to the establishment conditions, i.e. during the emergence and seedling life stages, was to exist in populations of *Pinus nigra*, seeds collected in the warmer, drier southern peripheral forests should exhibit higher recruitment in those sites than seeds from the less warm, dry central forests. The existence of this potentially adaptive character should be concealed under wet conditions. Therefore, we included an irrigation treatment in the experimental design in order to validate the hypothesis and to get more insights into the *Pinus nigra* regeneration process under a climate change scenario. The specific questions addressed by this study are: (a) Does *Pinus nigra* exhibit local adaptation to the emergence environment faced by its seedlings? (b) Does this result extend to the survival environment? (c) Which may be the determinants of the found local adaptation pattern?

2. Material and methods

2.1. Study species and sites

Pinus nigra is a tree species of economic and ecological interest. It thrives along the eastern mountain ranges of Spain on rich calcium and mainly shallow soils under a Mediterranean-type climate with cold, wet winters and hot, dry summers. Seeds of *Pinus nigra* mature at the

beginning of the second winter after pollination. Seed predation, herbivory by ungulates, the presence of unsuitable soil conditions, and the irregular production of seed crops are factors which negatively affect *Pinus nigra* regeneration in natural stands, but summer drought is considered the major cause of seedling mortality in this species (Tíscar and Linares, 2011).

The *Pinus nigra* distribution area is fragmented. We conducted the reciprocal transplant experiments between the mountains of Cuenca and the mountains of Cazorla, covering *Pinus nigra* populations in the core and the southernmost edge of the species' range (Fig. 1). The Cuenca and Cazorla mountains are located within two different *Pinus nigra* provenances: *Sistema Ibérico Meridional* and *Cordilleras Béticas*, respectively. Climate conditions differ between these two provenances (Alía et al., 2009), with summers being specifically longer, hotter and drier in the provenance of the Cazorla mountains (Table 1). Meteorological conditions during the study span were coincident with these general climatic differences (see Appendix A).

Two sites were selected in the Cazorla mountains, namely, "*Navillas*" and "*Puertollano*", and two sites were selected in the mountains of Cuenca: "*El Cardozo*" and "*La Fuenseca*". The *Navillas* site (37°56′N – 2°53′W, 1220 m a.s.l.; Peripheral-Low population hereafter) is a representative low-altitude stand of *Pinus nigra* from the species's southernmost populations. It consists of *Pinus nigra* trees intermixed with some individuals of *Quercus ilex* and *Pinus pinaster*. The site of *Puertollano* (37°48′N – 2°56′W, 1820 m a.s.l.; Peripheral-High population hereafter) is located near the forest limit in the area and consists of a pure stand of *Pinus nigra* with scattered individuals of *Acer opalus* and *Sorbus aria*. El Cardozo (39°54′N – 2°04′W, 1082 m a.s.l.; Central-Low population hereafter) is a mixed forest stand mostly composed of *Pinus nigra* with some *Pinus pinaster* individuals, while the site of *La Fuenseca* (40°20′N – 1°47′W, 1640 m a.s.l.; Central-High population hereafter) is almost a pure stand of *Pinus nigra*, although some individuals of *Pinus sylvestris* are present. Pairwise geographical distances between study populations ranged between 14 and 268 km (Fig. 1).

2.2. Plant and soil material

From each study population, we collected a minimum of 10 cones from at least 10 widely separated trees of *Pinus nigra* in December 2014. Cones were completely opened in an oven set at 40 °C during 48 h, and seeds from the same site were pooled together. As *Pinus nigra* seed mass varies depending on collection habitat (Tíscar, 2002) and seed mass might influence emergence and survival (Castro et al., 2006), we weighed seeds individually to the nearest 0.1 mg. Seeds of less than 14 mg were discarded as they probably would be empty (Tíscar and Lucas-Borja, 2010). A sub-set of the weighed seeds was used to check seed viability in a growth chamber in the laboratory. Seeds from the four study sites exhibited germination rates that exceeded 96%. Thus, we could obtained samples of sound seeds from every study site or origin population that were kept in the fridge until the sowing experiment began.

We also took a soil sample from each study site at the time of cone collection. Specifically, we removed the soil litter and took a big enough sample of soil from the upper layer in a representative place of the site. Soil samples were analyzed using standard analytical procedures, in order to determine texture (percentage of sand, silt and clay), pH, the total content of moisture, carbonates, nitrogen, phosphorous and potassium, percent organic matter, soil respiration and dehydrogenase activity (see Lucas-Borja et al., 2017 for details). We performed a principal components analysis (based on correlation matrix) to synthesize in independent factors the variation of these soil properties among destination habitats.

