

Stomatal behavior moderates water cost of CO₂ acquisition for 21 boreal and temperate species under experimental climate change

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Abstract

The linkage of stomatal behavior with photosynthetic processes is critical to understanding water and carbon cycles under global change. The slope (g_1) of stomatal conductance (g_s) versus CO₂ assimilation (A_{net}) serves as a proxy of the marginal water cost of carbon acquisition and the trade-off between carbon gain and water loss. Here we use g_1 to assess species differences in the response of stomatal behavior to experimental climate change manipulations, asking whether generalizable patterns exist across species and climate contexts. A total of 17,727 A_{net} - g_s measurements made in a long-term open-air experiment under ambient and +3.3°C warming, and ambient and ~40% summer rainfall reduction provided > 2,700 estimates of g_1 across 21 boreal and temperate tree species. All species became more conservative in their water use (lower g_1) in warming and/or reduced rainfall treatments because of lower soil moisture. In contrast to these phenotypic responses, species from warmer and drier habitats tended to have slightly higher g_1 and to be the least sensitive to the decrease in soil water. Overall, both warming and rainfall reduction consistently made stomatal behavior more conservative in terms of water loss per unit carbon gain across 21 species and a decade of experimental observation.

Stomatal behavior moderates water cost of CO₂ acquisition for 21 boreal and temperate species under experimental climate change

Short informative title:

Experimentally manipulated temperature and summer rainfall affects stomatal behavior

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Summary statement: Climate change may influence the trade-off between water loss and carbon gain of forest trees. Experimental warming and rainfall manipulation in southern boreal forest led to elevation of soil water deficits causing all 21 tree species studied to become more conservative in their use of water.

Abstract

The linkage of stomatal behavior with photosynthetic processes is critical to understanding water and carbon cycles under global change. The slope (g_1) of stomatal conductance (g_s) versus CO₂ assimilation (A_{net}) serves as a proxy of the marginal water cost of carbon acquisition and the trade-off between carbon gain and water loss. Here we use g_1 to assess species differences in the response of stomatal behavior to experimental climate change manipulations, asking whether generalizable patterns exist across species and climate contexts. A total of 17,727 A_{net} - g_s measurements made in a long-term open-air experiment under ambient and +3.3°C warming, and ambient and ~40% summer rainfall reduction provided > 2,700 estimates of g_1 across 21 boreal and temperate tree species. All species became more conservative in their water use (lower g_1) in warming and/or reduced rainfall treatments because of lower soil moisture. In contrast to these phenotypic responses, species from warmer and drier habitats tended to have slightly higher g_1 and to be the least sensitive to the decrease in soil water. Overall, both warming and rainfall reduction consistently made stomatal behavior more conservative in terms of water loss per unit carbon gain across 21 species and a decade of experimental observation.

Key words:

B4WarmED, boreal-temperate ecotone, drought, water-use efficiency, stomatal behavior, warming, Unified Stomatal Optimization

Introduction

The rate of photosynthesis is dynamically regulated by stomatal responses to environmental factors (e.g., temperature, water availability) in concert with leaf biochemical capacities. In doing so, stomata influence the marginal water cost of carbon acquisition at the leaf scale and more broadly affect the coupling between carbon and water cycles, which is especially important in light of a changing climate (Damour *et al.*, 2010; Duursma *et al.*, 2013; Gimeno *et al.*, 2016; Hérault *et al.*, 2013). However, it is not well understood whether the water-carbon trade-off will shift towards a more profligate or more conservative water use strategy in a changing climate. To address this knowledge gap, we used 11 years of data from an ecologically realistic long-term climate change experiment to test whether and how the trade-off between water loss and carbon gain changes with modest experimental warming and rainfall reduction in 21 tree species at the boreal-temperate forest ecotone.

One of the long-standing hypotheses about stomata is that their behavior follows optimization theories (Cowan, Farquhar, 1977; Medlyn *et al.*, 2011; Manzoni *et al.*, 2013; Wolf *et al.*, 2016) with stomatal behavior often modeled as a gain-cost trade-off that maximizes carbon gain through variation of stomatal conductance in response to environmental constraints (e.g., water availability and temperature). Cowan and Farquhar (1977) proposed a mechanistic model where the role of stomata is to maximize carbon acquisition (A) at the lowest water loss through transpiration (E), described as a marginal water cost of carbon gain (λ). Therefore, optimal stomatal behavior minimizes the integrated sum of the following expression – that effectively defines the marginal water cost of carbon gain – and can be written as:

$$\lambda = \frac{E}{A} \quad (1)$$

where:

A – photosynthesis,

E – transpiration,

λ – is a parameter representing the marginal water cost of carbon gain.

However, fully mechanistic models like the one proposed by Cowan and Farquhar (1977) (equation 1) are difficult to parameterize with typically measured field data, leading to models using proxies to represent λ . Following many important predecessors (e.g., Ball, Woodrow & Berry *et al.*, 1987; Leuning, 1995, and others), Medlyn *et al.*, (2011) developed the Unified Stomatal Optimization model (USO), that describes stomatal conductance as a function of carbon assimilation and environmental conditions ($\frac{A}{C_a \sqrt{D}}$ where: A – is net assimilation rate, C_a – is atmospheric CO₂ concentration at the leaf surface, D – is vapor pressure deficit (kPa) at the leaf surface). As derived by Medlyn *et al.*, (2011) the slope (g_1) of the USO model gains biological meaning by combining equations of standard leaf diffusion with optimum leaf internal CO₂ concentration (C_i) to link the g_1 parameter with λ (Arneeth *et al.*, 2002); for detailed description of the model see Medlyn *et al.*, 2011 and supplement). The slope of the USO model (g_1 parameter) is directly proportional to the combination of λ and CO₂ compensation point (Γ^*):

$$g_1 \propto \sqrt{\Gamma^* * \lambda} \quad (2)$$

where:

Γ^* – is CO₂ compensation point,

λ – is marginal water cost of carbon gain.

This linkage allows interpretation of the slope parameter g_1 , where low values represent conservative water use while higher g_1 indicates more profligate use, and the development of testable hypotheses, including about the response of stomatal conductance to novel environmental conditions such as elevated temperatures and reduced water availability.

Thus, the g_1 parameter should increase with λ and Γ^* , assuming that C_a is much larger than Γ^* (Ehleringer, 2005) and that stomatal behavior optimizes for RuBP (Ribulose 1,5-biphosphate) regeneration limitation but not for Rubisco limitation of photosynthesis (Outlaw *et al.*, 1979; Outlaw & De Vlieghere-He, 2001; Shimazaki, 1989). Because g_1 is proportional to the $\sqrt{\Gamma^* * \lambda}$ term it can be assumed that it will be sensitive to water availability, temperature, and CO₂ concentration, and will vary as those do. Thus, it is expected that g_1 will decrease with decreasing water availability, and because Γ^* is exponentially dependent on temperature (Bernacchi *et al.*, 2001), g_1 should increase with increasing growth temperature.

Despite theoretical predictions summarized above, and many studies of photosynthesis and stomatal conductance in relation to climate, empirical evidence about the trade-off between carbon gain and water loss remain limited (Lin *et al.*, 2015; Medlyn *et al.*, 2016) especially concerning individual species representing different biomes, plant types, and responses to potential future climates. Moreover, while there has been considerable research on the impacts of single climatic drivers on stomatal behavior, we lack research on multiple climatic drivers and multiple species (Atkinson & Urwin, 2012; Stevens *et al.*, 2021). As a result, the effects of climate warming and water availability are highly uncertain and poorly represented in many models (from leaf to global scale) and in particular are not well parameterized in terms of drought sensitivity. By changing evaporative demand and/or soil moisture, both temperature and rainfall variation might change the optimal water cost and thus stomatal conductance and net photosynthetic rates. Additionally, given that species differ in their adaptations and sensitivity to both warm temperatures and limited water availability, we might also expect plants to differ systematically in terms of their stomatal behavior, water use efficiency (WUE), and how they modulate these as air and soil moisture conditions vary as pushed by a changing climate (Medlyn *et al.*, 2011).

