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Stomatal behaviour moderates the water cost of CO_2 acquisition for 21 boreal and temperate species under experimental climate change

Artur Stefanski[1] | Ethan E. Butler[1] | Raimundo Bermudez[1] | Rebecca A. Montgomery[1] | Peter B. Reich[1,2,3]

[1]Department of Forest Resources, University of Minnesota, St. Paul, Minnesota, USA

[2]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, New South Wales, Australia

[3]Institute for Global Change Biology, and School for the Environment and Sustainability, University of Michigan, Ann Arbor, Michigan, USA

Correspondence

Artur Stefanski, Department of Forest Resources, University of Minnesota, St. Paul, MN 55108, USA.
Email: stefa066@umn.edu

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Abstract

The linkage of stomatal behaviour with photosynthesis is critical to understanding water and carbon cycles under global change. The relationship of stomatal conductance (g_s) and CO_2 assimilation (A_{net}) across a range of environmental contexts, as represented in the model parameter (g_1), has served as a proxy of the marginal water cost of carbon acquisition. We use g_1 to assess species differences in stomatal behaviour to a decade of open-air experimental climate change manipulations, asking whether generalisable patterns exist across species and climate contexts. A_{net}-g_s measurements (17 727) for 21 boreal and temperate tree species under ambient and +3.3°C warming, and ambient and ~40% summer rainfall reduction, provided >2700 estimates of g_1. Warming and/or reduced rainfall treatments both lowered g_1 because those treatments resulted in lower soil moisture and because stomatal behaviour changed more in warming when soil moisture was low. Species tended to respond similarly, although, in species from warmer and drier habitats, g_1 tended to be slightly higher and to be the least sensitive to the decrease in soil water. Overall, both warming and rainfall reduction consistently made stomatal behaviour more conservative in terms of water loss per unit carbon gain across 21 species and a decade of experimental observation.

KEYWORDS

B4WarmED, boreal-temperate ecotone, drought, g_1, stomatal behaviour, stomatal optimisation, warming, water-use efficiency

1 | INTRODUCTION

The rate of carbon gain in photosynthesis is dynamically regulated by stomatal responses to environmental factors (e.g., temperature and 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; Héroult 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 behaviour follows optimisation theories (Cowan & Farqhuar, 1977; Givnish, 1986; Manzoni et al., 2013; Medlyn et al., 2011; Wolf et al., 2016) with stomatal behaviour often modelled as a gain-cost trade-off that maximises carbon gain through variation of stomatal conductance in response to environmental constraints (e.g., water availability and temperature). Cowan and Farqhuar (1977) proposed an optimisation where the role of stomata is to maximise carbon acquisition (*A*) at the lowest water loss through transpiration (*E*), described as a marginal water cost of carbon gain (λ). Therefore, optimal stomatal behaviour minimises the integrated sum of the following expression—that effectively defines the marginal water cost of carbon gain—and can be written as:

$$E - \lambda A \quad (1)$$

where *A*—photosynthesis, *E*—transpiration, and λ—is a parameter representing the marginal water cost of carbon gain. Cowan and Farqhuar (1977) minimised this expression that produced optimisation control:

$$\frac{\partial E}{\partial A} = \lambda \quad (2)$$

However, stomatal optimisations like the one proposed by Cowan and Farqhuar (1977) (Equation 1) are difficult to parameterise with typically measured field data, leading to models using proxies to represent λ. In contrast, our collective understanding of stomatal behaviour was significantly advanced by important predecessors that developed empirical models (e.g., Ball et al., 1987; Leuning, 1995) that were easy to fit with field data. There have also been optimisation-oriented models that combined the empirical and optimisation approaches (e.g. Arneth et al., 2002; Lloyd, 1991). Following along with the same perspective, Medlyn et al. (2011) developed the Unified Stomatal Optimisation (USO) model, which describes stomatal conductance as a function of carbon assimilation and environmental conditions ($A/(C_a\sqrt{D})$ where: A—is net assimilation rate, C_a—is atmospheric CO_2 concentration at the leaf surface, D—is vapour 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 CO_2 concentration (C_i) to link the g_1 parameter with λ (Arneth et al., 2002); for a 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 CO_2 compensation point (Γ*):

$$g_1 \propto \sqrt{\Gamma^{**}\lambda} \quad (3)$$

where Γ*—is the CO_2 compensation point, λ—is the 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 Ca is much larger than Γ* (Ehleringer, 2005) and that stomatal behaviour optimises for RuBP (Ribulose 1,5-biphosphate) regeneration limitation but not for Rubisco limitation of photosynthesis (Outlaw & De Vlieghere-He, 2001; Outlaw et al., 1979; 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, and temperature, 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 the theoretical predictions summarised 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 remains 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 behaviour, 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 parameterised 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 behaviour, 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). There is also increasing effort devoted to bridging experimentally probed traits and physiological processes with modelling of responses of individual species, populations, and ecosystems to climate change (e.g., De Kauwe et al., 2015; Zhou et al., 2019). For example, a review by Zhou et al. (2019) emphasises the importance of informing processed-based models with experimentally obtained plant traits that characterise variability for a wide variety of species in the context of both short and long-term exposure to climate change drivers. Our report helps make such data available, Thus, the main goals were to explore whether and how

(i) stomatal behaviour changes (e.g., plants decrease g_1 indicating more conservative water use) in response to direct (e.g., temperature) or indirect (e.g. soil water deficit) effects of experimental climate warming under both ambient and reduced rainfall; (ii) stomatal behaviour varies with species identity, drought tolerance, and biome association; and to determine (iii) whether there are generalisable patterns across species, their associations (e.g., biome) and environmental conditions: that is, do species differ in response to climate treatments, and our responses to warming and rainfall reduction additive or interactive? To achieve those goals, we addressed the following issues and hypotheses.

First, consistent with optimisation theory that predicts a decrease of λ with declining water availability, we hypothesise 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 will decrease with reduced rainfall*.

