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RESEARCH ARTICLE

The memory of past water abundance shapes trees 7 years later

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Abstract

Premise: Tree structure and function are constrained by and acclimate to climatic conditions. Drought limits plant growth and carbon acquisition and can result in "legacy" effects that last beyond the period of water stress. Leaf and twig-level legacy effects of past water abundance, such as that experienced by trees that established under wetter conditions are unknown.

Methods: In an 18-year forest irrigation experiment, we explored the lasting structural impact of past water richness on leaves and twigs of *Pinus sylvestris* using synchrotron-based X-ray microscopy. We compared 47 anatomical traits among never-irrigated control trees, trees irrigated for 18 years, and formerly irrigated trees, 7 years after their return to control-level water availability in this naturally dry forest.

Results: We found that legacy effects induced by a period of experimental irrigation continue to shape the structure of new leaves and twigs long after a sharp decrease in water availability. Legacy effects shaping twigs were present but dissipating, while leaf traits displayed long-lasting effects on structural strategy, with extreme values for traits associated with high water stress and low productivity.

Conclusions: Mature trees acclimating to an increasingly dry world may be at a disadvantage compared to young trees that have known only chronic drought. However, these young trees may be capable of thriving in sites of drought-related forest decline especially if planted while larger individuals are still present to support tree establishment. Without a legacy of past water abundance, trees in future forests may be better equipped to cope with our rapidly changing climate.

KEYWORDS

functional traits, leaf anatomy, legacy effects, Pfynwald experiment, *Pineaceae*, *Pinus sylvestris*, tree mortality, water stress

The ways in which past conditions, such as drought, shape future tree performance may determine the impacts of climate change on forests (Anderegg et al., 2015; Müller and Bahn, 2022). Water scarcity limits plant growth and carbon acquisition and leads to post-drought "legacy" effects that limit tree productivity and stress tolerance for multiple years (Cosgrove 1987, 2005; Peltier et al., 2022; Blumstein and Furze, 2023). Droughts, especially extended hot droughts, can therefore have impacts on tree and ecosystem function that persist for several years, increasing during successive drought years (Anderegg et al., 2015; Kannenberg et al., 2020; Szejner et al., 2020; Müller and Bahn, 2022; Sillett et al., 2022) and possibly contributing to long-term acclimation (Gessler et al., 2020). Such climatic legacy effects have important implications for our ability to predict forest carbon assimilation and retention. For example, drought-legacy effects influence carbohydrate depletion and cause decreases in trunk growth (Anderegg et al., 2015; Kannenberg et al., 2019; Peltier and Ogle, 2019; Peltier et al., 2022; Blumstein and Furze, 2023). Whether or not carbohydrate depletion or chemical acclimation to drought periods occur, the anatomical structure of wood and evergreen leaves acclimates to water availability in the short term (Dobbertin et al., 2010; Oldham et al., 2010; Beier et al., 2012; Eilmann et al., 2013; Azuma et al., 2016; Chin and Sillett, 2019), in ways that could leave lasting performance effects across and beyond organ lifespan. Fortunately, forest-scale manipulative experiments (e.g., Beier

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et al., 2012; Barbeta et al., 2013; Paschalis et al., 2020) and long-term monitoring plots (e.g., Acker et al., 1998; Schaub et al., 2011; Thimonier et al., 2001; Wolf et al., 2009; Smith et al., 2015), which are increasingly common, can be leveraged to address these unknowns across scales, from climate to anatomy.

What happens to twig and leaf structure when water is no longer plentiful, and how long do trees take to produce structures that better reflect a water-limited anatomical baseline? In most forests experiencing climate change today, the trees were established in previous centuries and have experienced much wetter times. The lasting impact of past water availability on tree structure may thus be more important to forest functioning than the better-documented legacy effects of drought because the largest forest trees of today were established under water-rich conditions that no longer exist (Balting et al., 2021; Vicente-Serrano et al., 2022). The Pfynwald forest irrigation experiment, where natural precipitation was doubled for 20 years ("Irrigated" treatment), may be the only place in the world where it is possible to assess the impacts of both past and present water availability on the structure of mature trees in a natural forest (Zweifel et al., 2020; Bose et al., 2022). Irrigation-induced changes in *Pinus sylvestris* including increased radial growth, carbohydrate storage, fine root growth, and leaf expansion as well as decreased crown transparency, resulted in a "water-rich" tree syndrome that may be ill-suited for the low water availability of rain-fed "Control" conditions in this dry system (Dobbertin et al., 2010; Eilmann et al., 2013; Schönbeck et al., 2018; Brunner et al., 2019; Zweifel et al., 2020). Halting irrigation in this system after 11 years ("Irrigation-stop" treatment) resulted in a gradual return to control levels of radial trunk growth and leaf length over 4 years (Zweifel et al., 2020). However, this trend of slowing growth in formerly irrigated Irrigation-stop trees has continued to progress below the current Control growth rate, resulting in individual trees that grow less annually than they had before the irrigation period (Vitali et al., 2024). Perhaps this lack of vigor is because summertime transpiration in Irrigation-stop trees declines more abruptly than in Control trees (Zweifel et al., 2020), but it is also potentially due to persistent structural modifications to the photosynthetic organs and the twigs that support them. As the end of the hydraulic chain, leaves and twigs experience the lowest water potentials in the tree, while being responsible for all carbon assimilation; thus, it is imperative to explore anatomical-scale legacy effects on leaves and twigs. The capacity of these key organs to tolerate and adjust to reduced water availability defines the ability of individual trees and tree species to survive in an increasingly hotter and dryer world, shaping ecosystems through their success or failure under climate change. But whether and how past water abundance shapes these critical tree structures remains a complete mystery—and the subject of our study here.

Does a history of high water availability influence the structure and function of leaves and twigs through long-term legacy effects if dry conditions return or appear? Our objective was to broadly explore the anatomy and evidence of water stress in the form of acclimations for tolerance or indications of hydraulic limitation in recently produced leaves and twigs in relation to current and past water availability at Pfynwald, one of the driest forests in the Alps. Specifically, we ask, is there a structural legacy of high water availability in the distal organs of trees? If so, what trait syndromes are these manifested in? For leaves, or twigs, or both? We hypothesized that experimentally Irrigated trees would be structurally different from rain-fed Control trees. We also expected that previously irrigated, Irrigation-stop trees, which had returned to naturally low, Control-level water availability 7 years prior, would have current-year twigs and leaves that were indistinguishable from those of rain-fed Control trees. However, trees can be surprising, and we do not know how long legacy effects may linger. Such ecological legacy effects remaining from a pre-climate-change world could have important implications for our assessment of tree vulnerability to climatic factors and for predicting forest success and ecological change with a drier climatic baseline.

