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Phenotypic plasticity facilitates resistance to climate change in a highly variable environment

Sarah Richter · Tabea Kipfer · Thomas Wohlgemuth ·
Carlos Calderón Guerrero · Jaboury Ghazoul ·
Barbara Moser

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Abstract Increased summer drought will exacerbate the regeneration of many tree species at their lower latitudinal and altitudinal distribution limits. In vulnerable habitats, introduction of more drought-tolerant provenances or species is currently considered to accelerate tree species migration and facilitate forest persistence. Trade-offs between drought adaptation and growth plasticity might, however, limit the effectiveness of assisted migration, especially if introductions focus on provenances or species from different climatic regions. We tested in a common garden experiment the performance of *Pinus sylvestris* seedlings from the continental Central Alps under increased temperatures and extended spring and/or summer drought, and compared seedling emergence, survival and biomass allocation to that of *P. sylvestris* and closely related *Pinus nigra* from a Mediterranean seed source. Soil heating had only minor effects on seedling performance but high spring precipitation doubled the number of continental *P. sylvestris* seedlings present after the summer drought. At

the same time, twice as many seedlings of the Mediterranean than the continental *P. sylvestris* provenance were present, which was due to both higher emergence and lower mortality under dry conditions. Both *P. sylvestris* provenances allocated similar amounts of biomass to roots when grown under low summer precipitation. Mediterranean seedlings, however, revealed lower phenotypic plasticity than continental seedlings under high precipitation, which might limit their competitive ability in continental Alpine forests in non-drought years. By contrast, high variability in the response of individual seedlings to summer drought indicates the potential of continental *P. sylvestris* provenances to adapt to changing environmental conditions.

Keywords Assisted migration · Genotypic variation · Provenance · Soil heating · Summer drought

Introduction

Projections of increased temperatures and more frequent drought events question the persistence of many plant species in their current distributions. This has triggered a debate about whether long-lived organisms with slow regeneration turnover such as trees will be able to keep pace with changing climatic conditions and adapt to new conditions or migrate fast enough to colonise new suitable habitat (Aitken et al. 2008). Given the wide distribution range of most temperate tree species, it is evident that they are able to tolerate a variety of climatic conditions, through phenotypic plasticity, genetic adaptation or both (Savolainen et al. 2007). While genetic adaptation might be slow in long-lived individuals such as trees, genotypic variation among individuals allows the persistence of a species via

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S. Richter · T. Kipfer · T. Wohlgemuth · B. Moser (✉)
Disturbance Ecology, Swiss Federal Institute for Forest,
Snow and Landscape Research WSL, Birmensdorf, Switzerland
e-mail: barbara.moser@wsl.ch

C. Calderón Guerrero
Escuela de Ingeniería Forestal y del Medio Natural,
Universidad Politécnica de Madrid, Madrid, Spain

J. Ghazoul
Institute for Terrestrial Ecosystems, ETH Zürich,
Zürich, Switzerland

the expansion of genotypes better suited to a new climate (Kramer et al. 2010). Currently, phenotypic plasticity is regarded as a key mechanism for tree species adaptation to rapid environmental change (Berg and Ellers 2010; Nicotra et al. 2010). Growth plasticity might be particularly important for adaptation to increased temperatures and drought since a small leaf canopy reduces water loss due to transpiration (DeLucia et al. 2000) and a large root network enhances access to water and nutrients (Markesteijn and Poorter 2009). Accordingly, several Mediterranean *Quercus* species are known to increase biomass allocation to roots when exposed to drought stress (Valladares and Sánchez-Gómez 2006). The degree of plasticity in adapting root–shoot ratio to water availability is, however, unknown in most temperate tree species because studies have often been restricted to aboveground growth measures (Reich and Oleksyn 2008). Alternatively, species might avoid drought by timing their growth and reproduction to occur during periods of high water availability. For instance, it has been shown that early emergence of *Pinus sylvestris* seedlings at its southern distribution limit increased the time span available for seedling development before the onset of summer drought, which increased seedling survival irrespective of the severity of the drought (Castro 2006). Extended growing seasons as a result of rising temperatures might thus buffer negative impacts of summer drought on tree seedling establishment.

If the degree of climatic change exceeds the adaptive capacity of a tree species at a given location, the species might be replaced by others that are better adapted to the new climatic conditions at that locality. Observations of tree regeneration along altitudinal gradients suggest that migrational and compositional shifts are already underway (Lenoir et al. 2009). Migration of individual species might, however, be limited by dispersal rates (Aitken et al. 2008), by the lack of suitable habitat in fragmented landscapes (Jackson and Sax 2010), or by dispersal barriers such as high mountain ranges. In the Alps, low-elevation provenances of *Fagus sylvatica* have been shown to be more vulnerable to increased temperature and drought than mid- or high-elevation provenances (Vitasse et al. 2010). Because dispersal barriers might limit the arrival of new species in mountainous regions, forest persistence might be threatened at lower elevations. A potential decline of stand-forming tree species is of considerable concern in these regions since forests often serve as protection against natural hazards. Forest management might thus involve actively facilitating species migration, e.g. through the introduction of more drought-tolerant provenances of native species, or by introducing new species better adapted to the future climate of a region (McLachlan et al. 2007). Using assisted migration as a tool to enhance forest resistance to climate change at the landscape scale requires

better understanding of species adaptations to local climatic conditions (Chmura et al. 2011), especially if introductions focus on provenances or species from different climatic regions (Rehfeldt et al. 1999). For instance, it has been suggested that a high degree of drought resistance is associated with limited phenotypic plasticity (Sambatti and Caylor 2007; Sánchez-Gómez et al. 2008), which might be disadvantageous in regions with highly variable climate (Baumann and Conover 2011).