2.3. Reciprocal transplant

The reciprocal transplant was carried out sowing seeds in the field. *Pinus nigra* regeneration is better achieved in managed stands through
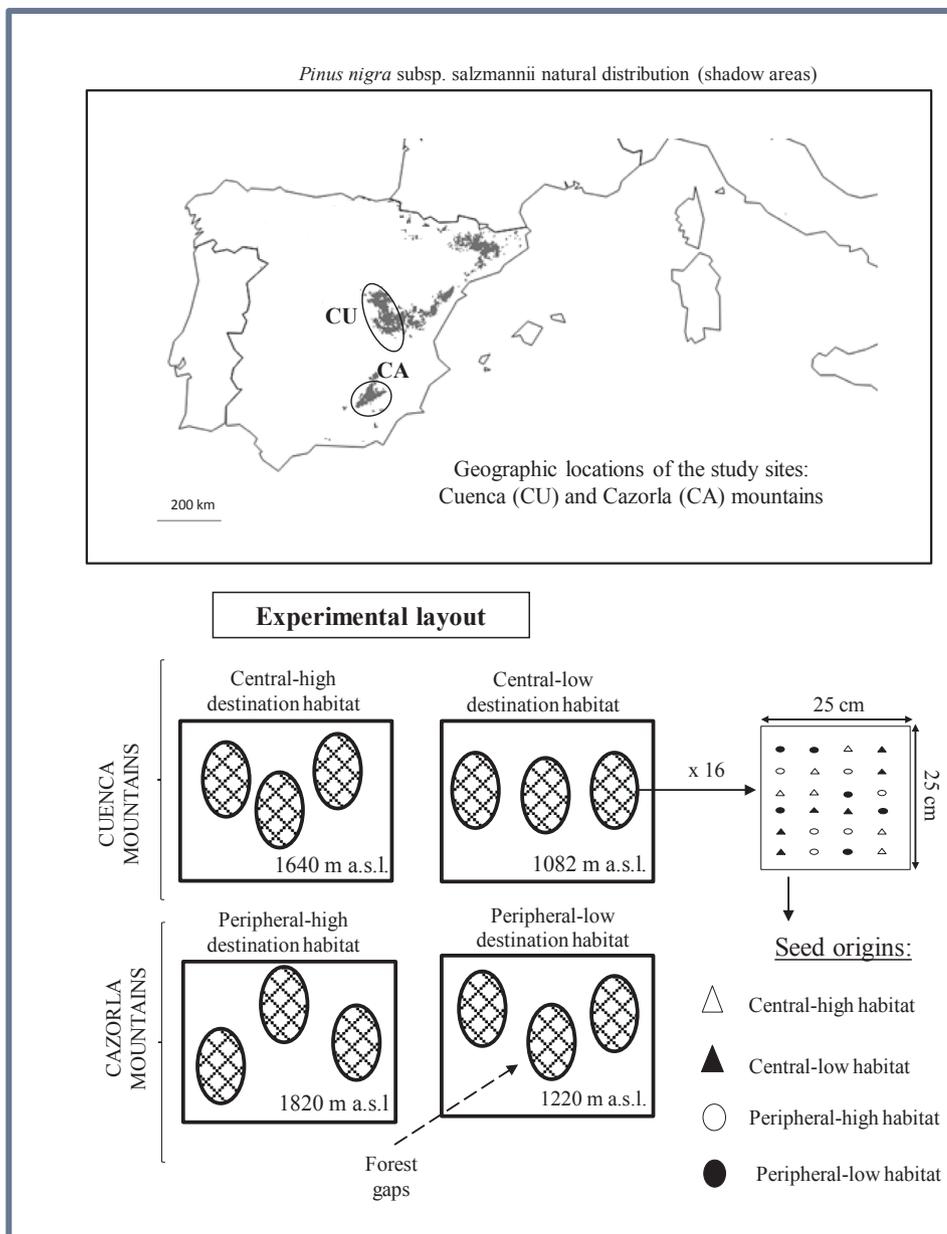
Fig. 1. Natural distribution of *Pinus nigra* subsp. *salzmannii* and geographical location of the study sites (*upper panel*), and experimental layout (*lower panel*).

Table 1

Characteristics of the average climatic conditions in the two *Pinus nigra* populations studied. Central populations belonged to the provenance 'Sistema Ibérico Meridional', whereas peripheral populations were located within the provenance 'Cordilleras Béticas'.

Climate variable	Central populations	Peripheral populations
Mean annual precipitation (mm)	700	830
Mean summer precipitation (mm)	109	73
Summer drought length (months)	1.8	2.7
Mean annual temperature (°C)	10.7	11.8
Mean maximum temperature warmest month (°C)	29.3	30.4
Mean minimum temperature coldest month (°C)	−2.0	−0.6
Annual mean daily thermic oscillation (°C)	17.9	18.4
Period of frost (months)	3.3	1.4

Source: Alía et al. (2009).

the opening of small scattered gaps in the forest canopy (Calama et al., 2017). In this sense, previous research has shown that the species frequently regenerates in forests gaps of about 300–400 m^2 in size (Tíscar, 2015). Considering this, we selected three forest gaps of approximately that size (distance between gaps ~ 200 m) in each study site (destination habitat) and homogeneously distributed sixteen 25 × 25 cm quadrats around the center of every gap over a surface c.a. 36 m^2. Quadrats consisted of 24 sowing points arranged in a grid of 4 × 6 cm. Each sowing point received one pine seed that was sown 1-cm deep at the end of March 2015. Specifically, we used 6 seeds from each one of the four origin populations that were randomly assigned to the 24 sowing points (Fig. 1). For each seed, we thus knew its origin population, its weight, its position (coordinates) within any given quadrat and the destination habitat where it had been sown. A total of 4608 seeds were used in the experiment. Quadrats were protected with 1 × 1 cm^2 mesh cages to prevent seed removal by birds and rodents. Before sowing, we removed the soil litter in order to expose the mineral soil, so we did not expect any interference from the pine seeds that had been naturally dispersed before the experiment began. Half of the quadrats

were randomly assigned to an irrigation treatment and the other to control. Seedling emergence of *Pinus nigra* is not usually limited by precipitation under natural conditions (Tíscar and Linares, 2011; Lucas-Borja et al., 2012). Therefore, we did not initiate the irrigation treatment until mid-June (80 days after sowing), when summer drought starts in an average year and seedling emergence is completed. Seedling emergence was considered to have occurred when any part of the seedling had broken through the soil surface. Irrigation consisted of adding ca. 2 L of water at each application time in a surface larger (30 × 30 cm) than the quadrat, in order to avoid border effects. Water was carefully sprinkled to prevent run-off every three weeks until early autumn. Thus, the irrigation treatment simulated a wet summer with storms (Matías et al., 2012). Seedling survival was monitored coinciding with the dates of irrigation, and approximately every five weeks after the end of the irrigation treatment. The experiment was considered to be concluded after ten months, in January 2016, as the experimental design was seriously disrupted by the trampling of wild boar and other animals. Yet we could note seedling emergence and total seedling survival after the whole, and most critical, first growing season.

