STANDARD PAPER

No carbon "bet hedging" in pine seedlings under prolonged summer drought and elevated CO_2

Christoph Bachofen[1,2] (iD) | Barbara Moser[1] (iD) | Günter Hoch[3] | Jaboury Ghazoul[2] | Thomas Wohlgemuth[1] (iD)

[1]Forest Dynamics, Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland

[2]Department of Environmental Sciences, Swiss Federal Institute of Technology, Zürich, Switzerland

[3]Department of Environmental Sciences - Botany, University of Basel, Basel, Switzerland

Correspondence
Christoph Bachofen
Email: christoph.bachofen@usys.ethz.ch

Abstract

1. More frequent drought episodes are expected to cause higher mortality in isohydric tree species such as pines, because individuals close their stomata early during drought in order to maintain constant needle water potentials. It has been suggested that trees delay the ensuing carbon starvation by actively storing carbon at the expense of growth ("bet hedging"). Because such a strategy is only adaptive in drought-prone regions, we hypothesise that the degree of carbon "bet hedging" should differ between ecotypes.

2. We repeatedly measured the allocation of biomass, starch and soluble sugars to needles, stem and roots in seedlings of nine populations of *Pinus sylvestris* and *Pinus nigra* along a gradient from Central Europe to the Mediterranean. During two consecutive growing seasons, seedlings grown from seed were exposed to factorial combinations of 4 months of drought (D1, D2) and ambient/elevated CO_2 (aCO_2/eCO_2).

3. Drought-stressed pine seedlings did neither increase starch concentrations, nor change biomass production or experience lower mortality under eCO_2 compared to aCO_2. By the end of D2, seedlings from drier origin had accumulated more starch but at the same time also more biomass than seedlings from wetter origin.

4. Surprisingly, seedlings acclimatised to dry conditions after D1 so that mortality dropped to zero and drought effects on needle starch (*P. sylvestris*) and overall starch (*P. nigra*), respectively, disappeared after D2.

5. *Synthesis.* The absence of a trade-off between carbon storage (starch) and growth (biomass), and the patterns of mortality observed in seedlings growing under combined drought and eCO_2 do not support the theory of carbon "bet hedging" in isohydric *Pinus sylvestris* and *Pinus nigra*. Results suggest that reduced growth and acclimatisation minimised seedling mortality in the second year. Acclimatisation might thus enable pine seedlings to resist a moderate increase in summer drought frequency expected in the future.

KEYWORDS
C-allocation, C-storage, CO_2, drought mortality, ecophysiology, non-structural carbohydrates, *P. nigra*, *P. sylvestris*, stomatal conductance

1 | INTRODUCTION

An increase in the frequency and duration of drought has been identified as principle factor for tree growth declines (Lindner et al., 2014), tree mortality (Anderegg, Kane, & Anderegg, 2013; Rigling et al., 2013) and impeded tree regeneration (Cochrane, Hoyle, Yates, Wood, & Nicotra, 2014) in many areas world-wide. Drought-induced stomatal closure and the consequential reduction of carbon assimilation may lead to insufficient carbohydrate supply for metabolism (carbon starvation), causing directly or indirectly tree death (McDowell et al., 2008). The carbon starvation hypothesis has raised a debate about the physiological mechanisms that link the storage of non-structural carbohydrates (NSC) with tree growth and mortality (Sala, Piper, & Hoch, 2010; Sala, Woodruff, & Meinzer, 2012; Wiley & Helliker, 2012). Isohydric species, including *Pinus*, which maintain relatively constant needle water potentials even during periods of water stress by reducing stomatal conductance (Roman et al., 2015), are expected to be at a higher risk of carbon starvation than anisohydric species such as juniper or oak, which have more variable leaf water potentials and maintain higher photosynthetic rates for longer during periods of water shortage (McDowell et al., 2008; Zweifel, Rigling, & Dobbertin, 2009). This expectation is supported by NSC decline in isohydric tree species subjected to prolonged drought (Hartmann, Ziegler, & Trumbore, 2013; Mitchell et al., 2013; Piper, 2011; Sevanto, McDowell, Dickman, Pangle, & Pockman, 2014). Some isohydric tree species have also, however, been observed to increase NSC concentrations under drought stress (see review in Duan et al., 2015; Gruber, Pirkebner, Florian, & Oberhuber, 2012; Muller et al., 2011), indicating that carbon allocation and storage of isohydric species during drought is not fully understood, yet.

There is still uncertainty about a minimum critical NSC threshold for tree survival (Martínez-Vilalta et al., 2016; Sala et al., 2012), and about how changes in NSC concentrations progress during prolonged drought (McDowell et al., 2011). Drought-induced increase in NSC might indicate carbon sink limitation due to nutrient or water deficiencies rather than impeded photosynthesis (Hsiao & Acevedo, 1974; Körner, 2003; Muller et al., 2011; Sala et al., 2010). Alternatively, NSC accumulation in drought-stressed trees could result from active carbon storage at the cost of reduced growth (Dietze et al., 2014; Sala et al., 2012; Wiley & Helliker, 2012), which would reduce the risk of carbon starvation during prolonged drought (O'Brien, Leuzinger, Philipson, Tay, & Hector, 2014; Wiley & Helliker, 2012) and enable faster recovery after drought (Piper, 2011; Yang, Huang, Zhang, & Cornelissen, 2015). Although higher NSC concentrations might lower the risk of drought-induced mortality, the concomitant reduction of growth also compromises the competitive ability of individual trees. Consequently, active carbon storage at the expense of growth might constitute a "bet hedging" strategy (Richardson et al., 2013; Yang et al., 2015). In view of the costs to growth of such a strategy, species and populations from regions with varying drought exposure risk should differ in the extent to which they actively store carbon (Wiley & Helliker, 2012; Yang et al., 2015). We thus hypothesise that populations of tree species from regions with prolonged summer drought are more likely to follow a "bet hedging" strategy, i.e. having higher carbon storage at the cost of reduced growth, while populations from wetter regions would preferably invest in growth and thus in their competitive ability.

A possibility to study effects of drought on carbon storage, growth and mortality is to subject trees to a combination of elevated atmospheric CO_2 (eCO_2) and drought conditions (Fatichi, Leuzinger, & Körner, 2014; McDowell, 2011). If drought limits sink activities, NSC concentrations will increase and eCO_2 will increase NSC levels still further, without any effect on growth or mortality. If, on the other hand, drought limits carbon sources, eCO_2 should mitigate drought effects by allowing sustained growth and reduced drought-induced mortality (Körner, 2003). In a "bet hedging" strategy, drought-stressed trees should reduce growth to save carbon even under eCO_2, which in turn should diminish mortality. Patterns of NSC allocation under combined drought and eCO_2 might therefore reveal climate related strategies of carbohydrate transport and utilisation.

We assessed whether isohydric pine species follow a carbon "bet hedging" strategy by studying whole plant carbon allocation in *Pinus sylvestris* and *Pinus nigra* seedlings. One-year-old seedlings from different climatic origin were exposed to factorial combinations of elevated CO_2 and 4 months of drought during two consecutive growing seasons. Different levels of summer precipitation and temperatures at the seed origin allowed to analyse the effect of ecotypic differentiation on carbon storage and growth during drought. Seedling biomass, starch and soluble sugar concentrations in needles, stems and roots were tracked seasonally during the two consecutive years. Soluble, low molecular weight sugars are used for metabolism and osmoregulation, while osmotically inactive starch serves solely for storage (Hoch, Richter, & Körner, 2003). We propose that the theory of carbon "bet hedging" is supported if all of the following patterns of carbon storage, seedling growth and mortality are detected:

- Drought-stressed seedlings store more carbon and have thus higher starch concentrations under eCO_2 than ambient CO_2 (aCO_2; premise 1).
- Drought-stressed seedlings do not grow more under eCO_2 than aCO_2 (premise 2).
- Drought-stressed seedlings experience less mortality under eCO_2 than aCO_2 (premise 3).

Assuming that "bet hedging" is adaptive, i.e. is an inherited, constitutive trait, we further propose that:

- Seedlings from drier origins grow less but accumulate more NSC than those from wetter origin irrespective of experimental conditions (premise 4).

2 | MATERIALS AND METHODS

2.1 | Species, populations and study site

Pinus sylvestris L. is a widespread species in forests ranging from Siberia to mountain ranges in the Mediterranean and showing

local adaptation to drought in southern populations (Richter et al., 2012). *Pinus nigra* Arnold thrives discontinuously in the northern Mediterranean and is regarded as more drought-tolerant than *P. sylvestris* (Richter et al., 2012). Seeds from five populations of *P. sylvestris* and four populations of *P. nigra* were collected in winter 2011/2012 (Figure S1). In each population, seeds from five maternal lineages were collected in order to standardise the amount of genetic variation. *Pinus sylvestris* populations were located at two Central Alpine locations in Switzerland, one at low and one at high elevation, as well as in Mediterranean regions in Spain, Greece and Bulgaria, following a gradient of decreasing climatic water balances during the driest month of the year. A negative water balance in the summer months is associated with higher drought-tolerances in *P. sylvestris* populations (Seidel, Schunk, Matiu, & Menzel, 2016). We therefore used the climatic water balance at the seed origin as an indicator for the degree of drought adaptation of the seed sources (Table 1). Cones of three Mediterranean *P. nigra* populations were collected at locations in close distance to the Mediterranean *P. sylvestris* populations. The fourth population originates from an Eastern Alpine location that corresponds to the northernmost natural occurrence of the species. The experiment was set up in a common garden in a Central Alpine valley near Leuk (Valais, Switzerland; 46°18′33″N, 07°41′10″E; 610 m a.s.l.), a region with a marked climatic water deficit and low relative humidity during the growing season (19.2°C average temperature in July; 603 mm annual precipitation, measured at the MeteoSwiss station in Sion, 1950–2000, −73.4 mm water deficit during the driest month of the year; Table 1).

