

1 **Biomass partitioning in a future dry and CO₂ enriched climate: shading aggravates**

2 **drought effects in Scots pine but not European black pine seedlings**

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4 Christoph Bachofen^{1,2}, Thomas Wohlgemuth¹, Barbara Moser¹

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6 ¹ Forest Dynamics, Swiss Federal Institute for Forest, Snow and Landscape

7 Research WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

8 ² Department of Environmental Systems Science, ETH Zurich, Universitätsstrasse

9 16, 8092 Zürich, Switzerland

10

11 Corresponding author:

12 Christoph Bachofen. Tel: +41 44 632 85 15. Email: christoph.bachofen@usys.ethz.ch

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13 Abstract

14 1. Climate change alters both water and CO₂ availability for plants, but it is
15 largely unknown how they interact with light to affect tree seedling establishment
16 and early growth. Light availability is often regulated by forest management, thus
17 understanding how these resources co-limit the regeneration success of tree
18 species and populations with contrasting drought tolerances is essential for
19 adaptive forest management and particularly for assisted migration.

20 2. We studied biomass partitioning of 3-year-old Scots pine (*Pinus sylvestris*) and
21 European black pine (*Pinus nigra*) seedlings in response to combined effects of
22 light (22% and 40% shade), soil water availability (moist and dry conditions) and
23 CO₂ (ambient and elevated), and examined the responses of seedlings from
24 Central Alpine and Mediterranean origin. Seedlings of nine populations with
25 varying drought tolerances were grown in a common garden in the European
26 Central Alps. Shoot height, vertical root length, shoot and root biomass of the
27 plants were assessed at the end of the third growing season.

28 3. Under 40% shade and dry conditions, *P. sylvestris* seedlings severely reduced
29 shoot biomass, resulting in an increased specific shoot height (SSH) compared

to seedlings under 22% shade and moist conditions. In contrast, *P. nigra* seedlings retained a constant shoot biomass under all treatment combinations. Seedlings from drier origin were generally larger, heavier, and had longer vertical roots than those from wetter locations. In order to keep up shoot height, seedlings from wetter origins disproportionately increased SSH under shaded conditions compared to populations from drier origin.

4. *Synthesis and applications.* Under high light availability, Scots pine (*Pinus sylvestris*) and European black pine (*Pinus nigra*) seedlings were well adapted to dry conditions. Moderate shading, however, substantially reduced Scots pine but not black pine growth, and potentially amplified the vulnerability of Scots pine seedlings to drought. Optimizing light conditions in forests, e.g. by thinning, may thus enhance early Scots pine regeneration in a drier future climate.

Keywords

assisted migration, elevated CO₂, light availability, optimal partitioning, root growth, shoot growth, water stress, thinning

1. Introduction

Climate change alters the availability of both water and CO₂ for plants, and interactive effects of these factors with light are expected to critically limit forest regeneration (Hartmann 2011; Valladares *et al.* 2016) and hence affect the succession dynamics and species composition of forests (Kim, Oren & Qian 2016; Wohlgemuth, Gallien & Zimmermann 2016). For instance, future climate conditions with more frequent heat waves and longer drought spells (Lindner *et al.* 2014) may hamper pine regeneration in dry valleys of the Central Alps (Moser *et al.* 2010; Rigling *et al.* 2013). In these regions, the persistence of forests may be facilitated by thinning competing tree and shrub species, which increases water availability for individual trees (Flathers *et al.* 2016; Giuggiola *et al.* 2016). But, thinning also increases light availability in the understory, which might affect the performance of tree seedlings during drought. At dry locations in the Mediterranean, where summer drought is one of the most limiting factors for seedlings establishment (Castro *et al.* 2005; Pardos *et al.* 2005; Moreno-Fernández *et al.* 2015), canopy shading from 'nurse plants' has been observed to enhance the regeneration of trees due to microclimatic amelioration (Gómez-Aparicio *et al.* 2005). Management practices that

concomitantly alter the availability of light and water might thus have very different effects on regeneration. Evaluating the growth responses of tree seedlings to concurrent changes in light, water and CO₂ availability is particularly important with respect to assisted migration of tree species. The translocation of species is a potential measure to sustain current ecosystem services under future climatic conditions (McLane & Aitken 2012; Bussotti *et al.* 2015; Aitken & Bemmels 2016). Mediterranean pine species and populations are, for instance, expected to be better adapted to the future climate in Central Europe (Hanewinkel *et al.* 2013) and might thus be considered for assisted migration to locations where Scots pine (*Pinus sylvestris* L.) regeneration might be at risk in the future. To ensure that the introduced species/populations are well adapted to projected future conditions and, at the same time, to minimise the risk of adverse effects on the recipient ecosystems, adaptive traits of potential migrants have to be evaluated carefully under a broad range of environmental conditions (Richter *et al.* 2012; Bachofen *et al.* 2016) and in conjunction with potential management measures.

79 Plants respond to changes in light, water and CO₂ availability with altered biomass
80 allocation to the shoot and roots, in order to optimise growth and survival
81 (McConnaughay & Coleman 1999; McCarthy & Enquist 2007; Poorter *et al.* 2012).
82 For instance, low water availability results in the development of larger root systems
83 for a better access to soil water (Hertel *et al.* 2013; Taeger, Sparks & Menzel 2015),
84 whereas low light availability increases the allocation of biomass to the shoot
85 (Poorter & Nagel 2000; Poorter *et al.* 2012). Mediterranean pine species or
86 populations may be better adapted to low water availability, because they invest a
87 larger fraction of their biomass to roots (Richter *et al.* 2012; Taeger *et al.* 2015). As
88 biomass partitioning underlies a trade-off (Craine 2009), a fundamental question is
89 how interactive effects of shade and drought affect biomass partitioning (Poorter &
90 Nagel 2000) and consequently forest regeneration (Sack & Grubb 2002), particularly
91 in a future enriched CO₂ environment (Poorter & Nagel 2000; Valladares *et al.* 2016).
92 It has, for instance, been hypothesised that drought has a stronger impact on shaded
93 tree seedlings, which tend to invest in leaf area at the expense of a smaller root
94 system, making them more susceptible to drought (Sack & Grubb 2002; Valladares &
95 Niinemets 2008; Laanisto & Niinemets 2015). However, in dry Mediterranean

96 climates, shading is known to mitigate drought effects (Gómez-Aparicio *et al.* 2005;
97 Quero *et al.* 2006), e.g. by reducing the vapour pressure deficit (Von Arx *et al.* 2013).
98 Higher atmospheric CO₂ concentrations, on the other hand, increase photosynthetic
99 carbon gain (Leakey *et al.* 2009) and therefore also affect the partitioning of biomass
100 to the shoot and roots (Poorter & Nagel 2000; Leuzinger & Hättenschwiler 2013).
101 Accordingly, elevated CO₂ (eCO₂) is expected to decrease root biomass fraction as a
102 result of an alleviated competition for soil water (Farrior *et al.* 2015). Rising CO₂
103 concentrations could therefore change the optimal partitioning and hence affect both
104 light usage and water uptake of tree seedlings. Experiments testing the co-limitation
105 of more than two resources are scarce (but see Danyagri & Dang, 2013; Pardos *et*
106 *al.*, 2006). Growth responses of tree seedlings to simultaneous changes in light ×
107 water × CO₂ concentrations are therefore not well understood.

108
109 We aimed at assessing adaptive traits of potential migrants to Central Alpine valleys,
110 and compared growth responses of Mediterranean pine species and populations with
111 those of Central European pines under current vs. future dry and CO₂ enriched
112 climate conditions in combination with high and low light availability. In particular, we

examined shoot and root growth responses of five populations of *Pinus sylvestris* and four populations of *Pinus nigra* J.F. Arnold seedlings to three-way interactive effects of light (light and moderate shade), water (dry and moist conditions) and CO₂ (390 ppm and 570 ppm) availability by manipulating all three factors in a full factorial common garden experiment. We analysed (1) whether moderate shading mitigates or aggravates the effect of drought on seedling growth and biomass partitioning, (2) if higher concentrations of atmospheric CO₂ alleviate water and/or light limitations, and (3) whether the growth advantage of more drought adapted species and populations under future climate conditions depends on light conditions.

2. Materials and Methods

2.1. Species and populations

Pinus sylvestris has become a model species for studying effects of future climatic conditions on forests (Martínez-Vilalta, Lloret & Breshears 2012) due to its wide distribution range from boreal Siberia and Scandinavia to mountainous regions in the European Mediterranean, and its considerable drought-tolerance, which today is

130 challenged by aggravated dry-hot periods (Reich & Oleksyn 2008). It requires bare
131 soil, light and water to establish, maintains high growth rates under non-shaded
132 conditions and forms dense stands on dry sites such as the Central Alps, but is
133 outcompeted on mesic sites by more shade tolerant species (Mátyás, Ackzell &
134 Samuel 2004; Pretzsch *et al.* 2015). *Pinus nigra* thrives around the northern
135 Mediterranean and exhibits a higher tolerance to drought (Herrero *et al.* 2013) and
136 shade (Niinemets & Valladares 2006) compared to *P. sylvestris*.

