Contrasting drought sensitivity and post-drought resilience among three co-occurring tree species in subtropical China

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ABSTRACT

The impact of drought stress on dominant tree species of subtropical China, and the capacity of these species to recover from drought, is relatively unknown. Three ecologically important tree species (*Syzygium rehderianum*, *Castanopsis chinensis* and *Schima superba*), with contrasting drought sensitivities, were grown in pots under well-watered conditions in the glasshouse before being subjected to two drought treatments (i.e. higher intensity and shorter-duration fast drought(FD);lower intensity and longer-duration slow drought(SD)) towards mortality, and then re-watered to assess the rate of physiological recovery. Dry mass production and physiological traits related to water and carbon relations were measured over the experimental period. FD led to faster declines in hydraulic and photosynthetic function than SD in all species, while hydraulic failure occurred towards mortality in all species under both drought treatments. Although NSC responses varied among drought treatments and species, carbon starvation was not observed. *S. rehderianum* exhibited more rapid declines in leaf water potential and gas exchange traits, and shorter time-to-mortality, followed by *C. chinensis* and *S. superba*. Following re-watering, all three species showed rapid recovery of leaf water potential, while rapid recovery in stem hydraulic conductivity was not evident in any species. Furthermore, *C. chinensis* exhibited full rapid recovery of photosynthesis, but photosynthetic recovery in *S. rehderianum* and *S. superba* was slower than recovery of water potential, reflecting differential stomatal and biochemical limitations. The response of the three species to drought was strongly correlated with xylem embolism resistance, but recovery following drought was complex and partially associated with stomatal and biochemical traits. Under future longer and severe droughts, these species may diverge in drought responses and recovery, perhaps driving shifts in forest community structure in subtropical China.

1. Introduction

Global forest die-off has been observed across all vegetated continents and in a variety of forest biomes (e.g. forests in tropical, temperate and boreal regions) over the last few decades,due to extreme drought and heat stress(see Phillips et al., 2009, 2010; van Mantgem et al., 2009; Peng et al., 2011; Allen et al., 2010, 2016). For instance, substantial mortality of tree species and significant changes in community structure has been evident in subtropical forests of southern China over the past 60 years, and highly correlated with air warming and increased soil dryness (Zhou et al., 2013, 2014a). Large-scale forest collapse can alter ecosystem structure and biodiversity, ecosystem

goods and services, carbon and water budgets, and feedbacks between the biosphere and climate (Metcalfe et al., 2010; Breshears et al., 2011; Hartmann, 2011; Anderegg et al., 2013).The subtropical forest ecosystems in southern China have relatively high carbon uptake and represent large carbon sinks (Yu et al., 2014).Thus, tree mortality in these ecosystems may have substantial impacts on local and/or global carbon balance, biogeochemical cycles and biodiversity conservation. Despite the importance of forests in the biosphere and the significant potential consequences of forest die-offs, the physiological mechanisms underpinning drought-induced tree mortality remain poorly understood (Choat et al., 2012; Choat et al., 2018; Hartmann et al., 2018). In particular, few studies have examined the drought strategies of tree

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Table 1

Summary of species background information and measured SLA, Root/shoot, wood density, *HV* and NSC concentration for the three species at the beginning of the experiment. Values are means with SE in the parentheses (n = 4) (where applicable). Ψ_{50}, the xylem water potential at which stem hydraulic conductivity was reduced by 50%; Ψ_{tlp}, turgor loss point; SLA, specific leaf area; *HV*, huber value.

Species	Family	Successional stage	Ψ_{50} (MPa)	Ψ_{tlp} (MPa)	SLA (cm^2 g^{-1})	Root/shoot (g g^{-1})	Wood density (g cm^{-3})	HV ($\times 10^{-4}$)	Leaf NSC (mg g^{-1})	Stem NSC (mg g^{-1})	Root NSC (mg g^{-1})	Whole plant NSC (mg g^{-1})
Syzygium rehderianum	Myrtaceae	Late	−2.20	−1.85	131.6(3.8)	0.15(0.03)	0.68(0.01)	1.03(0.17)	40.2(2.6)	27.1(0.8)	40.3(8.5)	35.6(2.7)
Castanopsis chinensis	Fagaceae	Mid	−2.44	−2.35	178.6(5.3)	0.52(0.04)	0.76(0.04)	0.81(0.14)	43.3(3.1)	25.8(1.2)	31.4(2.8)	33.4(2.4)
Schima superba	Theaceae	Mid	−4.30	−1.56	158.7(2.4)	0.25(0.01)	0.58(0.02)	2.57(0.39)	33.9(6.4)	27.3(2.4)	31.1(3.6)	31.0(2.7)

Note the species background information (i.e. Family, Successional stage, Ψ_{50} and Ψ_{tlp}) were derived from Zhu et al. (2013) and Li et al. (2015).

species of subtropical forests in southern China and the consequent drought-induced mortality. Under future climates characterized by droughts of increased intensity and duration (IPCC, 2014), better understanding of how subtropical tree species cope with drought stress will improve predictions of forest productivity and functioning in subtropical areas of China.

A comprehensive physiological framework that integrates two possible interrelated mechanisms (i.e. hydraulic failure and carbon starvation) has been proposed to describe physiological processes towards tree mortality associated with drought(McDowell et al., 2008, 2011). On one hand, under high intense but short duration drought stress, substantial loss of xylem hydraulic function inhibits water transport through xylem, thereby leading to rapid cessations of growth and photosynthesis and eventually desiccating organs through hydraulic failure. Species with higher xylem embolism resistance can sustain carbon assimilation at lower water potentials than species with lower xylem embolism resistance. On the other hand, under less intense but longer duration drought stress, carbon imbalance (i.e. between stomatal closure induced reduction in carbon assimilation and continued carbon demand) and failure of carbon transport systems to meet the metabolic, osmotic and defensive requirements may lead to plant death through carbon starvation (McDowell et al., 2008, 2011; Sala et al., 2010; Adams et al., 2017). Accordingly, species with tighter stomatal regulation save water at the expense of carbon input, thereby exhibiting higher risk of carbon starvation. Despite efforts over the last decade, the role of the two inter-related mechanisms have not been completely reconciled (Anderegg et al.,2012; Adams et al. 2009; Adams et al., 2013; Mitchell et al.,2013; Sevanto et al., 2014; Rowland et al., 2015; Duan et al., 2014; Duan et al., 2018). A very recent study, synthesizing data from 19 drought studies representing 26 species worldwide, has demonstrated that hydraulic failure is universal during tree mortality induced by drought, while reduction in non-structural carbohydrates (NSC) at mortality varied across or within species (Adams et al., 2017); these responses largely depended on the experimental conditions (e.g. variable intensity and duration of drought) and functional attributes and taxa of species (e.g. isohydry vs. anisohydry; gymnosperms vs. angiosperms).The notable lack of evidence of carbon starvation towards tree death in earlier studies has often been attributed to a short duration intense drought, which inhibits utilization of NSC. However, very few studies have experimentally compared differing responses of water and carbon traits towards mortality under varying intensities and durations of droughts within one experiment (Hartmann et al., 2013; Adams et al., 2017). Quantifying the effects of variable drought intensities and durations on physiological processes among species with contrasting drought sensitivity will improve mechanistic understanding of drought-induced tree mortality, and potentially provide insights into more accurate predictions for forest composition and structure under future changing rainfall patterns.

The capacity to recover from drought and avoid complete hydraulic failure is an important component of tree survival (Klein et al., 2018). On one hand, some species have been observed to restore hydraulic function rapidly via xylem refilling under sufficient soil water conditions (Ogasa et al., 2013; Martorell et al., 2014), or even diurnally

(Trifilò et al., 2015). On the other hand, some other species can repair embolism through new xylem growth (Brodribb et al., 2010), which will take from weeks to months. However, the degree of hydraulic recovery often varies across species and functional types (Brodribb et al., 2010; Ambrose et al., 2015; Klein et al., 2018). For instance, there is evidence from a study with seven temperate deciduous tree species that species with more vulnerable xylem exhibit faster xylem recovery (Ogasa et al., 2013). Furthermore, substantial lags between hydraulic recovery and gas exchange recovery occurred in some species (Brodribb and McAdam, 2013; Martorell et al., 2014; Creek et al., 2018), which may have important implications for subsequent productivity following the end of the drought. The degree of coupling or decoupling of hydraulic and gas exchange processes during drought recovery is complex and likely varies in species, particularly those representing a range of drought sensitivities.

