Ecophysiological responses of two closely related Magnoliaceae genera to seasonal changes in subtropical China

Hui Liu [1, 2], Liwei Zhu [1, 2], Qiuyuan Xu [1, 3], Marjorie R. Lundgren [4], Keming Yang [5], Ping Zhao [1, 2], Qing Ye [1, 2, *]

[1] *Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Guangzhou 510650, China*

[2] *Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China*

[3]*University of Chinese Academy of Sciences, Yuquan road 191, Beijing 100049, China*

[4] *Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK*

[5] *Horticulture Center, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510520, China*

[*] Correspondence address. Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Guangzhou 510650, China. Tel: +86-20-37083320; Fax: +86-20-37252615; E-mail: qye@scbg.ac.cn

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Abstract

Aims

Plants use a variety of hydraulic strategies to adapt to seasonal drought that differ by species and environmental conditions. The early-diverging Magnoliaceae family includes two closely related genera with contrasting leaf habits, *Yulania* (deciduous) and *Michelia* (evergreen), which naturally inhabit temperate and tropical regions, respectively. Here, we evaluate the hydraulic strategy of species from both genera that have been *ex situ* conserved in a subtropical region to determine how they respond to the novel cool-dry season climatic pattern.

Methods

We measured ecophysiological traits in five *Michelia* and five *Yulania* species conserved in the South China Botanical Garden in both wet and dry season conditions and monitored the whole-year sap flow for four of these species.

Important Findings

We found that Magnoliaceae species that have been *ex situ* conserved in a subtropical climate did not suffer from excessive water stress due to the mild drought conditions of the dry season and the ecophysiological adjustments the species made to avoid this stress, which differed by leaf habit. Specifically, deciduous species completely shed their leaves during the dry season, while evergreen species decreased their turgor loss points, dry mass based photosynthetic rates, stomatal conductance, and specific leaf areas compared to wet season measurements. In comparing the two distinct leaf habits during the wet season the leathery-leaved evergreen species had higher leaf hydraulic conductance and leaf to sapwood area ratios than the papery-leaved deciduous species, while the deciduous species had greater hydraulic conductivity calculated on both a stem and leaf area basis, dry mass based photosynthetic rates, leaf nutrients, specific leaf areas, and stomatal sizes than the evergreen species. Interestingly, species from both genera maintained similar sap flow in the wet season. Both photosynthetically active radiation and vapour pressure deficit affected the diurnal patterns of sap flow in the wet season, while only vapour pressure deficit played a dominant role in the dry season. This study reveals contrasting hydraulic strategies in *Yulania* and *Michelia* species under subtropical seasonal conditions, and suggests that these ecophysiological adjustments might be affected more by leaf habit than seasonality, thus reflecting the divergent evolution of the two closely related genera. Furthermore, we show that Magnoliaceae species that are *ex situ* conserved in a subtropical climate are hydraulically sound, a finding that will inform future conservation efforts of this ancient family under the threat of climatic change.

Keywords: hydraulic conductivity; leaf habit; leaf turgor loss point; phylogeny; photosynthesis; sap flow; stomata.

INTRODUCTION

Plants adjust many ecophysiological traits to adapt to seasonal drought, especially hydraulic traits, which are directly related to the degree of drought tolerance among species (Domac *et al.*, 2006, Fan *et al.*, 2011, Meinzer *et al.*, 2001). Under modest water stress, stomata will close to prevent unnecessary water loss and, in the process, also reduce xylem tension within the stems to avoid embolism (Arango-Velez *et al.*, 2011, Cochard *et al.*, 2002, Zhang *et al.*, 2013). For most tree species, leaves are more vulnerable to drought-induced embolism than branches, implying that leaves act as safety valves to protect the plant hydraulic pathway (Bucci *et al.*, 2012, Pivovaroff *et al.*, 2014). Moreover, plants may reduce their overall leaf area (*e.g.*, by shedding leaves) to protect stem functionality if the drought intensifies (Drake and Franks, 2003). However, Wolfe *et al.* (2016) found that leaf shedding does not likely stabilizing plant water potential during typical seasonal droughts, and will generally only do so during periods of intense hydraulic stress. While deciduous species can shed their leaves during periods of drought stress, evergreen species must maintain some level of water transport to their leaves and, as such, will avoid hydraulic failure through morphological and physiological adjustments (Calkin and Pearcy, 1984, Choat *et al.*, 2005). Evergreen species also reduce their leaf area to decrease total transpiration. For instance, *Eucalyptus kochii* trees growing in arid conditions have a lower leaf to sapwood area ratio (A_L/A_S) than those growing in wet conditions (Carter and White, 2009). However, evergreen leaves might also achieve lower leaf water potential at turgor loss point (Ψ_{tlp}) via osmotic adjustments in the dry season (Calkin and Pearcy, 1984). As water becomes less available, active solutes will accumulate in plant cells that enable leaves to maintain cell turgor pressure under these low water potentials (Bartlett *et al.*, 2012). Thus Ψ_{tlp} is an essential physiological determinant of plant drought tolerance within and across biomes (Bartlett *et al.*, 2012, Maréchaux *et al.*, 2015).

Sap flow measurements are useful to explore how water use varies among individual trees or across species at the whole tree level (Granier, 1985, O'Brien *et al.*, 2004, Oren *et al.*, 1999). Sap flows through stems when water deficits in the leaves induce greater water tension in the hydraulic pathway (Cochard *et al.*, 1996). Thus, under seasonal drought conditions when trees induce leaf stomatal closure to conserve water by limiting transpiration, sap flow will also decrease as a result (Ježík *et al.*, 2015, Pataki *et al.*, 2000). Quantifying the relationships between sap flow in tree species and environmental factors (*e.g.*, water availability and light level) is critical to assess the key limiting factors that affect species distribution and conservation (Pataki *et al.*, 2000).

Hydraulic responses to seasonal drought have been widely studied in tropical dry forests and Mediterranean woodlands (Choat *et al.*, 2005, Nardini *et al.*, 2014), where the hot-dry season (*i.e.*, characterized by high temperatures and little precipitation) is very stressful for plants. Furthermore, a convergent drought vulnerability for plants was found across the global forests (Choat *et al.*, 2012), however this excludes the subtropical forests of China, where the summer monsoon climate is characterized by contrasting hot-wet and cool-dry conditions. Hydraulic adaptations of plants in these Chinese subtropical forests likely differ from those reported in regions with hot-dry seasonal patterns.

