

Population-level differences in *Pinus monophylla* whole-plant seedling strategies under varying water pulse regimes

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Abstract

Forest persistence amid shifting climate conditions requires sufficient tree recruitment for sustaining populations. In dryland ecosystems where precipitation can be episodic and variable, changes in precipitation pulse regimes may be critically important for seedling establishment, as some populations may be locally adapted to variable resource availability. We conducted a greenhouse common garden experiment to evaluate *Pinus monophylla* seedling responses to varying watering pulse regimes. We used seeds from four sites representing endpoints of range-wide gradients of climatic water deficit and summer precipitation. Seedlings received the same amount of total water, but the water was applied in three different pulse patterns. We compared morphological and physiological traits in response to the three watering regimes, exploring whether responses were consistent with possible local adaptations. Seedlings demonstrated substantial trait differences among seed sources but few differences among water pulse treatments. Multivariate analysis of seedling traits suggested that seed source climate was related to two trade-offs in resource-use strategies: (i) acquisitive leaf traits versus seedling biomass and (ii) water use efficiency versus nitrogen use efficiency. A better understanding of intraspecific trait variation (ITV) can facilitate predictions of drought and temperature responses and help to identify suitable seed sources for *P. monophylla* restoration. While observed levels of ITV provide indirect evidence of local adaptation, this variation was not related to different precipitation pulse responses. However, the observed relationship between seed source climate and patterns of ITV suggests that local resource-use strategies underlying successful tree regeneration may constrain responses to a changing climate.

Keywords adaptive potential, increased temperature, drought, forest regeneration, intraspecific trait variation, plant resource-use strategies, precipitation pulses

Introduction

As anthropogenic increases in temperature accelerate, forests and woodlands become more vulnerable to drought-induced mortality as warmer and drier conditions and more varied precipitation regimes exceed species' tolerances (Liu *et al.* 2013, Allen *et al.* 2015, Petrie *et al.* 2017). Seedling recruitment represents a demographic bottleneck for forest and woodland persistence under future climate conditions, particularly in the hottest and driest locations within a species' range, where recruitment may already be constrained to years with more favourable conditions (Kursar *et al.* 2009, Lloret *et al.* 2009, Kueppers *et al.* 2017). Plants employ a range of adaptive strategies to tolerate

variability in climate and resource availability, and populations may exhibit adaptations to the local precipitation regimes that they experience (Schwinning and Sala 2004). Precipitation regimes drive many ecological processes (Stephenson 1990, Post and Knapp 2020) and, in arid ecosystems with frequent yet variable summer rainfall, changes to the frequency and magnitude of precipitation pulses can result in cascading effects on vegetation distributions, species interactions, and belowground processes (Collins *et al.* 2014, Zhang *et al.* 2021). One such change to precipitation patterns is the reduced frequency of precipitation events in the Western United States (Zhang *et al.* 2021). However, the importance of precipitation pulse frequency during the growing season is less well established for semiarid forests

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and woodlands with winter-dominated precipitation regimes than for those with summer-dominated precipitation regimes (Woods *et al.* 2014, Davis *et al.* 2022). These events may become more important as precipitation is predicted to change in the timing and duration of its delivery, not only in its overall annual amount (Snyder *et al.* 2019).

In dryland ecosystems, many plant species have developed strategies to balance resource acquisition during pulse events with resource conservation during intervening periods between pulses (de la Riva *et al.* 2016). These strategies represent a fundamental trade-off for dryland plant species that cannot simultaneously optimize traits for wet and dry conditions (Schwinning and Ehleringer 2001). Plant responses to precipitation pulses are driven by a suite of morphological and physiological traits on time-scales ranging from short-term responses to long-term acclimation (Ogle and Reynolds 2004, Schwinning and Sala 2004). Plants may shift strategies as they acclimate to different precipitation pulse regimes, as reflected in changes in leaf and root traits and in biomass allocation (Schwinning and Sala 2004). Plants prioritizing carbon acquisition over water conservation would be expected to increase photosynthetic capacity via higher specific leaf area (SLA), less strict stomatal control, lower root:shoot, lower wood density, and lower water use efficiency (WUE; McDowell *et al.* 2008, Markesteijn and Poorter 2009, Bu *et al.* 2018). Similarly, plants prioritizing nitrogen acquisition over water conservation are expected to have decreased WUE at the expense of nitrogen use efficiency (NUE) (C:N; Gong *et al.* 2011).

In water-limited environments, the seedling stage can be particularly vulnerable to changing climate conditions (Augustine and Reinhardt 2019). Intraspecific trait variation (ITV) can lead to differences in seedling performance that influence the potential for successful recruitment (Kerr *et al.* 2015, Augustine and Reinhardt 2019). Differing seedling strategies, often reflected in ITV, can arise from either genetic differences or trait plasticity (Moran *et al.* 2016). For species experiencing altered precipitation regimes, it is important to understand how ITV and associated local adaptations may influence seedling responses to future climate shifts (Atkins and Travis 2010, Stotz *et al.* 2022). Local adaptation to a specific precipitation regime may optimize seedling success in a fixed environment when trait plasticity is more costly (Bonser 2021), but this could become maladaptive as precipitation regimes change. On the other hand, phenotypic plasticity in functional traits allows seedlings to adjust in response to environmental variation, and plasticity itself is known to have a genetic basis (DeWitt and Scheiner 2004, Sniderhan *et al.* 2018).