Three, co-occurring dominant tree species (*Syzygium rehderianum* Merr. & Perry; *Castanopsis chinensis* Hance; *Schima superba* Chardn. & Champ.), from subtropical monsoon evergreen broad-leaved forests in southern China with contrasting drought sensitivities, were chosen for this study (Table 1).The frequency of drought has increased in this region since 1950 due to climate change and has already had significant impacts on tree survival and community structure (Zhou et al., 2011, 2014a).Hence, subtropical monsoon evergreen broad-leaved forests may be threatened by high risk of mortality under climate change (Zhou et al., 2013). Despite the ecological importance of subtropical forests (e.g. carbon sequestration, biodiversity), drought strategies of tree species in this forest in China are not well studied. Here, we investigated physiological processes, including dry mass production, water and carbon relations, and the time to mortality of three species subjected to varying drought conditions (i.e. higher intensity, shorter duration fast drought (FD) vs. lower intensity, longer duration slow drought (SD)) and recovery following re-watering after the drought. Our objectives were to test (i) impacts of FD and SD treatments on water and carbon processes during the drought towards mortality; and (ii) the recovery of hydraulic and photosynthetic traits following rewatering. Specifically, we hypothesized that (i) compared with SD seedlings, FD seedlings would exhibit lower growth, more rapid declines in water potential, hydraulic conductivity and photosynthesis, and more rapid mortality, but deplete less NSC; and (ii) if hydraulic and photosynthetic processes declined quickly in species during drought, that they would recover more rapidly following rewatering, representing a trade-off between drought resistance and drought resilience.

2. Materials and methods

2.1. Plant material and seedling establishment

Seedlings of three tree species (*Syzygium rehderianum* Merr. & Perry, *Castanopsis chinensis* Hance and *Schima superba* Chardn. & Champ.) from subtropical China, were chosen for this experiment (Table 1). These three species are dominant tree species in the subtropical monsoon evergreen broad-leaved forests in southern China, characterized by

different drought sensitivities. In May 2016, one year-old seedlings of these species, previously grown in the field, were purchased from a nursery near South China Botanical Garden, CAS, Guangzhou, Guangdong province, China (N23˚10'30.97", E113˚ 21'9.81"). The climate information can be found in Fan et al. (2019). Afterwards, forty seedlings per species with similar heights and basal diameters were transplanted into pots (20 cm diameter × 28 cm height) containing about 9 kg of air-dried red soil (Quaternary Red Earth) collected from plantations nearby; one seedling was planted into each pot with drainage holes at the bottom. All pots were randomly placed in a sun-lit glasshouse, which did not have environmental control, so plants were exposed to generally ambient outdoor growth conditions (i.e. air temperature, vapour pressure deficit (VPD), CO_2; however, ˜15% of direct sunlight was reduced by the glasshouse, at the research area of South China Botanical Garden. Seedlings were initially well irrigated daily and fertilized once a month with a commercial fertilizer (N ≥ 10%, P_2O_5 ≥ 8%, K_2O ≥ 6%, Micro nutrient element ≥ 5%, Amino acid ≥ 5%, Organic matter ≥ 16%). Seedlings were rotated within the glasshouse regularly to minimise potential differences in environmental conditions. However, *S. superba* seedlings were dead three months after planting, so we replanted *S. superba* seedlings in August 2016.

2.2. Watering treatments

Following four months of growth in the pots (i.e. September 2016), seedlings were randomly subjected to three watering treatments: well-watered treatment (WW), fast drought (FD; higher intensity, shorter duration) and slow drought (SD; lower intensity, longer duration), intending to investigate the seedling responses to droughts differing in durations and intensities. WW seedlings (i.e. twenty per species) were irrigated to field capacity (i.e. soil volumetric water content = 20%) throughout the experiment. FD seedlings (fifteen per species) received no water following the initiation of the drought treatment. SD seedlings (ten per species) were exposed to a controlled drought, which was implemented to slow the progression of the drought, thereby making it similar to field conditions. Pots in SD treatment were weighed in the afternoon (between 1700 and 1800 h) every second or third day to determine water loss. The controlled drought in SD treatment was imposed by replacing 95% of total water loss over these two or three days. Watering was discontinued when minimum water loss was less than 10 ml per day. SD seedlings were then allowed to desiccate completely (see Fig. 1). In this study, seedlings were considered 'dead' when photosynthesis was zero, there was 100% loss of hydraulic conductivity, and all leaves were brown, brittle and necrotic (see Duan et al., 2015). We considered leaf browning as the visual indicator of plant mortality by estimating the percentage of brown leaves and determined the day of mortality when 100% of leaves turned brown; however, we acknowledge that mortality may have occurred earlier than the observation of all brown leaves and that our estimate of the exact time of mortality is somewhat imprecise.

Furthermore, to determine the capacity of the three species to recover from drought, we re-watered a subset of four seedlings per species in FD treatment to field capacity when the leaf water potential was at the target water potential for a given species (i.e. approximately Ψ_{50}, the xylem water potential at which stem hydraulic conductivity was reduced by 50%). Re-watering occurred at 1900 h and soil water content was maintained at field capacity thereafter. There was no evidence that the plants exhibited foliar water uptake during the experiment, but a small amount may have occurred without our knowledge. We focused on the overnight recovery (12 h after re-watering) of hydraulics and photosynthesis in FD seedlings, which may have implications for daily embolism reversal and photosynthetic recovery, and the capacity to restore xylem function after a rain pulse.

2.3. Dry mass production, leaf carbon isotopic composition and nitrogen concentration

Four seedlings per species were randomly selected for destructive harvest prior to the drought (n = 4), whilst six seedlings per treatment per species were harvested at the end of the experiment (n = 6). Following 12 h of recovery from Ψ_{50} stress, four seedlings (n = 4) were harvested for determination of dry mass and hydraulic measurements. At each harvest, seedlings were separated into leaf, stem and roots, which were washed free of soil. All harvested fresh organs were immediately placed into an oven at 110 ˚C for 1 h to eliminate biological activity and then oven-dried at 70 ˚C for 72 h prior to determining dry mass. Leaf area (cm^2) was determined using a portable leaf area meter (LI-3100A, Li-Cor, Lincoln, NE, USA). Leaf area was estimated from leaf dry mass, based on leaf mass per unit leaf area (LMA) measurements, when leaves were dry and leaf area could not be measured at the final harvest. Leaf carbon isotopic composition ($\delta^{13}C$, ‰) and mass-based nitrogen concentration (N, %) were measured on dried samples, using a PE2400 elemental analyser (Perkin Elmer, USA) connected to an IsoPrime100 isotope ratio mass spectrometer (Elementar, Germany) at South China Botanical Garden. Leaf $\delta^{13}C$ was used to estimate the integrated, long-term leaf water-use efficiency. Two stem segments per seedling were used to determine the stem wood density (g cm^{-3}). The volume of the fresh segment (without bark) was determined gravimetrically by water displacement method and the dry mass was measured after 72 h oven-drying at 70 ˚C. Wood density was calculated as the stem dry mass divided by the stem volume.

2.4. Gas exchange measurements

Leaf gas exchange measurements were taken weekly on recent, fully expanded leaves from three or four seedlings per treatment per species (n = 3–4) between 0900 h and 1100 h on clear days, using a portable open path gas exchange system (Licor-6400, Li-Cor, Lincoln, NE, USA) equipped with a leaf chamber fluorometer (6400–40). In assessing recovery from drought, leaf gas exchange measurements were taken 12 h after re-watering. Leaf photosynthesis under saturating light (A_{sat}, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) were measured weekly throughout the experiment, at saturating photosynthetic photon flux density (PPFD) of 1500 μmol m^{-2} s^{-1}, [CO_2] of 400 μmolmol^{-1} and mid-day air temperature (29.5 ± 0.3 ˚C). The leaf-to-air VPD was 2.7 ± 0.8 kPa across species. Leaf instantaneous water-use efficiency (WUE_i, μmol mol^{-1}) was calculated as A_{sat}/g_s.

Prior to and post recovery, steady-state fluorescence (F_s) and maximum fluorescence (F'_m) were measured during a light-saturating pulse, enabling the calculation of photochemical efficiency of photosystem II (PSII) (Φ_{PSII}) as $\Phi_{PSII} = (F'_m - F_s)/F'_m$. The electron transport rate (ETR) (μmol m^{-2} s^{-1}) was calculated as $ETR = 0.5 \times \Phi_{PSII} \times PPFD \times \alpha$, where 0.5 is a factor accounting for the light distribution between the two photosystems and α is leaf absorptance (often assumed as $0.85 - 0.88$) (Zhou et al., 2016).