We studied the importance of phenotypic plasticity and genotypic variation for potential adaptation of *P. sylvestris* to future climatic conditions at the Central Alpine forest-steppe ecotone that are predicted to occur by approximately 2100. Concomitantly, we assessed the effectiveness of introducing more southern provenances or species from the Mediterranean to facilitate forest persistence in this ecosystem. Seedling emergence and establishment was studied in a common garden experiment in the Rhone Valley, Switzerland, simulating increased temperatures (+0, +2.5, +5°C) in combination with three precipitation regimes, which differed in seasonal distribution and amount of water added to experimental plots. We tested whether (1) *P. sylvestris* and *P. nigra* from a Mediterranean seed source perform better under warmer and/or drier conditions in terms of seedling emergence, survival and biomass production than continental seedlings; (2) differences between continental and Mediterranean provenances are linked to growth plasticity (aboveground vs. belowground allocation); and (3) the variation in seedling performance among individual seedlings could allow successful genetic restructuring of local tree populations.

Materials and methods

Simulated climate scenarios

Precipitation patterns at low elevations of the Central Alpine valleys are currently characterised by high year-to-year and low intra-annual variability (Online Resource 2) but are projected to change within the next century towards a more Mediterranean distribution with higher winter rainfalls and more extensive summer droughts (Schär et al. 2004; Beniston *in press*). In a common garden experiment located at the bottom of the upper Rhone valley near Leuk, Valais, Switzerland (46°18'33"N, 07°41'10"E; 610 m asl), we simulated three precipitation regimes in combination with three levels of soil heating corresponding to climate projections for the region likely to be reached by the end of the twenty-first century (Schär et al. 2004; Beniston *in press*). Precipitation regimes were applied by intercepting natural rainfall with automated transparent shelters and manually adding the desired amount of water weekly on

two consecutive days. The Central Alpine wet (CA_{wet}) precipitation regime simulated a wet variant of the current climate in the Rhone valley with a total of 433 mm of water added to experimental containers from 18 March to 17 September 2009 (i.e. 72 mm month⁻¹). This corresponds to the average April–September precipitation of the 10 wettest years of the past century at the meteorological station in Visp (156% of the average summer precipitation 1900–2007; MeteoSwiss) and simulates conditions where seedlings should not experience water stress (Online Resource 2). The Central Alpine dry (CA_{dry}) regime simulated a dry variant of the current Rhone valley climate with 218 mm of water added during the 6 months of the experiment (36 mm month⁻¹), i.e. 80% of the average April–September precipitation of the past century in Visp. In the Mediterranean (MED) regime, high rainfall during spring (72 mm month⁻¹ from 18 March to 31 May; equal to the CA_{wet} regime) was followed by a dry summer season (36 mm month⁻¹ from 1 June to 17 September; equal to the CA_{dry} regime; Online Resource 2). Volumetric water content of the top soil (0–12 cm) was measured on four occasions during the experiment with a Field Scout TDR 100 (Spectrum Technologies, Plainfield, USA; Online Resource 3). A constant increase of soil temperatures by +0°C (ambient), +2.5°C and +5°C was simulated with heating cables (Thermoforce, Cockermouth, UK) installed 0.5 cm beneath the soil surface. Temperature probes (thermistors; US Sensor, Orange, USA; resolution of 0.1°C) installed at the same soil depth measured soil temperature in both heating and ambient treatments at 1-s intervals and a computer based datalogger running a specially written control software (Simatic S7 300; Siemens, Zurich, Switzerland) switched the heating cables on and off as needed.

Seed material, experimental design and protocol

In continental Central Alpine valleys, *P. sylvestris* dominates forests between 600 and 1,300 m asl, whereas the species is stand-forming at slightly higher elevations (1,000–2,000 m asl) at its southern distribution limit in Spain. In February 2009, seeds were collected from autochthonous, low-elevation stands in the Rhone valley near Leuk, Switzerland (600–700 m asl; continental *P. sylvestris*) and from mid-elevation stands in the Penyagolosa Natural Park, Province of Castelló, Spain (1,100–1,400 m asl; Mediterranean *P. sylvestris* and *P. nigra*). The climate in the Rhone valley is continental with mean January temperature of -1.7°C, mean July temperature of 18.3°C and average annual precipitation of 607 mm (MeteoSwiss station Visp, 639 m asl, 1951–1969). At the origin of the Mediterranean seed

provenance, temperatures are slightly warmer during winter and colder during summer, and average annual precipitation exceeds that of the Rhone valley (mean January temperature 2.2°C, mean July temperature 17.3°C, average annual precipitation 748 mm; Observatorio Vistabella, 1,400 m asl, 1951–1969). Precipitation during summer months, however, is similar at both sites (June–August: 147 mm compared to 145 mm in Visp). Cones were collected at both sites from 10 individual trees, hereafter referred to as maternal lineages. Cones were dried, seed wings and empty seeds removed and seeds stored at 1°C. Seed characteristics of individual maternal lineages are given in Online Resource 1. Seeds were sown into containers of 50 × 60 cm surface area filled with 33 cm of sand and gravel from the local Rhone river bed and topped with 12 cm of organic material from a nearby *P. sylvestris* stand. The two layers were designed to simulate *P. sylvestris* forest soils of the Rhone valley, which have shallow organic top soil and low water retention capacity. To facilitate mycorrhization of the seedlings, chopped roots of mature *P. sylvestris* trees were added to the organic layer.

