

A meta-analysis of mesophyll conductance to CO₂ in relation to major abiotic stresses in poplar species

Raed Elferjani^{1, (*)}, Lahcen Benomar^{2, (*) (**)}, Mina Momayyezi³, Roberto Tognetti⁴, Ülo Niinemets⁵, Raju Y. Soolanayakanahally⁶, Guillaume Thérout-Rancourt⁷, Tiina Tosens⁵, Mebarek Lamara², Francesco Ripullone⁸, Simon Bilodeau-Gauthier⁹, Mohammed S. Lamhamedi⁹, Carlo Calfapietra¹⁰

1 Réseau Reboisement Ligniculture Québec, Université TÉLUQ, Montréal, QC H2S 3L5, Canada

2 Forest Research Institute, University of Québec in Abitibi-Témiscamingue, Rouyn-Noranda, QC, J9X 5E4, Canada

3 Department of Viticulture and Enology, University of California, Davis, CA95616 USA

4 Università degli Studi del Molise, Via De Sanctis, 86100, Campobasso, Italy

5 Estonian University of Life Sciences, Kreutzwaldi 1, 51006 Tartu, Estonia

6 Indian Head Research Farm, Agriculture and Agri-Food Canada, Indian Head, SK, S0G 2K0, Canada

7 Institute of Botany, University of Natural Resources and Life Sciences, Gregor-Mendel-Strasse 33, 1180 Vienna, Austria

8 University of Basilicata, Via Ateneo Lucano 10, 85100 Potenza, Italy

9 Direction de la recherche forestière, 2700 rue Einstein, Québec, QC, G1P 3W8, Canada

10 Institute of Agro-Environmental & Forest Biology (IBAF), National Research Council (CNR), Via Marconi 2, Porano (TR) 05010, Italy

(*) RE and LB have equally contributed to writing of the manuscript and are joint first-authors.

(**) Corresponding author: lahcen.benomar.1@gmail.com

30 **Highlight**

31 Our meta-analysis on the variation of mesophyll conductance under abiotic stresses
32 shows a noticeable response to light gradient, soil moisture and nitrogen availability and
33 a significant relationship with specific leaf area

34

Abstract

Mesophyll conductance (g_m) determines the diffusion of CO_2 from the substomatal cavities to the site of carboxylation in the chloroplasts and represents a critical limiting factor to photosynthesis. In this study, we evaluated the average effect sizes of different environmental constraints on g_m in *Populus* spp., a forest tree model. We collected raw data of 815 $A-C_i$ response curves from 26 datasets to estimate g_m , using a single curve-fitting method to alleviate method-related bias. We performed a meta-analysis to assess the effects of different abiotic stresses on g_m . We found a significant increase in g_m from the bottom to the top of the canopy that was concomitant with the increase of maximum rate of carboxylation and light-saturated photosynthetic rate (A_{\max}). g_m was positively associated with increases in soil moisture and nutrient availability, but insensitive to increasing soil copper concentration, and did not vary with atmospheric CO_2 concentration. Our results showed that g_m was strongly related to A_{\max} and to a lesser extent to stomatal conductance (g_s). Also, a negative linear relation was obtained between g_m and specific leaf area, which may be used to scale-up g_m within the canopy.

Key words

$A-C_i$ curve, abiotic stress, mesophyll conductance, meta-analysis, photosynthesis, poplar

Introduction

Carbon assimilation of plants is importantly determined by the diffusion efficiency of CO₂ from the atmosphere to the site of carboxylation. The rate of CO₂ diffusion is affected by two main diffusion limitations. The first limitation controls the CO₂ flux from the atmosphere to the sub-stomatal cavities through the stomata and is characterized by stomatal conductance (g_s). The second limitation determines the diffusion of CO₂ from the substomatal cavities to the sites of carboxylation in the chloroplasts and is characterized by mesophyll conductance (g_m). g_m is composed of gaseous and liquid phase resistances (Flexas et al., 2008; Evans et al., 2009; Niinemets et al., 2009). CO₂ diffusion inside the leaves is complex, facing a series of structural barriers coupled with biochemical regulations. It has been shown that g_m is typically limited by liquid phase conductance both in species with soft mesophytic leaves as well as in species with tough xerophytic leaves (Tomás et al., 2013; Tosens et al., 2012a,b). The liquid phase is a multi-components pathway that involves the mesophyll cell wall thickness and porosity, the plasmalemma, the chloroplast envelope, the chloroplast thickness and the mesophyll surface area exposed to intercellular air spaces per unit of leaf area (Evans et al., 2009; Tosens et al., 2012b; Tomás et al., 2013). After extensive study during the last two decades, g_m is now widely accepted as a critical limiting factor to photosynthesis, which has to be considered in characterizing plant carbon gain potentials and responses to future climate change (Evans et al., 2009; Niinemets et al., 2009; Niinemets et al., 2011; Flexas et al., 2016).

Mesophyll conductance has been shown to respond to environmental stress and may govern functional plasticity of photosynthesis and plant fitness under limited resources

(Galle et al., 2009; Barbour et al., 2010; Buckley and Warren, 2014; Th  roux-Rancourt et al., 2015; Flexas et al., 2016; Shrestha et al., 2018). However, recent findings on the response of g_m to abiotic stress are conflicting and inconclusive, demonstrating the complex nature of g_m variation (Flexas et al., 2008; Niinemets et al., 2009; Zhou et al., 2014; Shrestha et al., 2018). This suggests that the environmental and species-specific response (acclimation) of g_m to growth conditions should be considered to predict plant performance in the field. Among the contrasting environmental responses, growth temperature may (Warren, 2008; Silim et al., 2010) or may not (Dillaway and Kruger, 2010; Benomar et al., 2018) affect g_m . Similarly, the increase in soil nitrogen may (Warren 2004; Shrestha et al., 2018; Xu et al., 2020; Zhu et al., 2020) or may not (Bown et al., 2009) stimulate g_m . The magnitude of decrease in g_m under water stress and low light differed among studies (Warren et al., 2003; Niinemets et al., 2006; Montpied et al., 2009; B  gelein et al., 2012; Tosens et al., 2012a; Zhou et al., 2014; Peguero-Pina et al., 2015; Th  roux Rancourt et al., 2015). These discrepancies among studies result in part from (i) the absolute changes in anatomical, morphological (mesophyll structure) and biochemical (aquaporins and carbonic anhydrase) traits controlling g_m , as well as from changes in the relative contribution of these traits (Marchi et al., 2008; Tom  s et al., 2013), and from (ii) the level of coordination between g_m , g_s and leaf-specific hydraulic conductivity (K_L) (Flexas et al., 2013; Th  roux-Rancourt et al., 2014; Xiong et al., 2017). Given the complex interplay between different factors controlling g_m , it is important to examine its acclimation at the genus and species level to gain a general insight into the mechanistic basis of changes in g_m .

Five methods exist to estimate g_m : a) chlorophyll fluorescence coupled to gas exchange (Harley et al., 1992), (b) carbon isotope discrimination coupled to gas exchange (initially developed by Evans et al., 1986), c) oxygen isotope discrimination (Barbour et al., 2016), (d) $A-C_i$ curve fitting (Ethier and Livingston, 2004; Sharkey et al., 2007), and (e) 1D modeling of g_m from leaf structural characteristics (Evans et al., 2009, Tosens et al., 2012b, Tomas et al., 2013). All of these methods are based on assumptions and each one has its limitations (Flexas et al., 2013; Tosens and Laanisto, 2018). The standard deviation of the estimate of g_m may vary from 10% to 40%, which may limit our understanding of g_m acclimation to growth conditions, particularly when the variation between treatments or studies is less than the error of estimates (Sun et al., 2014a).

Populus spp., model crops in forestry characterized by high yield potential, have been the subject of numerous studies to understand the physiological response to environmental factors but research is still necessary to make assessment of effects sizes and to draw generalizations (Larocque et al., 2013). A general understanding of the CO_2 pathway through mesophyll and how it is affected by environmental factors would be beneficial in the efforts to (i) accurately predict canopy photosynthesis under different environmental conditions, particularly under warmer and drier climate, and improve global carbon assimilation models and (ii) develop and effectively select more resilient and productive cultivars for wood, bioenergy and bioremediation. Substantial data of $A-C_i$ response curves in the literature has been used to estimate photosynthetic parameters, not to estimate g_m , and such compiled dataset would provide a basis to make such assessments on the response of g_m to the environment.

