**Quantifying the effects of belowground intra- and inter-specific competition on nighttime stomatal conductance and transpiration in black cottonwoods**

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**Abstract**

Stomata are critical valves responsible for gas exchange and transpiration and play a crucial role in terrestrial water cycling. Many plants maintain partially open stomatal apertures and transpiration at night, even when carbon gain is zero, which is an enduring mystery in plant ecophysiology. We tested the hypothesis that nocturnal stomatal conductance may inhibit unfavorable hydraulic redistribution in the soil, keeping soil water proximal and away from competitors. We planted black cottonwood (*Populus trichocarpa*) seedlings in a range of competitive settings including intraspecific planting groups and interspecific pots with water birch (*Betula occidentalis*) and then quantified responses during well-watered conditions and a water dry-down experiment. Intra- and inter-specific competitive and non-competitive cottonwoods alike increased nighttime stomatal conductance as water supply decreased, and cottonwoods planted in intraspecific competition showed significantly greater increases in nighttime stomatal conductance than solo trees. We found mixed results in examining the linkages between hydraulic redistribution and nighttime stomatal conductance. Our results support the hypothesis that trees likely alter stomatal conductance at night in the presence of competition, although nocturnal stomatal conductance peaked at intermediate competition levels. The observed pattern of competitive stomatal behavior at night has considerable implications for models of transpiration and plant water cycling in ecosystems.

**Introduction**

Gas exchange of carbon dioxide for photosynthesis and water vapor loss is mediated by small stomatal pores on the leaf surface. Stomata control rates of transpiration (E) and as such play a crucial role in terrestrial water cycling (Berry *et al.*, 2010). Plants must pay the water “cost” of open stomata in order to gain carbon via photosynthesis when sunlight is present. The genetic and environmental controls over stomatal conductance during the day have long been studied and are well-documented (Wong *et al.*, 1979; Ball *et al.*, 1987; Buckley, 2005; McAdam & Brodribb, 2015; Brodribb & McAdam, 2017).

Optimal stomatal theory suggests that plants should close stomata during the night when photosynthesis halts, to reduce transpiration when CO2 uptake is unnecessary (Snyder *et al.*, 2003; Caird *et al.*, 2007). However, many non-CAM plant species across functional types and climate zones have been observed maintaining partial stomatal conductance and transpiration at night (Snyder *et al.*, 2003; Caird *et al.*, 2007; Dawson *et al.*, 2007; Ogle *et al.*, 2012; Hoshika *et al.*, 2018; Resco de Dios *et al.*, 2019; Yu *et al.*, 2019; Wang *et al.*, 2021). The underlying mechanism(s) driving this phenomenon are an enduring mystery in plant ecophysiology. Research has found that nighttime transpiration (EN) rates commonly range from 10-15% of daytime rates (Bucci *et al.*, 2004; Caird *et al.*, 2007; Sellin & Lubenets, 2010; Ogle *et al.*, 2012) and are often highest in plants from dry regions (Yu *et al.* 2019).

A number of adaptive (e.g. beneficial to the plant) and non-adaptive hypotheses have been proposed to explain nocturnal stomatal conductance and water loss, including related to water stress recovery, competition, plant hormone levels, circadian rhythms, nutrient supply, oxygen and CO2 balancing, leaf cooling, and morphological constraints (Snyder *et al.*, 2003; Daley & Phillips, 2006; Caird *et al.*, 2007; Dawson *et al.*, 2007; Marks & Lechowicz, 2007; Scholz *et al*., 2007; Oliviera *et al*., 2010; Kupper *et al*., 2012; Zeppel *et al.*, 2012, 2014; Neumann *et al.*, 2014; Cirelli, 2015; Couple-Ledru *et al.*, 2016; Resco de Dios *et al.*, 2016, 2019; Huang *et al*., 2017; Yu *et al.*, 2018; Schoppach *et al.*, 2020; Wang *et al.*, 2021). The primary nonadaptive explanation is the ‘leaky stomata’ hypothesis, which posits that stomata are unable to fully close, or that the energetic cost of completely closing stomata is too high to justify halting transpiration (Barbour *et al.*, 2005). This hypothesis, however, is not consistent with several recent lines of evidence: (1) Significant genetic variation across differing environmental conditions suggests that nighttime stomatal conductance (gsn) is subject to selection (Caird *et al.*, 2007; Zeppel *et al.*, 2014; Chieppa *et al.*, 2020). (2) Nocturnal conductance has been repeatedly observed following circadian patterns, suggesting that plants actively open stomata (Caird *et al.*, 2007; Resco de Dios *et al.*, 2019). (3) In arid environments, where the fitness cost of water loss is very high, many plants continue to exhibit significant nighttime transpiration (Ogle *et al.* 2012; Yu *et al.* 2019). A large-scale phylogenetic assessment of nighttime stomatal conductance found that plants from drier regions exhibited higher gsn than plants from wetter regions despite more extreme water loss-energy cost tradeoffs (Yu *et al.*, 2019).

Considering potential adaptive hypotheses, nighttime stomatal conductance has been studied for its potential benefits to nutrient acquisition (Snyder *et al.*, 2003; Zeppel *et al.*, 2014; Howard & Donovan, 2007, 2010; Christman *et al.*, 2009; Oliveira *et al.*, 2010; Kupper *et al.*, 2012; Eller *et al.*, 2017). Modifying a plant’s nutrient environment through increased mass flow could theoretically be an advantage of increased nocturnal transpiration. However, evidence supporting this hypothesis has been described as equivocal and inconsistent (Eller *et al.*, 2017; Wang *et al.*, 2018; Chieppa *et al.*, 2020). Other adaptive hypotheses suggesting that plants utilize nighttime stomatal conductance as a hydraulic strategy to restore capacitance, remove xylem embolism, or respond to water stress and ABA conditions have also shown mixed support (Caird *et al.*, 2007; Ogle *et al.*, 2012; Zeppel *et al.*, 2012; Zeppel *et al.*, 2014).

Recently, transpiration at night has been discussed as an indirect product of growth because circadian-driven transpiration could prime stomata for early-morning photosynthesis. If stomata are partially open at dawn, the time it takes to reach operating steady-state stomatal conductance is reduced (Resco De Dios 2015, 2019; Schoppach et al., 2020). However, the direct connection between this anticipation hypothesis and resultant growth is unclear. Cooling of leaf temperatures to reduce nighttime respiration rates is another recently proposed adaptive hypothesis that has some experimental support (Wang *et al.* 2021), but has not yet been widely tested.

