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

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

8

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25

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

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

30 **Abstract**

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

52

53 **Introduction**

54 The increase in temperature recorded during the last decades has the capacity to alter the
55 phenology, growth and biotic interactions of plant species worldwide (Parmesan 2006;
56 Walther 2010). These impacts are expected to be especially evident over relatively small
57 spatial scales in mountain ecosystems, where the elevational ranges and shifts of trees are to a
58 great extent controlled by temperature (Peñuelas and Boada 2003) which declines rapidly
59 with increasing elevation. In addition, temperature changes will be accompanied by an
60 alteration of current precipitation patterns, for instance with a generalised increase of the
61 length and intensity of summer drought in central and southern European regions (Giorgi and
62 Lionello 2008). These climatic alterations are likely to have important consequences for tree
63 species dynamics at local and regional scales (Peñuelas and Boada 2003; Van Mantgem et al.
64 2009; Matías and Jump 2015).

65

66 Changing climatic conditions are especially relevant close to the equatorial limit of
67 species distributions (the rear, or trailing edge), where climatic conditions often correspond to
68 the species drought-tolerance limits (Hampe and Petit 2005). In these locations, even small
69 variations in climate could result in profound demographic effects, such as lower relative
70 fecundity and reduction of local population densities (Case and Taper 2000). Such changes
71 could result in reduced resilience under adverse climate conditions and eventual alteration of
72 species distributions. In the warmest regions of species distributions, the intensity of
73 population responses to drought stress are likely higher than in colder areas, being more prone
74 to local extinction as a consequence of extreme climatic events (Jump et al. 2009; Carrer et al.
75 2010). Alternatively, although the historical isolation and fragmentation of these range edge
76 populations has often resulted in impoverished genetic diversity within populations (Hampe
77 and Petit 2005), their long exposure to the xeric conditions may have resulted in local

78 adaptations to drought stress, and those stands might represent genetic reservoirs holding
79 drought-resistant genotypes (Hampe and Petit 2005; Matías and Jump 2014). Thus, the study
80 of the response of rear-edge populations to the expected temperature and drought increases is
81 important to understand and to predict the possible negative consequences of climate change
82 on natural populations.

83

84 Traditionally, regeneration has been considered as an important feature driving
85 population dynamics in temperate tree species (Houle 1994; Rickebusch et al. 2007). Early-
86 life stages are more susceptible to environmental alterations than adults because of their
87 limited root system and above-ground height, constraining their competitive abilities (Lloret
88 et al. 2004, 2009). Therefore, seedlings often respond faster to climatic variations than adult
89 trees (Lloret et al. 2009). Thus, recruitment is expected to be a key bottleneck determining
90 community dynamics in forests during the coming decades (Lloret et al. 2004; Matías et al.
91 2011a). Consequently, information on regeneration and component processes such as
92 seedling growth and physiology under future conditions is urgently needed for modelling and
93 predicting forest dynamics under climate change scenarios (Morin and Thuiller 2009).

94

95 Silver fir (*Abies alba* Mill.) is a widely distributed conifer in mountain forests of
96 central, eastern and southern Europe (Jalas et al. 1999), where it becomes dominant in pure or
97 mixed forests with *Fagus sylvatica*. This species reaches its main south-western distribution
98 limit in the Spanish Pyrenees. Here silver fir has experienced dieback and growth decline that
99 disproportionately affects western populations in response to warming-related aridification
100 trends starting in the 1980s (Camarero et al. 2011; Linares and Camarero 2012a). In some of
101 these western populations ongoing dieback events are characterized by high defoliation levels
102 (over 50%) and standing mortality rates up to 30-40% of adult trees as a consequence of

103 episodic drought events (Camarero et al. 2015), similar to those observed in southern France
104 (Cailleret et al. 2014). Furthermore, silver fir regeneration is expected to be an important
105 bottleneck under predicted warmer and drier climate (de Andrés et al. 2014). Bioclimatic
106 models indicate important reductions of the Pyrenean silver-fir area due to intensified
107 warming at the end of the 21st century (Serra-Díaz et al. 2012).