137
138 We collected seeds from five *P. sylvestris* and four *P. nigra* populations in winter
139 2011/2012 in regions located along a gradient of decreasing climatic water balances
140 in the summer months (Bachofen *et al.* 2018). *P. sylvestris* populations were located
141 in two Central Alpine regions, one low and one high elevation, and in three
142 Mediterranean regions in Spain, Greece and Bulgaria (see Appendix S1 in
143 Supporting Information, Table 1). The *P. nigra* populations, were located
144 geographically close to the three Mediterranean *P. sylvestris* populations. A fourth
145 population was selected from the northernmost natural occurrence of the species in
146 the Eastern Alps, which is assumed to be more susceptible to drought. In each

population we collected seeds from five maternal lineages in order to standardise the amount of genetic variation (Bachofen *et al.* 2018).

2.2. Experimental design and treatments

The experiment was set up in a common garden located in the Valais valley (Switzerland; 46°18'33"N, 07°41'10"E; 610 m a.s.l.; 19.2 °C average temperature in July; 602 mm annual precipitation). In February 2012, 32 mesocosms (surface: 200 cm × 80 cm; height: 50 cm) were filled with 30 cm of sand and gravel from the local Rhone riverbed (subsoil) and covered with 15 cm of humus (Oekohum GmbH, Herrenhof, Switzerland; topsoil). We designed the two layers to simulate the natural forest soils of the Rhone valley, where *P. sylvestris* is stand forming (Moser *et al.* 2016). The mesocosms were placed on gravelly soil and closed at the bottom with a precision mesh fabric that is impenetrable to roots and mycorrhizae, while water passes through (<1 µm mesh size; Petex 07-1/2, Sefar AG, Heiden, Switzerland). Consequently, the saplings were not able to access water outside the mesocosms. The mesocosms were arranged in a split-split-plot design with four blocks (whole plots), two sub-blocks (split-plots) accommodating two levels of a CO₂ treatment

164 (ambient CO₂/elevated CO₂), and four mesocosms per sub-block (split-split-plots) to
165 which fully crossed combinations of a soil water conditions (moist/dry) and a shading
166 treatment (light/moderate shade) were applied (see Appendix S2a).

167

168 The CO₂ treatment consisted of the two levels 'ambient CO₂' (aCO₂: 390 ppm) and
169 'elevated CO₂' (eCO₂: 570 ppm). During the growing season in 2012 (June–
170 September), 2013 and 2014 (March–October), the eCO₂ was supplied to the
171 mesocosms using the same FACE equipment as in Hättenschwiler *et al.* (2002;
172 details see Bachofen *et al.* 2018). Concurrently with the CO₂ treatment, water
173 availability was manipulated to simulate a 'moist' and a 'dry' climate, corresponding to
174 the 90th and the 10th percentile of the summer season precipitation sums from 1864
175 to 2011 in the Rhone valley (416 mm and 189 mm, respectively, i.e. 151% and 67%
176 of the average March–September precipitation measured from 1864–2011 at the
177 MeteoSwiss station in Sion located 28 km to the East of the study site). We assigned
178 the two water availability levels randomly to two mesocosms in each sub-block.
179 Automatic mobile rain shelters intercepted the natural rainfall, and the mesocosms
180 were watered weekly on two subsequent nights with an automatic drop irrigation

system to a level of 8 mm ('moist') and 3.5 mm ('dry') per night, respectively (Bachofen *et al.* 2016). From June to September 2012, and March to October in 2013 and 2014, all mesocosms were covered with white knitted polypropylene shade cloths (GreenhouseMegastore, International Greenhouse Company, Danville IL, USA). Half of the mesocosms from each CO₂ and water treatment received 'light shade' that blocked 22% (S₂₀), and the other half 'moderate shade' that blocked 40% (S₄₀) of the sunlight, respectively. The three treatments CO₂, shading, and water availability were hence fully crossed within each block.

We divided each mesocosm into two halves, with 70 squares of 10 cm × 10 cm per half. In March 2012, we sowed 15 seeds per species, population (five *P. sylvestris* and four *P. nigra* populations) and maternal lineage in randomly selected squares (9 populations × 5 maternal lineages = 45 squares) in each half of the mesocosms (Moser *et al.* 2016; Bachofen *et al.* 2018). The remaining 50 squares contained conifers that were not part of the present study (details see Appendix S2b). To facilitate germination of the seeds, the soil was watered daily until May 2012.

198 *2.3. Growth measurements*

199 In October 2014, we randomly chose one of the two squares of each population and
200 maternal lineage per mesocosm and cut one randomly selected seedling at ground
201 level. We measured shoot height of the seedlings from the base to the bud and dried
202 the shoots at 60 °C to constant weight before measuring shoot dry weight. In
203 addition, we excavated seedling roots in one randomly selected block. We removed
204 the humus and sand from the roots and disentangled the root system of one
205 randomly selected seedling per square (two halfmesocosms per treatment × five
206 squares per population = ten replicates per treatment and population; Moser *et al.*
207 2016). We then measured the length of the longest vertical root (vertical root length,
208 for details see Moser *et al.* 2016) before drying the roots at 60 °C to constant weight
209 and measuring root dry weight. During the excavation, we detected that the
210 mesocosm with the treatment combination dry × S₂₀ × eCO₂ was heavily infested with
211 May beetle (cf. *Melolontha melolontha* L.) grubs, which had fed on the sapling's
212 roots. As a consequence, we omitted shoot and root data of this mesocosm from the
213 analyses. Aboveground measurements refer to all four blocks (N=1409 seedlings),

while root measurements were restricted to one block (N=622), because excavation of more blocks was not feasible.

2.4. Statistical analyses of growth and biomass partitioning

We analysed the effects of shading, water availability, and CO₂, on shoot biomass, shoot height, specific shoot height (shoot biomass/shoot height), root biomass, vertical root length, specific vertical root length (root biomass/specific vertical root length), and root biomass fraction (root biomass/total biomass) separately for each species with a linear mixed effects model (LMM) using the 'lmer' function in the lme4 package of the statistics program R (R Core Team 2016). The treatments and the population were treated as fixed effects, the block and maternal lineage as random effects. Due to the May beetle grub infestation in the dry × S₂₀ × eCO₂ mesocosm that was used for root biomass measurements, three-fold interactive effects were only analysed for the shoot, whereas root traits were analysed separately for each level of CO₂. In addition, we analysed the effect of the climatic water balance (CWB) at the seed origin on all shoot and root parameters using a linear model (LM). The treatments were considered as fixed effects and the CWB as a covariate. The CWB

231 at the origin of each population was calculated as the difference between
232 precipitation and potential evapotranspiration according to (Thornthwaite 1948) using
233 precipitation and temperature data (means of 1950–2000) from the MeteoSwiss
234 stations in Sion and Montana for the two Central Alpine populations in Switzerland
235 (low elevation and high elevation), and interpolated precipitation and temperature
236 data (means of 1950–2000) from the WorldClim database (Hijmans *et al.* 2005) for
237 the remaining populations. July as the month with the lowest CWB in all seed origins
238 was used as an indicator for the population's drought-tolerance (Bachofen *et al.*
239 2018).

240

241

242 3. Results

243 3.1. Irrigation and soil water status

244 Seedlings in dry mesocosms obtained 44% of the water of seedlings in moist
245 mesocosms during all three growing seasons (2012–2014). Consequently, the soil
246 water content (SWC) at aCO₂ was considerably lower in the subsoil of dry compared
247 to moist mesocosms, whereas the topsoils had a similar SWC in all treatment

combinations (Fig. 1). SWC of eCO₂ mesocosms were similar to aCO₂ mesocosms, except in the topsoil of S₂₀ mesocosms, where SWC was slightly lower under eCO₂. All seedlings produced both lateral roots in the shallow topsoil and long vertical roots reaching the bottom of the subsoil (see Appendix S3).

3.2. Drought effects on biomass partitioning

At light shading and ambient CO₂, reduced water availability led on average to 10.1% less shoot biomass in 'dry' *P. sylvestris* seedlings compared to 'moist' seedlings, whereas shoot of *P. nigra* seedlings produced similar amounts of biomass in both water availability treatments (Fig. 2). Seedlings of both species did not differ in shoot height, root biomass and vertical root length (Table 2; Figs. 2 and 3). In *P. sylvestris*, this resulted in 26.7% higher specific shoot height, i.e. longer shoots per gram aboveground biomass under dry compared to moist conditions (Fig. 4), and also a marginally higher root mass fraction (Fig. 5).