A final adaptive hypothesis suggests that nighttime stomatal conductance may be beneficial to plants when they experience belowground competition for water. It has been hypothesized that plants maintain nocturnal transpiration despite water loss as a strategy for reducing unfavorable hydraulic redistribution in the soil (Zeppel *et al.* 2014; Huang *et al.* 2017; Yu *et al.* 2019). Open stomata increase tension in the soil-plant-air water potential (ψ) gradient, drawing below-ground water closer to the plant’s roots (Zeppel *et al.*, 2014). Thus, partially open stomata at night would benefit the plant by keeping a water source in proximity and not diffusing along passive water potential gradients in the soil to competitors, thereby increasing plant carbon gain (Huang *et al.*, 2017). Some predictions of this hypothesis have been observed across plant functional types (Caird *et al.*, 2007; Howard *et al.*, 2009; Neumann *et al.*, 2014; Huang *et al.*, 2017; Yu *et al.*, 2019

Here, we aim to test how competition and water availability influence nighttime stomatal conductance of a deciduous tree species common to western North America, *Populus trichocarpa* (black cottonwood). In a controlled environment growth chamber experiment, we test how black cottonwood modulates stomatal conductance at night in the presence of interspecific, intraspecific, and no competition and under varying soil moisture conditions. We ask: (1) Does competition influence nighttime stomatal conductance? (2) Does decreased water supply influence stomatal behavior at night? (3) Does nighttime stomatal conductance confer a competitive advantage in black cottonwoods?

**Materials and Methods**

*Experimental Design*

Black cottonwood (*Populus trichocarpa*) and water birch (*Betula occidentalis*) seedlings were obtained from the University of Idaho Pitkin Forest Nursery. These species were selected because they both exhibit high water use with water birch showing exceptionally high transpiration rates and is thus likely to be a high water-use competitor (Wang *et al.* 2019) and they both naturally co-occur in riparian ecosystems across western North America at low to moderate (~2500 m) elevations.

We planted seedlings in a solo (no competition) treatment and three competition treatments that varied in strength or competitor (intra- versus inter-specific). Black cottonwood was the focal species that received all combinations, whereas water birch was only used in the inter-specific competition treatment due to space constraints in the growth chamber. Each treatment had five replicates. Treatments were: black cottonwood planted alone (C), black cottonwood planted with a black cottonwood competitor (C-C), black cottonwood planted with a water birch competitor (C-B), and black cottonwood planted with a black cottonwood competitor with extra space between plants to weaken competition effects (C-X-C), making 35 trees total. We aimed for varying degrees of competition to provide insight into whether black cottonwood increase stomatal conductance at night in response to the strength of belowground competition for water resources.

Solo cottonwoods (C) were planted in 25-liter plastic containers. Cottonwoods grown in moderate competition with other cottonwoods (C-C) and water birches (C-B) were planted in 50-liter containers, and cottonwoods planted in weak competition with cottonwoods (C-X-C) were planted in 75-liter containers. C-C and C-B trees were planted 25-30 cm apart. C-X-C trees were planted 51-52 cm apart. All seedlings were transplanted with peat/bark based growing media (LM-BK, Lambert Peat Moss, Rivière-Ouelle, Québec, CAN) and established in a greenhouse starting March 2020. All pots followed the guideline by Poorter *et al.* that plant biomass should not exceed 1 g L-1 soil (2012). At the time of transplanting, cottonwood seedlings were 10-14 cm tall and birch seedlings were 12-18 cm.

Each of the replicates was arranged with the same layout and positioned to minimize variation of growing conditions across each planting group and ensure roughly similar aboveground positioning and competition (Supporting Information, Figure S1). Pots were well- watered every other day in the greenhouse for a growing period from March to June 2020. On June 5, 2020, the 5 replicates were moved from the greenhouse into one Percival PR-915 (Percival 120 Scientific, Perry, IA, USA) controlled environment growth chamber. Plants were well-watered daily and given 25 days to acclimate to the growth chamber environment. The growth chamber was programmed to simulate a normal summer day in Utah, with a 5-hour time adjustment for measurement convenience. Seedlings received a 13-hour daylength, with light, temperature, and relative humidity linearly changing to peak at 800 µmol m-² sec-1, 25 °C, and 50% respectively at the chamber’s midday (Table S1).

Initial measurements of nighttime and daytime stomatal conductance, photosynthetic rate, day and nighttime transpiration, soil moisture, leaf area, tree height and diameter, and predawn and midday water potential were taken June 23-24, 2020. During this first week, plants were ‘well-watered’ 6 days of the week, receiving 220-230 mL of water per L of soil with each watering event. During the months of July and August 2020, drought conditions were simulated by steadily decreasing water supply. After 1 week of measurements, plants were watered 3 times per week, receiving the same amount of water per watering event, resulting in a 50% reduction in watering. Watering was reduced to twice per week during week 3, week 4, and week 5 of the experiment. Plants were watered once per week for weeks 6, 7, and 8. During the drought treatment, nighttime and daytime stomatal conductance, day and nighttime transpiration, photosynthetic rate, and water potential were measured each week. At the conclusion of the drought treatment, all trees were destructively harvested and data was collected on final height and diameter, and biomass distribution.

*Leaf Level Gas Exchange*

Predawn and midday measurements were made weekly using a portable photosynthesis system (Licor Li-6800, Li-Cor, Lincoln, NE, USA). Predawn conditions in the Li-6800 chamber were: leaf temperature was set to match ambient growth chamber temperature (~22-25⁰ C), photosynthetic photon flux density at 0 µmol m-2 s-1 of photosynthetically active radiation (PAR), RH was set to match ambient growth chamber RH (~50-75 % +\- 5%), flow was set to 600 µmol s-1, fan speed at 10,000 rpm, and ambient CO2 at 400 ppm. Midday Li-6800 chamber settings were the same as predawn settings other than PAR, which was set to 800 µmol m-2 s-1. Predawn gas exchange was measured between 08:00 and 10:00 (i.e 1-3 hrs before growth chamber “dawn” event) and midday gas exchange was measured between 17:00 and 19:00 (30 mins before “midday” to 1.5 hrs after). All measurements were taken on mature, healthy leaves.

*Leaf Water Potential*

To test the effect of the drought treatment on plant water status, predawn and midday leaf water potential (ψPD and ψMD) were measured weekly concurrent with gas exchange measurements. Mature healthy leaves were harvested from each tree after gas exchange measurements and stored temporarily (less than 2 hours) in sealed plastic bags on ice in a cooler to prevent desiccation. Leaves were then transported immediately to the lab (<30 m of transport distance) and water potentials were measured using a Scholander pressure chamber (PMS Instruments, Albany, OR, USA). Water potentials were assessed to the nearest half-bar (0.05 MPa).