The experimental design included factorial combinations of the treatments *precipitation regime* (CA_{wet} , CA_{dry} , MED) and soil heating (ambient, +2.5°C, +5°C), which were applied to individual containers arranged in a fully randomised block design replicated five times (split-plot design). Each of the 45 containers was sown with 4 seeds of each species-provenance (continental *P. sylvestris*, Mediterranean *P. sylvestris*, Mediterranean *P. nigra*) and maternal lineage, i.e. a total of 120 seeds container⁻¹. Seeds were sown on a 5 × 5 cm grid after positions had been randomly assigned to individual seeds.

Seeds were sown on 17 and 18 March 2009 and the watering started on 18 March 2009. Seedling emergence was recorded every second or third day with the first record on 7 April 2009. Seedling survival was assessed eight times during the growing season (24 April, 5 March, 19 March, 3 June, 24 June, 29 July, 25 August and 14 September). Seedlings were considered dead if they showed typical symptoms of seedling damping-off, i.e. lying on the ground with thinning of the stem near the soil surface, or if all needles were entirely brown, which was considered as a sign of drought-induced death. In September 2009, a subset of 967 seedlings was harvested and the root system of each seedling carefully excavated. In *P. sylvestris*, roots are usually defined as the tissue below the root collar, a swelling at the base of the stem. We were not able to identify root collars in all seedlings thus shoot biomass was defined as all plant parts growing above the soil surface. Shoot and root biomass of individual seedlings were measured after drying the plant material for 72 h at 60°C.

Data analyses

The layout of the experiment was a split-plot design with the whole-plot factors *precipitation regime* and *soil heating* and the split-plot factors *species-seed provenance* and *maternal lineage*. Effects of these factors on the proportion of seedling emergence, number of seedlings present at the end of the first growing season and root–shoot ratio were analysed by means of ANOVA (with post hoc pairwise Tukey HSD tests), effects on total seedling biomass (shoot and root biomass) by means of ANCOVA (with Bonferroni adjusted multiple comparisons) using seed mass and date of seedling emergence as covariates. Effects of maternal lineages on seedling biomass were analysed for each species–provenance separately. Only maternal lineages with >15 seedlings were included in these analyses. ANOVA/ANCOVA was performed with the univariate general linear model procedure of SPSS Statistics (Release 17.0.0, SPSS, 2008). Since randomization of the whole-plot factor is not complete in a split-plot design, the factors *precipitation regime* and *soil heating* were tested against the whole-plot error, *species-seed provenance* and its interactions against the residuals (Sahai and Ageel 2000). In cases where data did not meet assumptions of normality or homogeneity of variances, logarithmic transformation improved data structure satisfactorily. Partial eta-squared (η_p^2) was used as a measure of effect size (Gamst et al. 2008). To analyse seedling survival in respect to climate treatments and maternal lineages, a generalised linear mixed model with binomial errors was fitted using the R *lmer* function in the *lme4* library (Version 0.999375-33).

Results

Seedling emergence and survival

Seedling emergence started 21 days after sowing and 90% of the seedlings emerged within the following 10 days. The timing of emergence was similar in continental and Mediterranean *P. sylvestris*, but soil heating of 5°C above ambient led to earlier seedling emergence in both species, although it did not affect final emergence rate (Fig. 1). The proportion of seedling emergence was affected first and foremost by the *precipitation regime* (Table 1) with up to three times higher emergence under wet compared to dry conditions (Fig. 2a). During seedling emergence, water addition was equal in the CA_{wet} and MED regimes, thus no differences were found between the two treatments (Tukey's HSD, $P = 0.84$). The proportion of seedling emergence did not differ between Mediterranean *P. sylvestris* and *P. nigra* (Tukey's HSD, $P = 0.70$), but was 29% lower in the continental than the Mediterranean *P. sylvestris*

provenance under CA_{wet} conditions and 19% lower under CA_{dry} conditions (Tukey's HSD, $P < 0.001$; Fig. 2a). The lower proportion of emergence in continental compared to Mediterranean seedlings could be a result of inferior seed quality (Online Resource 1), but ANOVA results did not change after correction for lower germination rate under controlled conditions.

