

Original Article

Structural determinants of increased susceptibility to dehydration-induced cavitation in post-fire resprouting chaparral shrubs

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ABSTRACT

It is well established that transpiration and photosynthetic rates generally increase in resprouting shoots after fire in chaparral shrublands. By contrast, little is known about how plant hydraulic function varies during this same recovery period. We hypothesized that vascular traits, both functional and structural, would also shift in order to support this heightened level of gas exchange and growth. We examined stem xylem-specific hydraulic conductivity (K_s) and resistance to cavitation (P_{50}) for eight chaparral shrub species as well as several potential xylem structural determinants of hydraulic function and compared established unburned plants and co-occurring post-fire resprouting plants. Unburned plants were generally more resistant to cavitation than resprouting plants, but the two groups did not differ in K_s . Resprouting plants had altered vessel structure compared with unburned plants, with resprouting plants having both wider diameter vessels and higher inter-vessel pit density. For biomechanics, unburned plants had both stronger and denser stem xylem tissue than resprouting plants. Shifts in hydraulic structure and function resulted in resprouting plants being more vulnerable to dehydration. The interaction between time since disturbance (i.e. resprouting versus established stands) and drought may complicate attempts to predict mortality risk of resprouting plants.

Key-words: chaparral; embolism; modulus of rupture; pit area; pit membrane; vessel density; vessel diameter; vessel element length; vessel length; vulnerability to cavitation.

INTRODUCTION

Plant hydraulic functional and structural traits are linked to physiological performance, including drought survival (Pratt *et al.* 2008; Anderegg *et al.* 2013, 2014; Paddock *et al.* 2013; Pratt *et al.* 2014), community-level physiological properties (Jacobsen *et al.* 2007b, 2008, 2009; de Dios Miranda *et al.* 2010) and productivity (Brodribb 2009; Domec *et al.* 2010). Hydraulic trait plasticity and variation has been relatively little

studied (Holste *et al.* 2006; Schoonmaker *et al.* 2010; Plavcová & Hacke 2012) yet may be an important component of how species resist drought (Choat *et al.* 2012; Anderegg 2015).

Changes in hydraulic function that occur during development or following disturbance may lead to community-level changes in resilience over time. These changes complicate predictions of stand resilience with future changes in climate or with changes in disturbance or recruitment regimes. Some previous studies suggest that hydraulic traits may vary with the transition from seedling or juvenile to adult plants (Sperry & Saliendra 1994; Rice *et al.* 2004) or during post-disturbance resprouting (Utsumi *et al.* 2010; Ramirez *et al.* 2012; Pratt *et al.* 2014; Pausas *et al.* 2016; Schwilk *et al.* 2016). However, these studies have examined relatively few species, and patterns of trait change have varied by species and study (for instance, see Linton *et al.* 1998; Matzner *et al.* 2001; Rice *et al.* 2004).

In mediterranean-type shrubs, photosynthesis and growth rates are both increased in post-fire resprouting plants relative to unburned shrubs (Radosevich & Conard 1980; Oechel & Hastings 1983; DeSouza *et al.* 1986; Castell *et al.* 1994; Fleck *et al.* 1998; Clemente *et al.* 2005). Links between plant performance and plant hydraulics suggest that plant hydraulic structural and functional traits are also likely to vary between unburned and resprouting plants in order to accommodate these changes in plant performance. Thus, we hypothesized that vascular traits, both functional and structural, would differ between unburned and resprouting plants. As particularly related to photosynthetic and growth differences, we predicted that resprouting plants would have increased xylem-specific hydraulic conductivity relative to unburned plants. This was based on previously reported links between photosynthetic performance and leaf-specific hydraulic conductivity (Santiago *et al.* 2004; Campanello *et al.* 2008; Chen *et al.* 2009) and between growth and xylem-specific hydraulic conductivity (Vander Willigen & Pammenter 1998).

Southern California has seen an increase in anthropogenic fire ignitions (Syphard *et al.* 2007, 2009) and has similarly experienced a series of record-breaking droughts over the past several years (MacDonald 2007; Griffin & Anchukaitis 2015). In response to these events, studies have shown high mortality in some resprouting chaparral shrub species when drought

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has coincided with the period of post-fire recovery; similar mortality was not observed in the same species occurring in nearby unburned mature stands (Pratt *et al.* 2014; Pausas *et al.* 2016). In at least two species, mortality of post-fire resprouting plants was linked to high levels of embolism and presumable hydraulic failure and very low levels of hydraulic conductivity in resprouting plants (Pratt *et al.* 2014). These studies have suggested that resprouting plants may be particularly susceptible to drought during the post-fire recovery period (reviewed in Pausas *et al.* 2016). Thus, we predicted that resprouting plants would be less resistant to cavitation than unburned plants of the same species.

We examined the hydraulic structure, function and biomechanics of eight co-occurring chaparral shrub species in the stems of unburned plants compared with the stems of post-fire resprouting plants 1 year after fire. Changes in hydraulic conductivity and cavitation resistance were predicted to occur concomitant with shifts in xylem structural and biomechanical traits. Angiosperm xylem includes many different cell types that perform different functions within the xylem. Even within a single cell type or structure (i.e. vessel elements and vessels), some trait aspects may vary while others do not when the functional performance of the xylem tissue is altered (Plavcová & Hacke 2012; Plavcová *et al.* 2013). We measured a range of xylem traits in order to ascertain which traits may be potentially involved in the plastic response of the xylem during post-fire resprouting. These traits included mean vessel diameter, vessel element length, mean vessel length and several inter-vessel pit traits. We also examined the plant biomechanical traits of xylem density and modulus of rupture (MOR) because these had been reported previously to have varied between unburned and resprouting plants of a different chaparral species (Utsumi *et al.* 2010) and are correlated with chaparral plant hydraulic traits (Jacobsen *et al.* 2005, 2007a, 2007c).

MATERIALS AND METHODS

Study site and species

A field site was established in the San Gabriel Mountains within the Angeles National Forest in an area on the perimeter of a large fire that contained both unburned and burned areas immediately adjacent to one another and similar in aspect and species composition. The Sayre Fire burned approximately 4500 ha within the San Gabriel Mountains in mid-November 2008. We tagged 480 plants on 23 November 2008, before any plants were resprouting in the newly burned area and identified species by their root structure. A firebreak (approximately 1–2 m wide) at the site was mechanically cleared during the fire and protected a patch of plants from the fire. Within this unburned patch, we tagged 10 individuals of each of the eight dominant resprouting species occurring at the site: *Adenostoma fasciculatum* Hook. & Arn. (Rosaceae), *Arctostaphylos glandulosa* Eastw. (Ericaceae), *Ceanothus leucodermis* Greene (Rhamnaceae), *Heteromeles arbutifolia* (Lindl.) M. Roem. (Rosaceae), *Quercus berberidifolia* Liebm. (Fagaceae), *Quercus wislizeni* A. DC. (Fagaceae), *Rhamnus*

ilicifolia Kellogg (Rhamnaceae) and *Rhus ovata* S. Watson (Anacardiaceae).

