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**Modeled versus Experimental  
Photosynthetic Response  
to  
Light and Intercellular CO<sub>2</sub>  
concentration**

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## Abstract

The Earth Systems computer model CLASS has been used to predict land surface and atmospheric conditions, regionally, in the Netherlands. A portion of this model predicts vegetative response: the A- $g_s$  model – dealing with the relationship between photosynthesis and stomatal conductance – is this research's focus. The A- $g_s$  model was parameterized with only one plant species, *Vitis Vinifera*, in one growing location, central Spain. This limited parameterization suggests the model may not predict plant response accurately for other species, from other Plant Functional Types (PFTs) (De Kauwe et al., 2015; Kala et al., 2016). Through this research, the model's ability to predict experimental net photosynthesis data is considered. Experimental data were derived from gas exchange experiments on two common Dutch C3 grasses: *Triticum aestivum* and *Alopecurus pratensis*. Net photosynthesis was examined in response to changes in light intensity (PAR) and intercellular CO<sub>2</sub> concentration ( $C_i$ ), respectively.

Statistical analysis (through t-tests) revealed that the A- $g_s$  model predicted photosynthesis values which were, overall, significantly different than observed experimental photosynthesis. The A- $g_s$  model predicted the photosynthetic response to light and  $C_i$  in *T. aestivum* better than in *A. pratensis*. The model predicted observed values of *T. aestivum* reasonably well. To this point: the significant differences resulting from t-tests is thought to be due to low sample size rather than strong variation between data. Therefore, a more robust experimental analysis of *T. aestivum* is recommended, in terms of both A-PAR and A- $C_i$  response curves, using increased sample size.

It was expected, due to the similarity of the grass species studied, that the model would generate data which either explained the photosynthetic response of both grasses, or not. However, this was not the case. This result may be mitigated by species-specific parameterization (Prentice, et al., 2015). However, due to similarity of studied grasses, this divergent result more likely points to oversensitivity in the A- $g_s$  model to certain environmental factors, i.e. light. Different light levels were used in the A- $C_i$  experiments on *T. aestivum* and *A. pratensis*, and in the corresponding modeled environments. The low-light modeled photosynthetic response curve (used in the A- $C_i$  *A. pratensis* simulation) reached  $A_{max}$  at a low  $C_i$  and was shaped more logistically than commonly seen in literature (During, 1991; Greer, 2012; Jacobs, 1994) or in experiments. Under saturating light conditions (used in the A- $C_i$  *T. aestivum* simulation) the model generated an A- $C_i$  curve closer to curves observed experimentally and seen in literature.

KEYWORDS: Photosynthetic response, Land Surface Model Evaluation, A-PAR, A- $C_i$

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## *Preface*

While there is increasing reliance on computer models to predict detailed future land and atmospheric conditions, the accuracy of these predictions differs between models (Kala, et al., 2016, Prentice et al., 2015). We must question the predictive power of the computer models, some of which are used on an international scale and help to determine the movement of billions of Euros, the direction of policies globally and the fate of the climate and health of the Earth, at large (Allen, et al., 2014). And so, comparative studies using empirical data are necessary to determine the predictive powers of models (Li et al, 2018). This study is one these comparative analyses, which leads to an evaluation of the predictive powers of a vegetation model.

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## Terms

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$A(n)$	Net photosynthesis rate	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
$A_{\text{max}}$	maximal carbon dioxide assimilation rate	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
$C_a$	Ambient air CO <sub>2</sub> partial pressure	$\mu\text{mol mol}^{-1}$
$C_i$	Intercellular CO <sub>2</sub> partial pressure	$\mu\text{mol mol}^{-1}$
$E$	transpiration rate	$\text{mmol m}^{-2} \text{ s}^{-1}$
$g_s$	Stomatal conductance	$\text{mmol m}^{-2} \text{ s}^{-1}$
$I_{\text{inc}}$	Photon flux density incident to leaves	$\mu\text{mol photon m}^{-2} \text{ s}^{-1}$
$T$	Leaf temperature	$^{\circ}\text{C}$
$R_s$	Stomatal resistance	$1/g_s$
$V_{\text{cmax}}$	Maximum rate of Rubisco activity-limited carboxylation	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
$\Gamma$	CO <sub>2</sub> compensation point in the presence of $R_d$	$\mu\text{bar}$

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# 1 - Introduction

Computer modeling is widely used to predict the future state of the Earth system (Prentice et al., 2015). The Earth system includes wind patterns, cloud formation, water and water vapor budget, the carbon cycle and vegetation response. As a result of (anthropogenic) climate change, the climate today is changing faster than before and so Earth systems modeling is highly important to predict, mitigate and plan for our future (Allen et al., 2014; Stocker, 2014). Climate science relies heavily on these models for prediction. Specifically, the role of vegetation in the climate system is particularly ill constrained in current climate models due to a focus on equations rather than various parameterizations (Prentice et al., 2015). Many recent studies focus on the insufficient parameterization of vegetation response in models. For instance, De Kauwe et al. (2015) show how the use of a generic drought sensitivity level in modeling vegetation can lead to the overestimation of drought effects in arid areas, where plants are drought-tolerant (2015). They and others support the need for species-specific parameterization to account for differences (De Kauwe et al., 2015; Kala et al., 2016; Jacobs, 1994). Kala et al. (2016), point to the importance of species-specific parameterization of stomatal response to soil water. Kala et al. (2016) developed various stomatal response parameterizations for individual Plant Function Types (PFTs) (based on 316 plant species) to improve the accuracy of heat wave prediction in the CABLE model. Before their work, the CABLE model contained only dual parameterization: for C3 and C4 species. In summary, an important current issue in Earth systems modeling is the generic parameterization of vegetation response when this should be done on the PFT level or the species level (Prentice et al., 2015).

