bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Title: Temperate tree seedlings have similar drought vulnerability despite having different hydraulic drought responses in adults

Short title: Temperate tree seedling drought response

Authors: Benjamin R. Lee*[1#¶&] & Inés Ibáñez[1¶]

[1]University of Michigan – School for Environment and Sustainability

Ann Arbor, Michigan, USA

[#]**Current Address:** Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA

[¶]Contributed to the conceptualization, formal analysis, funding acquisition, resources, review, and editing of this manuscript

[&]Responsible for data curation, investigation, methodology, project administration, software, visualization, and original draft preparation

Corresponding Author* information:

ORCID: 0000-0002-5256-0515

E-mail: benrlee@umich.edu

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Abstract

Climate change is projected result in higher frequencies of drought events across the world and lead to reduced performance in many temperate tree species. However, many studies in this area focus specifically on adult tree drought responses and overlook how trees in other age classes might differ in their vulnerability. Evidence shows that seedling drought response can differ from that of adults and furthermore that demographic performance in the seedling age class will have disproportionately strong effects on the assembly dynamics of future forests, together suggesting that understanding seedling drought responses will be critical to our ability to predict how forests will respond to climate change. In this study, we measured four indices of hydraulic response to drought (leaf water potential, photosynthetic capacity, non-structural carbohydrate concentration, and hydraulic conductivity), as well as interaction effects with shade treatments, for seedlings of two temperate tree species that differ in their adult drought response: isohydric *Acer saccharum* and anisohydric *Quercus rubra*. We found a strong isohydric response in *A. saccharum* seedlings that included conservation of leaf water potentials (> -1.8 MPa) and reductions in non-structural carbohydrate concentrations consistent with reduction of stomatal conductance. *Quercus rubra* seedlings were able to survive to more negative water potentials, but only rarely, and they showed a similar reduction in photosynthetic capacity as was found for *A. saccharum*. Our results suggest that, although *Q. rubra* seedlings display some anisohydric responses to drought, they are more isohydric than adults. Both species seem to be relatively similar in their vulnerability to drought despite the differences predicted from adult drought response, and our results suggest that seedlings of both species will be similarly vulnerable to future drought events.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Keywords: leaf water potential, photosynthetic capacity, non-structural carbohydrate concentration, glucose, hydraulic conductivity, *Acer saccharum*, *Quercus rubra*

Introduction

Climate change is projected to increase temperatures and affect global patterns of precipitation, with many areas expected to become drier and hotter [1], and these environmental changes have the potential to strongly affect forest ecosystems [2]. Many studies have thus addressed the effects that water availability has on the performance of tree species, but primarily concentrating on adults [3–5]. However, in areas where projected climate may not significantly affect adult trees, relatively few studies address the effect that drought has on saplings and seedlings (but see Maguire and Kobe 2015, Kannenberg and Phillips 2020). This represents an important knowledge gap, as past research has demonstrated that drought response can significantly differ across ontogeny [8], suggesting that tree seedlings are likely to be affected by drought differently than their adult counterparts. Relatively small changes in water availability are likely to have profound effects on survival of younger life stages and could consequentially affect forest community assembly dynamics [2,9,10]. Scientists must therefore reconcile these differences to better predict the effects of climate change on forest demography.

Drought tolerance is a broad term that encompasses many plant traits including stomatal regulation behavior (e.g., iso/anisohydry), root morphology, xylem anatomy, and leaf abscission behavior [11–15]. Iso/ansiohydry, referring to whether plants close their stomata during drought to limit water loss [11], is an overarching concept that has been used to categorize a broad range of other drought-related traits. Plants are typically sorted along a gradient ranging from isohydric species which exhibit strong stomatal control on one end and anisohydric species which show

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

63 little or no stomatal regulation on the other. This difference in behavior has physiological
64 importance because prolonged stomatal closure (i.e., isohydry) can result in the over-depletion of
65 labile carbon reserves caused by reductions in photosynthetic capacity, i.e., carbon starvation
66 [16,17]. In contrast, anisohydric behavior can compromise the water column and result in
67 catastrophic embolism and reduce xylem conductivity, i.e., hydraulic failure [11,18,19].
68 Anisohydric species are typically considered to be more tolerant of drought than isohydric
69 species [11], but most species demonstrate some level of vulnerability to both carbon starvation
70 and hydraulic failure due to their overlapping effects [12,20]. Moreover, a recent study found
71 that plant vulnerability to drought is poorly predicted when using a singular plant hydraulic trait
72 [21], and it has been further suggested that plant hydraulics should be studied and classified
73 independently of the iso/anisohydry framework [22]. It is thus important that ecologists measure
74 multiple drought responses to understand and then predict the consequences of drought.

75 There are several indicators of hydraulic strategy that can be used to partially explain a
76 plant's response to drought stress; these include regulation of internal water potentials [23],
77 regulation of photosynthetic capacity [24], depletion of non-structural carbohydrates [16,25], and
78 loss of xylem conductivity [26]. Predawn leaf water potential (Ψ_{PD}) is a measure of plant
79 hydraulic stress that is more representative of soil moisture conditions compared to midday leaf
80 water potential, which is more strongly affected by hydraulic stress imposed by the atmosphere
81 and is thus more negative [8,27]. Ψ_{PD} is especially sensitive to soil water availability in drought
82 conditions for anisohydric species [28] and is therefore useful as a metric of plant water status in
83 stressful conditions. Water potential has strong effects on the regulation of stomatal conductance
84 [29], and stomatal regulation in turn affects Ψ_{PD} by allowing a plant to maintain internal water
85 pressures above critical thresholds [11,18], below which the plant's water column would snap,

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

resulting in hydraulic failure. Tree species with strict stomatal regulation exhibit relatively narrow ranges of Ψ_{PD} that are maintained by reductions in stomatal conductivity until that threshold is surpassed and the plant dies [30]. In contrast, species that exhibit anisohydric behavior have much wider variation in Ψ_{PD} and can survive to much lower water deficits [30]. This strategy is common in species that have wide xylem conduits that can be refilled easily after water availability is reestablished [31] and species with physiological adaptations that prevent the spread of cavitation once it is initiated [32]. Strict regulation of Ψ_{PD} tends to be associated with species which are more vulnerable to drought [11], although there is recent evidence that this is not a fully generalizable rule [21].

Plant photosynthetic capacity (i.e., A_{max}) is tightly linked to stomatal conductance, and thus plant water potential, during drought since stomatal closure limits CO_2 from entering leaves [33]. This causes plants with strict stomatal regulation to reach net negative photosynthetic rates at less extreme soil water potentials compared to species that exhibit a more anisohydric drought response [11]. There is evidence of a strong relationship between stomatal regulation and changes in photosynthetic capacity across a wide range of species and biomes [23,24,34,35].

Without being able to rely on the assimilation of new photosynthate while stomata are closed, isohydric species instead mobilize labile carbon sources to meet plant energy demands [16]. This source of carbon, commonly referred to as non-structural carbohydrates (NSC), includes starch as well as soluble sugars such as glucose [25]. Reductions in NSC concentrations have been linked to drought [11,16,36] and other stressful conditions such as shade [6,37], and extreme reductions would theoretically result in death via carbon starvation, although it is unlikely that plants would need to exhaust their carbon supply to experience negative effects [16,38]. For example, refilling xylem following cavitation often requires the expenditure of

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

energy [39] and soluble sugars are involved in plant osmoregulation such that reduced sugar content can lead to increased vulnerability to xylem cavitation [12,20]. Despite the difficulty associated with quantifiably measuring carbon starvation, the ability to increase or maintain [NSC] during drought is associated with drought-tolerant species that can afford to maintain stomatal conductivity due to other physiological and morphological traits [8,17].

Xylem conductivity (or hydraulic conductivity) is the rate at which fluid passes through a given stem or branch segment, and decreases with the amount of cavitation (i.e., gas embolisms) present within the segment [26,40]. Plants that conserve hydraulic conductivity through the closure of stomata are typically classified as isohydric and experience steep drop-offs in conductivity past a certain threshold [11]. Anisohydric plants are typically characterized by their more gradual decrease in conductivity that occurs across a wider gradient of water availability [11], a strategy that is commonly associated with drought tolerance. This strategy often coincides with wide xylem conduits (i.e., ring-porous anatomy), which are easier to refill following drought [31], and reduced stomatal regulation that provides the energy needed to expend in the refilling process [39].

Accurate classification of these traits is especially important for forest drought studies involving seedlings or saplings, which can substantially differ in hydraulic strategy compared to mature canopy trees. Cavender-Bares and Bazzaz (2000) showed that although adult *Quercus rubra* exhibit anisohydric behavior, seedlings were relatively isohydric, even when controlling for microenvironment differences between the two groups. The authors speculate that this is in part due to seedlings being unable to access deep water sources due to their shallow root profiles as well as to their higher vulnerability to environmental stress. Other recent research has also demonstrated that saplings received no tangible benefit from anisohydric behavior during

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

drought and took longer to recover after the drought ended [41], suggesting that small size classes will have different vulnerability to drought even if they follow the hydraulic strategies of adult trees. Together, results from these studies support the recent call for a full quantification of hydraulic responses to drought [21,22] that is more robust in addressing suites of traits and within the context of ontogenetic differences.

Seedlings are the size class most likely to experience directional mortality effects [10,42,43], meaning that differences in species' response to environmental drivers should be strongest in this phase of recruitment. Therefore, improving our understanding of ontogenetic differences in drought response will be critical to forecast changes to forest systems. Predicting future seedling performance will require an accurate estimation of hydraulic traits and behavior; if seedlings tend toward the same hydraulic strategies as their adult counterparts, climate change-related drought could accentuate differences in species performance based on these traits. However, if seedlings tend to be more isohydric than adult trees in general, as suggested by Cavender-Bares and Bazzaz (2000), drought effects could be more homogenous across species, increasing the relevance of other drivers of recruitment dynamics that affect community assemblage.

