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## Research paper

# Role of geographical provenance in the response of silver fir seedlings to experimental warming and drought

Luis Matías<sup>1,6,7</sup>, Patricia Gonzalez-Díaz<sup>1</sup>, José L. Quero<sup>2</sup>, J. Julio Camarero<sup>3</sup>, Francisco Lloret<sup>4,5</sup> and Alistair S. Jump<sup>1,4</sup>

<sup>1</sup>Biological and Environmental Sciences, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK; <sup>2</sup>Departamento de Ingeniería Forestal, Escuela Técnica Superior de Ingeniería Agronómica y de Montes, Universidad de Córdoba, Campus de Rabanales, Crta N-IV km 396, Córdoba 14071, Spain; <sup>3</sup>Instituto Pirenaico de Ecología (IPE-CSIC), Avda Montañana 1005, 50059 Zaragoza, Spain; <sup>4</sup>CREAF, Cerdanyola del Vallès, 08193 Barcelona, Spain; <sup>5</sup>Unitat d'Ecologia, Department of Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma Barcelona, Cerdanyola del Vallès, 08193 Barcelona, Spain; <sup>6</sup>Present address: Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), PO Box 1052, 41080 Sevilla, Spain; <sup>7</sup>Corresponding author (lmatias@irnas.csic.es)

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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* Mill.) reaches its south-western distribution limit in the Spanish Pyrenees, where recent climatic dieback events have disproportionately affected westernmost populations. We hypothesized 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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$ , nitrogen and content of soluble sugars. Our results demonstrate that, in *A. alba*, differences in the physiological response of this species to drought are also associated with differences in biogeographical history.

**Keywords:** climate warming, growth, Pyrenees, range-edge, recruitment, regeneration.

## 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 generalized 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 in order 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, Rieckebusch et al. 2007). Early-life stages are more susceptible to environmental alterations than adults because of their limited root system and aboveground 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 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 a

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 twenty-first century (Serra-Diaz 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 palaeo-ecological evidence that indicates a westward postglacial 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 palaeobotanic terms than eastern populations (Taberlet et al. 1998, Liepelt et al. 2009). Therefore, silver fir represents a highly valuable study species 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 hypothesized 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. (i) What are the consequences of the projected temperature rise and precipitation reduction on seedling growth and survival of seedlings from Pyrenean silver fir populations? (ii) Which physiological mechanisms affect the response of silver fir seedlings to climate? (iii) 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 13 natural populations located in northeastern Spain and southern France (Figure 1; see Table S1 available as Supplementary Data at *Tree Physiology* Online). In the Pyrenees, silver fir dominates subalpine