Pinyon-juniper woodlands cover a vast geographic area (40 million ha) in western North America, and their broad range of environmental conditions makes them a model system for studying the role of ITV in shaping plant responses among populations under varying climate conditions. The two most widespread pinyon pine species differ in seasonality of precipitation, with *Pinus monophylla* (singleleaf pinyon pine) occupying regions with winter-dominated precipitation regimes and *Pinus edulis* (two-needle pinyon pine) occurring in regions with higher summer precipitation related to the North American monsoon (Williams *et al.* 2018, Miller *et al.* 2019). For *P. monophylla*, low summer precipitation means that newly germinated seedlings often experience a prolonged dry season following spring snowmelt (Williams *et al.* 2018), making infrequent summer precipitation pulses potentially important for determining episodic seedling establishment.

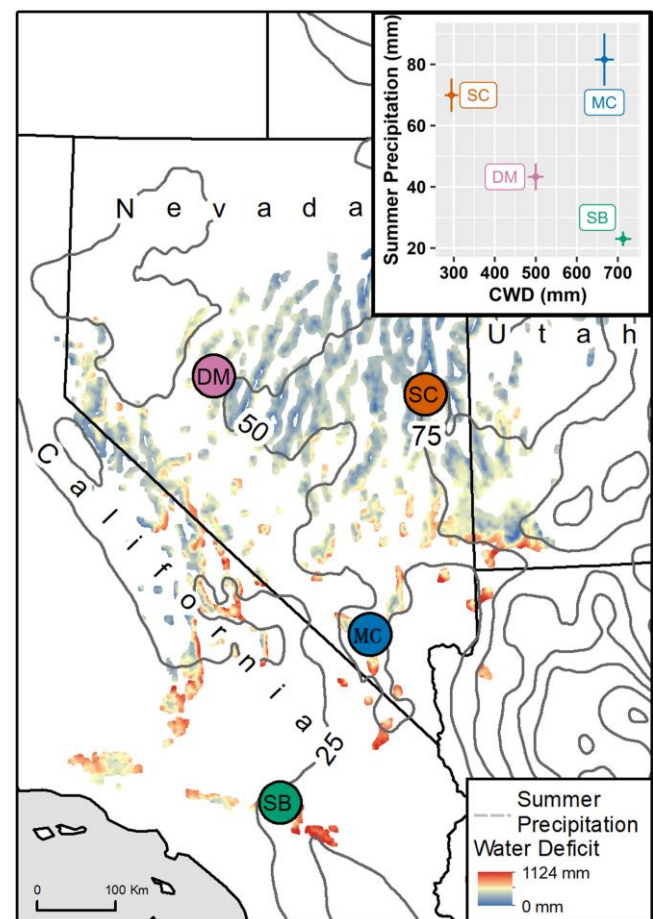


Figure 1 Seed sources were located at endpoints of range-wide regional gradients of climatic water deficit (CWD; mm) and amount of annual summer precipitation (June through August; mm). CWD is illustrated by a shading gradient from low CWD (wet) to high CWD (dry). Summer precipitation amount is represented by 25-mm contour lines. SB = San Bernardino (arid/summer-dry); MC = Mt. Charleston (arid/summer-wet); SC = Schell Creek (mesic/summer-wet); DM = Desatoya Mountains (intermediate). Inset: Cumulative CWD (mm) and summer precipitation amount (mm) in the four seed sources. The dots represent the mean 30-year climate normal (1990–2019), and the error bars represent the standard error.

Although the overall distribution of *P. monophylla* is characterized by a winter-dominated precipitation regime, the amount of precipitation falling in summer months varies along a regional gradient running approximately southwest-to-northeast (Fig. 1; Miller *et al.* 2019). Importantly, variation in summer precipitation does not align with the north-to-south gradient of climatic water deficit (CWD), a proxy of overall aridity (Dilts *et al.* 2015, Garbarino *et al.* 2020), resulting in populations that experience unique combinations of CWD and summer precipitation. Among populations of *P. monophylla*, these gradients of CWD and summer precipitation have been shown to be related to morphological trait variation for both mature trees and seedling offspring (Vasey *et al.* 2022, 2023).

Our research used a greenhouse common garden experiment to explore plant strategies and functional traits driving population-specific responses of *P. monophylla* seedlings to variations in precipitation pulse regime. Seedlings from four populations representing endpoints of gradients of CWD and

summer precipitation were subjected to three water pulse treatments. Pulse treatments simulated varying pulse regimes in which the pulse frequency varied, but the total amount of water across the treatment period remained constant. We were particularly interested in quantifying population-level variation in traits reflecting seedling ecophysiological responses. *Pinus monophylla* is generally understood to be an isohydric species that uses tight stomatal regulation to maintain stable xylem water potential across seasons (Drivas and Everett 1988), resulting in high WUE (DeLucia and Schlesinger 1991). In experimental studies, *P. monophylla* has exhibited several of the trade-offs commonly associated with increased WUE, including low photosynthetic nitrogen use efficiency (PNUE, the ratio of the photosynthetic rate to leaf nitrogen content) and low maximum photosynthesis (DeLucia and Schlesinger 1991, Jaindl et al. 1993), suggesting that it may be advantageous for populations experiencing more favourable climate conditions to use a less conservative strategy, reducing WUE in favour of resource acquisition.

We explored the following research questions: (i) Do resource-use strategies vary among populations from climate endpoints of *P. monophylla*'s range? (ii) Which individual traits best reflect the observed variation in acquisitive and conservative resource-use strategies, and how are these associated with seed source climate? (iii) How does the seed source climate relate to seedling responses to varying precipitation pulse dynamics? We hypothesized that seedlings would have different resource-use strategies among populations, and that this variation would mediate seedling responses to varying precipitation pulses. Specifically, we predicted that seedlings from more arid climates with lower summer precipitation would have traits associated with a resource-conservative strategy, particularly when grown under a low-frequency precipitation pulse regime. Conversely, we expected seedlings from less arid climates with higher summer precipitation would have traits associated with a resource-acquisitive strategy, particularly when grown under a high-frequency precipitation pulse regime.