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

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

122

123

124 Based on the previous evidence, we hypothesised that silver fir populations from the
125 eastern Pyrenees would be less vulnerable to the expected warming and drought increase than
126 the western populations. To test this hypothesis, we used controlled environment experiments
127 incorporating elevated temperature and drought. We analysed physiology, growth and

128 survival of silver fir seedlings collected in populations from eastern and western locations of
129 the Pyrenean range. The use of controlled environment growth chambers allowed precise
130 manipulation of soil moisture and temperature, while keeping all other climatic factors at
131 constant values. Specifically, we sought to answer the following questions: 1) what are the
132 consequences of the projected temperature rise and precipitation reduction on seedling growth
133 and survival of seedlings from Pyrenean silver fir populations? 2) which physiological
134 mechanisms affect the response of silver fir seedlings to climate? 3) is there any difference in
135 the response of seedlings from eastern and western Pyrenean lineages of silver fir to the
136 projected future climatic conditions?

137

138 **Materials and Methods**

139 *Field sampling*

140 The present study was conducted from January to June 2013 at the Controlled Environment
141 Facility of the University of Stirling (Stirling, UK). We used silver fir seed sources from
142 thirteen natural populations located in northeastern Spain and southern France (Fig. 1;
143 Supplementary material Appendix 1 Table A1). In the Pyrenees, silver fir dominates
144 subalpine and montane forests, mainly in sites on mesic and humid slopes with deep soils
145 (Vigo and Ninot 1987). We differentiated eastern and western provenances (Martín et al.
146 1998). Seeds were collected from six different populations at the eastern provenance and
147 from seven at the western provenance, selecting at least 15 mature trees per population and
148 collecting newly dispersed seeds from the soil surface, avoiding damaged or rotten ones.
149 Plant material from each provenance was pooled, and then stored at 4 °C until used for the
150 experiment.

151

152 *Experimental conditions*

153 Plants were grown in tubular pots with a diameter of 8 cm and a height of 45 cm (2.3 L
154 volume) to enable unrestricted vertical root growth. The pots were filled with a peat and river
155 sand mixture (proportion 2:1 in volume, respectively) above a layer of gravel to improve
156 drainage. Nutrient content available in the substrate mixture was nitrogen 0.7 %, phosphorous
157 0.4 % and potassium 0.3 %. In total 120 pots (60 per provenance) were placed inside each of
158 four Snijders Scientific MC1750E (Tilburg, Netherlands) controlled environment chambers
159 (inner space 1.8 m length × 0.75 m wide × 1.2 m high), giving a total of 480 pots. Before
160 sowing, all pots were irrigated with 0.2 L of a soil microbial inoculum to allow
161 mycorrhization and realistic microbial communities (Matías et al. 2014). This inoculum was
162 obtained from the maceration of soil sampled beneath adult silver fir, taken from the
163 respective locations where seeds were collected, in a proportion of 0.1 kg soil L⁻¹. Three
164 seeds were sowed per pot, randomly thinning to one seedling in case of multiple emergences.

165
166 Optimal conditions for emergence and early growth (16 h light / 8 h dark daily cycles
167 at 22 °C) were maintained for 3 weeks. Subsequently, we applied two experimental
168 treatments (temperature and water availability) crossed with the provenance factor in a full-
169 factorial design. As climate reference, we used the monthly mean temperature and
170 precipitation values obtained from six meteorological stations across the source area during
171 the 1990-2010 period (Table 1). The temperature treatment was applied as a whole plot
172 factor, and had two levels replicated in two chambers each: 1) *current temperature* (CT
173 hereafter), representing the mean monthly day and night temperatures during the growing
174 period (Table 1); 2) *future temperature* (FT hereafter), simulating the predicted increase in
175 temperature by 4 °C above *current* values, as projected for the 2090-2099 period in NE Spain
176 at the time of the experimental setup (A2 scenario; IPCC 2007). The water availability

177 treatment was applied as a subplot factor (within cabinets) and was also represented by two
178 levels, representing current and future scenarios: 1) *high availability*, based on the mean
179 monthly precipitation records during the growing season (May-September) at the reference
180 climatic database (100.5 L m⁻²); 2) *low availability*, simulating a reduction in precipitation by
181 30% from the previous level, as projected for NE Spain (70.3 L m⁻²). These monthly
182 precipitation amounts were split into 8 irrigation events (twice per week) from the 4th
183 experimental week to the 23rd. Consequently, the experimental design included three factors
184 (temperature, watering and provenance) with two levels each, fully crossed with 60 replicated
185 plants per combination ($2 \times 2 \times 2 \times 60 = 480$ plants).