MATERIALS AND METHODS

Study site and sampling

In a dry portion of the Rhône River Valley, the Pfyn-Finges Nature Park, in Valais, Switzerland is the site of the Pfynwald forest irrigation experiment (46°18′N, 7°3′E, 615 m a.s.l.). The Pfynwald Experiment began in June 2003 and covers 876 mature, naturally regenerated Scots pine (*Pinus sylvestris*) trees with an average age >100 years and a canopy height of ~10–11 m. The site is located at the dry edge of the distribution of Scots pine, and drought-induced tree mortality is common in that region (Hunziker et al., 2022). The experiment was established after tree mortality events of the 1990s that had been associated with patterns of decreasing regional rainfall (Bose et al., 2022; Hunziker et al., 2022). From April to October, four of eight 1000-m^2 experimental plots have been irrigated each year at a level consistent with local rainfall (3.8 mm per night, 600 mm added annually), approximately doubling water availability (Irrigation treatment), while the other four plots were monitored, but not irrigated (Control treatment), receiving water exclusively from rainfall. After 2013, the Irrigation treatment was halted in one half of each irrigated plot (Irrigation-stop treatment), and not resumed. In March 2022, we collected distalmost treetop twigs and leaves from 25 individual trees ($N = 9$ Control, 6 Irrigated, and 10 Irrigation-stop trees; Appendix S1) using canopy-access scaffolds. We used the most recent growth only, i.e., twigs and leaves produced in the previous 2021 growing season. That October we collected an additional leaf sample from each of five new trees to balance our study design to include 10 trees per treatment (i.e, $N = 1$ new Control, 4 new Irrigated; Appendix S1). Twigs were not analyzed from this second collection because they would have grown, whereas leaves are far more anatomically stable. Leaves used from the second collection were of the same 2021 cohort as the first collection.

Anatomical analyses

Following the initial March 2022 collection, samples were immediately transported to the Swiss Light Source at the Paul Scherrer Institute in Villigen, Switzerland (3 h by train) where they were scanned the same night using synchrotron-based X-ray tomographic microscopy (microCT) at the TOMCAT beamline (Stampanoni et al., 2006). All 50 scans (1 leaf and 1 twig from each of 25 individual trees; see Appendix S1) were taken with a 10× objective, which gives 0.65 μm resolution. Using a beam energy of 20 keV, we captured ~1 mm of sample height (volumes varied), with scans of 1501 projections of 100 ms exposure (i.e., ~2.5 min), with no repetitions made on the same sample in case of failed scans to reduce the risk of radiation damage or water loss. Each scanned twig was cut to a length of 2 cm (10–20× longer than the tracheids in this species) and scanned in the middle of this section to avoid any cutting artefacts such as embolisms or lost resin. Leaves were kept whole during scanning and sealed in polyimide tape to avoid water loss while keeping high X-ray transmittance. Images were reconstructed at TOMCAT using both absorption phase reconstruction (Marone and Stampanoni, 2012; pictured in Figure 1: twig) and phase-contrast enhancement reconstruction (Paganin et al., 2002; pictured in Figure 1: leaf). MicroCT allowed the measurement of the size and circularity of transfusion tracheids and counts of fibers and amyloplasts in leaves and measurement of tracheid length in twigs, none of which were possible with traditional histology. The final five leaf samples collected near the end of summer were sectioned fresh with a microtome using the method of Chin and Sillett (2017). For all 30 leaf samples (both collections), we made acrylic resin imprints of adaxial and abaxial leaf surfaces to capture stomatal anatomy; the resin (nail polish) was placed directly onto glass slides, and leaves were laid in the resin with the appropriate side down and then popped off when dry (Chin and Sillett, 2017).

We selected and measured a wide range of anatomical traits (29 leaf traits, and 18 twig traits; Appendix S2) that covered attributes associated with either hydraulic limitation or water stress tolerance and sets of traits that expressed ecological strategy such as productivity, local storage, size and surface to volume ratios, defense, and variations in water-use strategy (Appendix S2). All traits were measured using the program Fiji (Schneider et al., 2012). We relied on the linear measurement tool for lengths and diameters, areas were hand traced as needed, and transfusion tracheid circularity and size were measured with "analyze particles" after isolating the tissue. Traits extracted from microCT images were measured on the reconstruction where they were most distinct.

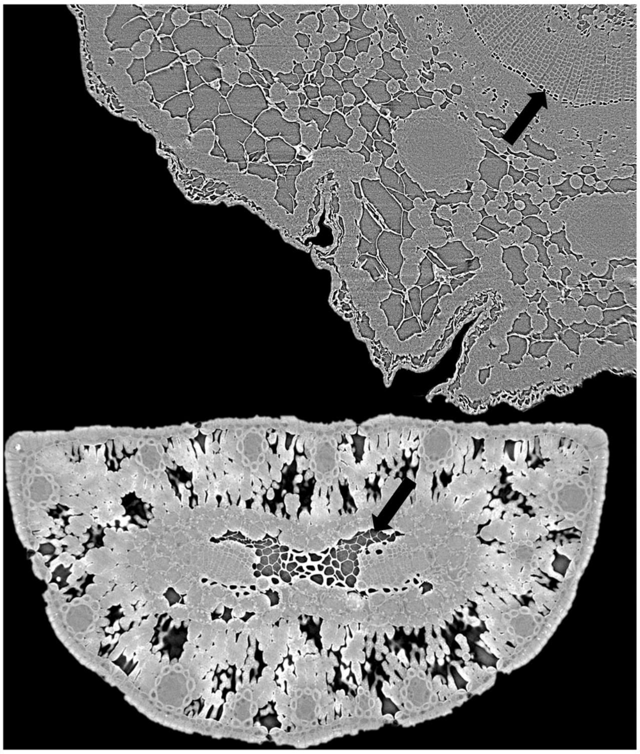


FIGURE 1 Synchrotron-based X-ray tomographic microscopy (microCT) images of intact *Pinus sylvestris* twig and leaf shown in cross section. These 0.65-μm-resolution images (50 total) were used to measure most of the 49 leaf and twig traits assessed (see details in Appendix S2). Vascular embolisms (arrows) were present in all samples due to a recent thaw event.

Data analyses

Our focus for this study was on broad patterns of multivariate trait differences; thus, univariate tests were not used for individual anatomical traits. (Means, standard deviations, and coefficients of variation [CVs] for traits are provided in Appendix S1.) Instead, we discuss the 47 individual anatomical traits in the context of their ranking among treatments and membership in functional groupings or "trait syndromes", rather than assigning probabilities to their differences. We took two complementary approaches to exploring the distance of the twigs and leaves from the three Pfynwald treatments in multivariate anatomical trait space: (1) analysis of space occupancy in reduced-dimensional trait space using the geometry of principal components analysis (PCA) axis scores, and (2) a probabilistic assessment of the differences among treatments using two complementary approaches; multiresponse permutation procedures (MRPP) and analysis of variance using distance matrices (Adonis). All multivariate analyses (PCA, MRPP, and Adonis) were implemented with the R package vegan (Oksanen et al., 2022).

