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Title: Role of geographical provenance on the response of silver fir seedlings to experimental warming and drought

**Running head**: WARMING AND DROUGHT EFFECTS ON SILVER FIR

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**Abbreviations**: CT: current temperature; FT: future temperature; iWUE: intrinsic water use efficiency; Ψ: plant water potential.

**Key-words**: Climate warming, drought, growth, Pyrenees, range-edge, recruitment, regeneration, silver fir.

## Abstract

Changes in climate can alter the distribution and population dynamics of tree species by altering their recruitment patterns, especially at range edges. However, geographical patterns of genetic diversity could buffer the negative consequences of changing climate at rear range edges where populations might also harbour individuals with drought-adapted genotypes. Silver fir (*Abies alba*) reaches its south-western distribution limit in the Spanish Pyrenees, where recent climatic dieback events have disproportionately affected westernmost populations. We hypothesised that silver fir populations from the eastern Pyrenees are less vulnerable to the expected changing climate due to the inclusion of drought-resistant genotypes. We performed an experiment under strictly-controlled conditions simulating projected warming and drought compared with current conditions and analysed physiology, growth and survival of silver fir seedlings collected from eastern and western Pyrenean populations. Genetic analyses separated eastern and western provenances in two different lineages. Climate treatments affected seedling morphology and survival of both lineages in an overall similar way: elevated drought diminished survival and induced a higher biomass allocation to roots. Increased temperature and drought provoked more negative stem water potentials and increased $\delta^{13}C$ ratios in leaves. Warming reduced nitrogen concentration and increased soluble sugar content in leaves, whereas drought increased nitrogen concentration. Lineage affected these physiological parameters, with western seedlings being more sensitive to warming and drought increase in terms of $\delta^{13}C$, nitrogen and content of soluble sugars. Our results demonstrate that, in *Abies alba*, differences in the physiological response of this species to drought are also associated with differences in biogeographical history.

## Introduction

The increase in temperature recorded during the last decades has the capacity to alter the phenology, growth and biotic interactions of plant species worldwide (Parmesan 2006; Walther 2010). These impacts are expected to be especially evident over relatively small spatial scales in mountain ecosystems, where the elevational ranges and shifts of trees are to a great extent controlled by temperature (Peñuelas and Boada 2003) which declines rapidly with increasing elevation. In addition, temperature changes will be accompanied by an alteration of current precipitation patterns, for instance with a generalised increase of the length and intensity of summer drought in central and southern European regions (Giorgi and Lionello 2008). These climatic alterations are likely to have important consequences for tree species dynamics at local and regional scales (Peñuelas and Boada 2003; Van Mantgem et al. 2009; Matías and Jump 2015).

Changing climatic conditions are especially relevant close to the equatorial limit of species distributions (the rear, or trailing edge), where climatic conditions often correspond to the species drought-tolerance limits (Hampe and Petit 2005). In these locations, even small variations in climate could result in profound demographic effects, such as lower relative fecundity and reduction of local population densities (Case and Taper 2000). Such changes could result in reduced resilience under adverse climate conditions and eventual alteration of species distributions. In the warmest regions of species distributions, the intensity of population responses to drought stress are likely higher than in colder areas, being more prone to local extinction as a consequence of extreme climatic events (Jump et al. 2009; Carrer et al. 2010). Alternatively, although the historical isolation and fragmentation of these range edge populations has often resulted in impoverished genetic diversity within populations (Hampe and Petit 2005), their long exposure to the xeric conditions may have resulted in local adaptations to drought stress, and those stands might represent genetic reservoirs holding drought-resistant genotypes (Hampe and Petit 2005; Matías and Jump 2014). Thus, the study of the response of rear-edge populations to the expected temperature and drought increases is important to understand and to predict the possible negative consequences of climate change on natural populations.

Traditionally, regeneration has been considered as an important feature driving population dynamics in temperate tree species (Houle 1994; Rickebusch et al. 2007). Early-life stages are more susceptible to environmental alterations than adults because of their limited root system and above-ground height, constraining their competitive abilities (Lloret et al. 2004, 2009). Therefore, seedlings often respond faster to climatic variations than adult trees (Lloret et al. 2009). Thus, recruitment is expected to be a key bottleneck determining community dynamics in forests during the coming decades (Lloret et al. 2004; Matías et al. 2011a). Consequently, information on regeneration and component processes such as seedling growth and physiology under future conditions is urgently needed for modelling and predicting forest dynamics under climate change scenarios (Morin and Thuiller 2009).

Silver fir (*Abies alba* Mill.) is a widely distributed conifer in mountain forests of central, eastern and southern Europe (Jalas et al. 1999), where it becomes dominant in pure or mixed forests with *Fagus sylvatica*. This species reaches its main south-western distribution limit in the Spanish Pyrenees. Here silver fir has experienced dieback and growth decline that disproportionately affects western populations in response to warming-related aridification trends starting in the 1980s (Camarero et al. 2011; Linares and Camarero 2012a). In some of these western populations ongoing dieback events are characterized by high defoliation levels (over 50%) and standing mortality rates up to 30-40% of adult trees as a consequence of episodic drought events (Camarero et al. 2015), similar to those observed in southern France (Cailleret et al. 2014). Furthermore, silver fir regeneration is expected to be an important bottleneck under predicted warmer and drier climate (de Andrés et al. 2014). Bioclimatic models indicate important reductions of the Pyrenean silver-fir area due to intensified warming at the end of the 21st century (Serra-Díaz et al. 2012).