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 (Héroult 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 hypothesise 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 hypothesise *H3: reduced rainfall and warming will have 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.*

Modelling shows that plants can be differentially acclimated to low soil moisture in ways consistent with so-called aniso- and isohydric behaviour (i.e., slow vs. fast decline of g_s in response to changing environmental conditions; Mrad 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 behaviour and conservative water use strategies, and thus, we hypothesise *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.

2 | MATERIALS AND METHODS

2.1 | 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 characterised by 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, respectively (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 analysed 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 Reich et al. (2015), Sendall et al. (2015), and Reich et al. (2016). The 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),

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).

		2009–2019 June 1st to September 30th			
		CFC		HWRC	
		Open	Closed	Open	Closed
Aboveground temperature (°C)	$T_{ambient}$	16.41 (7.63)	16.10 (5.92)	16.96 (7.65)	16.54 (5.66)
	$\Delta_{3.3°C}$	3.12 (1.27)	3.49 (0.88)	3.19 (1.28)	3.51 (0.87)
Belowground temperature (°C)	$T_{ambient}$	16.43 (2.71)	15.89 (2.80)	16.96 (3.20)	15.89 (2.84)
	$\Delta_{3.3°C}$	3.20 (0.79)	3.27 (0.71)	3.17 (0.92)	3.41 (0.67)
Soil volumetric water content (%)	$VWC_{Tambient*ambient\ rainfall}$	21.9 (3.70)	19.61 (4.39)	15.08 (4.28)	23.77 (6.20)
	$VWC_{+3.3°C*ambient\ rainfall}$	16.56 (3.90)	16.87 (4.04)	11.46 (3.30)	20.74 (5.05)
	$VWC_{Tambient*reduced\ rainfall}$	18.94 (5.00)	–	14.14 (3.24)	–
	$VWC_{3.3°C*reduced\ rainfall}$	12.98 (3.31)	–	11.07 (3.08)	–
2012–2019					
Precipitation (mm)	Total summer	454.5 (70.0)	454.5 (70.0)	376.8 (52.6)	376.8 (52.6)
	Total summer after reduction	269.5 (41.0)	–	222.9 (30.8)	–
	% Reduction	40.8 (1.1)	–	40.9 (1.8)	–
	40 years nearby weather stations	424.4 (101.9)		408.8 (100.6)	

Note: The comparison summaries represent means for each treatment based on hourly records for each experimental plot and averaged for the period from June 1st to September 30th (i.e., 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.

we used soil temperature probes randomly inserted on each plot at a 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 3.3°C (Tables 1 and 2, Supporting Information: Figures S1 and S2), but as they were close for the full period of warming treatments, we call the warming treatment +3.3°C 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 minimise the shading of tree seedlings, rainout shelters were typically deployed during overcast conditions or at night shortly before and closed shortly after (typically 0.5–1 h) 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 to September 30th). In each growing season, about half of this time occurred during night hours (for more information about treatments, see Table 1 and Supporting Information: Figures S1 and S2). Across the seven years of summer rainfall removal, we saw an average reduction of 40.7% in summer rainfall as compared to ambient plots (Table 1 and Supporting Information: 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 realised 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 a relatively wet summer (~70th percentile wettest) and rather a dry summer (~10th percentile driest) for ambient and reduced rainfall, respectively as compared to the broader temporal context of the 100 years 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—cm^3 water/cm^3 soil) was measured across 0-30 cm soil profile on an hourly basis throughout all years (see Table 1 and Supporting Information: 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 4 invasive tree species (a total of 21) in different combinations among years and canopies (for details, see Table 3 and Supporting Information: Table S2). Seedlings were sourced from local ecotypes, well suited for the research site's typical environmental conditions. We planted 1- or 2-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

TABLE 2 Mean ambient plant surface temperatures (±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).

Canopy	Treatment	During the measurement campaign Mean ambient 24 h air temperatures (°C)	Mean ambient 08:00–16:00 h air temperatures (°C)	June 1st to September 30th Mean ambient 24 h air temperatures (°C)	Mean ambient 08:00–16:00 h air temperatures (°C)
Closed	Ambient temperature – ambient rainfall	17.03 (3.75)	20.64 (4.35)	16.26 (5.78)	19.73 (5.30)
Closed	+3.3°C – ambient rainfall	20.51 (3.73)	23.91 (4.35)	19.17 (5.89)	22.51 (5.43)
Open	Ambient temperature – ambient rainfall	16.89 (4.30)	23.29 (5.46)	16.36 (7.43)	22.14 (6.53)
Open	Ambient temperature – reduced rainfall	17.24 (4.36)	24.38 (5.38)	16.95 (7.85)	23.44 (6.80)
Open	+3.3°C – ambient rainfall	20.09 (4.12)	26.18 (5.43)	19.30 (7.29)	24.75 (6.58)
Open	+3.3°C – reduced rainfall	20.50 (4.11)	27.33 (5.16)	19.58 (7.79)	25.93 (6.82)

Note: Means are shown for 24 h periods, as well as for the period of the day when most photosynthetic activity occurs (08:00–16:00 h) 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.

TABLE 3 List of species with their corresponding biome and phylogenetic associations and drought indices as described by Niinemets and Valladares (2006).