One such driver is light availability, which can severely limit photosynthetic rates even in species with high photosynthetic capacities [44]. Shade-tolerant species, i.e., species that can withstand prolonged periods in full shade, typically have relatively lower respiration demands than shade-intolerant species that allow them to persist on limited resources [45]. They may also exhibit phenological escape behavior that allows them to assimilate the resources they need in a short period of time when light availability is high [46–49]. Light limitations can lead to carbon deficits analogous to carbon starvation caused by stomatal limitations during drought [37,48] and

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

155 can strongly shape recruitment in forest understories [50–52]. Light availability is also likely to
156 affect seedling drought response [6,37]. Carbon starvation caused by deep shade can potentially
157 compound with carbon starvation caused by stomatal limitations during drought [12,53].
158 However, there is also evidence that shading can ameliorate drought stress [54], particularly for
159 drought-intolerant and shade-tolerant species [55]. These positive effects of shading are
160 projected to drive seedling recruitment in some systems [56,57], and should therefore be
161 considered alongside drought when studying the effects of climate change on forest demography.
162 In order to evaluate the extent to which drought response in tree seedlings differs from
163 their adult counterparts, we measured four ecophysiological drought responses (leaf water
164 potential, photosynthetic capacity, non-structural carbohydrate concentration [NSC], and xylem
165 conductivity) for seedlings of two temperate tree species that commonly occur throughout
166 eastern North America: isohydric *Acer saccharum* and anisohydric *Quercus rubra*. We used a
167 greenhouse experiment to combine artificial drought and shading treatments. Our research
168 questions were 1) Does seedling hydraulic strategy differ between species where adult hydraulic
169 strategies are different? 2) How is seedling hydraulic strategy affected by light availability? And,
170 3) what are the implications for seedling demographic performance under climate change? We
171 hypothesize that if seedlings of these species have hydraulic strategies similar to those used by
172 their adult counterparts, then: 1) the range of leaf water potential experience by *A. saccharum*
173 seedlings is much narrower than those observed in *Q. rubra* seedlings. 2) Under drought
174 conditions, reductions in photosynthetic capacity are steeper in isohydric *A. saccharum* than in
175 anisohydric *Q. rubra*. 3) The drop in NSC levels under drought is larger for *A. saccharum*
176 seedlings. And, 4) the magnitude of the decrease in xylem conductivity when exposed to drought
177 is higher for *A. saccharum*. Addressing these questions and hypotheses will contribute to our

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

178 knowledge on how climate change will affect the demographic processes that shape temperate
179 forest communities.
180
181 **Methods**
182 We studied two temperate deciduous tree species that commonly co-occur across eastern
183 North America, and that differ in their respective tolerances to shade and drought: *Acer*
184 *saccharum* (Marsh.) and *Quercus rubra* (L.). *Acer saccharum* is a strongly shade-tolerant species
185 [58] that is intolerant of drought [59]. It has relatively narrow xylem conduits (i.e., diffuse-
186 porous xylem; Ellmore et al. 2006) and has been shown to exhibit isohydric stomatal behavior in
187 response to drought [24]. In contrast, *Q. rubra* is moderately shade-tolerant [60] and moderately
188 drought-tolerant [59]. Trees of this species have large xylem conduits (i.e., ring-porous xylem;
189 Ellmore et al. 2006) and exhibit anisohydric stomatal behavior in response to drought [8].
190 Although wider xylem conduits are more vulnerable to embolism, there is also evidence that they
191 are easier to refill following drought [31], conferring the higher drought tolerance generally
192 associated with this trait. Adult *Q. rubra* trees also develop deeper roots than *Acer* species [61],
193 which may enhance their relative drought-tolerance by allowing them to access deeper water
194 sources.
195 Seeds of both species (from two different sources) were cold-stratified beginning in
196 December 2016 and sown in large plastic tubs filled with potting soil (SunGro Horticulture;
197 Agawam, MA, USA) at the Matthaei Botanical Gardens greenhouse (42.2996° N, 83.6630° W)
198 the following spring. Once seedlings developed their first true leaves, we carefully removed
199 them from the tubs and transplanted them into individual pots (volume = 313 cm^3),
200 supplementing new potting soil as needed. Sixteen seedlings of each species x source

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

combination were randomly assigned to each treatment group (control, shade, drought, shade and drought) for a total of 256 total seedlings; 191 of these seedlings survived to be used in our analyses. Initial height for each seedling was measured two weeks following transplantation in order to account for maternal effects [57].

Experimental Design

Following transplantation to individual pots, seedlings were immediately moved under moderate shade cloth (~40% ambient PAR) and allowed to grow for an entire growing season (summer of 2017) under well-watered conditions. This was done to minimize the effects of first-year transplant stress and to allow seedlings the ability to assimilate enough carbon to allocate photosynthate to storage tissue and other labile carbon pools. Seedlings were moved to an outdoor pit following the onset of leaf color change in fall to allow seedling foliar phenology to respond to natural climate conditions. The pit was used to help insulate seedling roots from frost conditions they would otherwise not experience, and seedlings were further insulated by surrounding the pots with potting soil.

Seedlings were removed from the pit and moved back into the greenhouse in early spring 2018 corresponding to when leaf bud expansion was noted for both species. All pots were moved under one layer of shade cloth and were regularly watered until treatments were implemented. Environmental sensors were added simultaneously with when the seedlings were moved back inside, measuring air temperature and relative humidity (HOBO U23 Pro v2 data loggers) and light (HOBO Pendant data loggers; Onset Computer Corporation; Bourne, MA, USA). Temperature, relative humidity, and light were all measured at 30-minute intervals. Soil moisture was also measured at the individual (pot) level coinciding with harvesting or gas exchange

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

measurement using a FieldScout TDR300 soil moisture meter (Spectrum Technologies; Aurora, IL, USA).

We took pre-treatment measurements of photosynthetic capacity and non-structural carbohydrate concentrations approximately four weeks following initial seedling leaf-out (June 14th), after which treatment conditions were initiated. Our four treatments were drought (D; no additional shade cloth added, seedlings are no longer watered), shade (S; extra shade cloth used to reduce PAR to 10% of ambient, seedlings remained well-watered), shade and drought (DS; extra shade cloth added, seedlings are no longer watered), and a control treatment (C; no additional shade cloth, seedlings remain well-watered). All seedlings acclimated to the study treatments for two weeks before three harvests were made at weekly intervals. Seedlings in each treatment combination were randomly assigned to be harvested for measurement of either xylem conductivity or [NSC]. Each harvest included six seedlings from each group: two for measurement of xylem conductivity and four for measurement of [NSC]. Predawn leaf water potential (Ψ_{PD}) was measured on the morning of each harvest before sunrise as an approximation for soil water potential. Water potential was measured using excised leaves and a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA).

Gas exchange measurements (A_{max})

The day before each harvest, for each treatment, we measured gas exchange in two of the four seedlings selected for the NSC analyses. We used an LI-6400 Portable Photosynthesis System equipped with a CO_2 mixer assembly, LI-02B LED red/blue light source, and LI-06 PAR sensor (Li-COR Biosciences, Lincoln, NE, USA). We constructed light curves (i.e., *A*-*Q* curves) for each plant by recording gas exchange at 1500, 1000, 750, 500, 250, 100, 50, 25, and 0 μmol

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

247 photons m^{-2} s^{-1} at CO_2 concentrations of 400 ppm, ambient temperature, and ambient humidity.
248 Maximum photosynthetic capacity (A_{max}) was calculated using equations published by Marshall
249 and Biscoe (1980) and using the *nls* command in the *stats* package in *R* v3.5.3. This parameter
250 represents the maximum photosynthetic rate that a leaf is capable of under saturating light
251 conditions. Reductions in A_{max} indicate limitations on the photosynthetic machinery, such as
252 from reduced stomatal conductance [24].
253
254 *Non-structural glucose concentrations [NSC_{Glu}]*
255 After Ψ_{PD} measurement, tissue from seedlings selected for the NSC_{Glu} analysis were
256 immediately separated into three pools: leaves (including petioles), stem (above root collar), and
257 roots (below root collar). Leaves were microwaved for 180 seconds at 800 watts to stop leaf
258 enzyme activity [25] and then all tissues were transferred to a drying oven and dried for 48 hours
259 at 70 °C. We weighed each sample, ground them using a ball mill, and then stored the samples at
260 20 °C in airtight containers. We measured out 50 mg of each sample into screw-top conical tubes
261 and soluble sugars were extracted according to Quentin et al. (2015) using repeated incubation
262 and centrifuging in 80% ethanol. We measured glucose concentrations using a phenol-sulfuric
263 acid colorimetric assay as described by Dubois et al. (1956). Glucose concentration was
264 measured against a glucose standard curve using absorbance measured at 490 nm. Measurements
265 were then converted to units of mg glucose per gram of dry tissue.
266
267 *Xylem conductivity (k)*
268 Following Ψ_{PD} measurement, seedlings harvested for xylem conductivity measurements
269 were removed from their pots and their root balls were soaked in water for 10 minutes to

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

alleviate stress on the water column. We then cut stem segments from each plant underwater, recording the length of each segment and its average diameter. Conductance was measured using protocol established by Kolb et al. (1996). We slightly modified this protocol by using 20 mM KCl solution filtered to 0.22 μm (used to prevent microbial growth within the system that could cause artificial xylem blockages). Flow rate of the solution through the stem segment was measured at pressures of 0, -8, -16, -24, and -32 KPa. Hydraulic conductance (*k*) was calculated as the slope of the relationship between flow rate and pressure [26,64]. Flow rate was then standardized by segment length and cross-sectional area. Our vacuum source was not strong enough to forcefully remove embolisms from the stem segments and so we were unable to calculate the percent loss of conductivity.