2.2 | Experimental design and treatments

In February 2012, 12 mesocosms (surface: 200 cm × 80 cm; height: 50 cm) were filled with 30 cm of sand and gravel from the local Rhone riverbed (subsoil), topped by 15 cm of humus (Oekohum GmbH, Herrenhof, Switzerland; topsoil), simulating natural forest soils of the Rhone valley (Moser, Bachofen, Müller, Metslaid, & Wohlgemuth,

2016). The mesocosms were arranged in a split–split plot design with three blocks (whole-plots), two sub-blocks (split plots) with ambient and elevated CO_2, respectively, and two mesocosms per sub-block (split–split plots) accommodating two levels of a drought treatment (Figure S2a). The CO_2 treatment consisted of the two levels "ambient CO_2" (aCO_2: 390 ppm) and "elevated CO_2" (eCO_2: target concentration of 570 ppm); the latter was applied from June to September in 2012, and from March to October in 2013 and 2014. The eCO_2 simulated the predicted concentration in 2100 assuming an increment of 20 ppm per decade (www.esrl.noaa.gov/gmd/ccgg/trends) and represents roughly twice the preindustrial CO_2 concentration. Instead of the hexagonal arrangement used in free-air CO_2 enrichment (FACE) experiments, we chose a rectangular arrangement of the CO_2 injection tubes that fitted the shape of the mesocosms. Otherwise the same FACE equipment as in Hättenschwiler, Handa, and Egli (2002) was used. To reduce costs, CO_2 release was interrupted when conditions were unfavourable for photosynthesis (air temperature <5°C, photon flux density <30 μmol m^{-2} s^{-1} and air temperature >35°C) or wind speed exceeded 2 m/s. The region where the common garden was located has a distinct diurnal wind regime. During summer months, winds prevail between 1100 and 1700 h, while the rest of the day is calm. Thus, CO_2 release was not affected by wind during the daily peak of photosynthetic activity in the morning hours (Figure 2b, July). Concurrently with the CO_2 treatment, automatic mobile rain shelters intercepted the natural rainfall and all mesocosms were irrigated weekly on two subsequent nights to a level of 16 mm, i.e. 416 mm from March to September (Moser, Bachofen, et al., 2016). This corresponds to the top decile of natural rainfall at low elevations in the Rhone valley simulating conditions of no water stress (151% of the average March–September precipitation, measured from 1864 to 2011 at the MeteoSwiss station in Sion located 28 km to the East of the study site). A drought treatment ("dry-out") was applied to one randomly selected mesocosm in each sub-block by completely stopping irrigation from June to October in 2013 (D1) and in 2014 (D2). Soil water content (v/v; SWC) was recorded in all treatments with

TABLE 1 Seed origin, respective precipitation sums and climatic water balance of the driest month of the populations sowed in the common garden at Leuk (Switzerland). 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 of driest month (mm)
Pinus sylvestris	Central Alpine	1. Visperterminen (Switzerland)	1,363	46.27	7.91	90	−13.8
	Mediterranean	2. Jundola (Bulgaria)	1,405	42.05	23.83	35	−51.9
	Mediterranean	3. Serres (Greece)	1,333	41.24	23.58	29	−71.9
	Central Alpine	4. Leuk (Switzerland)	570	46.29	7.61	49	−73.4
	Mediterranean	5. Ademuz (Spain)	1,542	40.08	−1.08	34	−77.7
Pinus nigra	East Alpine	6. Bad Fischau (Austria)	344	47.83	16.13	36	−26.1
	Mediterranean	7. Dobrostan (Bulgaria)	1,167	41.90	24.93	40	−56.6
	Mediterranean	8. Ademuz (Spain)	1,195	40.09	−1.38	26	−98.4
	Mediterranean	9. Parthenonas (Greece)	644	40.13	23.86	18	−113.9

EC-5 soil moisture sensors (Decagon Devices, Pullman, WA, USA), which were installed both in the topsoil (at 5 cm depth) and the subsoil (40 cm depth). Due to the different textures of the topsoil and the subsoil, SWC does not correspond to plant available water. The permanent wilting point (PWP) of organic soil (topsoil) lies above 20% SWC (Zuber, 2007) but is only between 2% and 4% in sandy/gravelly substrate (subsoil; Singer & Munns, 2006). Each mesocosm was divided into two halves, with 70 squares of 10 cm × 10 cm per half. In March 2012, 15 seeds per species, population (five *P. sylvestris* and four *P. nigra* populations) and maternal lineage were sown in randomly selected squares (9 populations × 5 maternal lineages = 45 squares) in each half of the mesocosms (Moser, Bachofen, et al., 2016). This resulted in two replicates per maternal lineage per mesocosm (2 × 45 squares = 90 squares) and hence 10 replicates per population per mesocosm. The remaining 2 × 25 squares in each mesocosm were sowed with other conifers that were not part of this study (for details, see Figure S2b). Until May 2012, the soil was watered daily to facilitate germination.

2.3 | Seedling emergence and mortality

Emergence of the seeds in the mesocosms was recorded in June 2012 and, to even out seedling densities, the number of seedlings per square was reduced to four in April 2013 by randomly removing seedlings. Due to the destructive nature of the NSC measurements (see below), seedling density decreased to three in May 2013, two in October 2013 and to one in May 2014. Seedling survival was determined in all squares in April 2013 by counting the number of living seedlings, defined as individuals with at least one green needle. In May and September 2013, we only recorded the number of living seedlings in "dry-out" mesocosms; in May 2014 and September 2014, all mesocosms were assessed. Seedling mortality was calculated as the difference in number of living seedlings between two dates, taking into account the number of seedlings removed since the last count as result of thinning or harvesting. As thinning and seedling harvests were carried out randomly, effects of seedling removal on mortality counts are considered random too.

2.4 | Biomass and NSC

In May and September 2013 and 2014 (Figure 1), we randomly chose one of the two squares of each species, population and maternal lineage per mesocosm and uprooted one randomly selected seedling. Only living seedlings were harvested for biomass and NSC measurements. Due to the destructive sampling for NSC measurements, mortality could not be assessed for the same individuals thereafter. We washed
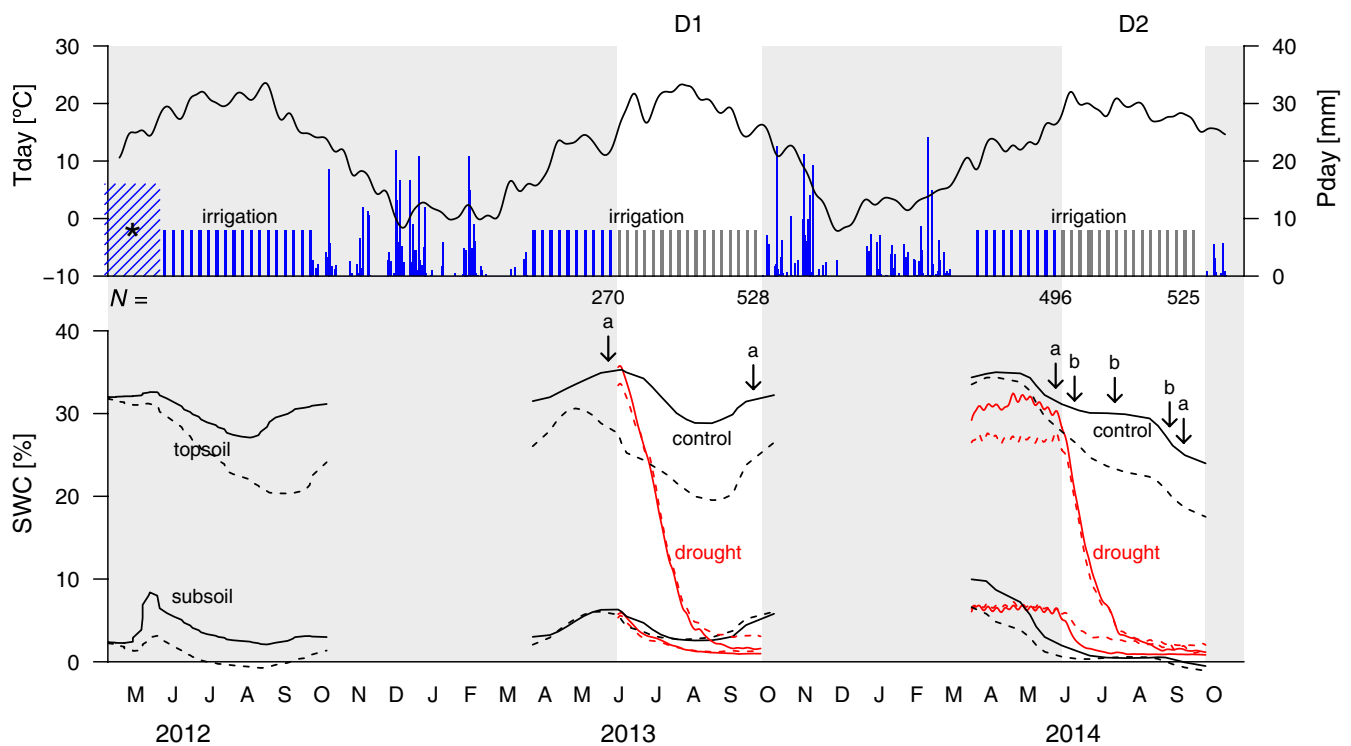

FIGURE 1 Daily air temperatures (Tday), natural precipitation/irrigation (Pday) at the experimental site (top) and volumetric soil water content (SWC) in the mesocosms during the experiment (bottom). D1 and D2 indicate the periods of drought treatments (4 months in 2013 and 2014, respectively). SWC is displayed for the topsoil (5 cm depth) and the subsoil (40 cm depth) of "moist" and "dry-out" mesocosms (black and red lines) and aCO_2 and eCO_2 (solid lines and dashed lines). The permanent wilting point is approximately 20% SWC for the topsoil and between 2% and 4% SWC for the subsoil. The amount of irrigation is depicted instead of natural precipitation when rain shelters were in operation. The asterisk denominates the period of daily watering until seedling emergence. Arrows refer to the biomass and non-structural carbohydrates samplings (a) and measurements of stomatal conductance (b)

the roots and shock heated the seedlings within 1 hr of uprooting with microwaves to stop enzymatic activity (two pulses of 20 s, 700 W; Popp et al., 1996). The five maternal lineages per population in every mesocosm were pooled in order to obtain enough dry biomass for NSC analyses in May 2013. This sampling scheme was continued throughout the experiment and resulted in 108 pooled samples per season (9 populations × 2 drought treatments × 2 CO_2 treatments × 3 replicates). Hence, biomass and NSC analyses were both performed on the population level, without considering maternal lineages. After drying the seedlings for 72 hr at 60°C to constant weight, we separated them into four parts: roots, green needles, brown needles and stem (including twigs and buds) and measured the dry weight of each part. Needle biomass included brown and green needles, while NSC analyses were conducted on green needles only. After grinding the plant material with a ball mill (Retsch M200; Retsch, Haan, Germany), total NSC and soluble sugars (SS; sucrose, fructose and glucose) concentration of the roots, green needles and stem were measured photometrically according to Hoch, Popp, and Körner (2002), except that amyloglucosidase was used instead of clarase for starch digestion. Starch (St) concentration was calculated as the difference between NSC and SS.