3.3. Shading effects on biomass partitioning

264 Under moist water and ambient CO₂ conditions, the moderate shading entailed a
265 12.0% reduction in investments in shoot biomass in both species (Fig. 2), but had,
266 similarly to the drought treatment, no effect on shoot height (Table 2; Fig. 3), which
267 lead to 19.1% increase in specific shoot height compared to light shaded seedlings.
268 At the same time, moderately shaded seedlings accumulated 23.4% less root
269 biomass and had 22.9% shorter vertical roots than seedlings under light shading
270 (Figs. 2 and 3). In *P. sylvestris*, this resulted in 1.9% less root biomass fraction in
271 moderate shade (Fig. 5).

272

273 3.4. Combined effects of drought and shading

274 *P. sylvestris* and *P. nigra* reacted differently to the combination of drought and
275 moderate shading at aCO₂. In *P. sylvestris*, dry × S₄₀, seedlings allocated even less
276 biomass to the shoot than seedlings that were exclusively droughted (dry × S₂₀) or
277 exclusively shaded (moist × S₄₀), corresponding to 28.8% less biomass than control
278 seedlings (Fig. 2). Surprisingly, *P. sylvestris* seedlings growing in the dry × S₄₀ still
279 had similar shoot height as the controls (moist × S₂₀; Fig. 3). Consequently, they
280 exhibited a 42% increase in specific shoot height under combined drought and

shading compared to controls. Similarly, they drastically cut on investments in root biomass (-42.6% compared to controls; Fig. 2), and as a consequence, the root biomass fraction remained unaffected by the combined drought and shading (Fig. 5). Moderately shaded and droughted *P. nigra* seedlings (dry × S₄₀), on the other hand, produced similar shoot biomass and grew similarly tall as controls (moist × S₂₀; Table 2; Figs. 2 and 3), but they reduced investments in roots similarly to shaded only seedlings: in comparison to controls, they produced 21.5% less root biomass (Fig. 2), and vertical roots grew shorter by 17.6% (Fig. 3), resulting in similar specific vertical root length (Fig. 4). Root biomass fraction remained, on the other hand, unaffected by combined drought and shade, as in *P. sylvestris* seedlings (Fig. 5).

3.5. Elevated CO₂

Increased atmospheric CO₂ concentration did not affect aboveground biomass partitioning of *P. sylvestris* and *P. nigra* seedlings in any combination of the drought and shading treatments (Table 2; Figs. 2, 3, and 4). Thus, the same growth restrictions by water and light limitations were observed in eCO₂ seedlings as well. But, vertical roots grew shorter under eCO₂ in both species, with an average of

26.0% reduction for *P. sylvestris* and 25.6% for *P. nigra* seedlings, leading to a 20.3% and a 21.2% decrease in specific vertical root length, respectively (Figs. 3 and 4).

3.6. Growth in relation to the seed origin

Both shoot and root biomass correlated negatively with the climatic water balance at the seed origin (CWB) in seedlings from all treatment combinations ($p < 0.001$, Figs. 6 a–d). Consequently, the root biomass fraction remained constant in relation to CWB (Figs. 6 e–f). In some treatment combinations, shoots grew taller and vertical roots longer in seedlings from drier origin (Figs. 6 g–j). The relationship between shoot height or vertical root length with CWB was, however, less pronounced than the relationship between shoot or root biomass and CWB. Consequently, specific shoot height and specific vertical root length were smaller in seedlings from dry origin ($p < 0.001$, Figs. 6 k–n).

4. Discussion

Our results on seedling growth of the two pine species *P. sylvestris* and *P. nigra* under drought revealed several mitigation and aggravation effects of shade, elevated CO₂ and seed origin that partly contrast with previous studies.

4.1. Shade aggravates drought effects to seedlings

While *P. sylvestris* and *P. nigra* seedlings reacted similarly to either drought or shading, their biomass allocation patterns differed considerably under concurrent shading and water shortage. *P. nigra* seedlings accumulated similar shoot and root biomass as in the shading alone and drought alone treatments (Fig. 2), whereas *P. sylvestris* disproportionately reduced both shoot and root biomass (Fig. 2), indicating a trade-off between investments in shade versus drought tolerance (Valladares & Niinemets 2008). This supports the theory that plants preferentially invest in the organ that accesses the most limiting resource ('optimal partitioning'; McConnaughay & Coleman 1999; McCarthy & Enquist 2007). Accordingly, droughted only seedlings increased their root biomass fraction while shaded only seedlings increased their investments in the shoot (Fig. 5). Concomitant drought and shade, however, resulted in the collapse of both shoot and root growth of *P. sylvestris* seedlings. Similar

332 reactions have been observed in Mediterranean *Quercus* (Sánchez-Gómez, Zavala
333 & Valladares 2006) and many other woody and non-woody plant species (Valladares
334 & Niinemets 2008; Laanisto & Niinemets 2015). In contrast to biomass, shoot height
335 of both species did hardly differ across treatments and populations (Table 2). Fast
336 height growth is crucial for interspecific competition among forest trees (Vizcaíno-
337 Palomar *et al.* 2016), especially for pioneer species such as *P. sylvestris* and *P.*
338 *nigra*, which often have to establish during a short window of opportunity after
339 disturbances (Moser *et al.* 2010). Maintaining shoot height under dry and/or shady
340 conditions while simultaneously decreasing investments in aboveground biomass
341 signifies that specific shoot height increases considerably (Fig. 4) at the cost of
342 secondary growth. Lower investments in secondary growth either translate to
343 reduced tracheid lumen diameter, limiting the capacity for water transport
344 (Mencuccini, Grace & Fioravanti 1995; Overdieck, Ziche & Böttcher-jungclaus 2007),
345 or to reduced wood density (Pittermann *et al.* 2006), increasing the vulnerability to
346 cell wall cavitation (Anderegg & Hille Ris Lambers 2016). This suggests that
347 moderate shade compromised the drought resistance of both *P. sylvestris* and *P.*
348 *nigra* seedlings. The considerably lower specific shoot height of *P. nigra* compared to

P. sylvestris across all treatments is congruent with the species higher intrinsic tolerance to low water availability (Niinemets & Valladares 2006; Richter *et al.* 2012; Bachofen *et al.* 2018), which may further explain the smaller effect of combined drought and shading on biomass partitioning of *P. nigra* seedlings in our experiment. Hence, under the climatic conditions of the Central Alps, shading was not beneficial for drought-stressed *P. sylvestris* seedlings, as suggested by experiments with nurse trees in the Mediterranean (Gómez-Aparicio *et al.* 2005) or southwestern U.S. (Flathers *et al.* 2016).

4.2. Elevated CO₂ does not mitigate effects of shade and drought

Responses of trees to elevated concentrations of CO₂ are complex, and have been described in many experiments during the last decades (see e.g. Leuzinger and Hättenschwiler, 2013; Norby and Zak, 2011 for FACE experiments). For instance, eCO₂ can mitigate negative effects of low water availability by decreasing plant water consumption and concomitantly increasing soil water availability (Leuzinger & Körner 2007; Franks *et al.* 2013). This is corroborated by the fact that soil water content was similar in 'dry' and 'moist' mesocosms at eCO₂ (Fig. 1). Plants might therefore prefer

366 to reduce investments in roots and allocate more resources to shoots (Farrior *et al.*
367 2015). By excavating the whole root system, we observed that eCO₂ severely
368 reduced vertical root length in all treatment combinations (Fig. 3), while root biomass
369 remained unaffected (Table 2). Even though vertical root growth was restricted by the
370 size of the mesocosms, it did not stop at the bottom of the containers but was rather
371 diverted and continued along the mesh fabric at 45 cm soil depth (see Appendix S3),
372 which indicates a continuous search for water in the subsoil (Moser *et al.* 2016;
373 Bachofen *et al.* 2018). Shorter vertical roots coincided with higher soluble sugar
374 concentrations in the roots (Bachofen *et al.* 2018), which are important for osmotic
375 water uptake (Brunner *et al.* 2015). Hence, the increase of root soluble sugar
376 concentrations might have allowed to maintain water uptake despite shorter vertical
377 roots. The similar shoot biomass of seedlings growing under eCO₂ and aCO₂ shows,
378 however, that the reduced investment in vertical root growth did not come at the
379 benefit of heavier shoots. Thus, there was no indirect effect of eCO₂ through soil
380 water content on shoot biomass. But, the significant interaction between water
381 availability and CO₂ on shoot height of *P. sylvestris* (W × C; Table 2) suggests that
382 seedlings growing under dry × eCO₂ conditions were able to produce slightly longer

shoots than those growing under dry \times aCO₂ conditions, even though corresponding post-hoc tests were not significant (Fig. 3).