2.4. Common garden experiment

In addition to the field reciprocal transplant, a common garden experiment was conducted in an unheated greenhouse during the same period of time (from March 2015 to January 2016) at *Las Peñas* tree-nursery, located in an intermediate position between the mountain of Cazorla and Cuenca (Albacete; 38°57′N – 1°52′W).

For this experiment, we used the soil obtained from each study site at the time of cone collection. The soil samples had been stored in plastic bags in the greenhouse before using them. In March 2015, the soil bulk from each site was homogenized. Then, 96 pine seeds from each origin were sown into four seedling trays per destination soil, summing up a total of 384 seeds (24 cells per tray × 4 origins × 4 destinations). The volume of each cell was 400 cm^3 (18 cm in height). By reciprocally transplanting both seed material and soils under identical climatic conditions in the greenhouse, we could directly test the role of soil factors on seedling emergence and survival.

Trays were frequently shuffled to minimize the influence of microclimates within the greenhouse. Seedlings were not fertilized during the experiment, but everything else was done according to standard tree-nursery procedures. For instance, irrigation was applied regularly. Emergence and survival of seedlings were monitored as in the field experiment until January 2016.

2.5. Statistical analysis

For the reciprocal transplant experiment, we computed Generalized Lineal Mixed Models (GLMM) with the *lme4* package in R software (R Development Core Team, 2014) to evaluate the effects of destination habitat (fixed factor), origin population (fixed factor), mean seed size (fixed factor) and quadrat (random factor, nested within forest gap, nested within destination habitat) on seedling emergence and survival. Seed size was modeled as a categorical variable with four levels, using the quartiles of the seed weight distribution as cutting points. This was done because we detected heterogeneity of slopes when seed size was introduced as a covariate. Specifically, there was a significant interaction of seed size with the categorical factor 'origin'. Seedling emergence and survival were adjusted to a binomial distribution ("emerged/total sown seeds per quadrat" and "survived/total emerged seedlings per quadrat", respectively) using logit as link function. Comparisons by means of the Akaike's Information Criterion between models with and without random effects warranted incorporating all the random factors in the GLMM (p < .001 in all cases) (Bates et al., 2010). Seedling survival in irrigated and control (non-irrigated) quadrats were analyzed separately.

Evidence for local adaptation was taken to be a significant destination x origin interaction for which native plants showed greater emergence and survival probabilities than non-native plants at any given site. When a significant destination x origin interaction was observed, we filtered the data and conducted pre-planned contrasts to test the destination x origin interaction under both the "local vs. foreign" and the "home vs. away" criteria (Kawecki and Ebert, 2004). Under the "local vs. foreign" criterion, we compared emergence and survival outcomes between seed origins within destination habitats. Specifically, the local origin would be expected to perform better than origins from other habitats, if local adaptation were to exist. Under the "home vs. away" criterion, we compared emergence and survival outcomes of each origin across destination habitats. Specifically, each origin would be expected to perform better in its own habitat than in other habitats to confirm local adaptation.

For the common garden experiment, the significance of the destination x origin interaction was tested by means of Generalized Linear Models (GLM) with a binomial error distribution and a logit link function for emergence (yes or no) and survival (yes or no) as binary response variables. Individual seed size was included in the model as in the reciprocal transplant analysis. Additional GLM were performed to analyze the relationship between the factor scores from the PCA analysis on soil properties and probability of seedling emergence and survival in the greenhouse. GLM were conducted with the *MASS* package of R software (R Development Core Team, 2014). Individuals (nursery tray cells) were considered as sample units in these analyses. Again, a significant destination x origin interaction was considered diagnostic of local adaptation. Throughout the paper, values are means ± SE.

3. Results

3.1. Soil properties

Table 2 summarizes results of the physical, chemical and biological properties analyzed in the soil samples collected at the different destination habitats. The first and second principal components extracted from the PCA analysis performed on correlation matrix of soil variables explained 46.3 and 39.2% of total variation, respectively. The first principal component separated central and peripheral sites, sorting soils according to pH and both silt and sand content. The second component separated high altitude from low altitude sites, sorting soils according to clay and phosphorous content (Table 2, Fig. 2).

3.2. Seedling emergence in the reciprocal transplant

Mean weight of seeds sown in the field varied amongst origin populations ($F_{3,4604} = 885.52$, P < .0001), seed sizes being

Table 2
Characteristics of the soil samples collected in the four destination habitats: Central-High (C-H), Central-Low (C-L), Peripheral-High (P-H) and Peripheral-Low (P-L), and component weights of the principal component analysis of them.