2.5 | Stomatal conductance and pre-dawn water potential

We measured stomatal conductance (g_S) of 48 seedlings (2 species × 2 populations × 2 drought treatments × 2 CO_2 treatments × 3 replicates), on 25/26 June, 24 July and 11/12 September 2014 (Figure 1) using a SC-1 leaf porometer (Decagon Devices). In every mesocosm, we randomly marked one seedling of two *P. sylvestris* and two *P. nigra* populations and used the same seedling for all measurements in that year. For *P. nigra*, we selected the population from the driest Mediterranean location (Greece) and the least dry East Alpine location (Austria). For *P. sylvestris*, we selected a Mediterranean population from the same region as for *P. nigra* (Greece) and the autochthonous population (Leuk, Switzerland). At each date, g_S was recorded over 24 hr at 2-hr intervals from 1500 h to sunset (*c.* 2100 h) and from sunrise (*c.* 0700 h) to 1300 h on the following day, except on 24 July when g_S was recorded between 0700 and 0900 h of the same day. We always attached the sensor head of the porometer to sections in the middle of the needle laminae, making sure that the entire area of the diffusion path was covered with needles. Consequently, normalisation for leaf area was not necessary (http://manuals.decagon.com/Manuals/10711_Leaf%25Porometer_Web.pdf). SC-1 leaf porometers start measurements automatically once relative humidity rises in the sensor head, which may happen before the sensor head is tightly attached to the needles. We made sure to abort and repeat the measurement process under these circumstances. Overall, precipitate measurements were rare as relative humidity is low in the study region. Analyses of needle oxygen isotope fractionation ($\partial^{18}O$) in September 2014, measured in the same needles as NSC, confirmed porometer results (Figure S3, Appendix S1).

In 2014, pre-dawn water potential (ψ_{stem}) was measured using a portable Scholander pressure chamber (M-600; PMS Instruments Inc.,

Corvallis, OR, USA; Scholand, Hammel, Bradstre, & Hemmings, 1965) on 3 June, 29 July and 10 September between 0100 and 0430 h. At each date, we randomly selected one seedling from each species and population per mesocosm (108 seedlings per date). Because seedlings had to be harvested for these measurements, only one seedling per block was measured and we were not able to use the same seedlings as for g_S. The seedlings were chosen in a Latin square design, i.e. a different maternal lineage was selected in each block. On each seedling, a randomly selected shoot from the current year was cut and measured within a minute.

2.6 | Data analysis

Effects of drought, CO_2 and populations on seasonal biomass, and St and SS concentrations were analysed separately for the two species and three plant parts with linear mixed-effect models using the statistics program R (R Development Core Team, 2016). Treatments and populations were considered as fixed effects, and the block as a random effect. Maternal lineages were pooled during harvest (see "Biomass and NSC") and thus not separately analysed. Multiple testing was corrected for with the "Hommel" method. In order to test how the degree of drought adaptation of individual populations affects seedling performance, we calculated the climatic water balance of the driest month at the seed origin according to Thornthwaite (1948) (precipitation–potential evapotranspiration). We used precipitation and temperature data (mean of 1950–2000) from the MeteoSwiss stations in Sion and Montana for the two Central Alpine populations in Switzerland (low elevation and high elevation), and interpolated precipitation and temperature data (mean of 1950–2000) from the WorldClim database (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) for the remaining populations. Seedling mortality across species was analysed with generalised linear mixed-effect models and binomial distribution, including species and treatments as fixed effects, and the block as a random effect. The daily cumulative g_S was estimated for each date by adding the area under the curve (i.e. the integrals of g_S over the time intervals) of the afternoon and subsequent morning measurements. g_S, daily cumulative g_S at each time interval and pre-dawn water potentials (ψ_{stem}) at each sampling date were analysed separately for the two species with linear mixed-effect models, analogous to seasonal biomass.

3 | RESULTS

3.1 | Soil moisture and physiological responses

The SWC in the "dry-out" mesocosms approached the estimated PWP in the topsoil (20%) sometime in July 2013 and June 2014, and in the subsoil (2%–4%) in August 2013 and July 2014 (Figure 1). Accordingly, pre-dawn water potentials (ψ_{stem}) of *P. sylvestris* and *P. nigra* seedlings were significantly lower in "dry-out" than in "moist" mesocosms from July to September 2014 (Figure 2a, Table S1). *Pinus sylvestris* "dry-out" seedlings reduced g_S already in June (Figure 2b), and later in July and September, as the drought continued, both *P. sylvestris* and *P. nigra*
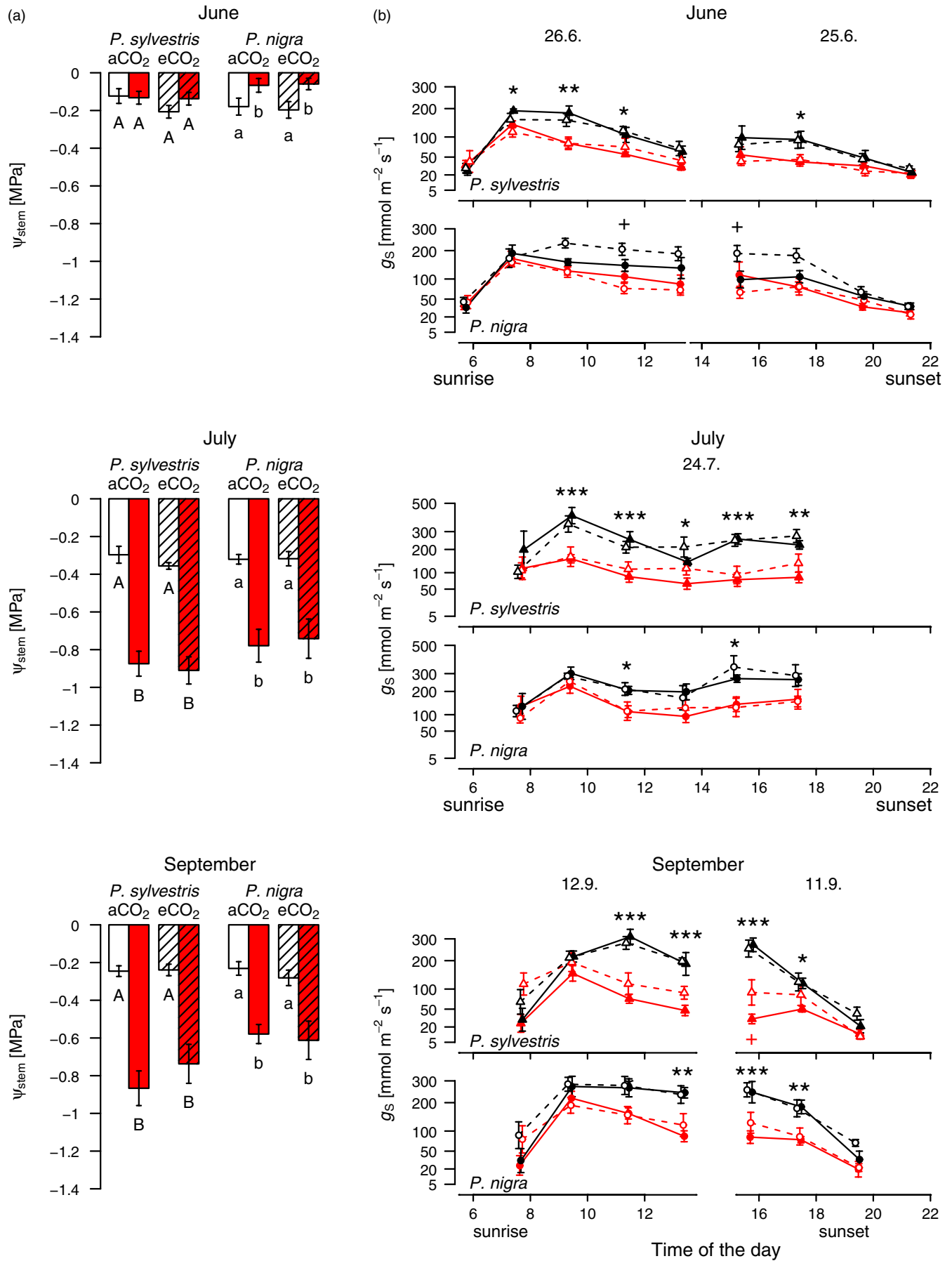
(a)

June

July

September

(b)

June

July

September

Time of the day

FIGURE 2 (a) Stem pre-dawn water potential (ψ_{stem}) of *Pinus sylvestris* (3 replications × 5 populations) and *Pinus nigra* (3 replications × 4 populations) seedlings in 2014 under factorial combinations of "moist" (white bars) and "dry-out" (red bars) conditions with ambient (aCO_2, unshaded bars) and elevated (eCO_2, shaded bars) atmospheric CO_2. Letters indicate significant differences between treatment combinations within species ($p < .05$). (b) Stomatal conductance (g_S) under the same conditions (solid lines: aCO_2; dashed lines: eCO_2), measured at 2-hr intervals between sunrise and sunset on 25 June (afternoon) and 26 June (morning), 24 July and on 11 September (afternoon) and 12 September (morning) 2014 on six seedlings per species and treatment combination. Significant hour-wise differences between "moist" and "dry-out" conditions (LMM): *$p < .05$, **$p < .01$ and ***$p < .001$; and between aCO_2 and eCO_2: +$p < .05$

"dry-out" seedlings severely lowered g_S around noon until late afternoon (Figure 2b). In both species and on all dates, the daily cumulative g_S was significantly smaller in "dry-out" than "moist" seedlings (Figure S4). Elevated CO_2 (eCO_2), on the other hand, did neither affect ψ_{stem} (Figure 2a) nor daily cumulative g_S (Figure S4). At individual time intervals of the day, g_S was largely unresponsive to eCO_2 as well, although g_S of "moist" *P. nigra* seedlings was higher under eCO_2 before noon and at early afternoon in June 2014 (drought × CO_2: $p < .05$), and "dry-out" *P. sylvestris* seedlings exhibited slightly higher g_S under eCO_2 at early afternoon in September 2014 (CO_2: $p < .05$; Figure 2b).

3.2 | NSC accumulation under combined drought and eCO_2

Soluble sugar (SS) concentrations were higher in all plant parts of both species, particularly in the roots, of "dry-out" compared to "moist" seedlings by the end of D1 (Tables 2 and 3, Figure 3). In *P. sylvestris*, this was also the case after D2, while in *P. nigra*, higher SS concentrations after D2 were restricted to roots (Figure 3). The first drought (D1) also led to an increase in stored carbon, i.e. starch concentrations, of "dry-out" compared to "moist" seedlings (September 2013, Tables 2 and 3, Figure 4). Under drought, both species exhibited higher starch concentrations in the needles (*P. sylvestris*: +184%, *P. nigra* +236%), *P. nigra* also in stem and roots (+91% and +39%). Surprisingly, starch concentrations did no longer differ between drought treatments after the second drought (D2; non-significant effect in September 2014 in both species, Tables 2 and 3), aside from an increased root starch concentration in "dry-out" *P. sylvestris* (+96%; Figure 4). Under eCO_2, SS concentrations in the roots of "moist" and "dry-out" *P. sylvestris* (significant main effect, Table 2), and "moist" *P. nigra* (significant CO_2 × drought interaction; Table 3) were higher than under aCO_2 (Figure 3). Similar to drought effects on starch concentrations, eCO_2 effects on SS concentrations had disappeared by the end of D2 (September 2014; Figure 3). By contrast, starch concentrations did not react to eCO_2, neither during D1 nor D2 (non-significant effect in September 2013 and 2014 in both species; Figure 4, Tables 2 and 3). As "dry-out" seedlings accumulated SS but not starch under eCO_2, their carbon storage pattern does not comply with a carbon "bet hedging" strategy (premise 1).