Methods

Study design

We conducted a greenhouse common garden experiment simulating multiple precipitation pulse regimes for *P. monophylla* seedlings from four seed sources representing endpoints of annual cumulative CWD and summer precipitation within the species' geographic range (Fig. 1; Vasey et al. 2022). Details on replication are described in Supplementary Table S1. At each site, we collected seeds from the lower-elevation limit of *P. monophylla*, which we expected to experience the greatest water stress and to potentially represent the trailing edge of the species distribution. Within the range of *P. monophylla*, lower-elevation sites are generally characterized by hotter temperatures, lower precipitation, and greater aridity (Urza et al. 2020). *Pinus monophylla* individuals were identified based on geographic origin and standard field identification criteria. Recent genomic work indicates that some populations in these regions may include admixture with closely related pinyon pine species, including *P. californiarum* and *P. edulis* (Buck et al. 2023). The San

Bernardino Mountains (SB) experience high CWD with low summer precipitation ('arid/summer-dry'). Mount Charleston (MC) also has high CWD but with high and variable summer precipitation ('arid/summer-wet'). The Desatoya Mountains (DM) are intermediate in water deficit and amount of summer precipitation ('intermediate'). The Schell Creek Mountains (SC) have relatively low CWD, with high and variable summer precipitation ('mesic/summer-wet'; Supplementary Table S2). Seeds were collected in September 2019, pooling seeds from six trees at each site. Seeds were stored in a refrigerator for 2 years at ~4°C.

In December 2021, seeds were imbibed for 36 h and flushed with cold water every 4 h to remove pathogens (Kildisheva et al. 2012). After seeds were imbibed, they were placed in cold stratification with wetted vermiculite (2800 ml vermiculite to 1000 ml water) at 1–5°C until ~10% of the seeds germinated (48 days).

In January 2022, a greenhouse common garden was established using 1396 seeds sourced from the Desatoya (278), Mount Charleston (287), San Bernardino (338), and Schell Creek (493) sites. Soil samples from four commercial soil vendors were analysed by A&L Western Agricultural Laboratories for soil texture and nutrients, and we used the Moana Nursery triple mix, the most similar soil to previously characterized woodland soil samples (Chambers 2001, Vasey et al. 2023, Supplementary Table S3). We used deep cylindrical pots measuring 6.35 cm by 35.56 cm, arranged in 20-pot support trays (Stuewe and Sons, Inc., Tangent, OR, USA). Seeds were randomly assigned to pots, with five replicates from each of the four populations in each tray. Pots were watered twice weekly for 40 days, after which the 440 seedlings that had emerged were gradually dried down for 60 days. Watering transitioned from twice weekly to once weekly, then to no water for the last 4 weeks prior to the onset of experimental treatments. This dry-down was designed to simulate the initiation of the post-snowmelt dry season commonly experienced by *P. monophylla* populations; e.g. our two arid sites (MC and SB) received no precipitation during the entire month of June in seven of the last 10 years (Supplementary Figure S1).

After the dry-down, each tray was assigned to one of three water pulse treatments (Supplementary Figure S2) using randomization with spatial stratification, ensuring the pulse treatments were evenly distributed within the greenhouse. The monthly treatment (W1) received the full amount of monthly water on the first day of each 28-day pulse cycle. The biweekly treatment (W2) received half of the water on the first day of the pulse cycle, and half on Day 14. The weekly treatment (W3) received one-quarter of the water on Days 1, 7, 14, and 21. The monthly water requirement was estimated to equal the water needed for the W1-monthly treatment to reach field capacity at the beginning of each pulse cycle, calculated using a gravimetric method (Dumroese 2015). Two full pulse cycles were applied from 12 May 2022 to 4 July 2022, followed by a final pulse event on 5 July 2022. The volumes for the three pulse cycles were: (i) 176, 88, and 44 ml; (ii) 181, 91, and 45 ml; and (iii) 187, 94, and 47 ml, for the monthly, biweekly, and weekly applications, respectively. Given a pot surface area of 31.67 cm², the W1 pulse volumes correspond to 55.6–59.0 mm of precipitation per 28-day cycle (with W2 and W3 receiving the same total per cycle split into 27.8–29.7 mm and 13.9–14.8 mm events, respectively), enabling comparison to growing-season monthly precipitation (Supplementary Figure S1).

Trait measurements

Physiological and morphological traits were measured on independent seedling replicates that were destructively sampled throughout the study period. Seedlings were randomly selected such that the total number of seedlings measured was 4 seedlings per population by treatment combination per measurement day. Our sample size was limited by our ability to measure gas exchange during the timeframe that the seedlings were photosynthetically active. We measured height (0.1 cm precision), stem diameter (0.01 mm), aboveground biomass (0.0001 g), leaf area (0.0001 cm²), leaf fresh and dry mass (0.0001 g), and maximum photosynthetic rate ($A_{\max}$; $\mu\text{mol m}^{-2} \text{s}^{-1}$) before the pulse treatments began (Day 0; $n = 15$ per seed source; 60 total), after two pulse cycles but before the final pulse (Day 54; $n = 4$ seedlings per population and treatment; 48 total), and on the three consecutive days after the final pulse (Days 56–58; $n = 4$ seedlings per seed source and treatment; 48 total per day). $A_{\max}$ was not measured on Day 57. Additionally, belowground biomass (0.0001 g), total biomass (0.0001 g), needle stable carbon isotope ratio ($\delta^{13}\text{C}$, ‰), needle stable nitrogen isotope ratio ($\delta^{15}\text{N}$, ‰), needle per cent carbon, and needle per cent nitrogen were measured before the treatment period (Day 0; $n = 15$ per seed source; 60 total), and before the final pulse (Day 54; $n = 4$ seedlings per seed source and treatment; 48 total).