Using just one species for parameterization means that a model is set to predict the response of only that species, not necessarily others. This is problematic because such models are often used to predict responses for a wide range of species. This study questions how values generated by a certain vegetation model (with limited parameterization) compare to empirical data involving multiple species.

This research focuses on the Chemistry Land Surface Atmosphere Soil Slab (CLASS) model (de Arellano, 2015). This broad mechanistic systems model was developed at Wageningen University by C.M Jacobs in 1994 and more recently by Jordi Vila-Guerau de Arellano and his team (de Arellano, 2015; Jacobs, 1994). CLASS is a coupled vegetation-Planetary Boundary Layer model, comprised of a complex nesting of models describing vegetation, clouds, aerosols, CO<sub>2</sub> budget and more (de Arellano et al., 2015; van den Dries et al., 2013; Jacobs, 1994). The model is used regionally (in the Netherlands) to predict responses of land surface and atmospheric fluxes. This research focuses solely on the vegetation portion of the CLASS model, the A-g<sub>s</sub> model, which deals with (A) photosynthesis and (g<sub>s</sub>) stomatal conductance, among other vegetative responses (de Arellano et al., 2015; Jacobs, 1994). The A-g<sub>s</sub> model is complex in and of itself, accounting for synergistic interactions between stimuli (de Arellano et al., 2015; Jacobs, 1994).

The A-g<sub>s</sub> model was parameterized with response data from only one experimental plant species: the common grapevine, *Vitis vinifera* (Jacobs, 1994). The individual *V. vinifera* plants used for parameterization were grown in Central Spain (Jacobs, 1994). These plants were grown as a part of a larger piece of research on desertification in Europe by the European International Project on Climatic and Hydrological Interactions between the Vegetation, the Atmosphere, and the Land Surface from 1991. A description of these experiments can be seen in Bolle, et al. (1993). This study uses the *V. Vinifera* L., cv. Airen variety of grapevine. These plants were grown under semi-arid conditions and were unstressed during growth. Jacobs (1994) determined that the A-g<sub>s</sub> model can accurately predict responses of the grapevine species with which it was parameterized “reasonably well”. However, it is unclear if the model can accurately predict responses for other species, especially those different phylogenetically to *Vitis*. This is problematic because the A-g<sub>s</sub> model is often applied generically to quantify gas exchange in other species. For instance, the CLASS model, within which the A-g<sub>s</sub> model is nested, is often used to predict water and carbon fluxes for the research fields around Wageningen University (Netherlands), which contain mostly C3 grasses.

This research compares the vegetation response predictions of the A-g<sub>s</sub> model to responses from certain common grasses in the Netherlands, specifically how net photosynthesis (also referred to as photosynthesis) responds to changes in environmental conditions. Two environmental conditions, intercellular CO<sub>2</sub> (C<sub>i</sub>) and light (irradiance), were manipulated to determine the photosynthetic response curves. Net photosynthesis is the focus because, in the A-g<sub>s</sub> model, net photosynthesis encompasses stomatal conductance, CO<sub>2</sub> compensation point and respiration (de Arellano, 2015; Jacobs, 1994). The equations in the model leading to the net photosynthetic rate are dependent on these other mechanisms and in this way, analyzing net photosynthesis data offers a more holistic comparison of modeled versus experimental data.

Photosynthesis/carbon assimilation was studied partly because it measures the productivity under the introduced experimental conditions. For agricultural and ecological purposes, having accurate prediction systems of photosynthesis is crucial, as it will help us to model net primary production and crop yield (Flexas et al., 2012) Two C3 Poaceae grasses, *Alopecurus pratensis* and *Triticum aestivum*, were used to examine vegetation response. *A. pratensis* was selected for experimentation because this was the main species found in the fields near Wageningen University. Wheat (*T. aestivum*) plants are chosen due to the abundance and importance of wheat as a global staple crop. To help accurately predict food abundance or scarcity, photosynthetic response data of *T. aestivum* is crucial.

*A. pratensis* and *T. aestivum* make up a significant portion of vegetation in the Netherlands (NDFP, 2018). *A. pratensis* is ubiquitous to grasslands throughout the Netherlands while *T. aestivum* is very common in the western half of the country (Figure 1, A & B) (NDFP, 2018). Given their abundance, using the photosynthetic response of these common Dutch plants as a comparison to the A-g<sub>s</sub> modeled output is appropriate. *V. vinifera*, on the other hand, is uncommon in the Netherlands (Figure 1 C), so its use to

parameterize the A-g<sub>s</sub> Model may limit accurate predictions of vegetation response in the Netherlands, for which it is now used.

