



Larger investment in roots in southern range-edge populations of Scots pine is associated with increased growth and seedling resistance to extreme drought in response to simulated climate change



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ABSTRACT

Extreme climate events such as heat waves or severe drought have the potential to fundamentally alter species dynamics, and are expected to increase in frequency in the coming decades. Early life stages of plant species are highly sensitive to these variations, with the potential for profound consequences for plant communities. In order to explore the response of Scots pine (*Pinus sylvestris* L.) seedlings to predicted increases in temperature and extreme drought, we performed a controlled-conditions experiment using growth chambers to simulate (1) current and future temperatures expected at the southernmost edge of the range, (2) current and drier growing seasons before an acute summer drought, and (3) drought alleviation by sporadic rainy events during summer. We analysed the response of seedlings by assessing survival, growth, biomass allocation and isotopic discrimination. Southern range-edge seedlings were compared under same conditions with those from the northern range edge, which experience cooler and wetter conditions throughout the year. The combination of extreme drought and the predicted temperature rise severely reduced overall survival for both provenances. A reduction in precipitation before the onset of drought diminished the survival and final biomass of seedlings, while the interruption of summer drought drastically increased survival probabilities. Southern seedlings invested a higher proportion of their biomass in roots, which conferred on them higher growth, higher survival probabilities, better nutritional status and lower drought stress. These results help us to understand the mechanisms of local adaptation at the southern range edge and indicate southern populations as a valuable genetic resource to buffer the response of Scots pine against such extreme climatic events.

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1. Introduction

Air temperature has increased during the last century at a global scale, and it is expected to continue rising to at least the end of the present century (IPCC, 2013). Furthermore, these changes will be accompanied by an alteration of precipitation patterns, with a generalised increase of summer drought in central and southern European regions (Giorgi and Lionello, 2008). However, although future precipitation regime is still uncertain, the frequency of extreme events (i.e. torrential rainfall, severe drought or extreme heat waves) is expected to increase (Kao and Ganguly, 2011; IPCC, 2013). This climatic variability might have profound consequences for plant communities through the alteration of current population and ecosystem dynamics. Indeed, the ecological effects of

extreme events have been identified as one of the main gaps in the knowledge of community ecology (Agrawal et al., 2007; Jentsch and Beierkuhnlein, 2008). Thus, the understanding of the effect of extreme climatic events under a global-change scenario is crucial for the accurate forecast of future plant-community dynamics.

Extreme climatic events may alter current dynamics of tree species at different scales, from the community to the species. Extreme drought events could result in biodiversity loss (Thomas et al., 2004; Thuiller et al., 2005; Matías et al., 2011), massive forest decline (Breshears et al., 2005; Allen et al., 2010), or in the reduction of net growth (Ogaya et al., 2003; Eilmann et al., 2006; Thabeet et al., 2009; Cavin et al., 2013). In the case of extreme heat waves, consequences vary from reductions in photosynthesis (Ameje et al., 2012) or growth (Bigler et al., 2006; Thabeet et al., 2009; Bauweraerts et al., 2013) to tree mortality (reviewed by Allen et al., 2010). Thus, these extreme events may result in strong alterations of the diversity and dominance patterns within communities (Tilman, 1998; Chapin et al., 2001; Matías et al., 2012a; Cavin et al., 2013). Given the increase in frequency of such extreme drought

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events in areas such as the Mediterranean, where 10 of the 12 driest years of the 20th century occurred in the last 20 years (Sousa et al., 2011), the study of their consequences is of particular importance. Although ongoing climatic changes will be experienced across the distribution range of the species, those populations located at the southern range are expected to be most at risk of negative effects since the conditions are currently at the species' limit of tolerance (Hampe and Petit, 2005) and the foreseen climate variability there is higher (Giorgi and Lionello, 2008; IPCC, 2013).