Gas exchange was measured on all sampled seedlings, except Day 57, using a portable open-system infrared gas analyzer (Li-6400, LI-COR Biosciences, Lincoln, NE, USA) with the narrow leaf chamber (2 cm × 3 cm). Measurements were taken on either the whole seedling canopy or a section at the top of the canopy if the growth was longer than the growth chamber. We set the chamber to control photosynthetically active radiation, block temperature, and carbon dioxide concentration in the reference cell. The carbon dioxide concentration was set 5 ppm above ambient. Block temperature was set 1–2°C above ambient, but not above 29°C; these temperatures were generally 27–29°C, except during the cooler sampling period in May, when block temperature was set to 22°C. Photosynthetically active radiation was set at a constant 1150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ throughout all measurement dates. Flow speed was set to either 250 or 350 $\mu\text{mol s}^{-1}$, and the fan speed was set to slow. Gas exchange data were corrected for leaf area by harvesting the needles measured in the leaf chamber. Total leaf area was measured for all needles from each seedling using a scanner (Epson Perfection V850, Suwa, Nagano, Japan) and image analysis software (WinSEEDLE, Regent Instruments, Québec City, Québec, Canada). Samples were weighed using an analytical balance (AL104, Mettler Toledo, Columbus, OH, USA). Needles were pooled for each seedling, weighed fresh, then dried in an oven at 65°C for 72 h and weighed again to obtain dry weight. Needle leaf moisture content (LMC) was calculated as fresh weight minus dry weight, divided by the dry weight, then multiplied by 100 to obtain a percentage. Only whole needles were used for LMC measurements. After dry weights were recorded, needle samples were ground in a ball mill (Spex 8000D dual mixer/mill, Metuchen, NJ, USA) and analysed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, per cent carbon, and per cent nitrogen at the Nevada Stable Isotope Laboratory. SLA was calculated as the ratio of leaf area to dry weight (Roderick *et al.* 1999). The root:shoot was calculated as the ratio of belowground to aboveground dry biomass. C:N was calculated as the

ratio of per cent carbon to per cent nitrogen in needle samples. For trait variables that were not ratios (i.e. height, stem diameter, and biomass), we calculated relative growth across the treatment period to standardize for initial size differences by subtracting the mean pretreatment trait value (for each population) from the individual post-treatment trait value, then dividing by the mean pretreatment trait value. Many traits required destructive sampling, so pretreatment trait values could not be measured on the same individual seedlings used in subsequent analyses.

Statistical analysis

Multivariate analysis

To elucidate whole-plant resource-use strategies that represent combinations of correlated traits, we conducted a principal component analysis (PCA). We used the end-of-treatment data from Day 54 to create a matrix of seedling trait variables (relative growth and final measurements of height, stem diameter, total biomass, and aboveground and belowground biomass; and final log-transformed LMC and SLA, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, carbon to nitrogen ratio, per cent nitrogen, and $A_{\max}$). The PCA was used to reduce the multivariate dimensionality of morphological and ecophysiological traits to an uncorrelated set of ordination axes representing the dominant patterns of seedling variation. We then explored the relationships between seedling traits, seed source population, and pulse treatment, through graphical display on the ordination biplots and by using a permutational multivariate analysis of variance (PERMANOVA) to formally test the multivariate significance ($\alpha \leq 0.05$) of population and treatment as grouping factors (Oksanen *et al.* 2022). For the PERMANOVA, we analysed Bray–Curtis dissimilarities of the trait matrix using a model with seed source population, pulse treatment, and their interaction, with significance assessed using 999 permutations. Homogeneity of multivariate dispersion among populations and treatments was evaluated using permutational multivariate analysis of dispersion (PERMDISP) with 999 permutations. All multivariate analyses used the vegan package in R (Oksanen *et al.* 2022, version 4.2.1, R Core Team 2022).

Univariate analysis

To evaluate baseline, pretreatment population-level differences in seedling traits (height, stem diameter, total biomass, aboveground biomass, belowground biomass, root:shoot, LMC, SLA, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N), we conducted separate one-way ANOVAs for each trait using population (factor with four levels) as a predictor variable ($n = 15$ seedlings per seed source; $n = 60$ total). Data were collected following the dry-down period but before the start of the pulse treatments.

Then, to evaluate seedling trait responses to varying pulse regimes, we conducted separate, two-way ANOVAs for each trait as a function of pulse treatment (factor with three levels), population (factor with four levels), and their interaction. Trait values measured after two full pulse cycles were used as response variables. We used post-treatment relative growth in height, stem diameter, and SLA from data collected on Days 54, 56, 57, and 58 ($n = 16$ seedlings per seed source per treatment; $n = 192$ total). We used growth of aboveground, belowground, and total biomass, root:shoot, isotope data, and LMC collected on a single

date (Day 54; $n = 4$ seedlings per seed source per treatment; $n = 48$ total). We explored adding a seedling tray as a random intercept; AIC scores were within 2 units for all variables except C:N, and model interpretations were unchanged, so we opted for the more parsimonious models without a random effect.

To evaluate population-level differences in instantaneous responses to the final precipitation pulses of different magnitudes, we conducted separate ANOVAs for each trait ($A_{\max}$ and LMC) as a function of treatment (factor with three levels), population (factor with four levels), date (factor with three levels; Days 54, 56, and 58), and all possible interactions ($n = 4$ seedlings per seed source per treatment per day; $n = 144$ total).

All analyses were conducted in R (version 4.2.1, R Core Team 2022). The Tukey HSD function was used to test for significant differences in predictor variables ($P < .05$) and to evaluate pairwise comparisons across groups (R Core Team 2022).