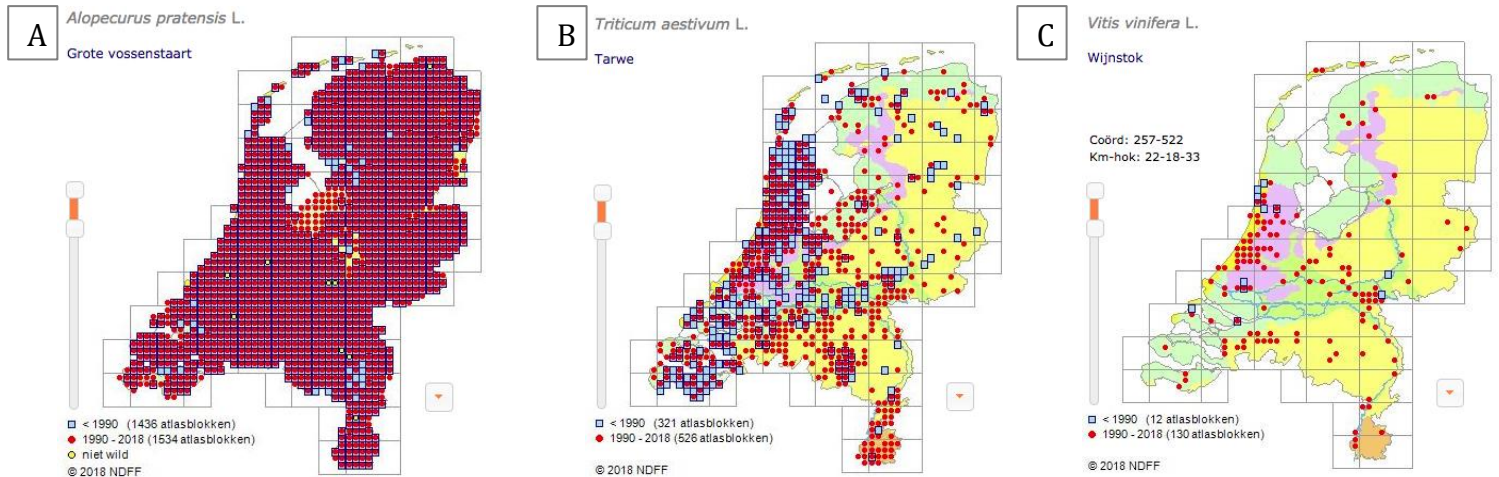


Figure 1 (A-C). Vegetation dispersion maps for the Netherlands. Blue squares show vegetation sightings before 1990, red squares show more recent sightings of vegetation (1990-2018). Map (A) shows C3 Poaceae grass, *Alopecurus pratensis*, Map (B) shows another C3 grass, *Triticum aestivum*. Map (C) shows Common grapevine, *Vitis vinifera*. *A. pratensis* is found nearly ubiquitously in the Netherlands, while *T. aestivum* is prominent in a small select region of the country and *V. vinifera* is scattered and quite uncommon (Dutch National Databank for Flora and Fauna, 2018).

## 2 - Research Questions and Hypothesis

The main question of this research is: what are the differences and similarities between: (1) the modeled net photosynthetic response of the A-g<sub>s</sub> model (parameterized using Spanish-grown *V. vinifera*), and (2) the experimental net photosynthetic response of C3 grasses *A. pratensis* and *T. aestivum*, to changes in light and CO<sub>2</sub>?

### 2.1 - Modeled v. Experimental Response to CO<sub>2</sub>

The first goal of this research is to compare the net photosynthetic rate of the A-g<sub>s</sub> vegetation model to the experimental net photosynthetic rate of the grasses: *A. pratensis* and *T. aestivum*, in response to changes in CO<sub>2</sub>.

Modeled data was expected to show similarities with the photosynthetic response of *V. vinifera*, with which it was parameterized. Therefore, the hypothesized model response (Figure 2 for model behavior) is based partially on the *V. vinifera* response from literature review (see Theoretical Framework). Though the following grapevine response patterns may not be significant to the modeled output, there may be possible similarities to experimental grapevine response and therefore are included.

**Sub-question 1.** What are the differences between modeled and experimental net photosynthetic response at low CO<sub>2</sub> levels?

It was hypothesized that the model, parameterized with grapevine, would generate higher photosynthesis values at low CO<sub>2</sub> than experimental grasses. The literature review performed on *V. vinifera* (see Theoretical Framework, 3.2), showed CO<sub>2</sub> assimilation increases dramatically with increases of C<sub>i</sub> at levels below ~300 μmol mol<sup>-1</sup> CO<sub>2</sub>. Poaceae grasses responded less strongly at such levels. For instance, *Bromus japonicas*, a C3 grass, responded linearly to increased CO<sub>2</sub>. This differs from the hyperbolic response seen in grapevine and supports the notion that grapevine responds more strongly at low CO<sub>2</sub> levels. Similar to the information gathered in the grapevine literature review, the A-g<sub>s</sub> model output (Figure 2) shows a strong photosynthetic response to increased C<sub>i</sub> at low levels.

Generalized research on plant type supports with the above hypothesis. Shrubs, like grapevine, may respond strongly to increased CO<sub>2</sub> due to the biological limitations of shrubs (e.g. the transfer rate of CO<sub>2</sub> from the air to chloroplasts). Therefore, increased CO<sub>2</sub> at low (limiting) concentrations should produce a stronger photosynthetic response in shrubs, compared with herbaceous species.