4.3. Growth responses of seedlings in relation to the seed origin

Larger investments in root biomass of more drought adapted tree populations have been observed for long (e.g. Hertel *et al.*, 2013; Moser *et al.*, 2014; Taeger *et al.*, 2015; Zimmer and Grose, 1958), but could not be directly related to climatic variables of the seed origin (see e.g. Taeger *et al.*, 2015). Here, we observed a strong negative relationship between the CWB at the seed origin and seedling biomass, i.e. the dryer the origin, the higher the shoot and root biomass (Fig. 6). Precipitation and evapotranspiration thus explained growth of the different populations in the common garden well, with the exception of biomass partitioning to roots and shoot, which was not related to the CWB at the seed origin (Figs. 6 e–f). The root biomass fraction is known to be inversely related to mean annual temperatures (MAT), which often changes along with elevation (Oleksyn *et al.* 1998). Due to the sampling design of our study with populations from different mountain ranges across central and southern Europe, MAT varies with both elevation and latitude, and we did not

400 observe any relationship between the root biomass fraction and the seed origin. In
401 contrast, specific shoot height and specific vertical root length exhibited an inverse
402 relationship with the CWB, i.e. seedlings from moister sites produced thinner stems
403 and roots. Hence, at the cost of a lower shoot and root biomass, a larger fraction of
404 the available biomass was invested in the corresponding plant part in order to
405 capture the limited resource. Assuming that smaller specific shoot height is
406 associated with higher water transport capacity and higher wood density (s. above),
407 this result implies that populations from drier origin are through their anatomical
408 adaptations less limited under low water availability than those from moister sites.
409 Moreover, specific shoot height of drought stressed seedlings from populations with a
410 high CWB at the seed origin was much more affected by shading than that of
411 seedlings from dry origin (Fig. 6). Pine seedlings from a drier origin thus exhibited
412 growth advantages under both water and light limitations compared to seedlings from
413 moist sites, which might allow them to better resist future climatic extreme events in
414 Central Alpine valleys.

415

416 As a means to ensure forest ecosystem services under future climate conditions, the

assisted migration of tree species/populations has been proposed (McLane & Aitken 2012; Bussotti *et al.* 2015; Aitken & Bemmels 2016). While Kreyling *et al.* (2012) suggested that higher frost sensitivity of Mediterranean pine populations in January could constrain their value for assisted migration to higher latitudes, measurements in our experiment showed sufficient cold hardening of the needles of all *P. sylvestris* and *P. nigra* populations to survive winter and late spring frost in the Central Alps (Bachofen *et al.* 2016). In the case of *P. nigra*, high susceptibility to the fungus *Sphaeropsis sapinea* after hailstorm damage has been observed, which potentially renders the species unsuitable for assisted migration to hailstorm-prone regions. However, in regions with low probability of hailstorm events such as Central Alpine valleys, *P. nigra* may be considered a valid option unless the species is not cultivated in pure stands, which increases the risk of insect outbreaks such as the pine processionary moth (*Thaumetopoea pityocampa* Denis et Schiffermüller, Aimi *et al.*, 2006).

4.4. Conclusions

Biomass partitioning of seedlings growing under three-way interactive effects of light,

434 water and CO₂ availability clearly corresponded to 'optimal partitioning', i.e. water
435 shortage led to higher, shading to a lower root biomass fraction. Shading and soil
436 water availability had the strongest effect on biomass, with best growing condition for
437 both *P. sylvestris* and *P. nigra* seedlings at light shading and high soil water. In
438 contrast to observations that show mitigating effects of shading, e.g. in the
439 Mediterranean (Gómez-Aparicio *et al.* 2005; Quero *et al.* 2006), our results
440 demonstrate aggravated drought effects under moderate shading under the
441 simulated future climatic conditions of the Central Alps. This result has important
442 implications for the management of older and/or only slightly managed pine forests,
443 where thinning may not only ameliorate soil water availability for adult trees by
444 reducing competition with other plants (Flathers *et al.* 2016; Giuggiola *et al.* 2016) but
445 also improve light conditions for pine regeneration. Even under future CO₂ enriched
446 conditions, the growth of pine seedlings is more limited by light than water
447 availability, which, in turn, confirms the wide regeneration niche of both *P. sylvestris*
448 and *P. nigra* regarding soil water conditions.

449

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463

464

465 **Authors' contributions**

466 T.W., B.M. and C.B. conceived and designed the experiment; field work and data
467 analyses were carried out by C.B., supported by B.M. and T.W.; C.B., B.M. and T.W.

468 wrote the manuscript. All authors contributed critically to the drafts and gave final
469 approval for publication.

470

471 **Data accessibility**

472 Data available via the Dryad Digital Repository

473 <https://doi.org/10.5061/dryad.h6q81d2> (Bachofen C, Wohlgemuth T, Moser B., 2018)

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671

Table 1. Climatic characteristics at the seed origin. The climatic water balance was calculated according to Thornthwaite (1948; precipitation - potential evapotranspiration) with precipitation and temperature data (mean 1950–2000; MeteoSwiss station Sion for Leuk, MeteoSwiss station Montana for Visperterminen, and www.worldclim.org for the other populations).

Species	Region	Population	Elevation (m a.s.l.)	Lat (°)	Long (°)	Precipitation (mm)	Climatic water balance in July (mm)
<i>P. sylvestris</i>	Central Alpine	1 Visperterminen (Switzerland)	1363	46.27	7.91	90	-13.8
	Mediterranean	2 Jundola (Bulgaria)	1405	42.05	23.83	35	-51.9
	Mediterranean	3 Serres (Greece)	1333	41.24	23.58	29	-71.9
	Central Alpine	4 Leuk (Switzerland)	570	46.29	7.61	49	-73.4
	Mediterranean	5 Ademuz (Spain)	1542	40.08	-1.08	34	-77.7
<i>P. nigra</i>	East Alpine	6 Bad Fischau (Austria)	344	47.83	16.13	36	-26.1
	Mediterranean	7 Dobrostan (Bulgaria)	1167	41.90	24.93	40	-56.6
	Mediterranean	8 Ademuz (Spain)	1195	40.09	-1.38	26	-98.4
	Mediterranean	9 Parthenonas (Greece)	644	40.13	23.86	18	-113.9

Table 2. LMM results (*F*- and *P*-value) for the effects of water availability (W), shading (S), CO₂ (C) and the population (P) on shoot biomass, shoot height and specific shoot height of 3-years old *P. sylvestris* and *P. nigra* seedlings, and for the effects of W, S and P on root biomass, vertical root length, specific root length, and root biomass fraction of seedlings grown at ambient CO₂.

P.
sylvestris

Source of variation	Shoot biomass	Shoot height	Specific shoot height	Root biomass	Vertical root length	Specific vertical root length	Root biomass fraction
W	21.99 (< 0.001)	1.08 (0.299)	34.78 (< 0.001)	3.36 (0.069)	0.00 (0.982)	2.77 (0.098)	4.26 (0.041)
S	11.95 (0.001)	0.42 (0.515)	28.40 (< 0.001)	30.16 (< 0.001)	50.24 (< 0.001)	0.40 (0.526)	7.63 (0.006)
C	0.87 (0.351)	0.39 (0.534)	0.89 (0.347)				
P	7.14 (0.001)	6.36 (0.002)	7.49 (0.001)	3.70 (0.021)	7.86 (0.001)	1.31 (0.300)	1.71 (0.186)
W x S	1.51 (0.219)	2.16 (0.142)	0.81 (0.369)	2.65 (0.105)	1.75 (0.188)	0.49 (0.486)	0.32 (0.573)
W x C	0.30 (0.584)	5.13 (0.024)	0.62 (0.432)				
W x P	0.35 (0.842)	0.21 (0.934)	0.58 (0.676)	0.07 (0.990)	0.69 (0.603)	0.41 (0.801)	1.62 (0.172)
S x C	0.21 (0.650)	1.53 (0.216)	1.69 (0.195)				
S x P	1.60 (0.173)	1.61 (0.169)	0.94 (0.442)	0.44 (0.780)	0.32 (0.862)	0.50 (0.733)	0.13 (0.972)
C x P	0.40 (0.807)	0.12 (0.974)	0.73 (0.570)				
W x S x C	2.41 (0.121)	0.12 (0.731)	4.57 (0.033)				
W x S x P	2.01 (0.092)	1.72 (0.144)	1.76 (0.134)	0.77 (0.549)	0.87 (0.482)	2.20 (0.072)	1.48 (0.209)
W x C x P	0.62 (0.651)	2.20 (0.067)	0.81 (0.522)				
S x C x P	2.14 (0.075)	0.96 (0.430)	2.30 (0.058)				