To address this knowledge gap, we evaluate the g_1 parameter for a suite of temperate and boreal tree species grown in a realistic experimental setting that mimics climate change drivers (i.e., warming and rainfall reduction). Our main goals were to explore whether and how (i) stomatal behavior changes (e.g., plants decrease g_1 indicating more conservative water use) in response to climate warming or soil water deficits induced by experimental treatments; (ii) stomatal behavior varies with species identity, drought tolerance, and biome association; and to determine (iii) whether there are generalizable patterns across species, their

associations (e.g., biome) and environmental conditions: i.e. do species differ in response to climate treatments, and are responses to warming and rainfall reduction additive or interactive? To achieve those goals, we addressed the following issues and hypotheses.

First, consistent with optimization theory that predicts a decrease of λ with declining water availability, we hypothesize that g_1 (which is proportional to λ , see equation 2) will decrease as soil moisture declines (e.g., Lu *et al.*, 2016). This leads to *H1: g_1 decreases with reduced rainfall for all species*.

Second, equation 2 suggests that g_1 will increase with warming because; i) it is proportional to Γ^* which is dependent on temperature (Bernacchi *et al.*, 2001), ii) temperature induced changes in wood density will affect hydraulic conductivity (H  roult *et al.*, 2013; McCulloh *et al.*, 2016), and iii) increasing temperature lowers the viscosity of water making it cheaper to transport (Yamamoto, 1995). However, we hypothesize that soil drying induced by warming treatments will cause g_1 to decrease. Thus, the ultimate influence of warming will depend upon a balance between the direct influences of temperature that should increase g_1 and the indirect influence of temperature on soil moisture that should decrease g_1 . We expect the direct warming effects on g_1 to be modest at best, and therefore the response of g_1 to warming to be dominated by soil moisture (Reich *et al.*, 2018). This leads to *H2: g_1 will decrease with climate warming due to soil moisture reduction induced by elevated temperature*.

The interactions of warming and rainfall reduction do not easily lend themselves to a simple hypothesis, due to the complexity of both direct and indirect effects of elevated temperature on factors that might influence g_1 (such as leaf temperature and soil moisture), and uncertainty about whether those effects are contingent on rainfall levels. However, because the effects of warming and reduced rainfall do not have a consistent interaction on VWC (Volumetric Water Content in soil) at our study sites (data not shown), we hypothesize

H3: reduced rainfall and warming will have an additive effects on g_1 because the primary mechanism of both warming and reduced rainfall effects on g_1 will be via the same pathway, of reduced VWC.

Modeling shows that plants can be differentially acclimated to low soil moisture in ways consistent with so-called aniso- and iso-hydric behavior (i.e., slow vs. fast decline of g_s in response to changing environmental conditions) (M  rad *et al.*, 2019). Since plants in warmer regions tend to experience greater evaporative demand and soil water deficits during periods of low precipitation, we expect them to have more isohydric behavior and conservative water use strategies and thus we hypothesize *H4: that in species adapted to either drier and/or warmer conditions g_1 will be both lower on average and less responsive to varying VWC than in species adapted to more mesic or cooler conditions*.

To test these hypotheses, we collected A_{net} and g_s data over the span of 11 years (2009–2019) in a warming and rainfall manipulation experiment (B4WarmED; Boreal Forest Warming at an Ecotone in Danger, e.g., Reich *et al.*, 2015, 2018; Rich *et al.*, 2015). This data set consists of 17,727 measurements that were collected from roughly mid-June to the end of September in each growing season during two to five independent survey campaigns each 1–2 weeks long. A minority of these data have been used in prior publications (Reich *et al.*, 2015, 2018), none of which examined questions of marginal water costs of carbon gain.

Materials and Methods

Site description and experimental design

This research was conducted *in situ* at the two sites of the B4WarmED experiment in northern Minnesota, USA established in 2008. The sites are located at the Cloquet Forestry Center (CFC, 46  40'46" N, 92  31'12" W, 382 m a.s.l.) near Cloquet, MN and the Hubachek Wilderness Research Center (HWRC, 47  56'42" N, 91  45'29" W, 415 m a.s.l.) near Ely, MN in the ecotone of the boreal-temperate forest. The research sites are characterized by a mean annual precipitation and temperature (1980–2019) of 824 mm and 4.9  C for the CFC and 715 mm and 2.8  C for the HWRC (based on nearby weather stations). At each site 24 research plots 3 m in diameter were established, half in relatively open areas that were recently cleared (open canopy) and half in the understory (closed canopy) of existing stands of [?]70 years old mixed aspen-pine-birch with scattered fir, spruce, and other species; in both sites on coarse-textured upland soil. The study includes an

incomplete factorial of sites, canopy types, warming and rainfall manipulation, which we analyzed as two overlapping factorial experiments. One experiment consisted of two sites, two canopy conditions (closed and open), and two temperature treatments (ambient and elevated), replicated in three blocks per canopy condition per site. In addition, rainfall was manipulated but only in open canopy plots; thus the second experiment consisted of two sites, one canopy condition (open), two temperature treatments (ambient and elevated), and two rainfall manipulation (ambient and reduced), replicated in three blocks per site.

An open air (chamberless) warming treatment was implemented simultaneously for the above- and below-ground part of the plot via an integrated microprocessor-based feedback control system (Rich *et al.*, 2015), designed to maintain a fixed temperature differential between ambient and warmed plots. Infrared ceramic heaters mounted above each plot in an octagonal pattern were used for the aboveground warming of plant surfaces, while resistance-type warming cables were buried (10 cm deep and spaced 20 cm apart) to achieve belowground warming. For more details about the project warming methodology see Rich *et al.*, (2015) as well as in Reich *et al.*, (2015), Sendall *et al.*, (2015), and Reich *et al.*, (2016). Aboveground temperature on each plot was measured at mid-canopy height (i.e., roughly the average for all planted tree seedlings in each plot). For temperature measurements below ground (i.e., soil temperature), we used soil temperature probes randomly inserted on each plot at the depth of 10 cm. During the mid-summer and daytime periods, across all 11 years, average temperature differentials between treatments specifically for the above ground were slightly different than the target of 3.3degC (Tables 1 and 2, Figures S1 and S2) but as they were close for the full period of warming treatments, we call the warming treatment +3.3degC throughout the paper.

The summer rainfall reduction treatment began in 2012 via rainout shelters installed only in the open canopy, on randomly selected plots across warming treatments at both sites. Rainout shelters were used to reduce both total summer rainfall and the number of rain events in each year from June 1st to September 30th (for details on rain shelter design and implementation see Stefanski *et al.*, (2020)). To minimize shading of tree seedlings, rainout shelters were typically deployed during overcast condition or at night shortly before and closed shortly after (typically 0.5-1h) the rain event. Over the course of seven seasons, rain shelters were deployed for an average total time of ~8% of the entire rainfall reduction period (i.e., June 1st – September 30th). In each growing season, about half of this time occurred during night hours (for more information about treatments see Table 1 and Figures S1 and S2). Across the seven years of the summer rainfall removal, we saw an average reduction of 40.7% summer rainfall as compared to ambient plots (Table 1 and Figures S1 and S2). That translated to a reduced mean summer rainfall of 269.5 mm (+15.5 SE) and 222.9 mm (+11.6 SE) as compared to ambient mean realized summer rainfall of 454.5 mm (+26.4 SE) and 376.8 mm (+19.9 SE) at the CFC and HWRC sites respectively. Consequently, our rainfall treatments were representative of relatively wet summer (~70th percentile wettest) and rather dry summer (~10th percentile driest) for ambient and reduced rainfall respectively as compared to the broader temporal context of the 100 year of the weather record (1912-2011 available for the CFC site). Soil moisture on the research plots was monitored over the course of this research using water reflectometers (Model CS616 from Campbell Scientific). Soil Volumetric Water Content (VWC - cm³ water/cm³ soil) was measured across 0-30 cm soil profile on an hourly basis through all years. The reduction of soil moisture by warming treatment is a result of increased evapotranspirative demand due to elevated temperatures that reduces soil moisture on a continuous manner as we warm 24/7 from early spring until late fall. In contrast to warming, rainfall reduction treatment is implemented on a cyclic demand basis that allows replenishing soil water in between rainfall removal events (see Table 1 and Figure S2 for more details).

Over the course of this experiment between both the open and closed canopy we grew seedlings of 17 native and four invasive tree species (a total of 21) in different combinations among years and canopies (for details see Tables 3 and S2). Seedlings were sourced from local ecotypes, well suited for the research sites typical environmental conditions. We planted one or two year old seedlings produced by MN DNR (Minnesota Department of Natural Resources) nurseries into an existing matrix of native vegetation. The chosen species represent dominant tree species from the boreal-temperate ecotonal region of northern Minnesota. Newly planted cohorts were given one year ([?]14 months) to acclimate after transplant before any gas exchange measurements were performed except for the 2012 and 2013 cohorts when plants were measured in the

same growing season following spring planting (but see below on requirements of foliage selection for the measurement). The observations reported in this study were made throughout all years of the experimental operation from 2009 to 2019 on different cohorts of seedlings that ranged from two to eight years of age (See Tables 3 and S2 for more details).