In this study, we compiled 815 $A-C_i$ response curves from 26 datasets of different poplar species and hybrids (Table 1). Published $A-C_i$ curve-fitting approaches differ broadly regarding the rectangularity of the hyperbola, segmentations of the model of photosynthesis and determination of the transition value of CO_2 from carboxylation to electron transport (Harley et al., 1992; Ethier and Livingston, 2004; Manter and Kerrigan 2004; Dubois et al., 2007; Sharkey et al., 2007; Pons et al., 2009; Gu et al., 2010). These approaches led to different fitted values (Miao et al., 2009, Sun et al., 2014a). Although $A-C_i$ curve fitting is unreliable for species with large g_m , it can provide results similar to those obtained from direct measurements for species with medium to low g_m (Niinemets et al., 2005, 2006; Qiu et al., 2017; Xu et al., 2020). Using the compiled $A-C_i$ response curves, we performed curve fitting using a single method (Ethier and Livingston, 2004) to alleviate the fitting method bias and to obtain uniformed estimates of g_m , maximum rate of carboxylation (V_{cmax}) and rate of electron transport (J). We further collected related variables like leaf nitrogen content, stomatal conductance and specific leaf area (SLA) when data were available. Our main goal was to find trends in the response of mesophyll conductance to prevalent abiotic stressors and to examine the relationship between g_m and other leaf physiological and morphological traits. We believe that a meta-analytical approach to analyze the accumulated data on the diffusion of CO_2 through the mesophyll diffusion pathway in relation to other photosynthesis-related traits provides key insight into physiological and structural controls on mesophyll conductance and into the environmental plasticity of mesophyll conductance. We aim at contributing to the efforts of improving poplar photosynthetic

efficiency in poplar breeding programs, and at improving modelling of global carbon assimilation of biomass and bioenergy crops under climate change.

Materials and methods

Data collection

Data were collected by a web search in Web of Science, Scopus, and Google Scholar using the following key words: (“*Populus*” or “poplar” or “hybrid poplar” or “aspen”) and (“ V_{cmax} ” or “maximum rate of electron transport (J_{max})” or “mesophyll conductance”). At this step, abstract of every item was checked to confirm the paper is actually about g_m . Then, we looked at the materials and methods section of selected papers where $A-C_i$ response curves of *Populus* spp. were measured.

To get raw data of $A-C_i$ response curves, we contacted the corresponding authors or co-authors of the targeted studies by e-mail and via ResearchGate. We obtained 23 data sets from published studies and three data sets from unpublished studies (Table 1). Collectively, they provided a total of 815 $A-C_i$ response curves.

The total data of 72 genotypes were collected from measurements on plants growing in plantations (5 studies), or under controlled conditions (greenhouse or growth chamber setups; 21 studies) with optimal and stressful conditions (Table 1). After compiling all $A-C_i$ curves, the quality of the data was assessed based on the following criteria: 1) only curves with at least 2 points in the saturation region (J region) were retained; 2) only curves with $P < 0.05$ for the p-value of fitted curves using the method of Ethier and Livingston (2004) were retained; Consequently, 65 curves that did not meet these conditions were removed; and 3) based on the literature, g_m values in *Populus* spp.

using at least two methods simultaneously never exceeded $1 \text{ mol m}^{-2} \text{ s}^{-1}$ (Singsaas et al., 2004; Flexas et al., 2008; Velikova et al., 2011; Tosens et al., 2012a; Thérour-Rancourt et al., 2014; Momayyezi and Guy, 2017; Xu et al., 2020). Then, $g_m > 1 \text{ mol m}^{-2} \text{ s}^{-1}$ were considered as non-available data (94 entries), and V_{cmax} and J values retained for further analyses. Furthermore, fewer data point and/or large interval in the intracellular concentration of CO_2 ($C_i \text{ } \mu\text{mol mol}^{-1}$) in the RuBisCO limited phase region are the major source of error and probability of failing g_m (reached the set bound during the grid search) frequently encountered during $A-C_i$ curve fitting (Warren, 2006; Miao et al., 2009, Sun et al., 2014a; Moualeu-Ngangue et al., 2016).

Data subsets

To examine the effect of a given abiotic factor on g_m , we estimated that a minimum of three studies is necessary to have reliable conclusions, regardless of the genotype used. Then, we could come up with subsets of data that focused on the same variable and performed analyses on them separately (identified in the column 'treatment' in Table 1). Our first goal was to examine the effect of variations in these factors on g_m , light-saturated photosynthetic rate (A_{max}), g_s , J , V_{cmax} and in a second step, the relationships between g_m and other photosynthetic characteristics (A_{max} , g_s , J , V_{cmax}). The data subsets included the following environmental factors:

- Canopy level: four studies addressed the photosynthetic activity of leaves at the bottom, middle and top of trees (Niinemets et al., 1998; Merilo et al., 2010; Calfapietra et al., 2005; Benomar, 2012).
- Ambient CO_2 : we examined the response of trees to elevated ambient CO_2 from Calfapietra et al. (2005), Merilo et al. (2010) and Tissue et al. (2010). We

considered 370 ppm as the control treatment in the three studies, while the elevated CO₂ was 550 ppm of CO₂ for the studies of Calfapietra et al. (2005) and Merilo et al. (2010), and 700 ppm for the study of Tissue et al. (2010).

- Copper stress: data sets from studies of Borghi et al. (2007) and Borghi et al. (2008) were used to examine the response of poplar trees to contamination of the substrate with copper (Cu). Treatments were assigned to three levels of Cu: 0 (0 to 0.4 µM), 20 (20 to 25 µM) and 75 (75 to 100 µM).
- Soil nitrogen (N) content: High vs. low soil N content treatments were reported in four studies: Benomar et al. (2018), Ripullone et al. (2003), Calfapietra et al. (2005) and Xu et al. (2020). In Merilo et al. (2010), no effect of nitrogen fertilization was observed by authors due to high background nutrient availability in the plantation site.
- Soil moisture: water status of trees was assessed and data from four studies was classified into two treatments: control (optimal watering) vs. water deficit (Tosens et al., 2012a; Li et al., 2012; Thérout-Rancourt, unpublished; Benomar et al., unpublished).

For Xu et al. (2020), we extracted data from the article (means and standard errors) and generated three replicates assuming a normal distribution using SURVEYSELECT procedure of SAS (SAS Institute, software version 9.4, Cary, NC, USA). The reason is that the authors used the same curve fitting approach (Ethier and Livingston 2004) we used in the meta-analysis (Table 1).

For studies with two or more investigated factors, we considered the different levels of the factor of interest and the control level of the rest of factors to avoid between-factors

interaction effects on results. For example, in Calfapietra et al. (2005), trees were subject to different levels of N and CO₂. When we focused on the effect of N, we selected trees exposed to ambient CO₂ only (control).

Curve analysis

Mesophyll conductance and photosynthetic capacity variables, V_{cmax} and J , were estimated by fitting $A-C_i$ curve with the non-rectangular hyperbola version of the biochemical model of C₃ plants (Farquhar et al., 1980). The model was fitted using non-linear regression techniques (Proc NLIN, SAS) following Dubois et al. (2007) and Sun et al. (2014a).

Briefly, the net assimilation rate (A_n) is given as:

$$A_n = \min \{A_c, A_j\} \quad (1)$$

with

$$A_c = V_{\text{cmax}} \frac{(C_c - \Gamma^*)}{C_c + K_c \left(1 + \frac{O}{K_o}\right)} - R_{\text{day}} \quad (2)$$

$$A_j = J \frac{C_c - \Gamma^*}{4(C_c + 2\Gamma^*)} - R_{\text{day}} \quad (3)$$

$$C_c = C_i - \frac{A_n}{g_m} \quad (4)$$

where V_{cmax} is the maximum rate of carboxylation ($\mu\text{mol m}^{-2} \text{s}^{-1}$), O is the partial atmospheric pressure of O₂ (mmol mol^{-1}), Γ^* is the CO₂ compensation point in the absence of mitochondrial respiration, R_{day} is mitochondrial respiration in the light ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$), C_c is the chloroplast CO₂ ($\mu\text{mol mol}^{-1}$), C_i is the intercellular air space concentration of CO₂ ($\mu\text{mol mol}^{-1}$), K_c ($\mu\text{mol mol}^{-1}$) and K_o (mmol mol^{-1}) are the Michaelis–Menten constants of RuBisCO for CO₂ and O₂, respectively, J is the rate of electron transport ($\mu\text{mol m}^{-2} \text{s}^{-1}$). The values at 25 °C used for K_c , K_o and Γ^* were

$\mu\text{mol mol}^{-1}$, $166 \text{ mmol mol}^{-1}$ and $37.4 \mu\text{mol mol}^{-1}$, respectively (Sharkey et al., 2007) and their temperature dependencies were as in Sharkey et al. (2007).

In four data sets, measurements were carried out under a temperature that was different from the reference (25°C). In this case, V_{cmax} and J were normalized to 25°C using the model of Kattge and Knorr (2007), which integrates the acclimation to growth temperature. However, the actual values of V_{cmax} and J were more often significant compared to normalized values, and this was true using both ANOVA and regression.

Statistical analyses

Data analysis assessing the effect of the environmental factors on g_m and the relationship between g_m and the other traits were carried out using SAS software (SAS Institute, software version 9.4, Cary, NC, USA).

When at least three studies focused on one factor (nitrogen, CO_2 , canopy level, copper), the effect of treatments on light-saturated photosynthetic rate (A_{max}), g_m , and g_s was assessed, separately for each response variable, through mixed model analyses of variance using the primary data (Riley et al., 2010; Mengersen et al., 2013). “Treatment” was the fixed effect while “study” and “genotype” nested within study were the random effects. The number of replicates was not necessarily balanced across treatments. The assumptions of normality of the residuals and homogeneity of variance were verified, and a log-transformation was made when necessary.