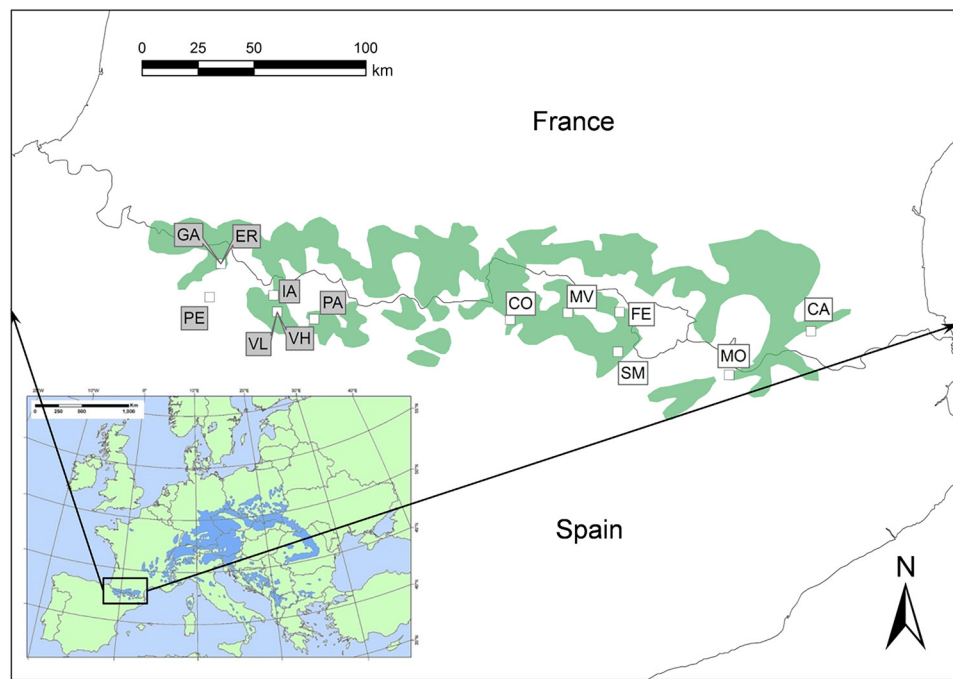


Figure 1. Distribution map of silver fir (inner map, source Euforgen; <http://www.euforgen.org/>) and selected populations across the western (shaded tags) and eastern (open tags) Pyrenean provenances. See sites codes in Table S1 available as Supplementary Data at *Tree Physiology* Online.

and montane forests, mainly in sites on mesic and humid slopes with deep soils (Vigo and Ninot 1987). We differentiated eastern and western provenances according to 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 °C 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 concentration in the substrate mixture was nitrogen 0.7%, phosphorus 0.4% and potassium 0.3% (expressed as a per cent mass of the bulk soil). In total, 120 pots (60 per provenance) were placed inside each of four Snijders Scientific MC1750E (Tilburg, The 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 firs, taken from the respective locations where seeds were collected, in a proportion of 0.1 kg soil l<sup>-1</sup>. Three seeds were sowed per pot, randomly thinning to one seedling in case of multiple emergences.

Table 1. Weekly temperatures (day/night, in °C) during the experiment development in the different scenarios: CT and FT. These values were obtained as monthly mean from three meteorological stations in the Eastern range (Espot, 42°34'33"N, 1°5'20"E, 1314 m above sea level (a.s.l.); Alp, 42°22'22"N, 1°53'10"E, 1166 m; La Molina, 42°19'57"N, 1°56'22"E, 1700 m) and three in the Western range (Linza, 42°54'N, 0°48'W, 1340 m a.s.l.; Ansó, 42°45'N, 0°50'W, 858 m; Canfranc, 42°43'N, 0°31'W, 1037 m). Optimal conditions for emergence were maintained up to the third experimental week.

Week	Equivalent	CT	FT
1–3	Optimal	20/20	20/20
4–7	May	20/8	24/12
8–11	June	24/10	28/14
12–15	July	27/12	31/16
16–19	August	27/12	31/16
20–23	September	23/9	27/13

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 it had two levels replicated in two chambers each: (i) 'current temperature' (CT hereafter), representing the mean monthly day and night temperatures during the growing period (Table 1); and (ii) 'future temperature' (FT hereafter), simulating the predicted increase

in temperature by 4 °C above 'current' values, as projected for the 2090–99 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: (i) 'high availability', based on the mean monthly precipitation records during the growing season (May–September) at the reference climatic database (100.5 l m<sup>-2</sup>); and (ii) 'low availability', simulating a reduction in precipitation by 30% from the previous level, as projected for NE Spain (70.3 l m<sup>-2</sup>). These monthly precipitation amounts were split into eight irrigation events (twice per week) from the 4th experimental week to the 23rd week. 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<sup>-2</sup> s<sup>-1</sup> during 16 h, rising progressively at dawn and decreasing at dusk for 1 h. This light intensity is representative of moderately open understorey where silver fir usually regenerates (Ameztegui and Coll 2011, de Andrés et al. 2014). Air relative humidity and CO<sub>2</sub> concentration were kept constant within cabinets at 65% and 950 mg m<sup>-3</sup> (490 p.p.m.) respectively. To avoid any possible chamber effect, all pots were rotated through the different chambers, spending at least 1 month in each one, while also randomizing pot position within chambers. Soil moisture (volumetric water content) 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 2 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 24), 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 ( $\Psi$ ) was measured around midday in the stem of each seedling using a pressure bomb (range: 0–15 MPa; Manófrígido, Lisbon, Portugal), and then seedlings were oven-dried at 45 °C for 72 h and weighed.