Early-life stages of plants are especially sensitive to climate alterations due to their limited root system and reserve storage capacity, so they respond faster than adults (Houle, 1994; Hampe and Arroyo, 2002). Consequently, recruitment is usually considered as the key stage in order to predict future consequences of climate change on plant communities (Lloret et al., 2004). However, differences in precipitation during the early stages of plant establishment could result in a differential resistance to extreme events through better seedling development before the onset of summer drought (Mendoza et al., 2009). In addition, sporadic rainy events during summer might also reduce the drought or temperature stress allowing seedlings to survive the summer (Matías et al., 2011, 2012a). Thus, variability in precipitation patterns might play an important role in the plant responses to climate alterations. A common way to explore these responses to environmental constraints is by the analysis of carbon stable isotopes ($\delta^{13}\text{C}$), which can be used to infer the drought stress experienced by plants during extreme events (Eilmann et al., 2010). $\delta^{13}\text{C}$ constitutes an integrated record of the ratio of intercellular to atmospheric CO_2 concentrations at the time of carbon fixation (Farquhar et al., 1982), making it a good indicator of intrinsic water-use efficiency, reflecting the balance between net photosynthetic rate and stomatal conductance. Complementing information on the water economy of plants derived from $\delta^{13}\text{C}$, nitrogen concentration and its isotopic concentration ($\delta^{15}\text{N}$) can inform us about the nutritional status of the plants and the source of nitrogen used (Högberg, 1997).

Scots pine (*Pinus sylvestris* L.) is an excellent species to test the consequences of climate alterations on growth and survival since it has a wide geographical range and, therefore, naturally occurs across a wide climatic gradient and so experiences very contrasted conditions at its latitudinal range edges (Matías and Jump, 2012). In a previous experiment (Matías and Jump, 2014), we determined that Scots pine seedlings from southernmost populations perform better under chronic drought simulations than northern range-edge seed sources, and that the recruitment pattern at both latitudinal range limits is susceptible to alteration by predicted climate-change scenarios. Building on this initial evidence, in the present study we explore the response of Scots pine seedlings from the southern range-edge to the extreme drought and increased temperature expected for the coming decades, and assess whether sporadic summer storms might elevate survival once drought has started. In addition, we explore the morphological and physiological mechanisms involved in this response and the role that local adaptations of southern populations play in the capacity to cope with the predicted increase in aridity and increased frequency of extreme events. We performed a controlled-conditions experiment using growth chambers to simulate current and future temperatures at the southern range edge of the species, and current and drier growing periods followed by a severe drought. In order to determine local adaptations to drought at the southern range edge, we compared responses with seedlings derived from northern range-edge populations that experience cooler and wetter conditions throughout the year and are expected to have evolved without drought constraints. Specifically, we asked: (1) what are the consequences of a combined rise in temperature and extreme drought for Scots pine recruitment? (2) Is there an effect of the precipitation during

the early growing season on the response to extreme drought? (3) Are Scots pine seedlings able to recover once the drought period has started? (4) Can the differences in response to the temperature and drought treatments between the two provenances explain patterns of local adaptation at the southern range edge?

2. Materials and methods

The present study was conducted from July to December 2012 at the Controlled Environment Facility of the University of Stirling (56°08'37"N, 3°55'09"W; Stirling, UK). We used Scots pine seed sources from the southern range edge of the species (Sierra Nevada and Sierra de Baza, SE, Spain). The response of southern provenance was compared with those from the northernmost range edge (Kevo, Finland; see Matías and Jump, 2014 for further details on seed provenances). Since Scots pine populations growing at Kevo are not limited by water availability, local adaptations to drought are expected to differ from those shown by southern range-edge populations. At both sites, mature cones were collected from three different populations, selecting at least 15 mature trees per population and 10 cones per tree. Seeds were extracted by oven-drying cones at 45 °C for 48 h. Seeds collected from southern provenance were heavier than northern ones (mean weight 0.011 ± 0.002 and 0.007 ± 0.001 , respectively; $F=674.4$, $P<0.0001$). Plant material from all populations within provenance were pooled, and then stored at 4 °C until used for the experiment.