Scientific name	Common name	Biome association	Phylogeny	Drought index
Abies balsamea L.	Balsam fir	Boreal	gymnosperm	1 (I)
Acer negundo L.	Box elder	Temperate	angiosperm	1 (I)
Tsuga canadensis (L.) Carriere	Canadian hemlock	Temperate	gymnosperm	1 (I)
Frangula alnus Mill.	Glossy buckthorn	Invasive	angiosperm	1.37 (I)
Populus tremuloides Michx.	Trembling aspen	Boreal	angiosperm	1.77 (I)
Acer rubrum L.	Red maple	Temperate	angiosperm	1.84 (I)
Picea mariana (Mill.) Britton	Black spruce	Boreal	gymnosperm	2 (M)
Betula papyrifera Marshall.	Paper birch	Boreal	angiosperm	2.02 (M)
Acer saccharum Marshall.	Sugar maple	Temperate	angiosperm	2.25 (M)
Pinus strobus L.	White pine	Temperate	angiosperm	2.29 (M)
Thuja occidentalis L.	White cedar	Temperate	gymnosperm	2.71 (M)
Picea glauca (Moench) Voss.	White spruce	Boreal	gymnosperm	2.88 (M)
Quercus rubra L.	Red oak	Temperate	angiosperm	2.88 (M)
Tilia americana L.	American basswood	Temperate	angiosperm	2.88 (M)
Betula alleghaniensis Britt.	Yellow birch	Temperate	angiosperm	3 (T)
Pinus resinosa Sol. Ex Aiton	Red pine	Temperate	gymnosperm	3 (T)
Lonicera morrowii A. Gray	Morrow's honeysuckle	Invasive	angiosperm	3.04 (T)
Lonicera tatarica L.	Tatarian honeysuckle	Invasive	angiosperm	3.04 (T)
Rhamnus cathartica L.	Common buckthorn	Invasive	angiosperm	3.46 (T)
Quercus macrocarpa Michx.	Bur oak	Temperate	angiosperm	3.85 (T)
Pinus banksiana Lamb.	Jack pine	Boreal	gymnosperm	4 (T)

Note: 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 follows: 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.

boreal-temperate ecotonal region of northern Minnesota. Newly planted cohorts of seedlings (i.e., groups of seedlings that were planted in the same year) were given 1 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 2 to 8 years of age (See Table 3 and Supporting Information: Table S2 for more details).

2.2 | Gas exchange measurements

Eleven years of surveys of net assimilation (A_{net}) of CO_2 at light saturation using Li-6400XT—infrared gas exchange analysers (LICOR) were conducted in situ on randomly selected individuals of target species (for details, see Table 3 and Supporting Information: S2), yielding 17 727 unique measurements. Measurements were typically conducted in 1–2 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 the scope and constraints of environmental conditions during which they were performed. Thus, all measurements were performed between 09:00 and 16:00 h 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 set for temperature, relative humidity (RH), and VPD to track ambient conditions within a range of average daily conditions allowing optimal gas exchange. Thus, RH in the leaf chamber was maintained within a range from 40% to 80% with the target goal of 60%. The airflow rate was set to 500 µmol $s^{-1,}$ and the leaf temperature, and VPD (Vapour Pressure Deficit) were unconstrained (aside from the control of RH) due to the limitations of the instrument to control temperature and VPD under field conditions. Across all measurements, leaf temperatures ranged from 19.3°C to 34.4°C (for the 10th and 90th percentile, respectively) with a mean of 27.4°C and $VPD_{chamber}$ (Vapour Pressure Deficit of the air inside the leaf chamber) ranged from 1.0 to 2.89 kPa (for the 10th and 90th percentile, respectively) and a mean of 1.89 kPa. On the other hand, to achieve saturating levels of irradiance, the Photosynthetically Active Radiation (PAR) was set to 1200 µmol m^{-2} s^{-1} for plants grown in the open canopy and 800 µmol m^{-2} 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. 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 Supporting Information: 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 parameterise the USO model and obtain estimates of g_1.

2.3 | Modelling

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 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 negative $C_{i,}$ etc.) or measured at unfavourable 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 the methodology of the initial evaluation of the carbon assimilation measurements used in the further modelling work, see Supporting Information.

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 analyse 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 Core Team, 2021) 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 × warming × rainfall reduction × treatment replicate (i.e., individual research plot) × block × canopy × site × year × 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:

1. closed canopy: species × warming × site × year × measurement campaign,
2. open canopy: species × warming × rainfall reduction × site × year × measurement campaign.

This yielded a total of 2732 estimates of g_1 by fitting the USO model. The mean number of data points used to fit the USO model was 6 (±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 (Supporting Information: Table S2 and Figures 1, 4, and 5) 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