2.5. Water relations measurements

Pre-dawn water potential (Ψ_{pd}, MPa) and mid-day leaf water potential (Ψ_l, MPa) were measured weekly, or after 12 h of recovery, following gas exchange measurements using a Scholander-type pressure chamber (PMS 1505D, PMS instruments, Corvalis, Oregon USA). On the evening prior to measurements, three seedlings per treatment per species were randomly selected. Each sampled seedling was double-bagged in a plastic bag overnight to ensure equilibration of the water potential between the soil and the seedling. Approximately one hour before sunrise, Ψ_{pd} of two leaves from each seedling was measured. After gas exchange measurements during the day, another two leaves from each seedling were sampled for the determination of Ψ_l.
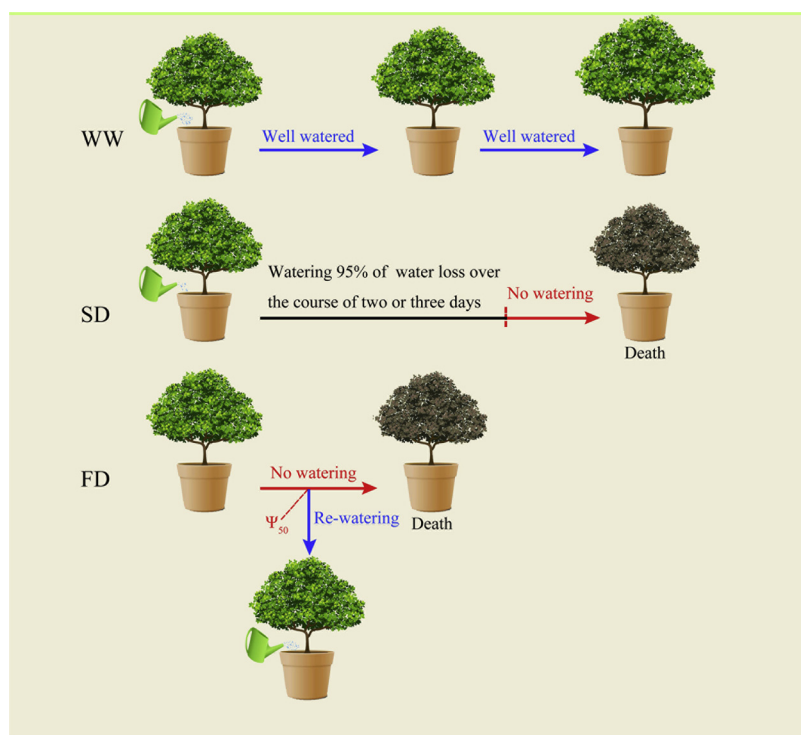
Fig. 1. Watering treatments for the experiment. WW (i.e. Well-watered control) seedlings were irrigated to field capacity throughout the experiment. SD (i.e. Lower intensity, longer duration drought- Slow drought treatment) seedlings were initially watered by replacing 95% of total gravimetrical water loss over the previous two or three days (Black line), but nowatering was added when minimum water loss was less than 10 ml until death (Red line). FD (i.e. Higher intensity and shorter-duration drought- Fast drought treatment) seedlings received no water following the initiation of the drought treatment. A subset of FD seedlings (5 seedlings) was re-watered to field capacity for 12 h when Ψ_{50} for a given species was achieved (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

2.6. Hydraulic measurements

Stem hydraulic measurements were conducted at each harvest. All harvests were conducted at predawn because xylem water potential was highest (i.e. least negative) and this will minimize problems associated with air entry into the cut surface. Therefore, approximately one hour before sunrise on the harvest day, stems of seedlings were cut close to the surface of the soil, sealed with parafilm and then sealed in a black plastic bag, humidified with wet paper towels, and returned immediately to the laboratory.

Hydraulic conductivity was measured using the pressure-flow method described by Sperry et al. (1988). Stems were recut under KCl solution (2 mM) immediately to prevent air entry into the xylem and stem segments of 20–30 cm in length were then cut for hydraulic measurements. The two ends of the segments were shaved smoothly with a razor. With a pressure head of 6 kPa, the initial/pre-flush hydraulic conductivity ($K_{initial}$) was estimated from the segment by measuring the flow rate of KCl solution using a hydraulic conductivity measurement apparatus (Li et al., 2015). $K_{initial}$ was calculated as the ratio of flow rate through the stem segment and the pressure gradient generating the flow. Then, the same segment was attached to a captive water tank with a pressure of 100 kPa and flushed with the same solution for 30 min to remove any embolism that may have been present before a hydraulic measurement was taken to establish K_{max}. The percentage loss of conductivity (PLC) of the stem segment was determined by:

$$\text{PLC} = 100 \times (K_{max} - K_{initial}) / K_{max}$$

Sapwood-specific hydraulic conductivity (K_s, kg m^{-1} s^{-1} MPa^{-1}) was calculated by $K_{initial}$ and the cross-sectional sapwood area of both ends of the segment (A_s). Huber value (HV, $\times 10^{-4}$) was calculated as A_s divided by the supported leaf area (A_l) (Zhou et al., 2016).

2.7. Non-structural carbohydrate (NSC) assay

Oven-dried plant organ samples were ground to fine powder in a ball mill. Organ samples (50 mg) were weighed and then extracted with 4 ml of 80% aqueous ethanol (v/v) in a polyethylene tube. The mixture was boiled in a water bath at 80 °C for 30 min, and then centrifuged at

3000 rpm for 5 min. The supernatant was collected and the pellet re-extracted once with 4 ml of 80% aqueous ethanol (v/v) and once with 4 ml of distilled water, then boiled and centrifuged as before. Total soluble sugars were determined on the supernatants colorimetrically at 620 nm using the anthrone-sulphuric acidmethod (Ebell, 1969). Starch was determined on the pellets remaining after perchloric acid extraction (35%;v/v) and also assayed colorimetrically at 620 nm following the modified anthrone method (Hansen and Møller, 1975; also see Quentin et al., 2017). NSC is defined as the sum of starch and soluble sugars. Whole-plant NSC concentration (mg g^{-1}) was calculated as the sum of the weighed concentrations (concentration multiplied by the proportion of organ dry mass to total dry mass) of the different organs (leaf, stem and roots).

2.8. Statistical analysis

All statistical analyses were conducted with the open-source statistical software platform R (version 3.4.1; R Foundation for Statistical Computing).Time-series of parameters (i.e. A_{sat}, g_s, Ψ_{pd}, PLC) were analyzed using repeated-measures mixed-effects with the 'nlme' package. The fixed factors were species, water and date. Seedling number was treated as a random factor in all analyses. We then used one-way analysis of variance (ANOVA) to test for water treatment effects on all parameters within each species on each sampling date followed with Tukey's HSD post-hoc test. Student t tests were used to compare difference between drought and well-watered treatments (i.e. parameters during recovery and NSC at mortality). Homoscedasticity and normality were checked prior to all statistical analyses. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Physiological traits at pre-drought

Based on previous studies, *S. superba* has more negative Ψ_{50} (i.e. the xylem water potential at which stem hydraulic conductivity was reduced by 50%) than the other two species (Table 1), while *C. chinensis* has the most negative Ψ_{tlp} (i.e. turgor loss point), indicating that the

Table 2
Changes of plant dry mass, leaf δ^{13}C and N concentration for the three species treated by different water conditions and mortality days in the drought treatments. Values are means with SE in the parentheses (n = 3–6). Pre-drought, at the beginning of the experiment; End, the time when mortality occurred; WW1, well-watered control for fast drought treatment; WW2, well-watered control for slow drought treatment; FD, fast drought treatment; SD, slow drought treatment.

Species	Treatment	Plant dry mass (g)		Root/shoot (g g^{-1})		δ^{13}C (‰)		N (%)		Mortality days
		Pre-drought	End	Pre-drought	End	Pre-drought	End	Pre-drought	End	
Syzygium rehderianum	WW1	31.70(4.80)	41.10(7.00)b	0.15(0.03)	0.19(0.04)a	−28.95(0.05)	−28.83(0.09)b	1.70(0.08)	1.55(0.10)a	
	FD	–	25.70(6.60)c	–	0.20(0.05)a	–	−28.61(0.46)b	–	1.58(0.09)a	50.0(0.8)
	WW2	–	90.90(3.60)a	–	0.15(0.01)ab	–	−27.26(0.12)a	–	1.53(0.05)a	
	SD	–	55.20(1.80)b	–	0.11(0.01)b	–	−28.22(0.28)b	–	1.51(0.10)a	121.0(2.2)
Castanopsis chinensis	WW1	52.20(4.10)	59.70(5.80)ab	0.52(0.04)	0.47(0.03)b	−28.54(0.06)	−28.48(0.11)b	2.29(0.08)	2.44(0.10)a	
	FD	–	48.60(2.70)b	–	0.44(0.04)b	–	−29.01(0.29)b	–	1.97(0.13)bc	71.0(0.9)
	WW2	–	69.40(7.70)a	–	0.65(0.04)a	–	−28.25(0.03)a	–	1.78(0.05)c	
	SD	–	50.10(2.50)b	–	0.64(0.07)a	–	−28.38(0.10)ab	–	2.08(0.03)b	151.0(3.5)
Schima superba	WW1	10.00(0.70)	13.10(0.60)a	0.25(0.01)	0.29(0.05)bc	−30.51(0.04)	−29.77(0.22)a	1.92(0.07)	2.55(0.20)a	
	FD	–	11.80(0.70)a	–	0.39(0.06)ab	–	−29.84(0.42)a	–	1.94(0.11)b	98.0(2.2)
	WW2	–	12.30(0.90)a	–	0.48(0.04)a	–	−29.10(0.21)a	–	2.77(0.45)a	
	SD	–	11.50(0.60)a	–	0.28(0.01)c	–	−29.03(0.61)a	–	2.75(0.37)a	170.0(2.1)

Note only well-watered plants were harvested or sampled for analyses at pre-drought, therefore values were only shown in WW1 treatment. Different letters denote significant differences among treatments for a given trait for each species ($P < 0.05$).

three species differ significantly in drought sensitivity and stomatal regulation. Additionally, SLA was highest in *C. chinensis*, followed by *S. superba* and *S. rehderianum*. The wood density was lower but *HV* value was higher in *S. superba* than the other two species. However, NSC of organs and the whole-plant did not differ among species. Leaf δ^{13}C was more negative in *S. superba*, whereas leaf N concentration was higher in *C. chinensis* (Table 2).