**Sub-question 2.** What are the differences between modeled and experimental maximum photosynthesis (A<sub>max</sub>) (at high C<sub>i</sub>)?

It was hypothesized that maximum photosynthesis levels of modeled output and experimental response data will be similar. Literature review shows that C3 grasses

reach higher maximum photosynthetic rates than grapevine (at high/saturating CO<sub>2</sub> concentrations). Though the A-g<sub>s</sub> model was parameterized with grapevine, the model produces photosynthesis values higher than data analyzed from experiments with grapevine; the model generates photosynthesis at levels of at ~30 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> at saturating C<sub>i</sub> (based on the preliminary A-g<sub>s</sub> model curve generated for hypothesis study, Figure 2). Therefore, there is contradicting information regarding the response of grapevine, thus, it was hypothesized the photosynthesis levels at high C<sub>i</sub> would be similar between modeled output and experimental grass data.

## 2.2 - Modeled v. Experimental Response to Light

The second goal of this research is to compare net photosynthesis of the A-g<sub>s</sub> vegetation model to the experimental net photosynthetic rate of *A. pratensis* and *T. aestivum* in response to changes in light.

**Sub-question 1.** What are the differences/similarities between modeled and experimental net photosynthetic response at low light levels?

The A-g<sub>s</sub> model and the experimental grasses were hypothesized to have similar photosynthetic rates at low light levels. Though, as described in the Theoretical Framework, increased light at low levels (<400 μmol PAR m<sup>-2</sup> s<sup>-1</sup>) causes a stronger (positive) response in C3 grasses than *V. vinifera*, this difference is minor. At levels of light from 0 to 400 μmol photons m<sup>-2</sup> s<sup>-1</sup>, the photosynthetic response of the studied grasses and grapevine studied was very strong. The rate of photosynthesis increased in grapevine from 0 to ~8.5 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> and in grasses from 0 to ~10.5 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>. Further, when looking to preliminary output of the A-g<sub>s</sub> model, generated for hypothesis study (Figure 3), a strong response to increased light at low levels can be seen. Here, photosynthesis reached a rate of ~12.5 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> by 400 μmol photons m<sup>-2</sup> s<sup>-1</sup> in Figure 3 (after converting units).

In summary, grasses have been shown experimentally to be more responsive at low light levels than grapevine, but only slightly. The model (parameterized with grapevine) response is even stronger than grasses studied in literature review. Thus, it was hypothesized that the model and experimental grasses would produce photosynthetic rates at low light levels (i.e. ≤ 400 μmol m<sup>-2</sup> s<sup>-1</sup>) that were *not* significantly different.

**Sub-question 2.** What are the differences between modeled and experimental net photosynthetic response at high light levels? What are the differences between modeled and experimental maximum photosynthesis (A<sub>max</sub>)?

It was hypothesized that at high light, photosynthesis would reach a higher rate in experimental grasses than in the model. Literature review, presented in the Theoretical Framework section (3.3.) provides supporting evidence using grapevine. In summary, grapevine photosynthesis is shown to reach ~16 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> while photosynthesis in the grasses reached ~23 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>.



## 3 – Theoretical Framework

### 3.1 - The A-g<sub>s</sub> Model

The A-g<sub>s</sub> model is empirical in nature, parameterized and tested using grapevine, *V. vinifera* (specifically, *V. vinifera* L., cv. Airen) grown in Central Spain (Jacobs, 1994). The A-g<sub>s</sub> model is most well described in the Ph.d. by C.M.J. Jacobs (1994) from Wageningen University. The A-g<sub>s</sub> model is a relatively simple representation of the observed correlation between A<sub>n</sub> and g<sub>s</sub> (Jacobs, 1994). The model couples photosynthesis and stomatal conductance to mirror their dependency in nature (Jacobs, 1994). And, further, predicts how these mechanisms are affected by environmental factors such as light, CO<sub>2</sub> and temperature.

The A-g<sub>s</sub> model is a simplified version of the Goudriaan et al. (1985) model. The Goudriaan model was then tuned for the A-g<sub>s</sub> model to the biochemical model of Farquhar et al. (1980) by Jacobs (1994). The more simplistic Goudriaan model was chosen (compared to Farquhar et al., 1980) owing to the computational efficiency and relatively simple parameterization (Jacobs, 1994). (See Appendix Table 10 for a list of parameter values used. Further, see Table 11 for the model code and equations). Important to the present research is the fact that the A-g<sub>s</sub> model is based on relationships between plant mechanisms (from the Goudriaan model) and is therefore considered to be more broadly applicable (to a variety of plant types) than a statistics-based model (such as Jarvis-Stewart models) (Jacobs, 1994). Further, some parameterization is said by Jacobs (1994) to be widely applicable to a broad range of plant species, yet the present research aims to question this assertion.