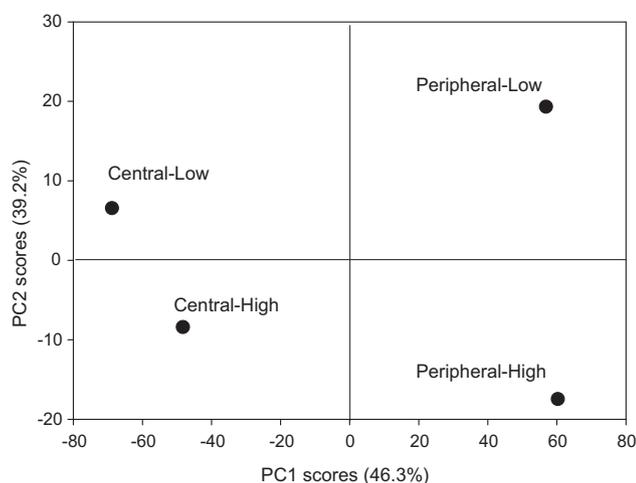| Soil parameter	Destination habitat				Component weights	
	C-H	C-L	P-H	P-L	PC 1	PC 2
Sand (%)	74.00	84	13.10	33.60	−0.3607	−0.2072
Silt (%)	8.00	12.00	56.60	56.30	0.4087	0.0812
Clay (%)	18.00	4.00	30.30	10.10	−0.0225	0.4379
Organic matter (%)	4.87	3.08	6.14	5.93	0.2759	0.3441
Moisture content	30.25	24.55	37.13	6.02	−0.2899	0.1931
Ph	8.48	8.34	8.30	6.94	−0.3908	0.0679
Carbonates	0.28	0.07	0.20	0.06	−0.2381	0.378
Nitrogen (ppm)	0.33	0.14	0.25	0.04	−0.3134	0.3028
Phosphorous (ppm)	0.05	0.16	0.09	0.08	−0.0498	−0.4212
Potassium (ppm)	165.00	185.00	70.25	80.05	−0.3463	−0.2143
Respiration (µg/ml)	16.57	6.86	11.52	16.94	0.1575	0.2971
Dehydrogenase (µg/ml)	0.07	0.02	0.02	0.01	−0.2954	0.2286

Fig. 2. The first two principal components of the twelve edaphic parameters measured in one sample of soil collected at each destination habitat.

$21.06 \pm 0.10 = 21.43 \pm 0.08 < 22.86 \pm 0.13 < 28.64 \pm 0.15$ mg, for the origins Central-Low, Peripheral-High, Central-High and Peripheral-Low respectively. Mean seed size also varied amongst destination habitats ($F_{3, 4604} = 3.61$, P = .0127), seed sizes being $23.08 \pm 0.13 < 23.60 \pm 0.12 = 23.66 \pm 0.15 = 23.66 \pm 0.18$ mg for the destinations Peripheral-High, Central-High, Central-Low and Peripheral-Low respectively.

Overall seedling emergence probabilities averaged 0.83 ± 0.01 across all destination habitats and seed origins. Both factors destination habitat and seed origin had a significant effect on emergence (Table 3). Across destination habitats, mean emergence probability ranged from 0.69 in the Peripheral-High destination habitat to 0.93 in the Central-High one (Fig. 3A). Differences between seed origins were smaller, but still significant. Mean emergence probability ranged from 0.80 for seeds from the Peripheral-Low origin to 0.86 for seeds collected at the Peripheral-High origin (Fig. 3B).

There was a non-significant destination × origin interaction effect on seedling emergence (Table 3). Consequently, seeds from the local populations did not germinate significantly better than seeds from the foreign populations (the "local versus foreign" criterion, Fig. 4A), and none of the seed origins performed significantly better at its native site compared with non-native sites either (the "home versus away" criterion, Fig. 4B). Seed size had no effect on seedling emergence (Table 3).

3.3. Seedling survival in the reciprocal transplant

Seedling survival probabilities differed between watered and control quadrats in the field ($0.31 \pm 0.02 > 0.07 \pm 0.01$, respectively; Mann-Whitney *U* test, n = 384, Z = 11.04, p < .0001).

In watered quadrats, survival probability differed between destination habitats, ranging from 0.10 in the Central-Low destination habitat to 0.51 in the Central-High destination habitat (Fig. 3A). However,
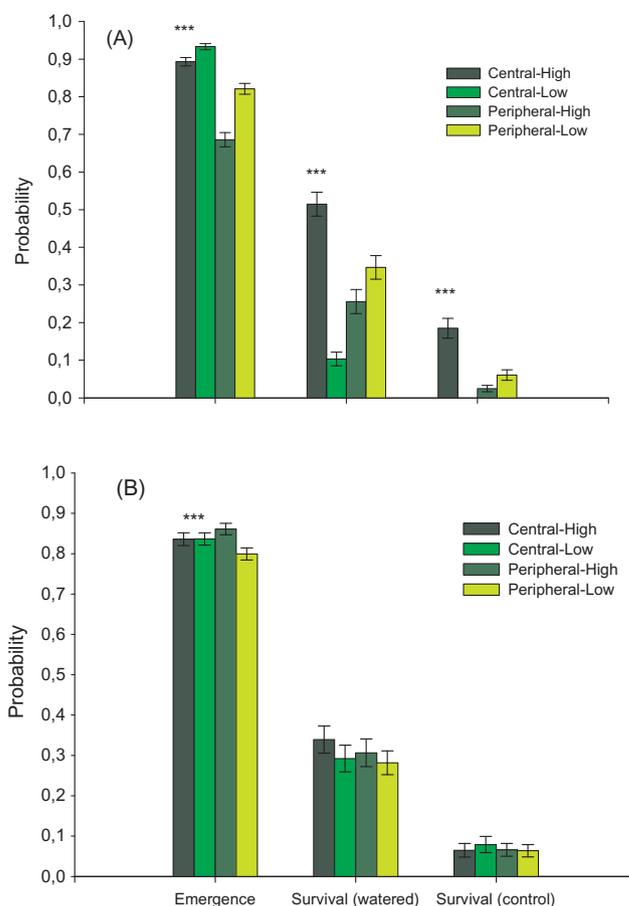

Fig. 3. Differences in the probability of seedling emergence and of seedling survival in watered and control quadrats across destination habitats (A) and among seed origins (B), based on results of Kruskal-Wallis tests. *, P < .05; **, P < .01; ***, P < .001.

there was no significant difference between seed origins (Fig. 3B). The destination x origin interaction effect was significant in these irrigated quadrats (Table 3), but it did not reveal a pattern of local adaptation. Thus, local origins never survived at their native sites better than foreign origins. In fact, all seed origins performed equally in the different destination habitats (Fig. 5A). Under the "home *versus* away" criterion, seeds from the Central-High origin performed significantly better in its own habitat than in other destination habitats, but this result did not reveal a pattern of local adaptation as it was rather the consequence of the Central-High destination habitat attaining a much higher overall survival probability (Fig. 5B).