3.2. Dry mass production, leaf δ^{13}C and nitrogen concentration at mortality

At mortality, both drought treatments (i.e. FD and SD) significantly reduced dry mass compared with the well-watered treatment for *S. rehderianum* and *C. chinensis*, but not for *S. superba* (Table 2). For *S. rehderianum*, the reduction was similar in FD (−37%) and SD (−39%), but the reduction was smaller in FD (−19%) and SD (−28%) for *C. chinensis*. Leaf δ^{13}C was more negative in SD than WW for *S. rehderianum*, but it was similar between WW and drought treatments for the other two species. Moreover, leaf N concentrations for *C. chinensis* and *S. superba* were lower in FD than WW. In both drought treatments, *S. rehderianum* exhibited the most rapid time-to-mortality, followed by *C. chinensis*, and then *S. superba*. For all species, FD led to shorter time-to-mortality than SD.

### 3.3. Hydraulic and gas exchange responses during drought stress and following recovery

Compared with WW, both drought treatments reduced Ψ_{pd} (more negative) in all species as drought progressed (Fig. 2a–c; $P < 0.001$), with steeper declines in FD than SD. Specifically, *S. rehderianum* showed faster decline in Ψ_{pd} than the other two species following the onset of drought, whereas *S. superba* maintained Ψ_{pd} until a sharp decrease near the end. At the pre-drought condition, A_{sat} and g_s were highest for *S. superba* (A_{sat} = 6.65 ± 0.27 μmol m^{-2} s^{-1}; g_s = 0.047 ± 0.003 mol m^{-2} s^{-1}), followed by *S. rehderianum* (A_{sat} = 4.02 ± 0.20 μmol m^{-2} s^{-1}; g_s = 0.029 ± 0.003 mol m^{-2} s^{-1}) and *C. chinensis* (A_{sat} = 3.63 ± 0.11 μmol m^{-2} s^{-1}; g_s = 0.026 ± 0.002 mol m^{-2} s^{-1}) (Figure S1). The dynamics of A_{sat} and g_s were in concert with changes in Ψ_{pd} (Fig. 2d–i; Fig. S1). A_{sat} and g_s declined to near zero more rapidly in FD than SD and rates of decline differed among species. For instance, it took about 20 days for *S. rehderianum*, 27 days for *C. chinensis* and 87 days for *S. superba* to achieve zero of A_{sat} and g_s in FD. The duration of negative carbon balance was estimated by the difference between the date when A_{sat} reached zero and the date of mortality. As such, the number of days for negative carbon balance was larger for *C. chinensis*

(44 days; 62% of experimental period), followed by *S. rehderianum* (30 days; 60%) and *S. superba* (9 days; 9%). These results indicate that different drought sensitivities among the three species, observed as physiological responses, affected the species difference in duration and intensity of stress. As Ψ_l dropped, the reduction in g_s occurred at less negative Ψ_l for *S. superba* than the other two species (Fig. 3), suggesting more highly regulated stomatal control in *S. superba* under drought stress.

During the 12-hour recovery post-drought stress, Ψ_l for the three species almost recovered to well-watered status (Fig. 4a; Fig. S2), suggesting that leaf water potential recovery from drought was fast in all species. Nonetheless, they exhibited different rates of recovery of xylem hydraulic conductivity. K_s of *S. superba* recovered significantly to *ca* 181% higher than the drought stress value, thereby leading to a substantial PLC recovery (Fig. 4b and c; Fig. S2). However, K_s and PLC of the other two species did not show significant recovery (Fig. 4b and c). Possible excision artefacts associated with the measurement of *S. superba* limit our ability to assess the capacity of *S. superba* to repair xylem (see discussion below). By contrast, photosynthetic recovery was substantially slower than water potential recovery and the degree of recovery differed among species (Figs. 4 and 5; Fig. S2). For example, *C. chinensis* exhibited full recovery of all gas exchange and fluorescence traits, but *S. rehderianum* and *S. superba* showed no recovery of those photosynthetic traits. Collectively, A_{sat} and g_s, particularly for *S. rehderianum* and *S. superba*, were lower during recovery than during dry-down at a given Ψ_l (Fig. 5), indicating that the trajectory describing gas exchange-Ψ_l relationship shifted during the recovery process. Moreover, *S. rehderianum* showed a lower WUE_i at a given Ψ_l during recovery compared to the dry-down, whereas the other two species had similar trajectories of WUE_i -Ψ_l relationships during dry-down and recovery.