Seedling mortality was highest between late June and early September with 99% of the dead seedlings showing signs of drought-induced death. Similar to emergence, seedling survival was primarily affected by precipitation: in the CA_{wet} treatment, 88% of the continental seedlings survived the first 6 months of establishment compared to 61% in the CA_{dry} treatment. High spring precipitation, however, did not increase survival of pine seedlings during subsequent summer drought; seedling mortality was indeed higher in the MED (62%) than the CA_{dry} (39%) treatment. Seedling survival was negatively affected by soil heating, but survival was slightly higher at 5°C than 2.5°C above ambient in continental *P. sylvestris* and *P. nigra* because higher temperatures advanced seedling emergence (Fig. 1) and early seedling emergence slightly increased seedling survival (Table 2). The survival rate of continental seedlings was lower than that of Mediterranean *P. sylvestris*, but no difference was found in comparison to *P. nigra* after taking seed mass and date of seedling emergence into account. The small variance attributed to maternal lineages indicates that seedling survival was independent of maternal seed origin.

Seedling establishment and biomass allocation

The number of seedlings present after one growing season was negatively affected by soil temperature but effect size (η_p^2) was 2.5 times smaller than that of precipitation (Table 1). Higher seedling emergence under wet compared to dry conditions more than offset high mortality in the MED treatment so that seedling numbers exceeded those in the CA_{dry} treatment in both species and provenances (Fig. 2b). Lower emergence and higher mortality resulted in a smaller number of continental seedlings compared to the Mediterranean provenances. No differences were found between Mediterranean *P. sylvestris* and *P. nigra* (Tukey's HSD, $P = 0.064$).

Total seedling biomass (shoot and root biomass) was not affected by soil heating but was positively related to early seedling emergence ($R^2 = 0.059$, $P < 0.001$) and average seed mass of the maternal lineage ($R^2 = 0.057$, $P < 0.001$; Table 3). Seedling biomass was two to three times higher under CA_{wet} than CA_{dry} conditions (Bonferroni, $P < 0.001$) and did not differ between species and provenances (Bonferroni, $P > 0.05$; Fig. 2c). In the Mediterranean provenances, seedling biomass did not differ between

Fig. 1 Cumulative number of seedlings emerged from *Pinus sylvestris* seeds from continental (Rhône valley, Switzerland) and Mediterranean (Castelló, Spain) provenances at two levels of soil heating (ambient, +5°C) and under two precipitation regimes: **a** Central Alpine wet regime, **b** Central Alpine dry regime. Circles and squares represent means ± SE of five containers, each of which was sown with 40 seeds of each species-provenance

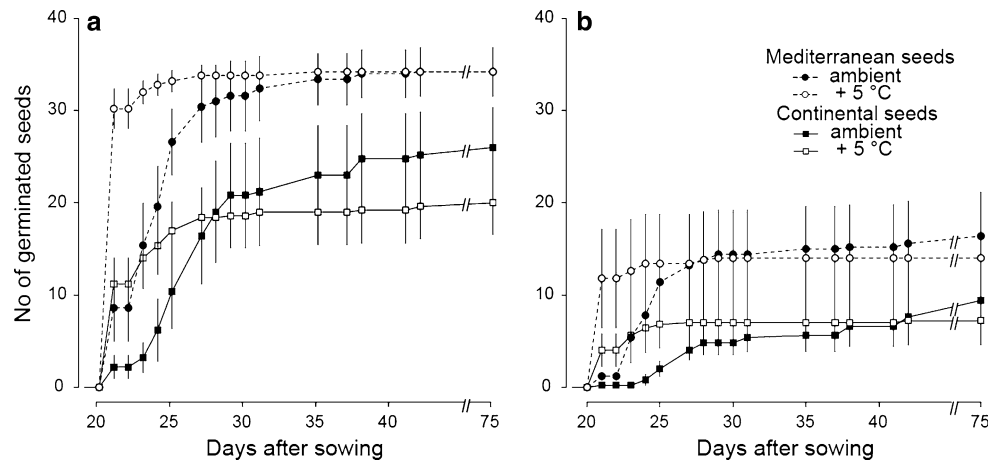


Table 1 Split-plot ANOVA results for the effects of the whole-plot factors precipitation regime (Central Alpine wet, Central Alpine dry, Mediterranean) and soil heating (ambient, +2.5°C, +5°C) and the split-plot factor species-seed provenance (continental *Pinus*

sylvestris, Mediterranean *P. sylvestris*, Mediterranean *P. nigra*) on the proportion of seedling emergence, number of seedlings present at the end of the growing season in September 2009 and root–shoot ratio of individual seedlings

Source of variation	Seedling emergence				Number of seedlings				Root–shoot ratio			
	df	F	P	η_p^2	df	F	P	η_p^2	df	F	P	η_p^2
Block	4, 32	22.65	<0.001	0.739	4, 32	22.62	<0.001	0.739	4, 32	3.96	0.009	0.288
Precipitation regime	2, 32	111.81	<0.001	0.875	2, 32	77.69	<0.001	0.829	2, 32	42.92	<0.001	0.705
Soil heating	2, 32	6.36	0.005	0.285	2, 32	7.91	0.002	0.331	2, 32	0.29	0.751	0.014
Precipitation × heating	4, 32	1.35	0.273	0.144	4, 32	1.38	0.263	0.147	4, 32	0.19	0.941	0.021
Whole-plot error	32				32				32			
Species-provenance	2, 72	116.38	<0.001	0.764	2, 72	143.57	<0.001	0.800	2, 904	23.82	<0.001	0.050
Species-provenance × precipitation	4, 72	1.55	0.196	0.079	4, 72	2.77	0.033	0.133	4, 904	0.62	0.652	0.003
Species-provenance × heating	4, 72	0.70	0.593	0.038	4, 72	0.23	0.923	0.012	4, 904	1.92	0.104	0.008
Species-provenance × precipitation × heating	8, 72	0.48	0.868	0.050	8, 72	0.60	0.777	0.062	8, 904	1.91	0.055	0.017
Residuals	72				72				904			