### Genetic analyses

To assess whether eastern and western sampled provenances 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 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 (simple sequence repeat) 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 1× of Type-it Multiplex PCR Master Mix (Qiagen), 0.2 µM of each primer and 20 ng of template DNA. Polymerase chain reactions were carried out in Veriti™ Thermal cycler (Applied Biosystems, Darmstadt, Germany), following conditions reported by Hansen et al. (2005). Polymerase chain reaction 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 isotope, 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  $\delta^{13}\text{C}$ . It is assumed that  $\delta^{13}\text{C}$  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 carbon (C), nitrogen (N) and <sup>13</sup>C. Determinations were conducted at the Stable Isotope Facility of the University of California at Davis (SIF, Davis, CA, 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 ( $\delta^{13}\text{C}$ ) in per million (‰) as relative deviation from the international standard V-PDB. From the same individuals, soluble sugars (SSs) were extracted from leaves with 80% (v/v) ethanol and their concentration determined colorimetrically, using the phenol–sulphuric method (Buysse and Merckx 1993). Soluble sugars 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 generalized 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,  $\delta^{13}\text{C}$  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). A 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 minimized by this rotation protocol.

Analyses of molecular variance (AMOVAs) 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  $\pm$  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 ( $31.0 \pm 0.1$  vs  $23.8 \pm 0.1\%$ , respectively;  $F = 821.9$ ,  $P < 0.0001$ ), and under CT than under FT conditions ( $28.9 \pm 0.1$  vs  $25.9 \pm 0.1\%$ , 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 ( $F = 519.1$ ,  $P < 0.0001$ ; see Figure S1a available as Supplementary Data at *Tree Physiology* Online). However, no systematic differences in soil moisture appeared between pots containing the two provenances (east and west;  $F = 1.52$ ,  $P = 0.21$ ), and there were no interactions between provenance and any of the other factors (provenance  $\times$  temperature:  $F = 0.62$ ,  $P = 0.43$ ; provenance  $\times$  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). Analysis of molecular variance 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, where the most probable number of genetic clusters was two (see Figure S2a available as Supplementary Data at *Tree Physiology* Online). 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 by the changes either in temperature or in watering (Table 3). Seedlings from the western provenance grew more than seedlings from the eastern provenance (Figure 2a), also producing deeper roots ( $290.9 \pm 7.9$  vs  $258.1 \pm 9.2$  mm) and taller shoots ( $32.1 \pm 0.5$  vs  $29.3 \pm 0.6$  mm). Although temperature had no effect on total biomass, higher temperature diminished root length (CT:  $329.3 \pm 8.1$  mm, FT:  $236.6 \pm 7.5$  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 (Figure 2b). Overall, higher temperature reduced biomass allocation to roots (CT:  $1.67 \pm 0.05$ , FT:  $1.41 \pm 0.03$ ), while reduced watering induced higher root investment (high:  $1.40 \pm 0.04$ , low:  $1.65 \pm 0.05$ ). 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:  $0.91 \pm 0.02$ , low:  $0.78 \pm 0.03$ ).

### 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 (Figure 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 CT or with higher watering had more negative  $^{13}\text{C}$  values than those under increased temperature or in

Table 2. Genetic diversity parameters for east and west population. The parameters given for each site are as follows: number of genotyped plants ( $N$ ) with six nuclear microsatellite loci, mean number of alleles per locus ( $N_a$ ), number of private alleles ( $A_p$ ), mean observed heterozygosity ( $H_o$ ), mean expected heterozygosity ( $H_e$ ), mean gene diversity ( $H_s$ ) and mean allelic richness ( $R_s$ ). Mean values and standard deviation ( $\pm$ SE) values are indicated.