P. nigra

Source of variation	Shoot biomass	Shoot height	Specific shoot height	Root biomass	Vertical root length	Specific vertical root length	Root biomass fraction
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W			15.07 (<				
	4.18 (0.041)	1.03 (0.311)	0.001)	2.18 (0.142)	0.13 (0.718)	1.24 (0.268)	5.43 (0.021)
S			18.44 (<		13.80 (<		
	7.21 (0.007)	1.23 (0.269)	0.001)	6.76 (0.010)	0.001)	0.30 (0.583)	0.28 (0.595)
C	0.08 (0.781)	0.72 (0.396)	1.60 (0.206)				
P	11.87 (<	13.93 (<			10.44 (<		
	0.001)	0.001)	8.58 (0.001)	8.88 (0.001)	0.001)	7.10 (0.003)	2.79 (0.071)
W x S	3.85 (0.050)	0.47 (0.493)	5.68 (0.017)	0.14 (0.713)	0.17 (0.682)	0.50 (0.483)	4.31 (0.040)
W x C	1.03 (0.312)	0.83 (0.362)	5.28 (0.022)				
W x P	2.32 (0.075)	0.99 (0.399)	2.39 (0.067)	3.08 (0.030)	1.07 (0.365)	2.23 (0.088)	0.36 (0.781)
S x C	0.73 (0.393)	0.36 (0.548)	0.73 (0.392)				
S x P	0.27 (0.850)	0.00 (1.000)	0.48 (0.694)	0.60 (0.615)	1.17 (0.325)	2.02 (0.115)	1.95 (0.125)
C x P	0.52 (0.671)	0.58 (0.627)	0.44 (0.723)				
W x S x C	1.31 (0.254)	0.00 (0.955)	3.23 (0.073)				
W x S x P	0.31 (0.816)	0.52 (0.667)	0.97 (0.406)	0.63 (0.595)	1.41 (0.243)	0.16 (0.921)	0.48 (0.698)
W x C x P	1.35 (0.258)	0.63 (0.597)	1.35 (0.257)				
S x C x P	1.61 (0.187)	0.10 (0.961)	3.52 (0.015)				

683

684 **Fig 1.** (a) Daily air temperatures (T_{day}), natural precipitation/irrigation (P_{day}) at the
685 experimental site and (b, c) volumetric soil water content (SWC) in the mesocosms
686 during the experiment: (b) aCO₂, (c): eCO₂. SWC refers to the topsoil (5 cm depth)
687 and the subsoil (40 cm depth) of 'moist' and 'dry' mesocosms (black and red lines)
688 and moderate shade and light shade (solid lines and dashed lines). During periods
689 when rain shelters were in operation (March–September), the amount of added water
690 is depicted instead of natural precipitation (blue bars refer to 'moist' and red bars to
691 'dry' conditions). The asterisk denominates the period of daily watering until seedling
692 emergence.

693

694 **Fig 2.** (a) Shoot biomass and (b) root biomass (means \pm se of populations, maternal
695 lineages and blocks) of *P. sylvestris* (shoot: n=100; roots: n=25) and *P. nigra* (shoot:
696 n=80; roots: n=20) seedlings grown under moist and dry soil conditions, ambient and
697 elevated CO₂ (aCO₂, eCO₂) and 22% and 40% shade (S₂₀ and S₄₀). Different letters
698 indicate statistical differences between the treatment combinations per species.
699 Root biomass of dry \times S₂₀ \times eCO₂ seedlings was omitted due to May beetle grub
700 infestation in the respective mesocosm.

701

702 **Fig 3.** (a) Shoot height and (b) vertical root length (means \pm se of populations,
703 maternal lineages and blocks) of *P. sylvestris* (shoot: n=100; roots: n=25) and *P.*
704 *nigra* (shoot: n=80; roots: n=20) seedlings grown under moist and dry soil conditions,
705 ambient and elevated CO₂ (aCO₂, eCO₂) and 22% and 40% shade (S₂₀ and S₄₀).
706 Different letters indicate statistical differences between the treatment combinations
707 per species. Vertical root length of dry \times S₂₀ \times eCO₂ seedlings was omitted due to
708 May beetle grub infestation in the respective mesocosm.

709

Fig 4. (a) Specific shoot height and (b) vertical root length (means \pm se of populations, maternal lineages and blocks) of *P. sylvestris* (shoot: n=100; roots: n=25) and *P. nigra* (shoot: n=80; roots: n=20) seedlings grown under moist and dry soil conditions, ambient and elevated CO₂ (aCO₂, eCO₂) and 22% and 40% shade (S₂₀ and S₄₀). Different letters indicate statistical differences between the treatment combinations per species. Specific vertical root length of dry \times S₂₀ \times eCO₂ seedlings was omitted due to May beetle grub infestation in the respective mesocosm.

Fig 5. Fraction of the total biomass (means \pm se of populations, maternal lineages and blocks) invested in roots of *P. sylvestris* (n=25) and *P. nigra* (n=20) seedlings grown under moist and dry soil conditions, ambient and elevated CO₂ (aCO₂, eCO₂) and 22% and 40% shade (S₂₀ and S₄₀). Different letters indicate statistical differences between the treatment combinations per species. Root biomass fraction of dry \times S₂₀ \times eCO₂ seedlings was omitted due to May beetle grub infestation in the respective mesocosm.

Fig 6. Shoot and root biomass, root biomass fraction, shoot height, vertical root

length, and specific shoot height and specific vertical root length (population means of the maternal lineages and blocks) of *P. sylvestris* (triangles) and *P. nigra* (circles) populations in relation to the climatic water balance in July at the seed origin. Seedlings were grown in moist and dry conditions (black and red symbols), ambient and elevated CO₂ (aCO₂, eCO₂) and light and moderate shade (S₂₀ and S₄₀, open and filled symbols, dashed and solid lines). Root biomass, root biomass fraction, vertical root length and specific vertical root length of dry × S₂₀ × eCO₂ seedlings was omitted due to May beetle grub infestation in the respective mesocosm.

Biomass partitioning in a future dry and CO₂ enriched climate: shading aggravates drought effects in Scots pine but not European black pine seedlings

Appendix Figure S1:

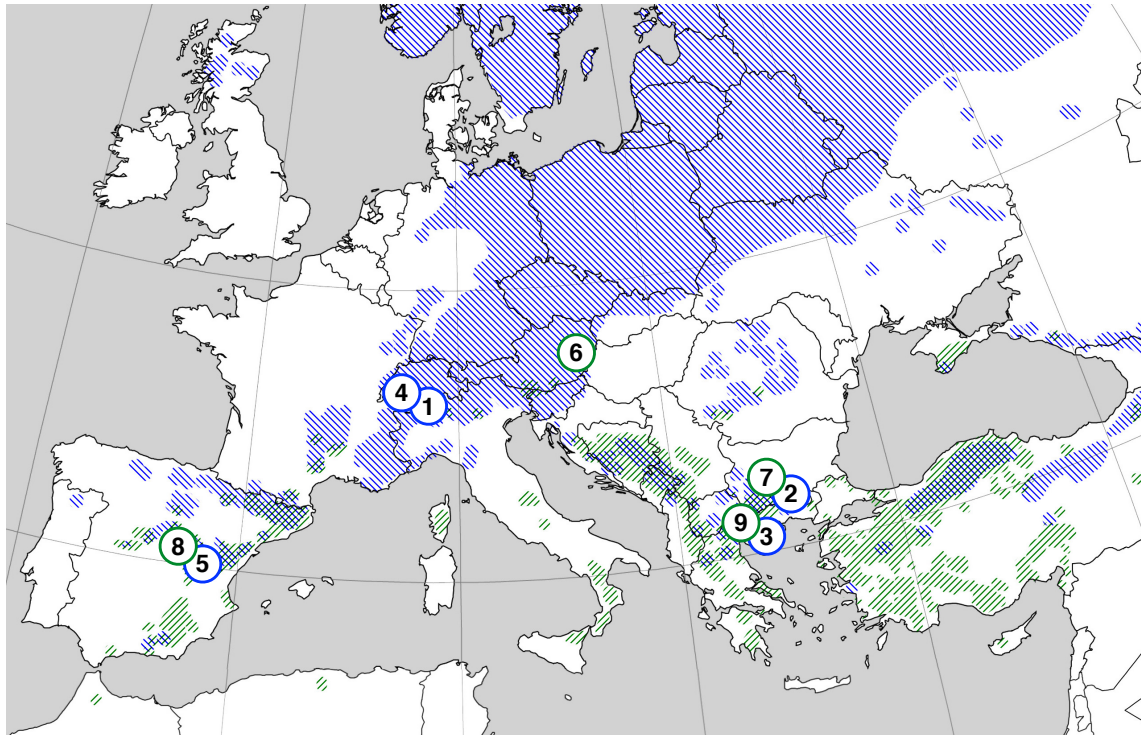


Fig S1. Distribution of *Pinus sylvestris* (///) and *Pinus nigra* (///) in Central and Southern Europe, edited after EUFORGEN (www.euforgen.org). The locations of the seed sources are indicated with blue circles (*P. sylvestris*) and green circles (*P. nigra*). Numbers correspond to the populations listed in Table 1.