In order not to restrict vertical root growth (maximum root depth tested in Matías and Jump, 2014), seeds from the two provenances were grown in tubular pots (8 cm in diameter and 45 cm high, 2.3 L volume) filled with a peat and river sand mixture (proportion 2:1 in volume, respectively) with a layer of gravel at the bottom to facilitate drainage. 120 pots (60 per provenance) were placed inside each of four Snijders Scientific MC1750E (Tilburg, Netherlands) controlled environment chambers (inner space 1.8 m length $\times$ 0.75 m wide $\times$ 1.2 m high), giving a total of 480 pots. Before sowing, all pots were irrigated with 300 mL of a soil microbial inoculum to allow mycorrhization. This inoculum was obtained from the maceration of roots and soil beneath adult Scots pine trees growing at the University of Stirling (Matías and Jump, 2014). Three seeds were sowed per pot, thinning to one seedling in case of multiple emergence. Optimal conditions for emergence and early growth (16 h light at 23 °C/8 h dark at 15 °C daily cycles) were maintained for 6 weeks and, after that, we applied two experimental treatments (temperature and water availability) fully crossed with the provenance factor in a factorial design. We used La Cortijuela area (Sierra Nevada, SE Spain, 1670 m a.s.l.) as a reference climatic population. *P. sylvestris* naturally establish in this area at its southernmost limit, and a meteorological station records climatic data (daily rainfall and temperature) since 1990, which allows for the simulation of realistic conditions for the experiment. The temperature treatment was applied as a whole plot factor, and had two levels replicated in two chambers each: (1) *current*, representing the mean day and night temperatures at the reference population (21.5 °C and 14.1 °C respectively for the 1990–2010 period) during the growing period; (2) *future*, simulating the predicted increase in temperature by 5 °C above *current* values, as projected for the 2090–2099 period in this area at the time of the experimental setup (A2 scenario; IPCC, 2007). The water availability treatment was applied as a subplot factor and was also represented by two levels, representing current and future scenarios: (1) *high availability*, based on the mean precipitation records during the early-growing season (May–June) at the reference population during the 1990–2010 series (110 L m^{-2}); (2) *low availability*, simulating the predicted reduction in precipitation

by 30% as projected for this area (77 L m⁻²). These total precipitation amounts were applied during 12 irrigation events (twice weekly during 6 weeks). From the 13th experimental week, all pots were divided into two drought levels in order to test the response to extreme drought and recovery capacity. Half of the seedlings were subjected to two 3 week dry-down cycles with one week recovery interval between each dry down, during which the same water amounts were applied as those during the watering period (Cregg and Zhang, 2001). This level simulated summer storms that occur infrequently in Mediterranean mountains (Matías et al., 2012a). For the other half of the seedlings, watering was completely stopped until the end of the experiment. These two drought levels will be referred hereafter as *recovering* and *terminal* droughts respectively. Summarising, the experimental design included four factors (temperature, precipitation, provenance and drought) with two levels each, fully crossed with 30 replicated plants per combination.

During the whole experiment, light intensity was fixed with a photosynthetic photon flux density of 210 μmol m⁻² s⁻¹ during 16 h, rising progressively at dawn and decreasing at dusk for 1 h. This light intensity is representative for forest understory (Valladares et al., 2004) and appropriate for pine seedlings which typically establish in the forest understory of beneath a canopy of dwarf shrubs (Castro et al., 2004). Air relative humidity and CO₂ concentration were kept constant within cabinets at 65% and 950 mg m⁻³ (490 ppm) respectively. To avoid any possible chamber effect, all pots were rotated through the different chambers, spending at least one month in each one, whilst also randomising pot position within chambers. Soil moisture was measured weekly during the experiment in all pots over the surface 5 cm by the time-domain reflectometry method (SM300; Delta-T devices, Cambridge, UK), values were recorded two days after irrigation. On 10-December-2012 (experimental week 21st), all surviving seedlings were harvested. Roots were carefully washed to remove soil remains, maximum root and shoot fresh length was measured, then seedlings were divided into aboveground and belowground parts, oven-dried at 60 °C for 72 h and weighed.