In control quadrats, overall seedling survival probabilities averaged 0.07 ± 0.01 across all destination habitats and seed origins. Survival probability differed between destination habitats, ranging from 0.00 in the Central-Low destination habitat to 0.18 in the Central-High destination habitat (Fig. 3A). However, there was no significant difference between seed origins (Fig. 3B). The destination × origin interaction
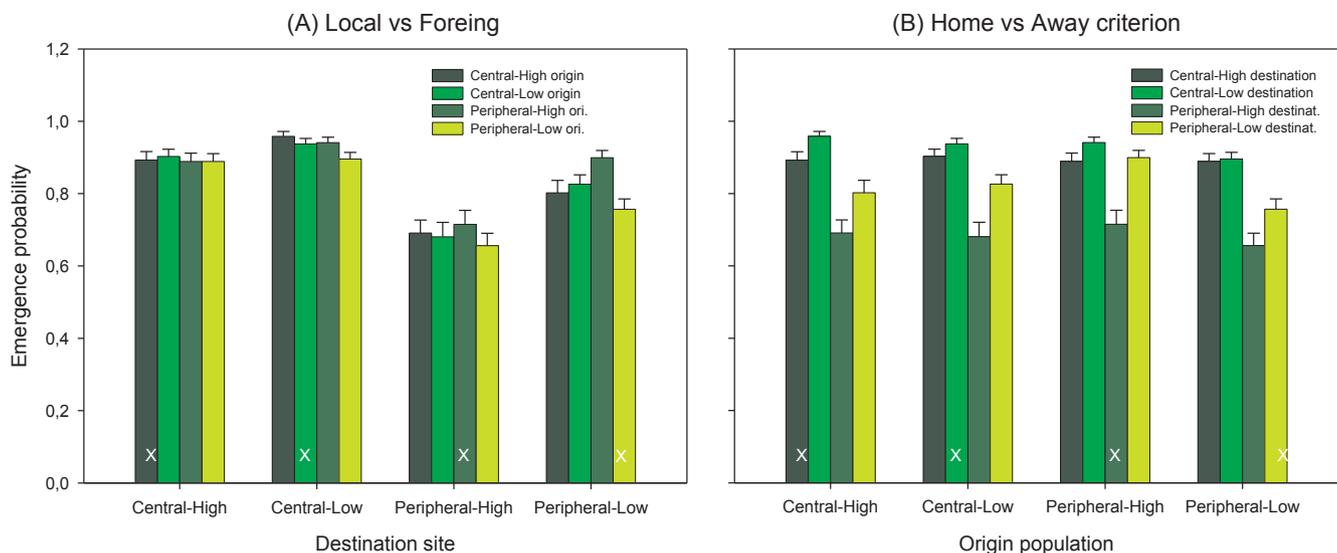
Table 3
Results of a Generalized Linear Mixed Model performed to explore the variation among seed origins and across destination habitats of seedling emergence and survival in the reciprocal transplant experiment with two treatments: irrigation and control. Seed size was included as a categorical factor with four levels. Significant values are shown in bold.

Fixed factors	Emergence			Survival (irrigation)			Survival (control)	
	df	χ^2	P	df	χ^2	P	χ^2	P
Destination (D)	3	14.08	**.0028**	3	7.89	**.0491**	15.02	**.0018**
Origin (O)	3	17.08	**.0007**	3	5.12	.1632	0.95	.8125
D × O	9	15.87	.0697	9	22.75	**.0068**	20.50	**.0151**
Seed size	3	0.21	.9759	3	3.25	.3550	5.36	.1476

Fig. 4. "Local versus foreign" (A) and "home versus away" (B) criteria assessing local adaptation of *Pinus nigra* to the environmental conditions of emergence. A white "X" marks the local deme in each comparison. [*], P < 0.05; [**], P < .01; [***], P < .001.

effect was significant in these non-irrigated quadrats (Table 3), but it did not reveal a pattern of local adaptation. Thus, local origins never survived at their native sites better than foreign origins. In fact, all seed origins performed equally in the different destination habitats (Fig. 5C). Under the "home *versus* away" criterion, seeds from the Central-High origin performed significantly better in its own habitat than in other destination habitats. However, this result did not reveal a pattern of local adaptation as it was rather the consequence of the Central-High destination habitat attaining a much higher overall survival probability (Fig. 5D). Seed size had no effect on seedling survival neither in watered or control quadrats (Table 3).

3.4. Seedling emergence and survival in the common garden experiment

Results from the common garden experiment did not show a destination × origin interaction effect, and destination habitat was the only factor affecting both emergence and survival of seedlings (Table 4). Here, seedling emergence probabilities were 0.92, 0.78, 0.37 and 0.89, and seedling survival probabilities were 0.73, 0.69, 0.50 and 0.86 for the seeds sown in soils collected at the Central-High, Central-Low, Peripheral-High and Peripheral-Low habitats, respectively. PCA1 scores had a significant negative effect on the probability of seedling emergence ($\beta = -0.075$, d.f. = 1, Wald $\chi^2 = 1.5\ 3$, p = .012). The effects of PCA1 scores on seedling survival and of PCA2 on both seedling emergence and survival were non-significant (p > .10 in all cases).

4. Discussion

Results from the present study indicate that *Pinus nigra* does not exhibit local adaptation to the conditions of seedling establishment. This conclusion is firstly based on the non-significant interaction found between origin and destination effects for seedling emergence and, secondly, on the fact that both the 'local *versus* foreign' and the 'home *versus* away' criteria did not provide support for local adaptation to the survival environment, although we detected significant origin x destination effects for this trait (Table 3).