*Soil Moisture*

To assess the effectiveness of the drought treatment on water availability, soil moisture was measured continuously. Soil moisture probes (Campbell Scientific, Logan, UT, USA) were placed within 6 cm of the tree bases of each tree in one replicate. Soil moisture measurements were converted from raw volumetric water content, which can vary across sensors and soils, to percentages for standardization. Soil moisture data were collected for all measurement periods (08:00-10:00 and 17:00-19:00 on weekly measurement days) and the highest value for each planting treatment were selected as the well-watered maximum soil moisture for that treatment. Data for each measurement period were averaged for each planting treatment, and these averages were assigned a percentage compared to the group’s maximum soil moisture (which were set to 100%).

*Plant Size and Growth*

Measurements of height and basal diameter were collected before the drought treatment and again before harvesting. Tree height was recorded using a standard meter stick from the base of the tree to the apical meristem, rounding to the nearest 0.1 cm. Basal diameter was measured using dial calipers. Two diameter readings were taken at perpendicular angles at the base of each seedling and averaged to the nearest millimeter.

*Whole Plant Transpiration*

We measured a stratified random subset of leaves from three size categories (n=3 leaves per tree) in order to estimate whole-tree leaf area and transpiration. For each tree, the number of small, medium, and large leaves were recorded. Small leaves ranged from 1 cm to 4 cm. Medium leaves ranged from 4 cm to 7 cm. Large leaves were longer than 7 cm. For each tree, one representative leaf was selected from each size category to be measured for surface area. The selected leaves were removed from the stem, attached without overlap to sheets of paper, and then scanned to create a digital image. The software ImageJ (National Institute of Health, Bethesda, MD, http://imagej.nih.gov/ij/, 1997-2014) was utilized to estimate the total one-sided leaf area of each sample. The area of each representative leaf was multiplied by the number of leaves in the category for that tree and added to the totals of the other size categories to estimate the total one-sided leaf area for each tree. Total tree leaf area was multiplied by average nighttime leaf transpiration rate to calculate nighttime whole tree transpiration rates, which was then summed across the nighttime period to yield total nighttime transpiration for each tree.

*Competition Indices*

Competition indices were calculated using the Hegyi index (1974):

Hegyi Index = (Db/Da)/DISTab

Db = Diameter of competitor

Da= Diameter of focal tree

DISTab = Distance between competing trees

Because the samples were young saplings, basal diameter was used in place of diameter at breast height in Hegyi’s equation. Distance between competitors was measured using a standard meter stick.

*Biomass*

At the conclusion of the 8-week drought period, all trees were harvested from the growth chamber for biomass measurements. Tree samples were separated into aboveground and belowground tissue. Root biomass was gently washed to remove debris and all samples were placed into brown paper bags to be dried. Biomass sample bags were kept in a drying oven at 50 C for at least 3 days or until a dry weight stabilized before being removed for measurement. Dry weights were recorded using a mass balance.

*Statistical Tests*

Two-factor ANOVA and mixed effects models were used to test the correlations between nighttime stomatal conductance, variables of competition (Hegyi indices, planting group), drought stressors (midday and predawn soil moisture, days in drought, predawn and midday water potentials) and growth aspects. Separate mixed effects models were run using data that reflected repeated measures from across all cottonwoods as well as within each planting group. For all two-factor ANOVAs, Tukey post-hoc tests were run to compare differences between planting groups. Akaike information criterion (AIC) tests were run on models to test the fit of each regression. We verified the assumptions of linear regressions by examining diagnostic plots, including Q-Q plots, of final models. All statistical analyses were conducted in R Studio using the “lme4”, “MuMIn”, and “lmerTest” packages (R Core Team; packages).

**Results**

*Does competition influence nighttime stomatal conductance?*

Competition for water was quantified for each tree using Hegyi competition indices. A low Hegyi score indicates that the focal tree is subject to less intense competition from its counterpart whereas a higher Hegyi index indicates a stronger competitive environment. In this experiment, Hegyi indices were well-partitioned between interspecific and intraspecific planting groups because the water birch competitors grew quickly and were quite large compared to the cottonwoods. However, there was substantial overlap between C-C and C-X-C indices, with the C-C group spanning over twice the range of the C-X-C range. The Hegyi indices for these intraspecific groups were not statistically different (*p*=0.068). The indices for each group were ranked as follows from least competition to most competition: C (solo trees; no competition) < C-X-C (0.023) < C-C (0.037) < C-B (0.107) (Fig. S2).

These planting groups of differing competition strengths differed in their nighttime stomatal conductance rates in well-watered conditions (*p*= 0.012; Fig. 1). Cottonwoods paired with water birch competitors had substantially lower average gsn values than the other competitive planting groups (C-B:C-C *p*=0.034; C-B:C-X-C *p*=0.009; C-B:C *p*= 0.366).

*Does decreased water supply influence stomatal behavior at night?*

Over time, decreases in watering frequency were reflected in significant decreases in nighttime and daytime soil moisture (day and night p <2 e-16; Fig. S3). As midday soil moisture decreased, gsn increased across planting groups (*p*=0.05; Fig. S4). Over the course of the graduated drought treatment, gsn increased significantly across planting groups as watering frequency decreased (*p*= 0.001; Fig. 2a). However, planting groups differed in their rates of gsn increase. Increases in gsn over time ranked as follows from least increase to greatest: C < C-B < C-C < C-X-C (Fig. 2a). During the well-watered period, Hegyi indices of competitive trees (*p*=0.001), planting group (*p*= 0.037), and competition type (interspecific, intraspecific, or no competition; *p*=0.008), were significant explanatory predictors of gsn. These patterns were maintained through the course of the drought treatment (*p*=0.01; *p*=0.007; *p*=0.004).

To investigate how gsn changed in competitive groups over time compared to the noncompetitive trees, we used the solo trees as a replicate-specific control reference for the gsn increase in competitive trees. When compared to the noncompetitive trees of their replicate, trees of competitive planting groups increased gsn over time at greater rates (*p*=0.035; Fig. 2b).. The amount in gsn difference from solo cottonwoods differed by planting group (*p*=0.001).

*Does nighttime stomatal conductance confer a competitive advantage in black cottonwoods?*

*Growth*

Within-species differences in size were not significant at the onset of the experiment. After the drought, plants subject to lower levels of competition (calculated from Hegyi indices) showed slightly smaller primary growth than trees under greater competition and slightly larger secondary growth, but these growth differences were not statistically significant (primary *p*=0.961; secondary *p*=0.156). There was also no significant relationship between gsn and primary or secondary growth in any treatment (primary *p*=0.304; secondary *p*=0.272; Fig. 3a). There were significant differences in whole tree leaf areas between all groups except between the intraspecific groups (*p*<2 e-16; Fig. S5). Solo cottonwoods had the largest average leaf areas with a mean of 0.552 m2. C-X-C trees averaged 0.457 m2. C-C trees averaged 0.425 m2, and C-B cottonwoods had a mean leaf area of 0.137 m2.