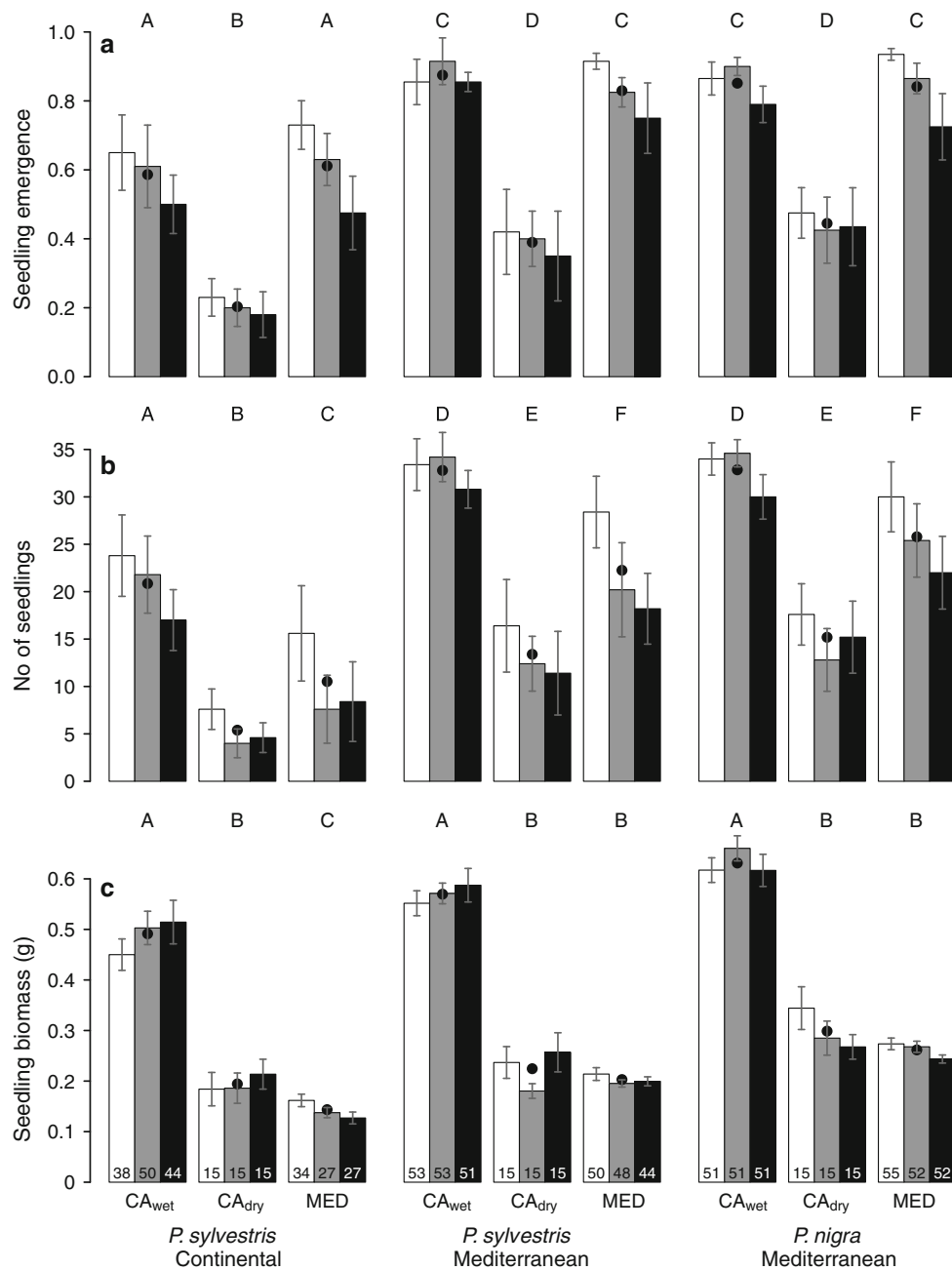
Seedling emergence and number of seedlings were calculated per container; root–shoot ratio refers to individual seedlings

Bold $P < 0.05$

the CA_{dry} and MED treatments (Bonferroni, $P > 0.05$). Continental seedlings grown under MED conditions, however, had on average lower biomass than in the CA_{dry} treatment. Biomass of continental MED seedlings was also lower than that of the Mediterranean MED seedlings (Bonferroni, $P < 0.001$; significant interaction species-provenance × precipitation; Table 3). Nevertheless, seedling biomass varied considerably between individuals so that some continental seedlings accumulated almost as much biomass as the heaviest *P. sylvestris* seedlings from the Mediterranean seed sources (Online Resource 4). In contrast to the Mediterranean provenances, this variation among individuals of the continental provenance could not be attributed to maternal lineages (split-plot ANOVA of 256 seedlings from 6 maternal lineages, $F_{5,170} = 1.21$, $P = 0.31$; Mediterranean *P. sylvestris*: $n = 343$, $F_{5,255} = 2.78$, $P = 0.018$; *P. nigra*: $n = 357$, $F_{5,268} = 7.71$,

