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# Differential ecophysiological responses and resilience to heat wave events in four co-occurring temperate tree species

Anirban Guha<sup>1,4</sup> , Jimei Han<sup>1,2</sup>, Cadan Cummings<sup>1,3</sup>, David A McLennan<sup>1</sup> and Jeffrey M Warren<sup>1</sup><sup>1</sup> Environmental Sciences Division and Climate Change Science Institute, Oak Ridge National Laboratory, Oak Ridge, TN, United States of America<sup>2</sup> Shihezi University, Xinjiang, People's Republic of China<sup>3</sup> University of Wisconsin-Madison, WI, Madison, United States of America<sup>4</sup> Author to whom any correspondence should be addressed.E-mail: [guha2009anirban@gmail.com](mailto:guha2009anirban@gmail.com)**Keywords:** temperate trees, heat wave, thermotolerance, photosynthesis, photosystem-II, photodamage, canopy necrosisSupplementary material for this article is available [online](#)

## Abstract

Extreme summer heat waves are known to induce foliar and stem mortality in temperate forest ecosystems, yet our mechanistic knowledge of physiological thresholds for damage is lacking. Current spatiotemporal simulations of forest growth responses to climate change fail to explain the variability between co-occurring tree species to climate extremes, indicating a need for new model frameworks that include mechanistic understanding of trait-specific responses. In this context, using manipulative heat wave (*hw*) experiments we investigated ecophysiological responses and physiological recovery in four co-occurring temperate tree species of the southeastern United States including three deciduous angiosperms: southern red oak (*Quercus falcata* Michx.), shumard oak (*Q. shumardii* Buckl.) and, tulip-poplar (*Liriodendron tulipifera* L.) and one evergreen conifer: eastern white pine (*Pinus strobus* L.). The objectives were to investigate inter-specific differences in ecophysiological responses to *hw* events to understand mechanistic differences in resilience that may be useful for future model development. Two-year-old, well-irrigated potted saplings were exposed to progressively increasing extreme *hw* diurnal cycles followed by a recovery cycle, with peak midday air temperature increasing from 37 °C to a maximum of 51 °C on the third day of the *hw*. Plants were assessed for various photosynthetic and water use responses, chlorophyll fluorescence and photosystem-II (PSII) activity, leaf temperature and foliar pigments. Intense heat caused progressive down-regulation in net photosynthesis, but the stomata remained operational, which helped cool leaves through loss of latent heat. Even though whole plant transpiration increased for all species, the rate plateaued at higher *hw* events that allowed leaf temperature to exceed 45 °C, well beyond the optimal range. A significant increase in non-photochemical quenching over the *hw* cycles was evident in all species though indications of both transient and chronic PSII damage were evident in the most heat sensitive species, pine and tulip poplar. The oaks, especially *Q. falcata*, showed greater thermotolerance than other species with a higher threshold for photodamage to PSII, rapid overnight recovery of photoinhibition and minimal heat-induced canopy necrosis. We conclude that these co-occurring tree species exhibit large variability in thermotolerance and in their capability to repair both transient and chronic photodamage. Our results indicate that extreme heat induced damage to PSII within the leaf chloroplasts may be a mechanistic trait that can be used to project how different species respond to extreme weather events.

## 1. Introduction

Global climate change is projected to increase peak summertime air temperatures and extreme heat wave (*hw*) events (Coumou and Rahmstorf 2012, Coumou *et al* 2013, IPCC 2013, Perkins-Kirkpatrick *et al* 2017). Broadly, *hw* events are periods of consecutive days where air temperatures are excessively hotter than normal summer days (Coumou and Robinson 2013, Perkins and Alexander 2013, Reichstein *et al* 2013). Periodic *hw* events are known to induce foliar and stem mortality in temperate forest ecosystems (Teskey *et al* 2015), yet we lack species-specific mechanistic knowledge of physiological thresholds for damage. An acute *hw* event can push different species beyond their critical response thresholds, which is a vulnerability of global concern. Current land surface models perform poorly in simulating ecological impacts of extreme heat events on forest ecosystems, partly due to the paucity of data on tree species responses (Gu *et al* 2015).

Extreme summer heat events in which air temperatures suddenly exceeds 35 °C–38 °C are expected to increase in duration and intensity throughout the United States, including the southeastern region (IPCC 2013, Diem *et al* 2017). In this region, mean maximum daily temperatures in summer often exceed 32 °C and have reached 44 °C during periodic extreme heat events (Luo and Zhang 2012). Such high temperature is well above the thermal optima of photosynthesis which generally peaks by ~30 °C (Blankenship 2014). Southeastern US forest ecosystems include scores of co-occurring temperate tree species that display a wide variety of phenological, biochemical and anatomical traits adapted to the mild-cool winters and hot humid summers, yet mechanistic understanding of species-specific responses to summer heat wave events is not well-characterized. To date, most field-based studies are restricted to post-*hw* productivity analysis due to the unpredicted nature of the stress. Only a few manipulative studies have provided some insight into tree responses before, during and after *hw* events (Teskey *et al* 2015). Responses of trees and forest ecosystems to environmental conditions are often non-linear, dependent on antecedent conditions (e.g. previous exposure to drought or heat) and different confounding variables like soil nutrient status, irradiation, etc (Sass-Klaassen *et al* 2016). Dynamic vegetation models such as the land surface component of earth system models (ESMs) (Wullschlegel *et al* 2014) provide predictive trajectories of future vegetation response to various climate change scenarios, including slowly warming temperatures. Many of these models include mechanistic, process-level photosynthetic biochemistry such as the maximum rates of carboxylation or electron transport, which depend on, and respond to changes in resources or environmental conditions as dependent on generalized plant functional types (PFTs) that group many species. Even so, while the models can respond to progressive drought, or progressive increases in heat,

there are currently no damage thresholds in response to acute heat wave events, which may be species-specific. This supports the need to move away from generic PFTs towards trait-based modeling (Verheijen *et al* 2013), where additional mechanistic thresholds might be introduced. Such a modification of existing ESMs would allow species- and trait-specific response thresholds for mechanistic damage to key processes during short-term climatic extremes like heat waves, chilling events etc (Sass-Klaassen *et al* 2016).

Forests in the southeastern United States are a diverse mosaic of temperate hardwood trees and conifers including different deciduous angiosperms and evergreen pines. In general, pines have shown tolerance to drought, but most species are sensitive to heat (Hu *et al* 2017, Arain 2017). A study on *Pinus taeda* seedlings exposed to repeated moderate or extreme heat stress reported significant growth reductions caused by the +12 °C, but not by the +6 °C heat stress treatment, which indicates a possible physiological threshold (Bauweraerts *et al* 2014a). Slightly elevated growth temperature (+2 °C) did not confer any thermotolerance to *P. taeda* seedlings exposed to a 47 °C event and resulted in net carbon loss (Bauweraerts *et al* 2014b). Warm summer and autumn temperatures reduced photosynthesis and inhibited effective quantum yield in *P. banksiana* due to altered photosystem-II/photosystem-I stoichiometry and decreased level of Rubisco protein (Busch *et al* 2007, 2008). In *P. radiata*, proline, soluble sugars, indole-3-acetic acid and cytokinins were found to be crucial to recover photosynthetic activity after heat stress (Escandón *et al* 2016). Unlike other native pines, response of *P. strobus* to climate extremes has been less documented. Forests dominated by *P. strobus* may be more sensitive to heat stress than drought, as illustrated by a large decrease in annual net ecosystem productivity during years experiencing heat waves (Arain 2017), however the underlying physiological responses and insights to recovery mechanisms following heat waves are missing.