Gas exchange measurements

Eleven years of surveys of net assimilation (A_{net}) of CO_2 at light saturation using Li-6400XT – infrared gas exchange analyzers (LICOR, Lincoln, Nebraska, USA) were conducted *in situ* on randomly selected individuals of target species (for details see Tables 3 and S2), yielding 17,727 unique measurements. Measurements were typically conducted in one to two weeks long campaigns from two to five times in each growing season starting late spring and ending in early fall (i.e., roughly mid-June to the end of September). We used the same measurement protocol for all gas exchange measurements performed across all years that defined their scope and constraints of environmental conditions during which they were performed. Thus, all measurements were performed between 09:00 hours and 16:00 hours on foliage from the upper part of the crown, using fully expanded, healthy leaves or current year needles, ensuring that the foliage we measured was fully mature and acclimated to growing conditions. Over the course of the day, we let measurement track ambient conditions for temperature, and VPD, but to achieve saturating levels of irradiance the Photosynthetically Active Radiation (PAR) was set to $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ for plants grown in the open canopy and $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ for plants grown in the closed canopy. Light levels were chosen based on a light response curve survey performed in the first year of the study and represent the light intensity needed to saturate A_{net} for all species. Relative humidity (RH) in the leaf chamber ranged from 40 to 80% with the target goal of 60%. The air flow rate was set to $500 \mu\text{mol s}^{-1}$ and the leaf temperature and VPD (Vapor Pressure Deficit) were unconstrained due to limitations of the instrument to control temperature and VPD under field conditions. Across all measurements, leaf temperatures ranged from $16.5 - 35.7^\circ\text{C}$ (for 90% of the data) with a mean of 27.2°C and $\text{VPD}_{\text{chamber}}$ (Vapor Pressure Deficit of the air inside the leaf chamber) ranged from 1.0 to 2.89 kPa (for the 10% and 90% quantile respectively) and a mean of 1.89 kPa. For the other meteorological background information related to conditions to which plants were exposed and during which measurements were taken refer to Tables 1 and 2 and Figures S1 and S2 that describe overall research plot level conditions that plants experienced. The environmental conditions under which foliage gas exchange was measured were used to parameterize the USO model and obtain estimates of g_1 .

Modeling

The large data set used for the g_1 estimates required careful evaluation and screening for erroneous data points. Thus, we used a methodical approach to screen, evaluate, test and if necessary, remove outlier and/or high leverage points as outlined below. We started screening of the data set for any potentially erroneous data points based on physiological and environmental constraints that were considered either physiologically unlikely (e.g., data points with extremely negative C_i while positive A_{net} , etc.) or measured at unfavorable chamber conditions (e.g., extremely high T_{leaf} , very low RH, etc.) or any points that were indicated by the operator of the instrument during measurement as potentially erroneous, all those points were removed. For additional details outlining methodology of initial evaluation of the carbon assimilation measurements used in the further modeling work see supplemental materials.

We used the subsequent data set to fit the USO model (see equation 1 in supplement and for its derivation details see Medlyn *et al.*, (2011)) and to estimate and effectively define g_1 for each species in accordance with their respective growing conditions (i.e., respective treatments and replication, see below). We used estimated g_1 values to analyze and quantify the size of the warming and rainfall reduction effects across spatial and temporal scales (i.e., site, canopy, and growing season). The g_0 parameter was set to zero as suggested (Duursma *et al.*, 2019, B Medlyn, pers comm), given its otherwise high correlation to g_1 and lack of precision. We used the “plantecophys” package (Duursma, 2015) in R (R Development Core Team, 2019) to fit the USO model (Medlyn *et al.*, 2011). In some cases, the final data set at the finest levels of factorial combinations (i.e., species \times warming \times rainfall reduction \times treatment replicate (i.e., individual research plot) \times block \times canopy \times site \times year \times measurement campaign) did not have a sufficient number

of replicates (i.e., at least 3 replicates are needed) to fit the USO model and/or available replicates were not enough to produce a fit with good confidence. Thus, the 17,727 collected observations were binned by experimental treatment, effectively pooling measurements across the same treatment combination by combining plot replicates of the same treatment together to achieve the following factorial combination by canopy:

closed canopy: species \times warming \times site \times year \times measurement campaign,

open canopy: species \times warming \times rainfall reduction \times site \times year \times measurement campaign.

This yielded a total of 2,732 estimates of g_1 by fitting the USO model. The mean number of data points used to fit the USO model was six (± 2 SD) with less than 2.5% of the model fits constructed based on the minimum of three data points and $>75\%$ based on six or more, with the maximum of 18 points. A data point was a unique instance where the four metrics needed to fit the USO model (A_{net} , g_s , D and C_a) were sampled on a single leaf.

Overall, estimates of g_1 values for our species (Table S2 and Figures 1-3) are within the range of those found by others (e.g., Franks *et al.*, 2017; Medlyn *et al.*, 2011; Zhou *et al.*, 2013). However, to further evaluate the quality of the g_1 estimates we implemented a two-step process. First, we compared values of observed stomatal conductance used in the USO model to estimate g_1 values, to the values of g_s predicted by the A - g_s coupled model (as described by Duursma, 2015). Used A - g_s coupled model predict A_{net} and g_s based on the environmental conditions that a given leaf experienced during measurement (i.e., leaf temperature, VPD, C_a , PAR), estimated g_1 values (based on USO model), and estimated photosynthetic capacity (i.e., rate of Rubisco carboxylation and photosynthetic electron transport, estimated based on one-point method (De Kauwe *et al.*, 2015)) (for more details on the A - g_s coupled model used here see Duursma, 2015). The overall linear fit for the entire data set minus outliers ($n = 17,040$) produced $R^2 = 0.71$ with nearly 1:1 slope adding confidence to the fits of the model. Second, we used a multivariate jackknife analysis of the g_1 estimates, performed in JMP statistical software (JMP 14.2, SAS Institute), that detected 146 ($\sim 5\%$) out of 2732 total g_1 estimates as potential outliers. Out of those 146 g_1 estimates $\sim 75\%$ of them were above the maximum values reported elsewhere in the literature (e.g., Medlyn *et al.*, 2011; Gimeno *et al.*, 2016; Hérault *et al.*, 2013; Franks *et al.*, 2017). Thus, we removed all 146 points indicated as outliers. We note that, in an analysis (not shown here) that excluded only the 12 most extreme values (g_1 > 500 ; orders of magnitude higher than average g_1 values reported here and elsewhere) the overall effects detected did not change.

To separate thermal effects from the indirect effect of warming on soil VWC we examined g_1 parameters for observations grouped by different VWC classes. To do this we used the 24h averages of the soil VWC on the day when the A_{net} measurements were made. The categorical values of VWC were created by binning 24h soil VWC averages into three categories as follows: i) low VWC $< 12.99\%$, ii) medium VWC $13 - 17.99\%$, and high VWC $> 18\%$ (for details on soil VWC measurements see Rich *et al.*, 2015).

Data analysis

We used mixed effects models to test g_1 separately for each canopy with the following independent variables: site, species, warming, rainfall reduction, and up to 4-way interactions; with measurement campaign (e.g., year and campaign during that year) as a random effect. To separate the direct effect of warming temperature from the indirect effect of warming treatment on soil moisture

we used soil VWC categories and tested the effect of experimental treatment when soil VWC was high in contrast to when it was low. We ran those tests separately for each canopy with independent variables: warming, rainfall reduction (for open only), and soil VWC category up to 3-way interactions; with measurement campaign set up as a random effect. In addition, we tested the effect of warming and rainfall reduction on g_1 for high soil VWC independently from other soil VWC categories. Moreover, we used soil VWC as a covariate in combination with fixed variables (as outlined above) and campaign measurement and site set as random variables. We tested whether different cohorts behaved differently in response to environmental drivers and found no evidence for this, so did not further consider those in analyses (but see Figure S3).