Analyses

Analyses were performed for each species independently. For each analysis we addressed our specific questions by trying different types of relationships between the variables involved (e.g., linear, exponential, additive, interactions), and tried several combinations of additional explanatory variables (i.e., initial seedling height and seed source). We describe below the models with the best fits based on Deviance Information Criterion for the photosynthetic capacity model (DIC; Spiegelhalter et al. 2002) and based on residual sum of squares comparisons for the other two analyses. Analyses used data from all harvests where seedlings from all relevant treatments were alive to be measured, with the exception being xylem conductivity measurements for *A. saccharum* where there were no data available for seedlings in the drought treatment.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

293 *Predawn leaf water potential*

294 Leaf water potential measurements (Ψ_{PD}) taken over the span of the experiment (Fig 1) were
295 used to assess the range of water potentials experience by seedlings of each species.
296 Measurements were pooled across all seedlings harvested for both NSC and xylem conductivity
297 experiments. We conducted a one-way ANOVA to assess whether Ψ_{PD} differed significantly
298 between the two species.
299

300 **Fig 1. Predawn plant water potential over duration of the study.**
301 Predawn plant water potential (Ψ_{PD}) plotted over the duration of the experiment for *A.*
302 *saccharum* (a) and *Q. rubra* seedlings (b). Differences in color represent the different
303 treatments: control (grey), shade (blue), drought (red), and combined drought and shade
304 (purple).
305

306 *Photosynthetic capacity*

307 We modeled maximum photosynthetic capacity (A_{max}) as a function of light treatment and of leaf
308 water potential (our proxy for drought effects); thus we combined seedlings from the two shaded
309 treatments (S and DS) as well as from the two unshaded treatments (C and D) to assess the two
310 light treatments and then used the full range of observed water potentials to account for drought
311 treatments. Photosynthetic capacity for seedling *i* was estimated from a normal likelihood:

312 $$A_{max,i} \sim N(\mu_i, \sigma^2)$$

313 And an exponential process model that described well the reductions in A_{max} observed in the data
314 (Fig 2a-b):

315 $$\mu_i = \beta 1_{shade(i)} * Exp(\beta 2_{shade(i)} * \Psi_{PD_i})$$

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

316 Parameter β1 represents the maximum photosynthetic rate at water potential equal to zero (i.e.,
317 full water availability). Parameter β2 indicates the decay rate at which A_{max} changes in response
318 to changes in Ψ_{PD}; we used this parameter to assess reductions in photosynthetic capacity. Both
319 parameters were estimated for each shade treatment. The model was also evaluated for the
320 effects of seed source and initial height, but they did not improve model fit and so we did not
321 include them in the final model.
322
323 **Fig 2. Relationship between Ψ_{PD} and maximum photosynthetic capacity**
324 (a-b) Observed (points) and modeled (lines) relationship between Ψ_{PD} and maximum
325 photosynthetic capacity (A_{max}) for (a) *A. saccharum* and (b) *Q. rubra* seedlings. Colors
326 represent seedlings in the non-shaded (grey) vs shaded (blue) treatments and dashed lines
327 represent 95% predictive intervals. (c-d) Posterior estimated means (± 95% credible
328 intervals) for (c) the intercept parameter β1 and (d) the decay parameter β2 relating Ψ_{PD}
329 to A_{max}. Parameters β2 are considered significantly positive if the 95% credible intervals
330 (CI) do not overlap with zero and are considered different from each other if their 95% CI
331 do not overlap.
332
333 Parameter β1 was estimated from non-informative normal priors constrained to be
334 positive, $\beta 1_* \sim N(0,1000)$, and parameter β2 was estimated from non-informative uniform prior
335 $\beta 2_* \sim$ *Uniform*(-1,1). Model variance was estimated from a non-informative gamma prior
336 distribution $1/\sigma^2 \sim Gamma(0.01, 0.01)$. We ran the model using two Monte Carlo chains for
337 40,000 iterations following a 60,000-iteration burn-in period. Analyses were completed using
338 OpenBugs statistical software v3.2.3 [66] and model convergence was assessed using the

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Brooks-Gelman-Rubin statistic [67]. Parameter values (means, variances, and covariances) were estimated from their posterior distributions.

Non-structural carbohydrates

Since seedling glucose concentrations did not change significantly over the duration of the harvest periods (Fig S1), data was pooled across harvest dates for which seedlings from all treatments were still alive (harvest 1 for *A. saccharum* and harvests 1 and 2 for *Q. rubra*). Non-structural glucose concentrations ($[NSC_{Glu}]$) were analyzed using ANOVA that estimated the effects of treatment on values pooled by tissue type (leaf, stem, and root), or averaged across all pools (using averages weighted by the mass of each pool). Analyses were conducted separately for each pool x species combination. We only included data for seedlings that were recorded as alive at the time of harvest because most of the dead seedlings appeared to have died from hydraulic failure, and therefore could skew the results.

Xylem conductivity

For each species, we analyzed the differences in xylem conductivity between seedlings harvested before and after the initiation of drought and shade treatments. We limited our analysis to compare differences only using seedlings harvested in the second harvest period (three weeks after the initiation of treatments) because there were no surviving seedlings in either of the two drought treatments past this harvest. We used initially carried out an ANCOVA to estimate the effects of treatment (C, D, S, DS), maternal effects (initial height), and of seed source. The effects of initial height and seed source did not improve the model fits and were therefore excluded from the final analysis.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Seedling conductivity and [NSC_{Glu}] analyses were conducted in R (v3.5.3) using the *aov* and *anova* commands in the *stats* package to fit and compare models, respectively. Significant differences between treatments were estimated using Tukey's HSD test, performed using the *TukeyHSD* command in *stats*.

Results

Average daytime light levels across the two light treatments (Fig S2a) were consistent with light levels measured in related field experiments [48]. Light levels in the deep shade treatments were 30% (± 0.05% s.d.) of the light levels in the control light treatment. Average daily temperature (Fig S2b) was also consistent with field observations [48], although maximum hourly temperatures were much higher in the greenhouse than what has been observed in the field (Fig S3), and well above the temperature increases predicted under extreme climate change conditions for the Great Lakes region [68]. Temperatures were consistent across all treatments. We found some significant differences in height and mass between populations for each species (summarized in Appendix S1), but they did not significantly affect any of our analyses and are not included in the following results.

Predawn leaf water potential (Ψ_{PD})

Leaf water potential decreased under the drought treatments for both species (Fig 1) with *Q. rubra* seedlings reaching more negative water potentials (-3.28 MPa) than *A. saccharum* seedlings (-1.76 MPa). However, we did not find any significant difference in Ψ_{PD} between the two species (Pr(>F) = 0.291). All *A. saccharum* seedlings in the D and DS treatments died before

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

384 the second harvest (~21 days after treatments were initiated) and all *Q. rubra* seedlings died
385 before the third harvest (~28 days after treatment initiation).
386
387 *Photosynthetic capacity*
388 Our model fit (r^2 of predicted vs observed) was 0.289 for *A. saccharum* and 0.305 for *Q.*
389 *rubra*. Posterior parameter estimates can be found in Table S1. There were no significant
390 differences in posterior estimates of intercept parameter β1 between light treatments for either
391 species (Fig 2c), although values were higher in the shade treatment for *Q. rubra*. Decay
392 parameters β2 were statistically significant, different from zero, for both shade treatments for *Q.*
393 *rubra* but was only significant for the unshaded treatment in *A. saccharum* (Fig 2d). These decay
394 parameters did not significantly vary between light treatments for either of the species. Predicted
395 A_{max} decreased from 3.375 μmol m^{-2} s^{-1} (± standard deviation of 0.692) at Ψ_{PD} = 0 MPa to 1.309
396 ± 0.413 μmol m^{-2} s^{-1} at Ψ_{PD} = -1 MPa for unshaded *A. saccharum* seedlings, a decrease of 61.2%
397 (Fig 2a). This drop was proportionally smaller in the shade treatment, with A_{max} decreasing from
398 2.936 ± 0.568 to 2.577 ± 0.929 μmol m^{-2} s^{-1} (12.3% decrease). This trend was the opposite for *Q.*
399 *rubra* seedlings (Fig 2b), where predicted A_{max} decreased in light conditions from 2.775 ± 0.383
400 to 1.893 ± 0.325 μmol m^{-2} s^{-1} (31.8% decrease) and in shade conditions from 4.934 ± 0.746 to
401 1.137 ± 0.411 μmol m^{-2} s^{-1} (77% decrease).
402
403 *Non-structural carbohydrates*
404 Non-structural glucose concentrations ([NSC_{Glu}]) showed a general decrease over time across all
405 treatments and pools for both species (Fig 3), but there were no significant differences between
406 treatments for any of the species x pool combinations (Table S2). Some carbon pools

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

significantly decreased from the pre-treatment values (Fig 3). There were no statistically significant drops in [NSC_{Glu}] between control and treatment seedlings in either of the two species, and in some cases mean [NSC_{Glu}] values were higher in the treatments than the control (particularly for *Q. rubra*, Fig 3e-h).

Fig 3. Non-structural glucose concentrations for seedlings in all treatment groups compared to pre-treatment controls.

Non-structural glucose concentrations ([NSC_{Glu}]; means + 2 s.d.) for *A. saccharum* (a-d) and *Q. rubra* seedlings (e-h) across the four experimental treatments. Rows show [NSC_{Glu}] in leaf (a, e), stem (b, f), and root (c, g) pools as well as averaged across all pools and weighted by dry mass of each pool (d, h). Colors correspond to treatments as described in Fig 1. Horizontal lines indicate means (solid) ± 2 s.d. (dashed) of [NSC_{Glu}] in seedlings harvested prior to the initiation of treatments.