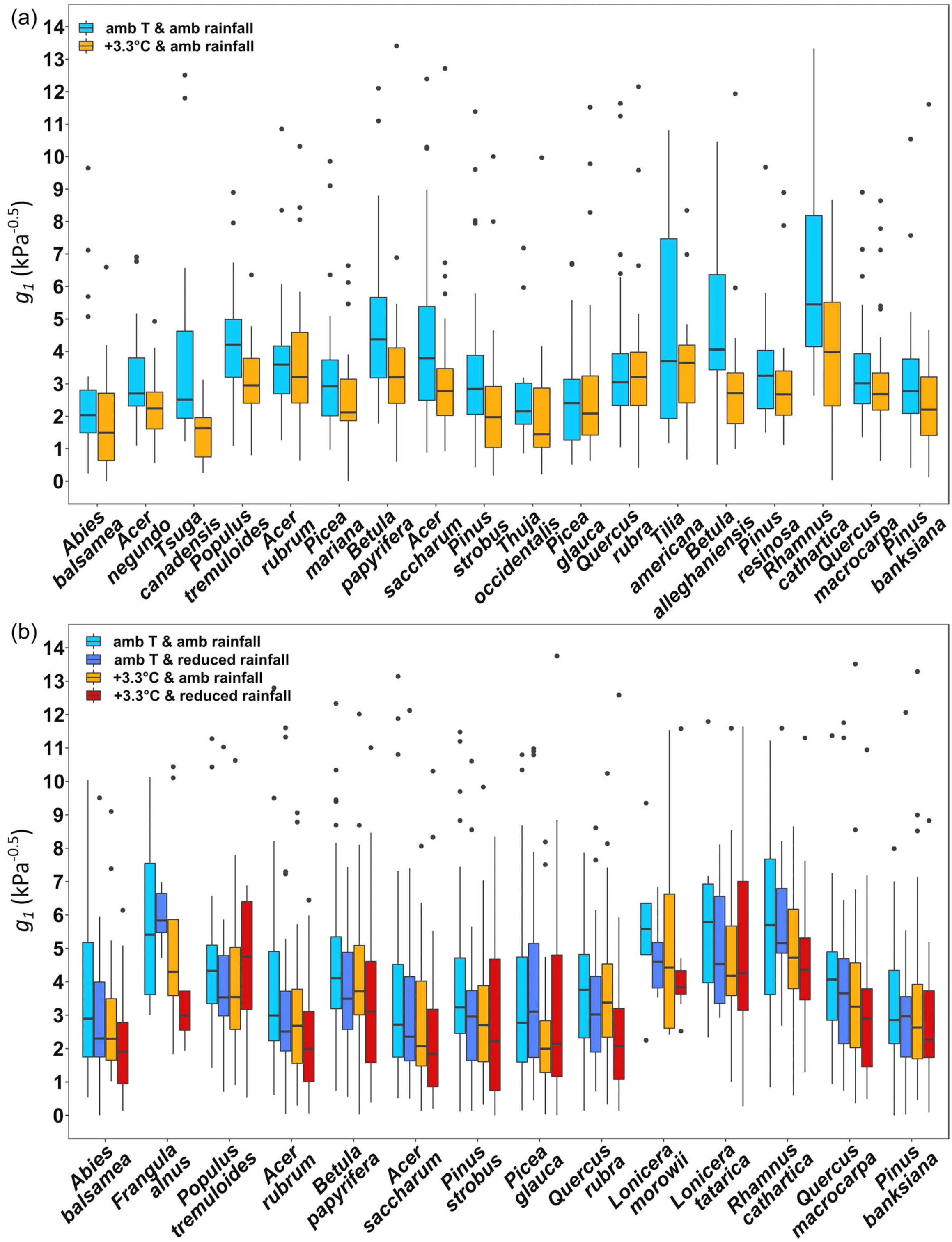

FIGURE 1 Effect of experimental treatments on g_1 estimates for all tested species. Panel (a) represents species grown in the closed canopy (n = 982), and panel (b) represents species from the open canopy (n = 1604). Species on each panel are organised with increasing drought tolerance from left to right 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. [Color figure can be viewed at wileyonlinelibrary.com]

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., 2016; 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 a 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 (~5%) out of 2732 total g_1 estimates as potential outliers. Out of those 146 g_1 estimates ~75% of them were above the maximum values reported elsewhere in the literature (e.g., Franks et al., 2017; Gimeno et al., 2016; Héroult et al., 2013; Medlyn et al., 2011). 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 ($g_1 \geq 500$; 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 24 h averages of the soil VWC on the day when the A_{net} measurements were made. The categorical values of VWC were created by binning 24 h 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).

2.4 | 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 four-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 three-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. Additionally, 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 of seedlings (i.e., groups of seedlings that were planted in the same year) behaved differently in response to environmental drivers and found no evidence for this, so we did not further consider those in analyses (but see Supporting Information: Figure S3). We also constructed additional mixed effects models to analyse 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 Table 3 and Supporting Information: Table S2). All statistical analyses were carried out in JMP statistical software (JMP 14.2, SAS Institute).

3 | RESULTS

Over more than a decade of experimental manipulation, growing conditions were altered in a consistent and significant way in our research plots. The warming treatment elevated temperature above and belowground by 3.3°C on average across all years, sites, and canopies (see Table 1 and Supporting Information: 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 Supporting Information: 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—λ; $p \leq 0.0087$, Table 4). In addition to being true on average among species, these results were generally consistent among species in the open plot conditions, as species did not differ in their responses to either driver. In the understory, species did not differ in responses of g_1 to rainfall reduction but did differ in their responses to warming (p = 0.0384, Table 4), because responses ranged from negligible to modestly negative to strongly negative among species. We organise the presentation of results around the hypotheses.

3.1 | H1: g_1 decreases with reduced rainfall

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 Supporting Information: 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 in the g_1 parameter was generally consistent across both sites and years (see Figure 2b). The role of VWC in these responses is presented below with respect to both rainfall and warming treatment effects.

3.2 | H2: g_1 decreases 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, years, and both

TABLE 4 Mixed effect models conducted on the g_1 estimates.

Source	Open canopy g_1 estimates $R^2 = 0.24$, $n = 1604$			Closed canopy g_1 estimates $R^2 = 0.31$, $n = 982$		
	df	*F ratio*	*Prob > F*	*df*	*F ratio*	*Prob > F*
Site	1	1.4351	0.2311	1	2.6153	0.1062
Warming	1	24.559	**<0.0001**	1	52.3635	**<0.0001**
Site * warming	1	8.5896	**0.0034**	1	6.9218	**0.0087**
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	**<0.0001**	17	7.8562	**<0.0001**
Site * species	13	0.6128	0.8453	17	0.9905	0.4665
Warming * species	13	0.5705	0.8789	17	1.6954	**0.0384**
Site * warming * species	13	1.6184	0.0736	17	1.4067	0.1251
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	–	–	–

Note: Square root transformation was applied to meet ANOVA assumptions. Campaign representing day of measurement was used as random variable. Experimental factors are represented as follows: 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 treatments (i.e., open and closed canopy) are analysed separately.

sites, with some differences as imposed by interannual and site variation in realised environmental conditions (see Table 4 and Figure 2). 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).

3.2.1 | 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 modelling and data analysis section of methods for additional details on VWC bins) showed interactions among warming and VWC bin, as g_1 declined when the soil water content was low due to elevated temperatures (Table 5 and Figure 3a,b). Thus, low VWC led to low g_1 in warmer treatments, and in any given VWC bin, warming tended to drive g_1 lower. In consequence, low VWC due to treatments is part of but not the only way in which g_1 was influenced by climate treatments. Alternatively, when we add soil VWC as a covariate (Table 6) to the initial model (Table 4), a similar interpretation results. Soil VWC had a significant ($p < 0.0124$, Table 6) effect on g_1 in both canopies and a significant interaction with warming ($p < 0.019$). In essence, the impacts of warming on g_1 were strong when the soil was drier.