3.4. K_s, PLC and NSC towards mortality

At the beginning of the experiment, K_s of *S. rehderianum* was significantly lower than *C. chinensis* and *S. superba* (Fig. 6a, c, e). As drought was intensified, K_s of the three species declined substantially in FD and SD treatments. Plants in SD maintained K_s longer than those in FD, irrespective of species. At the time of mortality (i.e. the last sampling points in the figure), K_s was zero for all species and drought treatments. By contrast, PLC of *S. rehderianum* was larger than *S. superba* at the beginning (Fig. 6b, d, f). PLC for the three species increased remarkably in both drought treatments as drought progressed, with earlier increases in FD than SD (Fig. 6). Overall, PLC reached 100% at mortality irrespective of species or drought treatments, reflecting that
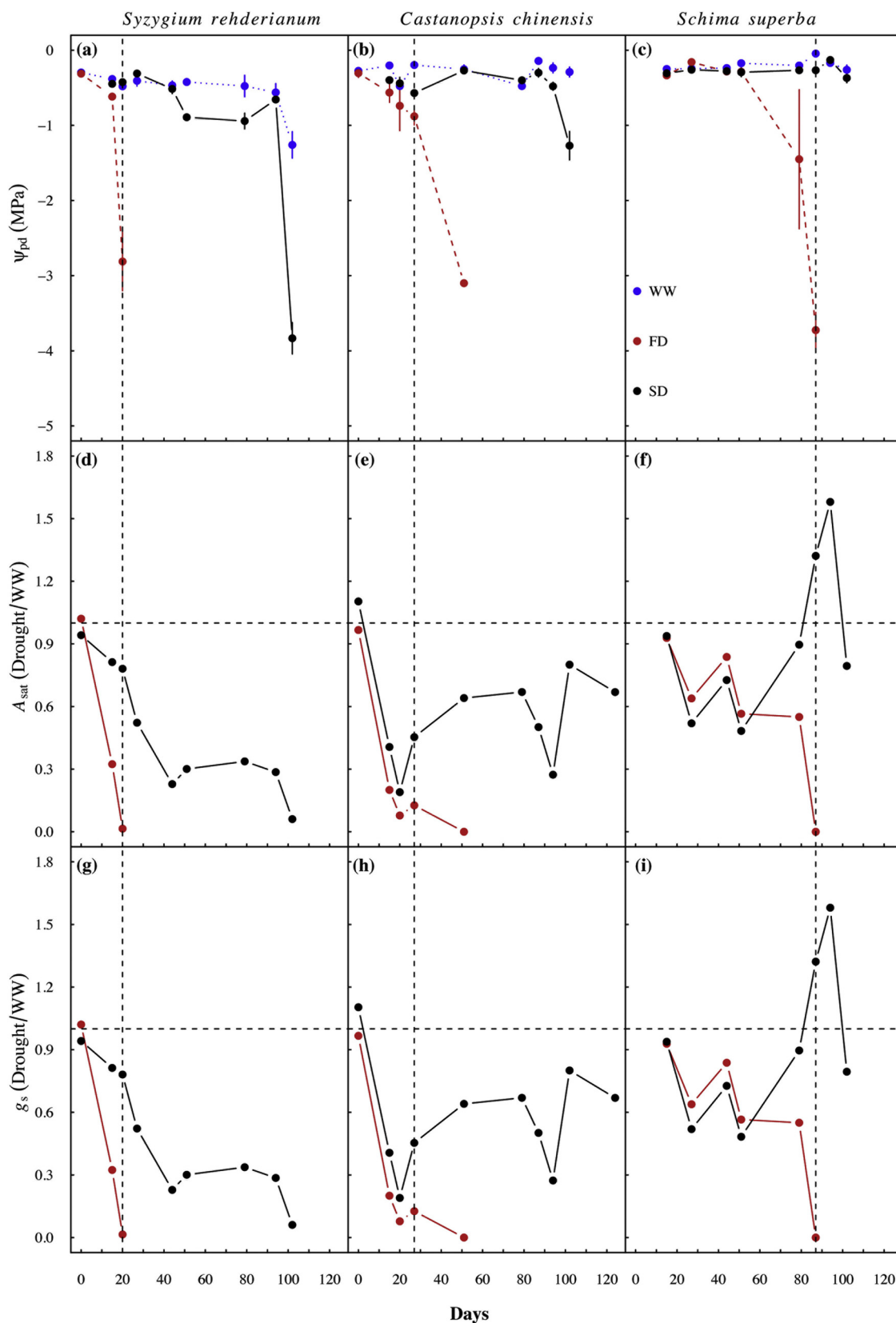
Fig. 2. Time course of predawn leaf water potential (Ψ_{pd}) and relative gas exchange ratios for the three species grown under different watering regimes. Values are Means ± SE (n = 3–4). WW-Well-watered control; FD-Fast drought treatment; SD-Slow drought treatment. Relative gas exchange ratios were calculated as gas exchange traits in drought treatments divided by those in well-watered control. The horizontal lines represent WW. The vertical lines represent the time when stomatal conductance was reduced to near zero.
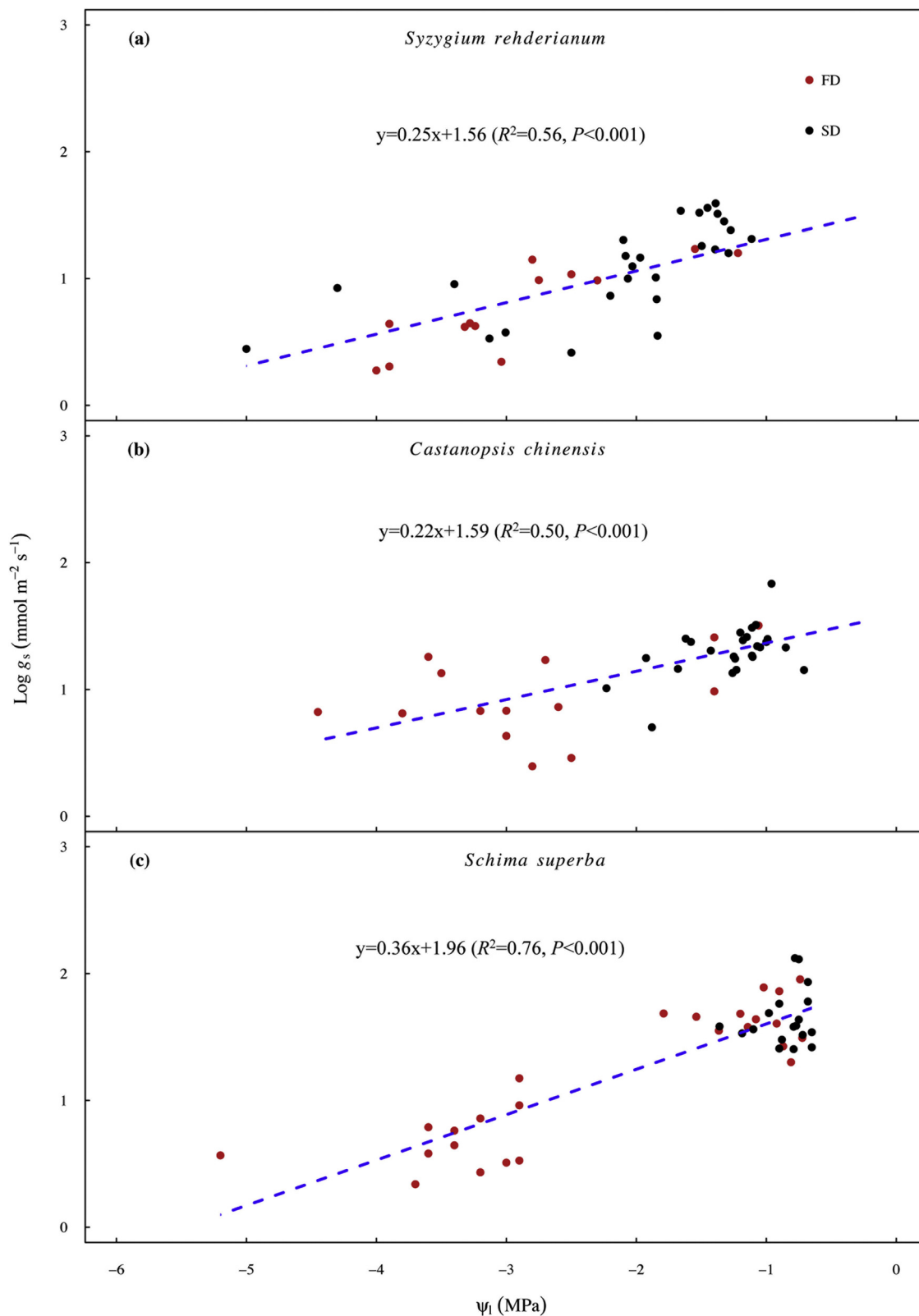
Fig. 3. Logged values of stomatal conductance (g_s) as function of midday leaf water potential for the three species grown under different watering regimes. Values are raw points. The fitted regressions are shown in the figure. The differences of regressions among species were tested by comparing the slope and 95% confidence interval (CI) values. The slope ± CI was 0.36 ± 0.06 for *S. superba,* 0.22 ± 0.06 for *C. chinensis* and 0.25 ± 0.05 for *S. rehderianum,* respectively. FD-Fast drought treatment; SD-Slow drought treatment.

hydraulic failure occurred during progression to mortality of the three species.

Changes in carbohydrate (starch, soluble sugars and NSC)

concentration of FD and SD plants at mortality, relative to WW plants, varied significantly across species and treatments (Fig. 7). For *S. rehderianum*, FD promoted significant depletion of leaf starch, but
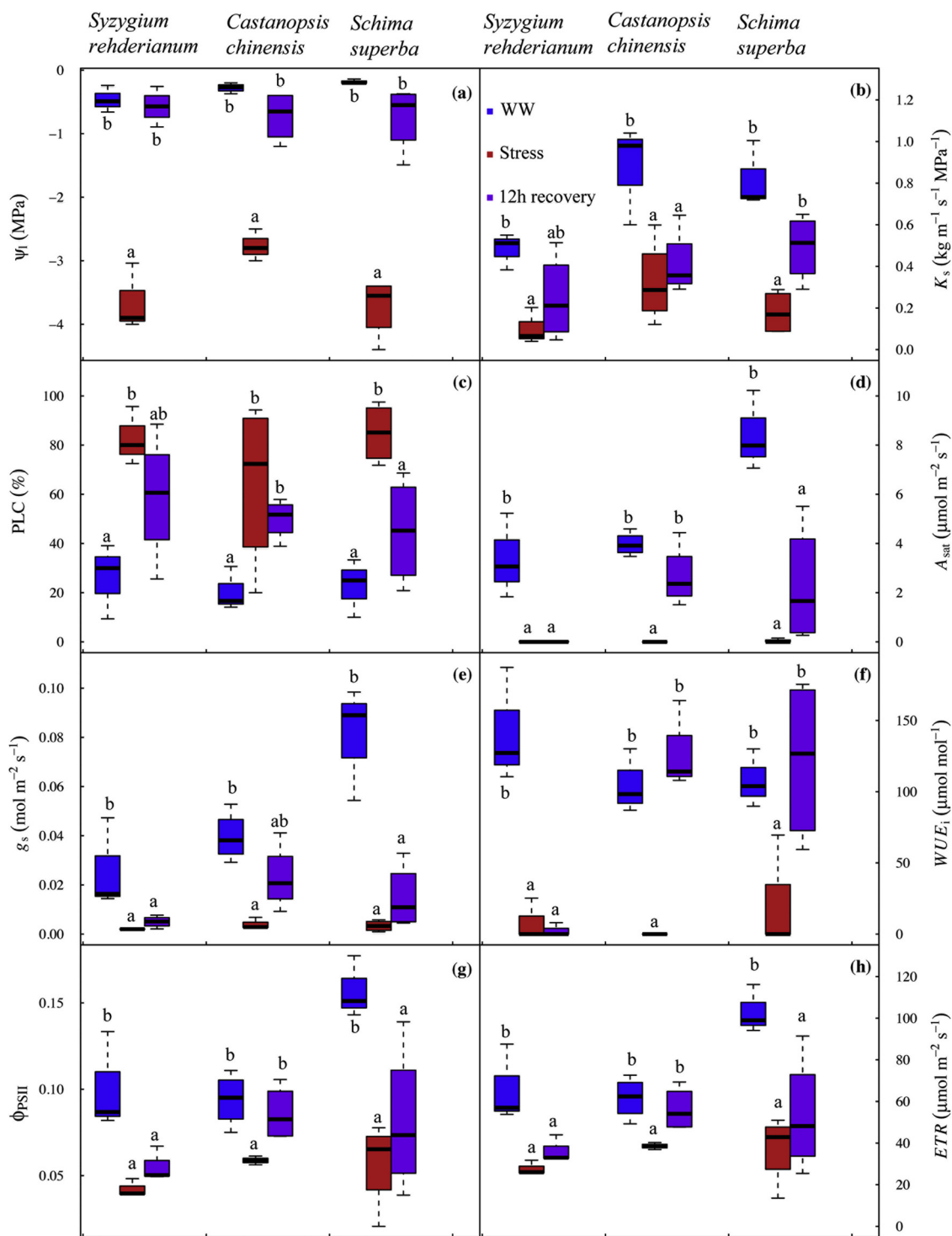
Fig. 4. Values of Ψ_l (a), K_s (b), PLC (c), A_{sat} (d), g_s(e), WUE_i (f), Φ_{PSII} (g) and ETR (h) for the three species during drought stress and recovery stages. Data are shown as boxplots (n = 3–4). WW, well-watered control (blue); Stress, FD treatment stress (red); 12 h recovery, 12 h recovery after the FD stress (purple); Ψ_l, leaf water potential; K_s, sapwood-specific hydraulic conductivity; PLC, percentage loss of hydraulic conductivity; A_{sat}, photosynthesis at saturating light; g_s, stomatal conductance; WUE_i, leaf instantaneous water-use efficiency; Φ_{PSII}, photochemical efficiency of photosystem II (PSII); ETR, electron transport rate. Different letters denote significant differences between treatments and stages for a given trait for each species ($P < 0.05$) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