Results

The number of studies on mesophyll conductance has rapidly increased since 2000, and more remarkably since 2013 (Fig. 1), suggesting a growing interest among plant

ecophysiologicals to understand the role of g_m in photosynthesis. This pattern was very similar to the increase of publication number on mesophyll conductance in *Populus* spp. (Fig. 2).

Canopy level

Light-saturated photosynthetic rate at an ambient CO_2 concentration (380-400 $\mu\text{mol mol}^{-1}$), A_{max} , significantly increased from $7.1 \pm 0.44 \mu\text{mol m}^{-2} \text{s}^{-1}$ on average at the bottom leaves to $13.0 \pm 0.45 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the mid-canopy, to $16.2 \pm 0.53 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the upper canopy (Fig. 3a). Similar to A_{max} , g_m had an ascending pattern, from the bottom ($0.12 \pm 0.01 \text{ mol } CO_2 \text{ m}^{-2} \text{s}^{-1}$) to the top of the canopy ($0.24 \pm 0.02 \text{ mol m}^{-2} \text{s}^{-1}$) (Fig. 3c). Stomatal conductance (g_s) was the lowest at the bottom canopy ($0.17 \pm 0.01 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$) and then increased to $0.36 \pm 0.02 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$ at the mid and the upper canopy (Fig. 3b). The g_m/g_s ratio was significantly greater at the upper canopy (1.17 ± 0.1), compared to the mid-canopy leaves (0.88 ± 0.1) and was not different everywhere else (Fig. 3d). While, V_{cmax} increased similarly to A_{max} and g_m from the bottom to the top of the canopy (Fig. 3e); however, SLA had an opposite trend (Fig. 3f).

Ambient CO_2

Increased air CO_2 had no effect on average A_{max} ($14.43 \pm 0.60 \mu\text{mol m}^{-2} \text{s}^{-1}$), g_m ($0.21 \pm 0.02 \text{ mol m}^{-2} \text{s}^{-1}$) and g_m/g_s (1.09 ± 0.1) (Fig. 4a, 4b and 4c). However, average g_s was higher ($0.40 \pm 0.03 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$) under “Ambient”, compared to “Elevated” CO_2 ($0.32 \pm 0.02 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$) (Fig. 4b).

Copper stress

A_{\max} was not affected when Cu soil concentration increased from 0 to 20 or 75 μM ($9.67 \pm 0.95 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 5a). It should be noted that at the highest Cu level (75 μM), A_{\max} ranged from 4 to 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Average g_s significantly decreased under medium (20 μM , $0.17 \pm 0.02 \text{ mol H}_2\text{O m}^{-2} \text{s}^{-1}$), and high Cu treatment (75 μM , $0.18 \pm 0.03 \text{ mol m}^{-2} \text{s}^{-1}$), compared to control treatment (Fig. 5b). Increasing Cu concentration in soil did not affect g_m (Fig. 5c). The g_m/g_s ratio was greater under 20 and 75 μM Cu, compared to control (Fig. 5d).

Soil nitrogen

A_{\max} was significantly greater ($16.1 \pm 0.61 \mu\text{mol m}^{-2} \text{s}^{-1}$) under the high soil nitrogen (HN, 250 kg N $\text{ha}^{-1} \text{y}^{-1}$ in field study or 20 mM for pot study), compared to the low nitrogen treatment (LN, $12.9 \pm 0.65 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 6a). The high supply of nitrogen increased g_s (from 0.29 ± 0.03 at LN to $0.36 \pm 0.03 \text{ mol m}^{-2} \text{s}^{-1}$ at HN) and g_m (from 0.19 ± 0.02 to $0.23 \pm 0.02 \text{ mol m}^{-2} \text{s}^{-1}$), but had no effect on g_m/g_s ratio (1.38 ± 0.16 on average) (Fig. 6b, 6c and 6d).

Soil moisture

Average A_{\max} decreased by drought (range of leaf predawn water potential under water deficit, $\Psi_{\text{leaf}} = -0.7$ to -0.8 , soil water content = 10%), dropping from $17.0 \pm 0.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $14.8 \pm 0.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ on average with minimum value ($3.8 \mu\text{mol m}^{-2} \text{s}^{-1}$) much lower than in watered trees ($8.9 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 7a). As expected, soil moisture deficit remarkably altered g_s , decreasing its average value from $0.33 \pm 0.02 \text{ mol m}^{-2} \text{s}^{-1}$ in control trees to $0.20 \pm 0.03 \text{ mol m}^{-2} \text{s}^{-1}$ under drought conditions (Fig. 7b). Drought had the same effect on g_m , but to a lesser extent than g_s . g_m decreased from 0.27 ± 0.02 to

0.19±0.02 mol m⁻² s⁻¹ under soil moisture deficit (Fig. 7c). In addition, g_m/g_s ratio increased by 37% when plants were subject to a drought (Fig. 7d).

Relationship between CO₂ diffusion and photosynthetic activity

A_{max} was strongly correlated to both g_s and g_m ($P=0.001$) and to V_{cmax} ($P=0.001$) over all the studies (Fig. 8a, 8b and 8c). Based on the collected data, g_m was significantly correlated to g_s ($P = 0.04$). However, the relationship was not linear. g_m was the highest (0.4-0.5 mmol m⁻² s⁻¹) when g_s values were intermediate (0.2-0.4 mol m⁻² s⁻¹), and lowest at high g_s values (Fig. 8e).

We found a significant negative correlation between SLA and g_m ($P=0.001$) (Fig. 8g) based on the collected data from studies that measured SLA ($n=12$). Leaf nitrogen content reported by three studies showed a significant correlation between g_m and N content per area (N_{area}) (Fig. 8f). g_m increased with N_{area} until a saturation point (~ 0.25 mol m⁻² s⁻¹).

Discussion

Canopy level

The scaling up of photosynthesis from leaves to the canopy and stands (using the model of Farquhar et al., 1980) requires a deep understanding of within-canopy variations in leaf morpho-physiology and the main drivers of foliage acclimation to the dynamic gradient of environmental conditions (light, temperature, vapor pressure deficit (VPD) and soil moisture) (Niinemets et al., 2006; Buckley and Warren, 2014; Niinemets et al., 2015). Unfortunately, pieces of knowledge regarding the variation of g_m within the canopy and its mechanistic basis are scarce, in particular for *Populus* spp. This situation

may explain why most global carbon cycle models remain “ g_m -lacking”, with possible consequences, such as overestimating the fertilization effect of CO_2 on global gross primary production and underestimation of water-use efficiency (WUE) and canopy gross photosynthesis under future climate (Sun et al., 2014b; Knauer et al., 2019).

The steep and parallel increase of g_m , A_{max} and V_{cmax} from the bottom to the top of the canopy found here for *Populus* spp. is in agreement with the findings of Niinemets et al. (2006) for *Quercus ilex* L., Montpied et al. (2009) for *Fagus sylvatica* L., and Warren et al. (2003) for *Pseudotsuga menziesii* (Mirbel) Franco. A decrease of g_m from the bottom to the top of the canopy was also reported (Bögelein et al., 2012; Cano et al., 2013). In absence of water deficit, g_m limitation is greater under high light (top of the canopy), compared to shade conditions (Niinemets et al., 2009; Cano et al., 2013).

We observed a significant inverse relationship between g_m and specific leaf area (SLA), comparable to previous studies (Montpied et al., 2009; Niinemets et al., 2006; Tosens et al., 2012b). This suggests that the increase in leaf thickness (lower SLA), e.g. in developing leaves and in leaves grown under higher light, may be associated with increased g_m (Tosens et al., 2012b). Tosens et al. (2012b) for leaves grown under low and high soil moisture, and Muir et al. (2014) for four species of *Solanum* spp. showed a positive relationship between SLA and g_m . In Tosens et al. (2012b), this relationship reflected increased density of leaves grown under lower water availability. This evidence collectively demonstrates the complex nature of the relationship between SLA and g_m , reflecting the circumstance that SLA is an inverse of the product of leaf thickness and density that can respond differently to environmental drivers (Niinemets, 1999; Poorter et al., 2009). The profile of g_m within the canopy observed here may be

partially attributable to the morphological acclimation of *Populus* spp. foliage to light availability within the canopy. Moreover, this inverse-relationship between SLA and g_m was used as an empirical model to estimate a maximum attainable g_m at different canopy layers for C_3 plant and was implemented in Community Land Model (CLM.4.5) (Sun et al., 2014b; Knauer et al., 2019).