3.3 | H3: Reduced rainfall and warming have 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 behaviour

Warming and reduced rainfall did not show significant interaction in any model ($p \geq 0.3621$, for details, see Tables 4–6 and Supporting Information: Table 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 an 8.3% decline, while warming alone resulted in a 15.6% decline of g_1, and both treatments acting together reduced g_1 by 26.5% (see Supporting Information: Table S2 and Figures 1b, 2b, 4b, and 5b).

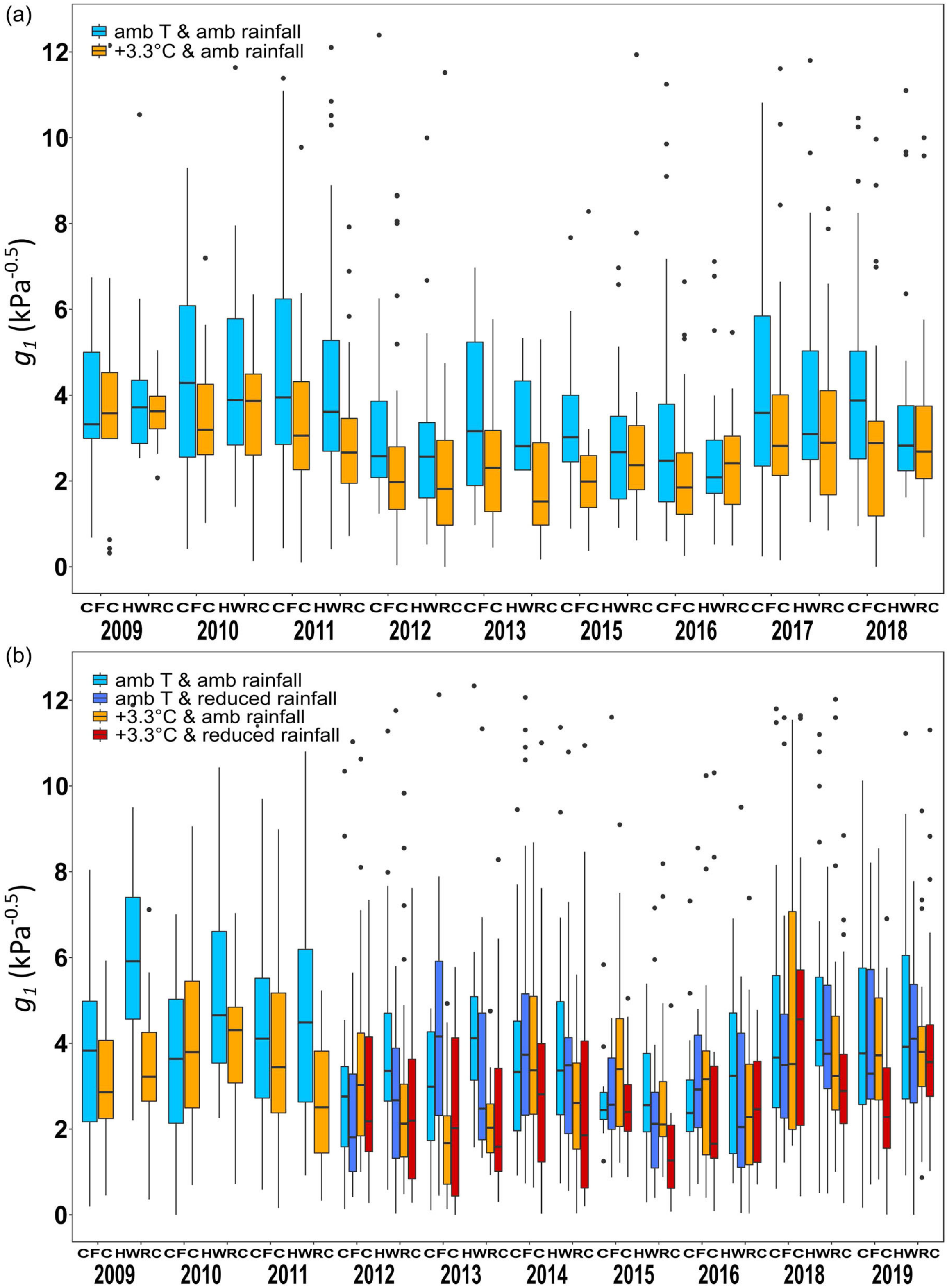

FIGURE 2 Comparison of g_1 estimates across years, sites, and canopies. Panel (a) depicts a closed canopy, and panel (b) open canopy. Whiskers extend to 1.5 times of interquartile range above and below the hinges of the box plot with the median. Any observations outside this range are denoted as individual points. The horizontal line inside the box denotes the median of the values. [Color figure can be viewed at wileyonlinelibrary.com]