induced accumulation of leaf soluble sugars, thereby resulting in higher leaf NSC (Fig. 7a–c). Despite notable depletion of root soluble sugars, root and stem NSC did not vary between FD and WW (Fig. 7a–c). SD led to depletion of stem and root starch, but accumulated leaf and stem soluble sugars, leading to accumulation of leaf NSC and depletion of root NSC. For *C. chinensis*, FD significantly depleted root soluble sugars,

while SD depleted root starch (Fig. 7d–f). However, only SD depleted stem and root NSC. For *S. superba*, starch accumulated in all organs in FD, but soluble sugars were depleted in all organs, eventually leading to depletion of leaf NSC (Fig. 7g–i). By contrast, depleted root starch under SD was offset by accumulation of root soluble sugars. At the whole-plant level, FD led to significant depletion of whole-plant NSC in *S.*
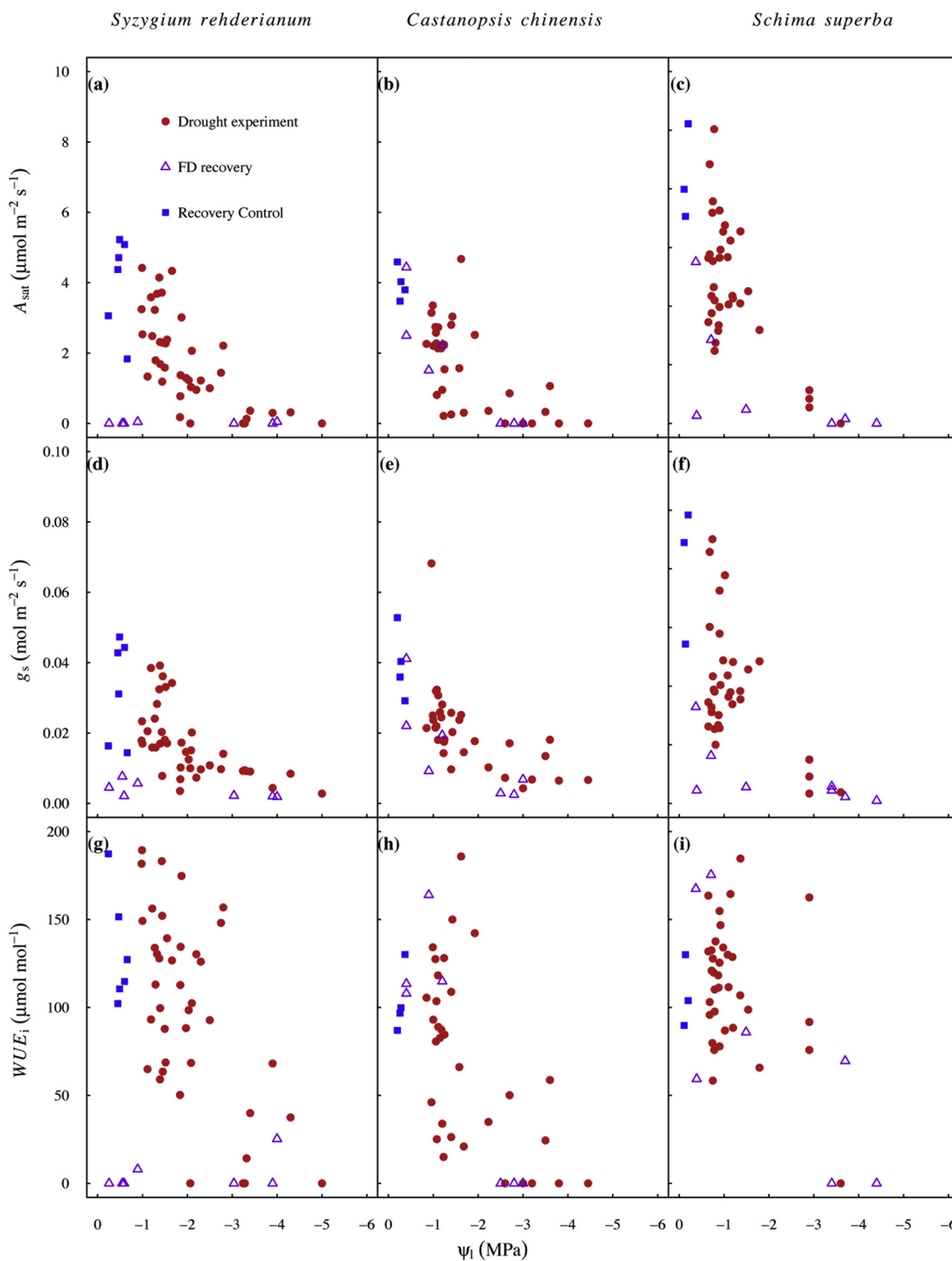
Fig. 5. Photosynthesisat saturating light(A_{sat}), stomatal conductance (g_s) and WUE_i as function of leaf water potential for the three species grown in WW and FD treatments during the dry-down and recovery stages. Values are raw points. WW-Well-watered control; FD-Fast drought treatment.

superba plants (-26%), whereas SD promoted notable accumulation of whole-plant NSC in *S. rehderianum* plants ($+14\%$) and depletion in *C. chinensis* plants (-33%) (Fig. S3).

4. Discussion

4.1. Effects of drought treatments on dry mass production and water and carbon relations

In this study, negative effects of drought on dry mass production were evident in *S. rehderianum* and *C. chinensis*, but not in *S. superba*. It is common that drought can inhibit plant growth (Hesiao 1973; Mitchell et al., 2016). Yet, negative effects of drought on growth are not
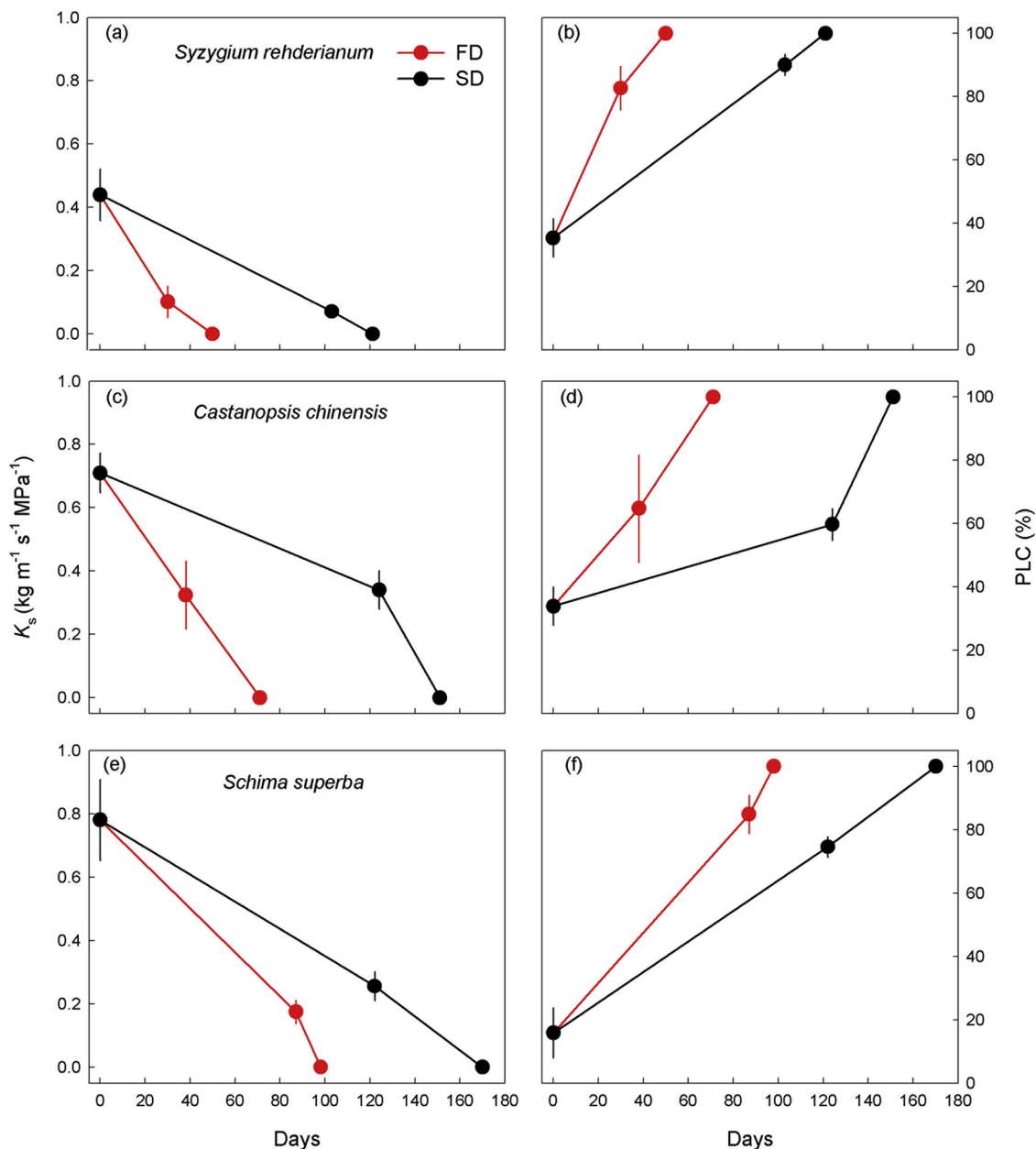
Fig. 6. Time course of K_s (sapwood-specific hydraulic conductivity) (a,c,e) and PLC (b,d,f) for the three species grown under different watering regimes. Values are Means ± SE (n = 3–4). The end points refer to the time to mortality for each species grown under FD or SD. Note that only well-watered plants were harvested for analyses at pre-drought, therefore values at the beginning were same between FD and SD. FD-Fast drought treatment; SD-Slow drought treatment.