First, for the two PCAs for twigs and leaves, we retained all 18 twig and 25 of 29 leaf traits (as noted in Appendix S2). Among these traits, we could not detect bivariate nonlinearities, and skewness and kurtosis of individual traits were minor. The four excluded leaf traits were those for which we only had microCT data (Appendix S2). The PCAs were done on the correlation matrix because of the different scales; for both twigs and leaves, we retained three axes. Individual, twigs or leaves were ordinated on these axes, and for each treatment, we calculated their three-dimensional volumes and their pairwise intersections using the R package hypervolume (Blonder et al., 2022). In the case of twigs, which had an uneven sample size, we corrected the three-dimensional volumes (hereafter, V_c) occupied by each treatment by dividing by N^3. This space-occupancy approach is geometric and not statistical in nature, so we also employed multivariate trait-based analyses of treatment-group identity.

For the same trait sets as for the PCA, we used MRPP to determine the probability that the treatments had more internal consistency than expected by chance, that is, the chance-corrected within group agreement among and between treatments. This Monte Carlo style test was done over 50,000 permutations using Euclidean distance. The MRPP was preferred because it has almost no assumptions and its low-sensitivity "name-tag swapping" approach should only detect practical differences. We then substantiated our MRPP results using Adonis (i.e., adonis2 in the R package vegan), again with Euclidean distance and 50,000 permutations. Adonis is a least squares regression-based ANOVA technique that adds to our MRPP interpretation by allowing us to consider the total trait variance explained by treatment class within a Euclidean distance matrix. In the case of twigs, Adonis was especially useful in addressing possible dispersion bias in the MRPP due to among-treatment volumetric differences in trait space revealed by the PCA and to sample size differences. We repeated the Adonis tests for the five twig traits (marked with an asterisk in Figure 2) that we considered to be directly linked to chronically low water availability.

RESULTS

Twigs

Irrigation-stop twigs appeared to be undergoing an active transition from Irrigated back to Control-like anatomical structure and were intermediate in nearly half of their traits (Figure 2, medium-colored bars). When all 18 twig traits were considered collectively, the traits did not differ significantly among treatments at Pfynwald, whether compared together or pairwise (Table 1). Even Control and Irrigation-stop trees were much less different from each other than seen at the leaf level, with 100× greater structural overlap in PCA trait space (Table 1, Figure 3: top left). Twig traits that stood out as clearly distinct in Irrigation-stop trees were related to chronically low water availability (marked with asterisks in Figure 2). These water-stress traits differed between Control and Irrigated tree twigs (Adonis: $R^2 = 0.3302$, $F = 5.9153$, $P = 0.0169$) and Control and Irrigation-stop trees ($R^2 = 0.207$, $F = 4.1626$, $P = 0.0354$), but not between Irrigated and Irrigation-stop trees. Unlike leaves, it was Control twigs that had the greatest relative global-trait plasticity, with a ~7.5× greater V_c than that of Irrigated twigs. Irrigation-stop twigs occupied an intermediate volume in the trait space, with a position that further suggested twig-level regression toward the Control (Table 1, Figure 3: top left).