$P < 0.001$). Maternal lineages, however, affected biomass allocation to shoot and roots in all species-provenances (continental *P. sylvestris*: $F_{5,170} = 6.36$, $P < 0.001$; Mediterranean *P. sylvestris*: $F_{5,255} = 5.69$, $P < 0.001$; *P. nigra*: $F_{5,268} = 3.51$, $P = 0.0043$). Seedlings of most but not all maternal lineages increased root–shoot ratio under CA_{dry} compared to CA_{wet} conditions (Fig. 3b). Consequently, *P. sylvestris* and *P. nigra* seedlings of both provenances were able to adjust biomass allocation in response to drought, but plasticity depended on maternal origin. The root–shoot ratio of individual seedlings further differed between precipitation regimes and species-provenances but not between soil heating treatments (Table 1). All species-provenances had a higher root–shoot ratio under CA_{dry} and MED conditions compared to CA_{wet} conditions, i.e. seedlings allocated more biomass to roots than shoots under dry conditions (Tukey’s HSD, $P < 0.001$; Fig. 3a). In the

Fig. 2 **a** Proportion of seedling emergence ($n = 5$ containers per treatment), **b** number of seedlings present per container at the end of the growing season in September 2009 ($n = 5$ per treatment), and **c** total biomass of individual seedlings in relation to precipitation regime [Central Alpine wet (CA_{wet}); Central Alpine dry (CA_{dry}); Mediterranean (MED)], soil heating, and species-seed provenance. Bars represent mean \pm SE of treatment combinations with soil heating by $+0^{\circ}\text{C}$ (open bars), $+2.5^{\circ}\text{C}$ (light shaded bars) and $+5^{\circ}\text{C}$ (filled bars). Circles show overall means of precipitation regimes; identical letters above two circles indicate no statistical differences between overall means (ANOVA followed by Tukey's HSD tests)



CA_{wet} treatment, Mediterranean seedlings produced more root biomass in relation to shoot biomass than continental seedlings, but no differences between species and provenances were found in the CA_{dry} treatment.

Discussion

Seedling emergence and establishment

Water availability is an important driver of germination and early seedling establishment (Pérez-Ramos and

Marañón 2009), which are both critical stages for the persistence of a species. In drought-prone habitats like the Central Alps, rising temperatures are likely to increase evapotranspiration and hence reduce soil moisture, effects that have already hampered tree regeneration of temperate species in southern Europe (Castro et al. 2004; Robson et al. 2009). Higher temperatures, on the other hand, might lead to earlier seedling emergence, which can be conducive to seedling establishment in Mediterranean areas by increasing the number of growing days until the onset of summer drought (Castro et al. 2006; Urbieto et al. 2008). Consistent with these theories, seedling emergence was

Table 2 Generalised linear mixed model with binomial errors on the effects of precipitation regime (CA_{wet}, CA_{dry}, MED), soil heating (ambient, +2.5°C, +5°C), species-seed provenance (continental *Pinus sylvestris*, Mediterranean *P. sylvestris*, Mediterranean *P. nigra*),

seed quality (in terms of germination rate under controlled conditions; Online Resource 1), date of seedling emergence and average seed mass of the maternal lineage on survival of individual seedlings

Fixed effects	All individuals			<i>P. sylvestris</i> Continental			<i>P. sylvestris</i> Mediterranean			<i>P. nigra</i> Mediterranean		
	Estimate	SE	Signif.	Estimate	SE	Signif.	Estimate	SE	Signif.	Estimate	SE	Signif.
Precipitation regime												
CA _{dry}	-1.448	0.16	***	-1.709	0.28	***	-1.046	0.27	***	-1.550	0.32	***
MED	-2.343	0.13	***	-2.779	0.23	***	-2.076	0.21	***	-2.276	0.27	***
Soil heating												
+2.5°C	-1.109	0.13	***	-1.328	0.22	***	-1.209	0.22	***	-0.883	0.22	***
+5°C	-1.074	0.14	***	-1.159	0.25	***	-1.381	0.23	***	-0.745	0.24	**
Species-seed provenance												
Mediterranean <i>P. sylvestris</i>	0.754	0.17	***	-	-	-	-	-	-	-	-	-
Mediterranean <i>P. nigra</i>	0.599	0.33		-	-	-	-	-	-	-	-	-
Seed quality	-0.008	0.01		0.005	0.01		-0.023	0.07		-0.105	0.19	
Date of seedling emergence	-0.052	0.01	***	-0.049	0.01	***	-0.052	0.02	**	-0.070	0.02	***
Average seed mass of maternal lineage	0.074	0.03	**	0.303	0.11	**	0.062	0.07		0.060	0.03	*
Random effects												
	Variance	SD		Variance	SD		Variance	SD		Variance	SD	
Block	0.278	0.53		0.498	0.71		0.257	0.51		0.155	0.39	
Maternal lineage	-	-		0.031	0.18		<0.001	<0.01		<0.001	<0.01	