Sancho-Knapik et al. (2014) demonstrated that the geographical variation in forest dieback is associated with differences in the genetic composition of populations. Rear-edge western populations of silver fir have lower genetic diversity and are more prone to drought-induced dieback than their eastern counterparts. This geographic difference in population genetic structure and dieback prevalence is reflected in paleocological evidence that indicates a westward post-glacial migration of silver fir through the Pyrenees from glacial refugia in the Balkans and in Central Italy with the consequence that western populations are younger in paleobotanic terms than eastern populations (Taberlet et al. 1998; Liepelt et al. 2009).

Therefore, silver fir represents a highly valuable study system due to (i) commonness and dominance at the rear edge of its distribution, (ii) occurrence in Mediterranean mountains, which constitute environments particularly vulnerable to climate change (Metzger et al. 2008), (iii) vulnerability to current drought episodes, as shown by tree mortality, and (iv) a rich biogeographical history that has resulted in a discontinuity in the origin of populations across its rear range edge.

Based on the previous evidence, we hypothesised that silver fir populations from the eastern Pyrenees would be less vulnerable to the expected warming and drought increase than the western populations. To test this hypothesis, we used controlled environment experiments incorporating elevated temperature and drought. We analysed physiology, growth and survival of silver fir seedlings collected in populations from eastern and western locations of the Pyrenean range. The use of controlled environment growth chambers allowed precise manipulation of soil moisture and temperature, while keeping all other climatic factors at constant values. Specifically, we sought to answer the following questions: 1) what are the consequences of the projected temperature rise and precipitation reduction on seedling growth and survival of seedlings from Pyrenean silver fir populations? 2) which physiological mechanisms affect the response of silver fir seedlings to climate? 3) is there any difference in the response of seedlings from eastern and western Pyrenean lineages of silver fir to the projected future climatic conditions?

## Materials and Methods

### *Field sampling*

The present study was conducted from January to June 2013 at the Controlled Environment Facility of the University of Stirling (Stirling, UK). We used silver fir seed sources from thirteen natural populations located in northeastern Spain and southern France (Fig. 1; Supplementary material Appendix 1 Table A1). In the Pyrenees, silver fir dominates subalpine and montane forests, mainly in sites on mesic and humid slopes with deep soils (Vigo and Ninot 1987). We differentiated eastern and western provenances (Martín et al. 1998). Seeds were collected from six different populations at the eastern provenance and from seven at the western provenance, selecting at least 15 mature trees per population and collecting newly dispersed seeds from the soil surface, avoiding damaged or rotten ones. Plant material from each provenance was pooled, and then stored at 4 ºC until used for the experiment.

*Experimental conditions*

Plants were grown in tubular pots with a diameter of 8 cm and a height of 45 cm (2.3 L volume) to enable unrestricted vertical root growth. The pots were filled with a peat and river sand mixture (proportion 2:1 in volume, respectively) above a layer of gravel to improve drainage. Nutrient content available in the substrate mixture was nitrogen 0.7 %, phosphorous 0.4 % and potassium 0.3 %. In total 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 × 0.75 m wide × 1.2 m high), giving a total of 480 pots. Before sowing, all pots were irrigated with 0.2 L of a soil microbial inoculum to allow mycorrhization and realistic microbial communities (Matías et al. 2014). This inoculum was obtained from the maceration of soil sampled beneath adult silver fir, taken from the respective locations where seeds were collected, in a proportion of 0.1 kg soil $L^{-1}$. Three seeds were sowed per pot, randomly thinning to one seedling in case of multiple emergences.

Optimal conditions for emergence and early growth (16 h light / 8 h dark daily cycles at 22 °C) were maintained for 3 weeks. Subsequently, we applied two experimental treatments (temperature and water availability) crossed with the provenance factor in a full-factorial design. As climate reference, we used the monthly mean temperature and precipitation values obtained from six meteorological stations across the source area during the 1990-2010 period (Table 1). The temperature treatment was applied as a whole plot factor, and had two levels replicated in two chambers each: 1) *current temperature* (CT hereafter), representing the mean monthly day and night temperatures during the growing period (Table 1); 2) *future temperature* (FT hereafter), simulating the predicted increase in temperature by 4 °C above *current* values, as projected for the 2090-2099 period in NE Spain at the time of the experimental setup (A2 scenario; IPCC 2007). The water availability treatment was applied as a subplot factor (within cabinets) and was also represented by two levels, representing current and future scenarios: 1) *high availability*, based on the mean monthly precipitation records during the growing season (May-September) at the reference climatic database (100.5 L $m^{-2}$); 2) *low availability,* simulating a reduction in precipitation by 30% from the previous level, as projected for NE Spain (70.3 L $m^{-2}$). These monthly precipitation amounts were split into 8 irrigation events (twice per week) from the 4th experimental week to the 23rd. Consequently, the experimental design included three factors (temperature, watering and provenance) with two levels each, fully crossed with 60 replicated plants per combination ($2 \times 2 \times 2 \times 60 = 480$ plants).

During the whole experiment, light intensity was fixed with a photosynthetic photon flux density of 210 µmol $m^2$ $s^{-1}$ during 16 hours, rising progressively at dawn and decreasing at dusk for one hour. This light intensity is representative for moderately open understory where silver fir usually regenerates (Ameztegui and Coll 2011; de Andrés et al. 2014). Air relative humidity and $CO_2$ 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 (volumetric water content, %VWC) 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). These values were recorded two days after irrigation. Microclimate conditions within the chambers were logged continuously and checked with periodic independent instrumental measurements to ensure comparability of treatments.