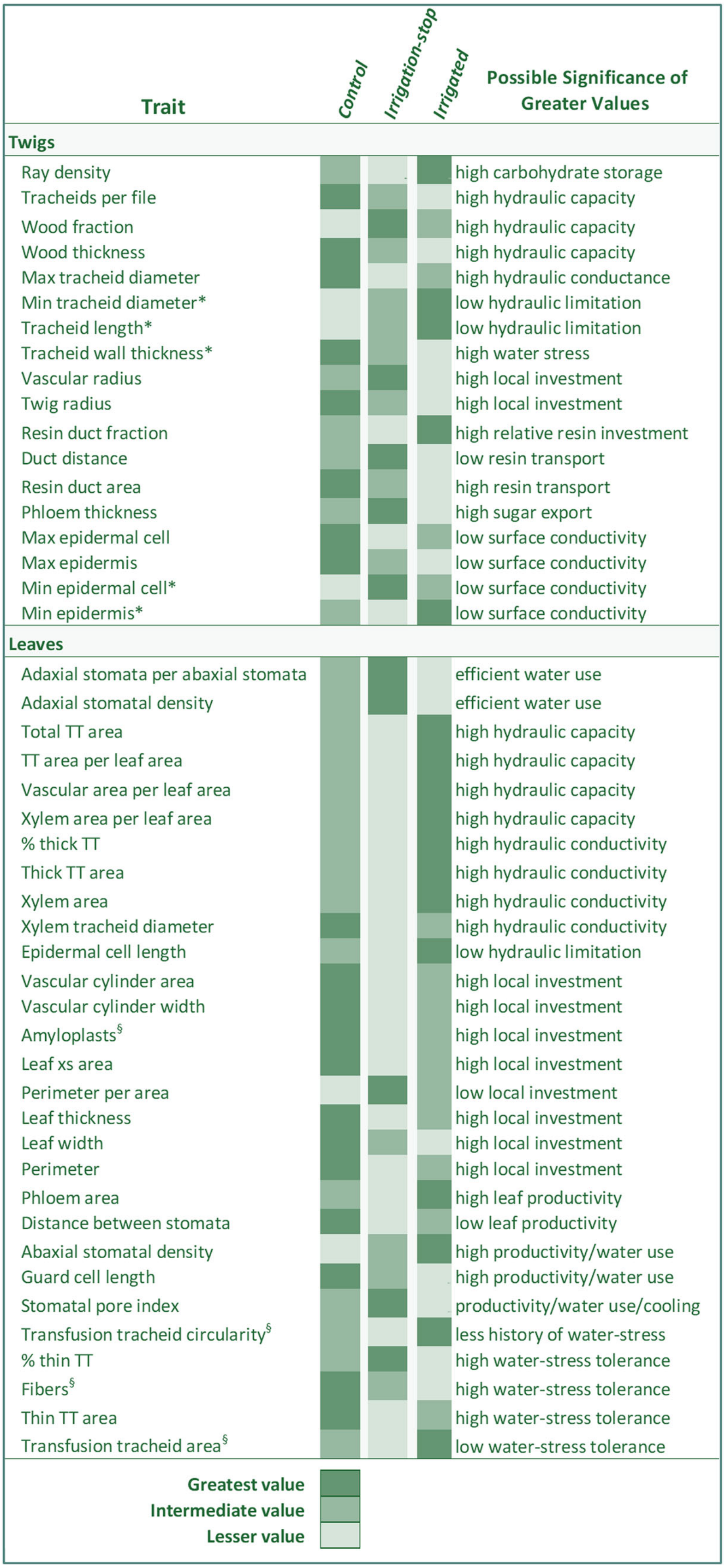
Leaves

In sharp contrast to twig traits, leaf traits (Figures 1, 2) had wide-ranging multivariate differences among the three treatments of the Pfynwald Experiment (Table 1). Leaves produced in 2021 on formerly irrigated (2003–2013) trees, i.e., "Irrigation-stop" trees, were relatively similar to leaves on continually "Irrigated" (from 2003–present) trees (MRPP: $A = 0.0371$, $P = 0.13$; Adonis: $R^2 = 0.13$, $F = 2.796$, $P = 0.0991$). Instead of returning to control conditions, Irrigation-stop trees remained significantly different from never-irrigated, rain-fed "Control" trees on a multivariate level (MRPP: $A = 0.2077$, $P = 0.0022$; Adonis: $R^2 = 0.36$, $F = 10.186$, $P = 0.0028$). Irrigation-stop trees exhibited pronounced trait differences linked to hydraulics, local investment, water stress, and productivity that collectively distinguished their leaf structure from both Control and Irrigation treatments (Figure 3: right side). The anatomical signature of the small Irrigation-stop leaves reflects an alternative, and generally extreme, structural strategy where <14% of traits were intermediate between Control and Irrigated trees (Figure 2: dark and light bars; Figure 3: top right; Appendix S1). On a multivariate level, Irrigated trees occupied ~3.5× more of the collective PCA trait space (sample-size corrected volume: V_c) than leaves from Control trees, suggesting that chronically low water availability decreased variability among individuals. In the multivariate trait space, Control and Irrigation-stop overlapped in only 0.3% of their unified V_c implying an absence of any reversion to their original condition (Table 1). While Control trees invested in large leaves and both local hydraulic capacity and conductivity as well as starch storage, Irrigation-stop trees were on the other extreme, with the lowest leaf-level investments (Figure 2: light bars).

DISCUSSION

The memory of water

Seven years after the 11 years of experimentally elevated water availability, mature trees in a natural forest continued to exhibit pronounced anatomical differences in new leaves and twigs. In contrast with studies of drought legacies, this developmental memory of ample water may be longer lasting, because, unlike drought legacies, it is not caused by dips in carbohydrate reserves, which can be replenished within months to a few years (Gessler et al., 2020). Developmental changes persisting after the climatic signal has



Trait	Control	Irrigation-stop	Irrigated	Possible Significance of Greater Values
Twigs				
Ray density	▨	□	■	high carbohydrate storage
Tracheids per file	■	▨	□	high hydraulic capacity
Wood fraction	□	■	▨	high hydraulic capacity
Wood thickness	■	▨	□	high hydraulic capacity
Max tracheid diameter	■	□	▨	high hydraulic conductance
Min tracheid diameter*	□	▨	■	low hydraulic limitation
Tracheid length*	□	▨	■	low hydraulic limitation
Tracheid wall thickness*	■	▨	□	high water stress
Vascular radius	▨	■	□	high local investment
Twig radius	■	▨	□	high local investment
Resin duct fraction	▨	□	■	high relative resin investment
Duct distance	▨	■	□	low resin transport
Resin duct area	■	▨	□	high resin transport
Phloem thickness	▨	■	□	high sugar export
Max epidermal cell	■	□	▨	low surface conductivity
Max epidermis	■	▨	□	low surface conductivity
Min epidermal cell*	□	■	▨	low surface conductivity
Min epidermis*	▨	□	■	low surface conductivity
Leaves				
Adaxial stomata per abaxial stomata	▨	■	□	efficient water use
Adaxial stomatal density	▨	■	□	efficient water use
Total TT area	▨	□	■	high hydraulic capacity
TT area per leaf area	▨	□	■	high hydraulic capacity
Vascular area per leaf area	▨	□	■	high hydraulic capacity
Xylem area per leaf area	▨	□	■	high hydraulic capacity
% thick TT	▨	□	■	high hydraulic conductivity
Thick TT area	▨	□	■	high hydraulic conductivity
Xylem area	▨	□	■	high hydraulic conductivity
Xylem tracheid diameter	■	□	▨	high hydraulic conductivity
Epidermal cell length	▨	□	■	low hydraulic limitation
Vascular cylinder area	■	□	▨	high local investment
Vascular cylinder width	■	□	▨	high local investment
Amyloplasts§	■	□	▨	high local investment
Leaf xs area	■	□	▨	high local investment
Perimeter per area	□	■	▨	low local investment
Leaf thickness	■	□	▨	high local investment
Leaf width	■	▨	□	high local investment
Perimeter	■	□	▨	high local investment
Phloem area	▨	□	■	high leaf productivity
Distance between stomata	■	□	▨	low leaf productivity
Abaxial stomatal density	□	▨	■	high productivity/water use
Guard cell length	■	▨	□	high productivity/water use
Stomatal pore index	▨	■	□	productivity/water use/cooling
Transfusion tracheid circularity§	▨	□	■	less history of water-stress
% thin TT	▨	■	□	high water-stress tolerance
Fibers§	■	▨	□	high water-stress tolerance
Thin TT area	■	□	▨	high water-stress tolerance
Transfusion tracheid area§	▨	□	■	low water-stress tolerance

Greatest value ■
Intermediate value ▨
Lesser value □

FIGURE 2 (See caption on next page).

TABLE 1 Multivariate among-treatment differences and trait-space volumes. Collective and pairwise differences among treatments were assessed with both multiresponse permutation procedures (MRPP, where A is the chance-corrected within-group agreement) and analysis of variance on the distance matrix (Adonis), both using Euclidean distance. Using the first three axes from PCAs done for both twigs and leaves, we geometrically assessed the pairwise unions and intersections of hulls occupied by each treatment in the 3D trait space. We also compared the 3D trait-space volumes of each treatment, correcting for sample size differences by dividing raw volume by N^3 (V_c = corrected volume × 10,000) to serve as an index of relative global trait plasticity.