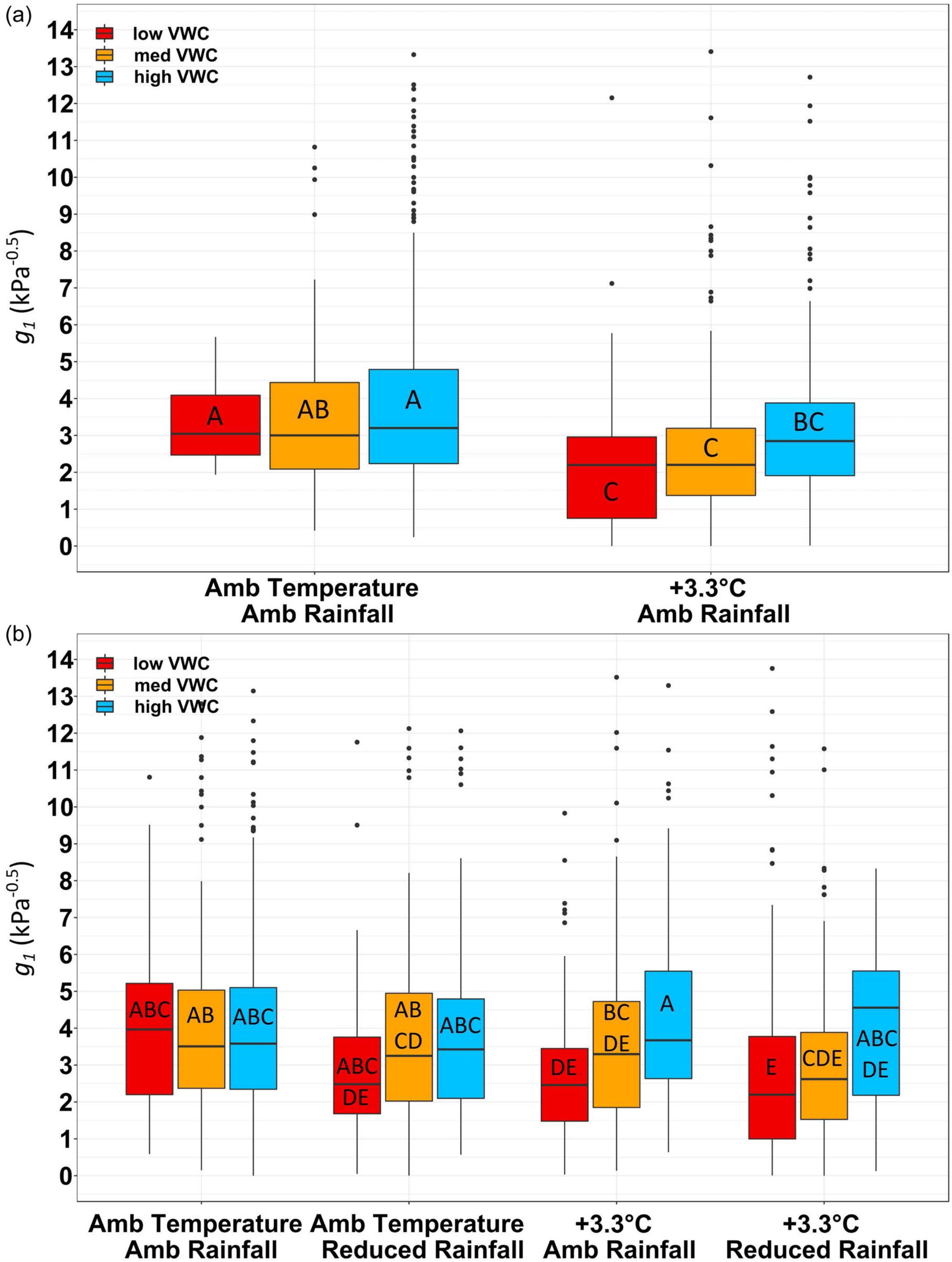

FIGURE 3 Effect of soil moisture on the mean estimates of g_1 in respect to soil VWC (soil Volumetric Water Content) categories (low VWC < 12%, medium VWC 12–16.99%, and high VWC ≥ 17% of 24 h average of the Volumetric Water Content on the day of measurement). Panel (a) represents a closed canopy with n = 982, and panel (b) represents an 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. Boxes not connected by the same letter are significantly different. [Color figure can be viewed at wileyonlinelibrary.com]