Our results would thus conform to the expectation that local adaptation is less common in plant populations than generally assumed (Leimu and Fischer, 2008) and would agree with previous research on Mediterranean pine species (Vizcaíno-Palomar et al., 2014). However, they would disagree with general findings coming from studies based on provenance trials, which have frequently proved the existence of

local adaptation in different tree species, including pines (Savolainen et al., 2007; Alberto et al., 2013). For instance, data-driven models based on provenance trials indicate that populations of *Pinus sylvestris* and *Pinus pinaster* from southern Spain could exhibit higher relative survival in northern territories, suggesting pre-adaptation of these populations to warmer climates (Benito Garzón et al., 2011). Similar results have been quoted for Spanish provenances of *Quercus suber* (Ramírez-Valiente et al., 2009) and *Fagus sylvatica* (Thiel et al., 2014). The apparent contradiction between this study and results from provenance trials can be explained by the fact that provenance trials have typically used transplanted seedlings, i.e. plants that had been previously nurtured in a tree-nursery and were conveniently placed into a hole dug in the ground. By using transplanted seedlings instead of seeds, the fitness components of the early life history stages are ignored and, thus, the overall consequences of local adaptation in the natural forests dynamics remain only partially understood.

It must be noted that demonstrating local adaptation can be difficult for plant traits related to early life history stages (Raabová et al., 2011; Garrido et al., 2012). Particularly, local adaptation for germination traits might be difficult to demonstrate in species that exhibit low germination fractions and prolonged dormancy, but none of these characteristics are present in *Pinus nigra*. On the contrary, we observed high germination and, subsequently, high emergence rates within a few days after sowing in the growth chamber, the greenhouse and the field. While seedling emergence was generally high, the mean survival rate was rather low (7%) in control quadrats, suggesting that stochastic factors acting at the microhabitat scale could be more important than natural selection at the early life stages of *Pinus nigra*, in which plants are intensely prone to desiccation due to their limited root system and the particular characteristics of the Mediterranean climate (Vizcaíno-Palomar et al., 2014; Tíscar et al., 2017). The fact that most seeds emerged regardless of their origin in the different habitats tested, although most seedlings subsequently died of desiccation along a typical Mediterranean summer in the field, could indicate that the action of directional selection is prevented during the life stages of seedling emergence and early survival. Some traits of *Pinus nigra*, such as being a long-lived species that exhibits a mast-seeding behavior (Tíscar and Linares, 2011), elevated genetic variation within populations (Rubio-Moraga et al., 2012) and local adaptation at the sapling life stage (Alberto et al., 2013) would additionally support this reasoning, in the sense that natural selection is more likely to filter the survival of well-established seedlings or saplings, while first-year seedling survival is
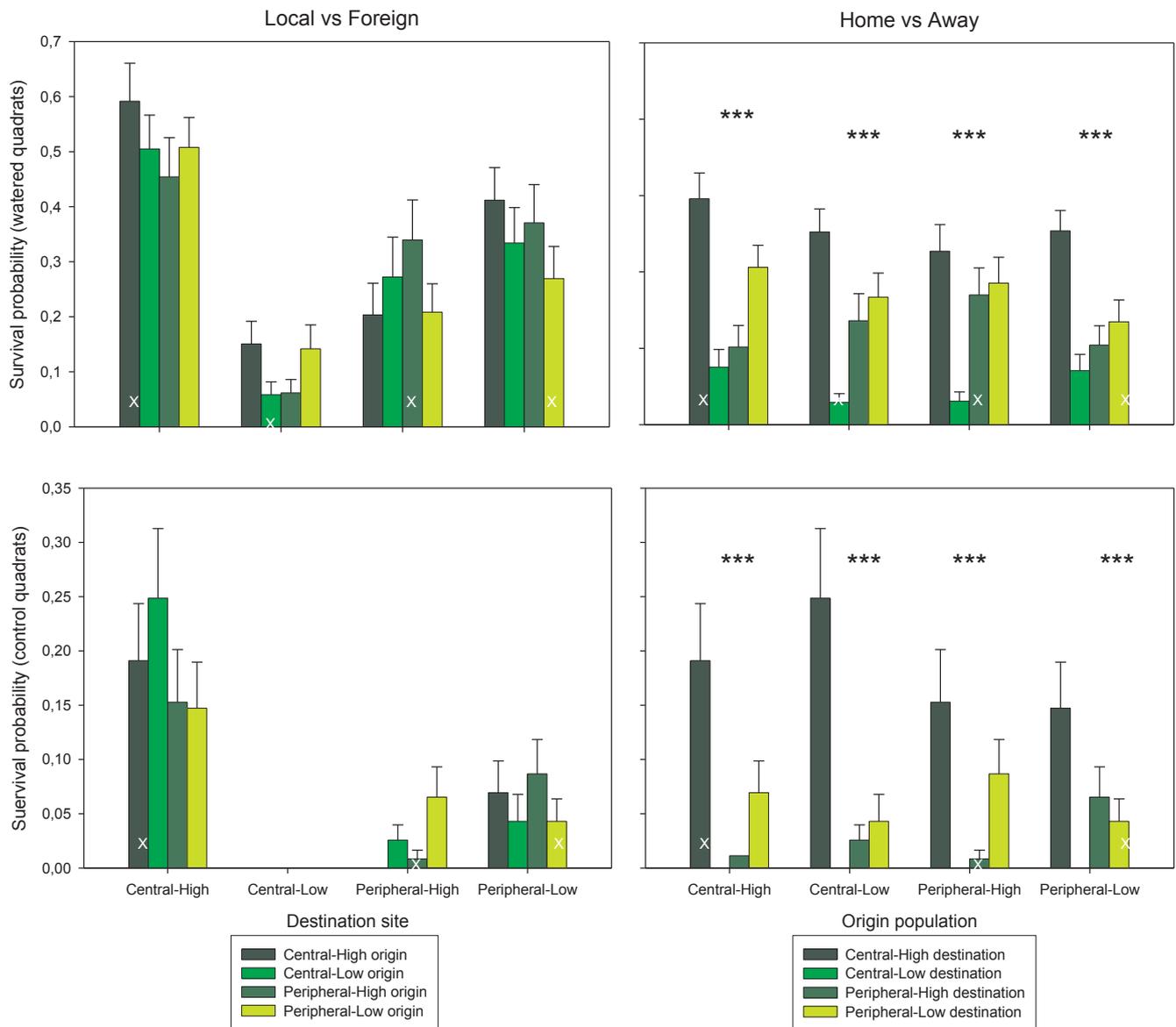
Fig. 5. "Local *versus* foreign" criterion assessing local adaptation of *Pinus nigra* to the environmental conditions of seedling survival in watered (A) and control (C) quadrats, and "Home *versus* away" criterion assessing local adaptation of *Pinus nigra* to the environmental conditions of seedling survival in watered (B) and control (D) quadrats. A white "X" marks the local deme in each comparison. *, P < .05; **, P < .01; ***, P < .001.