Higher values of gsn were associated with higher midday assimilation rates for all planting groups except C-C, which showed a non-significant negative relationship, although only the C-B relationship was statistically significant (*p*=0.001). Assimilation rates per mol m⁻² s⁻¹ of gsn were highest for C-B trees, followed by solo C, then C-X-C (Fig. 3b). To evaluate rates of gsn proportional to daytime stomatal conductance (gsd) rates, gsn measurements were divided by the gsd measurement of the following day. This variable was then compared to midday assimilation rates, which resulted in a negative correlation for all planting groups (*p*<2 e-16; Fig. S6).

*Drought Tolerance and Nighttime Transpiration*

(*p*=0.027 for the full mixed effects model; Fig. 4a). However, this relationship was not significant for any individual planting group (C *p*=0.688; C-C *p*=0.065; C-X-C *p*=0.844; C-B *p*=0.413). On every sampling day, the nighttime transpiration rates were substantial, averaging 36.7% of daytime transpiration rates. Of the trees’ total transpiration, 21.6% occurred at night. Nighttime transpiration as a proportion of total transpiration for each planting group ranked as follows from greatest to least: C-B > C-X-C > C-C > C (Fig. 4b).

**Discussion**

We tested here the role of how belowground competition for water affects nighttime stomatal conductance in a model tree species. We found that gsn tended to increase in competitive planting groups, although the relationship was not linear with competition strength because gsn tended to be highest in intermediate strengths of competition. Counterintuitively, we found that decreasing water availability tended to increase gsn with the strongest increases also apparent in intermediate competition groups (Fig. 2a, Fig. S4). Finally, we observed some evidence linking gsn to carbon uptake benefits, but limited evidence connecting gsn to. We conclude that competition very likely influenced gsn but extreme levels of competition (e.g. the C-B treatment) may have overwhelmed our hypothesized linear expected gradient of increasing gsn with increasing competition.

Intraspecific and interspecific trees’ average nighttime stomatal conductances diverged from those of the solo C group even without substantial water stress (Fig. 1). These patterns were conserved as water stress increased throughout the experiment. The significant difference between gsn ranges of the interspecific and intraspecific trees indicates that competition type did factor into stomatal behavior. However, contrary to the hypothesized expectation that plants subject to the most competition would have the highest nighttime stomatal conductances, average gsn ranges did not directly map to the competition indices for each planting group. Perhaps counterintuitively, the C-B cottonwoods showed the lowest rates of gsn of the cottonwoods. We hypothesize that this may be because their birch competitors had exceptionally high leaf areas that may have resulted in substantial above-ground competition for light and high daytime water use that led to persistent extreme water stress (as opposed to moderate water stress induced in most treatments by the end of the drought), as indicated by their low levels of soil moisture and high rates of mortality in the last 2 weeks of the experiment. The C-B cottonwoods may have thus had very small amounts of water in the root zone and closed their stomata more at night to limit water loss, consistent with nighttime stomatal closure during extreme water loss reported elsewhere (Ogle *et al.* 2012). This result indicates that there may be an upper limit of competition where the effects of light competition and severe water stress drive lower gsn.

The progressive drought treatment illustrated the divergent behaviors of the different planting groups with respect to gsn. When compared to solo cottonwoods, interspecific and intraspecific competitive groups all increased in gsn at greater rates over the course of the drought treatment (Fig. 2a). This suggests that nighttime stomatal conductance may be an adaptive behavior that is responsive to soil moisture content (Ogle *et al.* 2012). Although all competitive trees increased gsn more than the noncompetitive trees, trees planted in weak intraspecific competition (C-X-C) responded to decreasing water availability by increasing gsn at greater rates than trees under stronger competition for water in the root zone, counter to what was expected. This suggests that there may be an “intermediate” level of competition or root zone partitioning where elevated nocturnal stomatal conductance as a method of water sequestration is optimal. Compared to the other planting groups, the C-X-C planting group substantially increased gsn as predawn soil moisture decreased.

The smaller increases in gsn found in planting groups under strong competitive pressure could also be a result of ineffective separation of competitors’ root zones. Upon completion of the experiment, high levels of root zone overlap were observed in containers with C-C and C-B trees. Because of shared root zones, increasing rates of gsn may not have been an effective method for these trees to limit their competitors accessible water supply through hydraulic redistribution. C-X-C trees could have better utilized nighttime stomatal conductance as a means for competitive hydraulic redistribution because their roots did not occupy the same space as their competitors as closely as the trees in C-C and C-B groups. Thus, C-X-C plants could more effectively move water farther away from competitors’ roots. The patterns of high gsn rates among C-X-C trees under different drought variables supports the hypothesis that gsn was a competitive water-use strategy for the C-X-C trees. In this experiment, it appears that gsn increases with drought to a moderate stress level, then decreases in severe water stress treatments.

The observed gsn increases in response to moderate water limitation in this experiment are opposite of previously documented trends (Chowdhury *et al.*, 2022). Contrary to our findings, previous studies have reported decreasing gsn with extreme water stress (Cavender-Bares *et al.*, 2007; Cirelli et al., 2016). Increases in gsn throughout our drought experiment could potentially be the result of a leaf aging signal. Several studies have found decreasing stomatal responsiveness with age in *Populus* species (Reich, 1984; Marks & Lechowicz, 2007). This decrease in stomatal responsiveness could have resulted in greater stomatal apertures at night over time, despite increased water stress. One possible adaptive mechanism driving our observed increases in gsn with drought could relate to leaf cooling (Fig. S9). It has been proposed that leaf cooling through nighttime transpiration could benefit the plant by reducing carbon losses from nighttime respiration (Couple-Ledru *et al*., 2016, Wang *et al.*, 2021). As evaporative cooling lowers leaf temperatures, temperature-dependent respiration rates also decrease, which in turn saves carbon photosynthate for subsequent growth. Therefore, this carbon benefit could be maximized in water-stressed conditions by increasing transpiration-induced leaf cooling.

We found limited and mixed evidence between gsn and growth and plant performance metrics (Fricke, 2019). No significant correlation between gsn and growth metrics was observed in this experiment, although we note the duration of the experiment may partially account for this (Kupper *et al.*, 2021). Solo C trees had the largest shoot biomass and leaf area measurements of all the trees, indicating that competition likely limited tree growth in competition treatments. When trees exhibited high gsn, they tended to have higher rates of photosynthesis the following day (Fig. 3b), which is consistent with prior research of gsn across species (Yu *et al.* 2019). This pattern may be due to leaf or stomatal anatomy that favors stomatal conductance at night and during the day (Chieppa *et al.* 2021). When assimilation rate was compared to gsn/gsd, a negative relationship indicated that higher rates of gsn relative to gsd were correlated with less photosynthesis (*p*<2 e-16; Fig. S6). In sum, at least in this short-term experimental setting, we found relatively little evidence to suggest that higher rates of gsn during drought confer a growth advantage over competitors, although gsn may aide plants in surviving water stress.