3.3 | Growth responses to drought and eCO_2

All "dry-out" seedlings produced substantially less biomass compared to "moist" seedlings by the end of D1, independent of the species (Tables 2 and 3, Figure 5). Slow growth continued during D2 and resulted in lower biomass production of all parts in both species by the

end of D2 (Figure 5). eCO_2, on the other hand, did not affect biomass production, or biomass allocation of "moist" and "dry-out" *P. sylvestris* and *P. nigra* (non-significant effect in September 2013 and 2014 in both species, Figure 5). Biomass differed considerably between populations (significant population effect in both species, all plant parts and seasons, Tables 2 and 3). In September 2014, extraordinary high biomass of one *P. nigra* population (Spain; climatic water balance −98.4 mm; Figure 6) in the "dry-out" treatment led to overlapping *SE* bars between "moist" × eCO_2 and "dry-out" × eCO_2 *P. nigra* seedlings (Figure 5) even though the interaction drought × CO_2 was not significant (Table 2). Similar growth of "dry-out" seedlings under aCO_2 and eCO_2 indicates that the surplus of atmospheric carbon is not used for growth, which is consistent with premise (2) of the carbon "bet hedging" theory.

3.4 | NSC and growth responses in relation to the seed origin

Biomass of both "moist" and "dry-out" seedlings was related to the climatic water balance of the driest month at the seed origin (Figure 6). The climatic water balance at the seed origin accounted for more than 50% of the variance in total seedling biomass, both in "dry-out" (D1: $r^2 = .69$, $p < .001$; D2: $r^2 = .57$, $p < .001$) and "moist" seedlings (D1: $r^2 = .65$, $p < .001$; D2: $r^2 = .62$, $p < .001$).

A relationship between starch concentrations and seed origin was only found in "moist" seedlings and only at the end of D2 ($r^2 = .45$, $p = .014$; Figure 6). As seedlings from drier origin concurrently accumulated more starch and biomass than seedlings from wetter origin under "moist" conditions, a trade-off between NSC accumulation and seedling growth was absent, contrary to premise (4).

3.5 | Drought and eCO_2 effects on mortality

By the end of D1, only 6 out of 540 "dry-out" seedlings were dead (1.1%). By May 2014 of the following season, another 9.4% of the "dry-out" seedlings died, while the mortality of "moist" seedlings amounted to only 0.6%. In summary, D1 had a significant effect on mortality ($p < .001$), whereas no effect resulted from the CO_2 treatment ($p > .1$). Species-specific differences were considerable, with *P. sylvestris* exhibiting higher mortality under drought (44 individuals, 14.7%) than *P. nigra* (7 individuals, 2.9%; $p < .001$). In contrast, D2 had no effect on seedling mortality of either species in any treatments. Seedlings from "dry-out" mesocosms exhibited a significantly higher needle browning (D1: up to 32%; D2: up to 7% of the total needle biomass) compared to "moist" seedlings (D1: up to 9%; D2: up to 5%; $p < .001$). No differences between species and CO_2 treatments were

TABLE 2 LMM results (F- and p-value) for the effects of drought (D), CO_2 (C) and population (P) on biomass, starch and soluble sugar (sucrose, fructose and glucose) concentrations in different plant parts of *Pinus sylvestris* seedlings in September 2013, May 2014 and September 2014. Significant effects ($p < .05$) are shown in bold

	Biomass			Starch			Soluble sugars		
	September 2013	May 2014	September 2014	September 2013	May 2014	September 2014	September 2013	May 2014	September 2014
Needles									
D	**49.35 (<0.001)**	**53.73 (<0.001)**	**156.61 (<0.001)**	**73.79 (<0.001)**	**78.63 (<0.001)**	6.09 (0.073)	**47.07 (<0.001)**	**7.73 (0.025)**	**28.96 (<0.001)**
C	2.84 (0.697)	0.01 (0.944)	4.57 (0.312)	1.28 (0.852)	3.38 (0.516)	0.64 (0.944)	5.95 (0.137)	1.83 (0.735)	1.76 (0.771)
P	**24.29 (<0.001)**	**7.03 (<0.001)**	**18.65 (<0.001)**	1.21 (0.646)	2.74 (0.128)	3.07 (0.098)	3.19 (0.080)	3.04 (0.087)	1.84 (0.282)
D × C	1.28 (0.952)	0 (0.952)	0.02 (0.952)	0.78 (0.835)	7.41 (0.078)	0.04 (0.835)	0.03 (0.947)	0.6 (0.947)	0.18 (0.947)
D × P	0.44 (0.973)	2.84 (0.330)	0.47 (0.973)	0.43 (0.929)	0.21 (0.929)	0.7 (0.929)	0.57 (0.972)	0.53 (0.972)	0.14 (0.972)
C × P	0.61 (0.779)	0.53 (0.779)	1.26 (0.779)	0.75 (0.957)	0.89 (0.957)	1.15 (0.957)	0.41 (0.951)	0.29 (0.951)	0.63 (0.951)
Stem									
D	**113.61 (<0.001)**	**103.2 (<0.001)**	**311.63 (<0.001)**	**42.11 (<0.001)**	0.56 (0.457)	2.05 (0.32)	**131.15 (<0.001)**	1.03 (0.316)	**98.27 (<0.001)**
C	0.54 (0.944)	0.11 (0.944)	5.32 (0.213)	3.87 (0.452)	0.84 (0.944)	0 (0.944)	5.34 (0.184)	0.52 (0.893)	2.43 (0.510)
P	**24.09 (<0.001)**	**8.18 (<0.001)**	**10.09 (<0.001)**	0.33 (0.853)	**4.23 (0.044)**	**5.46 (0.013)**	4.2 (0.052)	2.79 (0.120)	2.95 (0.097)
D × C	0.45 (0.952)	0.13 (0.952)	1.53 (0.952)	7.46 (0.076)	0.05 (0.835)	3.59 (0.392)	2.53 (0.719)	1.25 (0.947)	0 (0.947)
D × P	0.12 (0.973)	1.69 (0.938)	0.48 (0.973)	0.37 (0.929)	2.26 (0.643)	1.04 (0.929)	0.56 (0.972)	0.55 (0.972)	0.13 (0.972)
C × P	0.88 (0.779)	0.5 (0.779)	1.47 (0.779)	0.96 (0.957)	0.27 (0.957)	0.16 (0.957)	0.22 (0.951)	0.17 (0.951)	0.33 (0.951)
Roots									
D	**125.4 (<0.001)**	**57.58 (<0.001)**	**120.83 (<0.001)**	4.16 (0.145)	5.88 (0.081)	**36.79 (<0.001)**	**276.42 (<0.001)**	**6.09 (0.036)**	**162.47 (<0.001)**
C	0.17 (0.944)	0.27 (0.944)	1.37 (0.944)	1.1 (0.903)	2.51 (0.642)	0.22 (0.944)	**11.83 (0.013)**	0.02 (0.893)	0.27 (0.893)
P	**13.25 (<0.001)**	**8.86 (<0.001)**	**9.11 (<0.001)**	2.64 (0.147)	2.81 (0.116)	4.09 (0.052)	2.98 (0.094)	0.58 (0.682)	3.14 (0.080)
D × C	1.48 (0.952)	0.06 (0.952)	0.4 (0.952)	1.73 (0.767)	3.14 (0.423)	2.11 (0.617)	1.59 (0.947)	1.2 (0.947)	3.91 (0.498)
D × P	0.96 (0.973)	1.4 (0.973)	0.8 (0.973)	3.92 (0.083)	0.6 (0.929)	0.53 (0.929)	0.26 (0.972)	0.66 (0.972)	0.14 (0.972)
C × P	0.44 (0.779)	0.57 (0.779)	1.47 (0.779)	1.21 (0.957)	0.25 (0.957)	0.79 (0.957)	0.61 (0.951)	0.7 (0.951)	1.2 (0.951)

TABLE 3 LMM results (F- and p-value) for the effects of drought (D), CO_2 (C) and population (P) on biomass, starch and soluble sugar (sucrose, fructose and glucose) concentrations in different plant parts of *Pinus nigra* seedlings in September 2013, May 2014 and September 2014. Significant effects ($p<.05$) are shown in bold