186

187 During the whole experiment, light intensity was fixed with a photosynthetic photon
188 flux density of 210 $\mu\text{mol m}^2 \text{s}^{-1}$ during 16 hours, rising progressively at dawn and decreasing
189 at dusk for one hour. This light intensity is representative for moderately open understory
190 where silver fir usually regenerates (Ameztegui and Coll 2011; de Andrés et al. 2014). Air
191 relative humidity and CO₂ concentration were kept constant within cabinets at 65% and 950
192 mg m⁻³ (490 ppm) respectively. To avoid any possible chamber effect, all pots were rotated
193 through the different chambers, spending at least one month in each one, whilst also
194 randomising pot position within chambers. Soil moisture (volumetric water content, % VWC)
195 was measured weekly during the experiment in all pots over the surface 5 cm by the time-
196 domain reflectometry method (SM300; Delta-T devices, Cambridge, UK). These values were
197 recorded two days after irrigation. Microclimate conditions within the chambers were logged
198 continuously and checked with periodic independent instrumental measurements to ensure
199 comparability of treatments.

200

201 *Seedling measurements*

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

208

209 *Genetic analyses*

210 To assess if eastern and western sampled populations corresponded to different lineages,
211 DNA was extracted from dried needles of 50 randomly selected seedlings per provenance
212 (populations pooled within provenance). Genomic DNA from each sample was extracted
213 using the DNeasy 96 Plant Kit (Qiagen, Manchester, UK) following the manufacturer's
214 protocol and then stored at -20 °C until use. All samples were genotyped using six nuclear
215 microsatellites markers (SSR) specific for silver fir: SF1, SFb4, SF78 (Cremer et al. 2006)
216 and NFF7, NFH3, NFF3 (Hansen et al. 2005). Reactions were carried out in a final volume of
217 10 μ L with 1X of Type-it Multiplex PCR Master Mix (Qiagen, Manchester, UK), 0.2 μ M of
218 each primer and 20 ng of template DNA. Polymerase chain reactions (PCR) were carried out
219 in a Veriti™ Thermal cycler (Applied Biosystems), following conditions reported by Hansen
220 *et al.* (2005). PCR products were analysed by DNA Sequencing and Services, Dundee, UK,
221 using a 3730 DNA Sequencer (Applied Biosystems) with reference to a LIZ 500 size
222 standard.

223

224 *Carbon isotopes, nitrogen and carbohydrate analyses*

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

227 is directly related to the intrinsic water use efficiency (iWUE), and represents a reliable
228 indicator of drought stress (Ehleringer 1993). Dried leaves were ground to powder and
229 analysed for total C, N and ^{13}C . Determinations were conducted at the Stable Isotope Facility
230 of the University of California at Davis (SIF, Davis, USA) using a PDZ Europa ANCA-GSL
231 elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon
232 Ltd., Cheshire, UK). Analyses were performed on whole-leaf tissue rather than extracted
233 cellulose since both are strongly correlated (Powers et al. 2008). The isotopic ratio was
234 expressed in delta notation ($\delta^{13}\text{C}$) in per mil (‰) as relative deviation from the international
235 standard V-PDB. From the same individuals, soluble sugars (SS) were extracted from leaves
236 with 80 % (v/v) ethanol and their concentration determined colorimetrically, using the
237 phenol–sulphuric method (Buysse and Merckx 1993). SS play a substantial role in higher
238 plant development, and its concentration is expected to increase in drought-tolerant plants
239 (Maguire and Kobe 2015).