The cut stumps of two non-resprouting species, which would have established as seedlings the year following the most recent prior fire at the site, were collected, and their annual growth rings counted to determine the age of the stand at the time of the fire. These samples confirmed that the unburned and pre-fire burned stands were mature. At least 27 years had passed between the studied fire and the prior fire (both cut samples contained 26 annual rings).

Most resprouting initiated between January and February 2009 during the onset of winter rains. Resprouts elongated rapidly following emergence (resprout height of 0.42 m in May averaged across all tagged and resprouted plants at the site), with elongation slowing with the onset of the dry season (average height of 0.52 m June, 0.71 m July, 0.80 m September and 0.80 m October). Plants were sampled for hydraulic, biomechanical and anatomical traits between May and October 2009.

Hydraulics: hydraulic conductivity and vulnerability to cavitation

Vulnerability to dehydration-induced cavitation curves were measured on six unburned and six resprouting plants per species in May–July 2009 using a standard centrifuge technique (Tobin *et al.* 2013). For unburned plants, large branches (0.5 to 1 m) were cut underwater from plants in the field. Plastic bottles or plastic centrifuge tubes containing water were affixed to the cut end to maintain the cut end underwater, and branches were wrapped in two large doubled plastic bags before being transported back to a laboratory at California State University, Bakersfield. For resprouts, ~0.5 m shoots were cut underwater from plants near where shoots were emerging from basal lignotubers and cut ends were placed in water-filled plastic centrifuge tubes that were affixed to stem ends in order to main the cut end underwater. Several shoots were placed together in two doubled large plastic bags for transport. Unburned branches and resprout shoots were refrigerated or stored in a cool room until they were measured. Samples were measured within 4 d of collection.

Stem samples 14 cm long were excised from larger branches and shoots by cutting samples successively from each end underwater until an approximately 15 cm long unbranched sample was obtained. The sample was then trimmed down to a final length of 14 cm using fresh razor blades. For resprouts, samples were composed entirely of current year growth. For unburned stems, samples of similar diameter to resprouts were measured, and these were usually more than current year growth because of annual elongation of less than 14 cm in adult plants.

Stem segments were flushed for 1 h at 100 kPa with an ultra-filtered (in-line filter Calyx Capsule Nylon 0.1 µm, GE Water and Process Technologies, Trevose, PA, USA) 20 mM KCl degassed solution. Maximum hydraulic conductivity (K_{hmax}) was measured gravimetrically using a conductivity apparatus under slight positive pressure (~2–4 kPa) and with

correction for background flows measured at 0 kPa pressure to correct for stem capacitance effects (Hacke *et al.* 2000). Maximum xylem-specific hydraulic conductivity (K_s) was calculated by dividing K_{hmax} by the xylem cross-sectional area of the distal end of the samples. Samples were then spun in a custom rotor (Alder *et al.* 1997; Tobin *et al.* 2013) at increasingly negative xylem pressure potentials, with hydraulic conductivity (K_h) measured between each spin. These data were used to construct a vulnerability to cavitation curve for each sample based on the maximum hydraulic conductivity of samples as measured at -0.5 MPa. This pressure was used as a maximum to account for potential cavitation fatigue, especially in unburned samples that may have contained multiple years of growth (Hacke *et al.* 2001). The pressure at which 50% loss in K_h occurred for each sample (P_{50}) was obtained for each sample using a Weibull curve (Microsoft Excel 2010, Microsoft, Redmond, WA, USA).

Biomechanics: xylem density and mechanical strength

Stem samples for biomechanics measures were collected from unburned and resprouting plants between August and October 2009. Straight, unbranched stems approximately 40 cm in length were collected from plants in the field and placed into a plastic bag with moist paper towels, with 6–12 samples measured per species and treatment (i.e. unburned and resprout). Samples were transported in a cooler to California State University, Bakersfield, and then refrigerated overnight before being measured the next day. A four-point bending test was conducted as described by Jacobsen *et al.* (2005) using an Instron mechanical properties tester (Model 3342, Instron, Norwood, MA, USA). The span length, the distance between the two supported ends, was 0.135 m. The load was applied at two points along the span length, 0.045 m apart, at a speed of 20 mm min^{-1} . Stems were stressed until they passed the point of mechanical failure. The maximum load value (F_{max}) from the bending tests, determined as the maximum force obtained in the bending test prior to stem failure, xylem cross-sectional dimensions of the xylem and bending test parameters were used to calculate the MOR using the equations included in Jacobsen *et al.* (2005).

Xylem density was measured on the same stems that were used to determine vulnerability to cavitation curves and K_s . Approximately 2 cm was removed from the basal portion of each stem. This segment was split longitudinally, and the bark and pith removed. Segments were submerged underwater in a vacuum desiccator, and a vacuum was pulled for ~1 h to degas the solution and to remove gas from xylem samples. Samples were then allowed to remain submerged overnight. The following day, saturated volume was determined by mass displacement of samples submerged in water on a balance. Volume was calculated according to Archimedes' principle and the temperature corrected density of the water. Samples were then dried at 60 °C for >72 h to a constant weight, and dry mass was determined. Xylem density was calculated as the dry mass per saturated volume.

Anatomy: vessel diameter, length and pit characteristics

All of the same stems that were used for determination of vulnerability to cavitation curves and K_s were also examined for vessel diameter and pit measures to minimize errors. Following hydraulic function measures, stems were frozen until sectioned. For vessel diameter measures, thin cross sections, approximately $40 \mu\text{m}$ thick, were made using a sledge microtome (Model 860 Microtome, American Optical Corp., Buffalo, New York, USA) and mounted on slides in glycerol. Images were taken of several wedge-shaped sectors extending from the pith and using ray parenchyma bands to delineate a sector (Axio Imager.D2 and AxioCam MRc, Carl Zeiss MicroImaging GmbH, Gottingen, Germany). Vessel lumen areas were measured for all vessels in each measured sector with at least 100 vessels measured per sample (AxioVision, AxioVs40, v. 4.8.2.0, MicroImaging GmbH, Gottingen, Germany), and vessel diameters were calculated from these areas using the assumption that vessels were circular. The hydraulic vessel diameter (D_h) was calculated for each sample using the formula $D_h = (\sum D^5) / (\sum D^4)$, based upon all the sampled vessels in a stem. Each of the vessels measured in cross section was scored for its connectivity (the number of other vessels that the measured vessel directly contacted) and the vessel-to-vessel contact fraction of its perimeter (the percentage of wall perimeter that contacted adjacent vessels).

For the measurement of vessel pit traits, thin longitudinal sections were made by hand from frozen hydraulic samples using Teflon-coated razor blades (GEM single edge stainless steel Teflon-coated blades, #71970 Electron Microscopy Sciences, Hatfield, Pennsylvania, USA). Sections were mounted on slides in glycerol and examined at 400 times magnification. Pit-field areas were identified within vessels and three representative pit fields were selected from different vessels within the section and photographed to be analysed (Axio Imager.D2, AxioCam MRc, and AxioVision AxioVs40, v. 4.8.2.0, Carl Zeiss MicroImaging GmbH, Gottingen, Germany). Within each pit-field area, a section was selected that was entirely in focus and included 11–14 pits. This area was measured, and the number of pits counted to determine the pit density (pit number per pit-field wall area). The pit membrane area for each of the individual pits was also measured (33–42 per sample). These values were used to calculate the mean individual pit membrane area and the percentage of the pit-field wall area that was composed of pit membrane. From these same slides, the length of 10 vessel elements was measured per sample.