Magnoliaceae is an early-diverging angiosperm family, originating 93.5~110 million years ago, that is important for phylogenetic and evolutionary studies (Azuma *et al.*, 2001, Kim and Suh, 2013, Massoni *et al.*, 2015). However, about half of the ~300 Magnoliaceae species alive today are threatened with extinction according to the IUCN red list due to habitat loss and over exploitation (IUCN, 2001). Nearly four fifths of Magnoliaceae species are currently distributed between eastern and south-eastern Asia, with the majority of species occurring in China where *ex situ* conservation efforts have been successful (Cicuzza *et al.*, 2007). Unfortunately, present-day climatic change is forcing many species to shift their geographical range or even become extinct (Parmesan, 2006, Thomas *et al.*, 2004), such that studies focused on ecophysiological adaptations within botanical gardens have become increasingly important for *ex situ* conservation efforts (Maunder *et al.*, 2001).

Previous phylogenetic and biogeographic work found that two Magnoliaceae lineages separated into temperate (*Yulania*) and tropical (*Michelia*) areas before the Oligocene (Azuma *et al.*, 2001, Nie *et al.*, 2008). Although *Yulania* and *Michelia* are the most closely related lineages among the Magnoliaceae genera, the temperate-centered *Yulania* species are deciduous, while tropical-centered *Michelia* species are evergreen (Liu *et al.*, 2016), suggesting that the two leaf habits may result from differing climatic regimes. When species originating from different climatic regions are conserved in a common subtropical location, their ecophysiological adaptations to the cool-dry season may differ between lineages, which is crucial evidence to help predict and manage the effects of climatic changes on native and *ex situ* conserved species (Davidson *et al.*, 2011).

To understand the ecophysiological differences between the two contrasting Magnoliaceae genera in this study, we measured wet and dry season physiological traits in five evergreen *Michelia* and five deciduous *Yulania* species conserved in the South China Botanical Garden, and also continuously monitored sap flow in four of these species over a year. Specifically, we ask the following three questions: (1) In the dry season, when deciduous species have shed their leaves, which hydraulic traits do evergreen species adjust to maintain water balance? (2) In the wet season, do deciduous species have higher hydraulic conductivities and consume more water than evergreen species to compensate for their growth losses from the dry season leaf shedding? (3) Will the key environmental factors that influence sap flow density differ between the two seasons? We hypothesized that 1) in the dry season, evergreen species might reduce stomatal conductance, A_L/A_S, and sap flow, but increase osmotic adjustment to release water stress compared to the wet season; 2) in the wet season, deciduous species might show higher hydraulic efficiency and higher sap flow to supply transpiration and photosynthesis due to their quick turnover strategy compared to their evergreen relatives; and 3) environmental indices related to water availability, such as vapour pressure deficit, will directly drive plant hydraulic strategies in the dry season.

MATERIALS AND METHODS

Study site and species

Experiments were conducted in the South China Botanical Garden (SCBG) (23°11'N, 113°21'E, 100 m altitude) at the Chinese Academy of Sciences in Guangzhou, China. SCBG is located in the south subtropical monsoon climatic region, where the average wet (April to September) and dry season (October to March) temperatures are 26.6 °C and 17.6 °C, respectively. While mean annual precipitation is ~1700 mm, 80% of this occurs in wet season. The monthly average air temperature, relative humidity (RH, %), and precipitation in Guangzhou between 1951 and 2014 were collected from the China Meteorological Data Sharing Service System (http://www.cma.gov.cn/2011qxfw/2011qsjgx/, last accessed June 2015). The soil water content (SWC, %) at 30 cm depth was monitored using three SM300 sensors (Delta-T Devices, Cambridge, UK) from the meteorological station in SCBG between 2013 and 2014 (Supplementary Fig. S1).

The Garden of Magnoliaceae at the SCBG is the largest conservation center for Magnoliaceae species in the world (Cultivated Flora of China, http://gardenflora.scbg.ac.cn/). Considering availability and accessibility, we selected five evergreen (*Michelia*) and five deciduous (*Yulania*) Magnoliaceae species to measure ecophysiological traits in both wet (August 2013) and dry seasons (January 2014) (Table 1). Two evergreen and two deciduous species from this list were also used for sap flow monitoring between April 2013 and April 2014. Three to five trees per species were selected, from which three replicate measurements per tree were made of leaf and stem hydraulic traits. All measured individuals were mature trees that had been transplanted to the SCBG Garden of Magnoliaceae between the 1960s and 1990s from the wild or other botanical gardens (Liu *et al.*, 1997).

Sap flow and environment monitoring

Sap flux density (J_S, g H_2O m^{-2} s^{-1}) was recorded continuously between 17 April 2013 and 20 April 2014 at 10 min intervals all the time, with a few interruptions caused by lightning, using a self-made thermal dissipation probes based on Granier's prototype (Granier, 1985). The four selected Magnoliaceae species are diffuse-porous species (Xu, 2008), meaning that vessels of these species are distributed evenly in the sapwood so that the calculation of J_S could follow the classic modeling under the ideal condition. Using core samples collected with an increment borer in May 2014, sapwood areas (A_S, m^2) were calculated for each tree as the difference between the heartwood area and stem cross-sectional area beneath the bark. Since sapwood thickness of the sampled trees ranged between 10 and 40 mm, we used 10 mm long sensors. Each sensor consisted of two cylindrical probes (2 mm in diameter); a continuously heated upper probe and an unheated lower probe. The upper probe included a heater that was continuously supplied with constant power at 0.15 W, while the lower unheated probe served as a temperature reference (James *et al.*, 2002). The probes were covered with a plastic box and insulated with aluminum foil to avoid direct solar heating and disturbance. Temperature differences between the probes were measured every 30 s, and 10 min means were recorded in a data logger (DL2e, Delta-T Devices, Cambridge, UK). J_S along the length of the heated probe was calculated as:

$$J_S = 119\times[(\Delta T_M - \Delta T)/\Delta T]^{1.231} \quad (1)$$

where ΔT is the temperature difference between the two probes, ΔT_M is the maximum value of ΔT under zero-flux conditions (*i.e.*, a period to meet such conditions needs at least two hours with nearly zero vapor pressure deficit (*VPD*, kPa) and basically no changes in ΔT), and 119 and 1.231 are constant factors in the classic equation (Granier, 1985). J_S was calculated by manually setting ΔT_M for each day using BaseLiner (v.3.0.7, C-H2O Ecology Lab, Duke University, NC, USA). For each species J_S was averaged as:

$$J_S = (J_{S1}\times A_{S1}+J_{S2}\times A_{S2}+J_{S3}\times A_{S3})/ (A_{S1}+A_{S2}+A_{S3}) \quad (2)$$

where S1, S2 and S3 are three replicate trees per species and A is the sapwood area for each of these replicate trees. The method of calculating J_S for each species by weighting several trees is conventional in previous studies (Herbst *et al.*, 2008).