always observed. In a previous study with seedlings of ten tropical tree species, eight of ten species did not exhibit less growth over150 d when plants from the drought treatment were compared with those from the well-watered control (O'Brien et al., 2015). In the present study, the relative change of growth of *C. chinensis* compared with control treatment was greater under SD treatment than FD treatment, mainly because drought seedlings were of similar size in SD and FD, while control seedlings for SD were larger than those for FD. For all species, FD treatment induced earlier declines in leaf water potential, hydraulic conductivity, photosynthesis and stomatal conductance than SD treatment. These results are reasonable because FD treatment imposed more intense drought stress to plants, thereby inducing earlier physiological stress for plants. At mortality, both drought treatments led to substantial xylem embolism (i.e. PLC reached 100%), which suggests that hydraulic failure occurred towards mortality of plants subjected to both drought treatments.

The NSC findings contrast with our expectation that FD treatment would deplete NSC to a lesser degree than SD treatment. Indeed, there was no clear pattern of NSC in response to FD and SD treatments across species or organs. NSC in response to drought is quite complex, largely dependent on the tradeoff between carbon supply (i.e. photosynthesis) and carbon demand (i.e. growth, respiration, defense and/or active requirements such as osmoregulation and maintaining hydraulic function) (Sala et al., 2012). Earlier evidence has shown that drought stress can increase, decrease or have minimal effect on NSC. For example, increased leaf NSC in *S. rehderianum* seedlings under both drought treatments is similar to other studies (Hsiao, 1973; Körner, 2003; Ayub et al., 2011; McDowell, 2011). The increase in NSC due to drought stress can be explained by the 'sink limitation hypothesis' that reductions in growth prior to reductions in photosynthesis resulted in NSC surplus. Furthermore, to respond to drought, *S. rehderianum* apparently converted about 60% of leaf starch into sugar, which indicates that
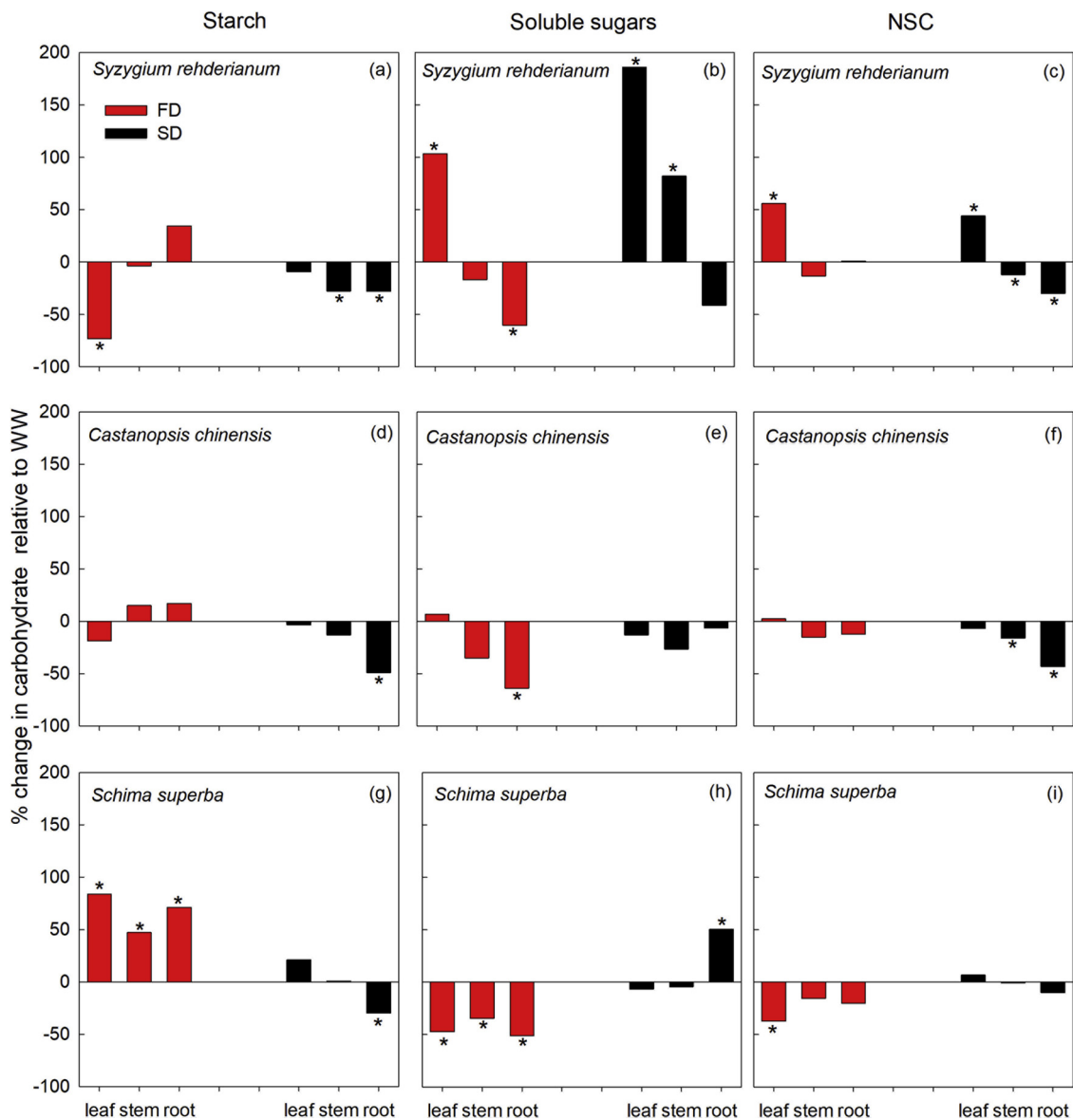
Fig. 7. Relative changes in starch, soluble sugars and NSC of all organs of drought seedlings compared with well-watered control at the end of the experiment. FD-Fast drought treatment; SD-Slow drought treatment. Asterisks represent significant difference between drought treatment and well-watered control.

osmoregulation may be a strategy for *S. rehderianum* to cope with drought stress (Sala et al., 2012). Conversely, increased starch and reduced soluble sugars were observed in FD *S. superba* seedlings. In a previous drought study using *Pinus edulis* seedlings (Adams et al., 2013), leaf starch was higher in drought seedlings than control seedlings, suggesting reduced phloem function for sugar translocation (Sala et al., 2010) and the shifts in source-sink relationships (Adams et al., 2013). Our finding with increased starch and reduced soluble sugars in all organs further demonstrates that sugar transport was largely inhibited within and/or among organs in *S. superba* seedlings. Collectively, NSC response to drought in this study diverged between drought treatments and among species as well as organs, suggesting that consideration of drought treatments and species contributes to the understanding of carbon relations in forest community experiencing extreme drought stress. However, evidence of carbon starvation is apparently absent across species in this study.