We also constructed additional mixed effects models to analyze the effect of warming and drought on higher groupings of the species following their biome association, drought tolerance, and phylogenetic affiliation (for details about mixed effects models for species and their respective groupings [drought tolerance, biome, phylogeny] see Tables 3 and S2). All statistical analyses were carried out in JMP statistical software (JMP 14.2, SAS Institute).

Results

Over more than a decade of experimental manipulation, growing conditions were altered in a consistent and significant way at our research plots. The warming treatment elevated temperature above and belowground by 3.3degC on average across all years, sites, and canopies (see Table 1, and Figure S1). Warming treatment had a significant effect on soil moisture; reducing VWC by 13% in the closed canopy plots and by 24% in open plots. In the open canopy plots reduced rainfall treatment caused an 11% decrease in VWC, and warming with reduced rainfall together reduced VWC by 35% (see Table 1 and Figures S1 and S2 for more details).

Analysis of 11 growing seasons of leaf gas exchange data across multiple species showed that rainfall reduction and warming treatments led to more conservative water use on average evidenced by decreased g_1 (the slope of the USO model serving as a proxy of the marginal water cost of carbon gain $-\lambda$) (P [?] 0.0087, Table 4). However, species differed in their responsiveness to both drivers. We organize the presentation of results around the hypotheses.

H1: g_1 decreases with reduced rainfall for all species. – Our hypothesis was supported as g_1 was lower in reduced rainfall treatments ($P = 0.006$; Table 4 and Figures 1-5). This effect was consistent in all tested models (for selected additional models see methods and Table S1). Overall, plants grown under the rain reduced treatment regime reduced g_1 by 10.5% on average compared to plants in ambient plots. This decrease of the g_1 parameter was generally consistent across both sites and all years (see Figure 5b). The role of VWC in these responses is presented below with respect to both rainfall and warming treatment effects.

H2: g_1 will decrease with climate warming due to soil moisture reduction induced by elevated temperature. – Mixed effect models showed that warming treatment strongly reduced g_1 in both canopies ($P < 0.0001$; Table 4 and Figures 1-5). This effect was generally consistent across all models and years (see Table 4 and Figure 5). Overall, plants grown in warmed treatments reduced g_1 by 25% in the understory and 18% in open canopy plots (see Table 4 and Figures 1-5). These responses support H2 (as further documented below).

Assessing soil moisture regulation of g_1 . – As both the warming treatment and reduced rainfall had significant effects on VWC (Table 1) we explored the role that soil moisture might play in regulating g_1 . Estimates of g_1 for plants experiencing different levels of soil moisture in each treatment (binned into three categories, i.e., low, medium, and high soil VWC – refer to modeling and data analysis section of methods for additional details on VWC bins) showed that g_1 declined when soil water content was low either due to reduced rainfall or elevated temperatures, or both (Table 5, and Figure 4a,b). Moreover, when we add soil VWC as a covariate the significance of the main effects (i.e., warming and rainfall reduction) is eliminated ($P > 0.3855$, Table 6) in open canopy but not in the closed ($P < 0.0001$, Table 6) while soil VWC alone has a significant ($P < 0.0124$, Table 6) effect on g_1 in both canopies. Also, a combination of soil VWC with warming becomes significant ($P < 0.019$) in both canopies that collectively demonstrates strong influence of soil moisture on g_1 that overtakes the effect of warming particularly in the open canopy. Slightly different response of plants in closed canopy is likely due to differences in microenvironments where plots in closed canopy have overall higher soil VWC on average that does not consistently become low enough for plants to drive changes in g_1 .

H3: reduced rainfall and warming will have an additive effects on g_1 because the primary mechanism of both warming and reduced rainfall effects on g_1 will be via the same pathway, of reduced VWC on stomatal behavior. – Warming and reduced rainfall did not show significant interaction in any model (P [?] 0.3621, for details see Table 4, 5, 6 and S1, and Figures 1b, 2b, 3b, 4b, and 5b) confirming our hypothesis. Across all other sources of variation (in open plots), reduced rainfall alone caused 8.3% decline while warming alone

resulted in 15.6% decline of g_1 , and both treatments acting together reduced g_1 by 26.5% (see Table S2 and Figures 1b, 2b, 3b, and 5b).

H4: species adapted to either drier and/or warmer conditions will on average have lower and less sensitive g_1 than species adapted to more mesic or cooler conditions. – Species (for details about species see Tables 3 and S2) differed in their g_1 parameter ($P < 0.0001$, Tables 4, 6, S1 and S2, and Figure 1). Species average g_1 in ambient growth conditions ranged in open canopy from 2.8 for *P. banksiana* to 5.5 in *F. alnus*, and in closed canopy from 2.3 for *A. balsamea* to 6.1 for *R. cathartica*. The four invasive species (i.e., *F. alnus*, *L. morrowii*, *L. tatartica*, and *R. cathartica*) and native *T. americana* had the highest g_1 of all species (Figure 1 and Table S2). The boreal species had on average the lowest g_1 with native temperate species in between invasive and boreal groups. Species with higher drought tolerance indices had slightly higher g_1 on average. For more details on the average g_1 values across species, their respective groupings (e.g., biome association, drought tolerance, etc.) and treatment effects, see Figures 1-3 and Table S2.

There were few differences among species and their respective higher groupings (i.e., drought tolerance, biome association, and phylogenetic associations) in sensitivity of g_1 , (that is the decline of g_1 in response to rainfall reduction or warming) (Figures 2-3, Table S1) as most species and groups responded to warming and reduced rainfall by significantly reducing g_1 (Table 4, Figure 1). For example, in closed canopy plots there was a large individualistic variation in responses ($P = 0.0384$, Figure 1) to warming, with change in g_1 ranging from a 10.6% increase in *P. glauca* to decreases for all other species that ranged from 3.3% for *Q. rubra*, to 60.5% in *T. canadensis*.

Discussion

More than a decade of measurements documented generally consistent ways that novel environmental conditions associated with climate change influenced the trade-off of water loss vs. carbon gain among 21 boreal and temperate species. Overall, g_1 decreased in response to both reduced rainfall and warming, driven largely by stomatal responses to soil drying in both cases, effectively increasing the water use efficiency of plants by maintaining stomata less open (H1, H2). The direction of these responses to experimental manipulations was uniform across all species despite differences in average g_1 associated with species-specific adaptations (i.e., drought tolerance) and associations (i.e., climate of origin or phylogeny) (H4). We also found that the combination of warming plus reduced rainfall (H3) had an additive effect. Moreover, the warming and reduced rainfall effects were consistent across years, sites, and species (Figure 1, 5) providing strong support for these responses as a general prediction. These results suggest that projected future warming and reduced summer rainfall will likely move boreal and temperate species to a more conservative water spending stomatal behavior; likely helping plants ameliorate drought stress but at a carbon cost.

The unified optimization theory predicts g_1 to have a small increase in response to warming, largely because it is related to Γ^* (Medlyn *et al.*, 2011), which is dependent on temperature (Bernacchi *et al.*, 2001). Hence, a neutral or near neutral effect of temperature on g_1 was found previously (Nijs *et al.*, 1997; Duursma *et al.*, 2013; or Gimeno *et al.*, 2016). Our results support that prior work but only when water was abundant (Table 5 and Figure 4). This is firm evidence that the weak neutral to positive direct effect of temperature on g_1 was overwhelmed by the stronger effect warming had on water limitation, through an increase in evapotranspiration demand (Seager *et al.*, 2014; Wang *et al.*, 2014; Reich *et al.*, 2018).

Species differ in g_1 but respond predictably in their response to soil water limitation and warming

We hypothesized that species would vary g_1 in ways largely related to their individual adaptations to drought. We found that g_1 did vary among species, but the responses to novel experimental conditions did not differ among them. Overall, g_1 was only modestly higher in species with greater drought tolerance and the g_1 of species with greater drought tolerance did not respond differently from less tolerant species to variation in VWC, in disagreement with prior reports in the literature (e.g., Gimeno *et al.*, 2016; Hérault *et al.*, 2013 and Zhou *et al.*, 2013). Differences in our result versus those previously reported could be due to the extent to which drought is a primary stress in these contrasting ecosystems, interspecific trait differences

that modulate the value of conservative stomatal behavior, or both. In boreal systems, although drought can occur, chronic low temperatures except for a short time window in mid-summer, and low nutrient availability, are also important, and may dampen the strength of selection for g_I in relation to drought adaptation. Additionally, trait differences and acclimation may increase some species tolerances to drought without a need of compromising water use. For example, oaks are known to develop deeper root systems (e.g., Abrams, 1990) and thus increase access to water. Oaks and maples have relatively higher wood density, compared to lower wood density species (e.g., gymnosperms), which has been associated with greater drought tolerance (Greenwood *et al.*, 2017). Moreover, modeling work by Mrad *et al.*, (2019) demonstrates that either aggressive or conservative behavior in water use might be related to acclimation of the rooting zone to competition for water, and little is known about such differences for our species.