Xylem conductivity

There were no significant differences in conductivity between any of the treatments for either species (Table 1a; Fig S4). Average *A. saccharum* conductivity was 0.075 ± 0.016 g s^{-1} MPa^{-1} mm^{-1} (mean ± s.d.) in the control treatment and 0.073 ± 0.027 g s^{-1} MPa^{-1} mm^{-1} in the shade treatment (n = 4 in each treatment). There were no *A. saccharum* seedlings that survived in the drought treatments that could be used in this analysis. *Quercus rubra* average conductance was 0.076 ± 0.024 g s^{-1} MPa^{-1} mm^{-1} in the control treatment, 0.041 g s^{-1} MPa^{-1} mm^{-1} in the drought treatment, 0.082 ± 0.011 g s^{-1} MPa^{-1} mm^{-1} in the shade treatment, and 0.043 ± 0.021 g s^{-1} MPa^{-1} mm^{-1} in the combined drought and shade treatment (n = 3, 1, 4, and 3, respectively). We

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

found a significant effect by drought treatment for *Q. rubra* seedlings when the different shade treatments were grouped together (Table 1b, Fig S5b), with seedlings of this species exposed to drought having significantly lower hydraulic conductivity (0.043 ± 0.017 g s^{-1} MPa^{-1} mm^{-1}) compared to those in the well-watered treatments (0.079 ± 0.021 g s^{-1} MPa^{-1} mm^{-1}). *Acer saccharum* seedling conductivity in the combined well-watered treatments was 0.074 ± 0.021 g s^{-1} MPa^{-1} mm^{-1}.

Table 1: ANOVA statistics describing (a) the differences in xylem conductivity between all four factorial treatments and (b) the differences between drought treatments for seedlings of both species. Drought treatment *A. saccharum* seedlings were not available to be used in this analysis and are thus excluded.

(a)	**Df**	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr(>F)**
A. saccharum	1	$7.5*10^{-6}$	$7.5*10^{-6}$	0.015	0.906
Q. rubra	3	$3.45*10^{-3}$	$1.15*10^{-3}$	3.307	0.087
(b)	**Df**	**Sum Sq.**	**Mean Sq.**	**F value**	**Pr(>F)**
A. saccharum	-	-	-	-	-
Q. rubra	1	$3.37*10^{-3}$	$3.37*10^{-3}$	12.1	*0.007*

Discussion/Conclusion

There is wide variation in the hydraulic strategies that plants use to avoid, tolerate, and recover from drought, and these strategies may be more nuanced than what is represented by broad categorization such as iso/anisohydry [22]. Previous research has demonstrated that common drought response indicators such as stomatal conductance regulation are often dependent on environmental context [22] and other trait axes [7,21], suggesting that adequate representation of drought tolerance requires the simultaneous quantification of multiple

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

indicators. Furthermore, there is evidence that hydraulic strategy in tree species can vary substantially along ontogeny [8], and there is a sizeable knowledge gap in the scientific literature for how seedling drought response differs from that of adults. Whether seedling hydraulic strategies differ from adult hydraulic strategies will affect our predictions of forest demography under climate change [69].

In this experiment we measured the hydraulic strategies used by seedlings of two dominant tree species that commonly co-occur across a wide range of eastern North American forests, and that differ in their response to drought (as measured in adults). We quantified four commonly measured indicators of drought tolerance (leaf water potential, photosynthetic capacity, non-structural carbohydrate concentrations, and hydraulic conductance) over the duration of a greenhouse dry-down experiment. We used two different light treatments to investigate the potential interaction effects between shade and drought on tree seedling performance, since shade can both exacerbate carbon starvation via low light levels and/or ameliorate drought stress via decreasing temperature.

Our results indicate that, as predicted from adult characteristics, *A. saccharum* seedlings appear to be slightly more vulnerable to drought due to their inability to survive past relatively moderate reductions in soil water potential (Fig 1a), whereas *Q. rubra* were recorded to survive to considerably lower levels (Fig 1b). *Quercus rubra* seedlings were also able to survive longer in the drought treatments, but only by about a week. Still, we did not find statistically significant differences between species in any of the drought indicators, suggesting that seedlings of these two species are still similarly vulnerable to drought. However, due to the relatively small sample sizes used in this study, it is important to be cautious with these results and the conclusions drawn from them. Overall, our results suggest that the increased drought frequency predicted for

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

the Great Lakes region [68] could negatively affect recruitment and demography of both species in approximately equal measure.

1) Does seedling hydraulic strategy differ between species where adult hydraulic strategies are different?

Tree performance during drought is strongly affected by stomatal regulation via two interacting processes [11,20]. Restricting stomatal conductance allows trees to avoid excessively low internal water pressures that can embolize xylem and lead to hydraulic failure [18]. However, reducing conductance comes at the cost of reducing photosynthetic capacity, which makes it necessary for trees to consume labile carbon pools and increases the risk of dying from carbon starvation [16]. Trees exhibit variation in other traits such as rooting depth [70,71] and xylem pit anatomy [32] that can help mitigate the negative effects associated with one process or the other (e.g., by giving trees access to more resources or by helping them resist cavitation), but there is strong evidence that tree performance in drought conditions is strongly determined by tradeoffs between these two processes [20]. Furthermore, juvenile trees may not be able to make use of the same mitigating strategies used by adults due their size and relative lack of access to resources (i.e., deep water sources; Cavender-Bares and Bazzaz 2000). Hydraulic outcomes at the seedling level may therefore be more similar among species than they are at larger size classes.

Our results do not fully support the idea that seedling hydraulic strategies are similar to those of the adults; while leaf water potentials reached lower levels in *Q. rubra* seedlings, as we expected, the other responses to drought did not differ between the two species. First, while adult *Q. rubra* (as well as other *Quercus* species in general) respond to drought stress by maintaining photosynthetic capacity at the cost of reduced leaf water potential [8,24], we found that seedlings

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

of this species exhibited declines in photosynthetic capacity that began at Ψ_{PD} < -1 MPa (Fig 2b). This was not significantly different from the trend found in *A. saccharum* seedlings (Fig 2a), which matched the photosynthetic response demonstrated in adults of this species [24]. This relatively isohydric response of *Q. rubra* seedlings agrees with previous work done by Cavender-Bares and Bazzaz (2000) and provides support for the idea that seedling hydraulic strategies will be more similar between species than they are in conspecific adults.

Adults of these two species have also been shown to have different wood densities, with *A. saccharum* having dense wood and diffuse-porous xylem and *Q. rubra* having ring-porous xylem and wood that is less dense [14]. Diffuse-porous xylem can help trees avoid embolism formation due to the narrower conduits, but they are also more difficult to refill after embolism occurs [31]. Narrow xylem conduits and strong stomatal control help *Acer* species maintain hydraulic conductance during drought [72,73]. In contrast, *Quercus* species are more prone to gradual but significant declines in conductivity [40,73,74]. We hypothesized that *A. saccharum* seedlings would demonstrate stricter control of internal water potentials compared to *Q. rubra*, and thus show little variation in the conductivity of living seedlings, whereas *Q. rubra* seedlings would show a gradual decline in conductivity associated with anisohydric stomatal regulation.

We found tentative support for this hypothesis with respect to *Q. rubra* seedlings, for which conductivity appeared to decrease gradually beginning at Ψ_{PD} < -1 (Fig 4), which closely resembles the pattern seen in conspecific adults [74] and agrees with previous work done on seedlings of this species [40]. However, we were not able to fully quantify this trend in a more complex analysis because we did not measure percent loss in conductivity (*sensu* Kolb et al. 1996) and because we had limited survival in our seedlings. There was high mortality for this species past the -1 MPa threshold, suggesting that these seedlings are still vulnerable to drought.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

519 *Acer saccharum* seedlings supported the hypothesis, with conductivity maintained at $\Psi_{PD} > -1$
520 and hydraulic failure past that point (Fig 4), which agrees with the strategy used by adults. We
521 observed seedlings of this species surviving to slightly more negative Ψ_{PD} in the NSC harvests
522 (Ψ_{PD} =-1.76 MPa, Fig 1), suggesting that reductions in conductivity may follow a trend more
523 similar to that of the *Q. rubra* seedlings, but conductivity was not quantified for these individuals
524 and thus we lack the evidence needed to better support this conclusion.
525
526 **Fig 4. Xylem conductivity for both species in relation to predawn plant water**
527 **potential.**
528 Xylem conductivity (k) for (a) *A. saccharum* and (b) *Q. rubra* seedlings plotted by
529 predawn plant water potential (Ψ_{PD}). These plots show data from across all three harvests
530 for seedlings that were recorded as alive at time of harvest. Colors correspond to
531 treatments as described in Fig 1.
532
533 Photosynthetic capacity and hydraulic conductivity are both strongly intertwined with
534 changes to non-structural carbohydrate concentrations. Reductions in photosynthetic capacity
535 limit the production of carbohydrates and make plants more dependent on labile carbon pools,
536 reducing [NSC] to maintain metabolic rates [16]. Conductivity directly affects a plant's ability to
537 transport carbohydrates from source tissue (leaves) to tissues where sugars are needed (stems and
538 roots). However, [NSC] also affects conductivity since energy is often required to refill xylem
539 following cavitation [39] and because soluble sugars are necessary for osmoregulation processes
540 [12,20]; thus, reduced sugar content can lead to faster hydraulic failure.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

We found no significant differences in non-structural glucose between treatment conditions for *A. saccharum* in any of the tissue pools, and limited differences between treatment and pre-treatment [NSC_{Glu}] (Fig 3a-d). This suggests that seedlings were generally able to maintain their labile glucose pools throughout the experiment. This is consistent with a previous study that worked with *Acer rubrum* seedlings (which are closely related to *A. saccharum* and typically exhibit a similar hydraulic strategy; Davies and Kozlowski 1977), which found reductions in soluble sugar concentrations over time in similar treatments [6]. We also found no significant differences between treatments in any of the *Q. rubra* NSC_{Glu} pools (Fig 3e-h), but there were more instances of [NSC_{Glu}] reductions relative to pre-treatment controls. This contradicts previous research by Maguire and Kobe (2015), which found general increases in soluble sugar concentrations for this species under similar drought and shade treatments and suggests that seedlings of this species experienced similar stress across all treatments.