Another dominant temperate forest trees are the oaks (*Quercus spp.*) (Wendel and Smith 1990, Box 2015), however, their responses to elevated summer temperatures and summer heat events are even less documented than pines. A growth chamber based study on *Q. rubra* demonstrated that elevated growth temperature caused reduction in seedling growth and this negative effect was linear and substantial, suggesting that any increase in air temperature will be detrimental to *Q. rubra* fecundity (Wertin *et al* 2011). Another study showed up to 39% biomass loss in *Q. rubra* seedlings when exposed to +12 °C heat stress treatment, however, the decrease was mitigated by elevated CO<sub>2</sub> (Bauweraerts *et al* 2014a). Few thermotolerance studies have shown that different Mediterranean and European oaks can display large plasticity of photosynthetic acclimation to increasing temperatures (Daas *et al* 2008) and some oaks like the winter-deciduous

*Q. pubescens* can be resilient to high temperature stress (Haldimann *et al* 2008).

In this study, we selected four contrasting temperate tree species of the southeastern United States including three deciduous angiosperms: southern red oak (*Q. falcata* Michx.), shumard oak (*Q. shumardii* Buckl.), tulip poplar (*Liriodendron tulipifera* L.) and one evergreen conifer: eastern white pine (*P. strobus* L.) (hereafter referred as QUFA, QUSH, LITU and PIST, respectively). These are prominent native species co-existing in the oak-hickory and oak-pine hardwood forests of the southeastern United States (Tankersley 1998). Regional distribution of these species can vary across microsites, with QUFA tending toward dry upland sites, QUSH tolerating a range of sites, from moist bottomlands to dry ridges, LITUs growing best in fertile moist soil, and PIST distributed along the full moisture gradient from wet bogs and moist stream bottoms to xeric sand plains and rocky ridges (Box 2015). Our study investigated the effects of extreme *hw* events on key ecophysiological processes in these four species, including leaf gas exchange, photosystem-II (PSII) activity and quenching mechanisms, whole plant transpiration, and leaf temperature. Our primary objectives were to determine: (1) the magnitude of interspecific differences in instantaneous ecophysiological processes under transient *hw* exposures and (2) differences in the short-term ecophysiological resilience following the *hw* events.

## 2. Materials and methods

### 2.1. Plant growth, maintenance and treatments

One-year old bare-root tree saplings of QUFA, QUSH, LITU and PIST were planted into 6.2 L plastic pots using standard potting soil (Sun Gro, Canada) in late spring. Ten months later, healthy uniform plants were carefully transplanted into 35 L pots and grown for 30 days before the *hw* experiments, which occurred in midsummer 2017 (July–August). The larger pots were used to eliminate potential pot artefacts, such as being root-bound or having nutrient or water limitations. The duration and intensity of *hw* treatments were designed based on current prediction of the southern United States *hw* trends as a function of increasing global temperature (Anderson and Bell 2011, Perkins-Kirkpatrick *et al* 2017). Initial pilot trials were undertaken to ensure that the *hw* cycles induced some physiological consequences without causing whole plant mortality. To achieve the *hw* treatments, well-watered saplings ( $n=5$ ) were transferred by block (batch) into a walk-in extreme growth chamber (Model BDW80, Conviron, Canada) where they were exposed to progressively increasing temperature cycles over a period of 6 d:  $T_{\max}$  37/24 °C (day-time highest maximum/night-time lowest maximum) (pre-*hw* cycle: 3 days), then  $T_{\max}$  47/24 °C (*hw*-d1 cycle: 1 day), then  $T_{\max}$  49/26 °C (*hw*-d2 cycle: 1 day), then

$T_{\max}$  51/28 °C (*hw*-d3 cycle: 1 day), and then back to  $T_{\max}$  37/24 °C (post-*hw*: 1 day) (figure 1). Exposure time to the peak temperature on each day was three hours. See appendix 1.1 (supplementary information available at [stacks.iop.org/ERL/13/065008/mmedia](http://stacks.iop.org/ERL/13/065008/mmedia)) for details on growth conditions and meteorological measurements.

### 2.2. Whole plant transpiration, relative water content and leaf mass per area

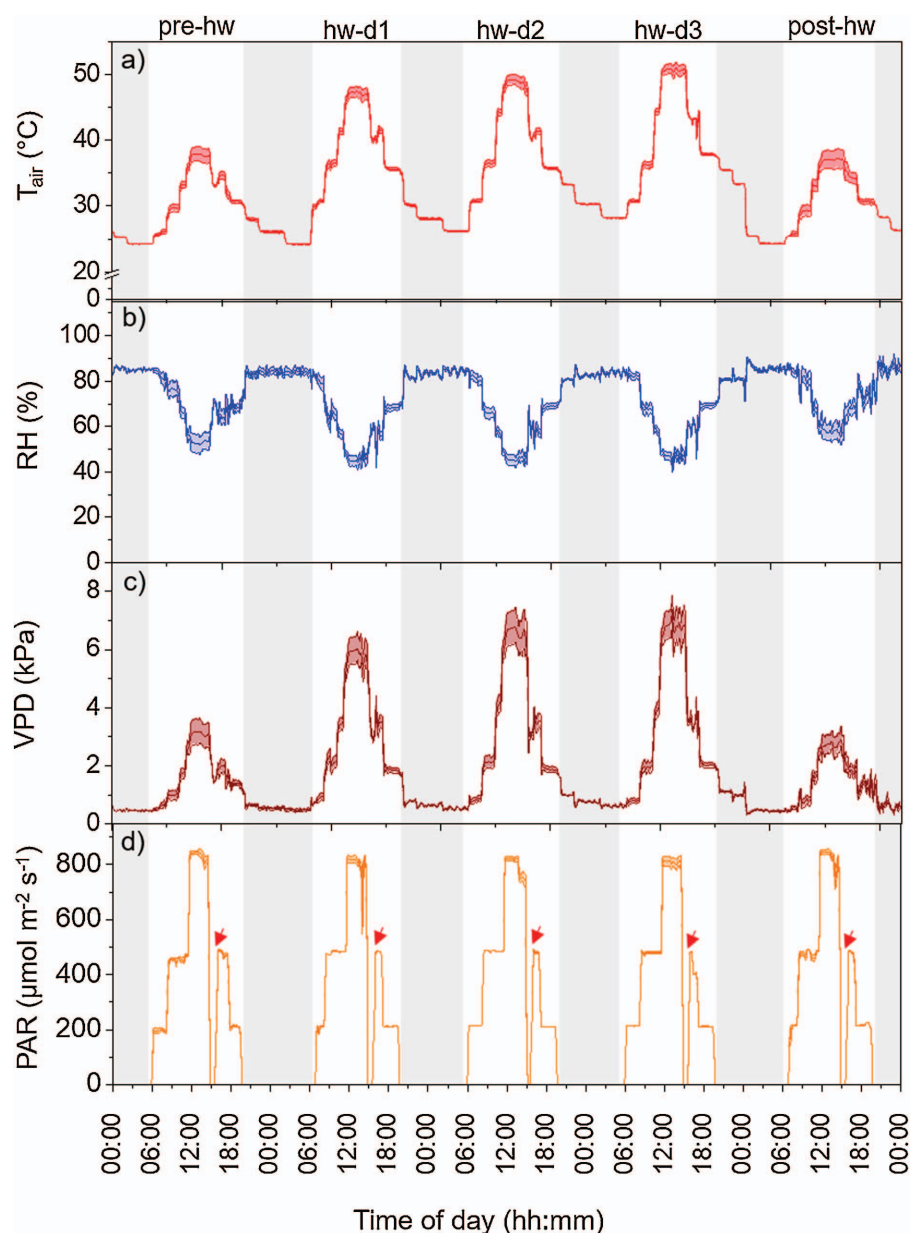
Absolute whole plant transpiration ( $WPT_A$ ) per plant was calculated based on daytime changes in pot weight, and to account for the differences in plant size and foliage area  $WPT_A$  was normalized ( $WPT_N$ ) based on pre-*hw* transpiration rate. Leaf tissue was collected on pre- and post- *hw* cycles for assessment of relative water content (RWC) and leaf mass per area (LMA) ( $n=3-5$ ). See appendix 1.2. for detailed methodology.