Despite individualistic variation in average g_I among species, there was consistent movement towards more conservative (i.e., water-saving) stomatal behavior in response to both rainfall reduction and warming, likely as a response to soil drying. This shift was consistent among species, sites, and canopy conditions, and observed across more than a decade of experimental responses. This strongly suggests a consistent acclimation by northern temperate and boreal species that would be beneficial in terms of ameliorating soil drought, but at a carbon cost. It is also unclear just how much additional soil drought this trade-off will offset, and whether those carbon costs translate into adverse impacts on growth or survival.

Conclusions

Our work documents the water-carbon trade-off response to long-term experimental manipulation for tree species common to the boreal-temperate ecotone in North America. Empirically quantifying those responses across a large number of species in a relatively realistic experimental context contributes to our understanding of whether and how stomatal behavior is expected to vary across species and their hierarchical affiliations (e.g., climate of origin or phylogeny) in response to climate change. In particular, we found that g_I was reduced in response to growth conditions that caused a decline of soil VWC (i.e., rainfall removal and warming). We also showed that there was a large variation among species intrinsic g_I ; however, their responses to reduced rainfall and warming did not depend on species identity or grouping. Our study also provides additional evidence that soil moisture will govern plants' response to future climate change: when soil moisture is abundant plants use it to facilitate greater carbon assimilation, but when soil water is limited plants will have diminished carbon assimilation but more efficient water use.

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Author Contribution

PBR, RAM and AS designed the study, RB and AS collected data, EEB and AS modeled the data, PBR, RAM, EEB, and AS analyzed the data and drafted the manuscript. All authors discussed and interpreted the results and substantially contributed to the final manuscript.

Conflict of interest

Authors declare no conflict of interest

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Table 1. Summary of the aboveground and belowground warming and summer rainfall reduction treatments for both research sites (Cloquet Forestry Center – in Cloquet, MN and Hubachek Wilderness Research Center – Ely, MN). The comparison summaries represent means for each treatment based on hourly records for each experimental plot and averaged for the period from June 1st – September 30th (as that is the period when rainfall removal occurred and represents the main part of the growing season when the A_{net} measurements were conducted) for all years combined. For the comparison of the rainfall removal, we show the summary of precipitation for the years when treatment was active in contrast to 40 years means for the same period. Standard deviation of the mean calculated for all years and all units of replication (i.e., all plots in each treatment combination) is shown in parentheses.

		2009- 2019	2009- 2019	2009- 2019	2009- 2019	2009- 2019	2009- 2019	2009- 2019
		June 1 st – Septem- ber 30 th CFC open	June 1 st – Septem- ber 30 th CFC	June 1 st – Septem- ber 30 th CFC closed	June 1 st – Septem- ber 30 th	June 1 st – Septem- ber 30 th HWRC open	June 1 st – Septem- ber 30 th HWRC	June 1 st – Septem- ber 30 th HWRC closed
Aboveground $T_{ambient}$		16.41		16.10		16.96		16.10
Tem- pera- ture (°C)		(7.63)		(5.92)		(7.65)		(5.92)
	Δ 3.3°C	3.12		3.49		3.19		3.51
		(1.27)		(0.88)		(1.28)		(0.88)
Belowground $T_{ambient}$		16.43		15.89		16.96		15.89
Tem- pera- ture (°C)		(2.71)		(2.80)		(3.20)		(2.80)
	Δ 3.3°C	3.20		3.27		3.17		3.49
		(0.79)		(0.71)		(0.92)		(0.71)
Soil	VWC	21.9		19.61		15.08		23.1
Volu- metric Water Con- tent (%)	$T_{ambient} \times \text{Rain}_{ambient}$	(3.70)		(4.39)		(4.28)		(6.1)
	VWC	16.56		16.87		11.46		20.1
	$+3.3^\circ\text{C} \times \text{Rain}_{ambient}$	(3.90)		(4.04)		(3.30)		(5.1)
	VWC	18.94		-		14.14		-
	$T_{ambient} \times \text{reduced rainfall}$	(5.00)		-		(3.24)		-
	VWC	12.98		-		11.07		-
	3.3°C	(3.31)		-		(3.08)		-
	$\times \text{reduced rainfall}$							
		2012- 2019	2012- 2019	2012- 2019	2012- 2019	2012- 2019	2012- 2019	2012- 2019
Precipitation Total (mm)	Summer	454.5 (70.0)		454.5 (70.0)		376.8 (52.6)		376.8 (52.6)

Total Sum- mer After Reduction %	269.5 (41.0)	-	222.9 (30.8)	-
Reduction 40 years nearby weather stations	40.8 (1.1)	-	40.9 (1.8)	-
	424.4 (101.9)	424.4 (101.9)	424.4 (101.9)	408.8 (100.6)
			408.8 (100.6)	408.8 (100.6)
				408.8 (100.6)

Table 2. Mean ambient plant surface temperatures (\pm SD) and the degrees above ambient achieved by the warming treatment from June 1st to September 30th (the portion of the growing season when the majority of photosynthesis occurs, and all our measurements were conducted). Means are shown for 24h periods, as well as for the period of the day when most photosynthetic activity occurs (08:00-16:00 hours) for the days when gas exchange measurements were conducted and overall means for the entire period between June 1st and September 30th across all years. All averages are pooled across years (2009 – 2019) and both sites (Cloquet Forestry Center – in Cloquet, MN and Hubachek Wilderness Research Center – Ely, MN), but separately for both canopies.

Canopy	Treatment	During the measurement campaign Mean ambient 24h air temperatures ($^{\circ}$ C)	During the measurement campaign Mean ambient 08:00-16:00 temperatures ($^{\circ}$ C)
closed	ambient temperature – ambient rainfall	17.03 (3.75)	20.64 (4.35)
closed	+3.3 $^{\circ}$ C – ambient rainfall	20.51 (3.73)	23.91 (4.35)
open	ambient temperature – ambient rainfall	16.89 (4.30)	23.29 (5.46)
open	ambient temperature – reduced rainfall	17.24 (4.36)	24.38 (5.38)
open	+3.3 $^{\circ}$ C – ambient rainfall	20.09 (4.12)	26.18 (5.43)
open	+3.3 $^{\circ}$ C – reduced rainfall	20.50 (4.11)	27.33 (5.16)

Table 3. List of species with their corresponding biome and phylogenetic associations and drought indices as described by Niinemets and Valladares (2006). Drought tolerance is expressed as an index where one denotes low and four high tolerance to drought. Category of the drought tolerance is denoted in parenthesis as follow: I – intolerant, M – moderately tolerant, T – tolerant. All species came from local ecotypes. Species are ordered by drought tolerance index from the most intolerant to the most tolerant.