*Seedling measurements*

On 24 June 2013 (experimental week 24th), all surviving seedlings were harvested. Roots were carefully washed to remove soil remnants, maximum root and shoot fresh length was measured, and seedlings were divided into aboveground and belowground parts. Water potential (Ψ) was measured around midday in the stem of each seedling using a pressure bomb (range 0–15 MPa; Manofrígido, Lisbon, Portugal), and then seedlings were oven-dried at 45 ºC for 72 h and weighed.

*Genetic analyses*

To assess if eastern and western sampled populations corresponded to different lineages, DNA was extracted from dried needles of 50 randomly selected seedlings per provenance (populations pooled within provenance). Genomic DNA from each sample was extracted using the DNeasy 96 Plant Kit (Qiagen, Manchester, UK) following the manufacturer's protocol and then stored at -20 °C until use. All samples were genotyped using six nuclear microsatellites markers (SSR) specific for silver fir: SF1, SFb4, SF78 (Cremer et al. 2006) and NFF7, NFH3, NFF3 (Hansen et al. 2005). Reactions were carried out in a final volume of 10 µL with 1X of Type-it Multiplex PCR Master Mix (Qiagen, Manchester, UK), 0.2 µM of each primer and 20 ng of template DNA. Polymerase chain reactions (PCR) were carried out in a Veriti™ Thermal cycler (Applied Biosystems), following conditions reported by Hansen *et al.* (2005). PCR products were analysed by DNA Sequencing and Services, Dundee, UK, using a 3730 DNA Sequencer (Applied Biosystems) with reference to a LIZ 500 size standard.

*Carbon isotopes, nitrogen and carbohydrate analyses*

A random subset of 10 individuals per treatment combination (80 seedlings in total) was selected for carbon isotopic discrimination analyses expressed as $\delta^{13}C$. It is assumed that $\delta^{13}C$

is directly related to the intrinsic water use efficiency (iWUE), and represents a reliable indicator of drought stress (Ehleringer 1993). Dried leaves were ground to powder and analysed for total C, N and $^{13}$C. Determinations were conducted at the Stable Isotope Facility of the University of California at Davis (SIF, 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 (Powers et al. 2008). The isotopic ratio was expressed in delta notation ($\delta^{13}$C) in per mil (‰) as relative deviation from the international standard V-PDB. From the same individuals, soluble sugars (SS) were extracted from leaves with 80 % (v/v) ethanol and their concentration determined colorimetrically, using the phenol–sulphuric method (Buysse and Merckx 1993). SS play a substantial role in higher plant development, and its concentration is expected to increase in drought-tolerant plants (Maguire and Kobe 2015).

### *Data analysis*

Variation in soil moisture among the different treatments was tested using repeated-measures analysis of variance (ANOVA), using temperature, watering and provenance as between subject factors and time as a within subject factor. Seedling survival was analysed using a Generalised Linear Model, with a binomial error distribution (1 for surviving and 0 for dead individuals) and logit link function, including the three experimental factors (temperature, watering and provenance) and their full-factorial interactions as independent variables. Differences in total biomass, root length, root:shoot biomass ratio, water potential, total N concentration, C:N ratio, $\delta^{13}$C and SS were tested across the different factors and interactions but using ANOVAs with the same model structure (provenance, temperature, watering and their interactions as independent variables). As a consequence of the rotation scheme of pots within and between chambers, chamber effect could not be specifically included in the models. Consequently, any possible confounding effect of the experimental chambers can not be detected. NOTE: Do you think this is the best place to state this? Yes, but I would rephrase if this doesn't cause problems with the requested revisions:

Possible confounding effect of the experimental chambers could not be detected via specific inclusion of chamber as a factor in the models since pots were rotated within and between chambers during the experiment. However, chamber effects should be minimised, via this rotation protocol.

Analyses of molecular variance (AMOVA) were used to perform hierarchical analyses of genetic variation within and among provenances based on all polymorphic sites. Allele frequencies and within-provenance genetic diversity parameters (mean number of alleles per locus, $N_a$; mean observed and expected heterozygosity, $H_O$ and $H_E$, respectively; mean genetic diversity, $H_S$; mean allelic richness, $R_S$) were estimated using FSTAT (Goudet 2002). Bayesian clustering was used to infer population structure using STRUCTURE v2.3.4 (Pritchard et al. 2000). We used an admixture model with correlated allele frequencies. Ten independent runs were performed per each number of clusters K from 1 to 5, with a burn-in period of 50,000 and 100,000 Markov chain Monte Carlo iterations. The optimum number of clusters, K, was determined using Structure Harvester (web version) (Earl and VonHoldt 2012) and Evanno method (Evanno et al. 2005). All values are given as mean ± SE through the text.

## Results

### *Soil moisture*

Watering levels imposed during the experiment resulted in different soil moisture across treatments. Soil moisture was affected by two of the three main factors during the experiment, being greater in the high- than in the low water availability treatment (31.0 % ± 0.1 vs. 23.8 % ± 0.1 respectively; $F = 821.9$, $P < 0.0001$), and under current than under future temperature conditions (28.9 % ± 0.1 vs. 25.9 % ± 0.1 respectively; $F = 147.2$, $P < 0.0001$). There was also a significant interaction between these two factors ($F = 12.9$, $P = 0.0004$) and a change in soil moisture over time ($F = 519.1$, $P < 0.0001$; Supplementary material Appendix 1 Fig. A1). However, no systematic differences in soil moisture appeared between pots containing the two provenances (east, west; $F = 1.52$, $P = 0.21$), and there were no interactions between provenance and any of the other factors (provenance × temperature: $F = 0.62$, $P = 0.43$; provenance × precipitation: $F = 1.16$, $P = 0.28$).