|	Biomass			Starch			Soluble sugars		
	September 2013	May 2014	September 2014	September 2013	May 2014	September 2014	September 2013	May 2014	September 2014
Needles									
D	**18.8 (0.001)**	**8.12 (0.016)**	**34.75 (<0.001)**	**112.76 (<0.001)**	**39 (<0.001)**	4.31 (0.184)	**77.03 (<0.001)**	1.84 (0.451)	0.99 (0.451)
C	1.02 (0.821)	1.77 (0.730)	0.96 (0.821)	4.73 (0.223)	5.63 (0.145)	2.08 (0.637)	8.86 (0.051)	1.76 (0.588)	0.57 (0.765)
P	**52.05 (<0.001)**	**22.03 (<0.001)**	**67.46 (<0.001)**	**14.11 (<0.001)**	**23.52 (<0.001)**	**7 (0.005)**	**18.97 (<0.001)**	**5.45 (0.021)**	**4.95 (0.033)**
D × C	0 (0.948)	2.11 (0.831)	2 (0.836)	1.94 (0.915)	0.1 (0.915)	0.12 (0.915)	2.79 (0.258)	5.42 (0.161)	3.82 (0.229)
D × P	1.46 (0.853)	1.62 (0.825)	0.26 (0.853)	2.47 (0.64)	3.73 (0.194)	0.25 (0.937)	2.85 (0.488)	1.18 (0.822)	1.07 (0.822)
C × P	0.3 (0.909)	0.75 (0.909)	3.44 (0.199)	0.9 (0.977)	0.91 (0.977)	0.07 (0.977)	0.64 (0.910)	0.51 (0.91)	0.69 (0.910)
Stem									
D	**51.17 (<0.001)**	**53.23 (<0.001)**	**72.54 (<0.001)**	**72.87 (<0.001)**	**23.71 (<0.001)**	1.32 (0.519)	**23.31 (<0.001)**	0.58 (0.451)	4.36 (0.227)
C	0.37 (0.824)	1.49 (0.730)	1.61 (0.730)	6.92 (0.097)	**16.21 (0.003)**	0.04 (0.849)	0.09 (0.765)	1.14 (0.687)	2.57 (0.490)
P	**39.42 (<0.001)**	**32.3 (<0.001)**	**56.76 (<0.001)**	**5.13 (0.022)**	1.88 (0.399)	**6.43 (0.008)**	3 (0.092)	**6.66 (0.010)**	1.01 (0.404)
D × C	0.24 (0.948)	0.8 (0.948)	4.15 (0.399)	0.5 (0.915)	6.03 (0.181)	2.8 (0.624)	1.96 (0.343)	**11.58 (0.015)**	0.68 (0.417)
D × P	1.41 (0.853)	1.78 (0.687)	1.84 (0.661)	0.97 (0.937)	0.88 (0.937)	1.55 (0.937)	0.47 (0.822)	0.44 (0.822)	2.16 (0.822)
C × P	0.28 (0.909)	0.54 (0.909)	4.05 (0.114)	0.25 (0.977)	0.66 (0.977)	0.95 (0.977)	0.32 (0.910)	0.99 (0.910)	0.18 (0.910)
Roots									
D	**37.53 (<0.001)**	1.93 (0.175)	**7.3 (0.022)**	**10.02 (0.018)**	0.23 (0.635)	1.37 (0.501)	**186.2 (<0.001)**	0.98 (0.451)	**41.41 (<0.001)**
C	0.05 (0.824)	0.71 (0.821)	2.78 (0.609)	0.43 (0.849)	3.16 (0.398)	0.31 (0.849)	1.77 (0.588)	1.64 (0.628)	6.7 (0.118)
P	**32.15 (<0.001)**	**46.08 (<0.001)**	**53.09 (<0.001)**	0.48 (0.697)	**6.75 (0.006)**	1.39 (0.531)	3.62 (0.069)	**12.23 (<0.001)**	3.72 (0.065)
D × C	0.68 (0.948)	0.08 (0.948)	4.21 (0.388)	0.03 (0.915)	0.01 (0.915)	3.42 (0.521)	**9.15 (0.035)**	**24.72 (<0.001)**	2.54 (0.258)
D × P	0.45 (0.853)	0.52 (0.853)	1.39 (0.853)	1.48 (0.937)	0.17 (0.937)	0.14 (0.937)	0.55 (0.822)	0.3 (0.822)	0.87 (0.822)
C × P	0.67 (0.909)	0.18 (0.909)	3.67 (0.156)	0.25 (0.977)	0.91 (0.977)	0.35 (0.977)	0.19 (0.910)	0.78 (0.910)	1.41 (0.910)
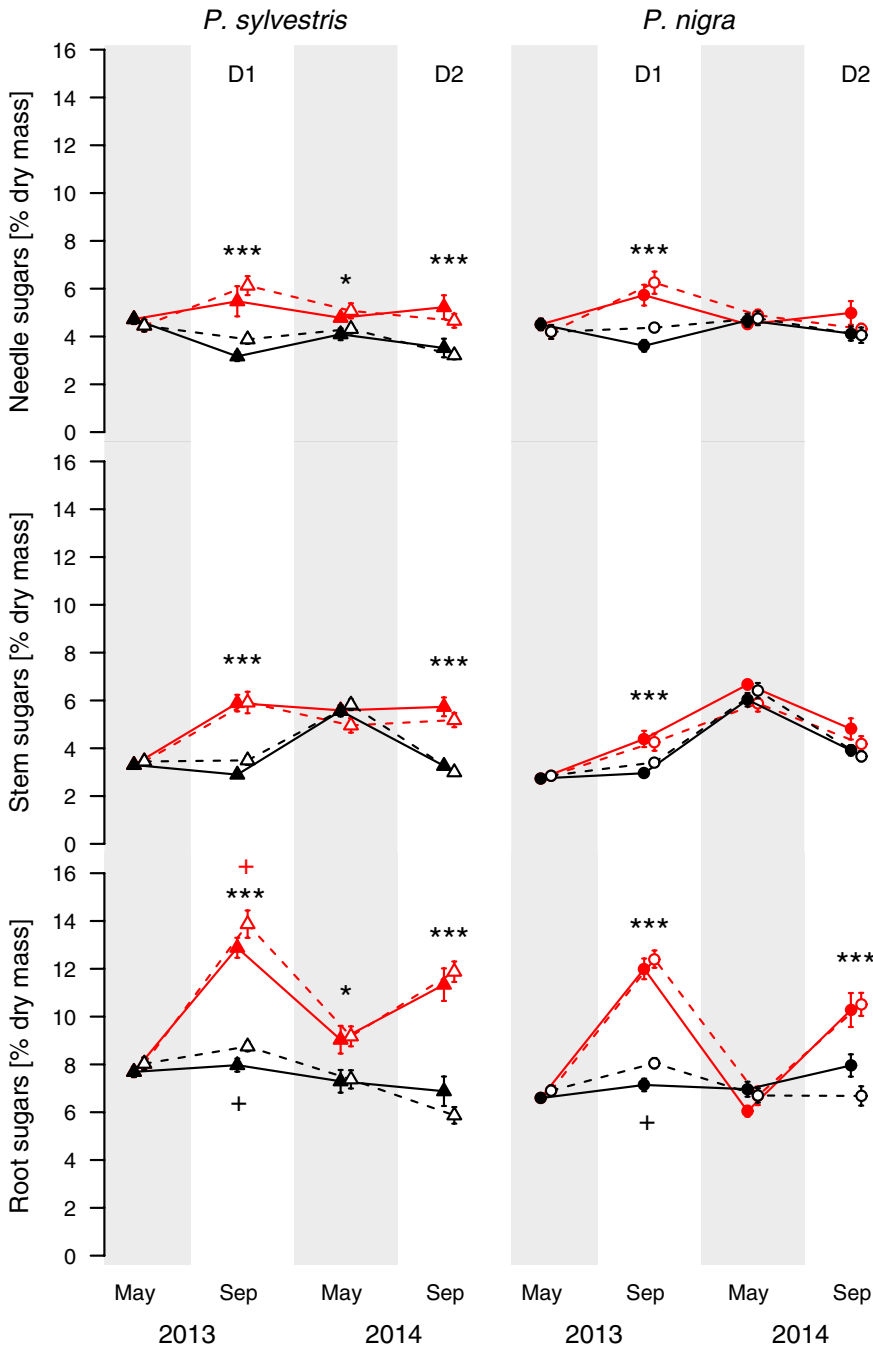
FIGURE 3 Soluble sugar concentrations (percentage of tissue dry mass; means ± *SE*) measured between May 2013 and September 2014 in different plant parts of *Pinus sylvestris* (3 replications × 5 populations) and *Pinus nigra* (3 replications × 4 populations) seedlings grown under factorial combinations of "moist" (black lines) and "dry-out" (red lines) conditions with ambient (aCO_2; filled symbols) and elevated (eCO_2; open symbols) atmospheric CO_2. Significant season-wise differences between "moist" and "dry-out" conditions (LMM): *$p < .05$ and ***$p < .001$, and between aCO_2 and eCO_2 (LMM): +$p < .05$ (see also Tables 2 and 3). D1 and D2 indicate the periods of drought treatments

observed ($p > .05$). Missing CO_2 effects on both seedling mortality and needle browning after D1 and D2 suggest that seedling mortality did not decline under combined drought and eCO_2, as expected in the presence of carbon "bet hedging" (premise 3).

4 | DISCUSSION

4.1 | "Bet hedging" with respect to carbon storage

Observations of NSC accumulation under drought stress (Duan et al., 2015; Gruber et al., 2012; Muller et al., 2011) have led to the hypothesis that trees follow a carbon "bet hedging" strategy,

according to which individuals cut investments in growth or reproduction, i.e. fitness, in order to actively build up carbon stores, which in turn are expected to lower the risk of carbon starvation during drought (Dietze et al., 2014; McDowell, 2011). Active carbon storage, and thus "bet hedging," should not only occur during drought but also during favourable growth conditions (Dietze et al., 2014). In our experiment, measurements of NSC concentrations in pine seedlings growing during two consecutive years under factorial combinations of aCO_2/eCO_2 and "moist"/"dry-out" conditions demonstrate that only one out of four premises of carbon "bet hedging" was met. In opposition to premise (1), "dry-out" seedlings did not increase carbon storage (starch; Figure 3) under eCO_2 compared to
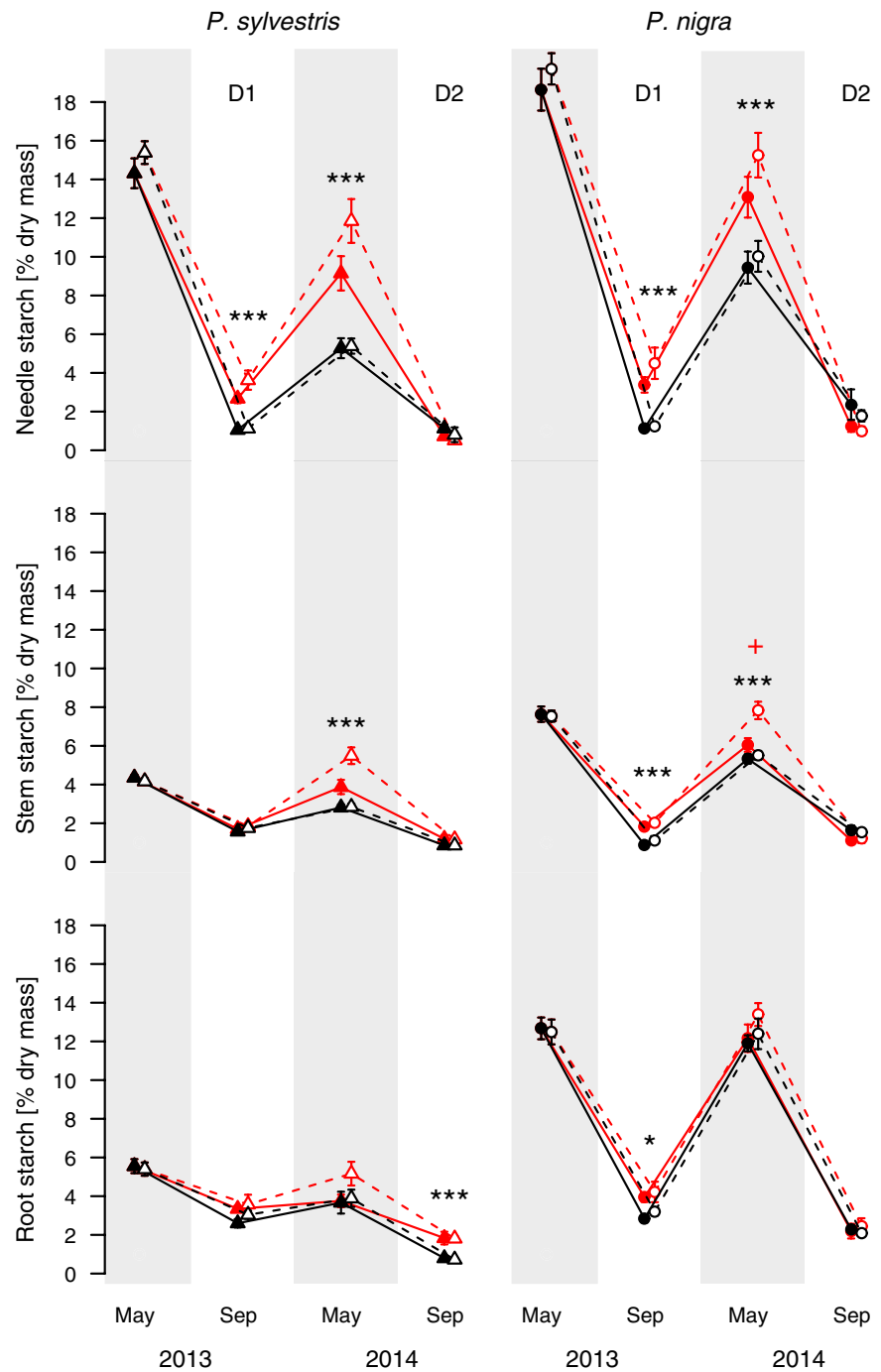
FIGURE 4 Starch concentrations (percentage of tissue dry mass; means ± *SE*) measured between May 2013 and September 2014 in needles, stem and roots of *Pinus sylvestris* (3 replications × 5 populations) and *Pinus nigra* (3 replications × 4 populations) seedlings grown under factorial combinations of "moist" (black lines) and "dry-out" (red lines) conditions with ambient (aCO$_2$; filled symbols) and elevated (eCO$_2$; open symbols) atmospheric CO$_2$. Significant season-wise differences between "moist" and "dry-out" conditions (LMM): *p < .05 and ***p < .001, and between aCO$_2$ and eCO$_2$ (LMM): +p < .05 (see also Tables 2 and 3). D1 and D2 indicate the periods of drought treatments