240

241 *Data analysis*

242 Variation in soil moisture among the different treatments was tested using repeated-measures
243 analysis of variance (ANOVA), using temperature, watering and provenance as between
244 subject factors and time as a within subject factor. Seedling survival was analysed using a
245 Generalised Linear Model, with a binomial error distribution (1 for surviving and 0 for dead
246 individuals) and logit link function, including the three experimental factors (temperature,
247 watering and provenance) and their full-factorial interactions as independent variables.
248 Differences in total biomass, root length, root:shoot biomass ratio, water potential, total N
249 concentration, C:N ratio, $\delta^{13}\text{C}$ and SS were tested across the different factors and interactions
250 but using ANOVAs with the same model structure (provenance, temperature, watering and
251 their interactions as independent variables). As a consequence of the rotation scheme of pots

252 within and between chambers, chamber effect could not be specifically included in the
253 models. Consequently, any possible confounding effect of the experimental chambers can not
254 be detected. **NOTE: Do you think this is the best place to state this?** Yes, but I would
255 rephrase if this doesn't cause problems with the requested revisions:
256 Possible confounding effect of the experimental chambers could not be detected via specific
257 inclusion of chamber as a factor in the models since pots were rotated within and between
258 chambers during the experiment. However, chamber effects should be minimised, via this
259 rotation protocol.

260

261 Analyses of molecular variance (AMOVA) were used to perform hierarchical
262 analyses of genetic variation within and among provenances based on all polymorphic sites.
263 Allele frequencies and within-provenance genetic diversity parameters (mean number of
264 alleles per locus, N_a ; mean observed and expected heterozygosity, H_O and H_E , respectively;
265 mean genetic diversity, H_S ; mean allelic richness, R_S) were estimated using FSTAT (Goudet
266 2002). Bayesian clustering was used to infer population structure using STRUCTURE v2.3.4
267 (Pritchard et al. 2000). We used an admixture model with correlated allele frequencies. Ten
268 independent runs were performed per each number of clusters K from 1 to 5, with a burn-in
269 period of 50,000 and 100,000 Markov chain Monte Carlo iterations. The optimum number of
270 clusters, K, was determined using Structure Harvester (web version) (Earl and VonHoldt
271 2012) and Evanno method (Evanno et al. 2005). All values are given as mean \pm SE through
272 the text.

273

274 **Results**

275 *Soil moisture*

276 Watering levels imposed during the experiment resulted in different soil moisture across
277 treatments. Soil moisture was affected by two of the three main factors during the experiment,
278 being greater in the high- than in the low water availability treatment ($31.0\% \pm 0.1$ vs. 23.8
279 $\% \pm 0.1$ respectively; $F = 821.9$, $P < 0.0001$), and under current than under future temperature
280 conditions ($28.9\% \pm 0.1$ vs. $25.9\% \pm 0.1$ respectively; $F = 147.2$, $P < 0.0001$). There was
281 also a significant interaction between these two factors ($F = 12.9$, $P = 0.0004$) and a change in
282 soil moisture over time ($F = 519.1$, $P < 0.0001$; Supplementary material Appendix 1 Fig. A1).
283 However, no systematic differences in soil moisture appeared between pots containing the
284 two provenances (east, west; $F = 1.52$, $P = 0.21$), and there were no interactions between
285 provenance and any of the other factors (provenance \times temperature: $F = 0.62$, $P = 0.43$;
286 provenance \times precipitation: $F = 1.16$, $P = 0.28$).

287

288 *Genetic diversity and structure*

289 Based on microsatellite information, seedlings from the eastern provenance showed a higher
290 number of private alleles than western seedlings. They also showed slightly higher values of
291 allelic richness, genetic diversity, and heterozygosity (Table 2). AMOVA results showed
292 significant genetic differentiation among provenances (8.1%, $P < 0.0001$), with the remaining
293 91.9% of genetic variability occurring within provenances. This differentiation was supported
294 by the results in STRUCTURE, where the most probable number of genetic clusters was two
295 (Supplementary material Appendix 1 Fig. SA2). These two clusters corresponded closely to
296 the two geographical sampling areas (east and west), with some admixed individuals.