A SKP215 quantum sensor (Sky Instruments, Powys, UK) and an AT2&RHT2 sensor (Delta-T Devices, Cambridge, UK) were mounted on a tower adjacent to the Garden of Magnoliaceae within the SCBG for monitoring photosynthetically active radiation (*PAR*, μmol m^{-2} s^{-1}), and air temperature (T, °C) and RH (%), respectively. Using these T and RH data, *VPD* was calculated as:

$$VPD = a\times\exp[b\times \mathrm{T}/ (\mathrm{T}+c)]\times(1-\mathrm{RH}) \quad (3)$$

where *a*, *b*, and *c* are constants of 0.611 kPa, 17.502 (unitless), and 240.97 °C, respectively (Campbell and Norman, 1998). Environmental factors were monitored concurrently with sap flow, at 30 s intervals, with 10 min means continuously recorded in a data logger (DL2e, Delta-T Devices, Cambridge, UK).

Stem hydraulic conductivity, leaf to sapwood area ratio, and sapwood density

Three to five branch stems (diameter 8~10 mm) from each sampled tree were excised using a tree pruner early in the morning. These stems were immediately recut under water to avoid embolism, leaves were sprayed with water and all stems were sealed in plastic bags with moist towels to prevent transpiration, and then rapidly transported to the laboratory. Stem segments were cut under water in ~20 cm lengths, using a razor blade to trim the ends. First, stem segments were flushed with filtered and degassed 20 mmol KCl solution (at 0.1 MPa for 10 min) to remove air embolisms. Next, a 50 cm hydraulic head was attached to one end to generate a fixed pressure with which to drive water flow in the segment. A pipette was fastened to the other end of the stem segment, and the time that it took for water flow to cross the 0.1 ml graduation mark on the pipette was recorded. These data were used to calculate hydraulic conductivity (K_h, kg m s^{-1} MPa^{-1}) as the value of water flow through the segment divided by the driving pressure gradient. Sapwood specific conductivity (K_S, kg m^{-1} s^{-1} MPa^{-1}) was calculated as K_h per sapwood cross-sectional area. Leaf specific hydraulic conductivity (K_L, kg m^{-1} s^{-1} MPa^{-1}) was calculated as K_h divided by the total leaf area on the stem segment (A_L).

A_L was determined by scanning all of the leaves on each stem with a leaf area meter (Li-3000A; Li-Cor, Lincoln, USA), and used to calculate the leaf to sapwood area ratio (A_L/A_S, m^2 cm^{-2}). Sapwood density (WD, g cm^{-3}) samples were taken from the same branches used for K_h measurements. With the bark completely removed, sapwood segments were submerged in water overnight, and then the saturated volume of each sample was measured by the water displacement method. Segments were then oven dried for 72 h at 70 °C and dry mass was weighed. WD is calculated as the ratio of dry mass to saturated volume in each sample.

Leaf water potential, hydraulic conductance (K_{leaf}), and turgor loss point (Ψ_{tlp})

Predawn leaf water potential (Ψ_{pre}, MPa) was measured between 06:00~07:00 and midday leaf water potential (Ψ_{mid}, MPa) was measured between 12:00~14:00. For each species, three replicate leaves per tree were measured using a pressure chamber with a portable pressure gas cylinder (PMS, Corvallis, Oregon, USA).

K_{leaf} (mmol m^{-2} s^{-1} MPa^{-1}) was measured according to the high pressure method (Franks, 2006). First, a single leaf was placed in the pressure chamber and the chamber pressure was increased enough to balance the pressure of the leaf (Ψ_1) and remained there to equilibrate for about 5 min. Chamber pressure was then increased rapidly to Ψ_2, with $\Delta\Psi$ (Ψ_2-Ψ_1) around 0.5 MPa. Pre-weighed Eppendorf tubes that had been stuffed with dry tissue were used to absorb exuded sap. The weight of exuded sap over the first 10 s was measured on an analytical balance (0.1 mg) as ΔW. Leaves were scanned to get leaf area (LA) with a leaf area meter (Li-3000A; Li-Cor, Lincoln, USA). K_{leaf} was calculated as:

$$K_{leaf} = \Delta W \times \text{LA}^{-1} \times \text{time}^{-1} \times \Delta\Psi^{-1} \qquad (4)$$

The bench-drying method was used to determine leaf turgor loss point (Ψ_{tlp}) (Tyree and Hammel, 1972). Three to five leaves for each tree were excised and rehydrated. When the first leaf water potential reached over -0.05 MPa, the drying process was started. Leaf weight and water potential were recorded periodically until the leaf wilted. Individual leaves were then oven dried and weighed to determine leaf dry matter content (LDMC, %). Ψ_{tlp} was calculated per pressure volume models (Schulte and Hinckley, 1985).

Leaf gas exchange, nutrients, specific leaf area (SLA), and stomatal traits

Maximum photosynthetic rate (A_{area}, μmol m^{-2} s^{-1}) and stomatal conductance (g_s, mol m^{-2} s^{-1}) of sun-exposed leaves were measured between 09:00~11:00 with a portable gas exchange system (Li-6400, LiCor, Lincoln, USA). Three to five leaves were measured per tree. The photosynthetic photon flux density (PPFD) in the measurement chamber was set to 1500 μmol m^{-2} s^{-1}, a level that should saturate photosynthesis based on previous experiments (Liu *et al.*, 2015). The reference chamber CO_2 and leaf temperature were set to 390 ppm and 28 °C, respectively. Leaves stabilized in the chamber environment for 5 minutes before recording a data point. Intrinsic water use efficiency (WUE_i) was calculated as A_{area}/g_s.