	MRPP		**Adonis**			**All-treatment 3-dimensional PCA hulls**				
	A	P	R^2	F	P	**Union**	**Intersection**	**% Shared**	**Treatment**	V_c
Twigs										
All treatments	0.0140	0.2441	0.11	1.274	0.2656	—	—	—	Control	1.71
Control vs. Irrigation-stop	−0.0139	0.6717	0.05	0.760	0.5687	0.1025	0.033389	32.58	Irrigation-stop	0.48
Control vs. Irrigated	0.0555	0.0702	0.16	2.291	0.0770	0.0879	0.004638	5.28	Irrigated	0.23
Irrigation-stop vs. Irrigated	0.0063	0.3123	0.07	0.977	0.3896	0.0508	0.002453	4.83	—	—
Leaves										
All treatments	0.1459	0.0041	0.29	5.417	0.0059	—	—	—	Control	0.13
Control vs. Irrigation-stop	0.2077	0.0022	0.36	10.186	0.0028	0.0382	0.00012	0.31	Irrigation-stop	0.25
Control vs. Irrigated	0.0785	0.0444	0.15	3.206	0.0769	0.0525	0.001494	2.85	Irrigated	0.40
Irrigation-stop vs. Irrigated	0.0371	0.1261	0.13	2.796	0.0991	0.0639	0.001421	2.22	—	—

passed imply that the epigenome has been altered, in line with the multifaceted water-rich tree syndrome of Irrigated trees, noted since early in the Pfynwald experiment (Dobbertin et al., 2010; Eilmann et al., 2013; Schönbeck et al., 2018; Brunner et al., 2019; Zweifel et al., 2020). Leaves had the most extensive evidence for persistence of a wide-ranging anatomical response to past water abundance (Table 1). The newest crop of leaves on formerly irrigated (2003–2013), Irrigation-stop trees remain much more like leaves on continually Irrigated (2003–present) trees than to those on never-irrigated, rain-fed Control trees. Moreover, the leaves of Irrigation-stop trees do not appear to be in the process of reverting back to Control-like leaf structure (Figure 2); instead, traits associated with hydraulics, local investment, water stress, and productivity collectively differ from both Control and Irrigation treatments (Table 1, Figure 3: right side). On the basis of their collective structural differences, we predict that the Irrigation-stop trees will continue to grow more slowly and have long-term differences in drought susceptibility from Control trees. We do not rule out a progressive functional decline in Irrigation-stop trees if they had been in any way still reliant on reserves from the Irrigation period. Although legacy effects on photosynthetic rate are well documented (e.g., Kannenberg et al., 2020), we are not aware of any previous work on leaf-level anatomical traits, making comparisons difficult. Regardless, the lack of reversion toward Control-form leaves 7 years after experimental irrigation was halted may be the longest-lasting legacy effect recorded in trees to date.

Divergence of Irrigation-stop leaves from the Control-form has become so extreme that they are even less similar to each other than the Irrigated and Control leaves are to each other (Table 1). The post-water Irrigation-stop leaves exhibited a unique, third structural strategy with only 4 of 29 traits intermediate between Control and Irrigated leaves (Figures 2 and 3: top right; Appendix S1); most of the structural traits were markedly smaller than in the other treatments (Figure 2; Appendix S3), with a large fraction of their limited cross-sectional area devoted to thin-walled transfusion tracheids, which can buckle to release emergency water supplies during drought stress (Chin et al., 2022). Higher soil water supply increases the allocation of new assimilates to the root-rhizosphere system in Scots pine (Joseph et al., 2020) and can over time expand the rooting zone (Gao et al., 2021). However, it is unlikely that Irrigation-stop trees still benefit from additional (deeper) water resources because transpiration in summer

FIGURE 2 Individual traits explored and their order among treatments. Greatest, intermediate, and lesser trait values are based on the raw means for each trait. Twigs from the Irrigation-stop treatment are often intermediate between Control and Irrigated trees, suggesting a shift back toward Control-like anatomical structure. Conversely, the leaves of Irrigation-stop trees mostly had trait values more extreme than either of the other treatments demonstrating both positive and negative legacy effects. Twig traits marked with "*" were used as proxies for water stress and compared among treatments, leaf-traits marked with "§" were excluded from the PCA due to missing values (see sample sizes and detailed trait explanations in Appendix S1). Consistently among both twigs and leaves, organs on Irrigation-stop trees showed signs of hydraulic limitation and water stress compared to Irrigated trees; however, in leaf traits, anatomical indicators of such stress were even more prominent than in the Control trees. TT: transfusion tissue. Trait means, CV, and standard errors are available in Appendix S2.

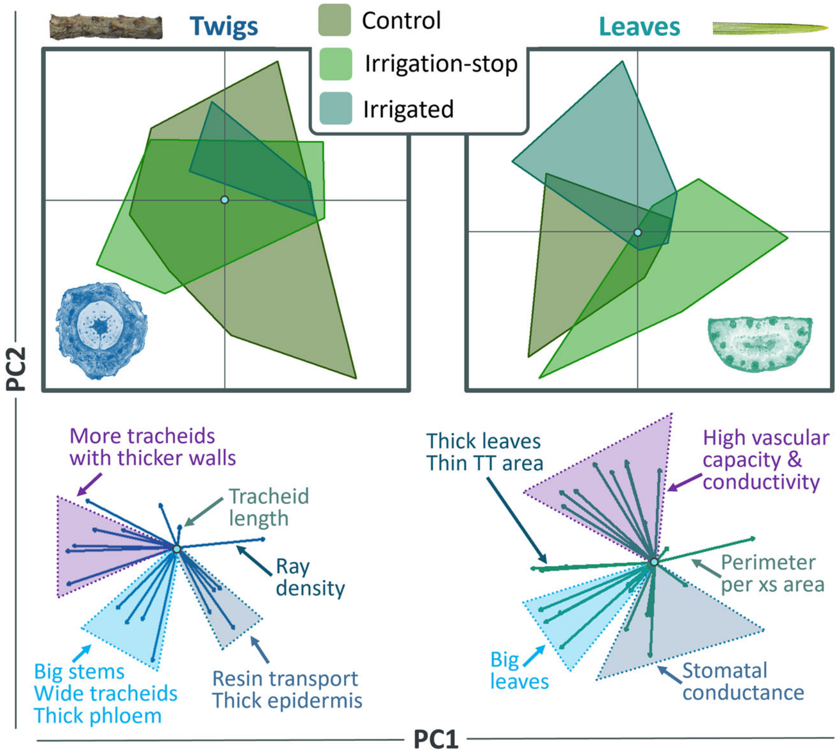


FIGURE 3 PCA results for twigs and leaves. Hulls show treatment-level overlap and trait-space occupancy on the first 2 PCs. The twig plot highlights trait-space conservation among Irrigated trees and the intermediate nature of twig structure in Irrigation-stop trees; on a multivariate level, these three treatments did not differ significantly. In contrast, the leaf plot shows substantial among-treatment differences in leaf structure and how leaves on Irrigation-stop trees occupy a unique and non-intermediate portion of the shared trait space. Trait loading plots are directly below each PCA plot; centroids (blue dots) indicate where they would be overlaid on the above plots. TT, transfusion tissue; xs, cross section. We interpret the loading vectors as clusters of related traits driving the spread of twigs and leaves within the trait space. Individually labeled vectors can be found in Appendix S1.

declined strongly compared to controls after irrigation was stopped (Zweifel et al., 2020), as further confirmed by the stress syndrome evident in their leaves.