### 2.3. Gas exchange, light-adapted *chl a* fluorescence and foliar pigments

Light-saturated foliar gas exchange and light-adapted steady-state *chl a* fluorescence (*ChlF*) were measured in tandem with a portable photosynthesis system (LI-6800, LI-COR Biosciences, Lincoln, NE, USA) equipped with leaf fluorometer (LI-6800). Measurements included net photosynthesis ( $P_n$ ), stomatal conductance ( $G_s$ ) and transpiration ( $T$ ) on the first day of pre-*hw* cycle and then on each *hw* cycle between 14:00 and 14:30 h, after 2 h of *hw* exposure. Measured *ChlF* parameters by LI-COR fluorometer (annotated by subscript *LCR*) included maximum efficiency of PSII in the light ( $F_V'/F_M'_{LCR}$ ) and PSII operating efficiency in the light ( $\phi PSII_{LCR}$ ) (Baker 2008, Maxwell and Johnson 2000, Murchie and Lawson 2013). Electron transport rate was determined (Genty *et al* 1989) as  $ETR_{LCR} = \phi PSII_{LCR} \times PAR \times 0.5 \times 0.85$  where, *PAR* is the intensity of actinic light, 0.5 is a factor assuming equal excitation of PSII and PSI and 0.85 is the leaf absorbance. Chlorophyll (*Chl*) *a*, *Chl b*, total *Chl* ( $T_{Chl}$ ) and total carotenoid ( $T_{car}$ ) concentrations were measured as Tait and Hik 2003 and calculated according to Wellburn 1994. For detailed methodology see appendix 1.3.

### 2.4. Diurnal measurements of leaf temperature and continuous *ChlF*

Abaxial leaf surface temperature ( $T_L$ ) was measured using fine wire thermocouples (0.762 mm diameter; Omega Engineering, Stamford, CT, USA) connected to a data logger (CR1000, Campbell Scientific, USA). Continuous measurements of *ChlF* were conducted using a Monitoring PAM (MONI-PAM) system (Heinz Walz, GmbH, Germany) consisting of independent measuring heads. MONI-PAM system uses blue LED light to measure pulse-amplitude modulated *ChlF* emitted from leaf sample at wave lengths longer than 625 nm, as well as PPFD in the range from



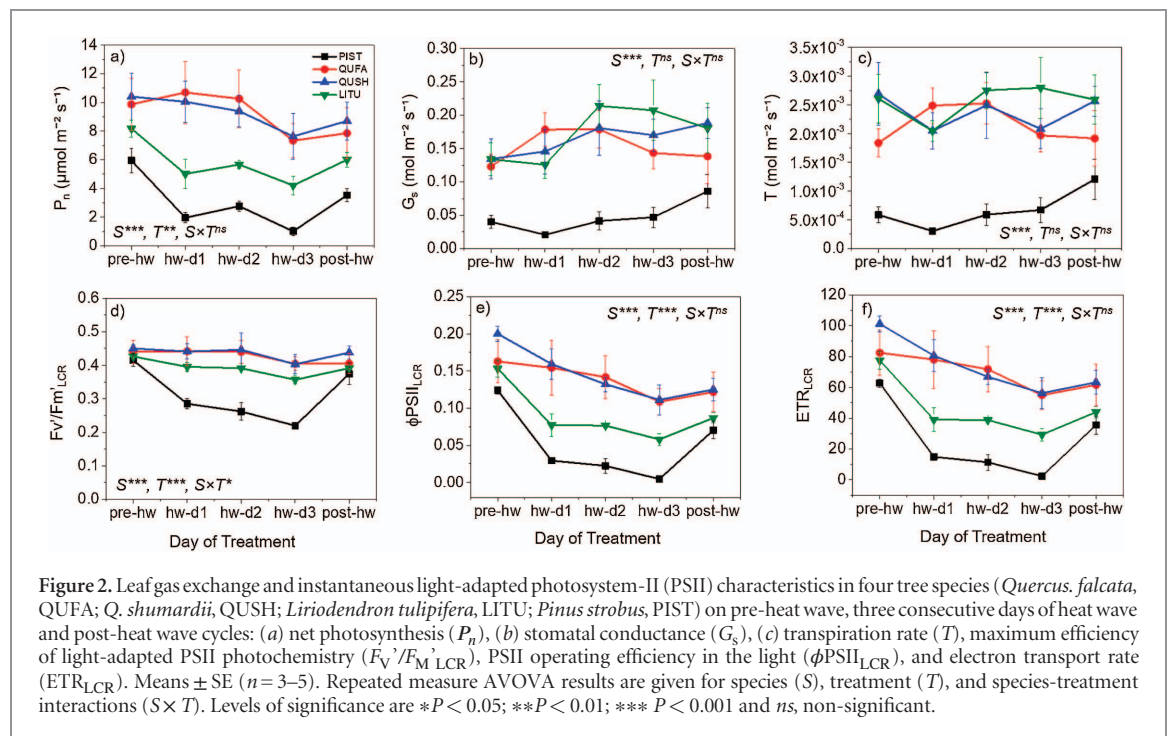
**Figure 1.** Growth chamber environmental conditions during the experiment recorded over pre-heat wave (pre-hw), three consecutive days of heat wave (hw-d1, hw-d2 and hw-d3) and post-heat wave (post-hw) cycles: (a) air temperature ( $T_{\text{air}}$ ), (b) relative humidity (RH), (c) vapour pressure deficit (VPD) and (d) photosynthetically active radiation (PAR). The grey areas depict times when all lights inside the growth chamber were switched off (ambient PAR = 0) to mimic night time. The lights inside the growth chamber were also switched off briefly for 40 min, every day between 15:00 and 15:40 h (red arrows in panel D) to measure post-midday dark-adapted chlorophyll *a* fluorescence after daily transient rise in  $T_{\text{air}}$ . Treatment means are given with SE, represented by the shaded areas.

400 to 700 nm (Porcar-Castell *et al* 2008). For logistic reasons, measurements were confined to three species, excluding QUSH. Recorded fluorescence signals by MONI-PAM (annotated by subscript MP) included steady-state fluorescence ( $F_{\text{tMP}}$  or  $F_{\text{oMP}}$  during the night) and maximal fluorescence ( $F'_{\text{mMP}}$  or  $F_{\text{mMP}}$  during the night) along with incident PPFD on leaf and air temperature (Porcar-Castell 2011). Operating quantum yield of PSII photochemistry was estimated (Genty *et al* 1989) as  $\phi\text{PSII}_{\text{MP}} = (F'_{\text{mMP}} - F_{\text{tMP}}) / F'_{\text{mMP}}$ . Day-time, electron transport rates ( $\text{ETR}_{\text{MP}}$ ) were estimated as Genty *et al* (1989). Total non-photochemical ( $\text{NPQ}_{\text{MP}}$ ) and photochemical quenching ( $\text{PQ}_{\text{MP}}$ ) were calculated as Porcar-Castell (2011).

See appendix 1.4. for detailed methodology and references cited therein.