Separate samples were collected for vessel length measures in July and August 2009 from the same individuals sampled for other measures. Separate samples were necessary because these measures required destructive sampling and longer samples than were used for hydraulic function measures. Shoots and branches were collected from the field in the same manner as those collected for vulnerability curves. In a laboratory, a 40 cm segment was cut under water from the larger samples harvested in the field, and the selected segments were matched in diameter to hydraulic samples. Segments were flushed for 1 h as described for hydraulic conductivity stems and were then

injected with a two-component silicone (RhodorsilRTV-141; Rhodia USA, Cranbury, NJ, USA) containing a ultraviolet (UV)-stain (Uvitex OB, Ciba Specialty Chemicals, Basel, Switzerland) dissolved in chloroform (1% by weight). One drop of the UV-stain was added per gram of silicone mixture. The two-component silicone and UV-stain were mixed thoroughly and left to sit for 2 h to allow the small air bubbles incorporated during mixing to come out of the silicone. The ends of the flushed stem segments were trimmed with a fresh razor blade, and the segments injected into their basal end at 50 kPa for 24 h. After the injection, stems cured for at least 48 h at room temperature. Prior to sectioning, stems were rehydrated to soften tissues by submerging them in water in a refrigerator for at least 24 h. Thin cross sections (40 μm thick) were obtained with a sledge microtome at distances of 0, 0.7, 1.4, 2.9, 5.8, 11.8 and 24.0 cm from the injection end and 1 cm from the terminus of the segment (~39 cm from the injection end). From each one of these cross sections, the number of filled vessels was counted from images captured with a fluorescence microscope attached to a digital camera (Zeiss Stereo Discover V.12 with Axiocam HRc digital camera, Carl Zeiss Microscopy, LLC, Thornwood, NY, USA) to determine the percentage of vessels that remained filled at each distance. For all samples, initial percentages of filled vessels at the injection end (i.e. 0 cm from the injection point) were high (generally >95%), indicating that emboli had been removed and most vessels within the cross section were active. The vessel length distribution and mean vessel length were calculated from these measures using the equations reported by Sperry *et al.* (2005). The vessel density was also measured from these sections and images by counting all the vessels present within the measured cross-sectional area of xylem.

Vessel-to-vessel pit area (A_P), calculated as described in Hacke *et al.* (2006), was also estimated for each sample. This parameter is an estimate of the total vessel-to-vessel pit membrane area contained within an average vessel. This was calculated using the mean vessel diameter, vessel-to-vessel contact fraction and the percentage of the pit-field wall area that was composed of pit membrane, which were all measured on the same samples as used for hydraulic measures and could be linked to an individual sample. Vessel length was measured on separate samples, and so the average mean vessel length across samples within a species and treatment was used in pit area calculations.

Analyses

Shifts in traits between unburned and resprouting plants were examined using ANOVA with treatment (unburned and resprout) as a fixed factor and species as a random factor. The primary goal of this study was to evaluate the impact of treatment (unburned and resprout), and pre-planned contrasts were run for each species pair to evaluate differences between treatments within species (JMP 9.0.0, SAS Institute Inc., NC, USA).

Correlations were used to determine if measured traits were related to either conductive efficiency (K_s) or cavitation resistance (P_{50}), because we were specifically interested in examining which structural traits were predictive of hydraulic

function. Variables were examined to evaluate if they met the assumptions of statistical models and, for A_P and connectivity, data were log-transformed prior to analysis. A principle components analysis was run to examine the relationships between the measured traits and the pattern of changes in traits between unburned and resprouting plants. For this analysis, all species were included even though *Q. berberidifolia* had values of zero for connectivity and pit area. These analyses were run using MINITAB 17 (v. 17.2.1, Minitab, Inc., State College, Pennsylvania, USA).

RESULTS

Unburned plants differed from resprouting plants in several hydraulic structural and functional traits (Tables 1 and 2). When analysed across treatments, resprouting plants were less resistant to water stress-induced xylem cavitation (P_{50}) than unburned plants, and six of eight species exhibited significant changes in P_{50} (Table 3). Hydraulic efficiency (K_s) did not differ between resprouting and unburned treatments, and only one species exhibited a change in K_s between resprouting and unburned plants. The change in P_{50} between unburned and resprouting plants coincided with changes in the shape of the vulnerability to cavitation curves for some, but not all species (Fig. 1), with the largest shifts tending to occur in species in which the unburned plants were highly cavitation resistant. Across treatments, resprouting plants exhibited altered vessel structure compared with unburned plants, with resprouting plants having both wider vessels and reduced pit density (Tables 1 and 2). Tissue-level biomechanical traits also varied, with unburned plants exhibiting both stronger and denser stem xylem tissue.

Vessel and pit structural traits were correlated with K_s , but tissue-level and biomechanical traits generally were not and relationships varied for burned and unburned plants. Vessel diameter, both mean and hydraulic mean (D_h), was correlated with the K_s of unburned plants (Fig. 2b and Table S1). Pit membrane area was correlated with K_s for both unburned and burned plants (Fig. 2d), and pit density was also correlated with K_s for burned plants (Table S1). Biomechanical traits, including xylem density (Fig. 2a), and other measured xylem and vessel-level traits, including vessel length (Fig. 2c), were not correlated with K_s .

Xylem and vessel structural traits, but not pit traits, were correlated with P_{50} . Vessel density, diameter and hydraulic mean diameter were correlated with P_{50} for both resprouting and unburned plants (Fig. 2f). Vessel length was correlated with P_{50} for unburned plants (Fig. 2g). Other traits, including xylem density (Fig. 2e) and pit membrane area (Fig. 2h), were not associated with P_{50} .

Traits related specifically to vessel-to-vessel connections, that is, connectivity and pit area (A_P), were not associated with hydraulic function (Fig. 3). Instead, these traits illustrated a major difference in hydraulic function between the measured species. Most species included in this study contain both vessels and tracheids within their xylem. This alters the structure of the hydraulic network and, in the most extreme case as found in *Q. berberidifolia*, may result in no direct contacts being

Table 1. The mean trait values for several hydraulic structural and functional traits and one SE (below each mean) for each of the treatment groups (unburned and resprout)

Treatment	K_s ($\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$)	P_{50} (MPa)	Xylem density (g cm^{-3})	MOR (N mm^{-2})	Vessel density ($\# \text{mm}^{-2}$)	Connectivity (#)	Vessel diameter (μm)	D_h (μm)	Vessel element length (μm)	Vessel length (m)	A_p (mm^2)	Pit membrane area (μm^2)	Pit density ($\# \mu\text{m}^{-2}$)
Unburned	1.214	-3.32	0.666	132.6	195.8	0.361	28.0	35.0	229.5	0.0904	0.436	18.73	0.0327
Resprout	0.216	0.52	0.023	9.9	44.3	0.170	2.5	3.7	7.2	0.0198	0.339	2.69	0.0042
	1.104	-1.88 **	0.607 **	107.9 *	181.2	0.394	31.9 *	37.4	236.9	0.0851	0.303	19.84	0.0293 *
	0.143	0.31	0.025	6.3	43.3	0.190	3.5	3.9	8.1	0.0170	0.194	2.55	0.0033

Trait abbreviations are included in Table 2. Within a column, asterisks indicate differences in that trait among treatments. Results for across species treatment comparisons are shown in Table 2.