The lack of a significant difference between our control treatments and the three stress treatments prevents us from being able to make strong conclusions about how drought will affect [NSC_{Glu}] for seedlings of either species. The difference between our results and the results from other studies that have found significant effects in similar experiments (e.g., Maguire and Kobe 2015) could be due to the extremely high temperatures experienced by seedlings in the greenhouse environment (Fig S3), which affected all seedlings equally and could have caused reductions in [NSC_{Glu}] associated with high respiration rates [76]. It is also possible that the trends we report do not tell the full story as we did not measure starch concentrations, nor did we measure the concentrations of other soluble sugars besides glucose. Recent evidence suggests that soluble sugar concentrations can be maintained through the conversion (and therefore

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

563 reduction) of starch [7], suggesting that measurement of total non-structural carbohydrate
564 concentrations may tell a more complete story.
565
566 **2) How is seedling hydraulic strategy affected by light availability?**
567 There is experimental evidence for both the mitigating [37,54] and exacerbating effects
568 [6,12,53] that shade can have on tree performance during drought. In temperate North American
569 forests, understory light availability under full canopies can be 2-3 orders of magnitude lower
570 than light availability in open canopy conditions [48], and access to light plays a significant role
571 in tree seedling demographic performance in these systems [77,78]. It is therefore important that
572 projections of tree recruitment under climate change account for any interactions between
573 drought and shade.
574 Contrary to other tree seedling studies (e.g., Piper and Fajardo 2016), we did not find any
575 significant effects associated with light availability in any of our drought response indicators.
576 Although including light treatments improved our photosynthetic capacity model's performance,
577 there were no significant differences between light condition effects for either β1 (photosynthetic
578 capacity at saturating water availability) or β2 (the decay rate parameter) (Fig 2c-d). There were
579 no significant shade effects in any of the glucose pools (Fig 3) and hydraulic conductance was
580 better predicted in an analysis that explicitly omitted light treatment and focused solely on
581 differences in drought treatment (Fig S5). The lack of a shade effect on glucose concentrations is
582 consistent with previous research that found no significant change in soluble sugar
583 concentrations over time in seedlings [6] and saplings [7] of temperate tree species common in
584 our study region. However, both studies found significant reductions in starch concentrations that
585 suggest that plants prioritize the mobilization of starch for use in metabolism over the

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

586 consumption of soluble sugars, potentially due to the importance of soluble sugars in
587 osmoregulation [12,20]. We did not measure starch concentrations, so we can only speculate that
588 this mechanism may have affected our results. The extreme temperatures experienced in the
589 greenhouse could have also created respiration demands across all treatments that overwhelmed
590 any signal that we might have otherwise observed with differences in light availability [76]. Still,
591 our results do not support the existence of either mitigating or exacerbating effects of shade on
592 hydraulic performance of temperate tree seedlings.
593
594 **3) What are the implications for seedling demographic performance under climate change?**
595 Altogether, the results from our experiment suggest a convergence in the vulnerability of
596 temperate tree seedlings to drought that is inconsistent with drought responses expected from
597 evidence collected in adult trees of the same species. There were no drought treatment *A.*
598 *saccharum* seedlings that survived longer than two weeks past the initiation of experimental
599 treatments and no water-limited *Q. rubra* seedlings that survived past three weeks. Although our
600 stress treatments were harsh (and also likely exacerbated by high temperatures) the lack of a
601 significant difference between seedling responses is consistent with results from previous
602 research [6]. Still, the relatively small sample size of seedlings surviving in this experiment could
603 have affected our ability to pick up on differences between the two species at finer scales of time,
604 temperature, and soil moisture, and it therefore prevents us from making stronger conclusions
605 about seedling drought dynamics.
606 Even so, with no clear distinction in seedling vulnerability to drought, potential
607 differences in seedling demography and recruitment between species within the context of
608 climate change are more likely to be driven by other factors. For example, we have previously

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

609 shown that access to spring light [48] and the capacity to track climate change with spring leaf
610 out phenology [47] differs between species and that this mechanism can help account for carbon
611 starvation dynamics expected under hotter and drier summers. The lack of a difference between
612 species further indicates that seedling drought vulnerability is decoupled from adult vulnerability
613 and suggests that future community assembly in temperate forests will not be strongly limited by
614 the vulnerability of tree seedlings to drought. This places a stronger emphasis on traits and
615 behaviors that do differ between species and that could lead to differential outcomes during
616 climate change.
617

618 **Acknowledgements:**

619 I. Ibáñez was funded by the NSF (DEB 1252664) and B. Lee was funded by the (Shrank
620 Summer Research Support Fund). We thank D. Zak for the use of an IRGA for gas exchange
621 measurements; DZ, R. Upchurch, and K. Wood for guidance and assistance on NSC analysis;
622 DZ and D. Goldberg for providing valuable feedback on preliminary drafts; and D. Peltier for
623 advice and guidance on modeling gas exchange measurements.
624

625 **Supporting Information Captions:**

626 **S1 Appendix 1.** Supplemental information concerning initial seedling height.

627 **S2 Supplemental tables and figures.** Tables S1 and S2 and Figures S1-S7
628
629

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

Citations

1. Meehl GA, Tebaldi C. More intense, more frequent, and longer lasting heat waves in the 21st century. Science (80-). 2004;305: 994–997. doi:10.1126/science.1098704
2. Bartlett MK, Zhang Y, Yang J, Kreidler N, Sun SW, Lin L, et al. Drought tolerance as a driver of tropical forest assembly: Resolving Spatial signatures for multiple processes. Ecology. 2016;97: 503–514. doi:10.1890/15-0468.1
3. Bréda N, Huc R, Granier A, Dreyer E. Temperate forest trees and stands under severe drought : a review of ecophysiological responses , adaptation processes and long-term consequences. Ann For Sci. 2006;63: 625–644. doi:10.1051/forest
4. Anderegg WRL, Klein T, Bartlett M, Sack L, Pellegrini AF a, Choat B. Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. Proc Natl Acad Sci. 2016. doi:10.1073/pnas.1525678113
5. Allen CD, Breshears DD, McDowell NG. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere. 2015;6: art129. doi:10.1890/ES15-00203.1
6. Maguire AJ, Kobe RK. Drought and shade deplete nonstructural carbohydrate reserves in seedlings of five temperate tree species. Ecol Evol. 2015;5: 5711–5721. doi:10.1002/ece3.1819
7. Kannenberg SA, Phillips RP. Non-structural carbohydrate pools not linked to hydraulic strategies or carbon supply in tree saplings during severe drought and subsequent recovery. Tree Physiol. 2020;40: 259–271. doi:10.1093/treephys/tpz132
8. Cavender-Bares J, Bazzaz FA. Changes in drought response strategies with ontogeny in Quercus rubra: implications for scaling from seedlings to mature trees. Oecologia. 2000;124: 8–18. doi:10.1007/PL00008865
9. Lebrija-Trejos E, Pérez-GarcíA EA, Meave JA, Bongers F, Poorter L. Functional traits and environmental filtering drive community assembly in a species-rich tropical system. Ecology. 2010;91: 386–398. doi:10.1890/08-1449.1
10. Green PT, Harms KE, Connell JH. Nonrandom, diversifying processes are disproportionately strong in the smallest size classes of a tropical forest. Proc Natl Acad Sci. 2014;111: 18649–18654. doi:10.1073/pnas.1321892112
11. McDowell NG, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, et al. Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? New Phytol. 2008;178: 719–739. doi:10.1111/j.1469-8137.2008.02436.x
12. Sevanto S, McDowell NG, Dickman LT, Pangle R, Pockman WT. How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. Plant, Cell Environ. 2014;37: 153–161. doi:10.1111/pce.12141
13. Markesteijn L, Poorter L. Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. J Ecol. 2009;97: 311–325. doi:10.1111/j.1365-2745.2008.01466.x
14. Ellmore GS, Zanne AE, Orians CM. Comparative sectoriality in temperate hardwoods: hydraulics and xylem anatomy. Bot J Linn Soc. 2006;150: 61–71. doi:10.1111/j.1095-8339.2006.00510.x
15. Munné-Bosch S, Alegre L. Die and let live: Leaf senescence contributes to plant survival under drought stress. Funct Plant Biol. 2004;31: 203–216. doi:10.1071/FP03236