### *Genetic diversity and structure*

Based on microsatellite information, seedlings from the eastern provenance showed a higher number of private alleles than western seedlings. They also showed slightly higher values of allelic richness, genetic diversity, and heterozygosity (Table 2). AMOVA results showed significant genetic differentiation among provenances (8.1%, $P$ <0.0001), with the remaining 91.9% of genetic variability occurring within provenances. This differentiation was supported by the results in STRUCTURE, were the most probable number of genetic clusters was two (Supplementary material Appendix 1 Fig. SA2). These two clusters corresponded closely to the two geographical sampling areas (east and west), with some admixed individuals.

### *Total biomass and survival*

Total biomass at the end of the experiment was determined only by the provenance factor, being unaffected either by the changes in temperature or in watering (Table 3). Seedlings from the western provenance grew more than seedlings from the eastern provenance (Fig. 2a), also producing deeper roots (290.9 ± 7.9 vs. 258.1 ± 9.2 mm) and taller shoots (32.1 ± 0.5 vs. 29.3 ± 0.6 mm). Although temperature had no effect on total biomass, higher temperature diminished root length (CT: 329.3 ± 8.1 mm, FT: 236.6 ± 7.5 mm; $F = 53.2$, $P < 0.0001$). Root:shoot biomass ratio was affected by the two climate factors, but not by the seed provenance (Table 3), showing a high plasticity in response to climatic variables (Fig. 2b). Overall, higher temperature reduced biomass allocation to roots (CT: 1.67 ± 0.05, FT: 1.41 ± 0.03), while reduced watering induced higher root investment (high: 1.40 ± 0.04, low: 1.65 ± 0.05). The proportion of surviving seedlings was affected only by the watering treatment (Table 3), with a decrease in the survival probability as watering diminished (high: 0.91 ± 0.02, low: 0.78 ± 0.03).

### *Ecophysiological variables*

At the time of harvesting, water potential of silver-fir seedlings was influenced by the changes in temperature and, especially, in watering (Table 3), but not by provenance. Both higher temperature and lower watering led to more negative water potentials (Fig. 3a), although the effect of watering was stronger than that of temperature (high watering: $\Psi = -1.68 \pm 0.04$ MPa; low watering: $\Psi = -2.25 \pm 0.04$ MPa). Carbon isotopic discrimination was similar between the two provenances, but it was also affected by changes in temperature and watering (Table 3). Overall, seedlings growing under current temperature or with higher watering had more negative $^{13}$C values than those under increased temperature or in low watering treatments (Fig. 3b). However, significant temperature- watering, temperature-provenance, and temperature-watering-provenance interactions appeared. Under current temperature conditions, seedlings from the eastern provenance increased $\delta^{13}$C concentration when watering was reduced, while under simulated future higher temperatures, western populations exhibited the higher $\delta^{13}C$ concentrations under reduced watering.

Nitrogen concentration in seedlings was also affected by the two climatic variables (Table 3). Warmer temperature reduced N concentration (CT: 11.5 ± 0.6 mg $g^{-1}$, FT: 9.6 ± 0.7 mg $g^{-1}$), whereas seedlings growing under the low watering treatment presented higher N concentration (13.2 ± 0.6 mg $g^{-1}$) than those under the high watering treatment (8.0 mg $g^{-1}$ ± 0.5). In a similar way, the C:N ratio increased with higher temperature, but diminished when watering was reduced (Fig. 4a). The effect of provenance was not significant, but the temperature-provenance interaction was significant for both N concentration and C:N ratio due to higher N concentration in seedlings from western provenances under higher temperatures (Fig. 4a).  Lastly, SS concentration was only affected by temperature, with higher values under the simulated future temperature (CT: 5.0 ± 0.2%, FT: 5.7 ± 0.2%). Although watering had no effect on SS concentration, there were significant watering-temperature and watering-provenance interactions on the SS content (Table 3; Fig. 4b). The increase of SS concentration under higher temperatures was much higher in seedlings from the eastern provenances when watering was reduced, while the opposite trend was observed in seedlings from the western provenances.

## Discussion

In this study we tested the effect of elevated warming and drought on early growth and survival of two geographic provenances of Pyrenean silver fir. We confirmed that the eastern and western Pyrenean provenances correspond to two different genetic lineages. Seedlings from the western lineage grew more than those from the eastern lineage. While climatic manipulations had no effect on total biomass, increased drought diminished survival across lineages. Warming induced more negative water potentials, higher $\delta^{13}C$ and SS values, higher C:N ratio and lower N content. Drought increase provoked more negative water potentials, higher $\delta^{13}C$ and N content and lower C:N ratio. Interestingly, eastern and western provenances presented different iWUE when only drought or warming and drought were simulated.