MOR, modulus of rupture.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 2. Stem xylem hydraulic functional, xylem tissue level and vessel and pit structural traits (abbreviations for selected traits included) were examined using ANOVA with treatment (unburned and resprout) as a fixed factor, species as a random factor

Trait categories and traits	Abbreviation	F	P
Hydraulic function			
Xylem-specific hydraulic conductivity	K_s	0.44	0.530
Pressure at 50% loss in hydraulic conductivity	P_{50}	17.74	0.004
Xylem tissue			
Xylem density		18.05	0.004
Modulus of rupture	MOR	9.39	0.018
Vessel density		0.49	0.509
Connectivity		1.39	0.292
Vessel structure			
Vessel diameter	D	6.01	0.044
Hydraulic vessel diameter	D_h	3.06	0.124
Vessel element length	E_L	0.13	0.728
Vessel length	V_L	0.48	0.511
Pit area per vessel	A_p	1.07	0.348
Pit structure			
Pit membrane area	A_m	1.05	0.339
Pit density		5.74	0.048

F -value and P -value shown for each trait; degrees of freedom = 1,7 for all traits except for connectivity and pit area per vessel where degrees of freedom = 1,5.

observed between any two vessels and vessel connections only occurring secondarily through tracheids. Thus, for these species, there are reduced numbers of vessel-to-vessel connections and reduced vessel-to-vessel contact fractions. This also reduces the vessel-to-vessel pit area, although vessels may still contain numerous vessel-to-tracheid pits. Only one species, *R. ovata*, contains only vessels, and this species strongly diverged from all others in pit area (Fig. 3b,d). A second species, *C. leucodermis*, contained more vessel-to-vessel connections than many of the other vessel and tracheid-bearing species (Fig. 3a,c), but still had low vessel-to-vessel pit area.

Many structural traits were highly correlated with one another. Although we were not specifically interested in correlations between structural traits and most of these are not discussed in detail, correlations were also run between all measured variables and a correlation matrix including Pearson correlation coefficients and P -values for these comparisons are included in the supplemental materials (Table S1). This generally showed correlations between traits that are functionally or structurally linked. For instance, the two biomechanical traits were correlated, and xylem density was strongly associated with biomechanical strength (Fig. 4a). For vessels, vessel density was strongly correlated with vessel diameter (Fig. 4b). And for pits, pit density was strongly associated with the pit membrane area of individual pits (Fig. 4c).

Examining the broader relationships between multiple traits highlights the links between xylem function and structure and how these are related to post-fire changes in stem xylem. Principle components analysis resulted in a first component (41% of variation) that was most strongly associated with hydraulic functional traits and vessel structural

Table 3. The resprouting chaparral shrub species included in the present study, their abbreviations as used in some figures, and the mean trait values for several hydraulic structural and functional traits and one SE (in parentheses below the mean) for each of the treatment groups (unburned and resprout) for each species

Species (family)	Abbreviation	Treatment	K_s ($\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$)	P_{50} (MPa)	Xylem density (g cm^{-3})	MOR (N mm^{-2})	Vessel density ($\# \text{mm}^{-2}$)	Connectivity (#)	Vessel diameter (μm)	D_h (μm)	Vessel element length (μm)	Vessel length (m)	A_p (mm^2)	Pit membrane area (μm^2)	Pit density ($\# \mu\text{m}^{-2}$)
<i>Adenostoma fasciculatum</i> Hook. & Arn. (Rosaceae)	Af	Unburned	0.492	-3.77	0.714	158.1	246.2	0.073	23.1	25.7	179.7	0.0629	0.044	7.97	0.0500
		Resprout	(0.078)	(0.43)	(0.011)	(10.4)	(12.2)	(0.016)	(0.5)	(0.5)	(8.0)	(0.0029)	(0.004)	(0.45)	(0.0032)
<i>Arctostaphylos glandulosa</i> Eastw. (Ericaceae)	Ag	Unburned	0.472	-4.64	0.630	91.1	212.3	0.190	24.7	28.5	252.7	0.0762	0.163	13.34	0.0394
		Resprout	(0.075)	(0.53)	(0.010)	(11.2)	(12.8)	(0.029)	(1.0)	(1.4)	(19.4)	(0.0060)	(0.028)	(1.32)	(0.0030)
<i>Ceanothus leucodermis</i> Greene (Rhamnaceae)	Cl	Unburned	1.589	-4.26	0.597	111.0	222.7	1.087	27.2	37.1	274.9	0.0345	0.381	28.17	0.0194
		Resprout	(0.206)	(0.35)	(0.016)	(8.5)	(21.8)	(0.118)	(2.0)	(1.4)	(13.9)	(0.0025)	(0.036)	(1.44)	(0.0009)
<i>Heteromeles arbutifolia</i> (Lindl.) M. Roem. (Rosaceae)	Ha	Unburned	0.889	-4.37	0.699	160.2	312.0	0.100	21.4	24.0	280.3	0.0606	0.046	12.47	0.0397
		Resprout	(0.145)	(0.63)	(0.014)	(10.3)	(9.6)	(0.015)	(0.9)	(0.8)	(20.6)	(0.0041)	(0.010)	(1.08)	(0.0037)
<i>Quercus berberidifolia</i> Liebm. (Fagaceae)	Qb	Unburned	2.211	-2.29	0.750	167.9	45.3	0	38.7	49.9	214.9	0.1891	0	24.27	0.0200
		Resprout	(0.129)	(0.83)	(0.024)	(17.6)	(2.2)	(0.012)	(0.7)	(0.5)	(15.6)	(0.0031)	(0.020)	(1.21)	(0.0026)
<i>Quercus wislizeni</i> A. DC. (Fagaceae)	Qw	Unburned	1.498	-1.47	0.680	122.7	49.7	0.103	36.8	46.6	277.0	0.1245	0.004	27.60	0.0204
		Resprout	(0.129)	(0.83)	(0.024)	(17.6)	(2.2)	(0.012)	(0.7)	(0.5)	(15.6)	(0.0031)	(0.020)	(1.21)	(0.0026)
<i>Rhamnus ilicifolia</i> Kellogg (Rhamnaceae)	Ri	Unburned	0.957	-4.66	0.705	140.4	389.3	0.148	20.5	25.4	209.0	0.0321	0.063	21.41	0.0294
		Resprout	(0.099)	(0.52)	(0.008)	(14.8)	(32.9)	(0.016)	(0.5)	(0.8)	(12.1)	(0.0016)	(0.008)	(1.58)	(0.0027)
		Resprout	(0.123)	(0.27)	(0.020)	(9.8)	(13.8)	(0.011)	(0.5)	(0.5)	(12.5)	(0.0028)	(0.004)	(2.42)	(0.0028)

