Appendix

1. Supplemental Materials and Methods

1.1. Plant growth, maintenance and treatments

Young tree saplings (1.5 y old) of QU FA, QUSH, LITU and PIST were obtained from the Tennessee Department of Agriculture’s Delano nursery and were planted in 6”x 16” vertical plastic tree pots using 5 kg potting soil mix (Sungro, Canada). The saplings were grown outside at Oak Ridge National Laboratory (35°54’N; 84°20’W) and maintained under natural sunlight, air temperature and humidity with periodic irrigation and NPK fertilization (2:1:2, Southern AG, USA) until they were two years old. Healthy uniform saplings were then transferred to 35L plastic pots with same soil mix and allowed to grow for 30 days before the experiments. Before experiments, the plants ranged from 66-113 cm tall with stem diameter ranging from 7.14-22.98 mm (at 15 cm stem height from soil surface).

Plants were evenly spaced and randomly rotated every day inside the growth chamber to minimize any potential chamber effects. To simulate a realistic heat event, the heat exposure treatments began with an increasing temperature gradient that peaked at noon and was maintained for three hours followed by a gradual decrease of chamber temperature into the night, though remained significantly higher than night-time pre-hw cycle (figure 1). Inside the growth chamber, ambient CO₂ (Cₐ) level was maintained at 400 µmol mol⁻¹, air temperature (Tₐ) and relative humidity (RH) were measured daily by type T thermocouples every 10 min, averaged and logged using a data-logger (Campbell, UT, USA). Each thermocouple was housed in a ventilated radiation shield (model SRS100; Ambient Weather, Chandler, AZ, USA) mounted on a pole 2 m above ground level. Potting soil temperature (T_soil) inside the pots was measured (at 10 and 20 cm soil depths) by soil temperature probes hooked inside pots through small drilled holes and were connected to the same data logger used for Tₐ and RH. Photosynthetically active radiation (PAR) inside chamber was measured with quantum radiation sensors (model LI-190SZ; Li-Cor Biosciences, Lincoln, NE, USA) mounted on top of the levelled radiation shields and connected to the Campbell 23X data logger. All pots were wrapped using multiple layers of reflective, double-sided insulated bubble-wrap (Reflectix, Inc., Markleville, IN, USA) to prevent any heat exposure of the rhizosphere. Saplings were watered to 80% field capacity, twice a day at 08:30 h and 17:30 h to avoid any drought effects and to buffer changes in belowground temperature during hw treatments.

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

After irrigating every day at 08:00 h, the soil surface of each pot was enclosed by a white plastic sheet (< 2 mm thickness) wrapped around the stem, tied to brim of the pots tightly with strong adhesive tape to prevent soil evaporation. After draining of extra water, each pot was weighed at 09:00 h and again re-weighed at 17:00 h with a digital scale (PS6600 ST, Befour, USA) of ± 100 g precision. Absolute whole plant transpiration (WPTₐ) per plant was calculated (for 8 hours) as the difference between the two pot weights in a day.

For relative water content (RWC), fresh leaf and needle tissues (n=3-5) were collected (adjacent foliage on the same branch used for gas exchange) on the pre-hw cycle and then on post-
Samples were collected between 08.30 to 09:00 h, weighed immediately, then rehydrated by immersing them in deionized water within small plastic screw cap bottles for 24 h at room temperature (25°C) in darkness and subsequently over-dried for 48 h at 70°C. RWC was determined as \( RWC = 100 \times \frac{(fw-dw)}{(tw-dw)} \) where, fw is the fresh weight of samples, tw is the turgid weight after re-hydrating the samples for 24 h, and dw is the oven-dried weight. While fresh, the foliage area (LA) was measured and dw of the same was used to calculate leaf mass per area (LMA) as dw/ LA.