The change in morphological traits and their role in the acclimation of g_m to a vertical gradient of environmental conditions within the canopy need additional investigations. For instance, shade acclimation of leaf morphology is associated with a lower surface area of chloroplasts exposed to intercellular air spaces (S_c/S) and thicker chloroplasts (Hanba et al., 2002; Niinemets et al., 2006; Tosens et al., 2012b; Peguero-Pina et al., 2015). Species-specific leaf development patterns (i.e., evergreen sclerophyllous vs. deciduous broadleaves) affect limitations to gas diffusion, thus determining the carbon balance of leaves (Marchi et al., 2007). However, light acclimation may be species-specific and altered by water and soil nitrogen, and leaf ontogeny (Niinemets et al., 2006; Tazoe et al., 2009; Peguero-Pina et al., 2015; Shrestha et al., 2018). It is still unclear whether g_m profile within the canopy is the result of the change in SLA.

Our results showing higher g_s and g_m/g_s at the top of the canopy are in disagreement with the findings of Montpied et al. (2009) and Bögelein et al. (2012), suggesting a species- and environment-specific gradient of g_m/g_s . Temperature and VPD responses of g_m and g_s are different (Cano et al., 2013), resulting in different diurnal patterns of g_m and g_s . Then, the gradient of g_m/g_s ratio along the canopy may drive the WUE at the canopy level and the midday depression of photosynthetic rate regardless of the level of isohydry of clones (Cano et al., 2013; Buckley and Warren, 2014; Stangl et al., 2019).

Ambient CO₂

The response of photosynthetic capacity and diffusion of CO₂ to free air CO₂ enrichment (FACE) considerably differed between species and experimental setups. The decrease in A_{\max} and g_s in response to elevated CO₂ showed in our meta-analysis is in agreement with numerous studies on *Populus* spp. and other species (Ainsworth and Rogers, 2007; Medlyn et al., 2013; DaMatta et al., 2016), but is in disagreement with the findings of some other studies, e.g., Sigurdsson et al. (2001) and Uddling et al. (2009). For g_m , the effect of growth CO₂ changed among studies and some species having an intrinsic low g_m are more likely to respond to elevated CO₂ than species with high intrinsic g_m (Niinemets et al., 2011). However, several studies have reported that g_m may decrease or be unresponsive to CO₂ enrichment (Singsaas et al., 2004; Zhu et al., 2012; Kitao et al., 2015; Mizokami et al., 2019). This suggests that the increase of A_{\max} under elevated CO₂ cannot be attributed solely to g_m variation (Singsaas et al., 2004). The absence of g_m response to elevated CO₂ complicates the research on mechanisms underlying this variation. Unlike g_m , researchers proposed some hypotheses like least-cost theory, nitrogen limitation and resources investment to explain the decrease of A_{\max} , $V_{c\max}$ and g_s under elevated CO₂ (Leakey et al., 2009; Smith and Keenan, 2020).

Copper stress

Similar to our findings, g_m remained unchanged in the herbaceous plant *Silene paradoxa* L., exposed to high Cu concentration, although g_s decreased significantly (Bazihizina et al., 2015). In other cases of exposure to other heavy metals, like nickel (Ni), Velikova et al. (2011) reported a significant decrease in chloroplast CO₂ content and mesophyll conductance in black poplar (*P. nigra* L.) exposed to 200 µM Ni under a

hydroponic setup (compared to control = 30 μ M Ni). This reduction of g_m might be attributed to an alteration of leaf structure by toxic effect of high concentrations of heavy metals in mesophyll cells Velikova et al. (2011). Hermle et al. (2007), reported an acceleration of senescence and necrosis of mesophyll cells in *P. tremula* L. leaves exposed to Cu, Zn, Cd and Pb at 640, 3000, 10 and 90 mg per kg of soil, respectively, and a decrease of chloroplast size from the early stages of exposure. The study of Hermle et al. (2007) also reported the thickening of cell walls and change of their chemical composition in damaged mesophyll cells, which might have affected permeability of cell walls and diffusion of CO₂ through them. Mercury (Hg) (HgCl₂ form) altered CO₂ diffusion through aquaporins, a membrane channel of CO₂ diffusion, in faba bean (*Vicia faba* L.) (Terashima and Ono, 2002) and significantly reduced g_m in *P. trichocarpa* Torr. & Gray. HgCl₂ may also decrease g_m indirectly by disrupting carbonic anhydrase activity, as reported by Momayyezi and Guy (2018), and demonstrated that carbonic anhydrase activity is strongly associated with g_m variation in *P. trichocarpa* Torr. & Gray (Momayyezi and Guy, 2017).

Soil Nitrogen

The increase of A_{max} by the enhancement of V_{cmax} in response to more available soil nitrogen has been established in the literature. However, the possible contribution of g_m to this augmentation remains unexplored for several species. Our results showed a concomitant increase of g_m with a higher supply of N. A positive correlation between the level of expression of AQPs genes (PIPs and TIPs) and g_m has been reported (Hanba et al., 2004; Flexas et al., 2006; Kaldenhoff et al., 2008; Perez-Martin et al. 2014), although it is still unclear whether this is a direct effect or a pleiotropic effect reflecting

simultaneous increase in A_{\max} , g_m and g_s (Flexas et al., 2012). Recent studies have demonstrated that an increase in g_m has coincided with an increase in the amount of AQPs after fertilization (Miyazawa et al., 2008b; Zhu et al., 2020).

Soil moisture

Although many studies showed a decline of g_m in response to soil water deficit (Flexas et al., 2009; Galle et al., 2009; Tosens et al., 2012a), it remains unclear if this limitation is happening within the mesophyll environment or occurs as a result of a stomatal limitation, which decreases intercellular CO_2 (C_i). Th  roux-Rancourt et al. (2015) showed that, in hybrid poplar, g_m remained unchanged ($\sim 0.3 \text{ mol m}^{-2} \text{ s}^{-1}$) following soil drying ($\Psi_{\text{leaf}} \sim -0.4$ to -1.2 MPa), while g_s decreased, until a threshold of $g_s \sim 0.15 \text{ mol m}^{-2} \text{ s}^{-1}$ from, which g_m decreased significantly as well. In a trial on *Quercus robur* L. and *Fraxinus angustifolia* Vahl grown in field, Grassi and Magnani (2005) reported a concomitant decrease of both g_s and g_m in a dry year ($\Psi_{\text{soil}} \sim -1.7$ MPa), compared with a wetter year ($\Psi_{\text{soil}} \sim -0.2$ MPa). In *P. tremula* L., g_m significantly declined when Ψ_{leaf} of saplings dropped from -0.3 to -0.7 MPa due to applied osmotic stress (Tosens et al., 2012a). Simultaneously, drought stress induced decrease in SLA accompanied with an increase in the cell wall thickness and a decrease in the chloroplast surface area exposed to intercellular air space per unit leaf area (Tosens et al., 2012a). Other studies have shown that biochemical changes induced by drought stress, like deactivation of aquaporins, could decrease CO_2 diffusion to carboxylation sites in the chloroplast (Miyazawa et al., 2008a).

Adaptation to the local environment might be a key driver of g_m variation among taxa, similarly to other morpho-physiological traits. Interspecific and intraspecific differences

in g_m from mesic *versus* xeric environments (*Quercus* spp. and *Eucalyptus* spp.) were reported by Zhou et al. (2014). Their study showed that g_m , as well as g_s , V_{cmax} and J of species from drier regions were less sensitive to water deficit which maintains transpiration and photosynthesis activity at higher rates under drought, compared to species from the mesic environment. Marchi et al. (2008) observed that structural protection of mesophyll cells had a priority over functional efficiency of photochemical mechanisms in *Olea europaea* L. (evergreen sclerophyllous) but not in *Prunus persica* L. (deciduous broadleaf), depending on age-related variation in mesophyll anatomy.

Conclusion and Future Directions

The present review shows that g_m in *Populus* spp. varies predictably along light gradients and that it responds to changes in soil moisture and nutrient availability, but is not affected by metal concentration and increasing atmospheric CO₂ concentration. Although metabolic processes noticeably influence the response of g_m to environmental changes, physical constraints through leaf development and ageing need to be considered in scaling photosynthesis from leaf to canopy, and in breeding programs for high WUE. Because fast-growing *Populus* spp. trees are important players in combating climate change, mitigating carbon emissions to some extent, comparisons of genotypes with different adaptations to changing environments and breeding for novel genotype-climate associations are urgently needed. This study shows that the variability of g_m in different experimental conditions offers a potential indicator for improving *Populus* spp. productivity and resilience. However, more research is yet needed, also combined with anatomical studies, to better understand the sources of variation of CO₂ diffusion through the mesophyll and their consequences on carbon assimilation and growth.

Moreover, determination of the efficiency and optimal age for early selection of fast-growing poplar clones require an understanding of the genetic control and age-based genetic correlations for traits related to g_m and growth. For that, a detailed evaluation of the genotypic control of the variances and clonal heritability of g_m is needed. Finally, the identification of molecular bases of the regulation of g_m is necessary to further refine a multi-criteria early selection approach of poplar clones dedicated to the future forestry capable of ensuring better productivity and increased resistance to environmental stresses (frost, drought, water logging, heavy metals, heat waves, etc.).

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Conflict of interests

The authors declare that there is no conflict of interest.

Data Availability

The datasets generated for this study are available on request to the corresponding author.