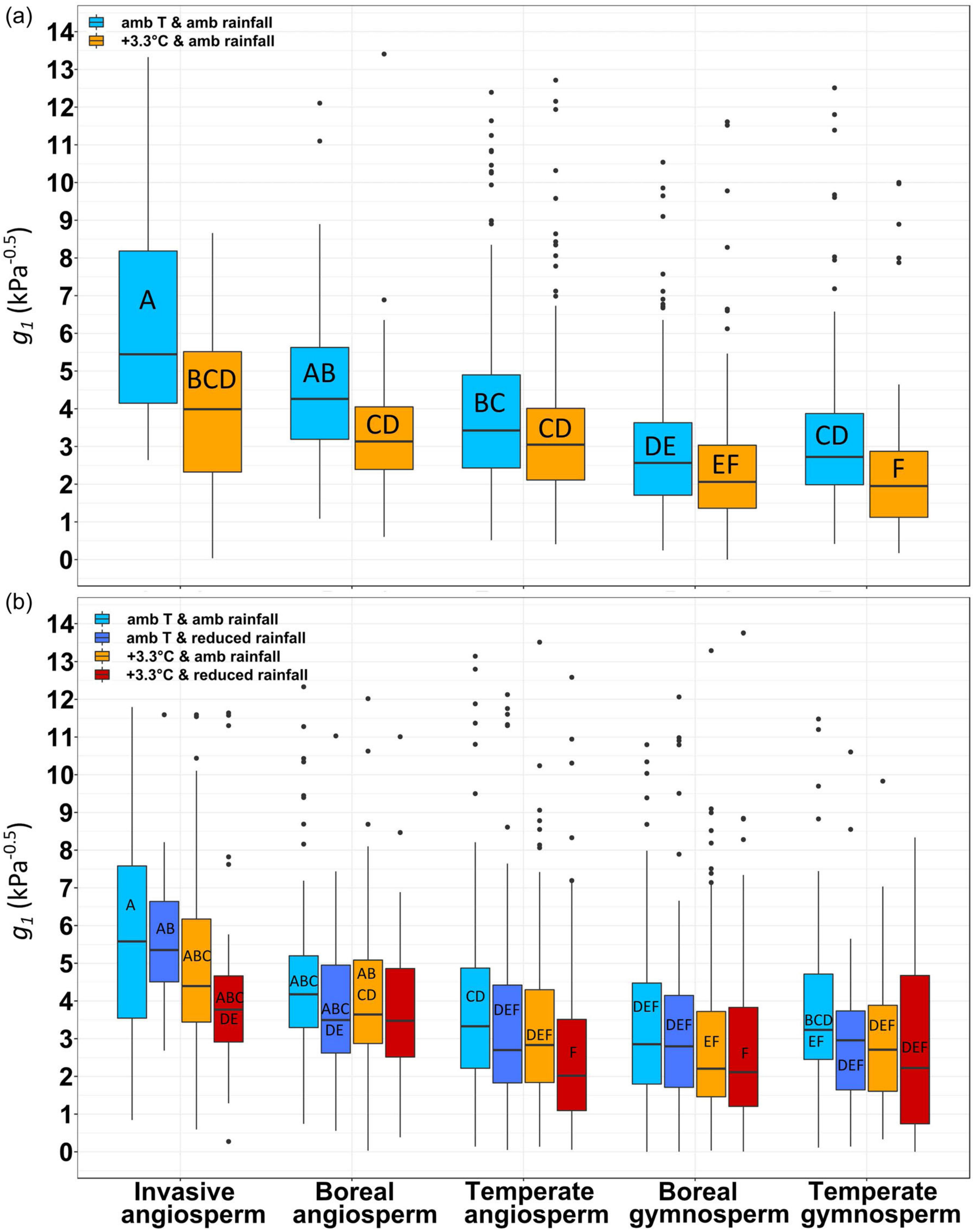

FIGURE 4 g_1 estimates for all species grouped by functional type and phylogenetic association. Panel (a) represents a closed canopy (n = 982), and panel (b) represents an open canopy (n = 1604). Species are grouped into one of five categories in accordance with their phylogenetic and biome association (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. Boxes not connected by the same letter are significantly different. [Color figure can be viewed at wileyonlinelibrary.com]

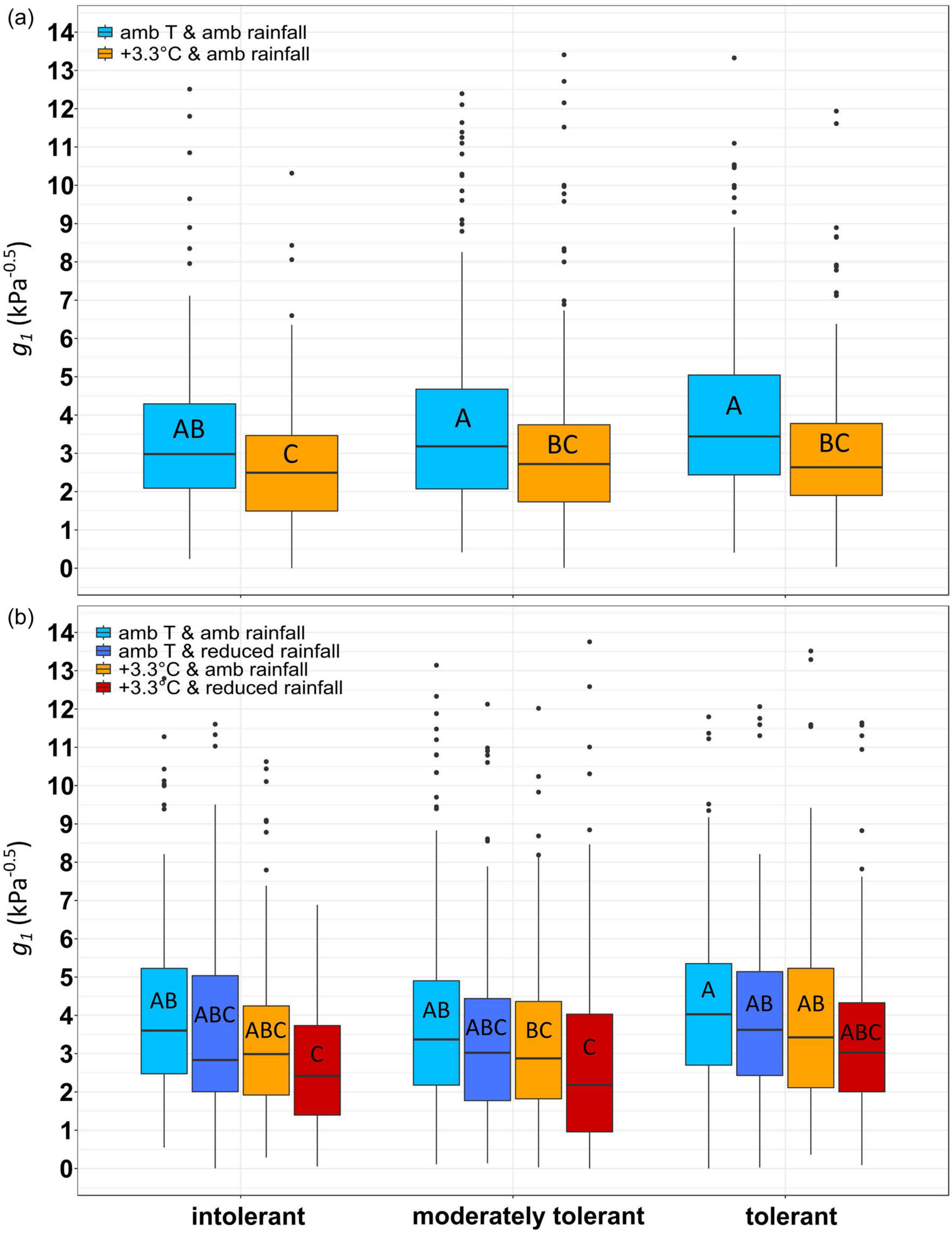

FIGURE 5 Effect of warming and reduced rainfall on estimates of g_1 for species grouped by their drought tolerance adaptations into three major groups: (i) intolerant (drought index from 1 to 1.84), (ii) moderately tolerant (drought index from 2 to 2.88), and (iii) tolerant (drought index from 3 to 4) in accordance with Niinemets and Valladares (2006) (for details about drought index see Table 4 and Supporting Information: S1). Panel (a) show a closed canopy (n = 982), and panel (b) represents an 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. Boxes not connected by the same letter are significantly different. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 5 Mixed effect models conducted on the g_1 estimates.

Source	Open g_1 estimates $R^2 = 0.15$, $n = 1604$			Closed g_1 estimates $R^2 = 0.15$, $n = 982$		
VWC bins	*df*	*F ratio*	*Prob > F*	*df*	*F ratio*	*Prob > F*
Warming	1	7.8423	**0.0052**	1	39.8832	**<0.0001**
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			

Note: Square root transformation was applied to meet ANOVA assumptions. Campaign representing the day of measurement was used as a random variable. Experimental factors are represented as follows: 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 Supporting Information: Methods). The environmental treatments (i.e., open and closed canopy) are analysed separately.