Continental *P. sylvestris* n = 842, Mediterranean *P. sylvestris* n = 1,251, *P. nigra* n = 1,283

* P < 0.05, ** P < 0.01, *** P < 0.001

Table 3 Split-plot ANCOVA results for the effects of the whole-plot factors precipitation regime (Central Alpine wet, Central Alpine dry, Mediterranean) and soil heating (ambient, +2.5°C, +5°C), the split-plot factor species-seed provenance (continental *Pinus sylvestris*,

Mediterranean *P. sylvestris*, Mediterranean *P. nigra*) and the covariates date of seedling emergence and seed mass of the maternal lineage on total biomass of individual seedlings at the end of the growing season in September 2009 (log-transformed)

Source of variation	df	F	P	η_p^2
Date of seedling emergence	1, 902	21.26	<0.001	0.023
Seed mass	1, 902	30.89	<0.001	0.033
Block	4, 32	7.65	<0.001	0.463
Precipitation regime	2, 32	187.23	<0.001	0.917
Soil heating	2, 32	0.80	0.459	0.041
Precipitation × heating	4, 32	0.68	0.613	0.074
Whole-plot error	32			
Species-provenance	2, 902	19.50	<0.001	0.041
Species-provenance × precipitation	4, 902	12.70	<0.001	0.053
Species-provenance × heating	4, 902	0.82	0.515	0.004
Species-provenance × precipitation × heating	8, 902	0.66	0.728	0.006
Residuals	902			

Continental *P. sylvestris* n = 266, Mediterranean *P. sylvestris* n = 344, *P. nigra* n = 357

Bold P < 0.05

more than 50% lower under CA_{dry} than CA_{wet} conditions and higher temperatures led to earlier seedling emergence in our study. Earlier seedling emergence did not affect emergence rate (Fig. 1) but slightly increased seedling

survival in both species and provenances (Table 2). Contrary to our expectations, seedling survival during summer drought was negatively affected by high spring precipitation (lower survival in the MED than the CA_{dry} treatment).

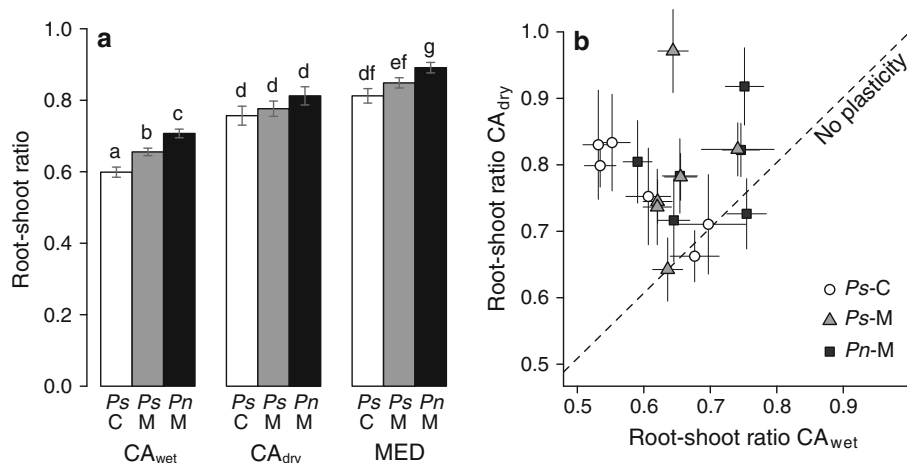


Fig. 3 Biomass allocation of *P. sylvestris* (*Ps*) and *P. nigra* (*Pn*) seedlings from continental (*C*) and Mediterranean (*M*) seed sources under different precipitation regimes [Central Alpine wet (CA_{wet}); Central Alpine dry (CA_{dry}); Mediterranean (*MED*)]. **a** Root–shoot ratio of individual seedlings (mean \pm SE). Identical letters above two bars indicate no statistical difference (ANOVA followed by Tukey’s HSD tests). **b** Plasticity of maternal lineages to adjust biomass

allocation in response to precipitation. Symbols represent average root–shoot ratios of maternal lineages (\pm SE) under CA_{dry} compared to CA_{wet} conditions. Points above the dashed 1:1 line indicate biomass allocation to roots, points below the line biomass allocation to the shoot in response to low precipitation; points near the line indicate absence of plasticity

We suggest that this is a consequence of higher resource investment in shoot than root biomass during wet spring conditions, which led to difficulties in sustaining shoot biomass during subsequent summer drought. Nevertheless, abundance of seedlings in the *MED* treatment was double that of seedlings in the CA_{dry} treatment at the end of the growing season (Fig. 2b). This was due to up to three times higher emergence rate in treatments with high spring precipitation. Although Mediterranean and continental seedlings responded similarly to water availability and soil temperature, seedlings from the Mediterranean seed source performed better than continental seedlings in all treatments, both in terms of emergence and survival. Cumulative effects of emergence and survival are key to the successful regeneration of *P. sylvestris* at the species southern range limit in Spain (Castro et al. 2005). High first year seedling density might become more important for tree regeneration in temperate forest ecosystems, too, especially after fire disturbance, where the window of opportunity for seedling establishment is short due to competition from understory vegetation (Greene et al. 2004; Moser et al. 2010). Large wildfires are expected to increase as a result of rising temperatures, not only at lower elevations in the Central Alps (Zumbrunnen et al. 2009) but worldwide (Pechony and Shindell 2010).