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477 **Table 1.** List of dataset sources used in the meta-analysis

Author	Journal	<i>Populus</i> species or hybrid parents	Number of genotypes	Treatment	Provenance of plant material	Growth Environment	Number of curves
Attia et al. 2015	Journal of Experimental Botany	- <i>P. balsamifera</i> L. - <i>P. simonii</i> Carrière - <i>P. balsamifera</i> L. x <i>P. simonii</i> Carrière	3	Water stress	Canada	Growth chamber	15
Benomar et al.	Unpublished data	<i>P. maximowiczii</i> A.Henry x <i>P. balsamifera</i> L.	2	Water stress	Canada	Growth chamber	12
Benomar 2012	PhD thesis	- <i>P. maximowiczii</i> A.Henry x <i>P. balsamifera</i> L. - <i>P. balsamifera</i> L. x <i>P. trichocarpa</i> Torr. & A.Gray	2	Spacing & canopy level	Canada	Plantation	52
Benomar et al. 2019	Plos one	- <i>P. maximowiczii</i> A.Henry x <i>P. balsamifera</i> L. - <i>P. maximowiczii</i> A.Henry x <i>P. nigra</i> L.	2	Temperature & nitrogen	Canada	Growth chamber	23
Borghi et al. 2007	Environmental and Experimental Botany	<i>P. x euramericana</i> (<i>P. deltoides</i> W.Bartram x <i>P. nigra</i> L.) (clone Adda)	1	Copper	Italy	Growth chamber	21
Borghi et al. 2008	Environmental and Experimental Botany	- <i>P. alba</i> L. - <i>P. x Canadensis</i> (<i>P. nigra</i> L. x <i>P. deltoides</i> W.Bartram)	2	Copper	Italy	Growth chamber	18
Calfapietra et al. 2005	Environmental pollution	<i>P. x euramericana</i> (<i>P. deltoides</i> W.Bartram x <i>P. nigra</i> L.)	1	Nitrogen & ambient CO ₂ & canopy level	Italy	Plantation	60
Castagna et al. 2015	Environmental Science and Pollution Research	- <i>P. x canadensis</i> (<i>P. nigra</i> L. x <i>P. deltoides</i> W.Bartram) - <i>P. deltoides</i> W.Bartram x <i>P. maximowiczii</i> A.Henry	2	Ozone & cadmium soil contamination	Italy	Greenhouse	16
Di Baccio et al. 2009	Environmental and Experimental Botany	<i>P. x euramericana</i> (<i>P. deltoides</i> W.Bartram x <i>P. nigra</i> L.) (clone i-214)	1	Zinc soil contamination	Italy	Growth chamber	12
Elferjani et al. 2016	Environmental and Experimental Botany	- <i>P. trichocarpa</i> Torr. & A.Gray x <i>P. balsamifera</i> L. (clone 747215) - <i>P. balsamifera</i> L. x <i>P. maximowiczii</i> A.Henry (clones 915004 and 915005) - <i>P. maximowiczii</i> A.Henry x <i>P. balsamifera</i> L. (clone 915319)	4	Latitudinal gradient	Canada	Plantation	24
Li et al. 2013	Tree Physiology	<i>P. euphratica</i> Oliv.	1	Ground water availability	China	In field under shelter (lysimeter)	9
Merilo et al. 2010	Baltic Forestry	- <i>P. nigra</i> L. - <i>P. alba</i> L.	2	Ambient CO ₂ (FACE) & nitrogen & canopy level	Italy	Plantation	104
Niinemets et al. 1998	Tree Physiology	<i>P. tremula</i> L.	1	Canopy level	Estonia	Natural forest stands	14
Ripullone et al. 2003	Tree Physiology	<i>P. x euramericana</i> (<i>P. deltoides</i> W.Bartram x <i>P. nigra</i> L.) (clone i-214)	1	Nitrogen	Italy	Greenhouse	14
Ryan et al. 2009	Plant, Cell and Environment	<i>P. deltoides</i> W.Bartram x <i>P. trichocarpa</i> Torr. & A.Gray	2	Ozone	United Kingdom	Greenhouse	118
Silim et al. 2010	Photosynthetic Research	<i>P. balsamifera</i> L.	1	Habitat & growth temperature	Canada	Greenhouse	30

Soolanayakanahally et al. 2009	Plant, Cell and Environment	<i>P. balsamifera</i> L.	1	Latitudinal gradient	Canada	Greenhouse	72
Théroux-Rancourt et al.	Unpublished data	- <i>P. deltoides</i> W.Bartram × <i>P. nigra</i> L. (clone 3570) - <i>P. maximowiczii</i> A.Henry × (<i>P. deltoides</i> W.Bartram × <i>P. trichocarpa</i> Torr. & A.Gray) (clones 505372 and 505508) - <i>P. maximowiczii</i> A.Henry × <i>P. trichocarpa</i> Torr. & A.Gray (clone 750361) - <i>P. maximowiczii</i> A.Henry × <i>P. balsamifera</i> L. (clones 915302, 915313, 915318) - (<i>P. deltoides</i> W.Bartram × <i>P. nigra</i> L.) × <i>P. trichocarpa</i> Torr. & A.Gray (clone 915508)	8	N/A	Canada	Greenhouse	38
Théroux-Rancourt et al. 2014	Journal of Experimental Botany	- Assiniboine: [(<i>P.</i> × ' <i>Walker</i> ': <i>P. deltoides</i> W.Bartram × <i>P. x petrowskiana</i> R.I. Schrod. ex Regel) × male parent unknown] - Okanese [(<i>P.</i> × ' <i>Walker</i> ') × <i>P. x petrowskiana</i> R.I. Schrod. ex Regel]	2	Water stress	Canada	Greenhouse and growth chamber	3
Théroux-Rancourt et al. 2015	Tree Physiology	- (<i>P. maximowiczii</i> A. Henry) × (<i>P. deltoides</i> W. Bartram × <i>P. trichocarpa</i> Torr. & A. Gray) - <i>P. maximowiczii</i> A.Henry × <i>P. balsamifera</i> L. - ' <i>Walker</i> ' [<i>P. deltoides</i> W.Bartram × (<i>P. laurifolia</i> Ledeb. × <i>P. nigra</i> L.)] × <i>P. deltoides</i> W.Bartram - ' <i>Walker</i> ' × <i>P. petrowskyana</i> Schr. - <i>P. balsamifera</i> L.	5	Water stress	Canada	Greenhouse and growth chamber	12
Tissue et al. 2010	Tree Physiology	<i>P. deltoides</i> W.Bartram	1	Phosphorous & ambient CO ₂	Australia	Growth chamber	76
Tognetti et al.	Unpublished data	<i>P. x euramericana</i> (<i>P. nigra</i> L. × <i>P. deltoides</i> W. Bartram) (clone i-214)		Zinc soil contamination	Italy	Greenhouse	24
Tognetti et al. 2004	Tree Physiology	- <i>P. deltoides</i> W.Bartram × <i>P. maximowiczii</i> A.Henry - <i>P. x euramericana</i> (<i>P. deltoides</i> W.Bartram × <i>P. nigra</i> L.) (clone i-214)	2	Heavy metals	Italy	Greenhouse	24
Tosens et al. 2012	Plant, Cell and Environment	<i>P. tremula</i> L.	1	Light & water stress	Estonia	Growth chamber	8
Velikova et al. 2011	Environmental Pollution	<i>P. nigra</i> L.	20	Nickel soil contamination	Italy	Growth chamber (climate chamber)	16
Xu et al. 2020	Tree Physiology	<i>P. x euramericana</i> (<i>P. deltoides</i> W.Bartram × <i>P. nigra</i> L.) (cv. '74/76')	1	Nitrogen & ozone	China	Growth chamber	6

Figure legends

Figure 1. Cumulative number of published studies for mesophyll conductance (g_m) between the years 2000 to 2020. Number of publications were determined using keywords (e.g. g_m) through database search available at the Web of Science Core Collection.

(<https://clarivate.com/webofsciencegroup/solutions/web-of-science-core-collection/>).

Figure 2. Cumulative number of published studies for mesophyll conductance (g_m) in *Populus* spp. between the years 2001 to 2020. Number of publications were determined using keywords (e.g. *Populus*) through database search available at the Web of Science Core Collection.

(<https://clarivate.com/webofsciencegroup/solutions/web-of-science-core-collection/>).

Figure 3. Effect of the leaf position in the canopy (Bottom: Bot, Middle: Mid, Upper: Upp) on light-saturated photosynthetic rate (A_{max} , a); stomatal conductance (g_s , b); mesophyll conductance (g_m , c); g_m/g_s ratio (d); maximum rate of carboxylation (V_{cmax} , e) and specific leaf area (SLA, f). In g_m/g_s ratio, g_s for water ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was divided by 1.6 to obtain g_s in $\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$. Means having the same letters are not significantly different at $\alpha = 0.05$.

Figure 4. Effect of the ambient air CO_2 concentration on light-saturated photosynthetic rate (A_{max} , a); stomatal conductance (g_s , b); mesophyll conductance (g_m , c) and g_m/g_s ratio (d). In g_m/g_s ratio, g_s for water ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was divided by 1.6 to obtain g_s in $\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$. Means having the same letters are not significantly different at $\alpha = 0.05$.