297

298 *Total biomass and survival*

299 Total biomass at the end of the experiment was determined only by the provenance factor,
300 being unaffected either by the changes in temperature or in watering (Table 3). Seedlings

301 from the western provenance grew more than seedlings from the eastern provenance (Fig. 2a),
302 also producing deeper roots (290.9 ± 7.9 vs. 258.1 ± 9.2 mm) and taller shoots (32.1 ± 0.5 vs.
303 29.3 ± 0.6 mm). Although temperature had no effect on total biomass, higher temperature
304 diminished root length (CT: 329.3 ± 8.1 mm, FT: 236.6 ± 7.5 mm; $F = 53.2$, $P < 0.0001$).
305 Root:shoot biomass ratio was affected by the two climate factors, but not by the seed
306 provenance (Table 3), showing a high plasticity in response to climatic variables (Fig. 2b).
307 Overall, higher temperature reduced biomass allocation to roots (CT: 1.67 ± 0.05 , FT: $1.41 \pm$
308 0.03), while reduced watering induced higher root investment (high: 1.40 ± 0.04 , low: $1.65 \pm$
309 0.05). The proportion of surviving seedlings was affected only by the watering treatment
310 (Table 3), with a decrease in the survival probability as watering diminished (high: $0.91 \pm$
311 0.02 , low: 0.78 ± 0.03).

312

313 *Ecophysiological variables*

314 At the time of harvesting, water potential of silver-fir seedlings was influenced by the
315 changes in temperature and, especially, in watering (Table 3), but not by provenance. Both
316 higher temperature and lower watering led to more negative water potentials (Fig. 3a),
317 although the effect of watering was stronger than that of temperature (high watering: $\Psi = -$
318 1.68 ± 0.04 MPa; low watering: $\Psi = -2.25 \pm 0.04$ MPa). Carbon isotopic discrimination was
319 similar between the two provenances, but it was also affected by changes in temperature and
320 watering (Table 3). Overall, seedlings growing under current temperature or with higher
321 watering had more negative ^{13}C values than those under increased temperature or in low
322 watering treatments (Fig. 3b). However, significant temperature- watering, temperature-
323 provenance, and temperature-watering-provenance interactions appeared. Under current
324 temperature conditions, seedlings from the eastern provenance increased $\delta^{13}\text{C}$ concentration

325 when watering was reduced, while under simulated future higher temperatures, western
326 populations exhibited the higher $\delta^{13}\text{C}$ concentrations under reduced watering.

327

328 Nitrogen concentration in seedlings was also affected by the two climatic variables
329 (Table 3). Warmer temperature reduced N concentration (CT: $11.5 \pm 0.6 \text{ mg g}^{-1}$, FT: 9.6 ± 0.7
330 mg g^{-1}), whereas seedlings growing under the low watering treatment presented higher N
331 concentration ($13.2 \pm 0.6 \text{ mg g}^{-1}$) than those under the high watering treatment ($8.0 \text{ mg g}^{-1} \pm$
332 0.5). In a similar way, the C:N ratio increased with higher temperature, but diminished when
333 watering was reduced (Fig. 4a). The effect of provenance was not significant, but the
334 temperature-provenance interaction was significant for both N concentration and C:N ratio
335 due to higher N concentration in seedlings from western provenances under higher
336 temperatures (Fig. 4a). Lastly, SS concentration was only affected by temperature, with
337 higher values under the simulated future temperature (CT: $5.0 \pm 0.2\%$, FT: $5.7 \pm 0.2\%$).
338 Although watering had no effect on SS concentration, there were significant watering-
339 temperature and watering-provenance interactions on the SS content (Table 3; Fig. 4b). The
340 increase of SS concentration under higher temperatures was much higher in seedlings from
341 the eastern provenances when watering was reduced, while the opposite trend was observed
342 in seedlings from the western provenances.

343

344 **Discussion**

345 In this study we tested the effect of elevated warming and drought on early growth and
346 survival of two geographic provenances of Pyrenean silver fir. We confirmed that the eastern
347 and western Pyrenean provenances correspond to two different genetic lineages. Seedlings
348 from the western lineage grew more than those from the eastern lineage. While climatic
349 manipulations had no effect on total biomass, increased drought diminished survival across

350 lineages. Warming induced more negative water potentials, higher $\delta^{13}\text{C}$ and SS values, higher
351 C:N ratio and lower N content. Drought increase provoked more negative water potentials,
352 higher $\delta^{13}\text{C}$ and N content and lower C:N ratio. Interestingly, eastern and western
353 provenances presented different iWUE when only drought or warming and drought were
354 simulated.