Biomass partitioning in a future dry and CO₂ enriched climate: shading aggravates drought effects in Scots pine but not European black pine seedlings

Appendix Figure S2:

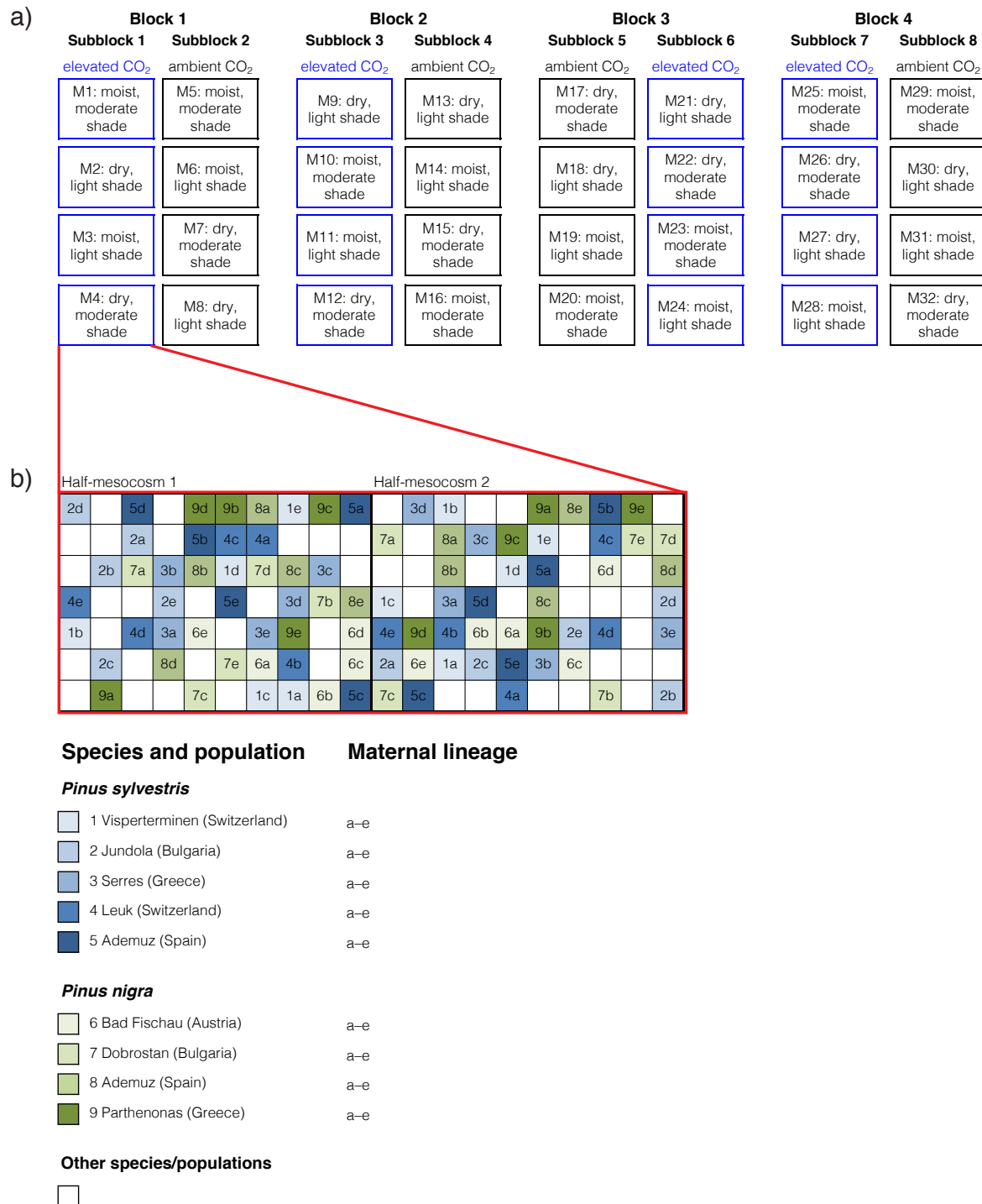


Fig S2. (a) Experimental design with four blocks (whole plots), each consisting of two sub-blocks (split-plots) accommodating two levels of a CO₂ treatment (ambient CO₂/elevated CO₂), and four mesocosms per sub-block (split-split-plots) to which fully crossed combinations of a soil water conditions (moist/dry) and a shading treatment (light/moderate shade) were applied. (b) Each mesocosm was divided into two half-mesocosms and 70 squares per half-mesocosm (10 cm x 10 cm). In each half-mesocosm, squares were randomly sown with 15 seeds of one species, population and maternal lineage. The populations are indicated by numbers (1–9) and colour coding, and maternal lineages by letters (a–e). The number of replicates per species and population thus amounts to 5 per half-mesocosm and 10 per mesocosm. Remaining squares in each mesocosm were sowed with *Pinus halepensis* Mill. and *Pseudotsuga menziesii menziesii* (Mirb.) Franco, which were not part of this study. Sampling took place in randomly selected squares of both half-mesocosms.

Biomass partitioning in a future dry and CO₂ enriched climate: shading aggravates drought effects in Scots pine but not European black pine seedlings

Appendix Figure S3:

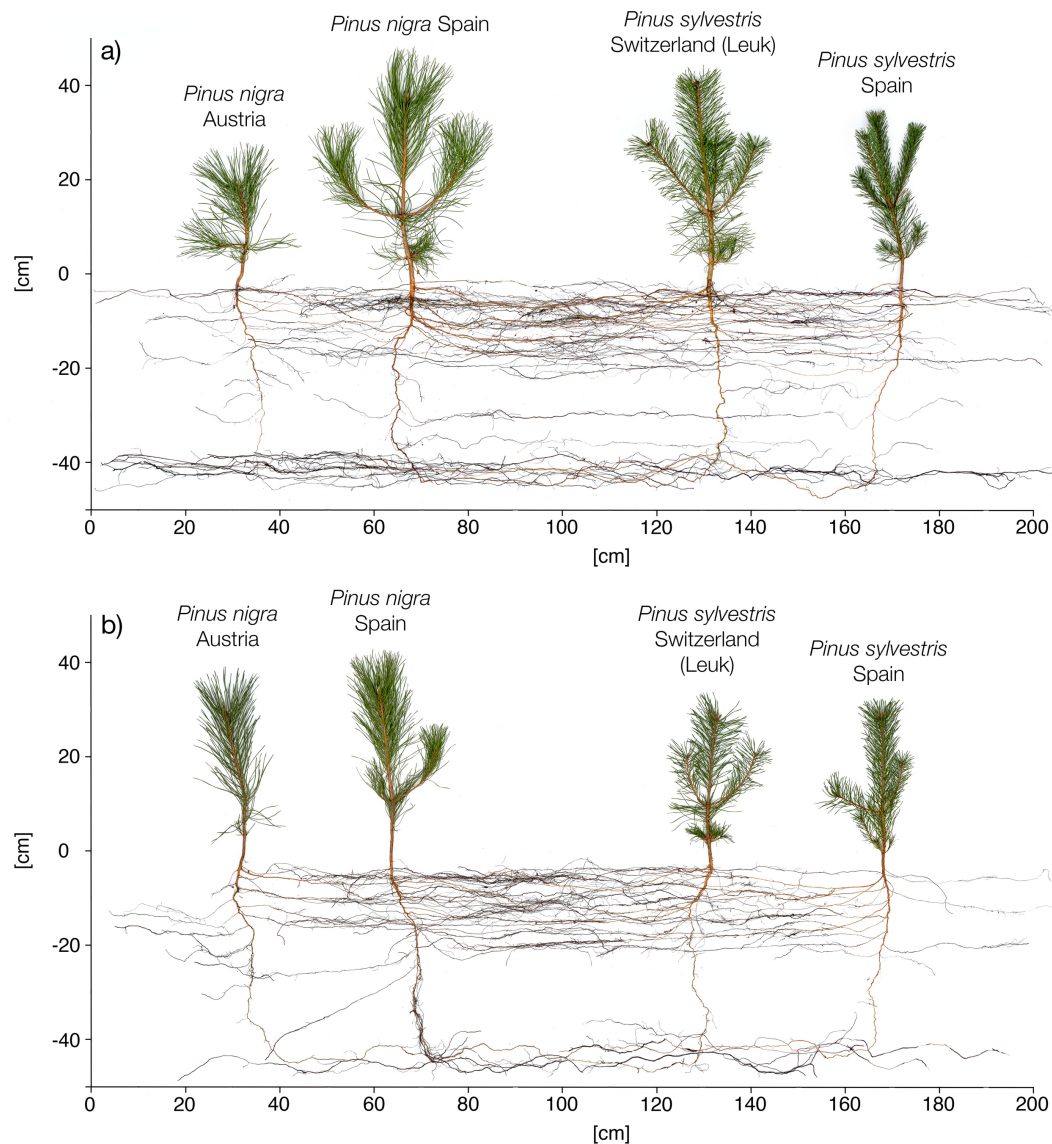
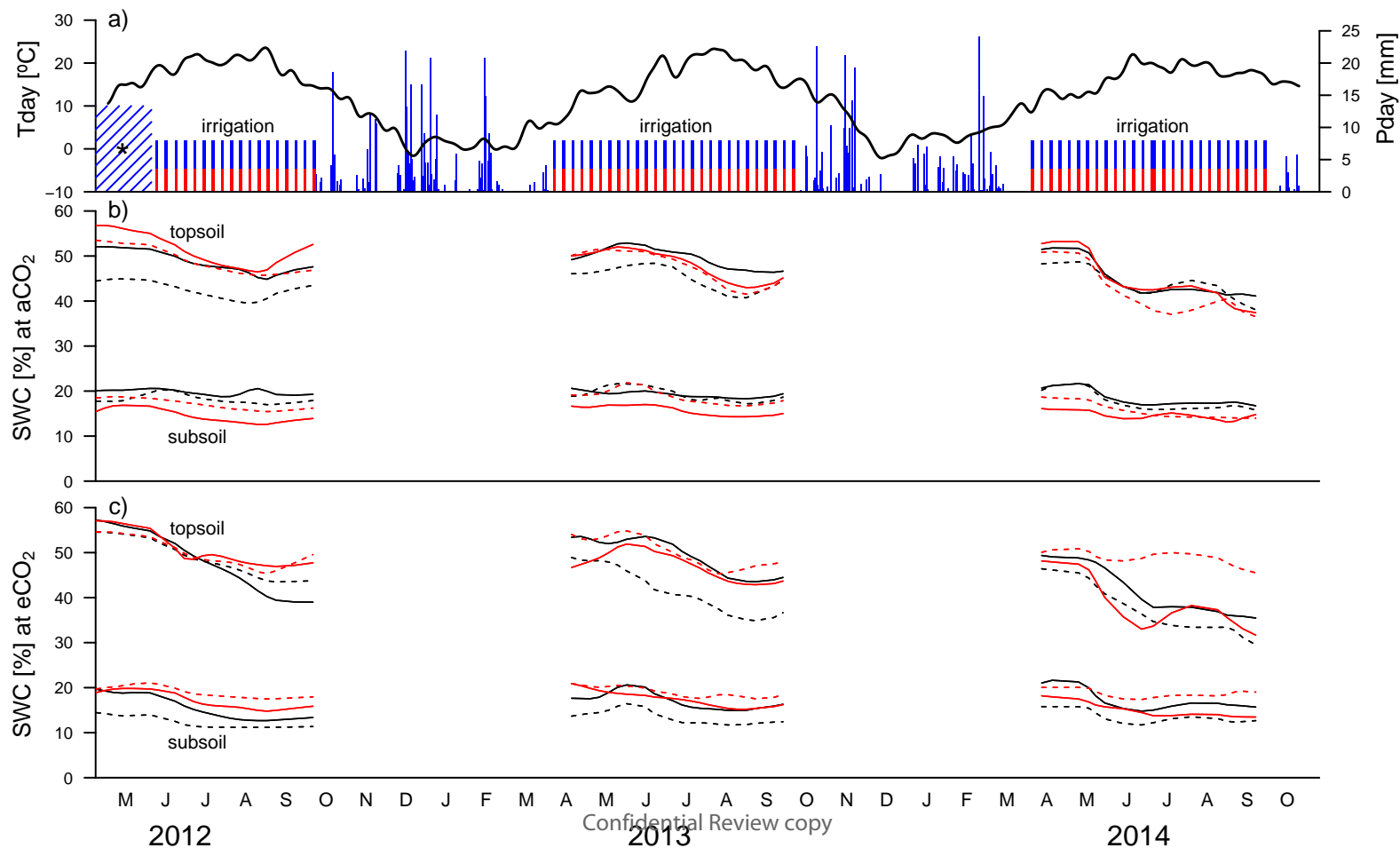
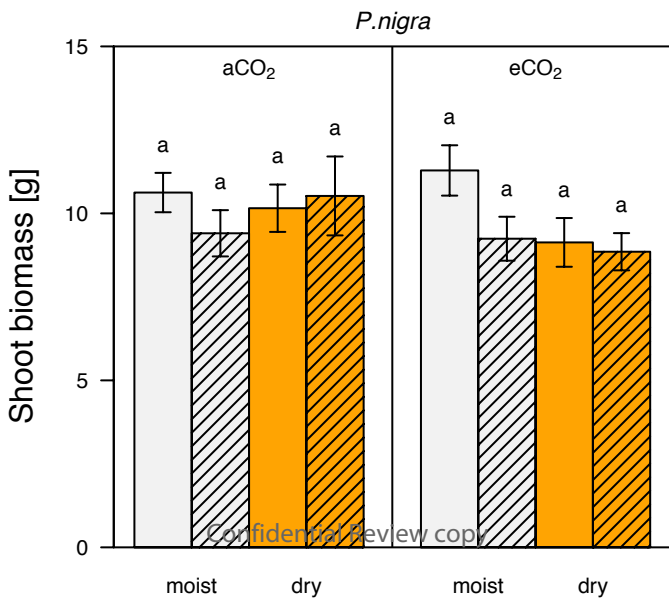
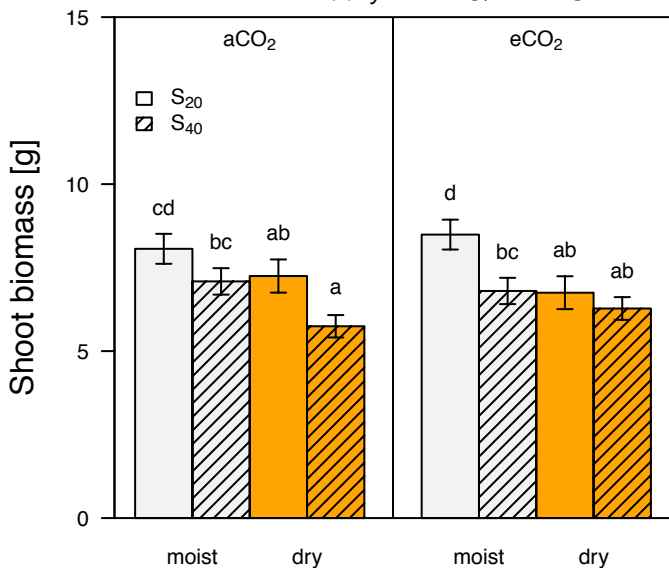
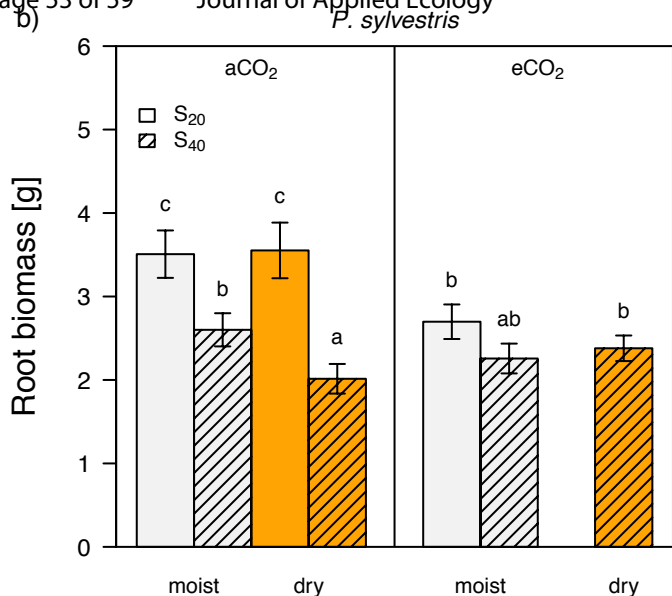
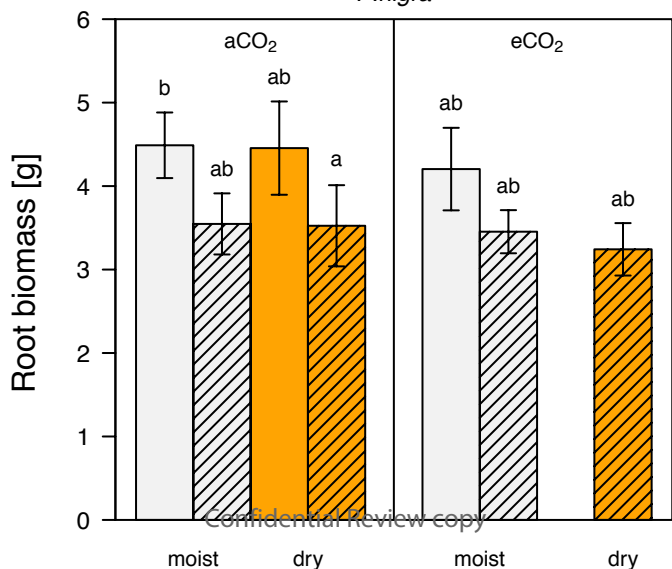