## 2.5. Post-midday dark-adapted fast OJIP kinetics

Fast *ChlF* induction kinetics (measured for 1 s), typically known as OJIP was measured by LI-6800 fluorometer (by continuous red LEDs) between 15:40 to 16:00 h after dark-adapting leaf for a short period of 40 min ( $n = 3-5$ ). An induction flash intensity of  $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$  was used to yield minimal ( $F_{\text{O-DC}}$ ) and maximal fluorescence ( $F_{\text{M-DC}}$ ) from which the maximum quantum yield of PSII was derived (Genty *et al* 1989) as  $F_{\text{VLCR}} / F_{\text{MLCR}} = (F_{\text{M-DC}} - F_{\text{O-DC}}) / F_{\text{M-DC}}$ . Subsequently, detailed analysis of the OJIP



**Figure 2.** Leaf gas exchange and instantaneous light-adapted photosystem-II (PSII) characteristics in four tree species (*Quercus falcata*, QUFA; *Q. shumardii*, QUSH; *Liriodendron tulipifera*, LITU; *Pinus strobus*, PIST) on pre-heat wave, three consecutive days of heat wave and post-heat wave cycles: (a) net photosynthesis ( $P_n$ ), (b) stomatal conductance ( $G_s$ ), (c) transpiration rate ( $T$ ), maximum efficiency of light-adapted PSII photochemistry ( $F_v'/F_m'_{LCR}$ ), PSII operating efficiency in the light ( $\phi_{PSII_{LCR}}$ ), and electron transport rate ( $ETR_{LCR}$ ). Means  $\pm$  SE ( $n=3-5$ ). Repeated measure AVOVA results are given for species ( $S$ ), treatment ( $T$ ), and species-treatment interactions ( $S \times T$ ). Levels of significance are \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  and ns, non-significant.

kinetics, different normalizations, calculation of kinetic differences or ratios as well as time-derivatives were undertaken (Strasser *et al* 2004, Ronde *et al* 2004, Tóth *et al* 2005, Oukarroum *et al* 2007, Redillas *et al* 2011, Guha *et al* 2013, Avenson 2017). See appendix 1.5. for detailed methodology.

## 2.6. Statistical analyses

Two-way analysis of variance (ANOVA) either repeated-measure or factorial were used to investigate species ( $S$ ), treatment ( $T$ ) and  $S \times T$  effects depending on various plant responses that were studied. Tukey's HSD post-hoc test was used to identify significantly different means at a significance level of 0.05. Linear and non-linear regressions were used to plot relationships between variables using independent fit mode. All statistical analyses were performed using STATISTICA ver. 10.0 (Stat Soft. Inc., USA).

## 3. Results

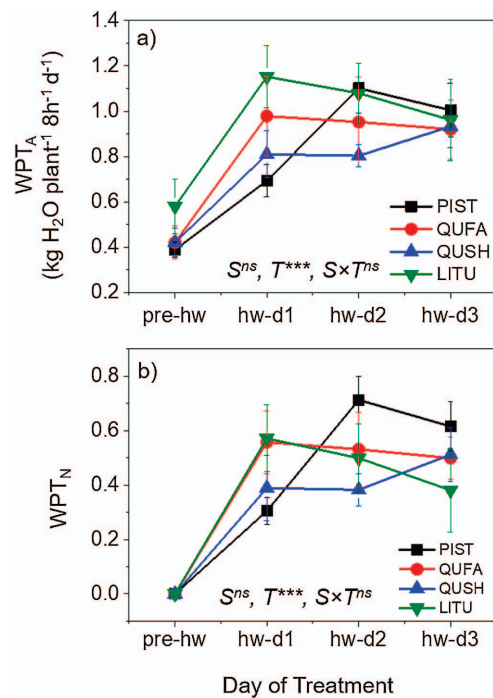
### 3.1. Diurnal change in plant growth conditions

Environmental conditions set inside the growth chamber resulted in diurnal variations and simulated realistic summer temperature patterns and  $hw$  events (figure 1(a)). Daily maximum air temperature ( $T_{air}$ ) recorded between 12:00 and 15:00 h were  $\sim 10$  ( $T_{max}$  47.4 °C), 12 ( $T_{max}$  49.1 °C) and 14 °C ( $T_{max}$  50.8 °C) higher on  $hw$ -d1, d2 and d3, respectively than pre- ( $T_{max}$  37.5 °C) and post- $hw$  ( $T_{max}$  37.3 °C) cycles. The large insulated pots successfully buffered the effects of extreme  $hw$  treatments on soil temperatures ( $T_{soil}$ ), keeping them within  $\sim 2$  °C of pre- $hw$  diurnal patterns, and limiting absolute  $T_{soil} < 33$  °C during  $hw$  events even as diurnal

$T_{air}$  rose  $20 \pm$  °C and approached 51 °C (figure S1). Relative humidity (RH) dropped significantly as  $T_{air}$  increased (figure 1(b)), resulting in maximum vapor pressure deficit (VPD) of 6.1, 6.8 and 7.3 kPa during  $hw$ -d1, d2 and d3, respectively (figure 1(c)). Photosynthetically active radiation (PAR) varied diurnally and showed a peak of  $850 \mu\text{mol m}^{-2} \text{s}^{-1}$  by noon (figure 1(d)).

### 3.2. Effects on leaf gas exchange and whole-plant transpiration

Net photosynthesis ( $P_n$ ) showed significant species effects, with the two oaks (QUFA, QUSH) having the greatest rates, and the pine the lowest (figure 2(a)). Over the  $hw$  cycles,  $P_n$  responded strongly to the treatments with strong initial decline in LITU (38%) and PIST (68%), and a delayed and muted decline in QUSH (22.9%), QUFA (28%) occurring on  $hw$ -d3. Post- $hw$  recovery of  $P_n$  was evident in all species. Both  $G_s$  and  $T$  were lowest in PIST in concurrence to its lowest  $P_n$  (figures 2(b) and (c)), and none were significantly affected by  $hw$  treatments. Both absolute ( $WPT_A$ ) and normalised ( $WPT_N$ ) whole-plant transpiration showed significant increase with  $hw$  treatments (figures 3 (a) and (b)). On  $hw$ -d1, whole plant water use increased significantly on an average by  $\sim 78.6$ , 90.4, 98.2 and 130.9%, in PIST, QUSH, LITU and QUFA, respectively relative to their pre- $hw$  control cycle. Although no significant  $S \times T$  effect was evident, some differential trends in whole plant use were evident, with increased water use by PIST on  $hw$ -d2 and then by QUSH on  $hw$ -d3. Interestingly, leaf RWC had a divergent species response to  $hw$  treatments; RWC increased in oaks, remained stable for the LITU, and declined for the PIST (table S1).



**Figure 3.** Absolute whole plant transpiration ( $WPT_A$ ) and normalized ( $WPT_N$ ) rates in four tree species measured on pre-heat wave and three consecutive days of heat wave between 09:00 h and 17:00 h. Means  $\pm$  SE ( $n=5$ ). The repeated measure AVOVA results are given for species ( $S$ ), treatment ( $T$ ), and species-treatment interactions ( $S \times T$ ). Levels of significance are \*\*\*  $P < 0.001$  and  $ns$ , non-significant.

### 3.3. Effects on light-adapted photosystem-II activity

Over the course of experiment,  $F_V'/F_M'_{LCR}$  decreased progressively from pre-*hw* to *hw*-d3, though marginally in LITU, QUSH and QUFA, and strongly in PIST ( $\sim 47\%$ ) compared to pre-*hw* control cycle. All species showed significant post-*hw*  $F_V'/F_M'_{LCR}$  recovery (figure 2(d)). As a response to *hw* treatments, both  $ETR_{LCR}$  and  $\phi PSII_{LCR}$  showed similar patterns of progressive decline over the course of *hw* cycles followed by partial recovery on post-*hw* cycle (figures 2(e) and (f)). Decline in  $\phi PSII_{LCR}$  and  $ETR_{LCR}$  was much steeper in PIST, followed by LITU than compared to QUFA and QUSH. Across species, the photosynthetic rates showed stronger dependencies on both  $\phi PSII_{LCR}$  and  $ETR_{LCR}$  than on  $F_V'/F_M'_{LCR}$  (except in PIST), as evident from the regression analysis (table S2).