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

16. Sala A, Woodruff DR, Meinzer FC. Carbon dynamics in trees: Feast or famine? Tree Physiol. 2012;32: 764–775. doi:10.1093/treephys/tpr143
17. Martínez-Vilalta J, Sala A, Asensio D, Galiano L, Hoch G, Palacio S, et al. Dynamics of non-structural carbohydrates in terrestrial plants: A global synthesis. Ecol Monogr. 2016;86: 495–516. doi:10.1002/ecm.1231
18. Sperry JS, Hacke UG, Oren R, Comstock JP. Water deficits and hydraulic limits to leaf water supply. Plant, Cell Environ. 2002;25: 251–263. doi:10.1046/j.0016-8025.2001.00799.x
19. Urli M, Porté AJ, Cochard H, Guengant Y, Burlett R, Delzon S. Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. Tree Physiol. 2013;33: 672–683. doi:10.1093/treephys/tpt030
20. Adams HD, Zeppel MJB, Anderegg WRL, Hartmann H, Landhäusser SM, Tissue DT, et al. A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. Nat Ecol Evol. 2017;1: 1285–1291. doi:10.1038/s41559-017-0248-x
21. Martínez-Vilalta J, Garcia-Forner N. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant Cell Environ. 2017;40: 962–976. doi:10.1111/pce.12846
22. Hochberg U, Rockwell FE, Holbrook NM, Cochard H. Iso/Anisohydry: A Plant–Environment Interaction Rather Than a Simple Hydraulic Trait. Trends Plant Sci. 2018;23: 112–120. doi:10.1016/j.tplants.2017.11.002
23. Thomas DS, Eamus D. The influence of predawn leaf water potential on stomatal responses to atmospheric water content at constant C(i) and on stem hydraulic conductance and foliar ABA concentrations. J Exp Bot. 1999;50: 243–251. doi:10.1093/jxb/50.331.243
24. Roman DT, Novick KA, Brzostek ER, Dragoni D, Rahman F, Phillips RP. The role of isohydric and anisohydric species in determining ecosystem-scale response to severe drought. Oecologia. 2015;179: 641–654. doi:10.1007/s00442-015-3380-9
25. Quentin AG, Pinkard E a., Ryan MG, Tissue DT, Baggett LS, Adams HD, et al. Non-structural carbohydrates in woody plants compared among laboratories. Tree Physiol. 2015;35: 1146–1165. doi:10.1093/treephys/tpv073
26. Sperry JS, Tyree MT. Water-stress-induced xylem embolism in three species of conifers. Plant Cell Environ. 1990;13: 427–436. doi:10.1111/j.1365-3040.1990.tb01319.x
27. Williams LE, Araujo FJ. Correlations among Predawn Leaf, Midday Leaf, and Midday Stem Water Potential in Grapevines. J Amer Soc Hort Sci. 2002;127: 448–454.
28. Bréda N, Granier A, Barataud F, Moyne C. Soil water dynamics in an oak stand - I. Soil moisture, water potentials and water uptake by roots. Plant Soil. 1995;172: 17–27. doi:10.1007/BF00020856
29. Anderegg WRL, Wolf A, Arango-Velez A, Choat B, Chmura DJ, Jansen S, et al. Plant water potential improves prediction of empirical stomatal models. PLoS One. 2017;12: 1–17. doi:10.1371/journal.pone.0185481
30. Breshears DD, Myers OB, Meyer CW, Barnes FJ, Zou CB, Allen CD, et al. Research communications research communications Tree die-off in response to global change-type drought: Mortality insights from a decade of plant water potential measurements. Front Ecol Environ. 2009;7: 185–189. doi:10.1890/080016
31. Ogasa M, Miki NH, Murakami Y, Yoshikawa K. Recovery performance in xylem hydraulic conductivity is correlated with cavitation resistance for temperate deciduous tree

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

species. Tree Physiol. 2013;33: 335–344. doi:10.1093/treephys/tpt010

32. Pittermann J, Sperry JS, Hacke UG, Wheeler JK, Sikkema EH. Inter-tracheid pitting and the hydraulic efficiency of conifer wood: The role of tracheid allometry and cavitation protection. Am J Bot. 2006;93: 1265–1273. doi:10.3732/ajb.93.9.1265

33. Cowan IR, Farquhar GD. Stomatal function in relation to leaf metabolism and environment. Symp Soc Exp Biol. 1977;31: 475–505.

34. Reich PB, Hinckley TM. Influence of pre-dawn water potential and soil-to-leaf hydraulic conductance on maximum daily leaf diffusive conductance in two oak species. Funct Ecol. 1989;3: 719–726.

35. Wilson KB, Baldocchi DD, Hanson PJ. Spatial and seasonal variability of photosynthetic parameters and their relationship to leaf nitrogen in a deciduous forest. Tree Physiol. 2000;20: 565–578. doi:10.1093/treephys/20.9.565

36. O'Brien MJ, Leuzinger S, Philipson CD, Tay J, Hector A. Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. Nat Clim Chang. 2014;4: 710–714. doi:10.1038/nclimate2281

37. Piper FI, Fajardo A. Carbon dynamics of Acer pseudoplatanus seedlings under drought and complete darkness. Tree Physiol. 2016; 1400–1408. doi:10.1093/TREEPHYS/TPW063

38. Sala A, Piper FI, Hoch G. Physiological mechanisms of drought-induced tree mortality are far from being resolved. New Phytol. 2010;186: 274–281. doi:10.1111/j.1469-8137.2009.03167.x

39. Trifilò P, Kiorapostolou N, Petruzzellis F, Vitti S, Petit G, Lo Gullo MA, et al. Hydraulic recovery from xylem embolism in excised branches of twelve woody species: Relationships with parenchyma cells and non-structural carbohydrates. Plant Physiol Biochem. 2019;139: 513–520. doi:10.1016/j.plaphy.2019.04.013

40. Tyree MT, Alexander J, Machado JL. Loss of hydraulic conductivity due to water-stress in intact juveniles of Quercus-rubra and Populus-deltoides. Tree Physiol. 1992;10: 411–415. doi:10.1093/treephys/10.4.411

41. Kannenberg SA, Novick KA, Phillips RP. Anisohydric behavior linked to persistent hydraulic damage and delayed drought recovery across seven North American tree species. New Phytol. 2019;222: 1862–1872. doi:10.1111/nph.15699

42. Harper JL. Population Biology of Plants. London, UK: Academic Press; 1977.

43. Grubb PJ. The Maintenance of Species-Richness in Plant Communities : The Importance of the Regeneration Niche. Biol Rev. 1977;52: 107–145. doi:10.1111/j.1469-185X.1977.tb01347.x

44. Farquhar GD, Caemmerer S Von, Berry JA. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta. 1980;149: 78–90. doi:10.1007/BF00386231

45. Boardman NK. Comparative Photosynthesis of Sun and Shade Plants. Annu Rev Plant Physiol. 1977;28: 355–377. doi:10.1146/annurev.pp.28.060177.002035

46. Jacques MH, Lapointe L, Rice K, Montgomery RA, Stefanski A, Reich PB. Responses of two understory herbs, Maianthemum canadense and Eurybia macrophylla, to experimental forest warming: Early emergence is the key to enhanced reproductive output. Am J Bot. 2015;102: 1610–1624. doi:10.3732/ajb.1500046

47. Lee BR, Ibáñez I. Improved phenological escape can help temperate tree seedlings maintain demographic performance under climate change conditions. Glob Chang Biol. 2021; gcb.15678. doi:10.1111/gcb.15678

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

48. Lee BR, Ibáñez I. Spring phenological escape is critical for the survival of temperate tree seedlings. Funct Ecol. 2021; 1365-2435.13821. doi:10.1111/1365-2435.13821
49. Kwit MC, Rigg LS, Goldblum D. Sugar maple seedling carbon assimilation at the northern limit of its range: the importance of seasonal light. Can J For Res. 2010;40: 385–393. doi:10.1139/X09-196
50. Coates KD. Tree recruitment in gaps of various size, clearcuts and undisturbed mixed forest of interior British Columbia, Canada. For Ecol Manage. 2002;155: 387–398. doi:10.1016/S0378-1127(01)00574-6
51. Rüger N, Huth A, Hubbell SP, Condit R. Response of recruitment to light availability across a tropical lowland rain forest community. J Ecol. 2009;97: 1360–1368. doi:10.1111/j.1365-2745.2009.01552.x
52. Wright SJ, Muller-Landau HC, Condit R, Hubbell SP. Gap-dependent recruitment, realized vital rates, and size distributions of tropical trees. Ecology. 2003;84: 3174–3185. doi:10.1890/02-0038
53. Holmgren M. Combined effects of shade and drought tulip poplar seedlings: Trade-off in tolerance or facilitation? Oikos. 2000;90: 67–78. doi:10.1034/j.1600-0706.2000.900107.x
54. Quero JL, Villar R, Marañón T, Zamora R. Interactions of drought and shade effects on seedlings of four Quercus species: Physiological and structural leaf responses. New Phytol. 2006;170: 819–834. doi:10.1111/j.1469-8137.2006.01713.x
55. Holmgren M, Gómez-Aparicio L, Quero JL, Valladares F. Non-linear effects of drought under shade: Reconciling physiological and ecological models in plant communities. Oecologia. 2012;169: 293–305. doi:10.1007/s00442-011-2196-5
56. Dobrowski SZ, Swanson AK, Abatzoglou JT, Holden Z a., Safford HD, Schwartz MK, et al. Forest structure and species traits mediate projected recruitment declines in western US tree species. Glob Ecol Biogeogr. 2015;24: 917–927. doi:10.1111/geb.12302
57. Ibáñez I, Mccarthy-Neumann S. Integrated assessment of the direct and indirect effects of resource gradients on tree species recruitment. Ecology. 2014;95: 364–375. doi:10.1890/13-0685.1
58. Lei TT, Lechowicz MJ. Shade adaptation and shade tolerance in saplings of three Acer species from eastern North America. Oecologia. 1990;84: 224–228. doi:10.1007/BF00318275
59. Coble AP, Vadeboncoeur MA, Berry ZC, Jennings KA, McIntire CD, Campbell JL, et al. Are Northeastern U.S. forests vulnerable to extreme drought? Ecol Process. 2017;6. doi:10.1186/s13717-017-0100-x
60. Crow TR. Reproductive mode and mechanisms for self-replacement of northern red oak (Quercus rubra): a review. For Sci. 1988;34: 19–40. doi:10.1016/S0378-1127(03)00108-7
61. Thomsen JE, Bohrer G, Matheny AM, Ivanov VY, He L, Renninger HJ, et al. Contrasting hydraulic strategies during dry soil conditions in Quercus rubra and Acer rubrum in a sandy site in Michigan. Forests. 2013;4: 1106–1120. doi:10.3390/f4041106
62. Marshall B, Biscoe P V. A model for c3 leaves describing the dependence of net photosynthesis on irradiance: II. Application to the analysis of flag leaf photosynthesis. J Exp Bot. 1980;31: 41–48. doi:10.1093/jxb/31.1.41
63. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric Method for Determination of Sugars and Related Substances. Anal Chem. 1956;28: 350–356. doi:10.1021/ac60111a017
64. Kolb KJ, Sperry JS, Lamont BB. A method for measuring xylem hydraulic conductance