Results

Multivariate analysis

The PCA showed variation among populations, which was supported by a PERMANOVA, which indicated that trait values were significantly grouped by population and treatment (population $P = .001$, treatment $P = .045$). The first two PCA axes explained 41.96% and 19.74% of the variation, respectively (Table 1). These two axes describe distinct axes of trait variation that represent potential trade-offs between resource-use strategies: acquisitive leaf traits vs. biomass/structure, and NUE vs. WUE (Fig. 2).

Table 1 Principal component analysis correlation coefficients for each principal component axis (PC1 and PC2).

	PC1	PC2	Trait category
Total biomass	0.351	-0.018	Size
Aboveground biomass	0.336	-0.087	Size
Belowground biomass	0.334	0.042	Size
Relative total biomass growth	0.313	-0.073	Growth
Relative belowground biomass growth	0.298	-0.027	Growth
Relative aboveground biomass growth	0.277	-0.125	Growth
Height	0.273	-0.238	Size
Stem diameter	0.194	-0.174	Size
Relative height growth	0.174	-0.332	Growth
C:N	0.119	0.49	Leaf
Relative stem diameter growth	0.087	-0.011	Growth
$\delta^{13}\text{C}$	0.063	-0.354	Leaf
$\delta^{15}\text{N}$	0.061	0.296	Leaf
Per cent N	-0.145	-0.458	Leaf
$A_{\max}$	-0.225	-0.064	Leaf
LMC	-0.247	-0.296	Leaf
SLA	-0.288	-0.128	Leaf

Each variable was assigned a trait category to help conceptualize the results.

The first principal component (PC1; Fig. 2 and Table 1) explained 41.96% of the variation and demonstrated differences in acquisitive leaf traits and biomass. Low values on PC1 were positively correlated with SLA, LMC, and $A_{\max}$, whereas high values on PC1 were positively correlated with seedling size and growth. PC1 generally separated the arid/summer-dry population (San Bernardino) from the remaining three populations that were either less arid or experienced greater summer rainfall.

The second principal component (PC2; Fig. 2 and Table 1) explained 19.74% of the variation and demonstrates a trade-off between WUE and NUE. Low values of PC2 were positively correlated with per cent nitrogen, $\delta^{13}\text{C}$ (‰), relative height growth, LMC, and height, whereas high values of PC2 were positively correlated with C:N and $\delta^{15}\text{N}$ (‰). The intermediate-climate (Desatoya) population had higher PC2 values than the other three populations.

Univariate analysis

Linear models of individual traits were consistent with the PCA results, showing important differences in seedling traits among

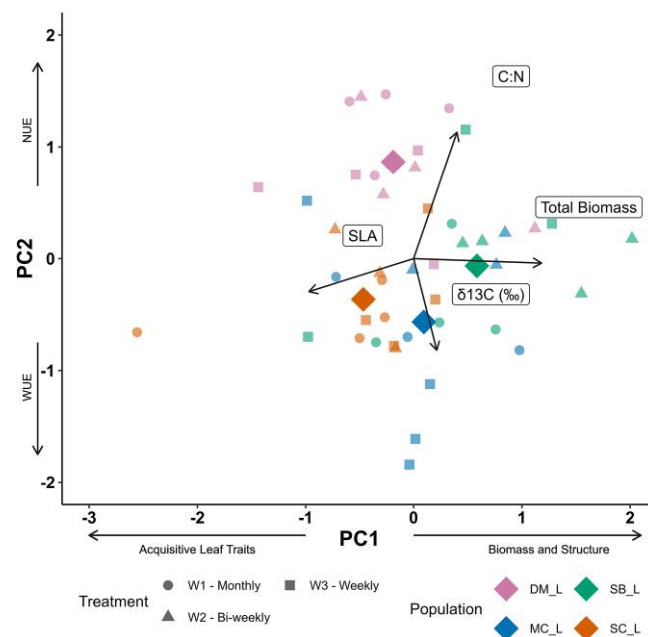


Figure 2 Principal component analysis biplot comparing post-treatment seedling traits for four *P. monophylla* seed sources grown in three pulse treatment groups. Smaller points represent individual seedlings, with colours representing seed sources and symbol shapes representing watering treatments. Large diamonds represent the population centroids. Arrows represent trait vectors for selected traits with relatively strong correlations to the first two principal components. PC1 explained 41.96% of the total variance and captured multivariate variation associated with seedling size, growth, and leaf traits commonly linked to resource acquisition (specific leaf area and maximum photosynthetic rate). PC2 explained 19.74% of the variance and reflected opposing contributions of traits related to nitrogen and water economy, including C:N ratio, $\delta^{15}\text{N}$, per cent nitrogen, and $\delta^{13}\text{C}$. For visual clarity, only a subset of contributing traits that represent the overall trade-offs is displayed as vectors in the biplot; complete trait loadings for all variables are reported in Table 1. SB = San Bernardino (arid/summer-dry); MC = Mt. Charleston (arid/summer-wet); SC = Schell Creek (mesic/summer-wet); DM = Desatoya Mountains (intermediate).

Table 2 ANOVA outputs for post-treatment trait linear models.