### 3.4. Diurnal changes in leaf temperature

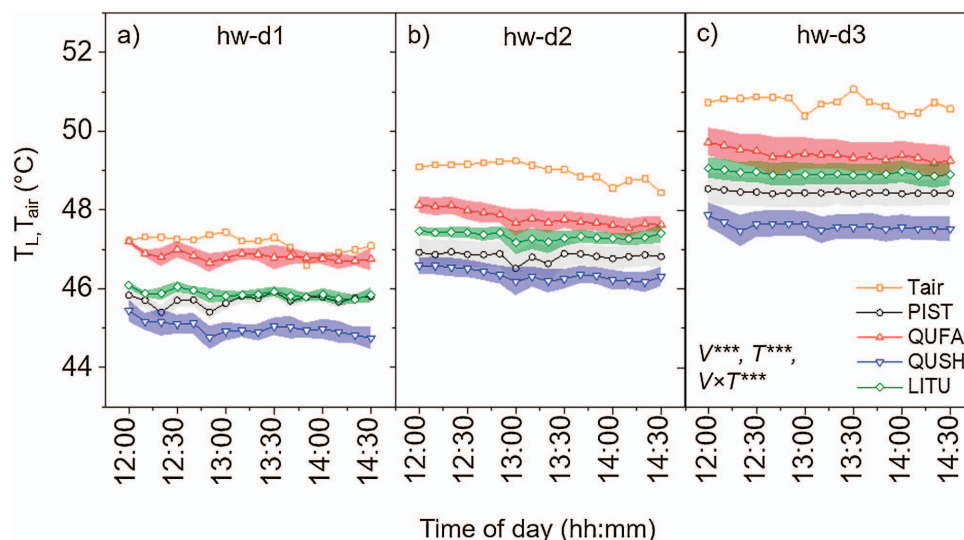
Diurnal dynamics in  $T_L$  tracked concurrent changes in  $T_{air}$  throughout the *hw* treatments, (figure S2). Despite the strong midday increase,  $T_L$  always remained considerably lower than midday  $T_{air}$  indicating continued loss of latent heat (figures 4(a)–(c)). Unlike rest of the diel cycle, there was a significant species-specific  $T_L$  response to *hw* treatments during midday where QUSH had the lowest  $T_L$  ( $\sim 44.9^\circ\text{C}$ ) followed by PIST ( $\sim 45.7^\circ\text{C}$ ), LITU ( $\sim 45.8^\circ\text{C}$ ) and QUFA ( $\sim 46.8^\circ\text{C}$ ), and this pattern was consistent during subsequent *hw* cycles 2 and 3.

### 3.5. Diurnal patterns in photosystem-II function

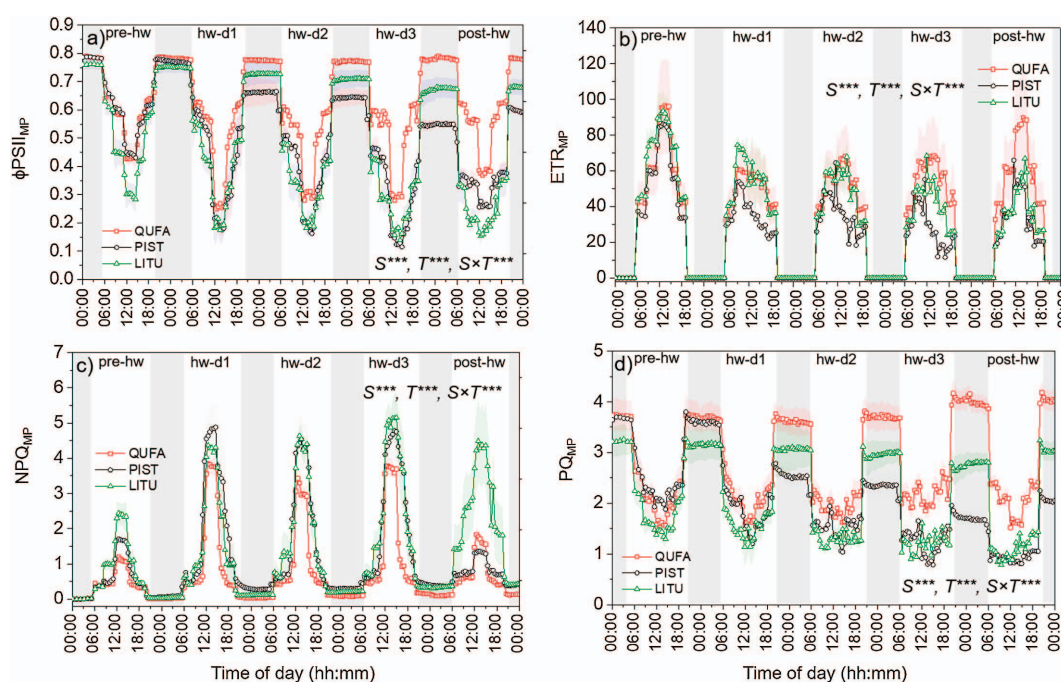
Diurnal *ChlF* included two core signals: instantaneous fluorescence,  $F_{tMP}$  (equivalent to  $F_{oMP}$  during night) and maximum fluorescence,  $F'_{mMP}$  (equivalent to  $F_{mMP}$  during night). Diurnal patterns observed in these core signals (described in appendix 2.1.) were translated into changes in  $\phi PSII_{MP}$ ,  $ETR_{MP}$ ,  $NPQ_{MP}$  and  $PQ_{MP}$  (figure 5). Highest, daytime  $\phi PSII_{MP}$  and  $PQ_{MP}$  were recorded for QUFA, intermediate values for LITU and lowest values for PIST (figures 5(a) and (d)). Predawn  $F_V'/F_{MMP}$  (equivalent to  $\phi PSII_{MP}$  before 06:00 h) showed no significant decline in QUFA, whereas significant gradual loss was recorded in PIST followed by LITU, resulting up to  $\sim 24$  and  $8\%$  loss on *hw*-d3, respectively (table 1). Progressive decline in  $ETR_{MP}$  was evident over the *hw* cycles in all species, but QUFA showed full post-*hw* recovery unlike others (figure 5(b)). The  $NPQ_{MP}$  levels showed strong mid-day rise during the *hw* cycles showing maximal values in LITU, followed by PIST and QUFA. (figure 5(c)). Dependency of  $NPQ_{MP}$  on  $T_{air}$  was significant (figure S4). Interestingly, similar quantitative rise in midday  $NPQ_{MP}$  was evident in all species during the *hw* cycles. Unlike QUFA and LITU, post-*hw* relaxation in  $NPQ_{MP}$  was not evident in LITU.

### 3.6. Midday photosystem-II maximum quantum efficiency and OJIP kinetics

No significant loss in post-midday  $F_V'/F_{MLCR}$  was evident in QUFA, whereas a  $\sim 26\%$  decrease was recorded in PIST on *hw*-d3 followed by no full post-*hw*



**Figure 4.** Variation in midday leaf temperature ( $T_L$ ) in four tree species on three consecutive days (a)–(c) of heat wave recorded between 12:00 to 14:30 h. Means are given with  $\pm$  SE ( $n = 3$ ), represented by the shaded areas (grey-PIST, pink-QUFA, blue-QUSH and green-LITU). Mean air temperature ( $T_{air}$ ) for the corresponding time of day is shown to compare the changing patterns between  $T_L$  and  $T_{air}$ . For repeated measure ANOVA, both species and  $T_{air}$  were used as dependent variable (V) and treatments (T) (hw-d1, hw-d2 and hw-d3) as categorical predictor. AVOVA results are given for variable (V), treatment (T), and variable-treatment interactions (V  $\times$  T). Levels of significance is \*\*\*  $P < 0.001$ .