Scientific name	Common name	Biome association	Phylogeny	Drought index
<i>Abies balsamea</i> L.	Balsam fir	Boreal	gymnosperm	1 (I)
<i>Acer negundo</i> L.	Box elder	Temperate	angiosperm	1 (I)
<i>Tsuga canadensis</i> (L.) Carriere	Canadian hemlock	Temperate	gymnosperm	1 (I)
<i>Frangula alnus</i> Mill.	Glossy buckthorn	Invasive	angiosperm	1.37 (I)
<i>Populus tremuloides</i> Michx.	Trembling aspen	Boreal	angiosperm	1.77 (I)
<i>Acer rubrum</i> L.	Red maple	Temperate	angiosperm	1.84 (I)
<i>Picea mariana</i> (Mill.) Britton	Black spruce	Boreal	gymnosperm	2 (M)
<i>Betula papyrifera</i> Marshall.	Paper birch	Boreal	angiosperm	2.02 (M)
<i>Acer saccharum</i> Marshall.	Sugar maple	Temperate	angiosperm	2.25 (M)
<i>Pinus strobus</i> L.	White pine	Temperate	angiosperm	2.29 (M)
<i>Thuja occidentalis</i> L.	White cedar	Temperate	gymnosperm	2.71 (M)
<i>Picea glauca</i> (Moench) Voss.	White spruce	Boreal	gymnosperm	2.88 (M)

<i>Quercus rubra</i> L.	Red oak	Temperate	angiosperm	2.88 (M)
<i>Tilia americana</i> L.	American basswood	Temperate	angiosperm	2.88 (M)
<i>Betula alleghaniensis</i> Britt.	Yellow birch	Temperate	angiosperm	3 (T)
<i>Pinus resinosa</i> Sol. Ex Aiton	Red pine	Temperate	gymnosperm	3 (T)
<i>Lonicera morrowii</i> A. Gray	Morrow's honeysuckle	Invasive	angiosperm	3.04 (T)
<i>Lonicera tatarica</i> L.	Tatarian honeysuckle	Invasive	angiosperm	3.04 (T)
<i>Rhamnus cathartica</i> L.	Common buckthorn	Invasive	angiosperm	3.46 (T)
<i>Quercus macrocarpa</i> Michx.	Bur oak	Temperate	angiosperm	3.85 (T)
<i>Pinus banksiana</i> Lamb.	Jack pine	Boreal	gymnosperm	4 (T)

Table 4. Mixed effect models conducted on the g_1 estimates. Square root transformation was applied to meet ANOVA assumptions. Campaign representing day of measurement was used as random variable. Experimental factors are represented as follow: sites (Cloquet Forestry Center – in Cloquet, MN and Hubachek Wilderness Research Center – Ely, MN), warming (ambient T and +3.3°C), reduced rainfall (ambient and ~40% of summer rainfall removed), species (see Table 3). The environmental treatment (i.e., open and closed canopy) are analyzed separately.

Source	Open canopy g_1 estimates $R^2 = 0.24$ $n = 1604$ df	Open canopy g_1 estimates $R^2 = 0.24$ $n = 1604$ F Ratio	Open canopy g_1 estimates $R^2 = 0.24$ $n = 1604$ $Prob > F$	Closed g_1 estimates $R^2 = 0.24$ $n = 1604$ df
site	1	1.4351	0.2311	1
warming	1	24.559	<.0001	1
site × warming	1	8.5896	0.0034	1
reduced rainfall	1	7.567	0.006	-
site × reduced rainfall	1	0.3235	0.5696	-
warming × reduced rainfall	1	0.2615	0.6092	-
site × warming × reduced rainfall	1	3.8608	0.0496	-
species	13	9.6242	<.0001	17
site × species	13	0.6128	0.8453	17
warming × species	13	0.5705	0.8789	17
site × warming × species	13	1.6184	0.0736	17
reduced rainfall × species	13	0.8304	0.6279	-
site × reduced rainfall × species	13	0.3396	0.9858	-
warming × reduced rainfall × species	13	0.4708	0.9413	-
site × warming × reduced rainfall × species	13	0.9076	0.5445	-

Table 5. Mixed effect models conducted on the g_1 estimates. Square root transformation was applied to meet ANOVA assumptions. Campaign representing day of measurement was used as random variable. Experimental factors are represented as follow: warming (ambient T and +3.3°C), reduced rainfall (ambient and ~40% of summer rainfall removed), soil Volumetric Water Content bins (low VWC, medium VWC and high VWC, for details see methods section). The environmental treatment (i.e., open and closed canopy) are analyzed separately.

Source	Open	Open	Open	Closed	Closed	Closed
VWC Bins						
	g_1 estimates $R^2 = 0.15$ $n=1604$	g_1 estimates $R^2 = 0.15$ $n=1604$	g_1 estimates $R^2 = 0.15$ $n=1604$	g_1 estimates $R^2 = 0.15$ $n = 982$	g_1 estimates $R^2 = 0.15$ $n = 982$	g_1 estimates $R^2 = 0.15$ $n = 982$

	<i>df</i>	<i>F Ratio</i>	<i>Prob > F</i>		<i>df</i>	<i>F Ratio</i>	<i>Prob > F</i>
warming	1	7.8423	0.0052		1	39.8832	<.0001
reduced rainfall	1	6.1062	0.0136				
warming* reduced rainfall	1	0.0506	0.822				
VWC bin	2	4.8495	0.008		2	0.8612	0.4231
warming*VWC bin	2	4.3745	0.0127		2	3.1289	0.0442
reduced rainfall *VWC bin	2	0.3366	0.7143				
warming* reduced rainfall *VWC bin	2	1.0629	0.3457				

Table 6 . Mixed effects models conducted on the g_1 estimates. Square root transformation was applied to meet ANOVA assumptions. Campaign representing day of measurement and site were used as random variable. Experimental factors are represented as follow: warming (ambient T and +3.3°C), reduced rainfall (ambient and ~40% of summer rainfall removed), species (see Table 3), and soil Volumetric Water Content (VWC). The environmental treatment (i.e., open and closed canopy) are analyzed separately.

Source	Open canopy g_1 estimates $R^2 = 0.26$ $n = 1604$ <i>df</i>	Open canopy g_1 estimates $R^2 = 0.26$ $n = 1604$ <i>F Ratio</i>	Open canopy g_1 estimates $R^2 = 0.26$ $n = 1604$ <i>Prob > F</i>	Closed canopy g_1 estimates $R^2 = 0.26$ $n = 1604$ <i>df</i>
warming	1	0.3495	0.5545	1
reduced rainfall	1	0.7534	0.3855	
warming* reduced rainfall	1	0.0046	0.946	
species	13	5.6022	<.0001	17
warming*species	13	1.0447	0.4049	17
reduced rainfall*species	13	0.2389	0.9975	
warming* reduced rainfall*species	13	0.4048	0.9687	
VWC	1	25.6956	<.0001	1
warming*VWC	1	7.7082	0.0056	1
reduced rainfall *VWC	1	0.0213	0.8841	
warming* reduced rainfall*VWC	1	2.4053	0.1211	
species*VWC	13	1.2885	0.2125	17
warming*species*VWC	13	1.0489	0.4009	17
reduced rainfall *species*VWC	13	0.4467	0.9526	
warming* reduced rainfall*species*VWC	13	0.7669	0.6962	