Provenance	$N$	$N_a$	$A_p$	$H_o$	$H_e$	$H_s$	$R_s$
East	48	$7.667 \pm 0.32$	17	$0.549 \pm 0.02$	$0.629 \pm 0.02$	$0.630 \pm 0.02$	$7.578 \pm 0.31$
West	45	$6.333 \pm 0.42$	9	$0.512 \pm 0.03$	$0.563 \pm 0.03$	$0.564 \pm 0.03$	$6.232 \pm 0.41$

Table 3. Summary of statistics ( $\chi^2$  or  $F$  and  $P$  values). Differences in survival were tested by a generalized linear model ( $\chi^2$  values) across the different experimental factors (temperature, T; provenance, Pr; watering, W), and all other variables were tested by using factorial ANOVA ( $F$  values) in a similar way. Significant results are in bold. df = 1 in all cases.

	$\chi^2/F$	$P$		$F$	$P$
Final biomass			N concentration		
T	0.02	0.89	T	<b>7.14</b>	<b>0.009</b>
W	0.12	0.73	W	<b>53.12</b>	<b>&lt;0.0001</b>
Pr	26.35	<b>&lt;0.0001</b>	Pr	0.50	0.48
T × W	0.02	0.88	T × W	0.21	0.65
T × Pr	3.24	0.07	T × Pr	<b>5.01</b>	<b>0.03</b>
W × Pr	0.01	0.91	W × Pr	1.33	0.25
T × W × Pr	0.16	0.69	T × W × Pr	0.48	0.83
Biomass allocation (R : S)			C : N ratio		
T	<b>9.00</b>	<b>0.003</b>	T	<b>14.30</b>	<b>0.0003</b>
W	<b>11.26</b>	<b>0.0009</b>	W	<b>54.87</b>	<b>&lt;0.0001</b>
Pr	0.11	0.74	Pr	0.04	0.84
T × W	0.73	0.39	T × W	0.90	0.34
T × Pr	1.01	0.32	T × Pr	<b>8.67</b>	<b>0.004</b>
W × Pr	0.01	0.91	W × Pr	0.87	0.35
T × W × Pr	0.01	0.95	T × W × Pr	1.45	0.23
Survival			$\delta^{13}\text{C}$		
T	0.01	0.96	T	<b>18.39</b>	<b>&lt;0.0001</b>
W	<b>9.57</b>	<b>0.002</b>	W	<b>13.78</b>	<b>0.0004</b>
Pr	0.05	0.83	Pr	0.62	0.43
T × W	0.31	0.58	T × W	<b>6.29</b>	<b>0.01</b>
T × Pr	0.19	0.66	T × Pr	<b>4.75</b>	<b>0.03</b>
W × Pr	0.02	0.90	W × Pr	0.07	0.79
T × W × Pr	0.76	0.38	T × W × Pr	<b>7.15</b>	<b>0.009</b>
Water potential			SSs		
T	<b>4.38</b>	<b>0.037</b>	T	<b>6.73</b>	<b>0.01</b>
W	<b>84.13</b>	<b>&lt;0.0001</b>	W	0.26	0.61
Pr	0.01	0.98	Pr	1.30	0.26
T × W	0.02	0.88	T × W	0.29	0.59
T × Pr	2.24	0.14	T × Pr	<b>6.04</b>	<b>0.02</b>
W × Pr	0.39	0.53	W × Pr	<b>4.28</b>	<b>0.04</b>
T × W × Pr	0.18	0.67	T × W × Pr	2.20	0.14

low-watering treatments (Figure 3b). However, significant temperature × watering, temperature × provenance and temperature × watering × provenance interactions appeared. Under CT conditions, seedlings from the eastern provenance increased  $\delta^{13}\text{C}$  concentration when watering was reduced, while under simulated future higher temperatures, western populations exhibited the higher  $\delta^{13}\text{C}$  concentrations under reduced watering.