A random subset of 10 individuals per treatment combination was selected for isotopic discrimination analyses. Due to the low survival in the terminal drought level, only plants from the recovering level were used. Dried leaves were ground to powder and analysed for total N, ¹³C and ¹⁵N. Determinations were conducted at the Stable Isotope Facility of the University of California at Davis (USA) using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Analyses were performed on whole-leaf tissue rather than extracted cellulose since both are strongly correlated (West et al., 2001; Sullivan and Welker, 2007; Powers et al., 2008). The isotopic ratio was expressed in delta notation (δ¹³C, δ¹⁵N) in per mil (‰) as relative deviation from the international standard V-PDB.

2.1. Data analysis

Variation in soil moisture among the different treatments was tested using a repeated-measures ANOVA. Seedling survival was analysed by a Generalised Linear Model, with a binomial error distribution and logit link function, including the four experimental factors (temperature, precipitation, provenance and drought) and their full-factorial interactions. Differences in total biomass, root length, root:shoot ratio, total N concentration, δ¹³C and δ¹⁵N were tested in a similar way across the different factors but using split-plot ANOVA. All variables were transformed (log, square root) to meet normality assumptions where necessary. Results are given as mean ± SE throughout this paper.

Table 1
Global effects of the different studied factors (temperature, T; precipitation, Pt; provenance, Pr; drought, D) over the main response variables. χ²-values are given for survival and F-values for all other variables. Since third and fourth order interactions were non-significant in most cases (only a survival T × Pt × D; χ² = 4.9, P = 0.03 significant interaction appeared), they are omitted for simplicity.

Response variable	Factor	d.f.	χ ² /F	P
(a) <i>Survival</i>	Temperature	1	26.11	<0.0001
	Precipitation	1	18.19	<0.0001
	Provenance	1	28.21	<0.0001
	Drought	1	127.31	<0.0001
	T × Pt	1	0.25	0.61
	T × Pr	1	2.29	0.13
	T × D	1	1.14	0.29
	Pt × Pr	1	0.43	0.51
	Pt × D	1	0.08	0.78
	Pr × D	1	0.68	0.41
(b) <i>Total biomass</i>	Temperature	1, 52	33.77	<0.001
	Precipitation	1, 113	5.03	0.03
	Provenance	1, 113	145.83	<0.0001
	Drought	1, 57	0.82	0.37
	T × Pt	1, 113	0.12	0.73
	T × Pr	1, 113	0.75	0.39
	T × D	1, 52	0.14	0.71
	Pt × Pr	1, 113	0.68	0.41
	Pt × D	1, 113	0.62	0.43
	Pr × D	1, 113	0.05	0.83
(c) <i>Root:shoot biomass ratio</i>	Temperature	1, 52	0.57	0.45
	Precipitation	1, 113	0.51	0.48
	Provenance	1, 113	51.30	<0.0001
	Drought	1, 57	0.07	0.79
	T × Pt	1, 113	2.57	0.11
	T × Pr	1, 113	0.36	0.55
	T × D	1, 52	0.55	0.46
	Pt × Pr	1, 113	1.04	0.31
	Pt × D	1, 113	1.10	0.30
	Pr × D	1, 113	0.74	0.39
(d) <i>Root length</i>	Temperature	1, 52	18.08	<0.0001
	Precipitation	1, 113	6.16	0.02
	Provenance	1, 113	88.23	<0.0001
	Drought	1, 57	0.17	0.68
	T × Pt	1, 113	1.51	0.22
	T × Pr	1, 113	6.14	0.01
	T × D	1, 52	0.03	0.87
	Pt × Pr	1, 113	0.11	0.74
	Pt × D	1, 113	0.08	0.78
	Pr × D	1, 113	0.02	0.90

3. Results

Soil moisture was affected by three of the four main factors during the experiment, being overall greater in the high than in the low availability treatment (14.0% ± 0.3 and 10.6% ± 0.2 respectively; F = 343.5, P < 0.0001), under current than under future temperature (14.8% ± 0.3 and 9.9% ± 0.2 respectively; F = 746.8, P < 0.0001), and under the recovering than terminal drought (16.6% ± 0.3 and 8.6% ± 0.3 respectively; F = 863.2, P < 0.0001). However, no differences in soil moisture appeared between the two provenances (F = 0.33, P = 0.57), and there were no interactions between provenance and any of the other factors (Appendix A).