**Figure 5.** Diurnal variations in (a) photochemical yield of photosystem-II ( $\phi PSII_{MP}$ ), (b) electron transport rates ( $ETR_{MP}$ ), (c) non-photochemical quenching ( $NPQ_{MP}$ ) and (d) photochemical quenching ( $PQ_{MP}$ ) measured in three tree species over pre-heat wave (pre-hw), three consecutive days of heat wave (hw-d1, hw-d2 and hw-d3) and post-heat wave (post-hw) cycles. Means are given with  $\pm$  SE ( $n = 3$ ), represented by the shaded areas. The grey areas depict times when all lights inside the growth chamber were switched off to mimic night time (ambient PAR = 0).

recovery (table 2). Progressive, though moderate loss in  $F_V/F_{MLCR}$  was evident in QUSH and LITU, but both showed post-hw recovery. Normalised OJIP kinetics revealed different heat induced changes in the shape (described in figure S4 legend). Kinetics normalized between  $F_O$  (0.05 ms) and  $F_k$  (0.3 ms) expressed as  $V_{OK}$  (figure S5 (a)–(d)) were used to construct  $\Delta V_{OK}$

and revealed large positive rise only in PIST over the hw cycles making the L-bands visible followed by partial post-hw restoration (figure 6(d)). However, the treatments did not induce any L-bands in other three species. Kinetics normalised between O and J (figures S5 (e)–(h)), clearly showed a prominent rise in the  $\Delta V_{OJ}$  peak (K-bands) in all species (figures 6(e)–(h))

**Table 1.** Pre-dawn maximum quantum yield of PSII ( $F_V/F_{MMP}$ ) during pre- and post-heat wave (*hw*) events, and during each heat wave cycle (*hw*-d1, *hw*-d2 and *hw*-d3) in three tree species. The values are translated from pre-dawn quantum yield of PSII photochemistry ( $\phi PSII_{MP}$ ) measured before 06:00 h after overnight dark-adaptation. Means  $\pm$  SE ( $n = 3$ ). The AVOVA results are given for species (*S*), treatment (*T*), and species-treatment interactions ( $S \times T$ ). Levels of significance are \*  $P < 0.05$  and \*\*\*  $P < 0.001$ .

|                            | QUFA            | PIST             | LITU            |
|----------------------------|-----------------|------------------|-----------------|
| pre- <i>hw</i>             | 0.78 $\pm$ 0.01 | 0.78 $\pm$ 0.001 | 0.76 $\pm$ 0.02 |
| <i>hw</i> -d1              | 0.77 $\pm$ 0.01 | 0.73 $\pm$ 0.03  | 0.74 $\pm$ 0.02 |
| <i>hw</i> -d2              | 0.77 $\pm$ 0.01 | 0.62 $\pm$ 0.05  | 0.72 $\pm$ 0.03 |
| <i>hw</i> -d3              | 0.76 $\pm$ 0.01 | 0.59 $\pm$ 0.07  | 0.70 $\pm$ 0.02 |
| post- <i>hw</i>            | 0.77 $\pm$ 0.03 | 0.52 $\pm$ 0.04  | 0.67 $\pm$ 0.04 |
| S***, T***, $S \times T^*$ |                 |                  |                 |

**Table 2.** Maximum quantum yield of PSII ( $F_V/F_{MLCR}$ ) during pre- and post-heat wave (*hw*) events, and during each heat wave cycle (*hw*-d1, *hw*-d2 and *hw*-d3) in four tree species measured post-midday, after 3.5 h of transient heat wave exposure. The measurements were made following a short dark-adaptation period of 40 min. Means  $\pm$  SE ( $n = 3-5$ ). The AVOVA results are given for species (*S*), treatment (*T*), and species-treatment interactions ( $S \times T$ ). Level of significance is \*\*\*  $P < 0.001$ .

|                                | QUFA            | QUSH            | PIST            | LITU             |
|--------------------------------|-----------------|-----------------|-----------------|------------------|
| pre- <i>hw</i>                 | 0.77 $\pm$ 0.02 | 0.79 $\pm$ 0.01 | 0.81 $\pm$ 0.01 | 0.79 $\pm$ 0.005 |
| <i>hw</i> -d1                  | 0.74 $\pm$ 0.01 | 0.75 $\pm$ 0.02 | 0.61 $\pm$ 0.01 | 0.73 $\pm$ 0.012 |
| <i>hw</i> -d2                  | 0.73 $\pm$ 0.01 | 0.71 $\pm$ 0.03 | 0.56 $\pm$ 0.05 | 0.73 $\pm$ 0.012 |
| <i>hw</i> -d3                  | 0.73 $\pm$ 0.01 | 0.69 $\pm$ 0.04 | 0.35 $\pm$ 0.03 | 0.69 $\pm$ 0.022 |
| post- <i>hw</i>                | 0.79 $\pm$ 0.01 | 0.82 $\pm$ 0.01 | 0.60 $\pm$ 0.05 | 0.80 $\pm$ 0.014 |
| S***, T***, $S \times T^{***}$ |                 |                 |                 |                  |

exhibiting distinct peaks of *K*-bands, more apparent in PIST and LITU. Unlike the oaks, PIST and LITU showed clear progressive accentuation in *K*-bands from *hw*-d1 to d3 followed by partial restoration only in LITU but not in PIST.

### 3.7. Visible damages to plant canopy

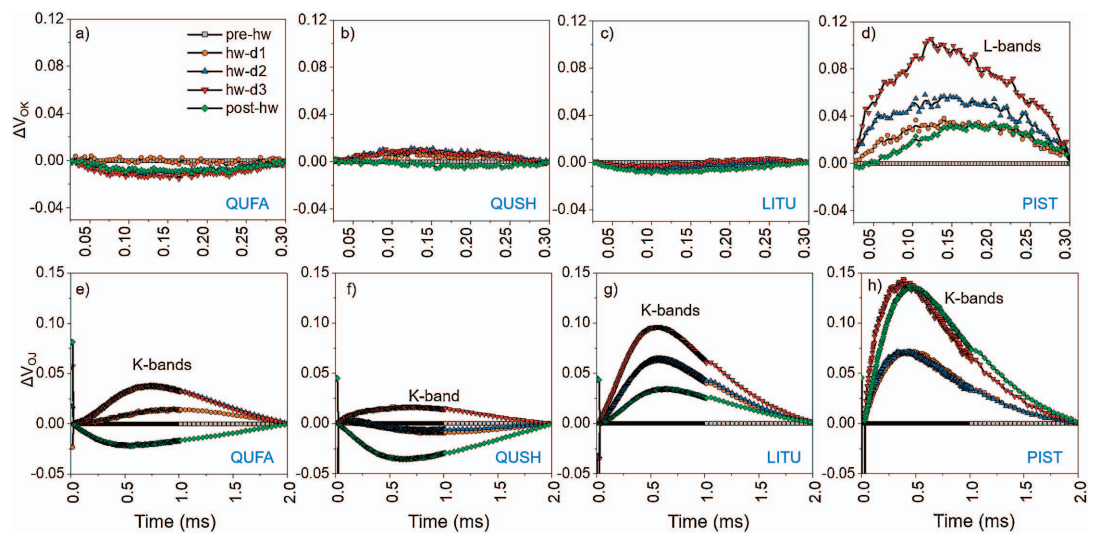
The terminal and lateral buds and terminal young needle fascicles of the lateral shoots in PIST showed significant post-*hw* necrosis and mortality (figures 7(d) and (e)), while differential damage was evident in LITU and QUSH with small young leaves exhibiting scorching yellowish-brown or black lesions, leaf curling, senescence, and abscission, but no visible damage to stem and branches was observed (figures 7(b) and (c)). In contrast, neither young nor mature foliage of QUSH showed any immediate *hw* induced damage or post-*hw* necrosis or mortality of leaves, branches or stem (figure 7(a)).