Nitrogen concentration in seedlings was also affected by the two climatic variables (Table 3). Warmer temperature reduced N concentration (CT:  $11.5 \pm 0.6 \text{ mg g}^{-1}$ , FT:  $9.6 \pm 0.7 \text{ mg g}^{-1}$ ), whereas seedlings growing under the low-watering treatment presented higher N concentration ( $13.2 \pm 0.6 \text{ mg g}^{-1}$ ) than those under the high watering treatment ( $8.0 \text{ mg g}^{-1} \pm 0.5$ ). In a similar way, the C : N ratio increased with higher temperature, but diminished when watering was reduced (Figure 4a). The effect of provenance was not significant, but the temperature × provenance interaction was significant for both N concentration

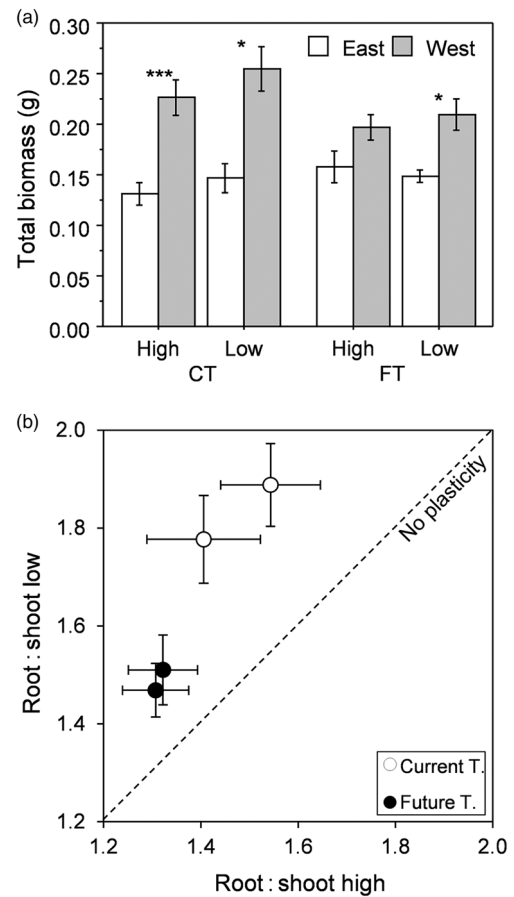


Figure 2. (a) Total biomass at the end of the experiment of silver fir seedlings from the eastern (open bars) and western (grey bars) Pyrenean provenances across the temperature (current, CT; future, FT) and watering (high, low) treatments. \*Significant at 0.01–0.05 level; \*\*\*significant at <0.001 level. (b) Plasticity to change in biomass allocation in response to watering. Circles represent mean root : shoot biomass ratios by temperature (open: CT; black: FT) 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. Error bars represent  $\pm$ SE.

and C : N ratio due to higher N concentration in seedlings from western provenances under higher temperatures (Figure 4a). Lastly, SS concentration was only affected by temperature, with higher values under the simulated FT (CT:  $5.0 \pm 0.2\%$ , FT:  $5.7 \pm 0.2\%$ ). Although watering had no effect on SS concentration, there were significant watering × temperature and watering × provenance interactions on the SS content (Table 3; Figure 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

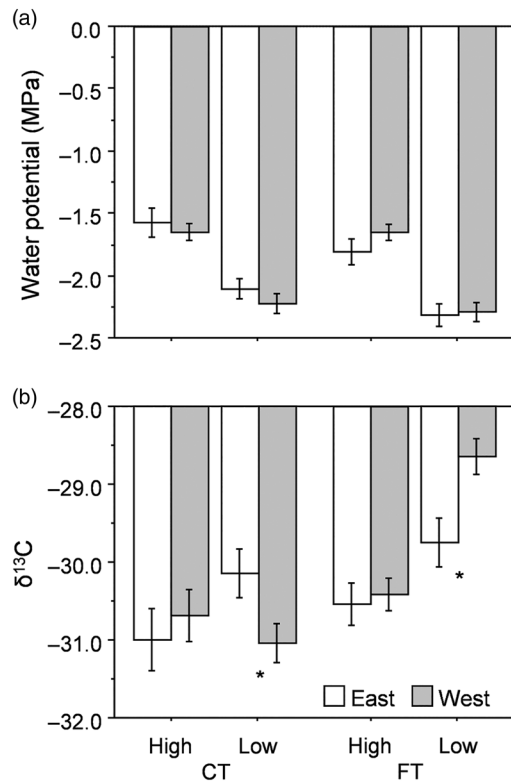


Figure 3. Water potential at harvesting time (a) and C isotopic discrimination ( $\delta^{13}\text{C}$ , b) of silver fir seedlings from the eastern (open bars) and western (grey bars) Pyrenean provenances across the temperature (current, CT; future, FT) and watering (high, low) treatments. Error bars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