3.1. Survival

Survival was strongly affected by the four main factors (Table 1a). Scots pine seedlings from the southern provenance showed higher survival than the northern ones (Fig. 1). Although this pattern was maintained across the different climatic conditions of temperature and precipitation, the greatest differences were observed under the expected future conditions of higher temperature and lower precipitation, with more than double survival in southern seedlings (16.6% vs. 38.3% for northern and southern

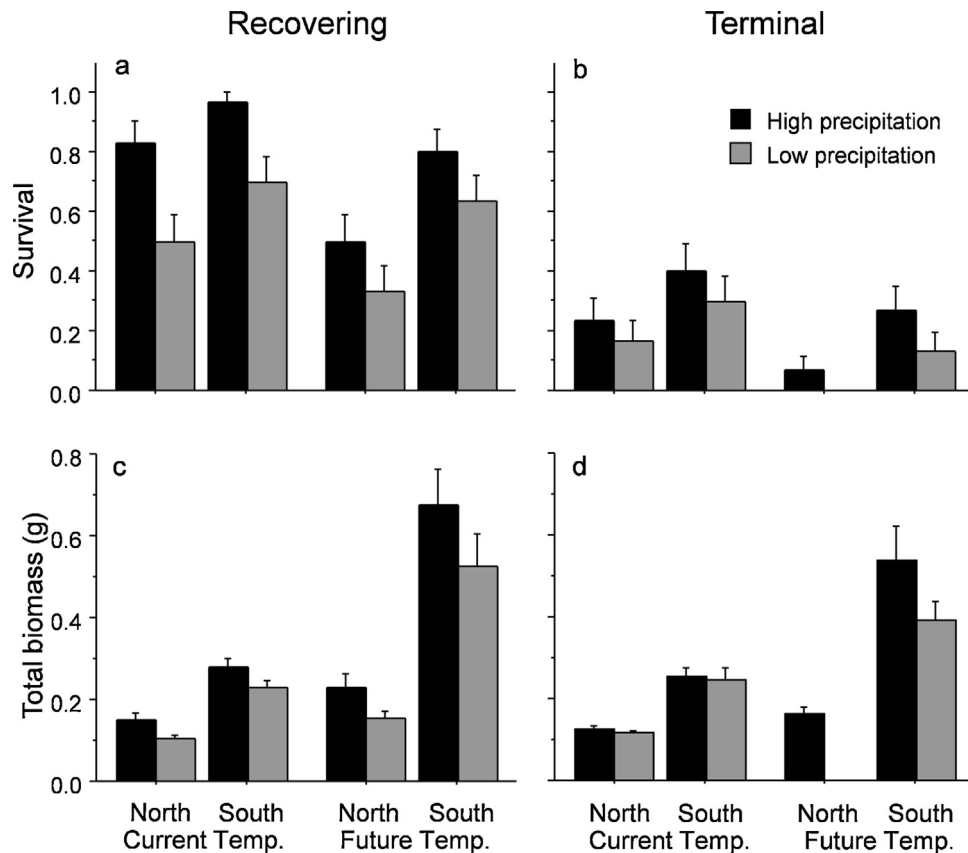


Fig. 1. Survival probabilities (a, b) and total biomass (c, d) at the end of the two drought treatments (a, c, recovering; b, d, terminal drought) across the different temperature (current, future), provenance (north, south) and precipitation (high, black bars; low, grey bars) levels. Error bars represents SE.

respectively, pooling the two drought levels). Overall, future temperature lead to a reduction in survival (34.2% vs. 51.2% for future and current temperatures respectively, provenances and precipitation treatments combined), although it was more evident in the terminal drought. Expected reduction in precipitation during the growing period also had a negative effect on survival (Fig. 1). Mortality reached 100% in northern plants with higher temperature and low precipitation in the terminal drought level. Nevertheless, a single recovering period strongly increased survival (overall 65.8% vs. 19.6% in the recovering and terminal drought levels respectively, combining temperature and provenance treatments; Appendix B).