The leaf remembers what the twig forgets

The dissimilarity between leaves and twigs in the magnitude and type of water-legacy effects reveals a previously unrecognized difference in sensitivity to climatic history between these key organs. The far greater post-water legacy effects in leaves compared to twigs is especially intriguing in terms of tree susceptibility to climatic variation. Our understanding of climatic legacy effects has recently expanded (Zweifel et al., 2020; Peltier et al., 2022; Sillett et al., 2022; Blumstein and Furze, 2023), but this work presents the first evidence that periods of high water-availability have enduring impacts on leaves—the structures that regulate tree performance and carbon assimilation. In sharp contrast to leaves, Irrigation-stop twigs were intermediate in 8 of 18 traits, suggesting an ongoing transition from Irrigated back toward Control-like anatomy. The even lower water potentials of leaves compared to twigs may have led toward selection for enhanced ecological memory at the leaf level.

The relative breadth of trait space occupied by a group of trees experiencing similar conditions may indicate either constraints or strategic options relating to optimum performance under these growing conditions, where trait space overlap provides a holistic view of similarity. Within the multivariate trait space, Control twigs showed the greatest plasticity, taking up ~7.5× greater trait volume than that occupied by Irrigated twigs, with Irrigation-stop twigs occupying an intermediate volume and position in the trait space (Table 1, Figure 3). The narrow anatomical strategy of Irrigated twigs is clearly dissimilar from leaves, where Irrigated trees occupied ~3.5× more of the collective trait space than leaves from Control trees. The conserved leaf-trait variation in both Control and Irrigation-stop trees is intriguing when considering that these two treatments overlap in only 0.3% of their unified volumes in the multivariate trait space—less overlap than between some conifer species (Chin and Sillett, 2019). In contrast, among twigs, Control and Irrigation-stop trees share >30% of their overall anatomical structure (Table 1).

The suite of twig traits that stood out as characteristic of Irrigation-stop trees was that most associated with chronically low water availability; these traits, especially those related to cell size, may therefore be considered constraints due to hydraulic limitation on cellular expansion, rather than acclimations (Figure 2). In pairwise comparisons, these water-stress traits were different between Control and Irrigated tree twigs and Control and Irrigation-stop trees, but not between Irrigated and Irrigation-stop trees. These traits include tracheid diameter, length, and wall thickness, all of which are related to hydraulic vulnerability, and epidermal features related to surface conductivity (Figure 2). This intriguing lack of return to reference conditions in traits that are key to water-stress tolerance may indicate a lasting hydraulic weakness in twigs of formerly water-rich Irrigation-stop trees. Bearing in mind that multivariate differences between Irrigated and Control trees were minor in all 18 twig traits measured here (Table 1), twigs had less structural distance to traverse to revert to the Control form. Regardless, the memory of water is held much more strongly at the leaf level, which may have profound implications for the productivity and sensitivity of trees that have known periods of abundant water.

Hard times may make strong trees

The lingering effects of formerly high water availability may result in trees that are potentially structurally mismatched to current climatic conditions. Thus, we must ask, what is the mechanism by which freshly produced twigs and, especially, leaves still retain a legacy of high-water availability? We suspect that unexplored variation in root, trunk, and branch anatomy, which would include active tissues produced during the irrigation period, continues to impact leaf development. Implicitly, the degree of responsiveness of trees to current conditions may be directly tied to the turnover time for both organs and reserves formed, renewed, or depleted under previous conditions (Zweifel and Sterck, 2018; Zweifel et al., 2020). If this is the case, then the legacy effects we see in Irrigation-stop trees may persist until all sapwood formed under irrigation is converted to hydraulically inactive heartwood (Zweifel et al., 2020). Although species with a fast turnover of carbohydrates and structures could have shorter ecological memories (Zweifel and Sterck, 2018; Zweifel et al., 2020), we expect conversion to heartwood to take ~60 years in *Pinus sylvestris* of this age (Gjerdrum, 2003) Potential post-irrigation legacy effects at the Pfynwald experiment were placed into two classes by Zweifel et al. (2020): (1) positive effects with trait values temporarily remaining intermediate between irrigated and control trees before returning to control levels and (2) negative effects with trait values in formerly irrigated trees initially more extreme than in control trees. Such negative effects were apparent in the 40% lower radial growth and smaller tracheid diameter (compared to control) found in trunks in these same trees during sampling efforts concurrent with ours (Vitali et al., 2024). Our work suggests that both positive and negative legacy effects occur in *P. sylvestris*, with positive effects most common in the generally intermediate twigs and negative effects dominating leaf anatomy (Figures 2 and 3 : bottom right). The importance of leaf level processes is evidenced by the importance of crown transparency, an index of tree vitality, in determining which individual trees showed negative legacy effects in trunk growth following after irrigation stopped (Vitali et al., 2024). Beyond influences from persistent organs, the observed leaf-level legacy effects may have endured due to epigenetic changes acquired during the 11 years of irrigation (Bose et al., 2020), that may continue to shape phenotypic expression. If epigenetic changes persist, then leaves on the Irrigation-stop trees will either never return to the control status or only do so after an indeterminate period of "epigenetic forgetfulness" (Sharma et al., 2022).

Importantly, trees are now establishing under "new normal" conditions that would have historically been labeled drought (Balting et al., 2021; Vicente-Serrano et al., 2022). Both Irrigated and Irrigation-stop trees had traits suggesting greater vulnerability to stressors, these included low investment in resin (which protects from pests), thinner leaves (which lower drought tolerance), and minimal local investment in starch storage as amyloplasts (Figure 2), which contributes to post-stress recovery. Trees regenerating under drought conditions may have a novel foliar trait syndrome that influences forest productivity, energy balance, and stress tolerance on a global scale. While we may expect greater climatic stress tolerance in such future forests, we can imagine scenarios where these forests are less productive or have lower cooling capacity. Conversely, chronically low water availability experienced by the parent-tree generation may enhance the resilience of new trees through heritable changes to the epigenome (Bose et al., 2020). It is important to note that during the Pfynwald experiment, the >100-year-old Control trees have had opportunity to acclimate to "new normal" dry conditions for longer than the trees in the other treatments, a natural component to the experiment that requires caution in our interpretations.