TABLE 6 Mixed effects models conducted on the g_1 estimates.

	Open canopy g_1 estimates $R^2 = 0.26$, $n = 1604$			Closed canopy g_1 estimates $R^2 = 0.31$, $n = 982$		
Source	*df*	*F ratio*	*Prob > F*	*df*	*F ratio*	*Prob > F*
Warming	1	0.3495	0.5545	1	22.5932	**<0.0001**
Reduced rainfall	1	0.7534	0.3855			
Warming * reduced rainfall	1	0.0046	0.946			
Species	13	5.6022	**<0.0001**	17	6.1986	**<0.0001**
Warming * species	13	1.0447	0.4049	17	1.1142	0.3343
Reduced rainfall * species	13	0.2389	0.9975			
Warming * reduced rainfall * species	13	0.4048	0.9687			
VWC	1	25.6956	**<0.0001**	1	6.3843	**0.0124**
Warming * VWC	1	7.7082	**0.0056**	1	5.525	**0.019**
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	1.4536	0.1045
Warming * species * VWC	13	1.0489	0.4009	17	0.7683	0.7312
Reduced rainfall * species * VWC	13	0.4467	0.9526			
Warming * reduced rainfall * species * VWC	13	0.7669	0.6962			

Note: Square root transformation was applied to meet ANOVA assumptions. Campaign representing the day of measurement and site was used as a random variable. Experimental factors are represented as follows: 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 treatments (i.e., open and closed canopy) are analysed separately.

3.4 | H4: Species adapted to either drier and/or warmer conditions on average have lower and less sensitive g_1 than species adapted to more mesic or cooler conditions

Species (for details about species, see Table 3 and Supporting Information: Table S2) differed in their g_1 parameter ($p < 0.0001$, Tables 4, 6, Figure 1; Supporting Information: S1 and S2). Species average g_1 in ambient growth conditions ranged in the open canopy from 2.8 for *P. banksiana* to 5.5 in *F. alnus*, and in the 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. tatatrica* and *R. cathartica*) and native *T. americana* had the highest g_1 of all species (Figure 1 and Supporting Information: 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, 4, and 5 and Supporting Information: Table S2.

There were few differences among species and their respective higher groupings (i.e., drought tolerance, biome association, and phylogenic associations) in sensitivity of g_1, (i.e. the decline of g_1 in response to rainfall reduction or warming; Figures 4 and 5 and Supporting Information: Table S1) as most species and groups responded to warming and reduced rainfall by significantly reducing g_1 (Table 4 and 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*.

4 | DISCUSSION

More than a decade of measurements documented generally consistent ways that warming and reduced rainfall conditions associated with future climate change influenced the trade-off of water loss versus 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 WUE of plants by maintaining stomata less open (H1, H2). The direction of these responses to experimental manipulations was uniform across all species despite differences on average g_1 associated with species-specific adaptations (i.e., drought tolerance) and associations (i.e., the 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 (Figures 1 and 2), 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 temperature species to more conservative water-spending stomatal behaviour, likely helping plants ameliorate drought stress but at a carbon cost.

The unified optimisation 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 (Duursma et al., 2013; Gimeno et al., 2016; Nijs et al., 1997). Our results support that prior work but only when water was abundant (Table 5 and Figure 3). This is firm evidence that the weak neutral to a 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 (Reich et al., 2018; Seager et al., 2014; Wang et al., 2014). Consequently, to untangle the direct effect of temperature on g_1, thermal effects need to be separated from the indirect effects of warming on soil moisture—that is, thermal effects should be assessed when VWC is not limiting.

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

We hypothesised 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 experimental conditions did not differ greatly 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; Héroult et al., 2013; 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 behaviour, 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_1 in relation to drought adaptation. Additionally, trait differences and acclimation may increase some species' tolerance to drought without the 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, modelling work by Mrad et al. (2019) demonstrates that either aggressive or conservative behaviour in water use might be related to the acclimation of the rooting zone to competition for water, and little is known about such differences for our species.

Despite individualistic variation on average g_1 among species, there was a consistent movement towards more conservative (i.e., water-saving) stomatal behaviour 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.

4.2 | Importance of experimentally quantifying stomatal behaviour

Continuing efforts at improving global land models and projections of the effects of climate change on species individual, population, ecosystem, and biome responses call not only for the improvement of representation of specific processed-based models but also for a better representation of specific processes parameterisation based on new data syntheses and/or experimental findings (De Kauwe et al., 2015; Medlyn et al., 2016; Zhou et al., 2019). For example, De Kauwe et al. (2015) shows that differences in plants sensitivities at an individual level have important implications at the ecosystem level, and that land surface models likely will overestimate impacts of drought unless variation and sensitivities across different representants of vegetation are incorporated. Moreover, Zhou et al. (2019) underscored the importance of observing plants' responses characterised by different adaptations from a short to long-term perspective. Our work should help such modelling work because documenting stomatal behaviour in response to climate change factors provides the needed work quantitative characterisation of stomatal behaviour and its variability that is based on a realistic long-term implementation of climate change drivers for multiple species representing contrasting adaptations and origins.

5 | 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 behaviour is expected to vary across species and their hierarchical affiliations (e.g., the climate of origin or phylogeny) in response to climate change. We found that there was a large variation among species intrinsic g_1; however, their responses to reduced rainfall and warming did not depend on species identity or grouping. In particular, we found that g_1 was reduced in general across species in response to growth conditions that caused a decline of soil VWC (i.e., rainfall removal and warming) but also further declined due to warming whenever soil moisture deficits were high. Thus, both indirect impacts of warming (through soil water declines) and other unidentified mechanisms will likely lead to increasingly conservative water-carbon trade-off behaviour for temperate and boreal tree species in a warmer world.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

SUMMARY STATEMENT

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

ORCID

Artur Stefanski http://orcid.org/0000-0002-5412-1014

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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