3.2. Final growth and biomass allocation

Total biomass at the end of the experiment was affected by three of the factors studied (Table 1b). Plants from the southern provenance showed higher biomass than the northern ones in all the different climatic conditions and treatments (Fig. 1c and d). Overall, increased temperature induced higher total biomass in seedlings from both provenances and deeper roots ($407.2 \text{ mm} \pm 4.4$ and $461.5 \text{ mm} \pm 9.9$ under current and future temperatures respectively; Table 1d). Reduced precipitation reduced growth consistently across all other factors, as denoted by the lack of significant interactions. The drought alleviation in the recovering level had no effect on final biomass. Root:shoot biomass ratio was only controlled by the provenance (Table 1c), with seedlings from the southern origin investing higher proportion of their biomass in roots than northern seedlings did (Fig. 2) irrespective of environmental conditions. Furthermore, roots from southern seedlings were not only heavier, but also longer than northern ones ($461.2 \text{ mm} \pm 5.7$ and $367.1 \text{ mm} \pm 6.8$ respectively;

Table 1d), although higher temperature and high precipitation also increased root length. There was no significant relationship between root:shoot ratio and survival, although a positive trend was evident between these two variables ($r = 0.4$, $P = 0.09$).

3.3. Total nitrogen and isotopic discrimination

Total nitrogen concentration significantly differed between provenances ($F = 8.4$, $P = 0.005$), being higher for the southern plants (Fig. 3), but was not affected by any of the other experimental factors. Likewise, $\delta^{13}\text{C}$ was only controlled by provenance ($F = 8.3$, $P = 0.005$), with southern plants showing more negative values than northern ones (Fig. 3). The same pattern appeared in the case of $\delta^{15}\text{N}$, with higher values for southern than for northern plants

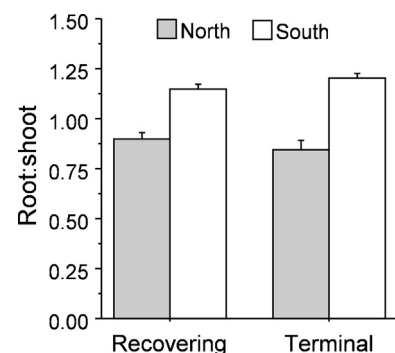


Fig. 2. Root:shoot ratio for the two provenances (north, grey bars; south, white bars) at the two drought levels (recovering and terminal drought). Error bars represents SE.

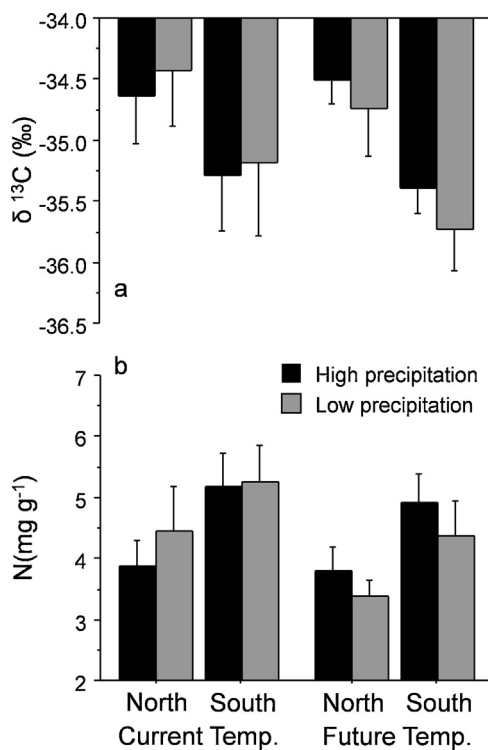


Fig. 3. $\delta^{13}\text{C}$ (a) and nitrogen concentration (b) across the different temperature (current, future), precipitation (high, low) and provenance (north, black bars; south, grey bars) levels in Scots pine leaves. Error bars represents SE.

($F = 19.3$, $P < 0.0001$; Appendix C). No other factors affected $\delta^{15}\text{N}$ apart from the significant interaction between temperature and provenance ($F = 5.1$, $P = 0.03$).