The model's equation for net photosynthesis is dependent on many factors. First of all is CO<sub>2</sub> compensation point. CO<sub>2</sub> compensation point is dependent on temperature and the associated Q10 parameter (Q10CO<sub>2</sub>). Stomatal conductance (g<sub>m</sub>) and the maximum photosynthetic rate (A<sub>max</sub>) are similarly dependent on temperature and their associated Q10 parameter (Q10g<sub>m</sub> and Q10A<sub>m</sub>, respectively). The CO<sub>2</sub> primary productivity rate (A<sub>m</sub>), or the gross photosynthetic rate is dependent on: stomatal conductance, the difference between C<sub>i</sub> and CO<sub>2</sub> compensation point and on the maximum photosynthetic rate. Finally, the net photosynthetic rate (A<sub>n</sub>) is dependent on irradiance (PAR) and respiration (which itself is dependent on A<sub>m</sub>).

Jacobs (1994) tested model performance against the experimental grapevine (similar to what was completed in this study). He found the model to simulate observed data “reasonably well”: yielding high correlation and linear regression lines with a slope near 1 (Jacobs, 1994). The model performance should be tested against empirical data from various species, to determine its ability to predict the response of other species (Prentice et al., 2015). It may need alternative parameterization to predict such responses (Kala, et al., 2016; Jacobs, 1994; Prentice et al., 2015).

According to Jacobs (1994), the A-g<sub>s</sub> model is adjusted (partly) to fit the grapevine response patterns, which are here elaborated. For one, the T1 and T2 values representing temperature optimum of photosynthesis in the model were adjusted for the high-temperature adaptation exhibited by the experimental grapevine (by Jacobs, 1994). At optimum light, photosynthesis is optimized at temperatures between 26-30 °C, a high temperature according to Jacobs (1994, Figure 4.9). If the model is adapted (through parameterization) to warm climates then it may be the case that Dutch- and German-grown grasses would not be predicted accurately by the model. Beyond the acclimatized species used for parameterization, hard-to-define parameters were sometimes given “standard” values for C3 plants (also according to Jacobs, 1994). Specifically, in the A-g<sub>s</sub> model, these parameters were A<sub>mmax</sub> at 25° C as well as the Q10 values (Jacobs, 1994). Though, he adds that the model would be improved through using non-standard (specific) values for parameters.

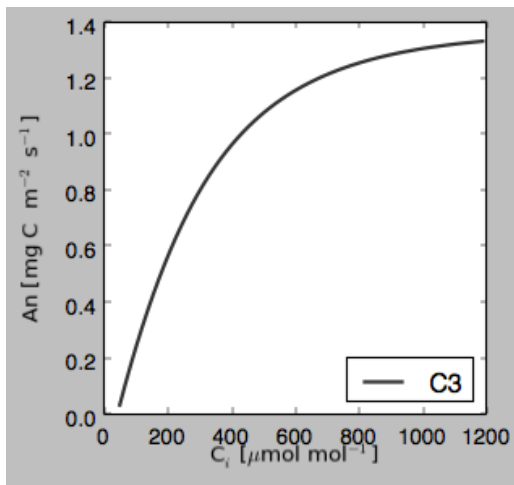


Figure 2. This figure shows a photosynthetic response curve to  $C_i$  and is an example of the modeled output from the A-g<sub>s</sub> model. The graph was created using the following variables in the A-g<sub>s</sub> model: PAR = 1500 and T = 20°C.

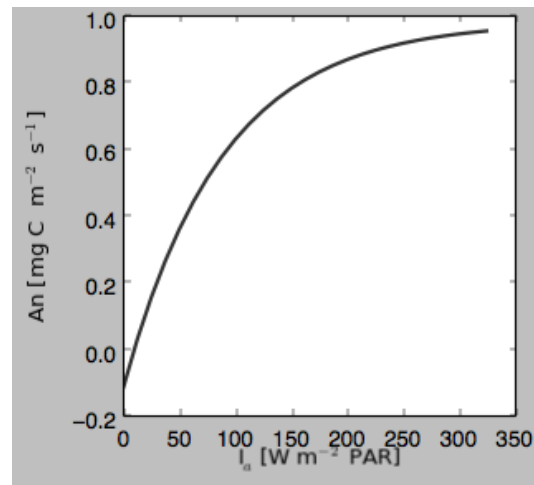


Figure 3. This figure shows a photosynthetic response curve to light and is an example of the modeled output from the A-g<sub>s</sub> model, showing an A-PAR response curve. The graph was created using the following variables in the A-g<sub>s</sub> model:  $c_i = 450$ , T = 20°C. The “x” axis is set to 328 W m<sup>-2</sup>, equal to 1500 μmol m<sup>-2</sup> s<sup>-1</sup>.

According to Prentice et al. (2015), a good land surface computer model is (1) reliable, (2) robust and (3) realistic. A reliable model is one that gives the correct predictions in most circumstances. A robust model does not rely greatly on the specification of unknown quantities. A realistic model is one that includes sufficient processes and complexity to simulate the response of the system to external variables of interest (Prentice et al., 2015). Prentice et al. (2015) argue that there is an increasing focus on realism through the introduction of additional parameters, but this comes at the expense



of robustness, as these additional parameters are often substantially uncertain. With these definitions, the A-g<sub>s</sub> model may need to be improved via robustness.