(Continues)

Table 3. (Continued)

Species (family)	Abbreviation	Treatment	K_s ($\text{kg s}^{-1} \text{m}^{-1} \text{MPa}^{-1}$)	P_{50} (MPa)	Xylem density (g cm^{-3})	MOR (N mm^{-2})	Vessel density ($\# \text{mm}^{-2}$)	Connectivity (#)	Vessel diameter (μm)	D_h (μm)	Vessel element length (μm)	Vessel length (m)	A_p (mm^2)	Pit membrane area (μm^2)	Pit density ($\# \mu\text{m}^{-2}$)
<i>Rhus ovata</i> S. Watson (Anacardiaceae)	Ro	Unburned	1.609 (0.319)	-1.08 (0.26)	0.555 (0.018)	109.6 (3.0)	89.1 (5.5)	1.185 (0.108)	31.2 (2.1)	42.9 (2.3)	172.5 (13.5)	0.1432 (0.0073)	2.790 (0.122)	14.62 (0.51)	0.0432 (0.0018)
		Resprout	1.064* (0.143)	-1.09 (0.13)	0.553 (0.014)	95.8 (9.3)	59.3 (2.5)	0.955 (0.056)	40.7*** (0.9)	48.2* (1.2)	227.7* (17.2)	0.1149 (0.0074)	1.644*** (0.140)	16.18 (0.92)	0.0379 (0.0028)

Trait abbreviations are included in Table 2. Within a column, asterisks indicate differences in a trait between the unburned and resprouting treatments.

MOR, modulus of rupture.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

traits, such as diameter, density and pit membrane area (Fig. 5a). The second principle component (25.5% of variation) was most strongly associated with biomechanical traits and vessel-to-vessel connection traits. For most species (five of eight), resprouting plants were shifted to the upper right compared with unburned plants (Fig. 5b). This indicates a shift to be more vulnerable to cavitation, have lower xylem density and MOR, wider vessels, a more connected vessel network, larger pit membrane areas and lower pit density (Table 3). The largest shift was seen in *H. arbutifolia* (Fig. 5b), and this species also exhibited the largest shifts in most individual traits (Table 3). For the remaining species (three of eight), traits did not generally vary between unburned and resprouting plants (Fig. 5b, Table 3), although vessel diameters were significantly wider in resprouting compared with unburned plants for all three of these species.

DISCUSSION

Post-fire shifts in hydraulic function

Xylem traits, including hydraulic function, biomechanics and anatomy, were altered in resprouting plants in the year post-fire relative to unburned mature plants. In general, plants shifted during the period of post-fire growth to be less resistant to water stress-induced cavitation. They also shifted to have reduced xylem density and weakened mechanical strength against stem rupture (i.e. lower MOR). These changes were consistent with prior studies that have found a link between xylem biomechanics and hydraulics (Wagner *et al.* 1998; Jacobsen *et al.* 2005, 2007c; Pratt *et al.* 2007). These shifts are consistent with the hypothesis that resprouting plants must rapidly re-establish their crown in a post-fire environment to compete for ephemeral abundant nutrients, light and space. Shifting to produce stem xylem tissue that is mechanically and structurally less robust may facilitate rapid growth (Poorter *et al.* 2008; Chave *et al.* 2009; Poorter *et al.* 2010) and competitive acquisition of abundant post-fire nutrients.

Xylem hydraulic efficiency (K_s) did not change significantly during the resprouting phase when analysed across species and for six of eight species. This was surprising given the significantly smaller vessel diameters of unburned plants relative to resprouting plants among treatments and for most species. It may be that small changes in other traits, although not significant individually, combined to balance hydraulic function and this change in vessel structure. For instance, although unburned plants had narrower vessels, they generally also had slightly increased vessel length and vessel density. Unburned plants also had significantly higher pit density with no change in the area of individual pit membranes, which would lead to an increase in available pit membrane surface area per conduit contact area and allow for increased flow between conduits of similar size and connectivity.

Greater K_s of stems could support greater levels of carbon acquisition and growth among resprouting plants; however, K_s was not greater. This is consistent with a recent study on

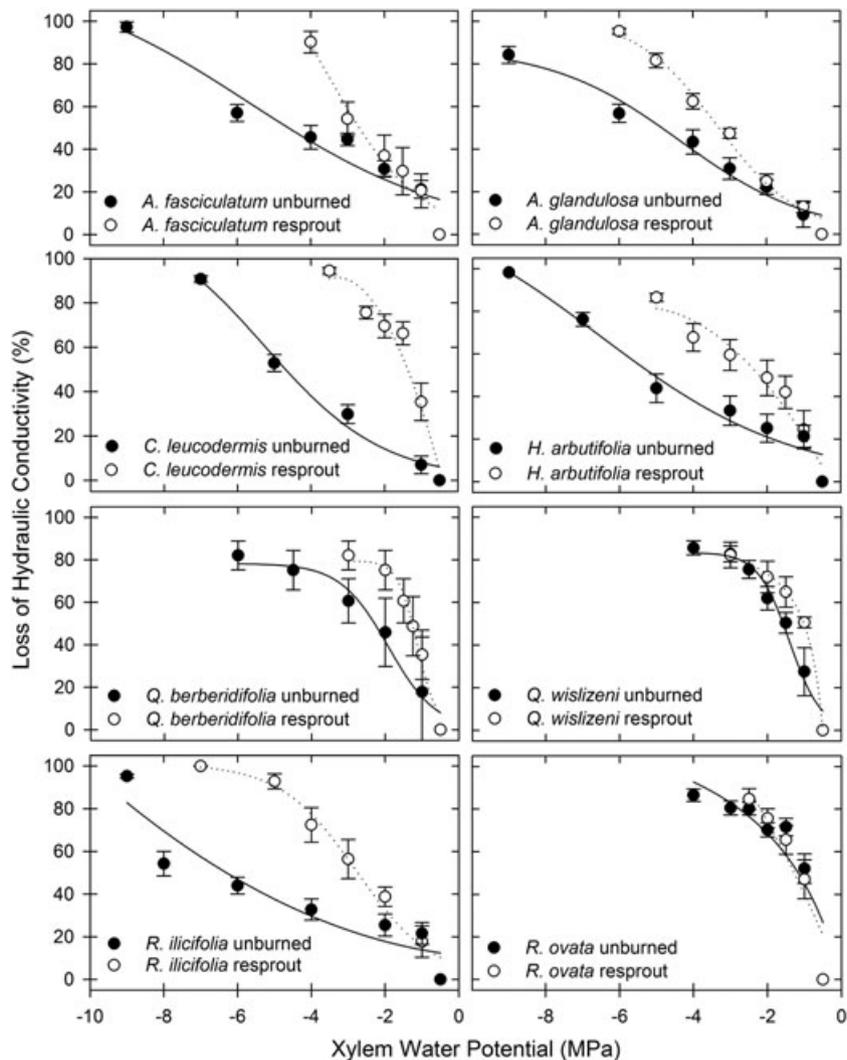


Figure 1. Vulnerability to cavitation curves of stems from unburned plants and post-fire resprouting plants of eight chaparral species.