Phenotypic plasticity

Phenotypic plasticity allows short-term adaptation of individual plants and populations in a variable environment and is thus thought to be crucial for the persistence of slow

growing tree species under climate change (Grulke 2010; Vitasse et al. 2010). The most common plastic responses of trees to drought are a reduction in leaf canopy to restrict water loss (DeLucia et al. 2000) and increased root proliferation to enhance access to water and nutrients (Marksteijn and Poorter 2009). Little is known, however, about the plasticity of tree root systems under natural conditions and in adult trees. In a greenhouse experiment, Cregg and Zhang (2001) found that Asian *P. sylvestris* seedlings allocated more biomass to roots and were hence more drought resistant than seedlings from mesic European seed sources. In our study, both species and provenances reacted plastically to drought and increased biomass allocation to roots under low precipitation (CA_{dry} regime). We also found elevated root–shoot ratio in the *MED* treatment, indicating that both *P. sylvestris* and *P. nigra* seedlings are able to change biomass allocation patterns within a few months. While total seedling biomass did not differ between species and provenances in the CA_{wet} and CA_{dry} treatments and seedlings invested similar proportions of biomass to roots under dry conditions, Mediterranean seedlings allocated more resources to roots under conditions of abundant precipitation (CA_{wet} regime; Fig. 3a). This suggests that southern provenances and species are less plastic than continental ones and corroborates the theory that limited phenotypic plasticity is beneficial in stressful environments (Chambel et al. 2007; Sánchez-Gómez et al. 2008). In the case of Mediterranean *P. sylvestris* and *P. nigra*, it might be an adaptation to regular summer drought in order to prevent futile investment into aboveground structures during seasons of high resource

availability (Valladares et al. 2007). Basically, however, high resource investment into belowground structures limits a species capacity for aboveground growth optimisation in years with moderate drought events (Sambatti and Caylor 2007), which are still likely to occur regularly in temperate forests under climate change. Although summer precipitation is similar at both sites of seed origin in the longer term, the climate in the Central Alpine valleys is characterised by high year-to-year variability (Online Resource 2). Since competition for light and nutrients from understory vegetation is substantial in temperate forests during seedling establishment, high aboveground growth capacity is essential in years with abundant precipitation. Thus it is likely that limited growth plasticity under optimal growth conditions would compromise the competitive ability of Mediterranean species and provenances in continental forests in the longer term.

Genotypic variation

Although phenotypic plasticity facilitates short-term adaptation to environmental change, genetic adaptation might ultimately be necessary for the persistence of species in extreme habitats like the forest-steppe ecotone. Micro-geographical genetic variation in response to water availability is known, e.g., in *P. edulis* (Cobb et al. 1994; Mitton and Duran 2004) and *P. ponderosa* (Beckman and Mitton 1984), but genotypic variation of *P. sylvestris* in the Alps is rather low and mainly concerns altitudinal differentiation related to phenology (Fournier et al. 2006; Labra et al. 2006). Accordingly, we found only marginal effects of maternal lineages on total seedling biomass and root–shoot ratio. Nevertheless, we detected substantial variability in the responses of individual seedlings to environmental conditions, which is not related to maternal origin (Online Resource 4). Although it has been questioned whether genetic adaptation of tree species might be fast enough under predicted climatic change, especially at the southern and lower altitudinal distribution limit of species (Rehfeldt et al. 2001, 2002), recent modelling studies argue that moderate genetic variability between individuals of a population will be sufficient to allow rapid genetic restructuring of tree populations (Jump et al. 2008; Kramer et al. 2010).

Conclusions

The results of our experiment suggest that a reduction in spring and summer precipitation is likely to limit early seedling establishment at the Central Alpine forest-steppe ecotone, whereas temperatures up to 5°C above current levels might be negligible. Although seedling emergence

and early survival were susceptible to a 20% reduction in long-term spring and summer precipitation, we also found potential for adaptation in the continental provenance, especially in terms of biomass partitioning between above- and below-ground structures, which was more plastic than that of the Mediterranean provenances. Given the high year-to-year variability of precipitation in Central Alpine valleys, long-term success of tree regeneration at the forest-steppe ecotone will depend on the frequency of consecutive years with spring and summer drought as well as their effects on seed production and masting (Martínez-Alonso et al. 2007). Consequently, assisted migration might have limited value as a management tool to accelerate species migration and facilitate forest persistence in temperate regions. Despite the long history of human mediated migration of tree species (Pollegioni et al. 2011), our results indicate that introducing more drought-tolerant species to mitigate climate change might not necessarily be successful due to trade-offs between drought tolerance and growth plasticity. Thus, we support recent views that autochthonous provenances have the potential for resistance to changes in climatic conditions as a function of both phenotypic plasticity and genotypic variation (Nicotra et al. 2010). Our study focused on earliest seedling stages and effects of limited growth plasticity might amplify with increasing seedling age.

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Conflict of interest The authors declare that they have no conflict of interest. The experiment described in this manuscript complies with the current laws of Switzerland.

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