Figure 5. Effect of the soil Copper (Cu) concentration on light-saturated photosynthetic rate (A_{max} , a); stomatal conductance (g_s , b); mesophyll conductance (g_m , c) and g_m/g_s ratio (d). In g_m/g_s ratio, g_s for water ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was divided by 1.6 to obtain g_s in $\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$. Means having the same letters are not significantly different at $\alpha = 0.05$.

Figure 6. Effect of the soil nitrogen content (high nitrogen: HN, low nitrogen: LN) on light-saturated photosynthetic rate (A_{max} , a); stomatal conductance (g_s , b); mesophyll conductance (g_m , c) and g_m/g_s ratio (d). In g_m/g_s ratio, g_s for water ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was divided by 1.6 to obtain g_s in $\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$. Means having the same letters are not significantly different at $\alpha = 0.05$.

Figure 7. Effect of the soil moisture on light-saturated photosynthetic rate (A_{max} , a); stomatal conductance (g_s , b); mesophyll conductance (g_m , c) and g_m/g_s ratio (d). In g_m/g_s ratio, g_s for water ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was divided by 1.6 to obtain g_s in $\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$. Means having the same letters are not significantly different at $\alpha = 0.05$.

Figure 8. Relationship between light-saturated photosynthetic rate (A_{max}), stomatal conductance (g_s), mesophyll conductance (g_m), maximum rate of carboxylation (V_{cmax}), electron transport rate (J), g_m/g_s ratio, specific leaf area (SLA) and per area leaf Nitrogen concentration (N_{area}). In g_m/g_s ratio, g_s for water ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was divided by 1.6 to obtain g_s in $\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$.

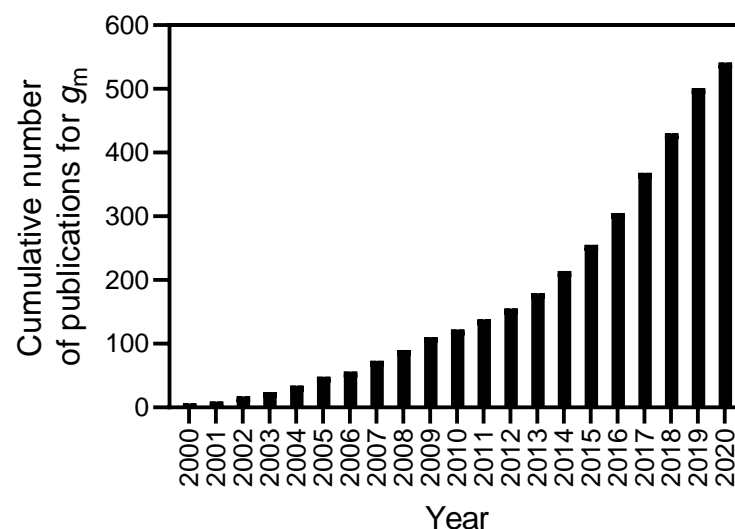


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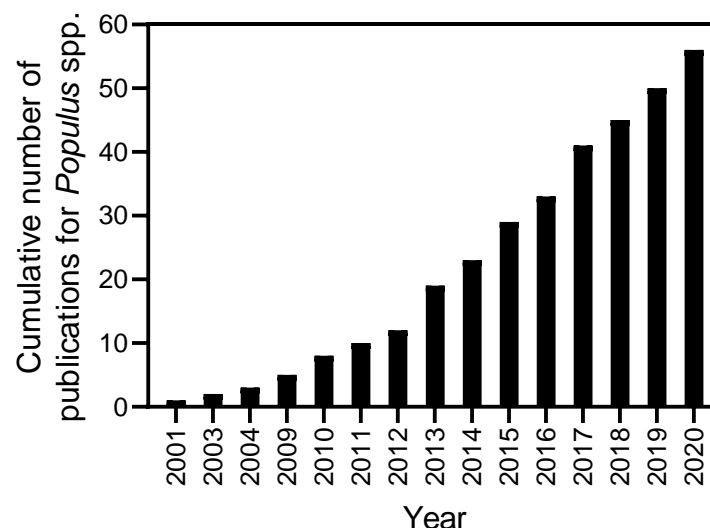


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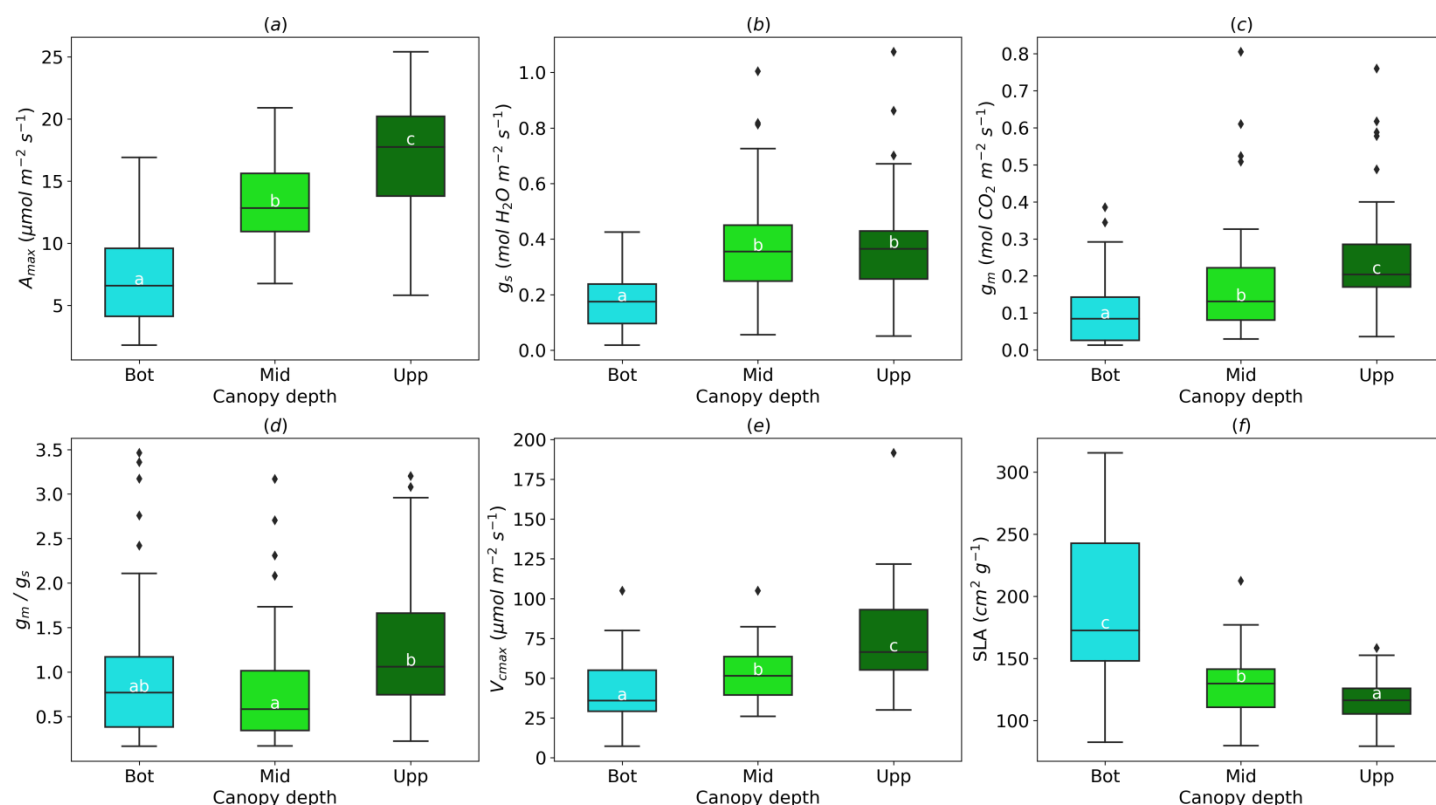