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

and embolism in entire root and shoot systems. J Exp Bot. 1996;47: 1805–1810. doi:10.1093/jxb/47.11.1805

65. Spiegelhalter DJ, Best NG, Carlin BP, Van Der Linde A. Bayesian measures of model complexity and fit. J R Stat Soc Ser B Stat Methodol. 2002;64: 583–616. doi:10.1111/1467-9868.00353

66. Lunn D, Spiegelhalter DJ, Thomas A, Best N. The BUGS project: Evolution, critique and future directions. Stat Med. 2009;28: 3049–3067. doi:10.1002/sim.3680

67. Gelman A, Rubin DB. Inference from interative simulation using multiple sequences. Stat Sci. 1992;7: 457–511. doi:10.1214/ss/1177011136

68. Handler S, Duveneck MJ, Iverson L, Peters E, Scheller RM, Wythers KR, et al. Michigan forest ecosystem vulnerability assessment and synthesis: a report from the Northwoods Climate Change Response Framework project. Gen Tech Rep NRS-129. Newtown Square, PA; 2014. doi:10.2737/NRS-GTR-129

69. McDowell NG, Beerling DJ, Breshears DD, Fisher RA, Raffa KF, Stitt M. The interdependence of mechanisms underlying climate-driven vegetation mortality. Trends Ecol Evol. 2011;26: 523–532. doi:10.1016/j.tree.2011.06.003

70. Loewenstein NJ, Pallardy SG. Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: A comparison of young plants of four temperate deciduous angiosperms. Tree Physiol. 1998;18: 421–430.

71. Lopez OR, Kursar TA, Cochard H, Tyree MT. Interspecific variation in xylem vulnerability to cavitation among tropical tree and shrub species. Tree Physiol. 2005;25: 1553–1562. doi:10.1093/treephys/25.12.1553

72. Sperry JS, Donnelly JR, Tyree MT. A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ. 1988;11: 35–40. doi:10.1111/j.1365-3040.1988.tb01774.x

73. Hoffmann WA, Marchin RM, Abit P, Lau OL. Hydraulic failure and tree dieback are associated with high wood density in a temperate forest under extreme drought. Glob Chang Biol. 2011;17: 2731–2742. doi:10.1111/j.1365-2486.2011.02401.x

74. Lo Gullo MA, Nardini A, Trifilò P, Salleo S. Diurnal and seasonal variations in leaf hydraulic conductance in evergreen and deciduous trees. Tree Physiol. 2005;25: 505–512. doi:10.1093/treephys/25.4.505

75. Davies WJ, Kozlowski TT. Variations among woody plants in stomatal conductance and photosynthesis during and after drought. Plant Soil. 1977;46: 435–444. doi:10.1007/BF00010099

76. Tjoelker MG, Reich PB, Oleksyn J. Changes in leaf nitrogen and carbohydrates underlie temperature and CO2 acclimation of dark respiration in five boreal tree species. Plant, Cell Environ. 1999;22: 767–778. doi:10.1046/j.1365-3040.1999.00435.x

77. Canham CD. An index for understory light levels in and around canopy gaps. Ecology. 1988;69: 1634–1638.

78. Beckage B, Clark JS, Clinton BD, Haines BL. A long-term study of tree seedling recruitment in southern Appalachian forests: the effects of canopy gaps and shrub understories. Can J For Res. 2000;30: 1617–1631. doi:10.1139/x00-075

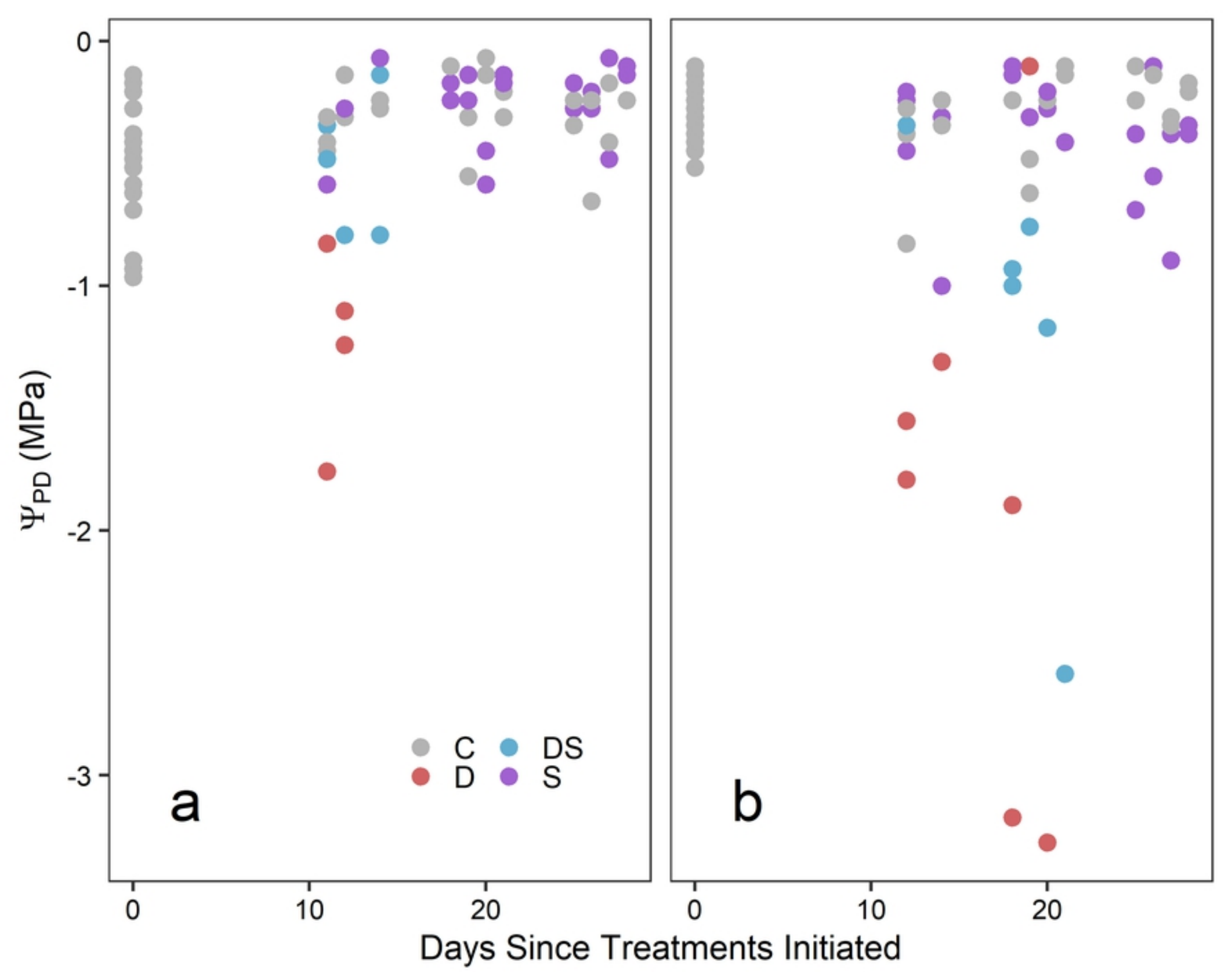

0
-1
-2
-3
Ψ_{PD} (MPa)
C
D
DS
S
a
b
0
10
20
0
10
20
Days Since Treatments Initiated