aCO$_2$. Biomass of "dry-out" seedlings was not enhanced by eCO$_2$ (Figure 5), which coincides with premise (2), but eCO$_2$ did not reduce mortality of "dry-out" seedlings, hence premise (3) was not met. Lastly, higher investment of carbon to storage did not come at the expense of growth in seedlings from drier origins (Figure 6), in contradiction to premise (4).

We expected that starch concentrations of "dry-out" seedlings would increase under eCO$_2$, because carbon fixation should exceed growth demand at low water availability (Runion, Entry, Prior, Mitchell, & Rogers, 1999), and seedlings applying a carbon "bet hedging" strategy should store excess carbon as starch. Studies investigating NSC

concentrations in tree species subjected simultaneously to drought and eCO$_2$ are scarce, and none have found an effect of eCO$_2$ on NSC concentrations of drought-stressed isohydric trees (Duan et al., 2015; Guehl, Picon, Aussenac, & Gross, 1994; Runion et al., 1999). Hence, trees may be well supplied with carbon and thus not be able to make use of increased CO$_2$ availability (Hoch et al., 2003). On the other hand, higher SS concentrations in eCO$_2$ seedlings suggest that additionally provided carbon was assimilated, but not converted to starch. Moreover, eCO$_2$ did not mitigate drought mortality, which was recently also observed by Duan et al. (2015), thus higher carbon availability may not be directly related to lower drought mortality.
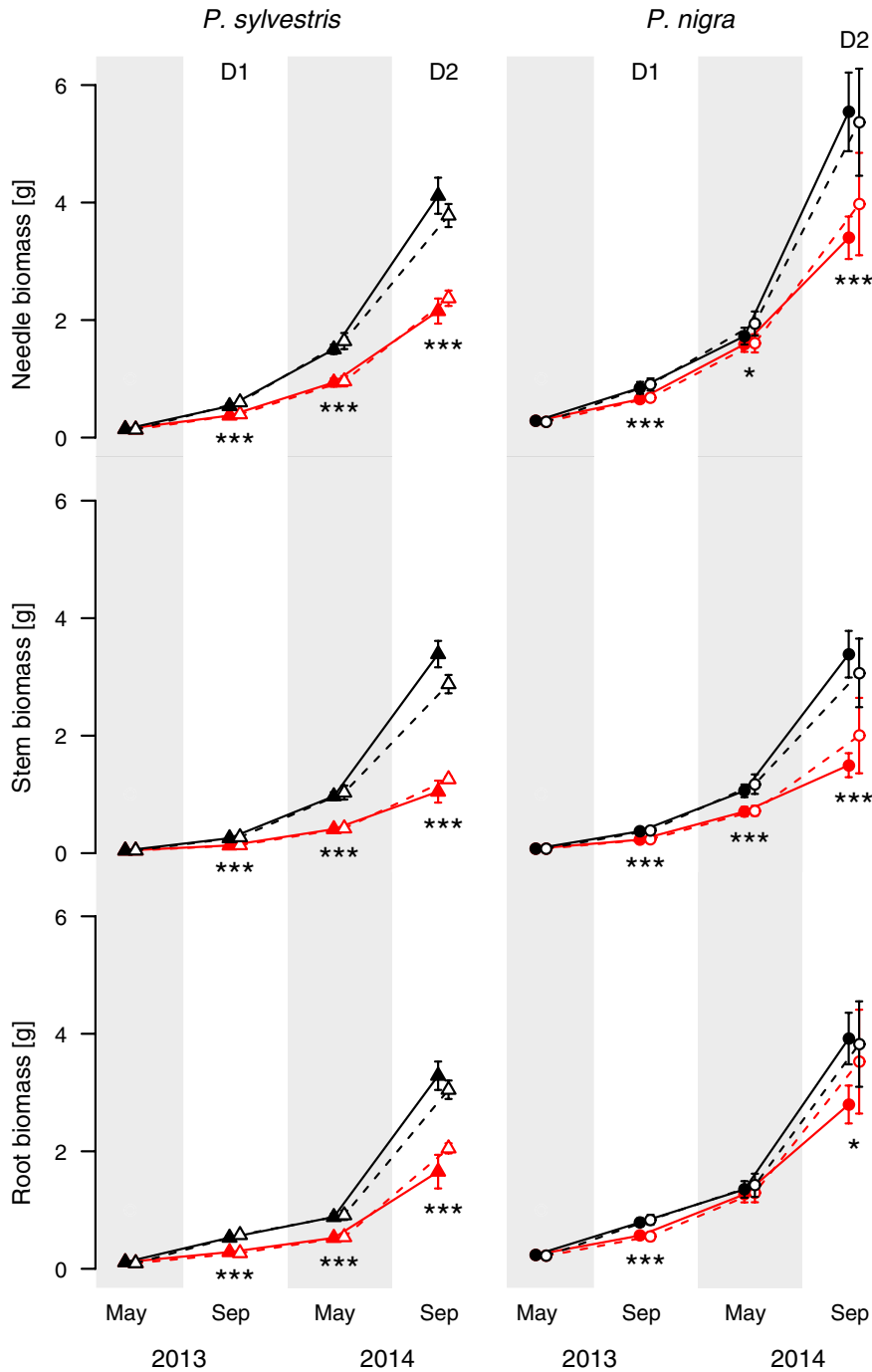
FIGURE 5 Biomass (means ± *SE*) measured between May 2013 and September 2014 in different plant parts of *Pinus sylvestris* (3 replications × 5 populations) and *Pinus nigra* (3 replications × 4 populations) seedlings grown under factorial combinations of "moist" (black lines) and "dry-out" (red lines) conditions with ambient (aCO$_2$; filled symbols) and elevated (eCO$_2$; open symbols) atmospheric CO$_2$. Significant season-wise differences between "moist" and "dry-out" conditions (LMM): *$p < .05$ and ***$p < .001$. The CO$_2$ treatment had no significant effect on season-wise biomass (see also Tables 2 and 3). D1 and D2 indicate the periods of drought treatments

Given the wide distribution of *P. sylvestris* along an extensive climatic gradient from the Mediterranean to Scandinavia and Siberia, it can be argued that the benefit of a carbon "bet hedging" strategy is restricted to drought-prone regions, where seedlings have to frequently tolerate reduced carbon uptake (Wiley & Helliker, 2012; Yang et al., 2015). In regions with intermittent and short drought episodes or high inter-annual variability of rainfall, fast height growth is crucial for interspecific competition for light among forest trees (Vizcaíno-Palomar, Ibáñez, González-Martínez, Zavala, & Alía, 2016). In such regions, prioritising storage over growth might hence be disadvantageous, particularly for pioneers such as *P. sylvestris*, which establish during short

windows of opportunity after disturbance events (Moser, Temperli, Schneiter, & Wohlgemuth, 2010). We found ecotypic differentiation in carbon storage between populations under "moist" experimental conditions, where drought-adapted populations accumulated more starch than those from wetter origin. Contrary to the premises of carbon "bet hedging," however, higher storage did not come at the cost of reduced growth. Indeed, it was even associated with a concurrent increase in seedling biomass. This indicates that carbon assimilation is more water-use efficient in drought-adapted pine populations than in wet ecotypes (Lévesque, Siegwolf, Saurer, Eilmann, & Rigling, 2014). Consequently, our results suggest that higher carbon accumulation in
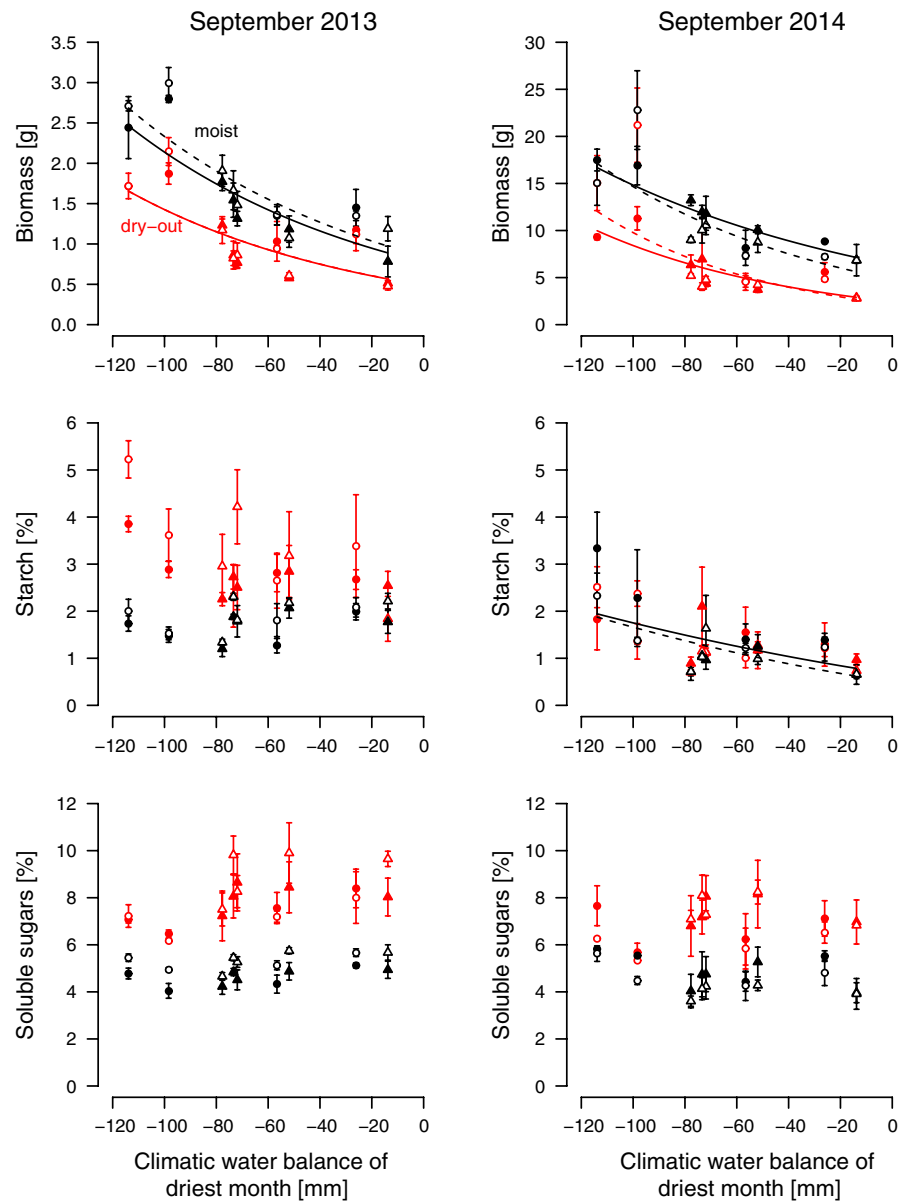
FIGURE 6 Whole-plant biomass, starch and soluble sugar concentrations of *Pinus sylvestris* (triangles, three replications) and *Pinus nigra* (circles, three replications) seedlings in relation to the climatic water balance of the driest month at the seed origin. Seedlings grown under factorial combinations of "dry-out" (red) and "moist" (black) conditions with aCO$_2$ (filled symbols, solid lines) and eCO$_2$ (open symbols, dashed lines), after the drought D1 in September 2013 and the drought D2 in September 2014