While some recent Land Surface Models (LSMs) are more robust through multiple parameterizations, representing differences in vegetation dynamics over Plant Functional Types (PFT) (Prentice et al., 2015), many, like the A-g<sub>s</sub> model, represent biologically diverse species with homogenous values (De Kauwe et al., 2015; Li et al., 2018). Such limited parameterization is a major cause of error in model performance (Li et al., 2018). Prentice et al. (2015) argues that points of improvement needed in LSMs include: (1) lack of variation in leaf photosynthesis, (2) uncertain parameterization of CO<sub>2</sub> controls (3) and water (vapor) controls by stomata. Even using parameterizations based on PFTs is not fully supported by the scientific community, as there is about 75% of variation in response occurring between plants within the PFT (Kattge et al., 2011). It is useful to include a quote from Prentice et al. (2015), “A vast amount of empirical work during the past decade has gone into the compilation of relevant trait measurements from many plant species (see Wright et al., 2004; Kattge et al., 2011), so the single-clause approach can no longer be justified by the paucity of available data (as was the case during the early years of LSM development).” The early years of LSM development were, in fact, when the CLASS (and underlying A-g<sub>s</sub> model) was developed. According to Prentice et al. (2015), there is, historically “insufficient and intermittent attention to model evaluation”. This research can be categorized as such: a vegetation model evaluation. And can be seen as relevant and crucial in the part of the attempt to increase predictive power of LSMs and Earth Systems models, overall.

### 3.2 - Photosynthesis-CO<sub>2</sub> Response

In this study we question whether the A-g<sub>s</sub> model (parameterized with Spanish grapevine) can provide photosynthetic response data similar to common plants of the Netherlands. For one, the climate in which a plant is grown has an effect on the response of the plant to environmental condition changes (Jacobs, 1994). And, two, the type of plant (e.g. plant functional type and species) will also have an effect on the plant response (Kala et al., 2016; Prentice et al., 2015).

For this study, it is important to note differences among plant responses between species. As the A-g<sub>s</sub> model was parameterized with Spanish-grown *V. vinifera*, gaining background knowledge of the photosynthetic response of *V. vinifera* is useful for understanding the species specificities. The grapevine response patterns presented here may not be significant to the modeled output, but similarities to experimental response may exist and are therefore included.

Grapevine is a member of the evergreen temperate/meadow shrub Plant Functional Type (PFT), whereas *A. pratensis* and *T. aestivum* are in the meadow C3 grass PFT (Xu & Zhou, 2011). Due to the different PFT of the grasses and grapevine, they likely would have different photosynthetic responses. Jiang et al., (1999) found meadow shrubs to have lower photosynthesis than C3 grasses, and literature review below concurs with this. These species may have a lower photosynthetic rate because of biochemical

limitation of woody species, such as Rubisco activity or the transfer rate of CO<sub>2</sub> from the ambient air to chloroplasts (Jiang et al., 1999). Grasses have potentially less limitation, and may therefore respond less strongly to C<sub>i</sub> at low levels (During, 1999). Literature review of photosynthetic response from C3 grasses and grapevine confer this difference, described below.

Grapevine in literature responded to increased C<sub>i</sub> with a stronger response at low levels of C<sub>i</sub> than grasses. Anderson (2001) found that *Bromus japonicas* responded to increased CO<sub>2</sub> in a linearly. This differs from the hyperbolic response seen in grapevine and supports the notion that grapevine responds more strongly at low CO<sub>2</sub> levels (Greer, 2012; Salazar-Parra et al., 2012). Considering response at higher C<sub>i</sub>, During (1991), Greer (2012) and Salazar-Parra et al. (2012) all had similar results with experimental grapevine reaching an A<sub>max</sub> of 17-20 CO<sub>2</sub> μmol m<sup>-2</sup> s<sup>-1</sup> in response to elevated CO<sub>2</sub>. This is compared to ambient CO<sub>2</sub> levels where grapevine carbon assimilation reached only 10-15 CO<sub>2</sub> μmol m<sup>-2</sup> s<sup>-1</sup> (During, 1991; Greer, 2012; Salazar-Parra et al., 2012).

A study of C3 grasses shows a general trend of higher maximum photosynthetic rates than grapevine. Though the grass species mentioned are generally different than those used for this research, they are from the same family. However, species variation in response is important to note. Morrison & Gifford (1983) found *Phalaris aquatica* grasses' photosynthetic rate to increase from ~16 μmol m<sup>-2</sup> s<sup>-1</sup> at ambient CO<sub>2</sub> to ~19 μmol m<sup>-2</sup> s<sup>-1</sup> in elevated conditions at A<sub>max</sub>. Yu et al. (2004) found *Triticum aestivum* to reach a higher rate of photosynthesis (A<sub>max</sub> = ~28 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) when manipulated with CO<sub>2</sub> concentrations of 1200 μmol mol<sup>-1</sup>. Experiments show that barley leaf reaches a photosynthetic rate of 20-25 CO<sub>2</sub> μmol m<sup>-2</sup> s<sup>-1</sup> (Tabalenkova and Golovko, 2014). Compared with maximum photosynthetic rates of grapevine, C3 grasses from literature review appear to reach higher photosynthetic levels in response to saturating CO<sub>2</sub>.