	<i>F</i> -statistic/ <i>P</i> -value	Post-treatment change			Post-treatment trait values		
		Population	Treatment	Pop × Treat	Population	Treatment	Pop × Treat
Height (cm)	<i>F</i> _{11,180}	6.82	1.69	1.2	41.4	1.36	1.02
	<i>P</i>	<.001	.19	.31	<.001	.26	.41
Stem diameter (mm)	<i>F</i> _{11,180}	3.16	3.08	1.21	20.01	2.49	1.01
	<i>P</i>	.03	.05	.3	<.001	.09	.42
Aboveground biomass (g)	<i>F</i> _{11,36}	1.74	2.72	0.44	19.21	3.47	0.74
	<i>P</i>	.18	.08	.85	<.001	.04	.62
Belowground biomass (g)	<i>F</i> _{11,36}	1.38	1.97	1.17	6.52	2.24	0.96
	<i>P</i>	.26	.15	.35	.001	.12	.47
Total biomass (g)	<i>F</i> _{11,36}	1.34	2.42	0.91	12.26	2.81	0.85
	<i>P</i>	.28	.1	.5	<.001	.07	.54
Root:Shoot	<i>F</i> _{11,36}	–	–	–	2.32	1.94	0.89
	<i>P</i>	–	–	–	.09	.16	.51
LMC (%)	<i>F</i> _{11,35}	–	–	–	4.06	3.28	0.51
	<i>P</i>	–	–	–	.01	.05	.80
SLA (cm ² g ^{−1})	<i>F</i> _{11,178}	–	–	–	22.00	7.84	1.60
	<i>P</i>	–	–	–	<.001	<.001	.15
δ ¹³ C (‰)	<i>F</i> _{11,33}	–	–	–	3.05	0.76	0.38
	<i>P</i>	–	–	–	.04	.47	.88
δ ¹⁵ N (‰)	<i>F</i> _{11,33}	–	–	–	102.14	1.03	0.74
	<i>P</i>	–	–	–	<0.001	0.37	0.62
C:N	<i>F</i> _{11,33}	–	–	–	9.54	1.44	2.89
	<i>P</i>	–	–	–	<.001	.25	.02
<i>A</i> _{max}	<i>F</i> _{11,36}	–	–	–	4.27	1.32	0.28
	<i>P</i>	–	–	–	.01	1.32	.36

Predictor variables included population, treatment, and their interaction. *F*-statistics (ndf, ddf) and *P*-values are provided, and *P*-values < .05 are bolded. Trait changes were calculated relative to pretreatment values. LMC and SLA were log-transformed.

populations but generally not among pulse treatment groups (Table 2). Overall, there were population-level effects on many traits during the treatment period, with fewer effects of treatment or population-by-treatment interactions. Univariate comparisons for the trait variables with the largest PCA coefficients are shown in Fig. 3, while other trait variables are shown in Supplementary Figures S3–S6, and pretreatment trait values are shown in Supplementary Table S4.

There were population-level differences in several measurements of seedling size and biomass. The seedlings sourced from the summer-wet populations (Schell Creek and Mount Charleston) exhibited the greatest relative height growth during the treatment period (Supplementary Figure S3a), with values 0.14–0.16 higher than those of the other populations. Overall, the seedlings from the arid seed source environments (San Bernardino and Mount Charleston) were the largest at the end of the treatment period (larger total biomass Fig. 3a, 0.44–1.59 cm taller, 0.17–0.47 mm wider in stem diameter, 0.06–0.17 g larger in aboveground biomass, and 0.13–0.16 g larger in belowground biomass; Fig. 3a, Supplementary Figure S3b, S3d, S4b, and S4d). No population-level differences existed in total, aboveground, or belowground relative biomass growth, or root:shoot during the treatment period (Table 2; Supplementary Figure S3e, S4a, S4c, and S4e).

There were population-level differences in post-treatment leaf traits (SLA, δ¹³C, C:N, and LMC) that were not clearly arranged along the ranges of seed source CWD and summer precipitation (Table 2; Supplementary Figure S5a, b, Supplementary Figure S6a, S6c, Fig. 3a and c, and Supplementary Figure S6b). However, seedlings from summer-wet seed sources had the lowest mean δ¹⁵N (Schell Creek: −2.67‰ ± 0.36, Mount Charleston: 2.98‰ ± 0.38; Supplementary Figure S6b), indicating higher discrimination against ¹⁵N.

The only significant pulse treatment effects were for relative stem diameter growth, aboveground biomass, and SLA (Supplementary Figures S3c, S4b, and Fig. 3a). The W1—Monthly treatment had the lowest relative stem diameter growth (0.045 cm ± 2.22; Supplementary Figure S3c), lowest final aboveground biomass (0.185 g ± 0.016; Supplementary Figure S4b), and highest SLA (71.5 cm² g^{−1} ± 2.22; Fig. 3a). The W2—biweekly treatment had the highest mean aboveground biomass (0.241 g ± 0.016; Supplementary Figure S4b). The W3—weekly treatment had the highest relative stem diameter growth (0.11 cm ± 0.019; Supplementary Figure S3c) and lowest SLA (60.5 cm² g^{−1} ± 2.22; Fig. 3a). There was only one population-by-treatment interaction effect, for C:N, where Desatoya seedlings had the highest C:N for the W1—monthly treatment, with non-significant population-level differences for the other treatments (Fig. 3d).