CONCLUSIONS

The newest cohort of establishing trees may be different from the present adults, not due to selection over time, but due to differences between the climatic contexts of future and adult-tree growth histories. Trees and forests underlie many proposed natural climate solutions (Griscom et al., 2017; Fargione et al., 2018); thus, accurate planning for forest carbon sequestration potential will require exploration of past, present, and future drivers of tree structure. The preservation of large, old trees in primary forests—the champions of carbon storage—remains the top priority, yet this work suggests that efforts focused on seedling-stage forest regeneration may yield crucial information on very long-term shifts in climatic tolerance. New and existing forest-scale manipulative experiments (e.g., Beier et al., 2012; Barbeta et al., 2013; Paschalis et al., 2020) and long-term monitoring plots (e.g., Acker et al., 1998; Schaub et al., 2011; Thimonier et al., 2001; Wolf et al., 2009; Smith et al., 2015) can be leveraged to address these unknowns and understand links across scales, from climate through anatomy to long-term ecosystem stability. Our work suggests that trees "remember" times of abundance and scarcity and provides a beacon of hope for the strength of future forests.

AUTHOR CONTRIBUTIONS

A.R.O.C. collected samples, performed the scans, planned the anatomical analyses, analyzed data, and wrote the first draft of the manuscript. A.G. and M.S. assisted with access to Pfynwald, coordinating sampling and collecting tree data, and interpretating results. O.L.L., K.V., and I.Ø. did scans, analyzed images, and created outreach materials. G.T.-R. did scans, helped with project coordination, and planned anatomical analyses. J.H.R.L. contributed to large portions of the data analysis, writing, and interpretation. All authors edited the manuscript.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material.

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REFERENCES

Acker, S. A., W. A. McKee, M. E. Harmon, and J. F. Franklin, 1998. Long-term research on forest dynamics in the Pacific Northwest: a network of permanent forest plots. *Man and the Biosphere Series* 21: 93–106.

Anderegg, W. R., C. Schwalm, F. Biondi, J. J. Camarero, G. Koch, M. Litvak, K. Ogle et al. 2015. Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models. *Science* 349: 528–532.

Azuma, W., H. R. Ishii, K. Kuroda, and K. Kuroda. 2016. Function and structure of leaves contributing to increasing water storage with height in the tallest *Cryptomeria japonica* trees of Japan. *Trees* 30: 141–152.

Balting, D. F., A. AghaKouchak, G. Lohmann, and M. Ionita. 2021. Northern Hemisphere drought risk in a warming climate. *NPJ Climate and Atmospheric Science* 4: 61.

Barbeta, A., R. Ogaya, and J. Peñuelas. 2013. Dampening effects of long-term experimental drought on growth and mortality rates of a Holm oak forest. *Global Change Biology* 19: 3133–3144.

Beier, C., C. Beierkuhnlein, T. Wohlgemuth, J. Penuelas, B. Emmett, C. Körner, H. de Boeck, et al. 2012. Precipitation manipulation experiments—challenges and recommendations for the future. *Ecology Letters* 15: 899–911.

Blonder, B., C. Babich Morrow, D. J. Harris, S. Brown, G. Butruille, A. Laini, and D. Chen. 2022. hypervolume: High dimensional geometry, set operations, projection, and inference using kernel density estimation, support vector machines, and convex hulls. R package version 3.0.2. Website: https://CRAN.R-project.org/package=hypervolume

Blumstein, M. J., and M. E. Furze. 2023. Interannual dynamics of stemwood nonstructural carbohydrates in temperate forest trees surrounding drought. *Journal of Forestry Research* 34: 77–86.

Bose, A. K., B. Moser, A. Rigling, M. M. Lehmann, A. Milcu, M. Peter, C. Rellstab, et al. 2020. Memory of environmental conditions across generations affects the acclimation potential of scots pine. *Plant, Cell & Environment* 43: 1288–1299.

Bose, A. K., A. Rigling, A. Gessler, F. Hagedorn, I. Brunner, L. Feichtinger, C. Bigler, et al. 2022. Lessons learned from a long-term irrigation experiment in a dry Scots pine forest: Impacts on traits and functioning. *Ecological Monographs* 92: p. e1507.

Brunner I., C. Herzog, L. Galiano, and A. Gessler. 2019. Plasticity of fine-root traits under long-term irrigation of a water-limited Scots pine forest. *Frontiers in Plant Science* 10: 1–10.

Chin, A. R., P. Guzmán-Delgado, S. C. Sillett, L. P. Kerhoulas, A. R. Ambrose, A. R. McElrone, and M. A. Zwieniecki. 2022. Tracheid buckling buys time, foliar water uptake pays it back: coordination of leaf structure and function in tall redwood trees. *Plant, Cell & Environment* 45: 2607–2616.

Chin, A. R., and S. C. Sillett. 2017. Leaf acclimation to light availability supports rapid growth in tall *Picea sitchensis* trees. *Tree Physiology* 37: 1352–1366.

Chin, A. R., and S. C. Sillett. 2019. Within-crown plasticity in leaf traits among the tallest conifers. *American Journal of Botany* 106: 174–186.

Cosgrove, D. J. 1987. Wall relaxation and the driving forces for cell expansive growth. *Plant Physiology* 84: 561–564.

Cosgrove, D. J. 2005. Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology* 6: 850–861.

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

Eilmann B., M. Dobbertin, and A. Rigling. 2013. Growth response of Scots pine with different crown transparency status to drought release. *Annals of Forest Science* 70: 685–693.

Fargione, J. E., S. Bassett, T. Boucher, S. D. Bridgham, R. T. Conant, S. C. Cook-Patton, P. W. Ellis, et al. 2018. Natural climate solutions for the United States. *Science Advances* 4: p.eaat1869.

Gao, D., J. Joseph, R. A. Werner, I. Brunner, A. Zürcher, C. Hug, A. Wang, et al. 2021. Drought alters the carbon footprint of trees in soils—tracking the spatio-temporal fate of ^{13}C-labelled assimilates in the soil of an old-growth pine forest. *Global Change Biology* 27: 2491–2506.

Gessler, A., A. Bottero, J. Marshall and M. Arend. 2020. The way back: recovery of trees from drought and its implication for acclimation. *New Phytologist* 228: 1704–1709.

Gjerdrum, P. 2003. Heartwood in relation to age and growth rate in *Pinus sylvestris* L. in Scandinavia. *Forestry* 76: 413–424.

Griscom, B. W., J. Adams, P. W. Ellis, R. A. Houghton, G. Lomax, D. A. Miteva, W. H. Schlesinger, et al. 2017. Natural climate solutions. *Proceedings of the National Academy of Sciences, USA* 114: 11645–11650.

Hunziker, S., M. Begert, S. C. Scherrer, A. Rigling, and A. Gessler. 2022. Below average midsummer to early autumn precipitation evolved into the main driver of sudden Scots pine vitality decline in the Swiss Rhône Valley. *Frontiers in Forests Global Change* 5: 874100.