Between 20 and 30 leaves of each species were scanned using the leaf area meter, then oven-dried for 72 h at 70 °C and dry mass weighed. Specific leaf area (SLA, $cm^2\ g^{-1}$) was calculated by dividing leaf area by leaf dry mass. To determine leaf nutrient content, dry leaves were ground to a powder and digested with concentrated H_2SO_4. Kjeldahl analysis was used to measure total nitrogen content (N, %). Atomic absorption spectrum photometry (UV-6000; Metash, Shanghai, China) was used to determine total phosphorus content (P, %). Photosynthetic rate per leaf mass (A_{mass}, nmol $g^{-1}\ s^{-1}$) was calculated as $A_{\mathrm{area}}\times$SLA.

Leaf epidermal peels were prepared from fresh leaves using a sharp razor blade, then imaged on a microscope fitted with a digital camera using image analysis software (OPTPro2012 4.0, Optec XTS20, Chongqing Optec Instrument, China). Three leaves were sampled from each species, from which three epidermal peels were taken from each leaf. Three randomly-located images from each epidermal peel were analyzed. Stomatal density (SD) was calculated and guard cell width (GW) and length (GL) were measured. The stomatal pore area index (SPI, %) was calculated as $\mathrm{SD}\times\mathrm{GL}^2$, which reflects stomatal pore area per leaf area (Sack *et al.*, 2003). Per Franks and Beerling (2009), the maximum diffusive conductance to water vapour (g_{max}), which estimates transpiration potential, can be calculated as:

$$g_{\mathrm{max}} = (d/v)\times\mathrm{SD}\times a_{\mathrm{max}}/[(l+\pi/2\times\sqrt{(a_{\mathrm{max}}/\pi)}] \qquad (5)$$

where d ($m^2\ s^{-1}$) is the water vapour diffusivity in air at 25 °C; v ($m^3\ mol^{-1}$) is the air molar volume at 25 °C; and SD is stomatal density. a_{max} is the maximum area of the open stomata pore, which was approximated by $\pi\cdot(p/2)^2$, where p is the stomata pore length, estimated as GL/2 based on Franks and Beerling (2009). l is the stomata depth for fully opened stomata, estimated as GW/2; and π is the geometric constant (Franks and Beerling, 2009). In Magnoliaceae species, stomata only exist on the abaxial surface of the leaf.

Data analyses

Data were analysed in R version 3.0.3 (R Development Core Team, 2013). Due to the lack of leaves for deciduous species in the dry season, t-tests on plant functional traits between evergreen and deciduous species were only carried out for data collected in the wet season, while differences between the wet and dry seasons were tested for evergreen species using *t*-tests.

To quantify the relationships between J_S and *VPD*, we partitioned the data into three categories by light level and performed boundary line analyses within each category (Chambers *et al.*, 1985). First, J_S values were filtered to remove data collected under limiting light (*PAR*=0 μmol m^{-2} s^{-1}) and low *VPD* (<0.1 kPa), conditions when empirical relationships between canopy stomatal conductance (G_s) and *VPD* are not well constrained (Oren *et al.*, 1999). This enabled the resulting boundary line to give the best estimate of hydraulic limitation to water flux because the boundary line occurred during conditions that lead to the highest G_s at any given *VPD*. Next the relationships between J_S and *VPD* were examined using the boundary line analysis separately for data grouped into three light levels: low light (LL; *PAR*<400 μmol m^{-2} s^{-1}), middle light (ML; *PAR*=400~800 μmol m^{-2} s^{-1}), and high light (HL; *PAR*=800~1200 μmol m^{-2} s^{-1}). There were no HL data in the dry season due to low *PAR* levels at that time. Different models to predict J_S from *VPD* were compared, including linear, exponential, power, and polynomial models, and then models with the lowest Akaike information criterion (AIC) values were selected. Due to the lack of leaves and very small J_S, the relationships between J_S and *VPD* in the two deciduous species during the dry season were not modelled.

RESULTS

Environmental conditions in the wet and dry seasons

In the study site, air temperature and precipitation are generally higher in the wet season than the dry season, while RH and SWC do not differ between the two seasons (supplementary Fig. S1). During the experimental year, *PAR* and air temperature in the wet season were higher than those in the dry season (supplementary Fig. S2a, b). In the wet season, *VPD* was higher in the day and lower in the night than those time points in the dry season (supplementary Fig S2c). SWC was slightly lower in the dry season compared to the wet season, yet consistently remained above 30%, indicating humid soil conditions throughout the year (supplementary Fig. S2c, d). Daily *PAR*, *VPD*, and RH readings fluctuated greatly throughout the experimental year due to irregular rainfall events (supplementary Fig. S3a, b).

Ecophysiological traits of evergreen and deciduous species under seasonal changes

Due to the lack of leaves for the deciduous species in the dry season, we only compared ecophysiological traits between evergreen and deciduous species in the wet season (Table 2). For hydraulic traits, evergreen species had lower K_S and K_L, and higher A_L/A_S than deciduous species. Evergreen species also had marginally higher K_{leaf} than deciduous species (P=0.07). There were no significant differences in WD, Ψ_{pre}, Ψ_{mid}, and Ψ_{tlp} between evergreen and deciduous species. Evergreen species had significantly lower A_{mass}, leaf N and P contents, SLA, and stomatal size, and higher LDMC than deciduous species (Table 2). All other leaf gas exchange and stomata traits were similar between wet season measurements in the two groups, including A_{area}, g_s, and WUE$_i$, as well as SD, SPI and g_{max}.

Very few traits differed between the wet and dry season measurements of the evergreen species (Table 2). Specifically, Ψ_{tlp}, A_{mass}, g_s, and SLA were significantly lower in the dry season, compared to the wet season. LDMC was marginally higher in the dry season, compared to the wet season, measurements of the evergreen species (P=0.08). All other traits did not differ with seasonality in the evergreen species.