355

356 *Geographical lineages of Pyrenean silver fir*

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

370

371 *Climatic effect on biomass and survival*

372 Warming and drought induced higher biomass allocation to roots, and drought reduced
373 seedling survival. Changes in climatic conditions simulated in this experiment altered growth,
374 survival and physiological performance of Pyrenean silver fir seedlings. Although western-

375 lineage seedlings grew more rapidly than those from the eastern lineage, climate treatments
376 generally affected seedling performance in a similar way, irrespective of provenance origin.
377 Thus, a reduction in watering diminished survival and altered the biomass allocation pattern
378 for both lineages. In addition, the combination of increased temperature and decreased
379 watering affected the water potential of seedlings from both lineages in a similar way.
380 However, the effects of temperature and watering varied between lineages for carbon isotopic
381 discrimination, nitrogen concentration, C:N ratio, and the concentration of soluble sugars.

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

400 have strong implications since, under a scenario of combined temperature and drought
401 increase, the negative temperature effect on root investment could cancel the positive effect
402 of higher root allocation in response to reduced precipitation, diminishing the plastic response
403 to climate when warmer and drier conditions occur.

404

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

411

412 *Physiological response to climate alterations*

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

425 plant water potential might be even more patent in natural systems, where the covariation
426 between temperature and VPD is stronger than under controlled conditions with constant
427 relative humidity.

428

429 Changes in temperature and watering simulated here also altered $\delta^{13}\text{C}$ in silver fir
430 seedlings. Both warmer and drier conditions augmented $\delta^{13}\text{C}$ concentration compared to
431 current climate values, indicating that seedlings are able to increase the carbon fixation per
432 water unit lost (i.e., higher iWUE) when temperatures increase or when precipitation
433 diminishes. This increase in iWUE implies that silver fir seedlings have the capacity to buffer
434 the effects of the predicted climate to some extent either by increasing the photosynthetic rate
435 and/or by diminishing the stomatal conductance (McDowell et al. 2005), maintaining the
436 water balance despite environmental alterations. However, this adjustment cannot be
437 maintained indefinitely and the thresholds where the capacity to improve iWUE is exceeded
438 by climatic alterations should be explored in more depth. We should note, however, that the
439 $\delta^{13}\text{C}$ results presented here are lower than values commonly found in nature for this species
440 (Linares and Camarero 2012b), likely due to the source of carbon available within our
441 experimental cabinets or because the cited studies analyzed tree-ring cellulose or shaded
442 needles. However, this difference does not alter our conclusions since these differences were
443 consistent among the different treatments.

444

445 The simulated climatic conditions affected the nutritional status of silver fir seedlings,
446 by diminishing the nitrogen concentration when warming was simulated but increasing under
447 drier conditions. Nutrients contained within microbial biomass under moist conditions are
448 usually released to the soil under drought conditions, allowing plants a higher uptake until a
449 critical threshold for the uptake of dissolved nutrients is surpassed (Matías et al. 2011b). Such

450 microbial nutrient release could explain the higher nitrogen concentration in the low-watering
451 scenario. However, the detected decrease in nitrogen concentration under warmer conditions
452 opens the possibility that warming-induced nutrient deficiency might play an additional role
453 in dieback of silver fir. We should take also into account that nutrient availability in the
454 experimental pots was higher than in natural populations (Supplementary Table A1), and the
455 reported differences might, therefore, be even higher in nature. Previous studies found an
456 increase in the soluble sugar content in response to drought in other fir species (Guo et al.
457 2010), associated with a decrease in other macromolecules such as starch, since soluble
458 sugars are efficient osmolytes (Ahmed et al. 2009). However, we did not find a clear climatic
459 effect on the soluble sugar concentrations in silver fir leaves.