1.3. Gas exchange, light-adapted chl \( a \) fluorescence and foliar pigments

Only current-year leaves were measured, and measurements were always performed on the same group of fully expanded upper canopy leaves (branch segment tagged for repeated measurements over time). For PIST, each measurement was taken on three fully developed fascicles (fifteen needles total) of the first growth flush. Needles were arranged in the cuvette on a flat plane to maximize light interception. Leaf cuvette conditions were set as follows: 25°C air temperature; 400 \( \mu \text{mol mol}^{-1} \) reference \( \text{CO}_2 \), 500 mL min\( ^{-1} \) flow rate, 60% relative humidity; and 1300 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) PAR (saturating). Gas exchange measurements were logged after steady-state conditions were achieved (typically in 5-7 min). Two LI-6800s were used concurrently to speed up measurements and avoid any over exposure effects on queued up plants. The broad leaves of QUFA, QUSH and LITU covered the entire leaf chamber cuvette area (6 cm\(^2\)), whereas the actual PIST needle area inside the cuvette was estimated from the length and width of each needles measured by a digital calliper, and gas exchange values were corrected accordingly. For simultaneous steady-state chl \( a \) fluorescence, the multiphase flash method (Loriaux et al 2013) was used with red actinic light intensity of 8000 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), initial peak, ramp and final peak set to 300 ms, and data output rate of 500 Hz. The fluorometer was also programmed to provide a semi-simultaneous dark pulse where the actinic light was briefly turned off and a far-red light was turned on to measure Fo’. The far-red light intensity was 25 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and dark phase length was 5 s.

For pigments, fresh leaf and needle tissues (n=7) were collected (from the same branch used for gas exchange) between 08.30 to 09:00 h on pre-and post-hw cycle. Collected tissues were immediately treated with 1ml of dimethyl sulfoxide (Fisher Chemical) inside air-tight 2 ml graduated polypropylene tubes (Fisherbrand™) and were kept in dark for a 3-day extraction period at room temperature (~25°C) (Tait and Hik 2003). Thereafter, absorbance of 200 µl extract was measured using a SpectraMax Plus 384 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 665, 649 and 480 nm wave lengths. Chlorophyll (Chl) \( a \), Chl \( b \), total chlorophyll (TChl) and total carotenoid (TCar) concentrations were calculated according to Wellburn 1994.

1.4. Diurnal measurements of leaf temperature and continuous \( \text{ChlF} \)

For diurnal, real-time monitoring of leaf temperature (\( T_L \)) and continuous measurements of \( \text{ChlF} \), a separate dedicated batch of all species (n=3) was used to facilitate un-interrupted monitoring of variables, otherwise not possible to accomplish in other batches for logistic reasons. Inside the extreme growth chamber, plants were placed in spots ensuring similar PAR, \( T_{air} \) and RH to avoid chamber effects. Values were recorded at a frequency of 5 min. In PIST, the thermocouples were routed to the dense tufts of current year first growth flush and were mounted on the abaxial surface.
of fully expanded needles. Leaf temperatures were also recorded with a handheld infrared thermometer (63 Fluke, USA) and yielded $T_L$ similar to those obtained with the fine wire thermocouples.

For monitoring continuous $ChlF$, three emitter-detector units or MONI-heads were installed on the saplings with the help of custom made aluminium supports ensuring the same foliage area was measured throughout the monitoring period. For PIST, measurements were made on fully developed fascicles of the second flush attached to the main stem. Three pairs of needles were clipped in each MONI-head with aluminium frame sample clips. Since the pine needles covered only a part of the sample holder, a black foam plate was placed behind the samples to exclude possible fluorescence from the background. The fiber-optic cables were wrapped with thermal insulation to minimize thermal effects on the signal and the MONI PAM was operated in stand-alone mode. Measurements were made every 30 min with a measuring pulse intensity of 6, frequency of 3 Hz and using a saturating pulse technique. The WinControl V3.25 was used to set the duration (0.8 s) and the intensity (~4000 μmol m$^{-2}$ s$^{-1}$) of saturating light pulse at the leaf surface (Porcar-Castell 2011). Due to limited number of MONI-heads, continuous $ChlF$ study was limited to only three species excluding QUSH. Both QUFA and QUSH belong to the oak family and showed nearly similar sensitivity to heat. Hence, we decided to eliminate one of the oak species (QUSH) to make sure that we capture the $ChlF$ responses of three major plant functional types.

1.5. Post-midday dark-adapted fast OJIP kinetics

To identify any photosystem-II (PSII) complex damage after each transient $hw$, dark-adapted OJIP induction kinetics were measured. We dark adapted the whole plant by turning off all growth chamber lights between 15:00 to 15:40 h and for conducting measurements, all plants were quickly moved to a custom-built temporary dark-room adjacent to the growth chamber without any light exposure. Two to three measurements were made per plant and each one was conducted on different fully developed upper canopy leaves covering the 6 cm$^2$ leaf chamber cuvette area of LI-6800 photosynthesis system integrated with LI-6800 leaf fluorimeter. For PIST, measurements were made on three fascicles (fifteen needles total) of the first growth flush and likewise 2-3 independent measurements were made per plant. The leaves and fascicles were carefully tagged to carry out repeated measurements across all temperature cycles.