### *Geographical lineages of Pyrenean silver fir*

Genetic data confirmed that the eastern and western provenances corresponded to the eastern and western genetic lineages identified in previous work (Liepelt et al. 2009; Sancho-Knapik et al. 2014). The Bayesian clustering analysis using Structure is based on attributing cluster membership at the individual level and is not reliant on pre-defined groupings, unlike AMOVA. Structure separated the individuals sampled broadly into two biogeographical lineages, east and west. Although some admixture occurred within each cluster, it is notable that there was no support for further differentiation within these lineages, such that the primary genetic differentiation of samples used in our experiment occurs at the lineage level rather than at the level of populations. Despite evidence for at least some ongoing gene-flow (as evidenced by admixture of some individuals), the clear signal of genetic differentiation between these lineages likely results from a persistent signal of differential postglacial history of the two lineages acting in conjunction with population fragmentation and isolation (Petit et al. 2003; Liepelt et al. 2009; Sancho-Knapik et al. 2014).

### *Climatic effect on biomass and survival*

Warming and drought induced higher biomass allocation to roots, and drought reduced seedling survival. Changes in climatic conditions simulated in this experiment altered growth, survival and physiological performance of Pyrenean silver fir seedlings. Although western-lineage seedlings grew more rapidly than those from the eastern lineage, climate treatments generally affected seedling performance in a similar way, irrespective of provenance origin. Thus, a reduction in watering diminished survival and altered the biomass allocation pattern for both lineages. In addition, the combination of increased temperature and decreased watering affected the water potential of seedlings from both lineages in a similar way. However, the effects of temperature and watering varied between lineages for carbon isotopic discrimination, nitrogen concentration, C:N ratio, and the concentration of soluble sugars.

Although the projected rise in temperature and reduction in precipitation have a long-term negative impact on growth and iWUE (inferred from carbon isotopic discrimination in tree-ring wood) for adult silver fir trees (Aussenac 2002; Camarero et al. 2011; Linares and Camarero 2012a, 2012b), the conditions simulated in this experiment had no effect on seedling growth. This lack of effect is in accordance with the pattern found by Lopushinsky and Max (1990), who determined a positive relationship between temperature and seedling growth for the fir species (*A. amabilis, A. procera* and *Pseudotsuga menziesii*), with a maximum at 20 °C and a growth cessation at 30 °C. Temperatures simulated here exceeded this optimal in both treatments, explaining this lack of effect. However, future conditions had a contrasting impact on the biomass allocation pattern, consistent with the trade-off mechanism for the acquisition of light and water (Smith and Huston 1989): whereas warmed plants allocate more to shoot, the capacity to invest in the root system is reduced under water stress. Root biomass is a key trait in the ability of seedlings to cope with drought (Markesteijn and Poorter 2009; Matías et al. 2014), since it determines the capacity to explore deeper soil layers for water and nutrient uptake. According to results presented here, root:shoot ratio is a very plastic trait in this species, since silver fir is able to alter this proportion when environmental conditions change, thus allowing seedlings to better cope with more stressful environmental conditions. However, the differential effect of temperature and watering may have strong implications since, under a scenario of combined temperature and drought increase, the negative temperature effect on root investment could cancel the positive effect of higher root allocation in response to reduced precipitation, diminishing the plastic response to climate when warmer and drier conditions occur.

A reduction in watering had a negative effect on seedling survival. Although this was a moderate reduction (about 12% less), it could, in combination with the inability to increase biomass allocation to roots under warmer, drier climate, have a net negative effect on the mid-term regeneration capacity of silver fir. This is especially important for western Pyrenean populations, since the declining trends currently recorded in adult trees raises the importance of recruitment for population maintenance in those forests experiencing dieback.

### *Physiological response to climate alterations*

Water potential, carbon isotopic discrimination, N content and SS concentration were implicated in the response of seedlings to modified climate. An increase in both temperature and drought resulted in more negative water potentials in silver fir seedlings from both provenances. Although this is a common response to harsher conditions (Quero et al. 2011), it should be noted that the effect of elevated drought was stronger than that of increased temperature. Across the soil-plant-atmosphere continuum, lower soil volumetric water content caused by decreased watering induces more negative soil water potentials in parallel with plant water potentials, since ascendant water flow has to be maintained from roots to leaves (Sperry et al. 2002). In the upper part of this continuum, warming provokes an increase in vapour pressure deficit (VPD) in the atmosphere surrounding the leaves and causes sudden stomatal closing (Schultz 2003), avoiding as large a reduction of plant water potential as that caused by soil water depletion in the case of low precipitation levels. This warming effect on plant water potential might be even more patent in natural systems, where the covariation between temperature and VPD is stronger than under controlled conditions with constant relative humidity.

Changes in temperature and watering simulated here also altered $\delta^{13}C$ in silver fir seedlings. Both warmer and drier conditions augmented $\delta^{13}C$ concentration compared to current climate values, indicating that seedlings are able to increase the carbon fixation per water unit lost (i.e., higher iWUE) when temperatures increase or when precipitation diminishes. This increase in iWUE implies that silver fir seedlings have the capacity to buffer the effects of the predicted climate to some extent either by increasing the photosynthetic rate and/or by diminishing the stomatal conductance (McDowell et al. 2005), maintaining the water balance despite environmental alterations. However, this adjustment cannot be maintained indefinitely and the thresholds where the capacity to improve iWUE is exceeded by climatic alterations should be explored in more depth. We should note, however, that the $\delta^{13}C$ results presented here are lower than values commonly found in nature for this species (Linares and Camarero 2012b), likely due to the source of carbon available within our experimental cabinets or because the cited studies analyzed tree-ring cellulose or shaded needles. However, this difference does not alter our conclusions since these differences were consistent among the different treatments.