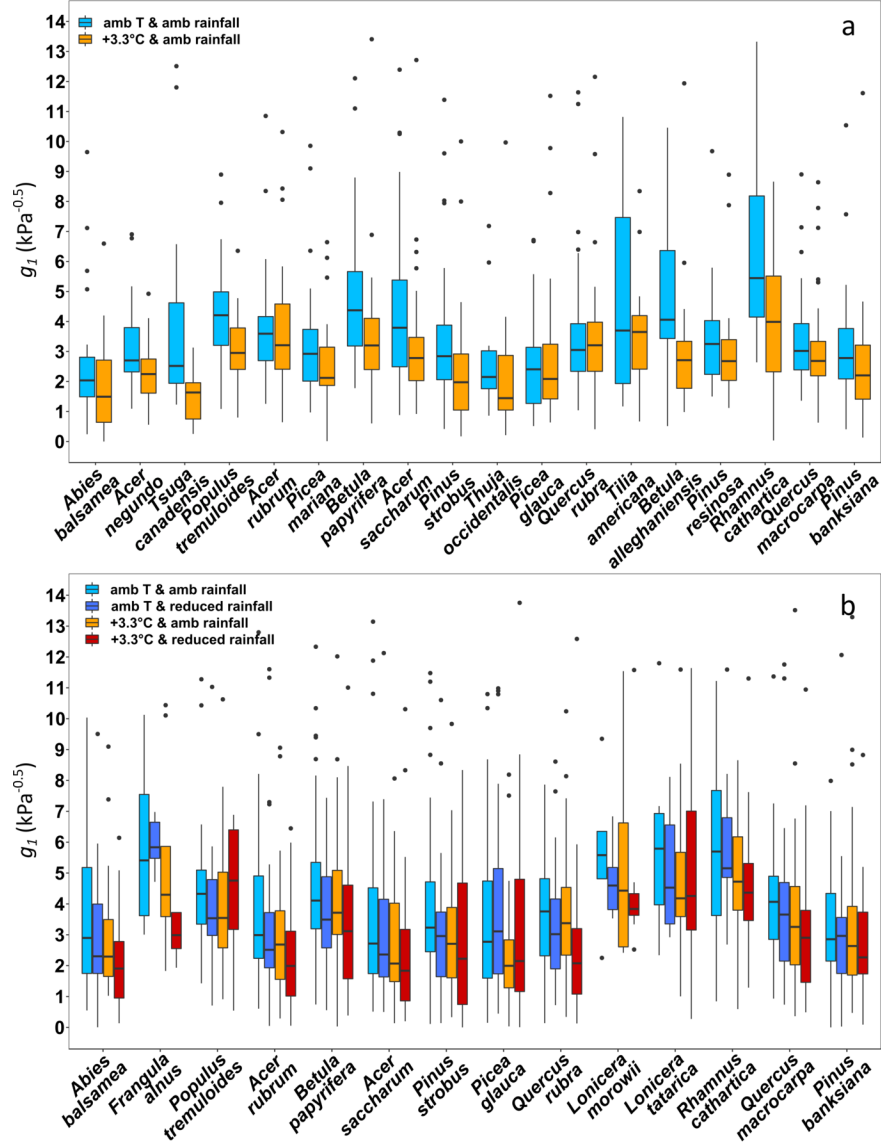


Figure 1. Effect of experimental treatments on g_1 estimates for all tested species. Panel a represents species grown in the closed canopy ($n = 982$) and panel b represents species from the open canopy ($n = 1604$). Species on each panel are organized with increasing drought tolerance from left to right in accordance with Niinemets and Valladares (2006). Whiskers extend to the largest or lowest value but no further than 1.5 times the interquartile range above and below the hinges of the box plot with the median. Any observations outside this range are denoted as individual points. The horizontal line inside the box denotes the median of the values.

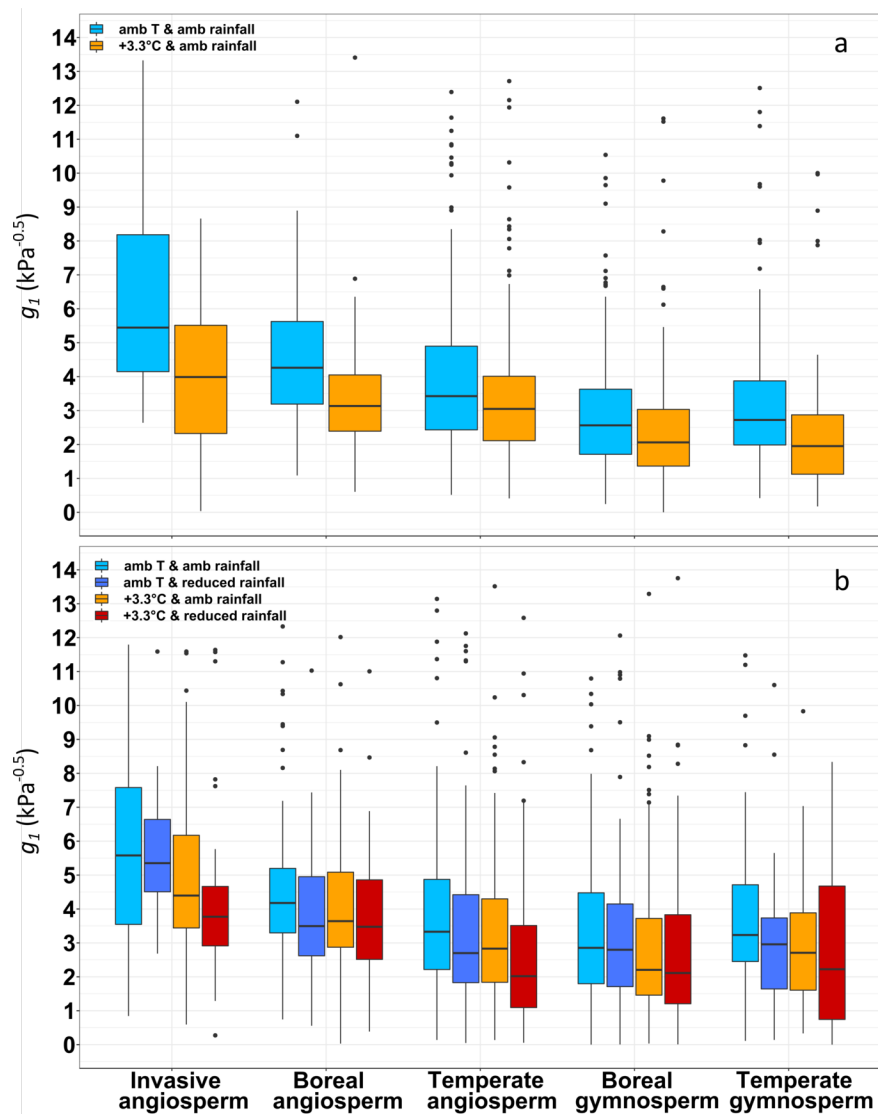


Figure 2. g_1 estimates for all species grouped by functional type and phylogenetic association. Panel a represents closed canopy ($n = 982$) and panel b represents open canopy ($n = 1604$). Species are grouped into one of five categories in accordance with their phylogenetic and biome association (i.e., invasive, boreal or temperate and angiosperms or gymnosperms). Whiskers extend to the largest or lowest value but no further than 1.5 times of interquartile range above and below the hinges of the box plot with the median. Any observations outside this range are denoted as individual points. The horizontal line inside the box denotes the median of the values. Important to note that invasive species as nonnative are not classified as either boreal or temperate.

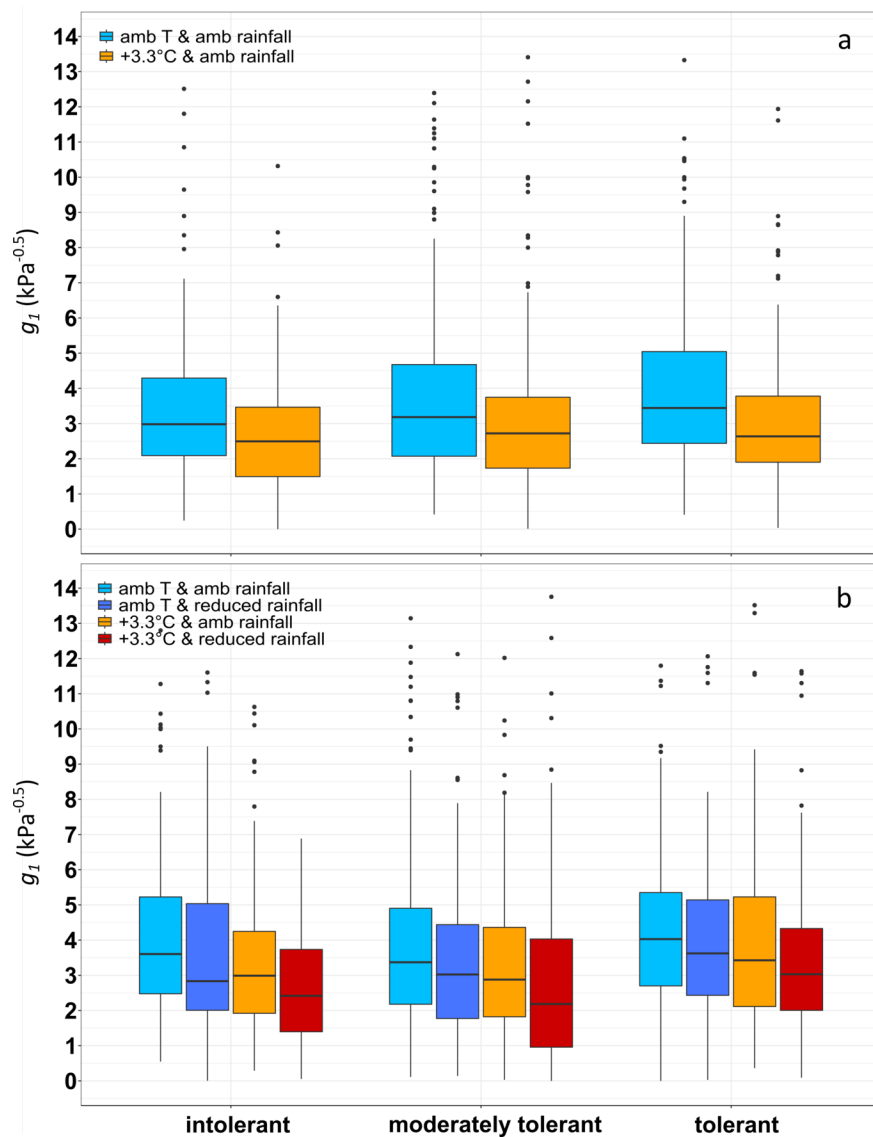


Figure 3. Effect of warming and reduced rainfall on estimates of g_1 for species grouped by their drought tolerance adaptations into three major groups: i) intolerant (drought index from 1 to 1.84), ii) moderately tolerant (drought index from 2 to 2.88) and iii) tolerant (drought index from 3 to 4) in accordance with Niinemets and Valladares (2006) (for details about drought index see Tables 4 and S1). Panel a show closed canopy ($n = 982$), and panel b represents open canopy ($n = 1604$). Whiskers extend to the largest or lowest value but no further than 1.5 times of interquartile range above and below the hinges of the box plot with the median. Any observations outside this range are denoted as individual points. The horizontal line inside the box denotes the median of the values.