resprouting oak species that also found that neither xylem-specific nor leaf-specific hydraulic conductivities changed in resprouting compared with unburned plants (Schwilke *et al.* 2016). At the scale of the whole plant and for many angiosperms, including chaparral shrubs (Pratt *et al.* 2010), the resistances of different organs are about 50% in roots, 25% in stems and 25% in leaves. If we assume that an early resprout and an unburned mature plant have the same root resistance (the root systems survive fire), then what changes post-fire is the resistance of the shoots. In the absence of changes in resistivity of individual stems, shoot resistance for the whole plant will likely be lower for resprouting plants simply because the shoot system is smaller and the overall transport path length is reduced compared with a larger mature plant that did not burn. Thus, even though individual stems were not different in resistivity (not different in K_s) between resprouts and unburned plants, the overall shoot resistance in unburned plants was likely higher. The effect of this would be that whole plant conductance is greater for resprouts and this is likely associated with the greater growth rates and stomatal conductance that is common for resprouting plants.

Relationships between xylem structure and function

Post-fire changes in resistance to cavitation, without a concomitant change in hydraulic efficiency, highlight the functional flexibility of angiosperm xylem and the weak relationship that exists between hydraulic safety and efficiency (Gleason *et al.* 2015). At the level of an individual pit membrane, larger pit membrane pores will allow for increased flow but will decrease resistance to air seeding; however, at the vessel or tissue levels, there are numerous potential arrangements and alterations to xylem structures that may decouple these traits (Brodersen 2016). In the case of the present study, hydraulic efficiency was related to the size of individual pit membranes and pit density. In contrast, cavitation resistance was linked to vessel length, and both traits were linked to hydraulic vessel diameter. Overall, there was little overlap between the traits that were predictive of hydraulic efficiency and hydraulic safety. Interestingly, Utsumi *et al.* (2010) also found this pattern, that is, significantly altered P_{50} post-fire relative to unburned plants, but

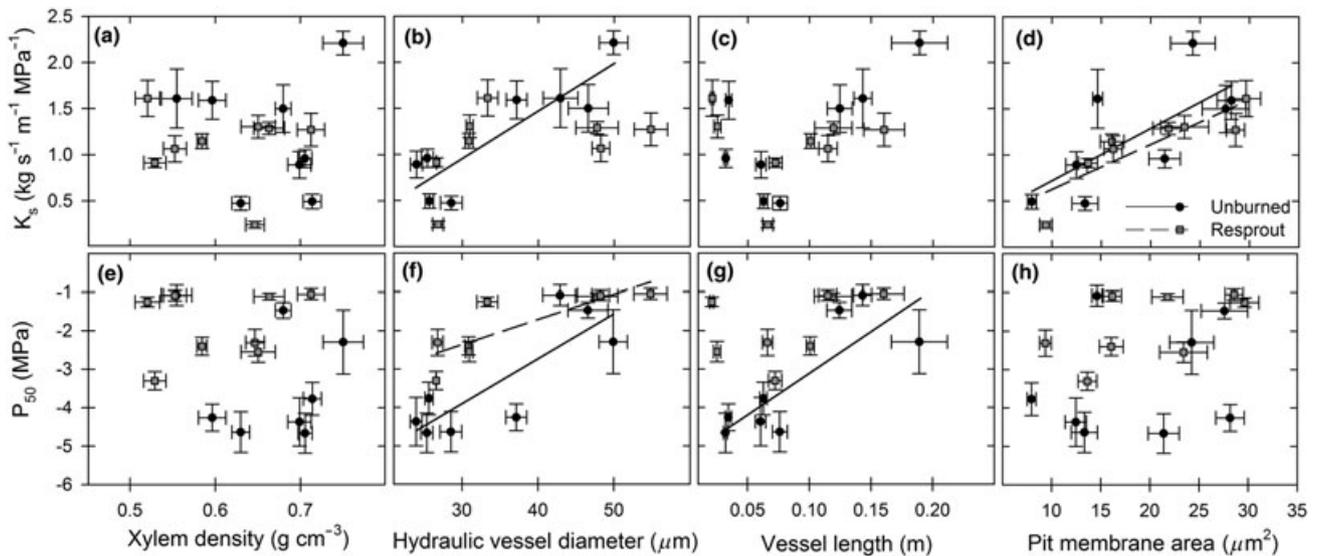


Figure 2. Xylem density, hydraulic vessel diameter (D_h), mean vessel length and pit membrane area as predictors for xylem-specific hydraulic conductivity (K_s) (a, b, c, d) and resistance to water stress-induced cavitation (P_{50}) (e, f, g, h). Lines are shown only for significant correlations ($P < 0.05$) and are only shown to highlight trends. See Table 3 for specific trait values and Table S1 for Pearson correlation coefficients and correlation P -values.

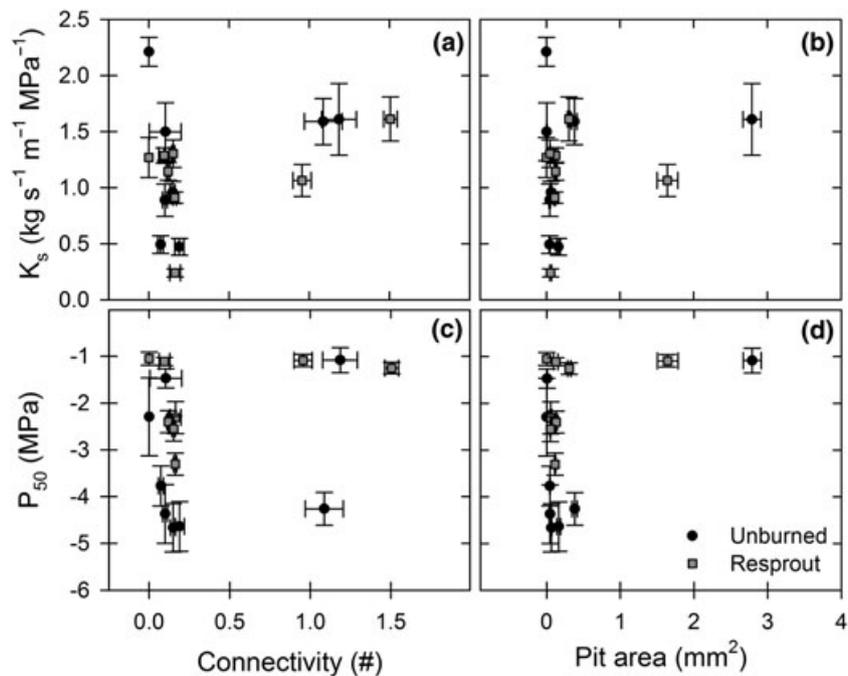


Figure 3. Traits related to vessel-to-vessel connections, that is, connectivity and pit area (A_p), were not predictive of xylem-specific hydraulic conductivity (K_s) (a, b) or resistance to water stress-induced cavitation (P_{50}) (c, d) ($P > 0.05$ for all; correlation analyses were conducted on log-transformed connectivity and pit area data). See Table 3 for specific trait values and Table S1 for Pearson correlation coefficients and correlation P -values. In panels a and c, the two species showing relatively high connectivity are *Ceanothus leucodermis* and *Rhus ovata*. In panels b and d, the species showing relatively high-pit area is *R. ovata*.

no change in K_s for a different southern California shrub species, *Juglans californica*. Differences between unburned and resprouting plants in structure and function may persist beyond the year immediately after fire (Kaneakua 2011; Pausas *et al.* 2016).