Thus, it is unclear if nighttime transpiration had clear impacts on this redistribution. This pattern may be consistent with the ‘two-water-worlds’ hypothesis which postulates that there are two distinct pools of soil water: bound and mobile (McDonell, 2014). The bound water pool occurs within a plant’s root zone and is assumed to be taken up by the plant, while mobile water percolates to groundwater

Plants of every group maintained remarkably high levels of nighttime transpiration through the drought treatment (~37% daytime E rates, 15-25% of total daily E), with increasing levels of water transpired even as soil moisture became very limited (Fig. 4b). Increased whole-tree nighttime transpiration in water-limited conditions occurred most in C-B trees, which were subject to the most competition. C-B trees transpired significantly lower amounts of water at night than the other planting groups (*p*<0.0001), but they had the highest proportions of water transpired at night compared to daytime transpiration (*p*=0.025; Fig. S8). In fact, nighttime transpiration rates proportional to daytime transpiration rates followed competition patterns, with C-B cottonwoods exhibiting the highest EN rates relative to ED, and solo C cottonwoods the lowest EN:ED across the course of the experiment. This points to a correlative relationship between competition and nighttime water loss.

Competition has substantial impacts on water and carbon fluxes in plant communities. For example, competition has been shown to alter trees’ stomatal sensitivity to water potential and vapor pressure deficit (Zenes *et al.,* 2021). Nocturnal transpiration is not negligible, but rather a substantial portion of a tree’s daily water loss (Snyder *et al.*, 2003; Dawson *et al.*, 2007; Novick *et al.*, 2009; Zeppel *et al.*, 2014; Padrón *et al.*, 2020), including up to 20-40% in this study. Zeppel *et al.* investigated the effects of environmental controls on nighttime stomatal conductance and subsequent effects; they similarly found nocturnal transpiration rates up to 40% of daytime rates (2014). The intraspecific competitive groups of this experiment showed relatively high EN rates and leaf areas, meaning that they transpired significantly large portions of water at night. This trend suggests that trees with moderate competition are likely very large contributors to nighttime water loss.

The patterns of competitive stomatal behavior at night observed in this experiment have considerable implications for models of transpiration and water cycling in ecosystems. Many current ecosystem models fail to account for dynamic nighttime water loss trends, either assuming low and/or constant rates of gsn and EN or ignoring nocturnal water loss completely (Lombardozzi *et al.*, 2017; Wang *et al.*, 2021). Improvements to these models will inform projections of water cycling as the climate changes. This research will also contribute to the growing interest in genetic modifications to improve water-use efficiency of crops and combat yield losses due to cropland aridification.

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**Author contributions**

KET and WRLA designed the study and KET collected the data; KET performed data analysis with inputs from WRLA; KET and WRLA wrote the manuscript.

**Data Availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Conflict of Interest**

None declared.

**References**

Anderegg, L.D.L., Anderegg, W.R.L., Abatzoglou, J., Hausladen, A.M., & Berry, J.A. (2013). Drought characteristics’ role in widespread aspen forest mortality across Colorado, USA. *Global Change Biology*, 19: 1526–1537.

Anderegg, W. R. L., Wolf, A., Arango-Velez, A., Choat, B., Chmura, D. J., Jansen, S., Kolb, T., Li, S., Meinzer, F. C., Pita, P., Resco de Dios, V., Sperry, J. S., Wolfe, B. T., & Pacala, S. (2018). Woody plants optimise stomatal behaviour relative to hydraulic risk. *Ecology Letters*, 21(7), 968-977. https://doi.org/10.1111/ele.12962.

Barbour, M.M., Cernusak, L.A., Whitehead, D., Griffin, K.L., Turnbull, M.H., Tissue, D.T., & Farquhar, G.D. (2005). Nocturnal stomatal conductance and implications for modeling d18O of leaf-respired CO2 in temperate tree species. *Funct Plant Biol*, 32: 1107–1121.

Ball, J. T., Woodrow, I. E., & Berry, J. A. (1987). A model predicting stomatal conductance and its contribution to the control of photosynthesis under different environmental conditions. In Progress in photosynthesis research (pp. 221-224). *Springer*, Dordrecht.

Bassman, J. H., & Zwier, J.C. (1991). Gas exchange characteristics of *Populus trichocarpa*, *Populus deltoides* and *Populus trichocarpa* × *P. deltoides* clones. *Tree Physiology*, Volume 8, Issue 2, 145–159. https://doi.org/10.1093/treephys/8.2.145

Berry, J.A., Beerling, D.J., & Franks, P.J. (2010). Stomata: key players in the Earth system, past and present. *Current Opinion in Plant Biology,* 13: 232–239.

Bonan, G. (2015). Ecological climatology: concepts and applications. *Cambridge University Press*.

Brodribb, T.J., & McAdam, S.A.M. (2017). Evolution of the stomatal regulation of plant water content. *Plant Physiology,* 174: 639–649.

Buckley, T.N. (2005). The control of stomata by water balance*. New Phytologist,* 168: 275–292.

Caird, M. A., Richards, J. H., & Donovan, L. A. (2007). Nighttime stomatal conductance and transpiration in C3 and C4 plants. *Plant physiology*, *143*(1), 4-10.

Casa, R., & Jones, H.G. (2002). Retrieval of crop canopy properties: a comparison between model inversion from hyperspectral data and image classification. Proceedings NERC meeting ‘Field spectral measurements in remote sensing’. *Southampton, UK*, 15–16.

Cavender-Bares, J., Sack, L., & Savage, J. (2007). Atmospheric and soil drought reduce nocturnal conductance in live oaks. *Tree Physiology*, 27(4), 611-620.

Chapin, F. Stuart., et al. (2012). Principles of Terrestrial Ecosystem Ecology. *Springer*.

Chaves, M.M., Costa, J.M., Zarrouk, O., Pinheiro, C., Lopes, C.M., & Pereira, J.S. (2016). Controlling stomatal aperture in semi-arid regions—The dilemma of saving water or being cool? *Plant Science*, Volume 251: 54-64, 0168-9452. https://doi.org/10.1016/j.plantsci.2016.06.015.