### 3.3 - Photosynthesis-Light Response

Presented here are both generalized and species-specific photosynthetic responses to light. Generally, at low irradiances, the maximum photon yield of photosynthesis is reached and so elevated light will strongly affect photosynthesis (Evans, 2013). At higher light levels, photosynthesis will plateau when chloroplasts are light-saturated (Evans, 2013). At that point, assimilation is dictated not by light but by CO<sub>2</sub> concentration and Rubisco limitation (Evans, 2013). Leaves at suboptimal irradiance respond within milliseconds to increased irradiance with increased photochemistry and electron flow (Kaiser, 2018). This is important because during experimentation, photosynthetic response was measured quickly after light levels were changed.

Intra-species variation in plants is caused by differences in internal anatomy and chlorophyll concentration, which are often dictated by environmental conditions during growth (Brown & Trlica, 1974). The leaves of high-light plants are normally thicker and have a higher stomatal density than low-light plants (Hager et al., 2016). Further, high-light plants have a higher Rubisco to chlorophyll ratio to fix more carbon while utilizing

the excess light (Wang et al., 2013). This is relevant here given the climatic differences between experimental locations: Spain, where the grapevine was grown compared with the Netherlands and Germany, where the grasses were grown. This suggests differences in responses between grasses and grapevine – used for parameterization.

The following focuses on results from photosynthesis-irradiance response literature that used experimental species similar (or equivalent) to those used for this study. Specifically, the following review compares grapevine photosynthesis-light response to experimental data of C3 grasses. Since the  $A-g_s$  model was parameterized using grapevine, the response of grapevine is relevant to this study, as there may be some similarities between modeled and real-world grapevine response.

Field-grown *Vitis vinifera* was analyzed for their photosynthetic response to changes in light at ambient  $CO_2$  (Greer et al., 2012). At 25° C, the species reached a maximum photosynthetic rate of  $\sim 16 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ . This occurred at an irradiance of  $\sim 800 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . Zufferey et al. (2000) found a similar result with grapevine however at a different irradiance level, finding  $A_{\text{max}}$  to reach  $\sim 15 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$  at  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (Zufferey et al., 2000). Based on this, the light saturation point for grapevine is reached somewhere between 750 and  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Light saturation point is similar for Poaceae grasses studied, e.g.  $1000 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$  for wheat plants (Evans, 2013).

Brown & Trlica (1974) studied the grasses, *Panicum laxum* and *Panicum boliviense* (at 30° C and a  $CO_2$  concentration of  $322 \mu\text{l l}^{-1}$ ). At a photon density of  $700 \mu\text{mol m}^{-2} \text{ s}^{-1}$  the assimilation response began to approach an  $A_{\text{max}}$  of  $\sim 18 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ . Yu et al., (2004) found *Triticum aestivum* to reach a higher rate of photosynthesis ( $A_{\text{max}} = \sim 23 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ ) at a photon density of  $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . In summary, grapevine photosynthesis is shown to reach  $\sim 16 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$  while photosynthesis in the grasses reached  $\sim 23 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$ . Therefore, the literature review shows that photosynthesis reaches a higher rate in Poaceae grasses than in grapevine.

At low light levels, grapevine experimental response data and C3 grasses display very similar response behavior and resulting photosynthetic values (based on literature). At levels of light from 0 to  $400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , the photosynthetic response of all grasses and grapevine studied was very strong. The rate of photosynthesis increased in grapevine from 0 to  $\sim 8.5 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$  (Greer et al., 2012; Zufferey et al., 2000) and in grasses from 0 to  $\sim 10.5 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$  (Brown & Trlica, 1974).

## 4 – Methods and Materials

Four separate experiments were completed: two regarding response to light i.e. (1) *T. aestivum* A-PAR, (2) *A. pratensis* A-PAR, and two regarding response to intercellular CO<sub>2</sub> (3) *T. aestivum* A-Ci, (4) *A. pratensis* A-Ci. As described in the Methods, the A-g<sub>s</sub> model simulated the respective experimental conditions of the described experiments, and its output was used for comparison.

### 4.1 - Experimental Plant Species

Data are derived from three sources: (1) the modeled data (parameterized with *V. vinifera*) from the A-g<sub>s</sub> model, (2) *T. aestivum* from Germany, obtained by Hugo de Boer of Utrecht University, and (3) *A. pratensis*, collected by the author at a farmland field site in eastern Netherlands. *A. pratensis* samples were then grown in the Utrecht University Botanic Gardens.

Experimental photosynthetic response data from two Poaceae grass species are analyzed in this study. *A. pratensis* is a perennial grass native to Europe and Asia, now spanning North America. It thrives in man-made habitats and prefers fertile clay and loamy soil, with its high demand for nutrients (Hilty, 2018).

*T. aestivum*, (Winter wheat/common wheat) is amongst the top three cereal crops grown worldwide. It is native to India, Iran and the surrounding areas but is now grown nearly everywhere, including Europe (Kew Science, N.d.). This crop is hugely important for global food production and the agricultural sector of the economy. Well drained, clayey-loam soils with high nutrients are ideal for this species (AgriInfo, 2015).

### 4.2 - Field Work

Grass samples (with soil core intact) were collected from a farmland field site of Wageningen University, the Netherlands. Samples were taken in three representative transects, from the river until the ditch. Ten samples were taken from each transect, taken at least one meter apart, randomly. This way, a gradient of soils were collected from more clayey near the river to sandier towards the ditch. Soil types were not differentiated during experimentation, only sampled as such to achieve variation and reduce the effect of soil type as a confounding variable.