The lack of a relationship between pit area and P_{50} may be linked to the presence of both tracheids and vessels within the xylem of seven of the eight species included in this study. Tracheids, with angiosperm-type homogenous pit membranes rather than gymnosperm-type torus-margo pits, are relatively

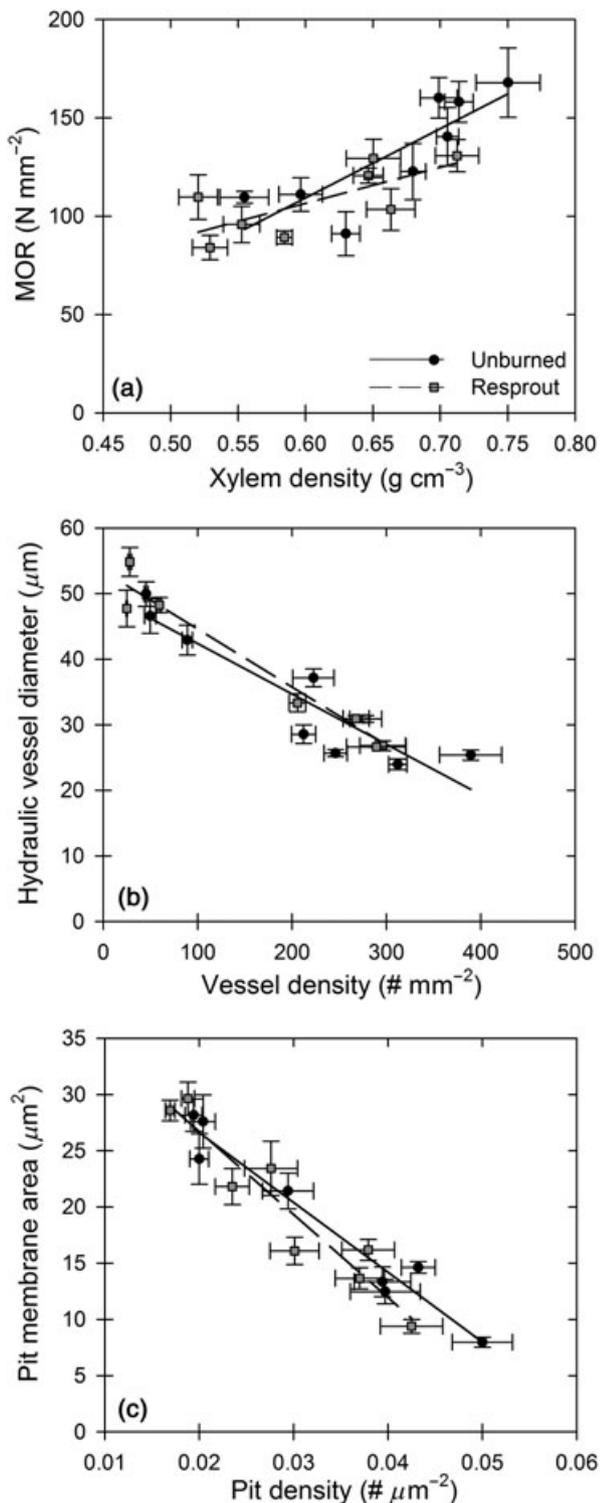


Figure 4. Structural traits measured on similar structures or functional levels were correlated with one another. This included biomechanical traits, where xylem density was strongly correlated with biomechanical strength (a), vessel-level structure, where vessel density was highly correlated with vessel diameter (b) and pit-level structure, where pit density was highly correlated with individual pit membrane area (c) for both unburned and resprouting plants. Linear regressions are shown only for significant correlations ($P < 0.05$). See Table 3 for specific trait values and Table S1 for Pearson correlation coefficients and correlation P -values.

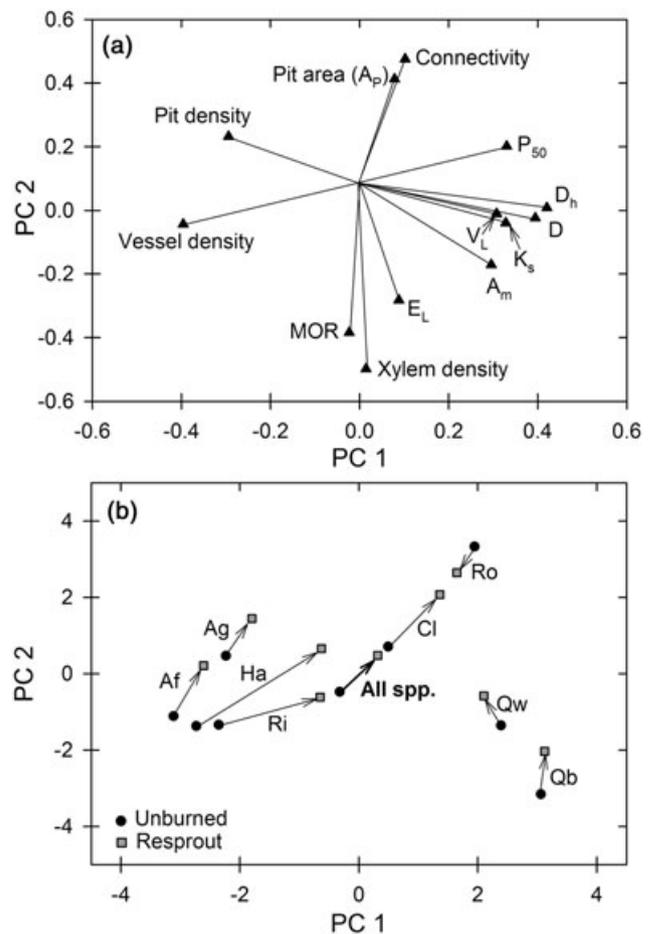


Figure 5. Principle components analysis of all traits and species included in the present study, illustrating the interrelationships among traits (a) and the shift in hydraulic function and structure between unburned and resprouting individuals for each species and averaged across all species (b). The first component (PC 1) was most strongly associated with hydraulic functional traits and vessel structural traits, such as diameter, density and pit membrane area, and accounted for 41.0% of the total variance, while the second component (PC 2) was most strongly associated with biomechanical traits and vessel-to-vessel connection traits and accounted for 25.5% of the variability. Trait abbreviations and units are shown in Table 2, and species abbreviations are shown in Table 3.