The simulated climatic conditions affected the nutritional status of silver fir seedlings, by diminishing the nitrogen concentration when warming was simulated but increasing under drier conditions. Nutrients contained within microbial biomass under moist conditions are usually released to the soil under drought conditions, allowing plants a higher uptake until a critical threshold for the uptake of dissolved nutrients is surpassed (Matías et al. 2011b). Such microbial nutrient release could explain the higher nitrogen concentration in the low-watering scenario. However, the detected decrease in nitrogen concentration under warmer conditions opens the possibility that warming-induced nutrient deficiency might play an additional role in dieback of silver fir. We should take also into account that nutrient availability in the experimental pots was higher than in natural populations (Supplementary Table A1), and the reported differences might, therefore, be even higher in nature. Previous studies found an increase in the soluble sugar content in response to drought in other fir species (Guo et al. 2010), associated with a decrease in other macromolecules such as starch, since soluble sugars are efficient osmolytes (Ahmed et al. 2009). However, we did not find a clear climatic effect on the soluble sugar concentrations in silver fir leaves.

### *Geographical differences*

In addition to the general effects described above, seedlings from the eastern and western Pyrenean provenances performed differently. Seedlings from the western provenance grew better than eastern ones, however, this higher growth rate of western-provenance seedlings was not linked to higher survival probabilities. The higher growth in the early life stage may grant seedlings a clear competitive advantage for establishment, which together with the relatively high survival across the different climatic scenarios could allow the existence of a good sapling bank in the understory able to maintain the populations after adult mortality, even in those populations suffering current declining trends. Nevertheless, the two provenances showed some relevant differences in seedling physiological response to the experimental climatic scenarios. The increase of $\delta^{13}$C under simulated warming was particularly high for western populations under drought conditions, while they exhibited diminished iWUE under current temperatures but with drought conditions. This result contrasts with higher $\delta^{13}$C values in seedlings from eastern populations under current temperatures and low watering when comparing them with western populations, which in fact agrees with less adult decline observed in the field. This higher physiological sensitivity to temperature and drought in the western provenance might imply contrasted responses depending on the yearly variations in temperature and precipitation. In the driest scenario simulated (future temperature and low watering) we also found geographical differences in the soluble sugar concentrations, with seedlings from the eastern range showing much higher values than western ones. This result concurs with the observed lower $\delta^{13}C$ values, suggesting reduced stomatal control, compared with western populations growing in the same conditions. Geographical provenance also influences patterns of N concentration in seedlings, with western populations showing higher values in warmer conditions. However, beside these differences, we should take into account that the source populations from two provenances differed in elevation (Supplementary table A1), which could imply some additional differences on seedling growth and physiology, as previously shown by Oleksyn et al. (1998).

We assessed differences in seedling performance between the eastern and western lineages of silver fir under controlled conditions. While the differences we report can inform on differences in plant performance in the field, they cannot fully explain the observed differences in adult dieback severity. Diverging patterns between adults and seedlings can occur due to ontogenetic shifts in niche requirements or in physiology (Mediavilla and Escudero, 2004; Quero et al. 2008) such that seedling and adult responses may not be directly comparable. However, the geographical variability in seedling performance that we report demonstrates high intraspecific variability in response to environmental change that is related to the postglacial colonisation history of the species. These differences would be even higher than the described here, since the different source populations differ on altitude and thus would have different thermal optima, while we subjected them to similar conditions (either current or future). Such a finding adds improved resolution to understanding the scale of intraspecific variation in plant performance whilst also challenging our ability to generalise population-level responses to predicted future climate.

### *Conclusions*

Predicted changes in climate including warmer and drier conditions will alter growth, physiology and survival of silver fir seedlings at the southern edge of the species geographical range. We did not find evidence for differences in seedling mortality rates in response to simulated future conditions.  However, our results suggest diverging physiological responses of seedlings from eastern and western Pyrenean lineages of silver fir in response to future climatic scenarios.  The plastic responses of biomass allocation and survival and the modulation of the physiological variables highlight regeneration as a relatively stable process under current levels of climatic variability. However, divergent physiological responses of eastern and western lineages in response to simulated future climates indicate the possibility that different regions of a species distribution may respond differently to environmental changes. If differences in physiological response at the landscape scale are widespread, this complicates our ability to forecast species-level responses to environmental change, however, it might also improve our ability to understand regional patterns of die-back and mortality such as those reported by Allen et al. (2010).

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## References

Allen CD, Macalady AK, Chenchouni H, et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Man 259: 660–684.

Ahmed CB, Rouina BB, Sensoy S, Boukhris M, Abdallah FB (2009) Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. Env Exp Bot 67: 345–352.

Ameztegui A, Coll L (2011) Tree dynamics and co-existence in the montane-sub-alpine ecotone: The role of different light-induced strategies. J Veg Sci 22: 1049-1061.

Aussenac G (2002) Ecology and ecophysiology of circum-Mediterranean firs in the context of climate change. Ann For Sci 59: 823-832.

Buysse J, Merckx R (1993) An improved colorimetric method to quantify sugar content of plant tissue. J Exp Bot 44: 1627–1629.

Cailleret M, Nourtier M, Amm A, Durand-Gillmann M, Davi H (2014) Drought-induced decline and mortality of silver fir differ among three sites in southern France. Ann For Sci 71: 643-657.

Camarero JJ, Bigler C, Linares JC, Gil-Pelegrín E (2011) Synergistic effects of past historical logging and drought on the decline of Pyrenean silver fir forests. For Ecol Man 262: 759-769.