Sap flow patterns of evergreen and deciduous species under seasonal changes

For evergreen species, the dry season J_S in *Michelia chapensis* was higher than that in the wet season, while J_S in *Michelia shiluensis* was similar in both seasons (Fig. 1a-d; supplementary Fig. S3c-d). For deciduous species, J_S was similar to evergreen species in the wet season, and it maintained its daily dynamic pattern even with very low values in the dry season (Fig. 1e-h; supplementary Fig. S3e-f). J_S in *Yulania glabrata* decreased to less than 10 g H_2O m^{-2} s^{-1} between 12-Dec-2013 and 22-Mar-2014, while J_S decreased to this same low level earlier in *Yulania jigongshanensis*, between 4-Nov-2013 and 17-Mar-2014 (supplementary Fig. S3e-f). These findings match our phenological observations of leaf longevity, where leaves of *Michelia chapensis*, *Michelia shiluensis*, *Yulania glabrata*, and *Yulania jigongshanensis* persist for 12, 13.5, 6.5 and 7 months, respectively.

In the wet season, J_S in the four species was sensitive to both *VPD* and light levels (Fig. 2a, c, e, and g). For all three of the assessed light levels, J_S climaxed when *VPD* was around 1.5 kPa and decreased when *VPD* was greater than 2.0 kPa. J_S also differed by light level, with higher J_S in ML than LL levels, while J_S was similar in ML and HL levels. In the dry season, J_S in the two evergreen species also increased with *VPD*, but they decreased less dramatically when *VPD*>2.0 kPa, than in the wet season. Moreover, they were not sensitive to light level, as J_S was similar in LL and ML levels (Fig. 2b, d).

DISCUSSION

Ecophysiological traits of evergreen and deciduous species under seasonal changes

Most of the morphological and hydraulic traits that we measured in the evergreen Magnoliaceae species did not differ between the wet and dry seasons. Only A_{mass}, g_s, Ψ_{tlp}, and SLA decreased for evergreen species in the dry season, which suggests that hydraulic adjustment mainly occurred at the leaf level when the tropical *Michelia* species were grown in a subtropical area, with a characteristic cool-dry season. A paucity of precipitation during the dry season reduced water availability, and yet we did not see simultaneous decreases in RH, SWC, or *VPD*. SWC remained sufficiently high as a result of the relatively high mean annual precipitation that year (~1700 mm) and the cool dry season temperatures that yielded low *VPD*, which together reduced drought stress during the experiment. Therefore, these species were not exposed to extreme water stress, as is indicated by our similar dry and wet season measurements of Ψ_{pre}, a parameter that indicates soil water potential around the root zone when nighttime transpiration is lacking and the internal water storage is recharged (Bucci *et al.*, 2004).

The evergreen Magnoliaceae species had lower g_s in the dry season because plants tend to close stomata to reduce transpiration, and consequently lower photosynthesis, during periods of short-term water stress (Arango-Velez *et al.*, 2011, Cochard *et al.*, 2002, Franks *et al.*, 2007, Zhang *et al.*, 2013). However, due to a sufficient soil water supply, Ψ_{mid} did not decrease, which suggests that the lower g_s measured in the dry season was likely caused by low temperature stress rather than water limitation (Zhu *et al.*, 2011). Meanwhile, leaf Ψ_{mid} remained greater than Ψ_{tlp} throughout the year because Ψ_{mid} remained fairly constant, while measurements of Ψ_{tlp} in the evergreen species strongly decreased in the dry season. The lower Ψ_{tlp} indicates that these species enhanced leaf desiccation tolerance in the dry season (Lenz *et al.*, 2006, Maréchaux *et al.*, 2015). Evergreen leaves tend to have lower SLA and higher LDMC in the dry season (Calkin and Pearcy, 1984), which are the structural basis for lower leaf elasticity and Ψ_{tlp} through osmotic adjustments (Bartlett *et al.*, 2012, Jacobsen *et al.*, 2007, Scholz *et al.*, 2012).

Our findings, that the deciduous Magnoliaceae species had higher branch level hydraulic conductivity (K_{S} and K_{L}) with lower $A_{\mathrm{L}}/A_{\mathrm{S}}$ and LDMC, higher A_{mass}, leaf nutrients (N, P), and SLA, and larger stomatal size in the wet season were expected, as they acquire more resources and achieve higher assimilation during the wet portion of the year compared to the dry season, as has been reported in other species (Carter and White, 2009, Chen *et al.*, 2009, Choat *et al.*, 2005). In addition, all ten species maintained Ψ_{mid} above Ψ_{tlp} in both seasons, indicating that they did not suffer from excessive water stress as a result of only mild drought conditions and their ecophysiological adjustments.

Sap flow patterns of evergreen and deciduous species in the wet and dry seasons

Contrary to our second hypothesis, the evergreen and deciduous species had similar J_{S} values in the wet season, although deciduous species did have higher K_{S} and K_{L} at this time of year. These findings, however, are not completely unexpected and are consistent with previous findings (Dünisch and Morais, 2002). First, K_{S} and K_{L} indicate the potential hydraulic conductivity, such that deciduous species might not achieve these maximum values under natural conditions. Second, the different branch architectures in evergreen and deciduous species might obscure whole-plant hydraulic differences (Givnish, 2002, Meinzer *et al.*, 2010). For example, higher $A_{\mathrm{L}}/A_{\mathrm{S}}$ and lower K_{S} and K_{L} in evergreen species may result in similar canopy-level conductivities and whole-tree water consumption levels as deciduous species. Third, Ψ_{mid} and g_{s} did not differ between evergreen and deciduous species in our study, which supports the J_{S} pattern, but conflicts with the idea that the average canopy-level transpiration and g_{s} values would be lower for evergreen than deciduous species (Meinzer *et al.*, 1993). Instead, we attribute this to a sampling bias, in that our Ψ_{mid} and g_{s} were measured on only sun-exposed leaves from the upper crown of the trees. While evergreen *Michelia* species have very dense crowns with most of their leaves shaded, the deciduous *Yulania* species have sparsely arranged branches, such that most leaves are exposed to the sun. Thus, at the whole tree level, similar J_S for the two Magnoliaceae genera represent an overall balance between leaf-, branch-, and tree-level water use strategies, of which the key linkages needed further investigation.