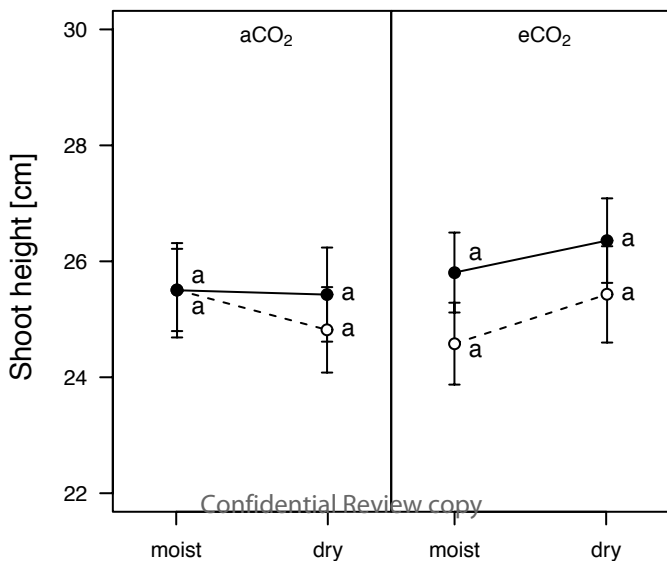
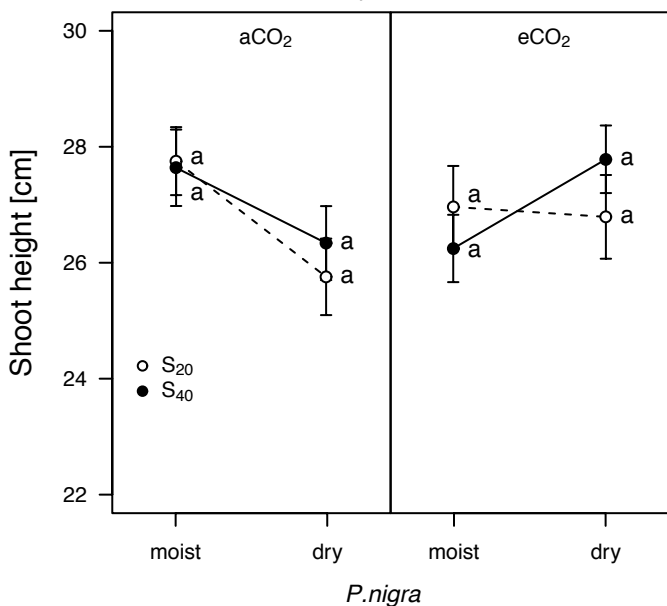
Fig S3. Photographs of a subset of *P. sylvestris* and *P. nigra* seedlings growing in the mesocosms under (a) moist \times S₂₀ \times aCO₂ and (b) moist \times S₄₀ \times aCO₂ conditions. The seedlings were excavated from mesocosms (surface: 200 cm \times 80 cm; height: 50 cm) of one randomly selected block at the end of the third growing season.

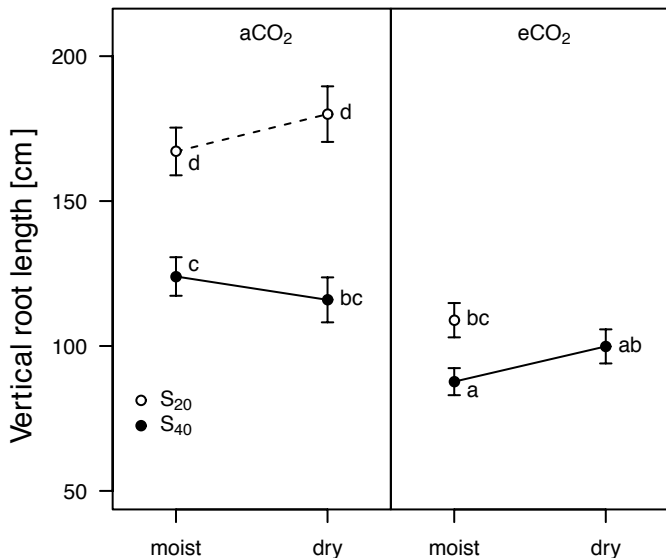
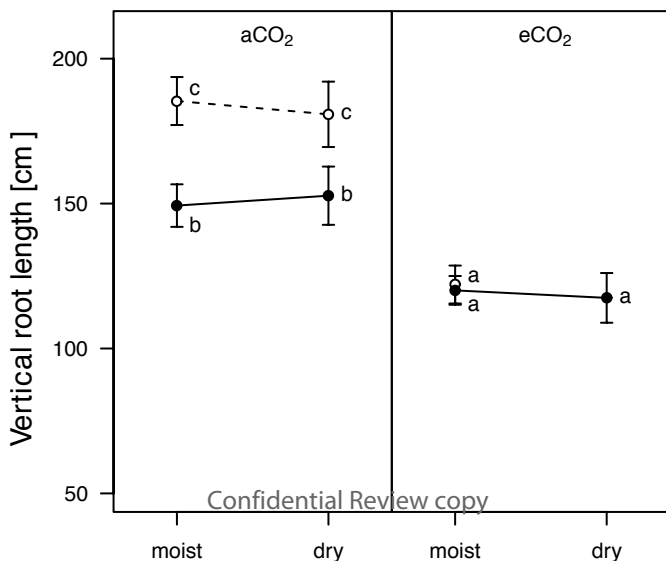


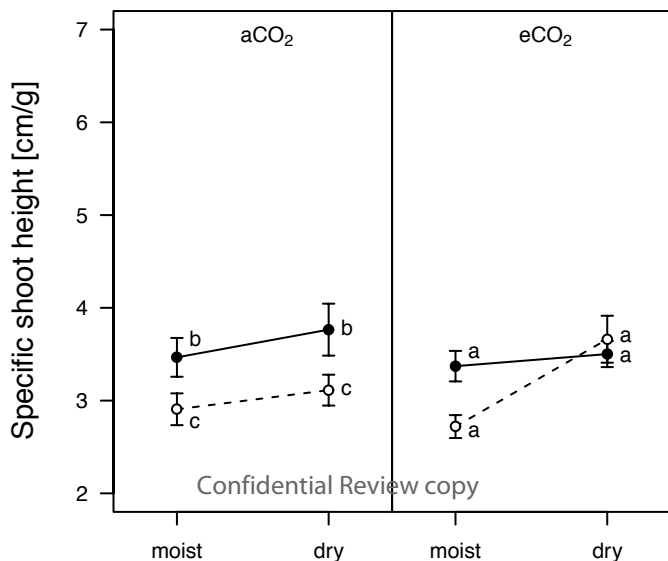
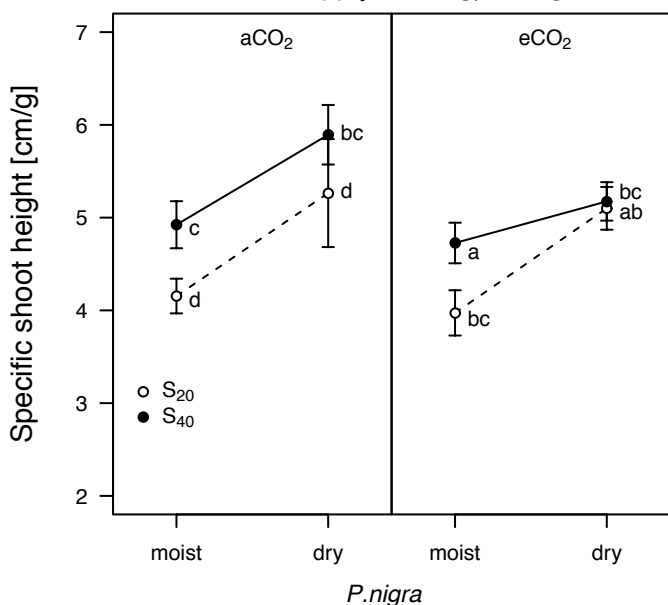


*P. nigra*

a)



*P. nigra*



b)