Camarero JJ, Gazol A, Sangüesa-Barreda G, Oliva J, Vicente-Serrano SM (2015) To die or not to die: Early warnings of tree dieback in response to a severe drought. J Ecol 103: 44-57.

Carrer M, Nola P, Motta R, Urbinati C (2010) Contrasting tree-ring growth to climate responses of *Abies alba* toward the southern limit of its distribution area. Oikos 119: 1515-1525.

Case TJ, Taper ML (2000) Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. Am Nat 155: 583–605.

Cremer E, Liepelt S, Sebastiani F, Buonamici A, Michalczyk IM, Ziegenhagen B, Vendramin GG (2006) Identification and characterization of nuclear microsatellite loci in *Abies alba* Mill. Mol Ecol Notes 6: 374-376.

de Andrés EG, Camarero JJ, Martínez I, Coll L (2014) Uncoupled spatiotemporal patterns of seed dispersal and regeneration in Pyrenean silver fir populations. For Ecol Man 319: 18-28.

Earl DA, VonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Cons Genet Res 4: 359–361.

Ehleringer JR (1993) Carbon and water relations in desert plants: an isotopic perspective. *Stable Isotopes and Plant Carbon-Water Relations* (eds. J.R. Ehleringer, J.R. Hall & G.D Farquhar), pp. 155–172. Academic Press, San Diego.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611–2620.

Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Glob Planet Chang 63: 90–104.

Goudet J (2002) FSTAT version 2.9.3.2 (Feb. 2002). Institute of Ecology. Biology Building, UNIL. CH-1015, Lausanne. Switzerland.

Guo J, Yang Y, Wang G, Yang L, Sun X (2010) Ecophysiological responses of *Abies fabri* seedlings to drought stress and nitrogen supply. Physiol Plant 139: 335-347.

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters. Ecol Lett 8: 461-467.

Hansen OK, Vendramin GG, Sebastiani F, Edwards KJ (2005) Development of microsatellite markers in *Abies nordmanniana* (Stev.) Spach and cross-species amplification in the *Abies* genus. Mol Ecol Notes 5: 784-787.

Houle G (1994) Spatiotemporal patterns in the components of regeneration of four sympatric tree species - *Acer rubrum*, *Acer saccharum*, *Betula alleghaniensis* and *Fagus grandifolia*. J Ecol 82: 39-53.

IPCC (2007) Climate Change 2007: *The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC*. Cambridge University Press, Cambridge.

Jalas J, Suominen J, Lampinen R (1999) Atlas Florae Europaeae: Distribution of Vascular Plants in Europe. Vol. 8 *Nymphaeaceae* to *Ranunculaceae*. Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanario.

Jump AS, Mátyás C, Peñuelas J (2009) The altitude-for-latitude disparity in the range retractions of woody species. Trends Ecol Evol 24: 694-701.

Liepelt S, Cheddadi R, de Beaulieu JL, Fady B, Gömöry D, Hussendörfer E, Konnert M, Litt T, Longauer R, Terhürne-Berson R, Ziegenhagen B. (2009) Postglacial expansion and its genetic imprints in *Abies alba* (Mill.) - A synthesis from palaeobotanic and genetic data. Rev Palaeobot Palynol 153: 139-149.

Linares JC, Camarero JJ (2012a) Growth patterns and sensitivity to climate predict silver fir decline in the Spanish Pyrenees. Eur J For Res 131: 1001-1012.

Linares JC, Camarero JJ (2012b) From pattern to process: linking intrinsic water-use efficiency to drought-induced forest decline. Glob Chang Biol 18: 1000-1015.

Lloret F, Peñuelas J, Estiarte M. (2004) Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. Glob Chang Biol 10: 248-258.

Lloret F, Peñuelas J, Prieto P, Llorens L, Estiarte M (2009) Plant community changes induced by experimental climate change: Seedling and adult species composition. Persp Plant Ecol Evol Syst 11: 53-63.

Lopushinsky W, Max TA (1990) Effect of soil temperature on root and shoot growth and on budburst timing in conifer seedling transplants. New Forests 4: 107-124.

Maguire AJ, Kobe RK (2015) Drought and shade deplete nonstructural carbohydrate reserves in seedlings of five temperate tree species. Ecology and Evolution 5: 5711-5721.

Markesteijn L, Poorter L (2009) Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. J Ecol 97: 311–325.

Martín S, Díaz-Fernández P, De Miguel J (1998) Regiones de Procedencia de Especies Forestales Españolas. Generos *Abies, Fagus, Pinus,* y *Quercus*. O.A. Parques Nacionales, Madrid.

Matías L, González-Díaz P, Jump AS (2014) 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. Env Exp Bot 105: 32-38.

Matías L, Jump AS (2014) Impacts of predicted climate change on recruitment at the geographical limits of Scots pine. J Exp Bot 65: 299-310.

Matías L, Jump AS (2015) Asymmetric changes of growth and reproductive investment herald altitudinal and latitudinal range shifts of two woody species. Glob Chang Biol 21: 882-896.

Matías L, Zamora R, Castro J (2011a) Repercussions of simulated climate change on the diversity of woody-recruit bank in a Mediterranean-type ecosystem. Ecosystems 14: 672-682.

Matías L, Castro J, Zamora R (2011b) Soil nutrient availability under a global change scenario in a Mediterranean mountain ecosystem. Glob Chang Biol 17: 1646-1657.