The dramatic decrease in J_S caused by the dry season leaf shedding of the deciduous species is natural. Indeed, it has been reported that the critical factor controlling seasonal stand transpiration is the degree of foliation present in temperate deciduous forests (Körner, 1995). Moreover, under drought conditions, deciduous leaves can serve as hydraulic fuses among seasonal dry tropical forest species (Wolfe *et al.*, 2016). J_S in our evergreen species did not differ between the two seasons, despite experiencing significant decreases in g_s and SLA in the dry season, which indicates that other ecophysiological regulations were at play. One possible explanation might be the specific phenology of the four species. We found that *Michelia chapensis* yields new leaves twice a year (*i.e.,* in late September and early March), while *Michelia shiluensis* only produces new leaves once a year (*i.e.,* in early March), and that both evergreen species bloom heavily in late February. As a consequence, the evergreen species require more water in January for flower and leaf bud growth. In contrast, the two deciduous species start to shed their leaves in the middle of September and do not grow new leaves until the middle of March. They also have very few flowers by early February (*i.e.,* fewer than ten flowers per individual tree; H. Liu, unpublished data). Therefore, J_S did not increase in the deciduous species during the dry season because the paucity of leaves and flowers at this time of year alleviates the need for significant water concentrations throughout the tree.

During the wet season, *PAR* and *VPD* controlled the diurnal patterns of J_S for evergreen and deciduous species in a similar way, suggesting that responses to these environmental cues is independent of leaf habit (Dünisch and Morais, 2002, Pataki *et al.*, 2000). *VPD* affected the shape of the diurnal patterns in J_S, while *PAR* influenced the amount of J_S, as has been reported in deciduous forests (Wullschleger *et al.*, 2001). The effect of *PAR* on the relationship between J_S and *VPD,* a trend that is reportedly related to low g_s during this season*,* was weak in the dry season due to low overall *PAR* levels (<800 μmol m^{-2} s^{-1}) (Williams *et al.*, 1996). However, at the whole tree level, J_S was likely prompted by more physiological processes in the evergreen species, such as flower and leaf growth.

Differences between evergreen and deciduous species reflect their divergent evolution

Most Magnoliaceae lineages are distributed in tropical and subtropical climates, however, the temperate-centered *Yulania* lineage was separated from its close relatives, the tropical-centered *Michelia*, before the Oligocene (Nie *et al.*, 2008). As we show here, this long period of divergent evolution (*i.e.*, ~40 million years) led to contrasting adaptive strategies. In the subtropical area with sufficient water availability, stems and leaves were hydraulically safe during the whole year of our study (*i.e.*, Ψ_{mid} was never lower than Ψ_{tlp}), as is also seen in species of the dry tropical forests (Hasselquist *et al.*, 2010), the evergreen species here did not need to strictly control for hydraulic conductivity, but instead increased investment in leaves via higher $A_{\mathrm{L}}/A_{\mathrm{S}}$ compared with their deciduous relatives, to compensate for the shady subtropical forest environment (*i.e.*, the natural conditions for *Michelia*). In contrast, the deciduous species employed high stem hydraulic conductivity (K_{S} and K_{L}) and leaf investment in the wet season, which allowed for greater leaf resources (*i.e.*, high N and P content, greater stomata area), to ultimately achieve higher photosynthetic rates compared to the evergreen species (Choat *et al.*, 2005). Overall, *ex situ* conservation and seasonality did not bring large ecophysiological variations, and instead leaf habit might more be decisive on how species would adapt to local environmental conditions. Furthermore, similar water consumption in evergreen and deciduous Magnoliaceae species also implies the rationality of *ex situ* conservation in subtropical China.

CONCLUSIONS

Magnoliaceae species *ex situ* conserved in a subtropical climate did not suffer from excessive water stress in the dry season due to their ecophysiological adjustments and only mild drought conditions during the study year. In the dry season, SWC and *VPD* did not significantly decrease, but precipitation, temperature, and light levels were low, so that evergreen species only reduced A_{mass}, g_{s}, Ψ_{tlp}, and SLA. Thus, we conclude that the divergent evolution between the evergreen *Michelia* and deciduous *Yulania* lineages led to different ecophysiological patterns: evergreen species have higher A_L/A_S, while deciduous species have higher K_S, K_L, A_{mass}, leaf nutrients, SLA, and stomatal size, but lower LDMC. All species showed similar sap flow patterns during the wet season. Furthermore, both *PAR* and *VPD* affected the diurnal patterns of J_S in the four species over the wet season, while in the dry season, only *VPD* was dominant in affecting J_S in evergreen species. Therefore, ecophysiological adaptations might be affected more by leaf habit than seasonality for the *ex situ* conserved Magnoliaceae species. These species showed good hydraulic status in the subtropical area, which provides a successful case for the *ex situ* conservation of this ancient family, and proves valuable for studying potential adaptations of Magnoliaceae species under climatic changes.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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Table 1: Description of the ten Magnoliaceae species used in this study. Names in bold indicate the four species used for sap flow monitoring. Data are mean ± SEM, *n* = 3~5 for each species. DBH, diameter at breast height. † indicates ground diameters for shrubs.

Species	Code	Growth form	Leaf texture	Plant height (m)	DBH (cm)
Michelia chapensis	**Mc**	Evergreen tree	Leathery	15.7±0.7	36.4±1.8
Michelia shiluensis	**Ms**	Evergreen tree	Leathery	8.5±0.2	19.2±0.9
Michelia maudiae	Mm	Evergreen tree	Leathery	10.4±0.4	18.5±0.6
Michelia figo	Mf	Evergreen shrub	Leathery	4.6±0.3	10.5±0.5 †
Michelia platypetala	Mp	Evergreen tree	Leathery	11.6±0.6	14.8±0.7
Yulania glabrata	**Yg**	Deciduous tree	Papery	7.8±0.2	17.4±0.5
Yulania jigongshanensis	**Yj**	Deciduous tree	Papery	6.7±0.3	11.6±1.2
Yulania cylindrical	Yc	Deciduous tree	Papery	7.6±0.4	13.5±1.1
Yulania denudate	Yd	Deciduous tree	Papery	6.3±0.2	15.0±0.7
Yulania liliiflora	Yl	Deciduous shrub	Papery	3.7±0.2	8.6±0.5 †

Table 2: Ecophysiological responses of the ten Magnoliaceae species in wet and dry seasons. Data are mean ± SEM, sample sizes (*n*) are given in brackets. Due to the lack of leaves for deciduous species in the dry season, *t*-tests on traits between evergreen and deciduous species are only carried out for the wet season data, with * and ** indicating $P<0.05$ and <0.01, respectively. Abbreviations: K_S, sapwood specific hydraulic conductivity; K_L, leaf specific hydraulic conductivity; K_{leaf}, leaf hydraulic conductance, A_L/A_S, leaf to sapwood area ratio; WD, sapwood density; Ψ_{pre}, leaf water potential at predawn; Ψ_{mid}, leaf water potential at midday; Ψ_{tlp}, leaf turgor loss point; A_{area}, maximum photosynthetic rate per leaf area; A_{mass}, maximum photosynthetic rate per leaf mass; g_s, stomatal conductance; WUE_i, intrinsic water use efficiency; N, leaf nitrogen content; P, leaf phosphorus content; SLA, specific leaf area; LDMC, leaf dry matter content; SPI, stomatal pore area index; g_{max}, maximum stomatal conductance to water vapor.