Table 4
Results of a Generalized Linear Model performed to explore the variation among seed origins and across destination habitats of seedling emergence and survival in the common garden experiment. Seed size was included as a categorical factor with four levels. Significant values are shown in bold.

Fixed factors	Emergence			Survival	
	df	Wald χ^2	P	Wald χ^2	P
Destination (D)	3	64.28	< **.0001**	16.18	**.0011**
Origin (O)	3	7.73	.0520	3.18	.3649
D x O	9	6.47	.6919	7.23	.6135
Seed size	1	6.29	.0983	2.50	.4750

more strongly dependent on stochastic factors acting at the microhabitat scale (Tíscar and Linares, 2011; Vizcaíno-Palomar et al., 2014). Congruently, the present study showed significant random factors at the quadrat and gap scales and accentuated differences across destination habitats for the probability of seedling survival (Fig. 3).

Extremely high mortality at the seedling stage that would prevent

the action of directional selection could be, as just stated, the main cause impeding local adaptation to the establishment conditions in *Pinus nigra* populations. Yet, other reasons could have led us to refute the initial study hypothesis, for instance: strong destination effects, gene flow, non-genetic effects and the scale of the experimental design. We will discuss them henceforth.

Some studies have related no detection of local adaptation to strong destination effects that may limit weaker origin ones (Garrido et al., 2012 and references therein) and, in fact, we report here strong effects of the destination habitat. We found reason to suspect that quality differences among the study habitats were mostly related to soil texture. First, emergence percent was high (above 69%) in the four destination habitats, suggesting that weather conditions were suitable to promote seedling emergence in all of them. Second, seedling emergence was negatively related to silt content and, congruently, the Peripheral-High habitat attained the lowest emergence rate in both the greenhouse and the field. The Peripheral-High habitat also had the highest clay content, resulting in a 'silty clay loam' soil texture for this site. Together, these results would indicate that emergence probability

declined with the increasing content of fine soil particles, particularly clay, because of an increment in the soil mechanical impedance to root penetration and hypocotyl elongation (Herrera, 2002; Garrido et al., 2012). Third, recruitment was absent after the first summer in the sandy soils of the control Central-Low sowing quadrats (Table 2; Fig. 3A), whereas seedling survival was 69% in the regularly watered greenhouse. Consequently, it is reasonable to conclude that poor water availability, due to low water retention, and extreme temperatures at the soil surface could have caused the death of the majority of emerged seedlings in those quadrats from the Central-Low site (Vizcaíno-Palomar et al., 2014). Despite this important effect of soil type on seedling emergence and survival, results of the common garden experiment (carried out under identical climatic conditions for all the growing seedlings) did not show evidence for adaptation to soil conditions. We cannot dismiss that collected soils may have changed during storage. Yet, a strong spatial variability of the soil properties may hamper local adaptation (Macel et al., 2007) and, indeed, the soil characteristics of the study sites differ over relatively small distances due to their broad range of physiographic and environmental conditions as shown by Herrera (2002) and Lucas-Borja et al. (2017). The fact that seedling survival ranked equally in watered and control quadrats (Fig. 3A) indicates that edaphic properties acted concomitantly with climate conditions.

Another reason that could justify the observed lack of local adaptation could be the existence of gene flow between the study populations (Kawecki and Ebert, 2004). It is known that some artificial gene flow has existed among the study areas through the occasional interchange of seeds and seedlings of *Pinus nigra*, used to regenerate the managed forests of the species. This management practice has been relatively frequent in Spanish forests during the past and ought to be considered in any plan of assisted migration, but it is difficult to track. Indeed, we cannot assess the influence of this artificial gene flow on our results, although we carefully located the study sites in mature, long-existing stands. Otherwise, the natural gene flow of our study system would have been limited between the central and peripheral populations, isolated for more than 30,000 years (Afzal-Rafii and Dodd, 2007; Kremer et al., 2012), as well as diminished by altitudinal ecological gradients between the low- and the high-altitude study populations (Herrera and Bazaga, 2008).