Chieppa, J., Brown, T., Giresi, P., Juenger, T.E., Resco de Dios, V., Tissue, D.T., & Aspinwall, M.J. (2021). Climate and stomatal traits drive covariation in nighttime stomatal conductance and daytime gas exchange rates in a widespread C4 grass. *New Phytol*, 229: 2020-2034. <https://doi.org/10.1111/nph.16987>

Chowdhury, F.I., Arteaga, C., Alam, M.S., Alam, I., Resco de Dios, V. (2022). Drivers of nocturnal stomatal conductance in C3 and C4 plants. *Science of The Total Environment*, Volume 814, 151952. https://doi.org/10.1016/j.scitotenv.2021.151952.

Christman, M.A., Richards, J.H., McKay, J.K., Stahl, E.A., Juenger, T.E., & Donovan, L.A. (2008). Genetic variation in Arabidopsis thaliana for night-time leaf conductance. *Plant, Cell & Environment*, 31: 1170–1178.

Christman, M.A., Donovan, L.A. & Richards, J.H. (2009). Magnitude of nighttime transpiration does not affect plant growth or nutrition in well-watered *Arabidopsis*. *Physiologia Plantarum*, 136: 264- 273. https://doi.org/10.1111/j.1399-3054.2009.01216.x

Cirelli, D., Equiza, M., Lieffers, V., & Tyree, M. (2015). Populus species from diverse habitats maintain high night-time conductance under drought. *Tree physiology*, 36. 10.1093/treephys/tpv092.

Costa, J.M., Monnet, F., Jannaud, D., Leonhardt, N., Ksas, B., Reiter, I.M., Pantin, F., & Genty, B. (2015). Open all night long: the dark side of stomatal control 1. *Plant Physiology,* 167: 289–294.

Coupel-Ledru, A., Lebon, E., Christophe, A., Gallo, A., Gago, P., Pantin, F., Doligez, A., & Simonneau, T. (2016). Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proceedings of the National Academy of Sciences of the United States of America*, 113(32), 8963– 8968. https://doi.org/10.1073/pnas.1600826113

Cramer, M.D., Hoffmann, V., & Verboom, G.A. (2008). Nutrient availability moderates transpiration in *Ehrharta calycina*. *New Phytologist,* 179: 1048-1057*,* https://doi.org/10.1111/j.1469-8137.2008.02510.x

Daley, M.J., & Phillips, N.G. (2006). Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiology,* 26: 411–419

Dawson, T.E. (1993). Hydraulic lift and water use by plants: implications for water balance, performance and plant-plant interactions. *Oecologia* 95, 565–574. https://doi.org/10.1007/BF00317442

Dawson, T.E., Burgess, S.S.O, Tu, K.P, Oliveira, R.S., Santiago, L.S., Fisher, J.B., Simonin, K.A., & Ambrose, A.R. (2007). Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiology*, Volume 27, Issue 4: 561–575. https://doi.org/10.1093/treephys/27.4.561

Eller, F., Jensen, K., & Reisdorff, C. (2017). Nighttime stomatal conductance differs with nutrient availability in two temperate floodplain tree species. *Tree Physiology*, Volume 37, Issue 4: 428–440. <https://doi.org/10.1093/treephys/tpw113>

Fricke, W. (2019). Night-Time Transpiration – Favouring Growth? T*rends in Plant Science*, Volume 24, Issue 4: 311-317. https://doi.org/10.1016/j.tplants.2019.01.007.

Gutschick, V.P. (2016) Leaf Energy Balance: Basics, and Modeling from Leaves to Canopies. In: Hikosaka K., Niinemets Ü., Anten N. (eds) Canopy Photosynthesis: From Basics to Applications. Advances in Photosynthesis and Respiration (Including Bioenergy and Related Processes), vol 42. *Springer*. https://doi.org/10.1007/978-94-017-7291-4\_2

Hegyi, F. (1974). A simulation model for managing jack-pine stands simulation. *RoyalColl. For, Res. Notes*, 30, 74-90.

Hoshika, Y., Osada, Y., de Marco, A., Penuelas, J., & Paoletti, E. (2018). Global diurnal and nocturnal parameters of stomatal conductance in woody plants and major crops. *Global Ecology and Biogeography,* 27: 257–275.

Howard, A. R., & Donovan, L. A. (2007). Helianthus nighttime conductance and transpiration respond to soil water but not nutrient availability. *Plant physiology*, 143(1), 145–155. https://doi.org/10.1104/pp.106.089383

Huang, C. W., Domec, J. C., Ward, E. J., Duman, T., Manoli, G., Parolari, A. J., & Katul, G. (2017). The effect of plant water storage on water fluxes within the coupled soil–plant system. *New Phytologist*, 213(3), 1093-1106.

Kerr, K., Zenes, N., Trugman, A., & Anderegg, W.R.L. (2019). Competition and functional traits mediate tree seedling drought response physiology and biomass allocation. *Unpublished manuscript, University of Utah, Salt Lake City, UT*.

Knoerr, K.R. & Gay, L.W. (1965). Tree Leaf Energy Balance. *Ecology*, 46: 17-24. https://doi.org/10.2307/1935254

Kupper, P., Rohula, G., Saksing, L., Sellin, A., Lõhmus, K., Ostonen, I., Helmisaari, H.S., & Sõber, A. (2012). Does soil nutrient availability influence night‐time water flux of aspen saplings? *Environmental and Experimental Botany*, 82: 37–42.

Kupper, P., Kaasik, A., Kukumägi, M., Rohula-Okunev, G., Rusalepp, L., Sõber, A. (2021) Predawn leaf conductance depends on previous day irradiance but is not related to growth in aspen saplings grown under artificially manipulated air humidity. *Functional Plant Biology* 48, 422-433. https://doi.org/10.1071/FP20254

Lambers, H., Chapin, F. S., & Pons, T. L. (2006). *Plant physiological ecology (Vol. 1)*. New York: Springer.

Li, S., Meinzer, F.C., Pita, P., Resco de Dios, V., Sperry, J.S., Wolfe, B.T. & Pacala, S. (2018). Woody plants optimise stomatal behaviour relative to hydraulic risk. *Ecol Lett*, 21: 968-977. 10.1111/ele.12962

Lombardozzi DL, Zeppel MJB, Fisher RA, Tawfik A. (2017). Representing nighttime and minimum conductance in CLM4.5: global hydrology and carbon sensitivity analysis using observational constraints. *Geoscientific Model Development,* 10: 321–331.

Marks, C. O., & Lechowicz, M. J. (2007). The ecological and functional correlates of nocturnal transpiration. *Tree Physiology*, 27(4), 577-584.

McAdam, S.A.M., & Brodribb, T.J. (2015). The evolution of mechanisms driving the stomatal response to vapor pressure deficit. *Plant Physiology*, 167: 833– 843.