## 4. Discussion

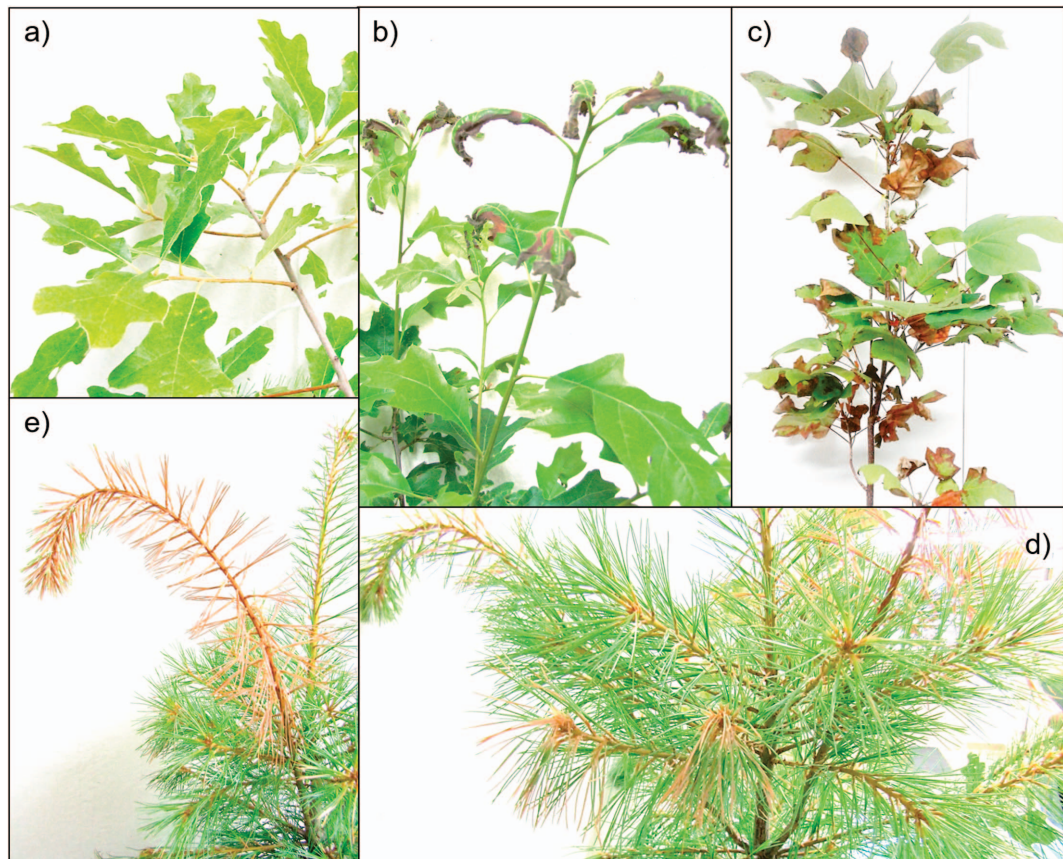
The studied species clearly differed in their resilience to the *hw* cycles with differential damage to photosynthetic capacity. Both the pine (PIST) and tulip-poplar (LITU) experienced a sharp decline in light-saturated  $P_n$ , whereas less reduction was seen for the oaks, QUFA and QUSH. Differential relative loss in photosynthetic capacity among the species was further substantiated by similar, concurrent losses in underlying PSII efficiency at light ( $\phi PSII_{LCR}$ ) and electron transport rate

of PSII ( $ETR_{LCR}$ ), which provided the first indication of potential damage to photosynthetic machinery. Some indication of LMA loss (table S1) was also evident in PIST and LITU that might be related to multiple days of reduced photosynthesis leading to loss in non-structural carbohydrates or forced recycling of metabolites. Reduced photosynthesis during heat stress can be mediated by stomatal or non-stomatal limitations. Stomatal limitation to  $CO_2$  fixation has largely focused on light,  $[CO_2]$ , water or VPD (Farquhar and Sharkey 1982, Buckley *et al* 2003), but the functional response of stomata directly to high or extreme temperature events remains unresolved (Urban *et al* 2017). Some reports indicate that  $G_s$  increased with high temperature (Lu *et al* 2000, Mott and Peak 2010, Bauweraerts *et al* 2014b, Urban *et al* 2017), others found that  $G_s$  was indifferent to increasing temperature (Teskey *et al* 1986, Ameye *et al* 2012, Cerasoli *et al* 2014, von Caemmerer and Evans 2015, Bauweraerts *et al* 2013, Bauweraerts *et al* 2014b), while other reports showed high temperature induced stomatal closure (Ghouil *et al* 2003, Weston and Bauerle 2007, Lahr *et al* 2015, Wang *et al* 2016). These conflicting results might be due to the synergistic effects of the VPD, water availability and the nature of leaf turgor regulation (iso- versus anisohydric characteristics) that largely dictate stomatal response. In our study, plants were always maintained under well-watered conditions and the daily observations of whole plant water use, and point measurements of  $G_s$  indicated no restriction in conductance, which allowed continued thermoregulation by latent heat loss and maintained  $T_L$  below  $T_{air}$  (Mahan and Upchurch 1988).

Few reports have shown that evaporative cooling of the leaf can mitigate the negative effects of supra-optimal  $T_{air}$  during heat wave events and contribute to thermotolerance (Lu *et al* 1994, Ameye *et al* 2012, Yu *et al* 2015). It is likely that differential hydraulic conductivities, stomatal responses to VPD, and trait-dependent boundary layer conductance (due to e.g. leaf shape, display, pubescence) led to the species-specific differences in maximum leaf cooling: 3.2 °C for QUSH, followed by PIST (2.3 °C), LITU (1.7 °C) and QUFA (1.7 °C). Often, summer heat events are associated with concurrent drought, which can limit water availability for evaporative leaf cooling and lead to xylem embolism. Under such conditions, differential species-specific hydraulic strategies may buffer or exacerbate leaf thermal stress and confound understanding of causal factors to stress-related reductions in net carbon uptake. In our study, high pot water availability allowed all species to enhance water uptake and maintain leaf hydration as temperature increased, which enabled the ability to focus on heat stress in the absence of desiccation. Nevertheless, constitutive hydraulic architecture and cavitation phenomena under high VPD can decouple foliar water availability from soil water availability, leading to hydraulic failure (Tyree 2003), and subsequent foliar



**Figure 6.** Change in amplitude of relative variable fluorescence in OJIP kinetics in (a)–(d) first 300  $\mu\text{s}$  between  $F_0$  and  $F_K$  ( $\Delta V_{OK} = L$ -band) influenced by the excitation energy transfer between photosystem-II units, commonly denoted as connectivity or grouping; and (e)–(h) first 2000  $\mu\text{s}$  between  $F_0$  and  $F_J$  ( $\Delta V_{OJ} = K$ -band) indicating connectivity of oxygen evolving complex with photosystem-II electron transport chain. For every heat wave (*hw*-d1, *hw*-d2 and *hw*-d3) and post-heat wave (*post-hw*) cycles, changes of  $\Delta V_{OK}$  and  $\Delta V_{OJ}$  are shown with respect to pre-*hw* cycle (black line with grey square) ( $n = 3$ –5).



**Figure 7.** Visual symptoms of heat wave-induced damage in the four studied temperate tree species: (a) southern red oak (*Quercus falcata* Michx.), (b) shumard oak (*Q. shumardii* Buckl.), (c) tulip-poplar (*Liriodendron tulipifera* L.) and (d)–(e) eastern white pine (*Pinus strobus* L.).

drought and heat stress during *hw* events. In our study, after the observed initial sharp rise in  $WPT_A$ , all species except PIST, showed plateauing or marginal decline in  $WPT_A$  indicating thresholds (either inherent or VPD driven) beyond which a further increase in water uptake was not achievable despite enough soil water availability.

It was interesting to notice that despite the highest  $T_L$ , QUFA did not undergo a stronger decline in photosynthetic efficiency ( $F_v/F_m'_{LCR}$ ,  $\phi PSII_{LCR}$  and  $ETR_{LCR}$ ) compared to other species, whereas, LITU and PIST demonstrated increased photoinhibition despite relatively lower  $T_L$ . This clearly indicates different temperature thresholds to photoinhibition among the four co-occurring species. The reversible effects of moderately high leaf temperature on photosynthetic functions include increased photo- and dark-respiration, decreased activity of Rubisco activase, decrease of  $CO_2$  solubility, and inhibition of electron transport capacity (von Caemmerer and Quick 2000, Salvucci and Crafts-Brandner 2004, Haldimann and Feller 2005). Optimal  $T_L$  for these temperate tree species is generally in the range of 20 °C–35 °C, moderate heat stress may be realized at 35 °C–40 °C (Sharkey 2005, Hozain *et al* 2010), and severe heat stress (for this study, 47 °C–51 °C) potentially exceeded thresholds of reversible photoinhibition (Yordanov 1992, Ducruet *et al* 2007, Daas *et al* 2008). Extreme heat stress can impose long-standing inactivation damage to PSII (also called photoinhibition) (Zhang *et al* 2009). Therefore, we focused on identifying potential photoinhibition by examining the dynamics of photochemical and non-photochemical activities of PSII. We particularly, monitored PSII as this complex has been considered the primary site of heat damage to photosynthesis (Enami *et al* 1994, Yamane *et al* 1998, Tóth *et al* 2005).