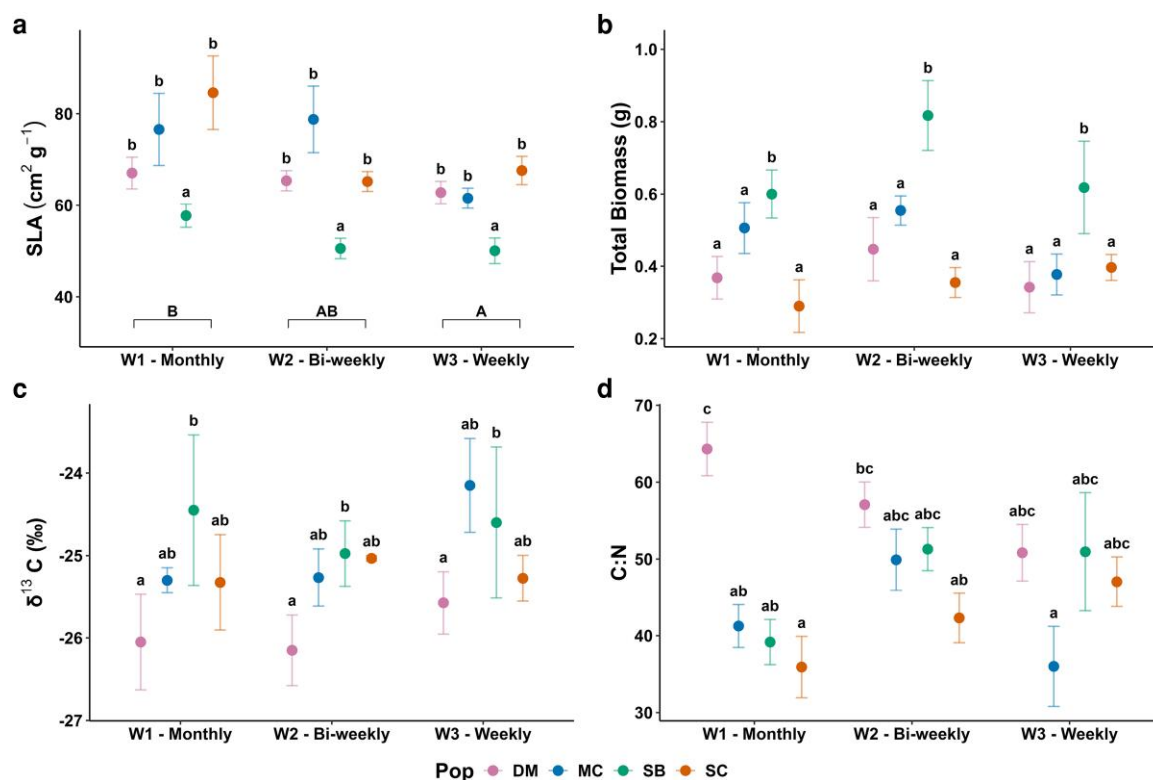


Figure 3 Post-treatment univariate comparisons for trait variables driving *P. monophylla* seedling resource use strategies (i.e. trait variables with the largest PCA coefficients). The first row (a, b) shows the trade-off between SLA and total biomass. The second row (c, d) shows the trade-off between WUE ($\delta^{13}\text{C}$) and NUE (C:N). Dots show the mean trait value, and the error bars represent standard error. Different lowercase letters represent significant population-level differences in traits. Different uppercase letters represent significant treatment-level differences. The *F*-statistics and *P*-values are shown in Table 2. All measured trait variables are shown in Supplementary Figures S3–S6. SB = San Bernardino (arid/summer-dry); MC = Mt. Charleston (arid/summer-wet); SC = Schell Creek (mesic/summer-wet); DM = Desatoya Mountains (intermediate).

Discussion

Distinct seedling resource-use strategies were related to seed source environments

Functional traits related to acquisition and conservation strategies may be crucial for understanding seedling responses to environmental changes, especially with regional warming and changing precipitation patterns that shift seasonal water availability (Reich *et al.* 2003, Ramírez-Valiente *et al.* 2022). Resource-use strategies may change across life history stages in some species (Solé-Medina and Ramírez-Valiente 2023). The trade-off framework between resource-use strategies explains how plants maximize their success in a specific climate (Reich and Cornelissen 2014). However, the presence of alternative resource-use strategies does not necessarily indicate life-history trade-offs. For example, a meta-analysis of Mediterranean *Pinus* and *Quercus* species found no consistent evidence for the hypothesized trade-off between stress tolerance and growth potential (Ramírez-Valiente *et al.* 2022). We found strong population-level differences in *P. monophylla* seedling traits in our experiment. The observed pattern of ITV was associated with the seed source environment, suggesting that there may be local adaptations to climate conditions such as precipitation amount, seasonality, and water deficit. However, given the lack

of observed treatment by population interactions, these adaptations do not appear to be driven by precipitation pulse frequency and magnitude if total precipitation is held constant, as was simulated in our experiment.

Our study identified multiple resource-use strategies in *P. monophylla* seedlings that were effectively partitioned by seed source environments at endpoints of range-wide climatic gradients. Our multivariate analysis, which facilitates exploration of how multiple correlated traits varied across source populations and pulse watering treatments, highlights that, when considering seedling ITV, it is important to examine individual traits as components of an integrated resource-use strategy (Carvalho *et al.* 2020, Zhang *et al.* 2020, Welles and Funk 2021). These patterns are consistent with the broader literature on integrated strategies. *Pinus sylvestris*, e.g. has demonstrated correlated functional traits, including leaf and stem traits and plant size (Carvalho *et al.* 2020). In our study, we assessed whole-plant trait responses in leaves, stems, and roots, as well as plant size and growth. Our analyses showed that *P. monophylla* seedlings had two distinct resource-use trade-offs, represented by the two axes of variation identified through a PCA: (i) acquisitive leaf traits vs. biomass/structure and (ii) NUE vs. WUE.

The first principal component described a trade-off between acquisitive leaf traits and biomass/structure associated with the seed source CWD. Consistent with a prior *P. monophylla* study (Vasey *et al.* 2023), seedlings from our driest site with low

summer precipitation (San Bernardino) grew larger, prioritizing structure with the highest total biomass and lowest SLA. In contrast, seedlings from seed sources with higher summer precipitation (Schell Creek and Mount Charleston) demonstrated a more resource-acquisitive strategy, with higher SLA and rapid height growth. This trade-off between carbon capture and biomass production may relate to the storage and production of non-structural versus structural carbohydrates (Long *et al.* 2021). For many pine species, including *P. monophylla*, SLA has a negative relationship with aridity and seasonality of precipitation (Letts *et al.* 2009, Lopez *et al.* 2013, Putz *et al.* 2021, Vasey *et al.* 2022).