dry ecotypes constitutes a passive process rather than a "bet hedging" strategy.

4.2 | Accumulation of soluble sugars

In contrast to stored carbon (starch), SS temporarily accumulated in the roots of eCO$_2$ seedlings. SS in roots are important for osmotic adjustment in order to maintain water uptake during drought (Brunner, Herzog, Dawes, Arend, & Sperisen, 2015) and have previously been observed to increase under eCO$_2$ during drought (Tschaplinski, Norby, & Wullschleger, 1993). More recently, SS were shown to be important for post-drought recovery of *Fagus sylvatica* L. seedlings (Hagedorn et al., 2016). In our study, the increase of SS with eCO$_2$ was not particularly associated with the drought treatment, but primarily occurred in "moist" seedlings (*P. sylvestris* also in "dry-out" seedlings; Figure 3, September 2013). This increase of SS concentrations in the seedling's roots may be due to the fact that g_S was largely unresponsive

to eCO$_2$ (Figure S4), which is supported by $\partial^{18}O$ levels in the needles (Figure S3). Unchanged g_S at eCO$_2$ allowed for additional carbon assimilation, thereby increasing root sugar concentrations during D1 (Figure 4). Generally a down-regulation of g_S is expected in response to eCO$_2$ in order to sustain the C source-sink balance, and reduce water loss (Ainsworth & Long, 2005; Leuzinger & Körner, 2007). We can only speculate that the seedlings rapidly acclimatised to the eCO$_2$ treatment, which already started in 2012. This phenomenon is known from many long-term eCO$_2$ enrichment studies with both conifer and deciduous tree species (Streit, Siegwolf, Hagedorn, Schaub, & Buchmann, 2014; Vaz et al., 2012).

4.3 | Acclimatisation to drought

While CO$_2$ effects on biomass and starch were similar in both years, drought effects on starch and seedling mortality could only be observed after D1 but not D2, except of higher root starch

concentrations in *P. sylvestris* after D2. Similarly, needle browning was severe after D1, but only slightly higher in "dry-out" compared to "moist" seedlings after D2. High crown defoliation is associated with increased risk of mortality in conifer species (Galiano, Martínez-Vilalta, & Lloret, 2010; Guada, Camarero, Sánchez-Salguero, & Cerrillo, 2016), hence lower needle browning in "dry-out" seedlings after D2 compared to D1 suggests lower mortality risk. There may be several reasons for the different responses of seedlings to D1 and D2. First, pine seedlings exhibit a pronounced change from primary to secondary needles during the first 1–3 years. Such ontogenetic changes in leaf morphology may result in different carbon demands as needles differ both in investment costs and photosynthetic efficiency (Pardos, Calama, & Climent, 2009). Second, acclimatisation of trees to drought also heavily relies on changes in leaf morphology (Limousin, Misson, Lavoir, Martin, & Rambal, 2010). Environmental conditions during the time of bud formation affect shoot and leaf morphology in the subsequent year (Bréda, Huc, Granier, & Dreyer, 2006). Accordingly, third-year needles, which initiated towards the end of D1, were potentially acclimatised to drought, while second-year needles were not. Because seedling growth is exponential during the first 3 years of establishment (Figure 5), seedlings had a larger fraction of acclimatised third-year than non-acclimatised second-year needles during D2. Consequently, phenotypic plasticity is much higher during exponential growth phases of the juvenile stage than later in adult trees (Valladares, Gianoli, & Gomez, 2007).

5 | CONCLUSIONS

By supplying drought-stressed *P. sylvestris* and *P. nigra* seedlings with elevated atmospheric CO_2, we were able to show that seedlings from populations with different degrees of drought adaptation do not exhibit a carbon "bet hedging" strategy. Tree seedlings have often been considered more vulnerable to drought than adult trees, both due to their small root system (Lyr & Hoffmann, 1967) and the low amount of carbon reserves (O'Brien et al., 2014). We demonstrate, however, that already 2- and 3-year-old pine seedlings tolerate, and acclimatise to, repeated summer droughts. We simulated 4 months of drought in two consecutive years, an extreme weather event that has not been observed in Central Europe since the beginning of continuous large-scale records (Figure S5, Serra, Martínez, Lana, & Burgueño, 2014) and is not projected to regularly happen up to 2050 (Heinrich & Gobiet, 2012). Despite the long duration of the artificial drought, seedling mortality was low with 1.1% after D1 and 9.4% by end of May of the following spring. It might thus be argued that the simulated drought was not severe enough, especially given the fact that needle water potential was still similar in "dry-out" and "moist" seedlings 1 month after the onset of the drought in June. Accordingly, seedlings reduced g_S relatively late during the growing season when carbon demands for growth may already have been declining (Swidrak, Gruber, & Oberhuber, 2014). Even though early season drought might be more decisive for pine trees and seedlings than summer drought (Lévesque et al., 2013; Moser, Walthert, Metslaid, Wasem, & Wohlgemuth, 2016), climate change scenarios have been projecting a decrease of summer rather than spring precipitation across Central Europe (CH2011, 2011). We hypothesise that after successful establishment in sporadic wet years (Moser, Walthert, et al., 2016), increased frequency of summer droughts is unlikely to result in widespread seedling die-off in regions with annual precipitation well above 300 mm, as e.g. the Central Alps (Benavides et al., 2015). Although increased summer drought might constrain future forest productivity (Feichtinger, Eilmann, Buchmann, & Rigling, 2014), our results suggest that *P. sylvestris* is not only highly resilient to drought during adult life stages (Dobbertin et al., 2010) but also has remarkable acclimatisation potential during the seedling stage.

AUTHORS' CONTRIBUTIONS

T.W., B.M., C.B., G.H. and J.G. conceived and designed the experiment; field work and data analyses were carried out by C.B., supported by B.M. and T.W.; C.B., B.M. and T.W. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.005sk (Bachofen, Moser, Hoch, Ghazoul, & Wohlgemuth, 2017).

REFERENCES

Ainsworth, E. A., & Long, S. P. (2005). What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist*, *165*, 351–372.

Anderegg, W. R. L., Kane, J. M., & Anderegg, L. D. L. (2013). Consequences of widespread tree mortality triggered by drought and temperature stress. *Nature Climate Change*, *3*, 30–36.

Bachofen, C., Moser, B., Hoch, G., Ghazoul, J., & Wohlgemuth, T. (2017). Data from: No carbon 'bet hedging' in pine seedlings under prolonged summer drought and elevated CO_2. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.005sk.

Benavides, R., Escudero, A., Coll, L., Ferrandis, P., Gouriveau, F., Hódar, J. A., … Valladares, F. (2015). Survival vs. growth trade-off in early recruitment challenges global warming impacts on Mediterranean mountain trees. *Perspectives in Plant Ecology, Evolution and Systematics*, 17, 369–378.

Bréda, N., Huc, R., Granier, A., & Dreyer, E. (2006). Temperate forest tree and stands under severe drought: A review of ecophysiological responses, adaptation processes and long-term consequences. *Annales des Sciences Forestières*, 63, 625–644.

Brunner, I., Herzog, C., Dawes, M., Arend, M., & Sperisen, C. (2015). How tree roots respond to drought. *Frontiers in Plant Science*, 6, 1–16.

CH2011 (2011). *Swiss climate change scenarios CH2011* (pp. 88). Zurich, Switzerland: C2SM, MeteoSwiss, ETH, NCCR Climate, and OcCC.

Cochrane, J., Hoyle, G., Yates, C., Wood, J., & Nicotra, A. (2014). Climate warming delays and decreases seedling emergence in a Mediterranean ecosystem. *Oikos*, 124, 1–11.

Dietze, M. C., Sala, A., Carbone, M. S., Czimczik, C. I., Mantooth, J. A., Richardson, A. D., & Vargas, R. (2014). Nonstructural carbon in woody plants. *Annual Review of Plant Biology*, 65, 667–687.

Dobbertin, M., Eilmann, B., Bleuler, P., Giuggiola, A., Pannatier, E. G., Landolt, W., … Rigling, A., (2010). Effect of irrigation on needle morphology, shoot and stem growth in a drought-exposed *Pinus sylvestris* forest. *Tree Physiology*, 30, 346–360.

Duan, H., O'Grady, A. P., Duursma, R. A., Choat, B., Huang, G., Smith, R. A., … Tissue, D. T. (2015). Drought responses of two gymnosperm species with contrasting stomatal regulation strategies under elevated CO_2 and temperature. *Tree Physiology*, 35, 756–770.

Fatichi, S., Leuzinger, S., & Körner, C. (2014). Moving beyond photosynthesis: From carbon source to sink-driven vegetation modeling. *New Phytologist*, 201, 1086–1095.

Feichtinger, L. M., Eilmann, B., Buchmann, N., & Rigling, A. (2014). Growth adjustments of conifers to drought and to century-long irrigation. *Forest Ecology and Management*, 334, 96–105.

Galiano, L., Martínez-Vilalta, J., & Lloret, F. (2010). Drought-induced multifactor decline of Scots Pine in the Pyrenees and potential vegetation change by the expansion of co-occurring Oak species. *Ecosystems*, 13, 978–991.

Gruber, A., Pirkebner, D., Florian, C., & Oberhuber, W. (2012). No evidence for depletion of carbohydrate pools in Scots pine (*Pinus sylvestris* L.) under drought stress. *Plant Biology*, 14, 142–148.