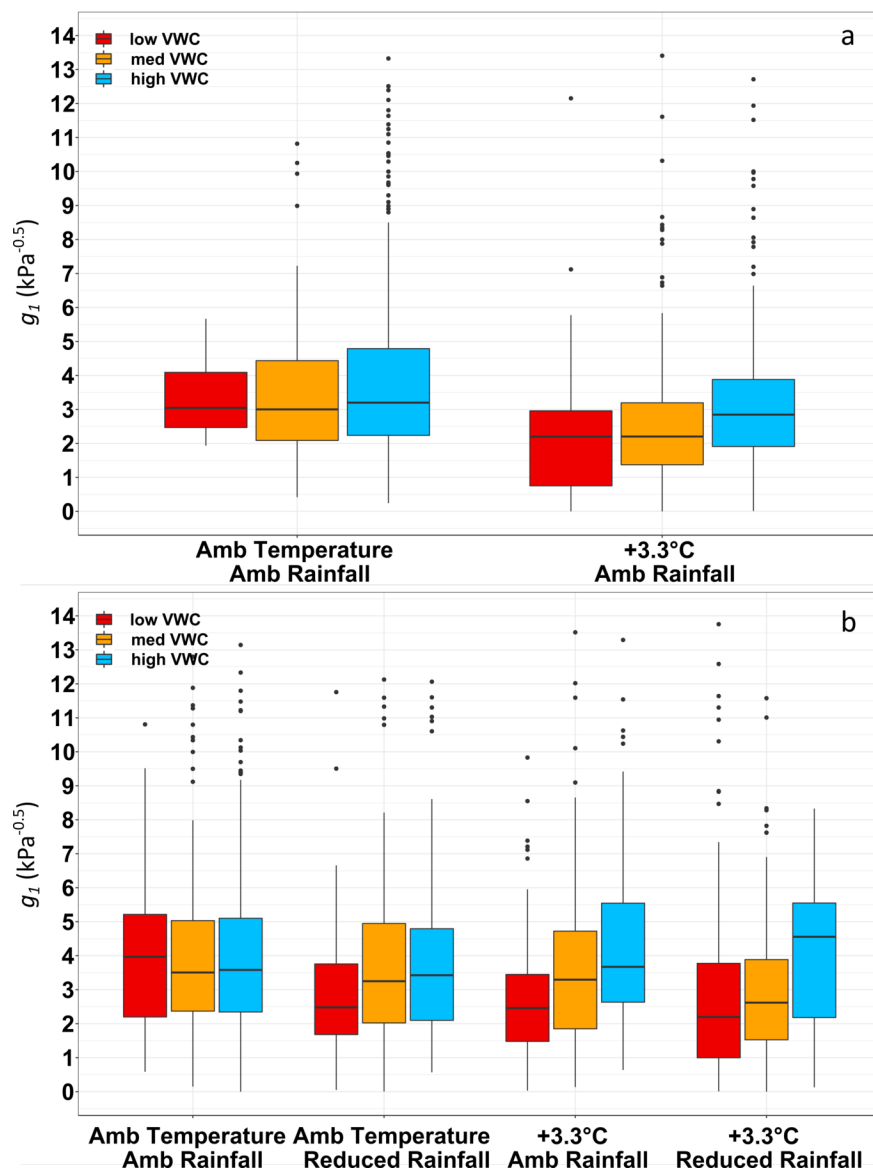


Figure 4. Effect of soil moisture on the mean estimates of g_1 in respect to soil VWC (soil Volumetric Water Content) categories (low VWC < 12%, medium VWC 12 – 16.99% and high VWC [?] 17% of 24h average of the Volumetric Water Content on the day of measurement). Panel a represents closed canopy with $n=982$ and panel b represents open canopy with $n=1604$. Whiskers extend to the largest or lowest value but no further than 1.5 times of interquartile range above and below the hinges of the box plot with the median. Any observations outside this range are denoted as individual points. The horizontal line inside the box denotes the median of the values.

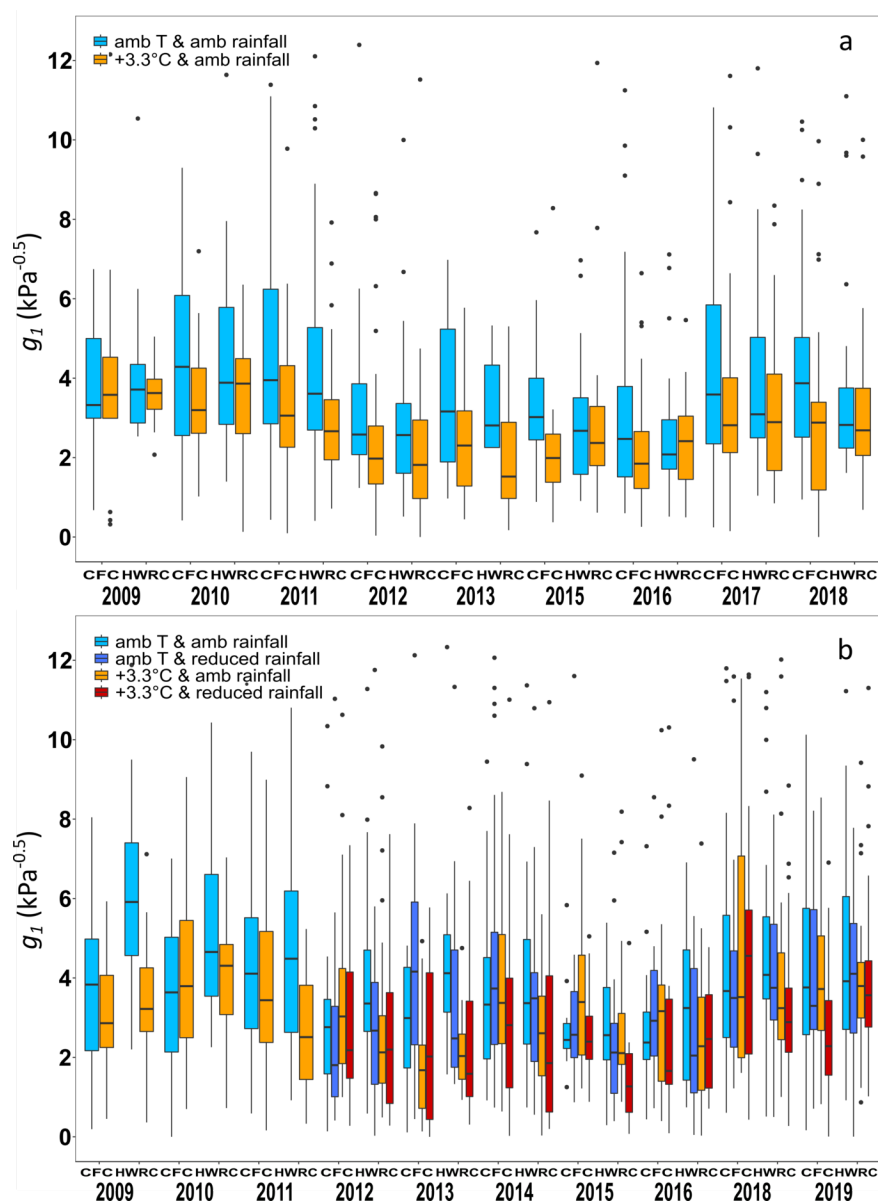


Figure 5 . Comparison of g_1 estimates across years, sites, and canopies. Panel a depicts closed canopy and panel b open canopy. Whiskers extend to 1.5 times of interquartile range above and below the hinges of the box plot with the median. Any observations outside this range are denoted as individual points. The horizontal line inside the box denotes the median of the values.