Joseph, J., D. Gao, B. Backes, C. Bloch, I. Brunner, G. Gleixner, M. Haeni, et al. 2020. Rhizosphere activity in an old-growth forest reacts rapidly to changes in soil moisture and shapes whole-tree carbon allocation. *Proceedings of the National Academy of Sciences, USA* 117: 24885–24892.

Kannenberg, S. A., K. A. Novick, M. R. Alexander, J. T. Maxwell, D. J. Moore, R. P. Phillips, and W. R. Anderegg. 2019. Linking drought legacy effects across scales: from leaves to tree rings to ecosystems. *Global Change Biology* 25: 2978–2992.

Kannenberg, S. A., C. R. Schwalm, and W. R. Anderegg. 2020. Ghosts of the past: how drought legacy effects shape forest functioning and carbon cycling. *Ecology Letters* 23: 891–901.

Marone F., and M. Stampanoni. 2012. Regridding reconstruction algorithm for real-time tomographic imaging. *Journal of Synchrotron Radiation* 19: 1029–1037.

Müller, L. M., and M. Bahn. 2022. Drought legacies and ecosystem responses to subsequent drought. *Global Change Biology* 28: 5086–5103.

Oldham, A. R., S. C. Sillett, A. M. Tomescu, and G. W. Koch. 2010. The hydrostatic gradient, not light availability, drives height-related variation in *Sequoia sempervirens* (Cupressaceae) leaf anatomy. *American Journal of Botany* 97: 1087–1097.

Oksanen, J., G. L. Simpson, F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, et al. 2022. vegan: Community ecology package. R package version 2.6-2. Website: https://CRAN.R-project.org/package=vegan

Paganin, D., S. C. Mayo, T. E. Gureyev, P. R. Miller, and S. W. Wilkins. 2002. Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. *Journal of Microscopy* 206: 33–40.

Paschalis, A., S. Fatichi, J. Zscheischler, P. Ciais, M. Bahn, L. Boysen, J. Chang, et al. 2020. Rainfall manipulation experiments as simulated by terrestrial biosphere models: Where do we stand? *Global Change Biology* 26: 3336–3355.

Peltier, D. M., J. Guo, P. Nguyen, M. Bangs, M. Wilson, K. Samuels-Crow, L. L. Yocom, et al. 2022. Temperature memory and non-structural carbohydrates mediate legacies of a hot drought in trees across the southwestern USA. *Tree Physiology* 42: 71–85.

Peltier, D. M., and K. Ogle. 2019. Legacies of more frequent drought in ponderosa pine across the western United States. *Global Change Biology* 25: 3803–3816.

Schaub, M., M. Dobbertin, N. Kräuchi, and M. Dobbertin. 2011. Preface—long-term ecosystem research: understanding the present to shape the future. *Environmental Monitoring and Assessment* 174: 1–2.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.

Schönbeck, L., A. Gessler, G. Hoch, N. G. McDowell, A. Rigling, M. Schaub, and M. H. Li. 2018. Homeostatic levels of nonstructural carbohydrates after 13 yr of drought and irrigation in *Pinus sylvestris*. *New Phytologist* 219: 1314–1324.

Sharma, M., P. Kumar, V. Verma, R. Sharma, B. Bhargava, and M. Irfan, 2022. Understanding plant stress memory response for abiotic stress resilience: molecular insights and prospects. *Plant Physiology and Biochemistry* 179: 10–24.

Sillett, S. C., M. E. Antoine, A. L. Carroll, M. E. Graham, A. R. O. Chin, and R. Van Pelt. 2022. Rangewide climatic sensitivities and non-timber values of tall *Sequoia sempervirens* forests. *Forest Ecology and Management* 526: 120573.

Smith, J. M., J. Paritsis, T. T. Veblen, and T. B. Chapman. 2015. Permanent forest plots show accelerating tree mortality in subalpine forests of the Colorado Front Range from 1982 to 2013. *Forest Ecology and Management* 341: 8–17.

Stampanoni, M., A. Groso, A. Isenegger, G. Mikuljan, Q. Chen, A. Bertrand, S. Henein, et al. 2006. Trends in synchrotron-based tomographic imaging: the SLS experience. *In* U. Bonse [ed.], Proceedings of the SPIE 6318, Developments in X-ray tomography, V: 63180M, 193–206, 2006, San Diego, CA, USA. SPIE, Bellingham, WA, USA.

Szejner, P., S. Belmecheri, J. R. Ehleringer, and R. K. Monson. 2020. Recent increases in drought frequency cause observed multi-year drought legacies in the tree rings of semi-arid forests. *Oecologia* 192: 241–259.

Thimonier, A., M. Schmitt, P. Cherubini, and N. Kräuchi. 2001. Monitoring the Swiss forest: building a research platform. *In* T. Anfodillo and V. Carraro [eds.], Monitoraggio ambientale: metodologie ed applicazioni. Atti del XXXVIII Corso di Cultura in Ecologia, 121–134, 2001, Padua, Italy. Department of Territory and Agro-Forestry Systems, Università degli Studi di Padova, Padua, Italy.

Vicente-Serrano, S. M., D. Peña-Angulo, S. Beguería, F. Domínguez-Castro, M. Tomás-Burguera, I. Noguera, L. Gimeno-Sotelo, and A. El Kenawy, 2022. Global drought trends and future projections. *Philosophical Transactions of the Royal Society, A, Mathematical, Physical and Engineering Sciences* 380: p.20210285.

Vitali, V., P. Schuler, M. Holloway-Phillips, P. D'Odorico, C. Guidi, S. Klesse, M. M. Lehmann, et al. 2024. Finding balance: Tree-ring isotopes differentiate between acclimation and stress-induced imbalance in a long-term irrigation experiment. *Global Change Biology* 30: p. e17237.

Wolf, A., S. Davies, and C. Richard. 2009. Ecological insights from long-term research plots in tropical and temperate forests. *Bulletin of the Ecological Society of America* 90: 519-525.

Zweifel, R., S. Etzold, F. Sterck, A. Gessler, T. Anfodillo, M. Mencuccini, G. von Arx, et al. 2020. Determinants of legacy effects in pine trees—implications from an irrigation-stop experiment. *New Phytologist* 227: 1081–1096.

Zweifel, R., and F. Sterck. 2018. A conceptual tree model explaining legacy effects on stem growth. *Frontiers in Forests and Global Change* 1: 1–9.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Traits, functional type, and methods.

Appendix S2. Trait means, standard errors, and coefficients of variation (CV: standard deviation/mean).

Appendix S3. Loading plots for twig and leaf PCAs.

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