	Michelia (Ever)		*Yulania* (Deci)	Ever-Deci (**wet season only**)	Wet-Dry (**evergreen only**)
	Wet (5)	Dry (5)	Wet (5)	P of t-test	P of t-test
K_S ($kg\ m^{-1}\ s^{-1}\ MPa^{-1}$)	1.6±0.3	1.3±0.2	2.8±0.4	*	0.25
$K_L \times 10^{-4}$ ($kg\ m^{-1}\ s^{-1}\ MPa^{-1}$)	2.2±0.3	2.2±0.5	7.1±0.6	*	0.89
K_{leaf} ($mmol\ m^{-2}\ s^{-1}\ MPa^{-1}$)	4.5±0.6	4.1±0.5	3.3±0.3	0.07	0.55
A_L/A_S ($m^2\ cm^{-2}$)	0.7±0.1	0.6±0.0	0.4±0.1	*	0.11
WD ($g\ cm^{-3}$)	0.5±0.0	0.5±0.0	0.4±0.0	0.68	0.46
Ψ_{pre} (MPa)	-0.08±0.0	-0.11±0.0	-0.09±0.0	0.73	0.26
Ψ_{mid} (MPa)	-0.90±0.08	-1.00±0.17	-0.94±0.15	0.83	0.63
Ψ_{tlp} (MPa)	-1.46±0.12	-1.88±0.11	-1.40±0.10	0.52	*
A_{area} ($\mu mol\ m^{-2}\ s^{-1}$)	9.2±0.5	8.6±0.9	8.8±1.6	0.83	0.56
A_{mass} ($nmol\ g^{-1}\ s^{-1}$)	107.6±9.5	76.4±8.2	169.0±18.1	*	*
g_s ($mol\ m^{-2}\ s^{-1}$)	0.18±0.04	0.11±0.03	0.16±0.04	0.64	*
WUE_i ($\mu mol\ mol^{-1}$)	65.0±5.3	75.9±3.9	49.4±5.0	0.16	0.68
N (%)	1.6±0.2	1.6±0.1	2.1±0.1	*	0.94
P (%)	0.09±0.01	0.10±0.01	0.13±0.00	*	0.38
Leaf area (cm^2)	54.7±13.1	47.3±11.6	63.4±10.4	0.21	0.68
SLA ($cm^2\ g^{-1}$)	116.8±9.3	89.8±5.1	203.8±11.1	*	*

LDMC (%)	33.0±2.0	38.7±2.1	25.1±0.8	**	0.08
Stomatal size (μm^2)	399.9±67.5	390.9±65.4	597.1±46.6	*	0.63
Stomatal density (mm^{-2})	294.9±26.0	283.3±16.7	256.2±18.4	0.26	0.72
SPI (%)	11.2±2.9	10.5±2.4	15.3±2.8	0.11	0.72
g_{max} (mol m^{-2} s^{-1})	2.5±0.2	2.6±0.3	2.7±0.2	0.56	0.92

Figure legends

Figure 1: Daily changes in sap flux density (J_S) for four species during the wet (left) and dry (right) seasons. Evergreen (Ever) and deciduous (Deci) types are labeled after the species code as in Table 1 for each panel. Data are based on typical sunny days in the wet (9-12 Aug and 14-16 Sep) and dry (16-19 Jan and 29-31 Jan) seasons.

Figure 2: Sap flux density (J_S) in relation to daytime vapor pressure deficit (*VPD*) for four species during the wet (left) and dry (right) seasons. Raw data, collected in ten minutes intervals as in Fig. 1, are shown as grey crosses. The results of boundary line analyses selected the maximum J_S at three *PAR* levels: low light (LL, black circles, solid thin lines), *PAR*<400 μmol m^{-2} s^{-1}; middle light (ML, white circles, dash lines), *PAR*=400~800 μmol m^{-2} s^{-1}; and high light (HL, black triangles, solid thick lines), *PAR*=800~1200 μmol m^{-2} s^{-1}. Due to low *PAR* levels, there are no HL data for the dry season. See the coefficients and Akaike information criterion (AIC) values for each curve in supplementary Table S1.