Guada, G., Camarero, J. J., Sánchez-Salguero, R., & Cerrillo, R. M. N. (2016). Limited growth recovery after drought-induced forest dieback in very defoliated trees of two pine species. *Frontiers in Plant Science*, 7, 1–12.

Guehl, J. M., Picon, C., Aussenac, G., & Gross, P. (1994). Interactive effects of elevated CO_2 and soil drought on growth and transpiration efficiency and its determinants in two European forest tree species. *Tree Physiology*, 14, 707–724.

Hagedorn, F., Joseph, J., Peter, M., Luster, J., Pritsch, K., Geppert, U., … Arend, M. (2016). Recovery of trees from drought depends on belowground sink control. *Nature Plants*, 2, 16111.

Hartmann, H., Ziegler, W., & Trumbore, S. (2013). Lethal drought leads to reduction in nonstructural carbohydrates in Norway spruce tree roots but not in the canopy (ed A Knapp). *Functional Ecology*, 27, 413–427.

Hättenschwiler, S., Handa, I., & Egli, L. (2002). Atmospheric CO_2 enrichment of alpine treeline conifers. *New Phytologist*, 156, 363–375.

Heinrich, G., & Gobiet, A. (2012). The future of dry and wet spells in Europe: A comprehensive study based on the ENSEMBLES regional climate models. *International Journal of Climatology*, 32, 1951–1970.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978.

Hoch, G., Popp, M., & Körner, C. (2002). Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. *Oikos*, 98, 361–374.

Hoch, G., Richter, A., & Körner, C. (2003). Non-structural carbon compounds in temperate forest trees. *Plant Cell and Environment*, 26, 1067–1081.

Hsiao, T., & Acevedo, E. (1974). Plant responses to water deficits, water use efficiency and drought resistance. *Agricultural Meteorology*, 14, 59–84.

Körner, C. (2003). Carbon limitation in trees. *Journal of Ecology*, 91, 4–17.

Leuzinger, S., & Körner, C. (2007). Water savings in mature deciduous forest trees under elevated CO_2. *Global Change Biology*, 13, 2498–2508.

Lévesque, M., Saurer, M., Siegwolf, R., Eilmann, B., Brang, P., Bugmann, H., & Rigling, A. (2013). Drought response of five conifer species under contrasting water availability suggests high vulnerability of Norway spruce and European larch. *Global Change Biology*, 19, 3184–3199.

Lévesque, M., Siegwolf, R., Saurer, M., Eilmann, B., & Rigling, A. (2014). Increased water-use efficiency does not lead to enhanced tree growth under xeric and mesic conditions. *The New Phytologist*, 203, 94–109.

Limousin, J.-M., Misson, L., Lavoir, A. V., Martin, N. K., & Rambal, S. (2010). Do photosynthetic limitations of evergreen *Quercus ilex* leaves change with long-term increased drought severity? *Plant, Cell and Environment*, 33, 863–875.

Lindner, M., Fitzgerald, J. B., Zimmermann, N. E., Reyer, C., Delzon, S., van der Maaten, E., … Hanewinkel, M. (2014). Climate change and European forests: What do we know, what are the uncertainties, and what are the implications for forest management? *Journal of Environmental Management*, 146C, 69–83.

Lyr, H., & Hoffmann, G. (1967). Growth rates and growth periodicity of tree roots. *International Review of Forestry Research*, 2, 181–236.

Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., … Lloret, F. (2016). Dynamics of non-structural carbohydrates in terrestrial plants: A global synthesis. *Ecological Monographs*, 86, 495–516.

McDowell, N. G. (2011). Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology*, 155, 1051–1059.

McDowell, N. G., Beerling, D. J., Breshears, D. D., Fisher, R. A., Raffa, K. F., & Stitt, M. (2011). The interdependence of mechanisms underlying climate-driven vegetation mortality. *Trends in Ecology and Evolution*, 26, 523–532.

McDowell, N. G., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., … Yepez, E. A. (2008). Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist*, 178, 719–739.

Mitchell, P. J., O'Grady, A. P., Tissue, D. T., White, D. A., Ottenschlaeger, M. L., & Pinkard, E. A. (2013). Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytologist*, 197, 862–872.

Moser, B., Bachofen, C., Müller, J., Metslaid, M., & Wohlgemuth, T. (2016). Root architecture might account for contrasting establishment success of *Pseudotsuga menziesii* var. *menziesii* and *Pinus sylvestris* in Central Europe under dry conditions. *Annals of Forest Science*, 73, 959–970.

Moser, B., Temperli, C., Schneiter, G., & Wohlgemuth, T. (2010). Potential shift in tree species composition after interaction of fire and drought in the Central Alps. *European Journal of Forest Research*, 129, 625–633.

Moser, B., Walthert, L., Metslaid, M., Wasem, U., & Wohlgemuth, T. (2016). Spring water deficit and soil conditions matter more than seed origin and summer drought for the establishment of temperate conifers. *Oecologia*, 183, 519–530.

Muller, B., Pantin, F., Génard, M., Turc, O., Freixes, S., Piques, M., & Gibon, Y. (2011). Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *Journal of Experimental Botany*, 62, 1715–1729.

O'Brien, M. J., Leuzinger, S., Philipson, C. D., Tay, J., & Hector, A. (2014). Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nature Climate Change*, 4, 710–714.

Pardos, M., Calama, R., & Climent, J. (2009). Difference in cuticular transpiration and sclerophylly in juvenile and adult pine needles relates to the species-specific rates of development. *Trees*, 23, 501–508.

Piper, F. I. (2011). Drought induces opposite changes in the concentration of non-structural carbohydrates of two evergreen *Nothofagus* species of differential drought resistance. *Annals of Forest Science*, 68, 415–424.

Popp, M., Lied, W., Meyer, A. J., Richter, A., Schiller, P., & Schwitte, H. (1996). Sample preservation for determination of organic compounds: Microwave versus freeze-drying. *Journal of Experimental Botany*, 47, 1469–1473.

R Development Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Richardson, A. D., Carbone, M. S., Keenan, T. F., Czimczik, C. I., Hollinger, D. Y., Murakami, P., … Xu, X. (2013). Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytologist*, 197, 850–861.

Richter, S., Kipfer, T., Wohlgemuth, T., Calderón Guerrero, C., Ghazoul, J., & Moser, B. (2012). Phenotypic plasticity facilitates resistance to climate change in a highly variable environment. *Oecologia*, 169, 269–279.

Rigling, A., Bigler, C., Eilmann, B., Feldmeyer-Christe, E., Gimmi, U., Ginzler, C., … Dobbertin, M. (2013). Driving factors of a vegetation shift from Scots pine to pubescent oak in dry Alpine forests. *Global Change Biology*, 19, 229–240.

Roman, D. T., Novick, K. A., Brzostek, E. R., Dragoni, D., Rahman, F., & Phillips, R. P. (2015). The role of isohydric and anisohydric species in determining ecosystem-scale response to severe drought. *Oecologia*, 179, 641–654.

Runion, G. B., Entry, J. A., Prior, S. A., Mitchell, R. J., & Rogers, H. H. (1999). Tissue chemistry and carbon allocation in seedlings of *Pinus palustris* subjected to elevated atmospheric CO_2 and water stress. *Tree Physiology*, 19, 329–335.

Sala, A., Piper, F., & Hoch, G. (2010). Physiological mechanisms of drought-induced tree mortality are far from being resolved. *New Phytologist*, 186, 274.

Sala, A., Woodruff, D. R., & Meinzer, F. C. (2012). Carbon dynamics in trees: Feast or famine? *Tree Physiology*, 32, 764–775.

Scholand, P., Hammel, H., Bradstre., E., & Hemmings, E. (1965). Sap pressure in vascular plants. *Science*, 148, 339–346.

Seidel, H., Schunk, C., Matiu, M., & Menzel, A. (2016). Diverging drought resistance of Scots Pine provenances revealed by infrared thermography. *Frontiers in Plant Science*, 7, 1247.

Serra, C., Martínez, M. D., Lana, X., & Burgueño, A. (2014). European dry spell regimes (1951-2000): Clustering process and time trends. *Atmospheric Research*, 144, 151–174.

Sevanto, S., McDowell, N. G., Dickman, L. T., Pangle, R., & Pockman, W. T. (2014). How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant, Cell & Environment*, 37, 153–161.

Singer, M. J., & Munns, D. N. (2006). *Soils: An introduction*, 6th edn. Upper Saddle River, NJ: Pearson Prentice Hall.

Streit, K., Siegwolf, R. T. W., Hagedorn, F., Schaub, M., & Buchmann, N. (2014). Lack of photosynthetic or stomatal regulation after 9 years of elevated CO_2 and 4 years of soil warming in two conifer species at the alpine treeline. *Plant, Cell & Environment*, 37, 315–326.

Swidrak, I., Gruber, A., & Oberhuber, W. (2014). Xylem and phloem phenology in co-occurring conifers exposed to drought. *Trees: Structure and Function*, 28, 1161–1171.

Thornthwaite, C. W. (1948). An approach toward a rational classification of climate. *Geographical Review*, 38, 55–94.

Tschaplinski, T. J., Norby, R. J., & Wullschleger, S. D. (1993). Responses of loblolly pine seedlings to elevated CO_2 and fluctuating water supply. *Tree Physiology*, 13, 283–296.

Valladares, F., Gianoli, E., & Gomez, J. M. (2007). Ecological limits to plant phenotypic plasticity. *New Phytologist*, 176, 749–763.

Vaz, M., Cochard, H., Gazarini, L., Graça, J., Chaves, M. M., & Pereira, J. S. (2012). Cork oak (*Quercus suber* L.) seedlings acclimate to elevated CO_2 and water stress: Photosynthesis, growth, wood anatomy and hydraulic conductivity. *Trees*, 26, 1145–1157.

Vizcaíno-Palomar, N., Ibáñez, I., González-Martínez, S. C., Zavala, M. A., & Alía, R. (2016). Adaptation and plasticity in aboveground allometry variation of four pine species along environmental gradients. *Ecology and Evolution*, 6, 7561–7573.

Wiley, E., & Helliker, B. (2012). A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. *New Phytologist*, 195, 285–289.

Yang, X., Huang, Z., Zhang, K., & Cornelissen, J. H. C. (2015). Geographic pattern and effects of climate and taxonomy on nonstructural carbohydrates of *Artemisia* species and their close relatives across northern China. *Biogeochemistry*, 125, 337–348.

Zuber, T. (2007). *Untersuchungen zum Wasserhaushalt eines Fichtenwaldstandorts unter Berücksichtigung der Humusauflage*. PhD thesis, University of Bayreuth, Bayreuth.

Zweifel, R., Rigling, A., & Dobbertin, M. (2009). Species-specific stomatal response of trees to drought – A link to vegetation dynamics? *Journal of Vegetation Science*, 20, 442–454.

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