Non-genetic effects such as differences in plasticity and maternal effects could also prevent the detection of local adaptation (Kawecki and Ebert, 2004). It has been argued that phenotypic plasticity, i.e. the ability of a given genotype to render different phenotypes under different environmental conditions, may override evidence of local adaptation (Gimeno et al., 2009). However, we could not confirm the presence of phenotypic plasticity in the early life history stages of *Pinus nigra*, since pre-planned contrasts showed that all the seed origins performed similarly at all the destination habitats, and this occurred irrespectively of water being added or not to the sowing quadrats (Fig. 5). We also excluded the possibility that maternal effects could be hindering the detection of local adaptation in the present study. Maternal effects are frequently mediated by seed size as, generally but not always, larger seeds germinate better and produce more competitive seedlings (Castro et al., 2006). Tíscar and Lucas (2010) showed, in this respect, that seed mass acts concomitantly with mother plant identity in determining first-year seedling performance in *Pinus nigra*. Consequently, we considered non-significant effects of seed size (Tables 3 and 4) as an indirect evidence of the absence of maternal effects in the final outcome of seedling emergence and survival.

Would we have found evidence of local adaptation at a larger scale than that used in the present study? Although local adaptation may occur within short distances (Giménez-Benavides et al., 2007; Bastida et al., 2015), populations are likely to be less well adapted to sites increasingly distant from their home, since the scale of environmental heterogeneity should determine the scale of local adaptation (Galloway and Fenster, 2000; Raabová et al., 2011). Consequently, we cannot rule out the possibility that local adaptation could be detected between the more distant southern and northern peripheral populations of *Pinus nigra*, since they represent more disparate environmental conditions within the species' range (Alía et al., 2009). This question might deserve further research in the future. However, if this were the case, assisted migration would still be limited as an operational approach to adapt *Pinus nigra* forests to ongoing climate change.

Finally, we found that irrigation clearly increased *Pinus nigra* seedling survival, although seedling mortality was still rather high in all the destination habitats (Fig. 3A). The Iberian Peninsula suffered 26-days of continuous heat wave at the beginning of summer in 2015 (AEMET, 2017) and, probably, this elevated temperature was the cause of the unexpected increase in seedling mortality observed at the watered sowing quadrats. Moreover, Matías et al. (2017) found that warming reduces the root-to-shoot ratio and the root length in seedlings of *Pinus nigra*. This physiological constraint will make this species very vulnerable to the future hotter conditions predicted by climate change models. As an example, results from this study showed that *Pinus nigra* natural regeneration can collapse under these stressful conditions in the lowland areas currently inhabited by the species.

5. Conclusions

This study shows negative evidence of local adaptation to the emergence and survival environment (either climatic or edaphic) faced by *Pinus nigra* seedlings. Seedling emergence and, particularly, subsequent seedling mortality are high in all habitats. Both facts could prevent the action of directional selection at those two early life stages. As seedling mortality is extremely high in all habitats irrespective of seed origin, Spanish black pines translocated from warmer, drier populations to accomplish assisted migration would not necessarily enter more seedlings into the sapling stage in naturally regenerated forests. Consequently, translocated genes would not eventually enter the genetic pool of the recipient population either, even though they were better adapted to more arid conditions. This would reduce the utility of assisted migration as a tool to adapt *Pinus nigra* forests to ongoing climate change.

Alternatively, forest managers could rely on the high levels of genetic variance observed in the species, which can by itself constitute a genuine form of pre-adaptation to new climates (Rubio-Moraga et al., 2012), as well as taking advantage of the microhabitat variation typically found in the mountainous terrain inhabited by the species (Benavides et al., 2016), while applying in an adaptive manner a varied and flexible silviculture based on natural processes (O'Hara, 2016).

Appendix A. Temperature and precipitation during the study span
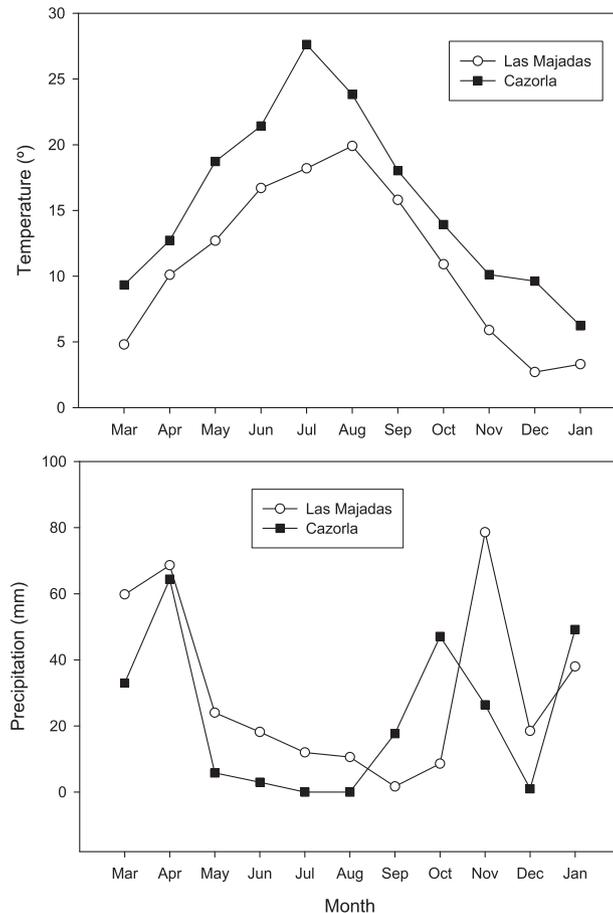
Monthly temperature and precipitation data from March 2015 to January 2016 obtained from meteorological stations (MS) nearest to the experimental sites. Las Majadas MS (40°17′51″ N – 2°01′21″W, 1400 m a.s.l.) is located 28 km from the Central-Low study site and 14 km from the Central-High site. Cazorla MS (37°54′35″ N – 3°00′07″ W, 885 m a.s.l.) is located 8.6 km from the Peripheral-Low study site and 12 km from the Peripheral-High site. In the Figure, temperature data from Cazorla are represented after applying an adiabatic lapse rate of -0.65 °C for every 100 m of elevation in order to overcome altitudinal differences between the two MS.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2017.12.014.

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