Real-time monitoring of PSII activity provided insights into diurnal acclimation of PSII operation (see appendix 3.1. describing normal diurnal PSII activity and references cited therein). In our experiment, under progressively higher midday *hw* temperatures,  $NPQ_{MP}$  progressively increased in all species. Though species level differences were observed in absolute  $NPQ_{MP}$  rise, the quantitative increase with respect to pre-*hw* cycle was quite similar for all species indicating a similar non-photochemical quenching escalation to stronger electron sinks in response to *hw* treatments. The progressive decline in midday rates of  $\phi PSII_{MP}$  over the *hw* cycles as observed in all three species corresponded to increasing magnitude in  $NPQ_{MP}$  as more energy was dissipated as heat rather utilized in photochemistry (Haldimann *et al* 2008). Excess un-utilizable energy leads to a decrease in thylakoid lumen pH and causes induction of non-photochemical quenching through protonation of the light harvesting complex (LHC) proteins associated with PSII and activation of xanthophyll cycle (Maxwell and Johnson 2000, Zarter *et al* 2006). Overnight recovery of

$\phi PSII_{MP}$  and relaxation of  $NPQ_{MP}$  were not similar for all species. It has been reported that photoinhibition of PSII can be quickly repaired at low light intensity unless photosystem-I (PSI) activity is severely damaged (Zhang and Scheller 2004). It was shown that cyclic electron flow can help ATP synthesis under low light which in turn help in fast repair of PSII photoinhibition (Allakhverdiev *et al* 2005, Huang *et al* 2010). In QUFA, the rates of  $\phi PSII_{MP}$  recovered overnight to pre-*hw* level after each *hw* cycle (unchanged predawn  $F_v/F_{MMP}$ ) indicating lack of chronic damage to PSII complex. We speculate that post-midday low light inside the growth chamber might stimulated cyclic electron flow to generate necessary ATP and helped repairing PSII photoinhibition as reported by Allakhverdiev *et al* (2005). In PIST and LITU, reduced levels of predawn  $\phi PSII_{MP}$  which further corresponded to lower  $PQ_{MP}$  observed over the *hw* cycles can be primarily attributed to partial but not a full relaxation of  $NPQ_{MP}$  and possibly also due to overnight retained reduced state of PSII caused by chronic PSII damage. Post-midday, when  $T_{air}$  relatively declined along with PAR intensity, the observed recovery in  $\phi PSII_{MP}$  was more apparent in QUFA than in PIST and LITU indicating the capacity of QUFA to resume linear electron transfer and concurrent shutting down of heat dissipation mechanisms ( $NPQ_{MP}$  dropped) following the heat waves. However, in LITU and PIST, the post-midday rise in  $\phi PSII_{MP}$  and subsequent drop in  $NPQ_{MP}$  were quite lingering which substantiated the lack of significant post-midday recovery in  $PQ_{MP}$  indicating failure to resume electron flow, possibly due to existing transient damage to PSII.

The long-standing loss in PSII activity as observed in PIST and LITU could be related to direct photosystem-level damages which are known to affect the redox state of thylakoid complex, thylakoid membrane structure, protein homeostasis and quenching reactions (Yamane *et al* 1998, Takahashi and Murata 2008, Boksaczanin and Fragkostefanakis 2013). The OJIP kinetics were used to identify different damages in PSII assembly and recovery from damage. A gradual decline in  $F_v/F_{MLCR}$  from *hw*-d1 to d3 indicate aggravated photoinhibition in PIST and LITU as compared to QUFA and QUSH. The shape of OJIP kinetics between 50–300  $\mu s$  (*L*-band) is influenced by the excitation energy transfer between PSII units, denoted as connectivity or grouping (Strasser and Stirbet 1998, Oukarroum *et al* 2007). Except PIST, no other species demonstrated rise in *L*-band indicating their ability to resist or recover heat-induced connectivity loss between PSII reaction centers (RC) and LHCII—a measure of tolerance against the thylakoid membrane damage (Oukarroum *et al* 2007). Occurrence of the *K*-band between 0–2000  $\mu s$  was reported in plants growing naturally in hot dry environments (Srivastava *et al* 1997). It is primarily attributed to inhibition or dissociation of the oxygen evolving complex (OEC) resulting in an imbalance between the

electron flow from OEC to PSII RC, which can be caused by heat stress (Strasser 1997, Bukhov *et al* 2001, Tóth *et al* 2005). Appearance of K-band was observed in all species as a response to *hw* treatments, but more apparently in PIST and LITU showing gradual rise in band attenuation with increasing *hw* intensity. This indicates strong OEC damage, more pronounced in PIST and LITU compared to oaks. It was interesting to witness rapid restoration of K-band in QUFA and QUSH, but the band persisted in PIST and LITU even after the *hw* ended indicating more chronic PSII damage (Oukarroum *et al* 2007, Chen *et al* 2016). Loss of connectivity between PSII RC and LHCII and damage to OEC under heat stress might have contributed to the poor post-*hw* recovery of maximum yield of photochemistry in PIST and LITU as reflected by signals like  $\phi\text{PSII}_{\text{MP}}$  and  $\text{PQ}_{\text{MP}}$ . Overall, the comparative dynamics of OJIP kinetics reveals that the four temperate species differed largely in photobiological fitness due to varying innate thermotolerance (due to e.g. varying photo-pigment levels, table S3) or efficiency in damage repair mechanisms, or both (Bokszczanin and Fragkostefanakis 2013).

While our experiments could identify and isolate heat related damage to foliar biochemistry and sapling-level responses under well-watered conditions, mesocosm-based experiments cannot replicate natural conditions, or reflect all of the heat stress responses of mature trees in a natural forest where the responses are mooted by far more complex internal and external cues. The four studied species not only exist in different microsites of the regional temperate forest, but vary widely in phenology, physiognomy and hydraulic architecture (Box 2015), which can influence the response of mature stands under realistic heat (and often co-occurring drought) event scenarios. However, our growth-chamber based study allowed mechanistic insight of physiological responses to heat stress without having the confounding influence of other factors. In summary, we found that *hw* events had large effects on photosynthetic  $\text{CO}_2$  fixation and bioenergetics of the PSII complex but not on stomatal conductance. Although leaf thermoregulation was functional during all *hw* cycles, the mechanism has limits, and could not prevent some of the major heat-related damages to PSII that have important limitations to post-stress recovery. The two studied oak species QUFA and QUSH appeared to have a greater tolerance against *hw* events, while PIST and LITU endured higher transient and chronic photoinhibitions due to their vulnerable photobiology. The observed visual damage to the plant canopy further demonstrated that the oaks, particularly QUFA had greater tolerance to extreme high temperature which not only avoided damage to photosynthetic apparatus but also prevented heat induced tissue damage, necrosis and death. In PIST and LITU, observed necrosis and mortality of young foliage and shoots might lead to large carry-over effects on future plant growth and continued performance.

Our study suggests a strong variability in thermotolerance existing among these four southeastern temperate forest species that indicates physiological and growth responses to future extreme events will not be uniform across this biome.

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## ORCID iDs

Anirban Guha  <https://orcid.org/0000-0002-6202-0045>

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