McDonnell. (2014). The two water worlds hypothesis: ecohydrological separation of water between streams and trees? *Wiley Interdisciplinary Reviews. Water*, 1(4), 323–329.

Muir, C.D. (2019). tealeaves: an R package for modelling leaf temperature using energy budgets. *AoB PLANTS,* 11: plz054. 0.1093/aobpla/plz054

Neumann, R.B., Cardon, Z.G., Teshera-Levye, J., Rockwell, F.E., Zwieniecki, M.A., & Holbrook, N.M. (2014). HR model with night‐time transpiration fits data. *Plant Cell Environ*, 37: 899-910. https://doi.org/10.1111/pce.12206

Novick, K.A., Oren, R., Stoy, P.C., Siqueira, M.B.S., Katul, G.G. (2009). Nocturnal evapotranspiration in eddy-covariance records from three co-located ecosystems in the Southeastern U.S.: implications for annual fluxes. *Agricultural and Forest Meteorology*, 149: 1491–1504.

Ogle, K., Lucas, R.W., Bentley, L.P., Cable, J.M., Barron-Gafford, G.A., Griffith, A., Ignace, D., Jenerette, G.D., Tyler, A., & Huxman, T.E., *et al.* (2012). Differential daytime and night-time stomatal behavior in plants from North American deserts. *New Phytologist,* 194: 464–476.

Oliveira, E., Ruiz, H., Hugo, V., Paulo, F., Costa, F., & Almeida, I. (2010). Nutrient supply by mass flow and diffusion to maize plants in response to soil aggregate size and water potential. *Revista Brasileira De Ciencia Do Solo - REV BRAS CIENC SOLO,* 34. 10.1590/S0100-06832010000200005

Padrón, R. S., Gudmundsson, L., Michel, D., & Seneviratne, S. I. (2020). Terrestrial water loss at night: global relevance from observations and climate models. *Hydrology and Earth System Sciences*, 24(2), 793-807.

Pathan, A.K., Bond, J., & Gaskin, R.E. (2010). Sample preparation for SEM of plant surfaces. *Materials Today*, Volume 12, Supplement 1: 32-43. 1369-7021. https://doi.org/10.1016/S1369-7021(10)70143-7

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., & Postma, J. A. (2012). Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, 39(11), 839-850.

R Core Team (2022). R: A language and environment for statistical

computing. R Foundation for Statistical Computing, Vienna, Austria.

URL https://www.R-project.org/.

Reich, P. B. (1984). Loss of Stomatal Function in Ageing Hybrid Poplar Leaves. *Annals of Botany*, 53(5), 691–698, https://doi.org/10.1093/oxfordjournals.aob.a086734

Resco de Dios, V., Loik, M. E., Smith, R., Aspinwall, M. J., & Tissue, D. T. (2016). Genetic variation in circadian regulation of nocturnal stomatal conductance enhances carbon assimilation and growth. *Plant, Cell & Environment*, 39(1), 3-11.

Resco de Dios, V., Chowdhury, F.I., Granda, E., Yao, Y. & Tissue, D.T. (2019). Assessing the potential functions of nocturnal stomatal conductance in C3 and C4 plants. *New Phytol*, 223: 1696-1706. https://doi.org/10.1111/nph.15881

Scharwies, J.D., Dinneny, J.R. (2019). Water transport, perception, and response in plants. *J Plant Res* 132: 311–324. [https://doi.org/10.1007/s10265-019- 01089-8](https://doi.org/10.1007/s10265-019-%2001089-8)

Schoppach, R., Sinclair, T.R., Sadok, W. (2020). Sleep tight and wake-up early: nocturnal transpiration traits to increase wheat drought tolerance in a Mediterranean environment. *Functional Plant Biology* 47, 1117-1127. https://doi.org/10.1071/FP20044

Scholz, F. G., Bucci, S. J., Goldstein, G., Meinzer, F. C., Franco, A. C., & Miralles-Wilhelm, F. (2007). Removal of nutrient limitations by long-term fertilization decreases nocturnal water loss in savanna trees. *Tree physiology*, 27(4), 551– 559. https://doi.org/10.1093/treephys/27.4.551

Snyder, K.A., Richards, J.H., & Donovan, L.A. (2003). Night-Time Conductance in C3 and C4 Species: Do Plants Lose Water at Night? *Journal of Experimental Botany,*  Volume 54, Issue 383: 861-865. https://doi.org/10.1093/jxb/erg082

Sparks, J.P., & Black, R.A. (1999). Regulation of water loss in populations of *Populus trichocarpa*: the role of stomatal control in preventing xylem cavitation. *Tree Physiology*, Volume 19, Issue 7: 453–459, https://doi.org/10.1093/treephys/19.7.453

Strong, T., & Hansen, E.A. (1991). Response of three Populus species to drought. Research Paper NC-302. *U.S. Dept. of Agriculture, Forest Service, North Central Forest Experiment Station*

Vialet-Chabrand, S., & Lawson, T. (2019). Dynamic leaf energy balance: deriving stomatal conductance from thermal imaging in a dynamic environment, *Journal of Experimental Botany*, Volume 70, Issue 10: 2839–2855. https://doi.org/10.1093/jxb/erz068

Wang, Q., Gao, J., Zhao, P., Zhu, L., Ouyang, L., Ni, G., & Zhao, X. (2018). Biotic- and abiotic-driven variations of the night-time sap flux of three co-occurring tree species in a low subtropical secondary broadleaf forest. *AoB PLANTS*, Volume 10, Issue 3. https://doi.org/10.1093/aobpla/ply025

Wang, Y., Anderegg, W.R.L., Venturas, M.D., Trugman, A.T., Yu, K. and Frankenberg, C. (2021), Optimization theory explains nighttime stomatal responses. *New Phytol*, 230: 1550-1561. https://doi.org/10.1111/nph.17267

Weemstra, M., Eilmann, B., Sass-Klaassen, U.G.W., & Sterck, F. J. (2013). Summer Droughts limit tree growth across 10 temperate species on a productive forest site. *Forest Ecology and Management*, 306: 142- 149.https://doi.org/10.1016/j.foreco.2013.06.007

Wong, S., Cowan, I. & Farquhar, G. (1979). Stomatal conductance correlates with photosynthetic capacity. *Nature*, 282, 424–426. https://doi.org/10.1038/282424a0

Yu, T., Feng, Q., Si, J., et al. (2018). Depressed hydraulic redistribution of roots more by stem refilling than by nocturnal transpiration for Populus euphratica Oliv. in situ measurement. *Ecology and Evolution,* (5):2607-2616. DOI: 10.1002/ece3.3875.