460

461 *Geographical differences*

462 In addition to the general effects described above, seedlings from the eastern and western
463 Pyrenean provenances performed differently. Seedlings from the western provenance grew
464 better than eastern ones, however, this higher growth rate of western-provenance seedlings
465 was not linked to higher survival probabilities. The higher growth in the early life stage may
466 grant seedlings a clear competitive advantage for establishment, which together with the
467 relatively high survival across the different climatic scenarios could allow the existence of a
468 good sapling bank in the understory able to maintain the populations after adult mortality,
469 even in those populations suffering current declining trends. Nevertheless, the two
470 provenances showed some relevant differences in seedling physiological response to the
471 experimental climatic scenarios. The increase of $\delta^{13}\text{C}$ under simulated warming was
472 particularly high for western populations under drought conditions, while they exhibited
473 diminished iWUE under current temperatures but with drought conditions. This result
474 contrasts with higher $\delta^{13}\text{C}$ values in seedlings from eastern populations under current

475 temperatures and low watering when comparing them with western populations, which in fact
476 agrees with less adult decline observed in the field. This higher physiological sensitivity to
477 temperature and drought in the western provenance might imply contrasted responses
478 depending on the yearly variations in temperature and precipitation. In the driest scenario
479 simulated (future temperature and low watering) we also found geographical differences in
480 the soluble sugar concentrations, with seedlings from the eastern range showing much higher
481 values than western ones. This result concurs with the observed lower $\delta^{13}\text{C}$ values, suggesting
482 reduced stomatal control, compared with western populations growing in the same conditions.
483 Geographical provenance also influences patterns of N concentration in seedlings, with
484 western populations showing higher values in warmer conditions. However, beside these
485 differences, we should take into account that the source populations from two provenances
486 differed in elevation (Supplementary table A1), which could imply some additional
487 differences on seedling growth and physiology, as previously shown by Oleksyn et al. (1998).

488

489 We assessed differences in seedling performance between the eastern and western
490 lineages of silver fir under controlled conditions. While the differences we report can inform
491 on differences in plant performance in the field, they cannot fully explain the observed
492 differences in adult dieback severity. Diverging patterns between adults and seedlings can
493 occur due to ontogenetic shifts in niche requirements or in physiology (Mediavilla and
494 Escudero, 2004; Quero et al. 2008) such that seedling and adult responses may not be directly
495 comparable. However, the geographical variability in seedling performance that we report
496 demonstrates high intraspecific variability in response to environmental change that is related
497 to the postglacial colonisation history of the species. These differences would be even higher
498 than the described here, since the different source populations differ on altitude and thus
499 would have different thermal optima, while we subjected them to similar conditions (either

500 current or future). Such a finding adds improved resolution to understanding the scale of
501 intraspecific variation in plant performance whilst also challenging our ability to generalise
502 population-level responses to predicted future climate.

503

504 *Conclusions*

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

519

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526

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681

682 **Figure legends**

683 **Figure 1:** Distribution map of silver fir (inner map, source Euforgen;

684 <http://www.euforgen.org/>) and selected populations across the western (shaded tags) and

685 eastern (open tags) Pyrenean provenances. See sites codes in Supplementary Table A1.

686 **Figure 2:** a) Total biomass at the end of the experiment of silver fir seedlings from the eastern

687 (open bars) and western (grey bars) Pyrenean provenances across the temperature (current,

688 CT; future, FT) and watering (high, low) treatments. * Significant at 0.01–0.05 level; **

689 Significant at 0.001–0.01 level; *** Significant at <0.001 level. b) Plasticity to change in

690 biomass allocation in response to watering. Circles represents mean root:shoot biomass ratios

691 by temperature (open: current temperature; black: future temperature) and provenance under

692 high and low precipitation treatments. Points above the dashed 1:1 line indicate higher

693 biomass allocation to roots in response to decreased watering. E and W by each point

694 represent east and west provenances respectively. Error bars represent \pm SE.

695 **Figure 3:** Water potential at harvesting time (a) and C isotopic discrimination ($\delta^{13}\text{C}$, b) of

696 silver fir seedlings from the eastern (open bars) and western (grey bars) Pyrenean

697 provenances across the temperature (current, CT; future, FT) and watering (high, low)

698 treatments. Error bars represents \pm SE. * Significant at 0.01–0.05 level; ** Significant at

699 0.001–0.01 level; *** Significant at <0.001 level.

700 **Figure 4:** C:N ratio (a) and soluble sugar concentration (b) of silver fir seedlings from the

701 eastern (open bars) and western (grey bars) Pyrenean provenances across the temperature

702 (current, CT; future, FT) and watering (high, low) treatments. Error bars represents \pm SE. *

703 Significant at 0.01–0.05 level; ** Significant at 0.001–0.01 level; *** Significant at <0.001

704 level.