Both continuous (DC, measured by continuous red LEDs) and modulation (AC, by modulated LED) fluorescence induction kinetics were measured using an induction flash intensity of 3000 μmol m$^{-2}$ s$^{-1}$ for one second. The DC signals were only considered in the present study to derive OJIP induction kinetics as the signals were much larger, smooth and less noisy in compared to AC signals (Avenson 2017). The fast induction kinetics was recorded from 4μs to 1s (at 250 kHz) and the time versus fluorescence intensity data for each kinetics were automatically logged in an excel file after filtering signals by an in-built fluorometer software (MPF-551025, LI6800, 1.0.0 ver.).

Original OJIP transients were double normalised between O ($F_O$, 4μs) and P ($F_M$, 500 ms-1s) phases and the variable fluorescence between OP ($V_{OP}$) was determined. Further, induction kinetics were double normalised between $F_O$ and $F_K$ (300 μs) expressed as $V_{OK}$ [$V_{OK} = (F_T-F_O)/(F_K-F_O)$] to unfold any fluorescence rise at an early step at about 300 μs. For every $hw$ cycle, the difference in transients ($ΔV_{OK}$) with respect to the pre-$hw$ cycle was calculated to unfold the characteristic $L$-band. Further, the induction kinetics were double normalized between $F_O$ and $F_J$ (2 ms) expressed as $V_{OJ}$ [$V_{OJ} = (F_T-F_O)/(F_J-F_O)$] and the difference in transients ($ΔV_{OJ}$) was
determined similarly for every $hw$ cycle with respect to pre-$hw$ cycle to unfold any fluorescence rise and assess the characteristic $K$-band.

2. Supplemental Results

2.1. Diurnal patterns in photosystem-II function

The general pattern shows three distinct stages in a single diurnal cycle: firstly, with morning illumination, $F'_{mMP}$ decreased and $F_{MP}$ increased, secondly, during the daytime, both $F'_{mMP}$ and $F_{MP}$ dropped initially ($F_{MP}$ later peaks up after midday) and thirdly, before night, $F'_{mMP}$ increased and $F_{MP}$ decreased (figure S3(A,B)). These three clearly distinguishable stages were carefully monitored over the course of the experiment and the changing patterns were compared among three studied species.

3. Supplemental Discussion

3.1. Normal diurnal PSII activity

Over a diurnal time scale, leaf-level $ChlF$ signals provide dynamic processes of energy partitioning in PSII with changes in environmental variables like light, temperature etc (Porcar-Castell et al 2008, Porcar-Castell 2011, Kolari et al 2014, Pieruschka et al 2014). Within a normal diel cycle (pre-$hw$), with morning illumination, rise in $F_{MP}$ and subsequent decline in $F'_{mMP}$ were observed in all species denoting onset of photosynthetic electron transport as reflected by $ETR_{MP}$. This process of energy utilization also concurrently starts reducing electron transport chain (ETC) and photochemical efficiency, builds up ΔpH and de-epoxidation state to generate non-photo chemical quenching as reflected by progressive decline in $\phi_{PSII_{MP}}$ and $PQ_{MP}$ tracked by parallel increase in $NPQ_{MP}$ (Porcar-Castell et al 2008). At midday, during maximum $T_{air}$ and PAR intensity, $F_{MP}$ declined and $F'_{mMP}$ declined even more indicating maximal reduction of ETC (maximum $ETR_{MP}$ achieved) and further shift towards non-photochemical quenching mediated dissipation of thermal energy which is a strong competing process with photochemical quenching as tracked by simultaneous maximal decline in $PQ_{MP}$. Post-midday, with decline in PAR intensity, $F'_{mMP}$ started peaking up and $F_{MP}$ decreased, indicating relaxation of non-photochemical energy dissipation (drop in $NPQ_{MP}$), re-oxidation of ETC and beginning of recovery of PSII maximal quantum efficiency (rise in $\phi_{PSII_{MP}}$) (Porcar-Castell et al 2008).