Yu, K., Goldsmith, G.R., Wang, Y. & Anderegg, W.R.L. (2019). Phylogenetic and biogeographic controls of plant nighttime stomatal conductance. *New Phytol*, 222: 1778-1788. <https://doi.org/10.1111/nph.15755>

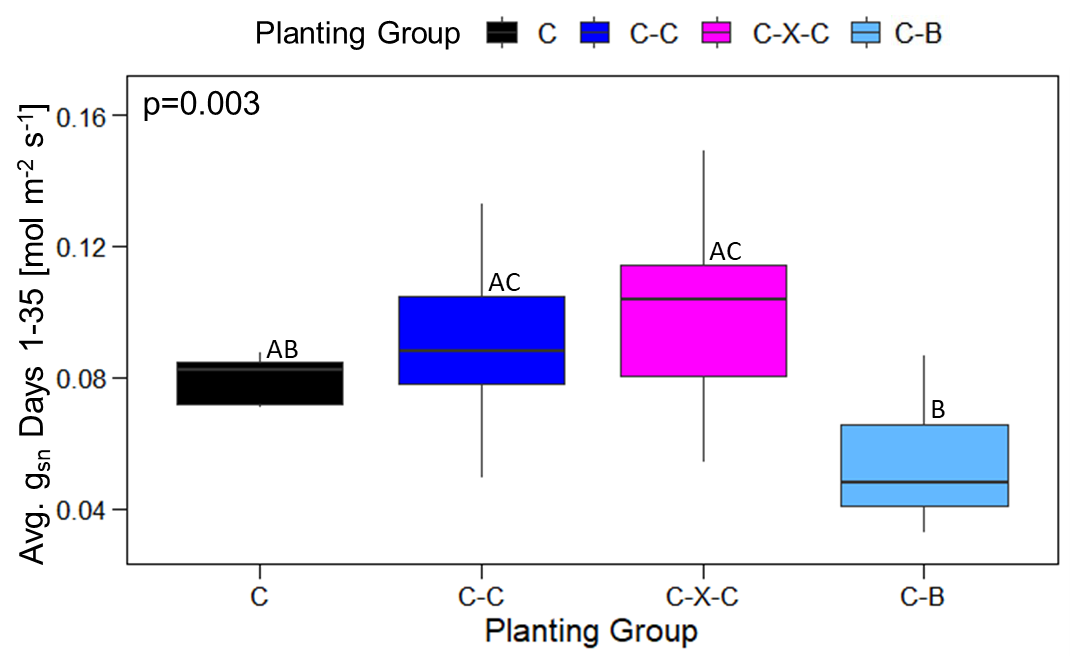
Zenes, N., Kerr, K., Trugman, A., Anderegg, W.R.L. (2020). Competition and Drought Alter Optimal Stomatal Strategy in Tree Seedlings. *Frontiers in Plant Science,* 11. 478. 10.3389/fpls.2020.00478.

Zeppel, M., Tissue, D., Taylor, D., Macinnis-Ng, C., & Eamus, D. (2010). Rates of nocturnal transpiration in two evergreen temperate woodland species with differing water-use strategies. *Tree Physiology*, Volume 30, Issue 8: 988– 1000. https://doi.org/10.1093/treephys/tpq05

Zeppel, M., Lewis, J., Chaszar, B., Smith, R., Medlyn, B., Huxman, T., & Tissue, D. (2012). Nocturnal stomatal conductance responses to rising [CO2], temperature and drought. *The New Phytologist,* 193. 929-38. 10.1111/j.1469- 8137.2011.03993.x.

Zeppel, M., Lewis, J.D., Phillips, N.G., & Tissue, D.T. (2014). Consequences of nocturnal water loss: a synthesis of regulating factors and implications for capacitance, embolism and use in models. *Tree Physiology*, Volume 34, Issue 10: 1047–1055, https://doi.org/10.1093/treephys/tpu089

**Figures and Figure Legends**

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**Figure 1** Distribution of average gsn for each tree during the first 35 days (i.e. well-watered conditions) of the experiment are displayed in boxes for each planting group. Letters indicate significant differences between planting groups and the between the binned intraspecific (C-C and C-X-C) competitive group.

Chart, scatter chart

Description automatically generated

**Figure 2** Competitive gsn change over time. **a)** The difference in gsn increase over time between competitive and non-competitive groups. Each data point represents the gsn of a tree in a given planting group for a single night. Solid lines represent significant relationships (α=0.05) and dashed lines represent non-significant relationships. The p-value shown represents the significance of the full mixed effects model across all planting groups. **b)** For each day of measurement, the gsn value recorded for each solo cottonwood was subtracted from the recorded gsn values of each competitive tree in its respective replicate. Replicate gsn differences were averaged for each planting group. Note that replicate- specific measurements were averaged rather than bulk gsn averages for each group. The p-value shown represents the significance of the full mixed effects model across all planting groups.

Chart, scatter chart

Description automatically generated

**Figure 3 a)** Each dot represents the average gsn and height increase for each tree in the experiment. Dashed lines represent the non-significant relationship between gsn and primary growth for each planting group. The p-value shown represents the significance of the full mixed effects model across all planting groups. **b)** Assimilation rates (A) compared to nighttime stomatal conductance for each planting group. Each dot represents the midday assimilation rate for one tree of a given planting group compared to its predawn stomatal conductance value for that day. Lines represent the relationship between gsn and A for each planting group. Solid lines represent significant relationships (α=0.05), and dashed lines represent non-significant relationships. The p-value shown represents the significance of the full mixed effects model across all planting groups.

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**Figure 4** **a)** Minimum leaf water potential levels of trees plotted against the value of their slope in a regression where gsn is the dependent variable of the day of the experiment. Thus, higher values on the x-axis represent trees that increased gsn at a greater rate over the course of the experiment. Each dot represents the slope of one tree and its most extreme minimum leaf water potential. Dashed lines represent the non-significant relationship between gsn increase rate and minimum water potential for each planting group. The p-value shown represents the significance of the full mixed effects model across all planting groups. **b)** Dots represent nighttime transpiration (EN) as a proportion of total transpiration in a 24-hour period plotted against soil moisture for each tree. Lines represent the relationship between EN proportion and soil moisture for each group and across all groups (purple line). Solid lines represent significant relationships (α=0.05) and dashed lines represent non-significant relationships. The p-value shown represents the significance of the full mixed effects model across all planting groups.

Chart, line chart

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**Figure 5** Each line represents the isotopic enrichment of leaf water for a sampled tree over the course of the 72-hour pulse-chase experiment. Line color represents the strength of the “water pull” of the sampled tree from the labeled competitor tree. Red lines indicate a sampled tree with a weak water pull relative to its competitor, with negative values meaning that it transpired less water than its competitor. Blue lines indicate a sampled tree that transpired much more than its competitor and has a relatively strong water pull.