common within angiosperm chaparral shrub species (Carlquist & Hoekman 1985; Carlquist 1989; Pratt *et al.* 2015a) as well as drought-tolerant lineages elsewhere globally (Carlquist 1985). Pit area (A_P) estimates are calculated using vessel dimensions and prevalence of vessel-to-vessel contact areas and do not include potential vessel-to-tracheid connections (Wheeler *et al.* 2005; Hacke *et al.* 2006), which leads to an underestimation of total pit area in tracheid-bearing angiosperms as discussed by Hacke *et al.* (2009). Tracheid-to-vessel connections may contribute potential air-seeding sites. This would increase vulnerability to cavitation making their prevalence in arid land species seem paradoxical; however, Carlquist (1989) noted that tracheids are prevalent in shrubs from semi-arid regions and suggested that they might facilitate continued hydraulic function after more vulnerable vessels had embolized. Precisely how tracheids may function during

dehydration of tissues and how they related to the air-seeding hypothesis in angiosperms remains an interesting question (Pratt *et al.* 2015a).

Many trait relationships appear to be constrained by scaling, are very tightly correlated and are not different between resprouting and unburned plants. This includes the relationships between biomechanics (MOR) and xylem density, vessel diameter and vessel density, and individual pit membrane area and pit density. The relationship between xylem density and MOR has been previously reported (Jacobsen *et al.* 2005; Jacobsen *et al.* 2007a; Chave *et al.* 2009). The relationship between vessel diameter and vessel density has also been previously found (Poorter *et al.* 2010; Zanne *et al.* 2010) and is likely related to a packing limit that restricts the density possible for large vessels. A similar packing limit is also likely for the relationship between pit membrane area and pit density, although this relationship has not been reported previously.

Relevance for methods

There has been recent debate about methods used to construct vulnerability to cavitation curves (Jacobsen & Pratt 2012; Sperry *et al.* 2012; Hacke *et al.* 2015), and some of the data collected in this study are relevant in that context. It has been suggested that centrifuge methods generally lead to erroneous *r*-shaped vulnerability curves in long-vesselled species (Cochard *et al.* 2010). In the present study, vulnerability curves of one unburned species (*C. leucodermis*) shifted from *s*-shaped in unburned plants to *r*-shaped in resprouting plants without a significant change in vessel length. This result shows that *r*-shapes are not dependent on vessel length. Some have suggested that xylem that is cavitation fatigued is more vulnerable (Hacke *et al.* 2001; Feng *et al.* 2015) and that sampling stems that contain multiple growth rings are artificially vulnerable (*r*-shaped). This was not supported in the present study, where un-fatigued new growth in relatively hydrated 1-year-old stems was more likely to be *r*-shaped than the potentially fatigued stems of unburned plants, although fatigue may be important for other species or sites (Hacke *et al.* 2001; Christensen-Dalsgaard & Tyree 2014).

Resprouts are generally more hydrated than unburned plants in the field (Oechel & Hastings 1983; DeSouza *et al.* 1986; Saruwatari & Davis 1989) and may generally avoid extensive cavitation even though they are more vulnerable. This is likely due to a greatly increased root:shoot in resprouting plants, which may maintain much of their pre-fire root system and have a much smaller newly forming crown. The general shift to increased vulnerability, including displaying a more '*r*-shaped' vulnerability to cavitation curve, is therefore consistent with their typical water relations. Thus, as has already been shown for chaparral roots, which are also typically more hydrated than stems (Pratt *et al.* 2015b), *r*-shaped curves appear to describe a relatively common hydraulic strategy that can be found in some organs, species and, now, recovery stages of woody plants.

Interactions between fire and drought

Many of the changes in hydraulic structure and function between unburned and resprouting plants were species-specific. The diversity of post-fire resprouting responses in chaparral shrubs was also described previously for three chaparral shrub species that varied in their post-fire hydraulic and carbon traits and also in their ability to survive a drought during the period of post-fire recovery (Pratt *et al.* 2014). This may make it particularly challenging to predict the impact of drought on chaparral communities because the susceptibility of species and communities will vary depending on the time since fire. Beyond chaparral, a study from a mediterranean-type ecosystem in South Africa found that six species did not differ in cavitation resistance or K_s between co-occurring resprouts and unburned plants suggesting that species in other similar systems may not conform to the patterns described here (Pratt *et al.* unpublished data from individuals measured as part of Pratt *et al.* 2012).

In unburned mature chaparral stands, extreme drought appears to impact non-resprouting species more heavily than resprouting species, potentially because of less extensive rooting for many non-resprouting species (Paddock *et al.* 2013; Pausas *et al.* 2016). This was unexpected because non-resprouting chaparral species are some of the most cavitation resistant angiosperms globally (Maherali *et al.* 2004; Jacobsen *et al.* 2007a; Pratt *et al.* 2007; Choat *et al.* 2012).

In burned stands, seedlings of non-resprouting species, which maintain their high resistance to cavitation, are most able to survive drought, and they survive in greater numbers than the seedlings of resprouting species (Frazer & Davis 1988; Davis 1989; Thomas & Davis 1989; Pratt *et al.* 2008). Many resprouting shrub species appear to shift to become more vulnerable to cavitation (Ramirez *et al.* 2012; present study) and have exhibited drought associated mortality during the period of post-fire recovery (Pratt *et al.* 2014). Thus, extreme drought would be predicted to most impact non-resprouting species in unburned stands and resprouting species in burned stands, although such generalizations are likely complicated by the species-specific nature of many plant responses as described earlier. Drought regime is also an important factor in making such predictions (Pausas *et al.* 2016).

In summary, during the period of post-fire recovery, resprouting plants show altered xylem hydraulic function, biomechanics and anatomy as compared with unburned mature plants. These changes are species-specific, but a majority of species experience shifts to become more vulnerable to water stress-induced hydraulic failure because of changes in tissue, vessel and pit-level traits within the xylem. These results confirm the complicated patterns of resprouting vulnerability to drought described in the recent global review by Pausas *et al.* (2016) and contrast with the recent suggestion that resprouting plants may not be as susceptible to drought as non-resprouting plants (Zeppel *et al.* 2015). The interaction between time because disturbance, drought and life history may complicate attempts to predict mortality risk globally (Choat *et al.* 2012) because of temporal changes in community

drought tolerance and limited information on hydraulic structural and functional traits across multiple developmental and recovery stages. Continued examination of the plasticity and ontogeny of plant hydraulic traits is key to developing realistic models of community change over time and to understanding community resilience and composition dynamics.

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SUPPORTING INFORMATION

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Table S1. Correlation matrix showing the Pearson correlation coefficient above and the *P*-value below for all traits measured in the present study. The upper right values display results for correlations run using species means for unburned (blue, italics) and resprouting (red) plants separately. The lower left figures (black text) show the results from the correlations run when unburned and resprout data were pooled. Values in bold indicate significant correlations.