4. Discussion

The combination of extreme drought and the predicted temperature rise severely reduced overall survival in Scots pine seedlings. In addition, the expected reduction in precipitation during the early growth stages before the starting of the drought period is also able to diminish the survival and final biomass, but the alleviation of the summer drought by a short period with simulated precipitation drastically increased the survival probabilities. We also found differences between seed sources from both latitudinal limits in the N concentration and in the isotopic discrimination.

Projected temperature increases are expected to improve growth rates for many tree species in cold regions (Körner, 1999; Salminen and Jalkanen, 2007; Mathisen and Hofgaard, 2011). However, such increased temperature also implies a higher evaporation from both soil and leaves, thus amplifying the summer-drought stress in warm areas and resulting in decreased survival probabilities (Barber et al., 2000; Rehfeldt et al., 2004; Wilmking et al., 2004; Reich and Oleksyn, 2008; Martínez-Vilalta et al., 2008; Matías et al., 2012a). In this study, Scots pine grew faster in response to the expected future temperature, which represents a competitive advantage in early-life stages (Castro, 2006). However, it has been proven that higher summer and autumn temperatures are negatively related to growth in adult Scots pines at their southern range (Bogino et al., 2009; Thabeet et al., 2009). In addition, survival was strongly reduced with the expected temperature increase in both provenances and precipitation scenarios as a consequence of the reduction in soil moisture, which may counteract any positive effect of this early growth. Thus, data presented here suggest that besides the potential positive effect on growth, either extreme

heat waves or a progressive temperature rise, might induce a substantial reduction in Scots pine recruitment. This finding, together with the strong adult mortalities associated with heat waves (Rebetez and Dobbertin, 2004; Van Mantgem and Stephenson, 2007; Adams et al., 2009; Allen et al., 2010), can drastically alter the natural dynamics of this species.

The extreme drought simulated in this study strongly reduced survival probability (overall 11% when combined with future temperature), and could hamper recruitment completely under natural conditions (Mendoza et al., 2009). However, there are two scenarios under which drought stress might be ameliorated, and thus seedling survival increased. First, high precipitation during late spring or early summer, as simulated in this study, is able to influence final seedling fate. Contrary to the pattern found by Richter et al. (2012), higher precipitation during the early growth stage significantly increased growth and the survival probability of both provenances in response to summer drought. The faster growth under the high precipitation treatment allowed seedlings to develop deeper roots, able to access water from deeper parts of the soil profile, and also increased leaf biomass, enhancing the photosynthetic capacity of seedlings. A faster early development has many benefits in terms of inter- and intraspecific competitive advantages (Verdú and Traveset, 2005; Castro, 2006) and, as shown here, in the capacity to cope with extreme summer drought. Second, sporadic summer storms such as that simulated in the recovering treatment have the capacity to strongly boost survival. This species maintains strict control of stomata in order to avoid hydraulic failure and desiccation (Poyatos et al., 2007; Zweifel et al., 2007) but at the cost of carbohydrate depletion through decreasing photosynthesis and carbon assimilation, ultimately causing death by starvation under prolonged drought (McDowell et al., 2005; Mitchell et al., 2013). A single rainy event at the centre of summer drought might allow enough carbon acquisition to survive this stressful period, here tripling the final survival with respect the terminal drought treatment (Fig. 1). Hence, all these results suggest spring and early-summer precipitation as an important factor controlling Scots pine recruitment, as well as indicating the significance of rare summer storms in reducing drought stress. In a global change scenario where the frequency of extreme drought periods is expected to increase, but also the stochasticity of rainy events at the southern range of this species (IPCC, 2013; Giorgi and Lionello, 2008), not only the total amount of precipitation is an important factor to be taken into account when predicting future species dynamics, but also the increased evaporation as a response to temperature rise, and the distribution of precipitation over time seems to be crucial. Although tree regeneration is naturally a pulse phenomenon, a progressive reduction in the frequency and/or magnitude of regeneration events might interact strongly with the increased mortality rates already registered in adult trees (Allen et al., 2010) in the determination of the final composition of the community. The idea that variation in water availability may be a critical factor driving response to warming at the southern range of Scots pine is also supported by other field, modelling and dendrochronological studies (Benito-Garazón et al., 2008; Martínez-Vilalta et al., 2008; Matías et al., 2012b; Sanchez-Salguero et al., 2012).