The second principal component described a trade-off between NUE (represented by C:N) and WUE (represented by $\delta^{13}\text{C}$). Multiple studies have demonstrated that the trade-off between NUE and WUE depends on which resource is more limiting (Field *et al.* 1983, Gong *et al.* 2011). Compared to other drought-adapted pine species from the Great Basin and Sierra Nevada, *P. monophylla* maintains higher WUE at similar levels of PNUE (DeLucia and Schlesinger 1991). In arid conditions, seeds from drier areas often produce seedlings with higher WUE (Gouveia and Freitas 2009, Salazar *et al.* 2018). Similarly, some plant species demonstrate ITV related to nitrogen availability, such as through modifications in nitrogen uptake strategies (Spasojevic and Weber 2021). In our study, the Desatoya seedlings had trait values that did not reflect their intermediate climatic conditions. Desatoya seedlings had the lowest $\delta^{13}\text{C}$ (WUE), the highest $\delta^{15}\text{N}$, and relatively high C:N (NUE), especially under infrequent watering pulses. Strong intraspecific differences in NUE are unexpected for seedlings grown in the same soil medium (Snyder *et al.* 2022). While we found little literature to guide interpretation of this result, this finding may be related to seed source differences in edaphic or biotic conditions that influence nitrogen availability and fractionation (van der Sleen *et al.* 2017).

Pulse dynamics did not drive intraspecific trait variation

Counter to our predictions, precipitation pulse treatments had little influence on seedling traits, suggesting that *P. monophylla* seedlings may not be sensitive to short-term pulse frequency or magnitude when total precipitation is held constant. We expected *P. monophylla* to exhibit plastic responses to pulse dynamics because other dryland woody species (e.g. *Ephedra fragilis*, *Lycium intricatum*, and *Retama sphaerocarpa*) have demonstrated changes in drought-related traits under varying pulse regimes (Padilla *et al.* 2009). However, Davis (2020) found that for another dryland tree species, *Prosopis velutina*, the mean amount of water addition impacted the response more than the pulse frequency. While the low sample size in this study limits power to detect differences, the few treatment-level effects we observed suggest weakly plastic responses: seedlings of all populations showed varying stem diameter and SLA across pulse regimes, and instantaneous LMC differed following pulses of different magnitudes (Supplementary Table S5). Seedlings receiving frequent, small watering pulses had lower SLA than those experiencing less frequent but higher magnitude pulses, which may indicate drought conditions in the frequent watering treatment, as seedlings never received large quantities of water at any one time. A study of planted *Pinus ponderosa* seedlings found

that dry periods were alleviated only by more frequent or larger pulses, and that a moderate dry period reflected consistent drought rather than a pulse regime (Koehn *et al.* 2022).

Implications for *P. monophylla* seedling regeneration

Demonstrated variations in resource acquisition and conservation strategies are important because, as the Great Basin continues to warm and precipitation regimes become more variable, local strategies may not match the adaptive landscape. Furthermore, climatic changes are not likely to be uniform across *P. monophylla*'s range. The northern portion of *P. monophylla*'s range is predicted to experience the greatest increase in mean annual temperature, whereas the southern portion is predicted to experience the greatest increase in precipitation (Snyder *et al.* 2019). Overall, these changes are likely to contribute to increased drought duration, invasive grasses, and wildfire, as well as changes to winter snowpack, likely increasing ephemeral snowpack at the expense of seasonal snowpack (Dennison *et al.* 2014, Bradley *et al.* 2016, Petersky and Harpold 2018, Snyder *et al.* 2019).

Based on the results of this study, we might expect rising temperatures to increase selective pressure towards conservative resource strategies in the dry regions with low summer precipitation, as warming could fundamentally alter hydrology and water availability through increased atmospheric water demand and changes in the timing of snowmelt (Peterson 1998, Snyder *et al.* 2019). Conversely, warming temperatures may favour resource acquisition strategies in regions with higher summer precipitation, regardless of overall aridity, as increasing growing season length coinciding with summer precipitation may make conditions increasingly favourable for growth. *Pinus sylvestris* seedlings sourced from hotter, drier environments, e.g. had higher survival than other populations in common gardens grown in their local environment (Spain) as well as at a cooler, wetter site (Germany), during years that were drier and hotter than normal (Ramírez-Valiente *et al.* 2021). This supports the prediction that in cooler and wetter environments that are becoming drier, seedlings from hotter and drier sites could perform better than local seedlings. Although we found relationships between the seed source environment and whole-plant trait trade-offs, our study design cannot confirm instances of local adaptation. As such, future studies could focus on (i) determining to what extent demonstrated trait variation is genetically determined or a result of phenotypic plasticity, (ii) exploring how populations with different resource-use strategies perform in contrasting environments, and (iii) identifying if these ecologically important traits are heritable.

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Author contributions

Jeremy S. Adkins, Alexandra K. Urza, Keirith A. Snyder, and Peter J. Weisberg conceived and designed the experiment, collected the data, and wrote the paper. Jeremy S. Adkins performed the data analysis and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Supplementary material

Supplementary material is available at [AoB Plants](https://doi.org/10.1093/aob/plb13282) online.

Conflicts of interest

The authors have no conflict of interest to declare.

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Data availability

Raw data and R code are available online at <https://doi.org/10.5281/zenodo.20114113>

Ethics approval

The species sampled is not endangered or protected. The work was conducted in a greenhouse and no permission was required.

Consent for publication

Not applicable.

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