4.2. Hydraulic and gas exchange responses among species during drought stress

It took a longer time to reach the critical threshold of leaf water potential and hydraulic conductivity contributing to mortality in *S. superba* compared to the other two species. On one hand, as leaf water potential declined, g_s of *S. superba* exhibited decline at less negative Ψ_l, which indicates higher stomatal regulation of water loss. On the other hand, *S. superba* showed higher xylem embolism resistance which allowed it to maintain water transport at a greater degree of drought stress, promoting favorable water status for longer periods of time. Consequently, the time taken for Ψ_l of *S. superba* to reach critical leaf water potential was longest among the three species. In contrast, *S. rehderianum* and *C. chinensis* exhibited similar sensitivity of g_s to drought stress, while *S. rehderianum* had lower xylem embolism resistance than *C. chinensis*; subsequently, the decline in leaf water potential and xylem hydraulic conductivity were more rapid in *S. rehderianum*. Thus, plant water status in response to drought seems to correlate with stomatal behavior and xylem embolism resistance. Indeed, previous studies have shown that leaf water potential is not

only related to g_s, but also with whole plant water transport (Nolan et al., 2017). A recent study also argues that hydraulic traits (e.g. xylem embolism resistance) play an important role in regulating whole plant water use (Hochberg et al., 2018).Therefore, linking stomatal sensitivity and xylem embolism resistance will provide more accurate understanding of species-specific water status in response to drought stress.

4.3. Mechanisms underlying drought-induced mortality

Under both drought treatments, hydraulic failure occurred in all species prior to mortality. However, carbon starvation did not seem to play roles under either drought treatment or in either species. These results support many previous studies that hydraulic failure is common during drought-induced tree mortality (see Adams et al., 2017; Choat et al., 2018). Among one of the first studies investigating effects of drought characteristics (e.g. intensity and duration) on tree mortality, we found that intensity and duration of drought may have limited roles in determining mechanisms of drought-induced mortality within or among species, at least for seedlings. It is worthwhile to note that the duration of negative carbon balance in SD treatment may not be long enough to drive changes in NSC. In future experiments, the time period of negative carbon balance should be considered to more fully understand whether carbon starvation can occur during much longer duration, but much less intense droughts.

4.4. Hydraulic and gas exchange responses during recovery from drought

The three species displayed similar patterns of water potential recovery following re-watering, showing fast recovery of leaf water potential in the first 12 h. By contrast, the degree of xylem embolism was partially reduced in *S. superba*, but not in the other two species, which suggests that the capacity to restore xylem function may be species dependent; however, our results may be due to possible excision artefacts. We conducted hydraulic measurements following standard protocol, but did not relax stem tension or trim off sufficient stem length prior to the measurement, which would have minimized potential excision artefacts (Wheeler et al., 2013; Torres-Ruiz et al., 2015). Higher embolism levels could have been generated by the excision artefact if the tension of drought stressed plants was not relaxed prior to sampling (Wheeler et al., 2013; Torres-Ruiz et al., 2015; Choat et al., 2019). In practice, it would have been difficult to conduct several cuts (i.e. at least 1.7 times the maximum vessel length; Torres-Ruiz et al., 2015) in seedlings prior to measurements because of the relatively small seedling size. Therefore, the apparent xylem refilling observed in *S. superba* following re-watering could be due to an artefact related to excision and the overestimation of embolism level at the stress stage. Thus, to be conservative, we acknowledge that xylem repair was not evident for any species in this study. This finding agrees with current knowledge that rapid xylem repair after drought through xylem refilling may not occur (Brodribb et al., 2010; Choat et al., 2015, 2019). Furthermore, it is not well understood why tree species differ substantially in their ability to repair embolized xylem. For example, rapid xylem repair (hours to days) after drought was mostly observed in angiosperm tree species across forest biomes (Klein et al., 2018), but much less observed in gymnosperm tree species (e.g. Brodribb et al., 2010; Laur and Hacke, 2014). *Eucalyptus pauciflora* seedlings restored their hydraulic capacity within 6 h (Martorell et al., 2014), but *Sequoia sempervirens* saplings were not able to repair embolism after 2 weeks following re-watering (Choat et al., 2015). In angiosperms, the capacity to repair xylem embolism also differs remarkably, in that the degree of hydraulic recovery was negatively correlated with xylem embolism resistance (i.e. Ψ_{50} and/or Ψ_{88}) (Ogasa et al., 2013; Trifilò et al., 2015). Species with lower xylem embolism resistance were further shown to have lower wood density and higher wood capacitance, which can favour xylem reversal by providing more potential sugar and water sources (Salleo et al.,

2004; Brodersen et al., 2010; Trifilò et al., 2015).However, we found that the lower wood density of *S. superba* did not support more rapid xylem recovery, and additionally *S. superba* had the most negative Ψ_{50}. Our study did not support the functional links between wood density and Ψ_{50}, although it should be noted that the correlation between wood density and Ψ_{50} is not always observed (e.g. Zhu et al., 2013). Nevertheless, *S. superba* is a species with relatively high water storage in branches and leaves (Zou et al., 2010), which can buffer effects of drought to some extent. Future studies are required to examine the functional links between wood structure and physical or metabolic processes during recovery from drought.

The three species exhibited different rates of recovery of gas exchange following re-watering. *C. chinensis* showed coordinated recovery of gas exchange and fluorescence traits with water potential recovery, while *S. superba* and *S. rehderianum* exhibited limited photosynthetic recovery, which suggests that water potential recovery was decoupled from photosynthetic recovery in these two species. Photosynthesis responses during drought stress and following recovery can be co-determined by stomatal and non-stomatal limitations (Zhou et al., 2014b, 2016). On one hand, results show that photosynthesis and stomatal conductance were coordinated (Fig. S4). Our study demonstrated that stomatal conductance in *S. superba* and *S. rehderianum*did not fully recover despite complete recovery of water potential, indicating that factors other than hydraulic status contributed to the re-opening of stomata. Similar results were found in *Eucalyptus pauciflora* (Martorell et al., 2014) and *Pinus radiata* (Brodribb and McAdam, 2013). Earlier evidence showed that accumulation of ABA in leaves can substantially delay stomatal re-opening after drought stress, even when leaf water potential was favorable (Brodribb and McAdam, 2013; Brodribb et al., 2014). On the other hand, biochemical process such as electron transport rate may regulate photosynthesis during drought and recovery, highlighting the importance of stomatal and biochemical factors on photosynthetic responses under cycles of drought stress. Despite minimal recovery observed in A_{sat} and g_s for *S. superba*, WUE_i exhibited full recovery, mainly due to faster recovery of A_{sat} than g_s, which has been observed in other tree species (Creek et al., 2018). This finding suggests that *S. superba* has the ability to restore photosynthesis even if g_s did not fully recover from drought stress, thus restoring WUE_i to achieve carbon and water balance following water stress. Increases in WUE_i due to low stomatal conductance during recovery from drought stress may indicate an acclimation to drought stress (Ruehr et al., 2015).

5. Conclusions

We found that *S. rehderianum* was the most drought-sensitive species associated with earlier declines in leaf water potential, gas exchange and hydraulic conductivity, and more rapid time to mortality. On the other hand, *S. superba* was the most drought-resistant species and survived for the longest, associated with highest xylem embolism resistance and earliest stomatal closure at a given water potential. Hydraulic failure primarily contributed to mortality of all species irrespective of FD or SD treatment. Although NSC responses varied among drought treatments or species, there was no apparent evidence of carbon starvation, indicating the efficacy of predicting tree survival via plant hydraulic properties. Regarding recovery from drought, the three species did not exhibit rapid recovery in hydraulic conductivity. Despite fast recovery in leaf water potentials in all three species following re-watering, full recovery of photosynthetic and fluorescence traits were only observed in *C. chinensis*. Thus, water potential recovery and photosynthesis recovery were decoupled in *S. rehderianum* and *S. superba*. We further demonstrate that the decoupled relationship between water potential and photosynthesis can be co-determined by stomatal and biochemical limitations, but the relative importance of each factor requires future study.

To our knowledge, this is among the first studies to provide eco-

physiological information on drought response strategies of dominant tree species in forests of subtropical China, by linking sensitivity to variable drought conditions and the capacity to recover from drought. *S. rehderianum* is the most drought-sensitive species and did not exhibit overnight recovery in xylem hydraulic conductivity or gas exchange. *C. chinensis* showed higher drought-tolerance than *S. rehderianum* and exhibited the capacity to recover gas exchange rapidly to pre-stress values. However, *S. superba* was apparently most resistant to drought stress and had the ability to recover leaf-level water-use efficiency. Yet, none of these species were able to repair xylem embolism overnight, suggesting that full restoration of hydraulic function via xylem refilling may take more time. If more frequent severe droughts occur in subtropical China in the future, these species may diverge in drought responses and recovery, potentially leading to changes in community structure and ecosystem functioning.

Contributions by the authors

H.D. and Juxiu L. conceived this study, H.D., Y.L. and Y.X. conducted the experiment, H.D.,Y.L.,Y.X., S.Z. and Juan L. analyzed the results, D.T.T. contributed to the analysis and discussion of results, H.D. wrote the manuscript with input from the other authors.

Conflict of interest

None declared.

Acknowledgments

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agrformet.2019.03.024.

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