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  $\delta^{13}\text{C}$  and SS values, higher C : N ratio and lower N content. Drought increase provoked more negative water potentials, higher  $\delta^{13}\text{C}$  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 broadly separated the individuals sampled into two biogeographical lineages, east and west. Although some admixture occurred within each cluster, it is notable that

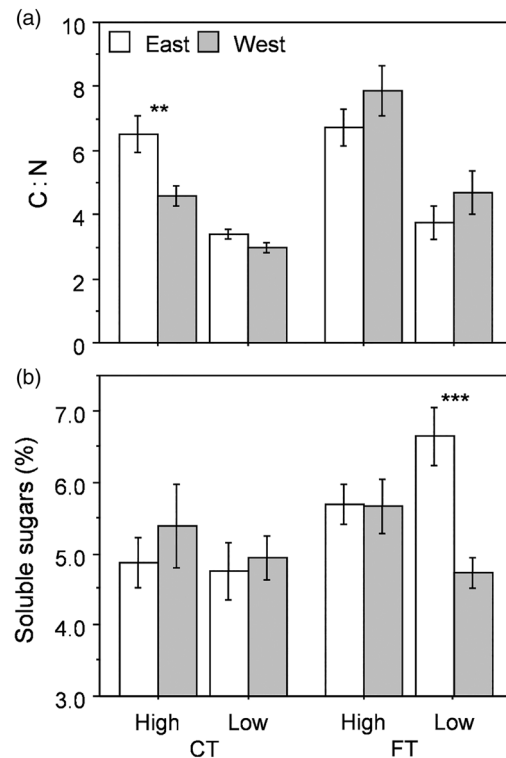


Figure 4. C : N ratio (a) and SS concentration (b) of silver fir seedlings from the eastern (open bars) and western (grey bars) Pyrenean provenances across the temperature (current, CT; future, FT) and watering (high, low) treatments. Error bars represent  $\pm$ SE. \*\*Significant at 0.001–0.01 level;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

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 SSs.

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 when warming and water stress are combined. 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 offset the positive effect of higher root allocation in response to reduced precipitation, diminishing the net plastic responses 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 (~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 emphasized 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  $\delta^{13}\text{C}$  in silver fir seedlings. Both warmer and drier conditions augmented  $\delta^{13}\text{C}$  concentration compared with 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 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  $\delta^{13}\text{C}$  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 analysed 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 also take into account that nutrient availability in the experimental pots was higher than in natural populations



(see Table S1 available as Supplementary Data at [Tree Physiology Online](#)), and the reported effects of climate on nitrogen concentration might, therefore, be even higher in nature. Previous studies found an increase in the SS content in response to drought in other fir species (Guo et al. 2010), associated with a decrease in other macromolecules such as starch, since SSs are efficient osmolytes (Ahmed et al. 2009). However, we did not find a clear climatic effect on the SS 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 understorey 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  $\delta^{13}\text{C}$  under simulated warming was particularly high for western populations under drought conditions, while they exhibited diminished iWUE under CTs but with drought conditions. This result contrasts with higher  $\delta^{13}\text{C}$  values in seedlings from eastern populations under CTs 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 (FT and low watering), we also found geographical differences in the SS concentrations, with seedlings from the eastern range showing much higher values than western ones. This result concurs with the observed lower  $\delta^{13}\text{C}$  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 (see Table S1 available as Supplementary Data at [Tree Physiology Online](#)), 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 colonization history of the species. In the field, these differences could be even higher since the source populations differ in altitude and thus might have different thermal optima, while we subjected them to similar conditions (either current or future) in this experiment. Such a finding adds improved resolution to understanding the scale of intraspecific variation in plant performance while also challenging our ability to generalize 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. 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 dieback and mortality.

### Supplementary data

Supplementary data for this article are available at [Tree Physiology Online](#).

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### Conflict of interest

None declared.

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