Fig 1

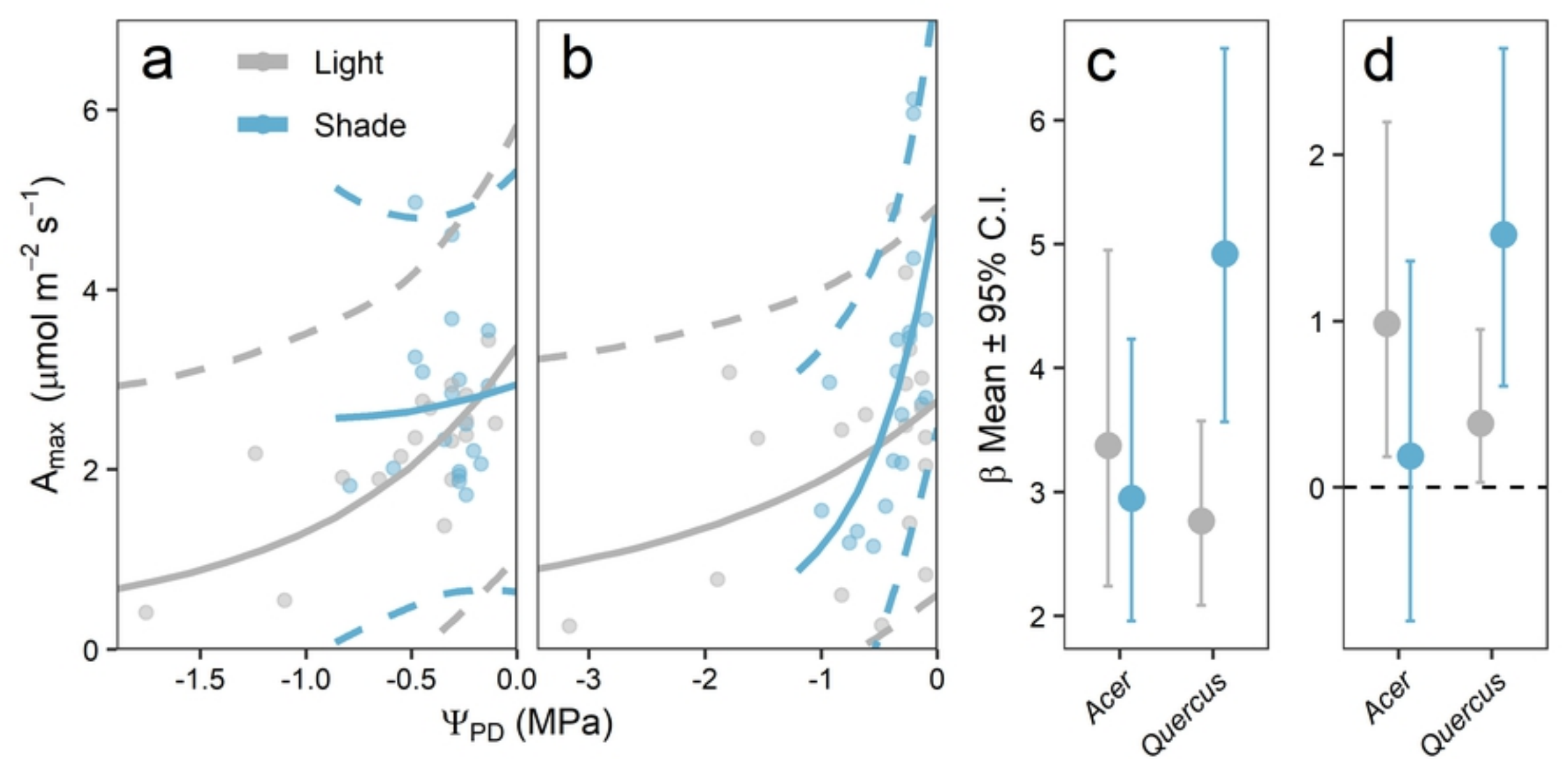

a
b
c
d
Light
Shade
A_{max} (μmol m^{-2} s^{-1})
Ψ_{PD} (MPa)
β Mean ± 95% C.I.
Acer
Quercus
Acer
Quercus

Fig 2

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.04.455116; this version posted August 4, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a CC-BY 4.0 International license.

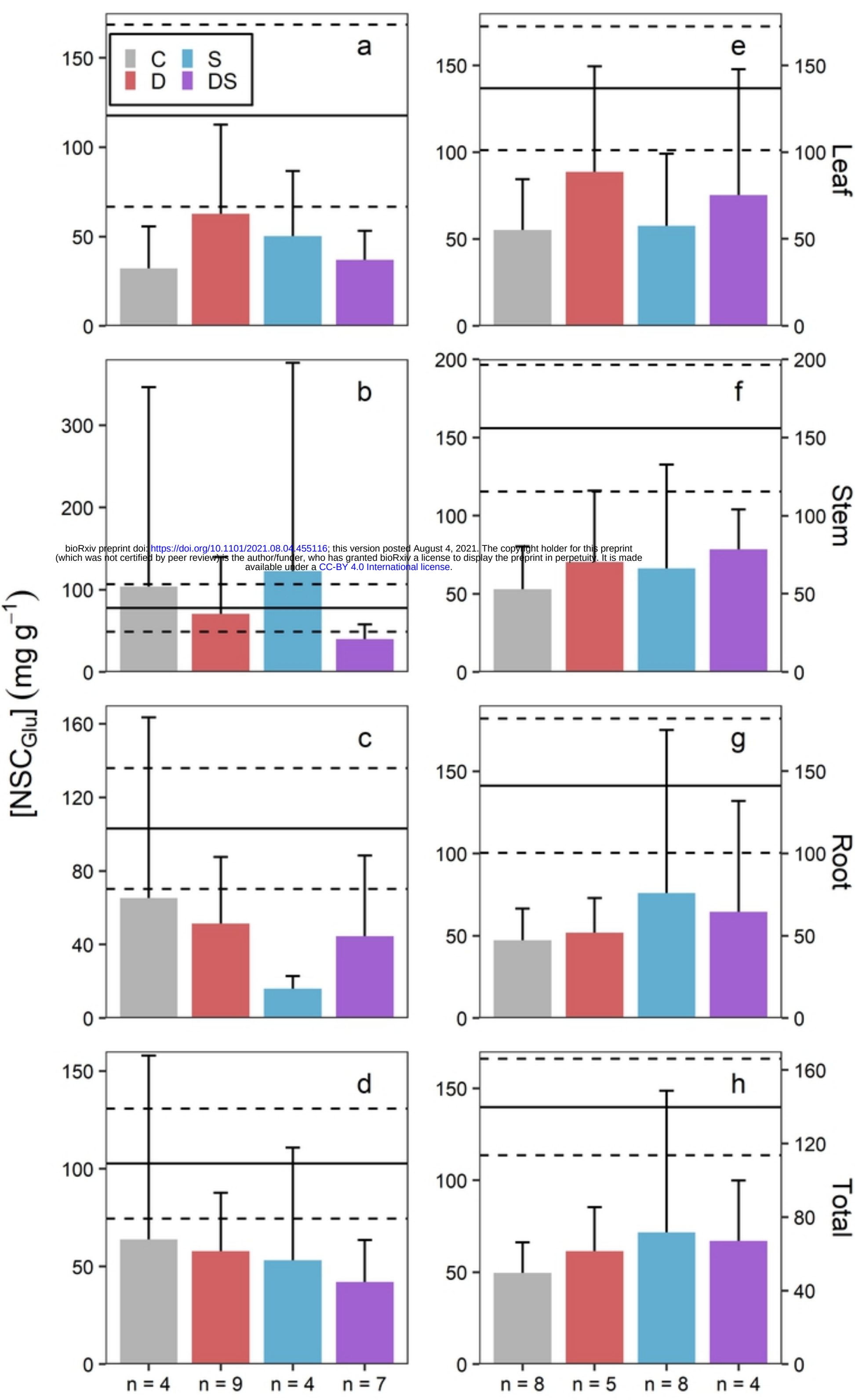


Fig 3

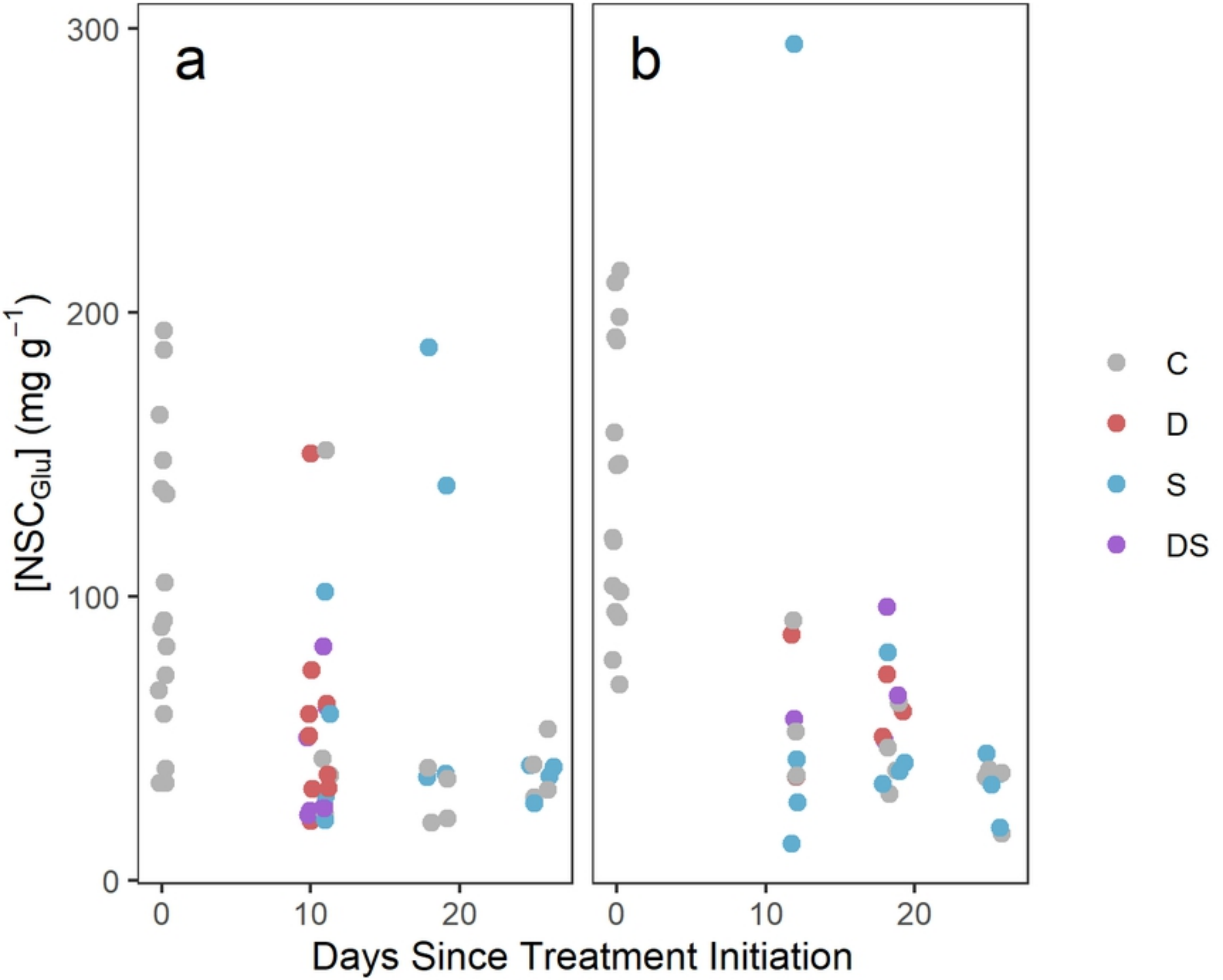

a
b
300
200
100
0
[NSC$_{Glu}$] (mg g^{-1})
0
10
20
Days Since Treatment Initiation
C
D
S
DS

Fig 4