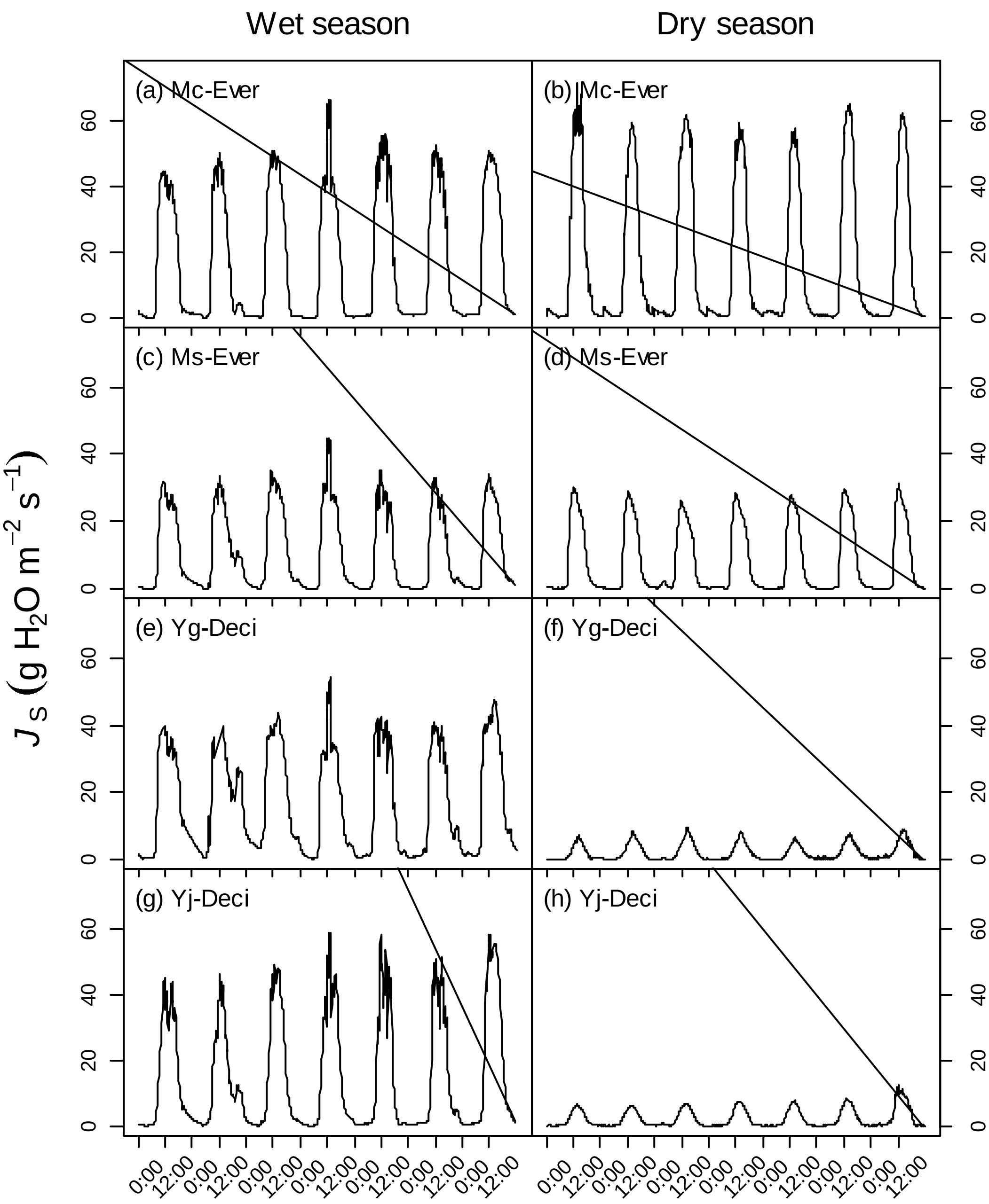

Fig. 1. Liu *et al*.

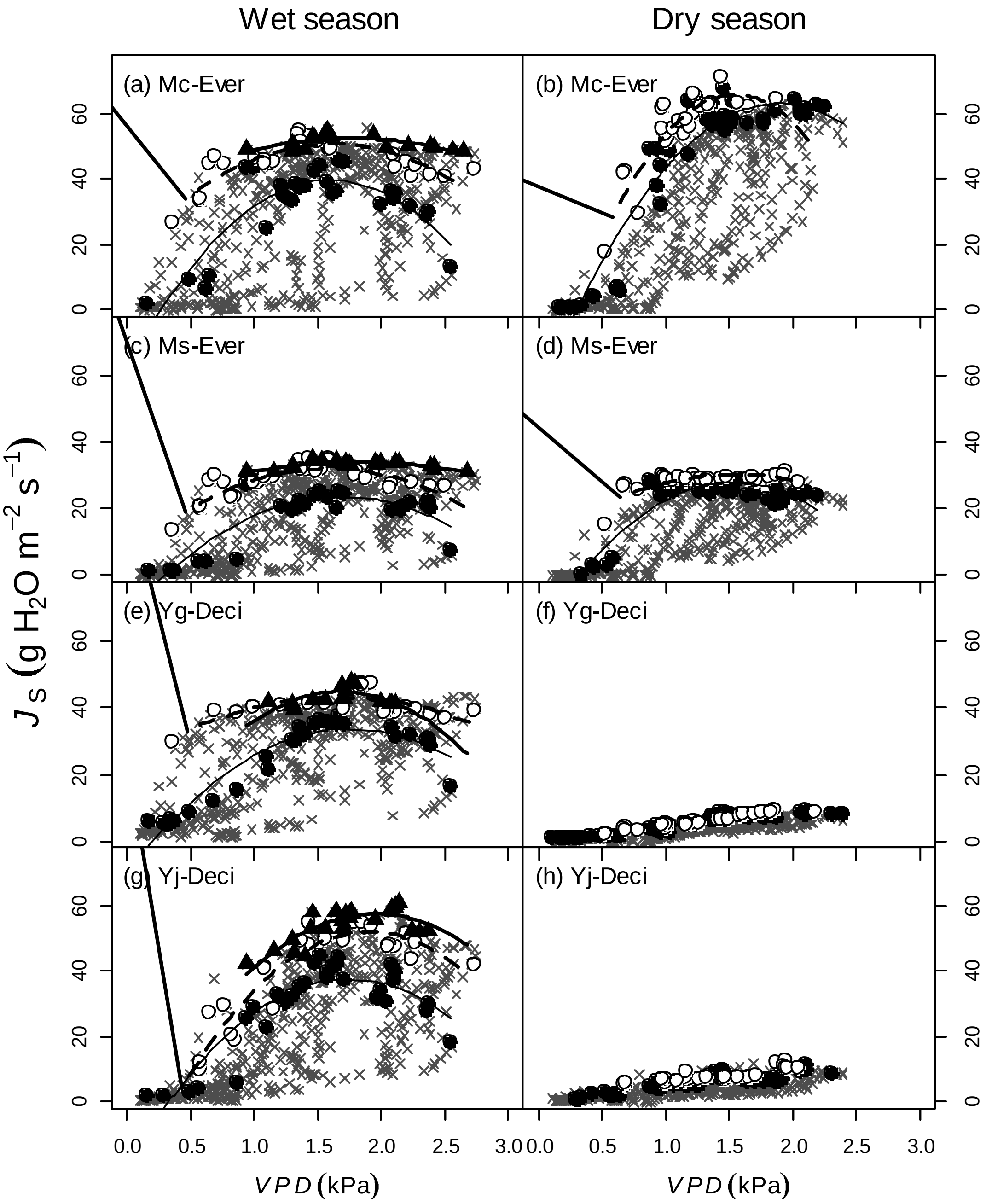

Fig. 2. Liu *et al*.