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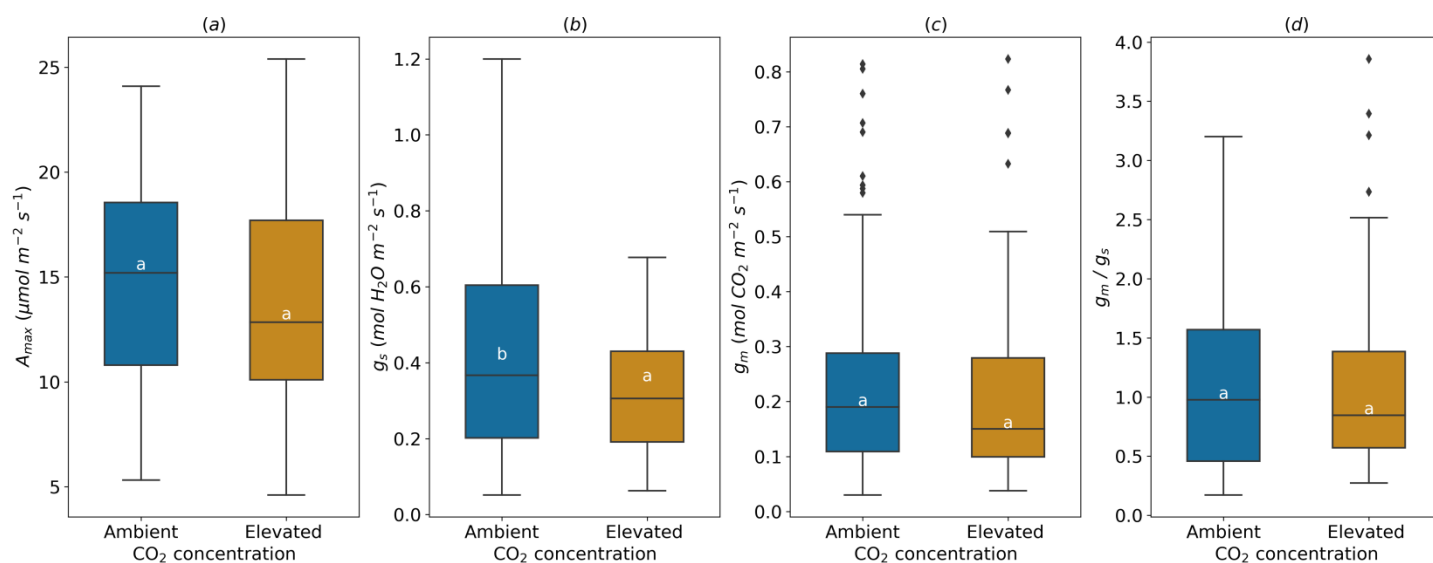


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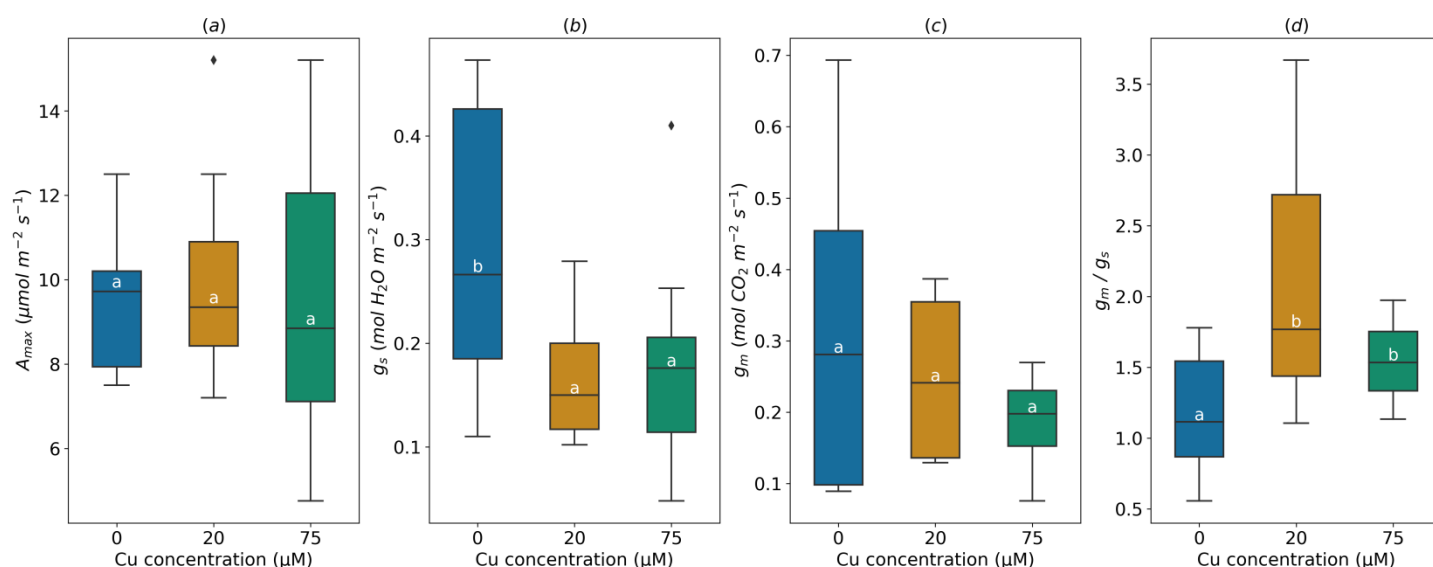


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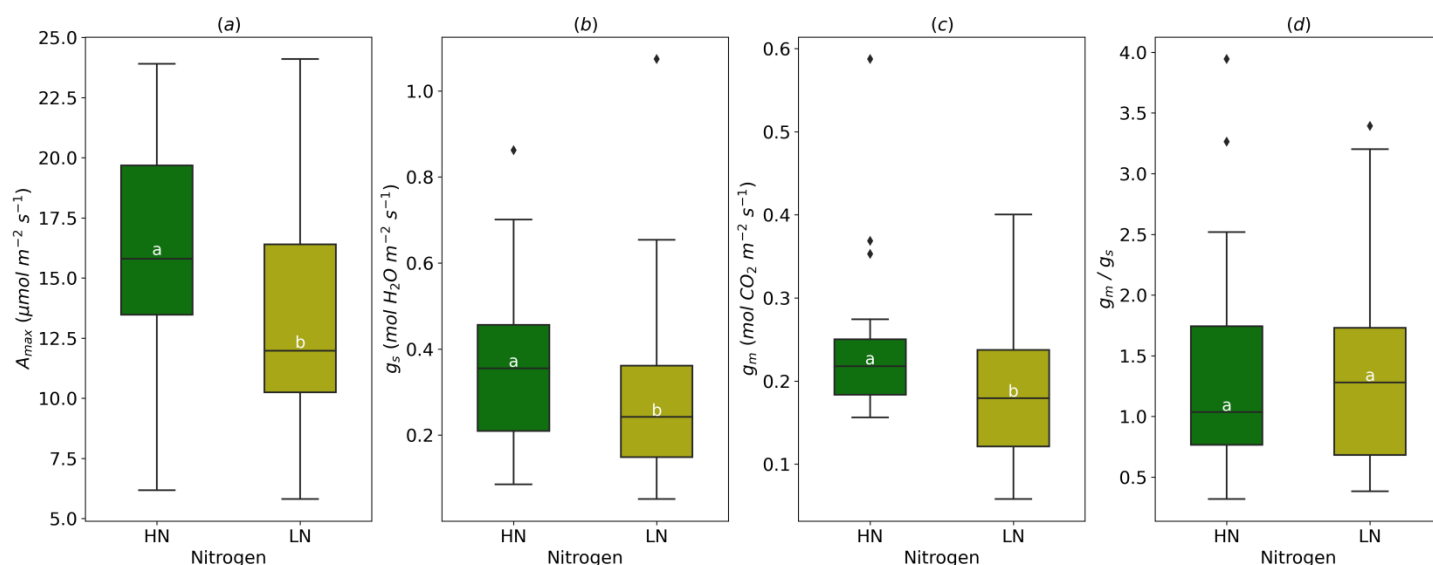