Although the response to the experimental variation in temperature and drought of the seedlings from the two range limits generally followed the same pattern, southern-limit seed sources performed better in all conditions. This superior performance can be explained in terms of the higher proportion of biomass allocated to roots in southern seedlings. A more developed radicular system allows exploration of deeper soil layers for water and nutrient uptake (Lloret et al., 1999; Paula and Ojeda, 2009), and has been identified as a key trait related to drought resistance (Collins and Bras, 2007; Markesteijn and Poorter, 2009). Even though we

did not find a significant relationship between root investment and survival in this study, we must take into account that both variables were consistently higher in southern than in northern seedlings in all treatment combinations and that the variability induced in survival by the other studied factors may be masking this relationship. Additionally, it should be noted that southern populations produced bigger seeds than northern ones, and it has been proven that this represent a clear advantage for early growth in this species (Reich et al., 1994; Castro, 1999; Castro et al., 2008). This local adaption to summer drought not only allowed higher growth and biomass, but also surviving seedlings were less stressed than northern ones as denoted by the differential values in $\delta^{13}\text{C}$. The higher (less negative) values of $\delta^{13}\text{C}$ in northern seedlings can be explained by the reduction of stomatal conductance in response to drought at the cost of carbon acquisition (McDowell et al., 2005). On the contrary, the higher investment in deeper roots of southern plants allowed them to maintain a favourable enough hydric balance to keep their photosynthetic system active, despite losing water by transpiration. Thus, these results suggest that at the same level of water availability, northern seedling suffered drought stress earlier, closed their stomata and stopped growing, explaining also the differences found in total biomass with respect to the southern provenance. It should be noted, however, that the $\delta^{13}\text{C}$ results presented here are lower than values commonly found in nature (Eilmann et al., 2010; Beghin et al., 2011), most likely due to the source of carbon available within our experimental cabinets. However, this does not alter our conclusions since the identified differences were consistent among the different treatments.

Scots pine populations from colder environments often show higher tissue nitrogen concentrations and higher metabolic rates than populations from warmer environments when grown in common gardens (Reich et al., 1996; Oleksyn et al., 2003). Nevertheless, we found an opposite pattern, with higher values of both total N concentration and $\delta^{15}\text{N}$ in southern seedlings. These differences between provenances can be also explained by the differential biomass allocation pattern. A more developed radicular system not only allowed better access to deep water, but also permitted a wider exploration of soil for nutrients. Total N and $\delta^{15}\text{N}$ are commonly related in plant tissue (Högberg, 1997), and indicate a better nutritive status of southern plant when compared to northern ones. Here we focussed on adaptation at the southern range edge of Scots pine, where conditions are expected to become increasingly unfavourable for the growth of this species over coming decades. We do not focus on local adaptation at the northern range edge. Consequently, it should be noted that, although southern seedlings performed better throughout this experiment, other sources of stress such as frost and the continued low temperatures experienced at the northern range are not tested in this study, and the resistance capacity of northern populations to these stressors is probably strongly important in the performance of this species at the northern limit of the distribution.

4.1. Conclusions

According to previous field and experimental studies (Castro et al., 2005; Mendoza et al., 2009; Matías et al., 2012a,b; Richter et al., 2012; Matías and Jump, 2014) and results presented here, Scots pine is very sensitive to climate alterations at its southern range edge. However, despite the strong negative effects of extreme drought and elevated temperature, southern populations show morphological and physiological adaptations that increase resistance to extreme drought events. These differences highlight the importance of the conservation of genetic sources in marginal populations as potential resources to ameliorate future consequences of ongoing climatic change. Furthermore, the presence of either a rainy period during the early growth stage or sporadic

summer storms can strongly modify the probability that seedlings will survive these extreme drought events. Consequently, models that aim to forecast alterations in species distributions based primarily on mean climatic projections are likely to be associated with high errors of prediction, and a better description of rainfall variability and local adaptations should be taken into account for more accurate prediction of future population dynamics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.envexpbot.2014.04.003>.

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