McDowell NG, Licata J, Bond BJ (2005) Environmental sensitivity of gas exchange in different-sizes trees. Oecologia 145: 9-20.

Mediavilla S, Escudero A (2004) Stomatal responses to drought of mature trees and seedlings of two co-occurring Mediterranean oaks. For Ecol Man 187: 281-294.

Morin X, Thuiller W (2009) Comparing niche- and process-based models to reduce prediction uncertainty in species range shifts under climate change. Ecology 90: 1301–1313.

Oleksyn J, Modrzynski M, Tjoelker MG, Zytkowiak R, Reich PB, Karolewski P (1998) Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for altitudinal ecotypes and cold adaptation. Functional Ecology 12: 573-590.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Ann Rev Ecol Evol Syst 37: 637-669

Peñuelas J, Boada M (2003) A global change-induced biome shift in the Montseny mountains (NE Spain). Glob Chang Biol 9: 131–140.

Petit RJ, Aguinagalde I, de Beaulieu JL (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. Science 300: 1563-1565.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945–59.

Powers MD, Pregitzer KS, Palik BJ (2008) Delta C-13 and delta O-18 trends across overstory environments in whole foliage and cellulose of three *Pinus* species. J Am Soc Mass Spec 19: 1330-1335.

Quero JL, Gómez-Aparicio L, Zamora R, Maestre FT (2008) Shifts in the regeneration niche of an endangered tree (*Acer opalus* ssp. *granatense*) during ontogeny: Using an ecological concept for application. Basic Appl Ecol 9: 635-644.

Quero JL, Sterck FJ, Martínez-Vilalta J, Villar R (2011) Water-use strategies of six co-existing mediterranean woody species during a summer drought. Oecologia 166: 45-57.

Rickebusch S, Lischke H, Bugmann H, Guisan A, Zimmermann NE (2007) Understanding the low-temperature limitations to forest growth through calibration of a forest dynamics model with tree-ring data. For Ecol Man 246: 251-263.

Sancho-Knapik D, Peguero-Pina JJ, Cremer E, Camarero JJ, Fernández-Cancio A, Ibarra N, Konnert M, Gil-Pelegrín E (2014) Genetic and environmental characterization of *Abies alba* Mill. populations at its western rear edge. Pirineos 169 doi: http://dx.doi.org/10.3989/Pirineos.2014.169007.

Schultz HR (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. Plant Cell Env 26: 1393-1405.

Serra-Diaz JM, Ninyerola M, Lloret F (2012) Coexistence of *Abies alba* (Mill.) – *Fagus sylvatica* (L.) and climate change impact in the Iberian Peninsula: a climatic-niche perspective approach. Flora 207: 10–18.

Smith T, Huston M (1989) A theory of the spatial and temporal dynamics of plant communities. Vegetatio 83: 49-69.

Sperry JS, Hacke UG, Oren R, Comstock JP (2002) Water deficits and hydraulic limits to leaf water supply. Plant Cell Env 25: 251-263.

Taberlet P, Fumagalli L, Wust-Saucy AG, Cosson JF (1998). Comparative phylogeography and postglacial colonization routes in Europe. Mol Ecol 7: 453-464.

Van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fulé PZ, Harmon M E, Larson AJ, Smith JM, Taylor AH, Veblen TT. (2009) Widespread increase of tree mortality rates in the western United States. Science 323: 521-524.

Vigo J, Ninot JM (1987) Los Pirineos. *La Vegetación de España* (eds. M. Peinado Lorca, M., S. Rivas-Martínez), pp. 351-384. Publicaciones Univ. Alcalá de Henares, Alcalá de Henares, Spain.

Walther G-R (2010) Community and ecosystem responses to recent climate change. Philos Trans R Soc B 365: 2019-2024.

## Figure legends

**Figure 1**: Distribution map of silver fir (inner map, source Euforgen; http://www.euforgen.org/) and selected populations across the western (shaded tags) and eastern (open tags) Pyrenean provenances. See sites codes in Supplementary Table A1.

**Figure 2**: a) Total biomass at the end of the experiment of silver fir seedlings from the eastern (open bars) and western (grey bars) Pyrenean provenances across the temperature (current, CT; future, FT) and watering (high, low) treatments. * Significant at 0.01–0.05 level; ** Significant at 0.001–0.01 level; *** Significant at <0.001 level. b) Plasticity to change in biomass allocation in response to watering. Circles represents mean root:shoot biomass ratios by temperature (open: current temperature; black: future temperature) and provenance under high and low precipitation treatments. Points above the dashed 1:1 line indicate higher biomass allocation to roots in response to decreased watering. E and W by each point represent east and west provenances respectively. Error bars represent ±SE.

**Figure 3**: Water potential at harvesting time (a) and C isotopic discrimination ($\delta^{13}$C, b) of silver fir seedlings from the eastern (open bars) and western (grey bars) Pyrenean provenances across the temperature (current, CT; future, FT) and watering (high, low) treatments. Error bars represents ±SE. * Significant at 0.01–0.05 level; ** Significant at 0.001–0.01 level; *** Significant at <0.001 level.

**Figure 4**: C:N ratio (a) and soluble sugar concentration (b) of silver fir seedlings from the eastern (open bars) and western (grey bars) Pyrenean provenances across the temperature (current, CT; future, FT) and watering (high, low) treatments. Error bars represents ±SE. * Significant at 0.01–0.05 level; ** Significant at 0.001–0.01 level; *** Significant at <0.001 level.