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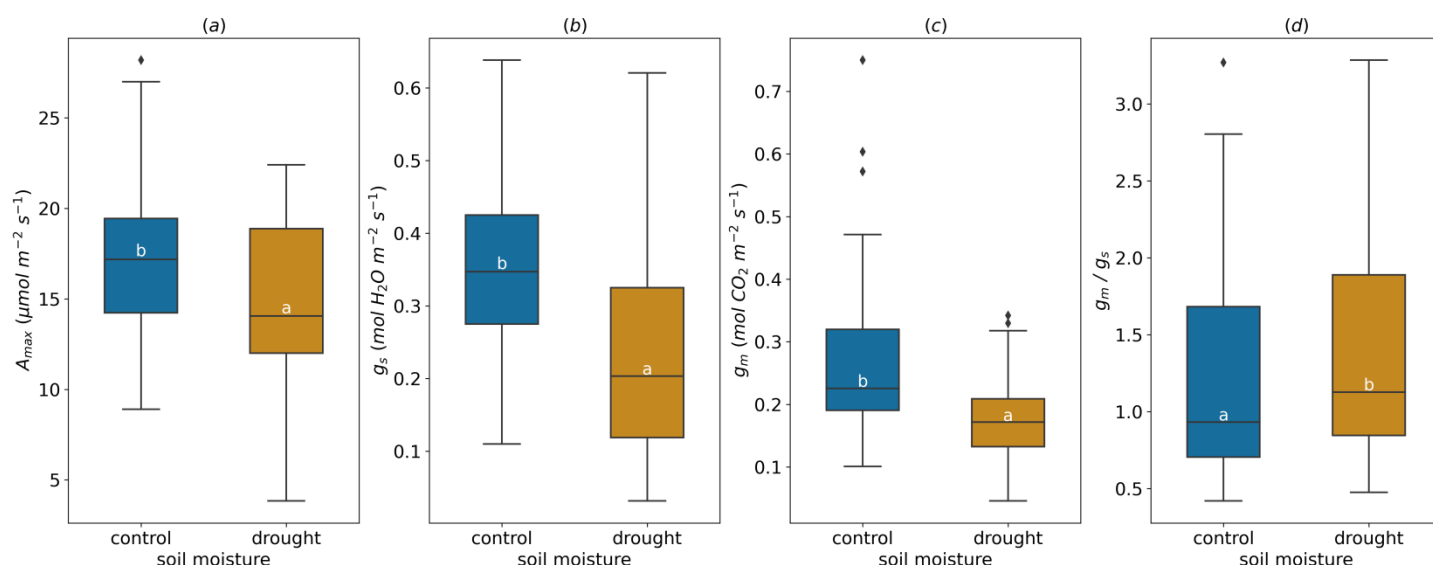
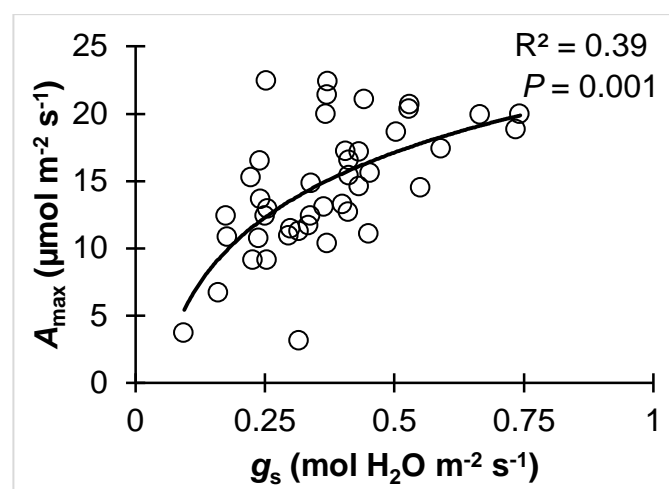
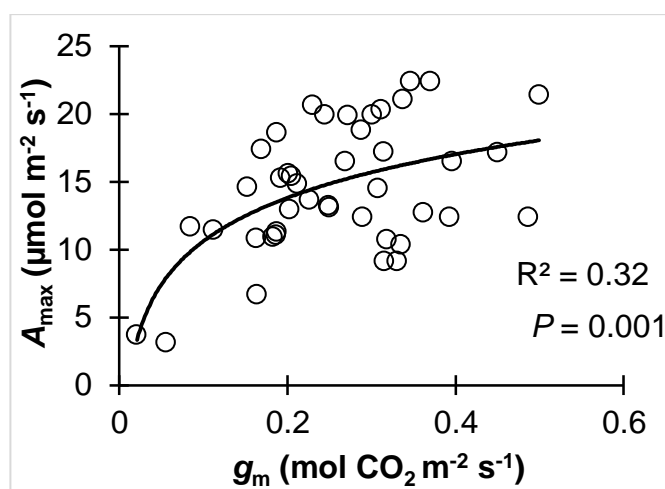


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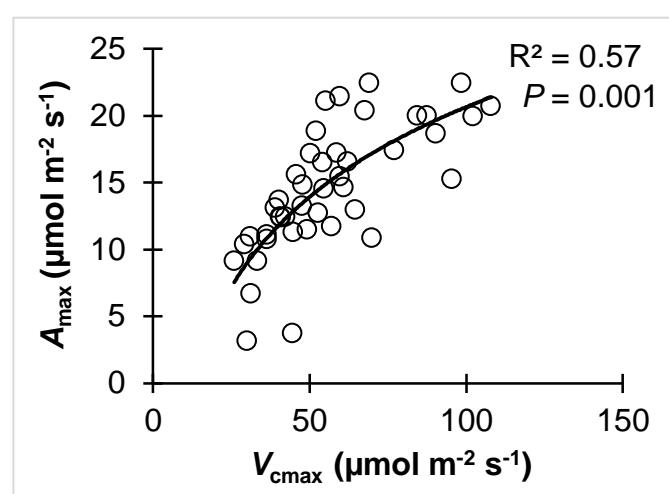
a)



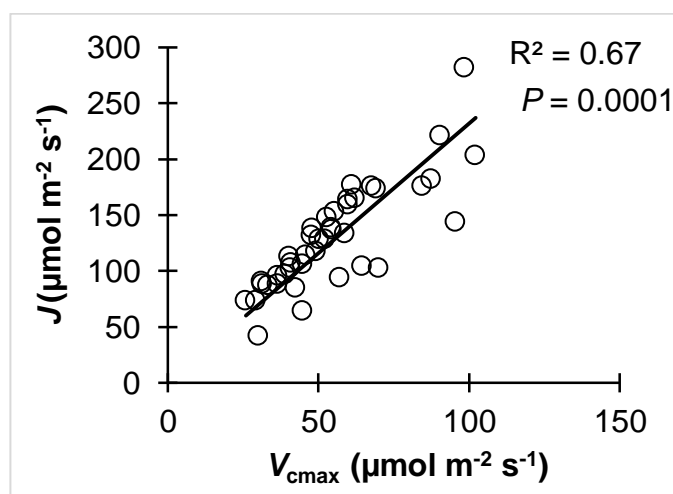
b)



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d)



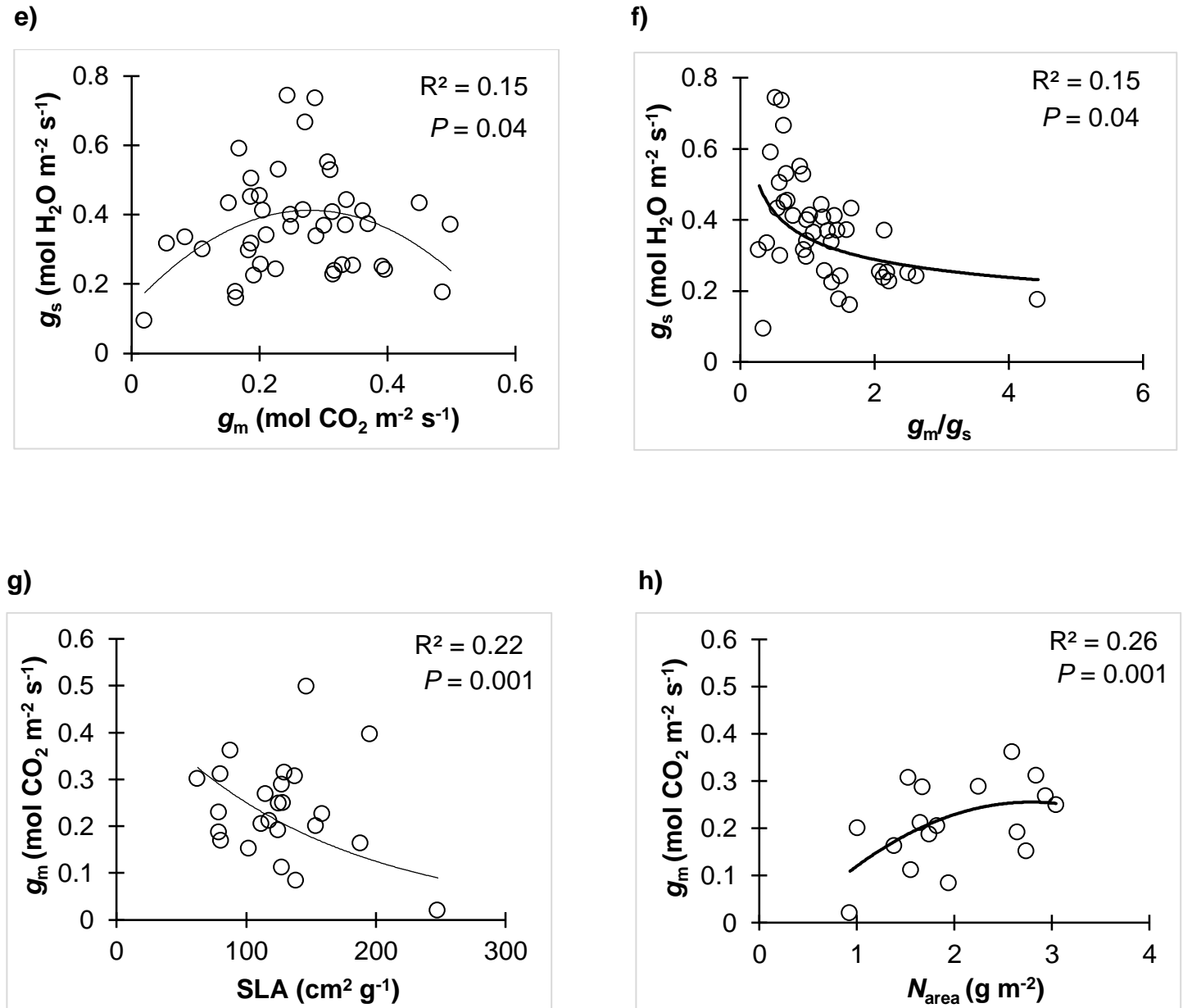


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