Samples contained mainly two grass species (*A. pratensis* and *Holcus lanatus*) as well as other herbaceous species. However, only *A. pratensis* was used for experimentation because of its abundance, representing ~85% species cover for the samples. Potted samples were brought to the Botanic Garden greenhouses at Utrecht University for experimentation. The plants were exposed to natural wind and sunlight and were watered every couple of days, when they were randomly rearranged to reduce confounding variables related to exposure to wind and sunlight.

### 4.3 – Gas Exchange Device: LI-6400XT

To complete the experimental portion of this research, gas exchange parameters were measured using the LI-6400XT (LI-COR, Lincoln, Nebraska, USA). Curves generated from experimentation were then used to illustrate the effects of changes in environmental condition to varying  $C_i$  and light.

The LI-6400XT is an open system, which works by measuring differences in  $CO_2$  and  $H_2O$ , which flow through the leaf chamber (LI-COR, Inc. 1999). Infrared Gas Analyzers (IRGAs) are used to determine the amount of  $CO_2$ , via the IR wavelengths transmission reducing with increasing  $CO_2$  concentration (Majer, 2013). Sample IRGAs are compared to Reference IRGAs (Figure 4) (LI-COR, Inc. 1999). The equations used by the LI-6400XT are derived from Caemmerer and Farquhar (1981) (LI-COR, Inc, 1999). A summary of symbols is included in the Appendix, showing the measurements and units with which the device stores its data (Table 8).

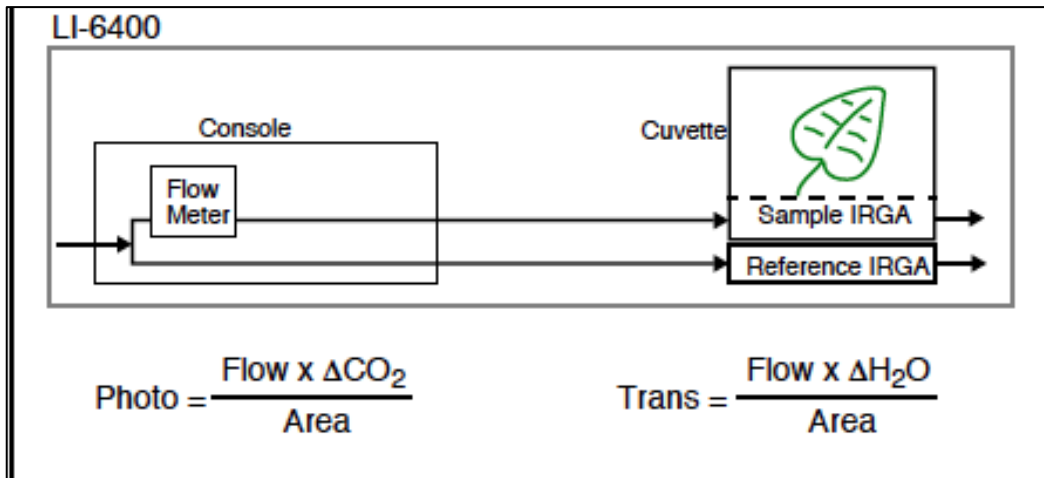


Figure 4. The LI-6400 gas exchange measurement system mechanism. The Sample IRGA is compared to the Reference IRGA and then the photosynthetic rate and transpiration rate are determined as a function of the flow and the leaf area (LICOR, Inc., 1999).

During experimentation, two leaves of *A. pratensis* were analyzed together from each sample plant. For this, the LI-6400XT gas exchange device was used under the protocol of Evans & Santiago (2014). The LI-6400XT was first set at desired initial environmental conditions. Leaves were then put into the gas exchange chamber. Leaf temperature was set at 25° C for optimum conditions (Brown & Trlica, 1974; Greer et al., 2012). Light and  $C_i$  were manipulated separately for each experiment using the LI-6400XT, ceteris paribus (Table 1). Plants will normally adapt to their changed conditions within minutes or seconds and gas exchange response data can be recorded (Hager et al., 2016), important to experimental design.

Table 1. Experimental design of experiments completed by the researcher to obtain the photosynthetic response of *Alopecurus pratensis* to changes in light and  $C_i$ . The following shows the environmental settings of the LI-COR 6400XT. There are two response experiments completed (one on light and one on  $C_i$ ) on each set of two leaves, from one individual *A. pratensis* plant.

Experiment No.	Leaf temp C ( $T_{leaf}$ )	Intercellular $CO_2$ $\mu mol mol^{-1}$ (Leaf $C_i$ )	Sample $CO_2$ $\mu mol mol^{-1}$ ( $CO_2S$ )	IRR ( $\mu mol m^{-2} s^{-1}$ ) (Parin)	Flow ( $\mu mol s^{-1}$ )
1	25		400	0	500
1	25		400	50	500
1	25		400	100	500
1	25		400	150	500
1	25		400	200	500
1	25		400	250	500
1	25		400	500	500
1	25		400	750	500
1	25		400	1000	500
2	25	0		500	500
2	25	20		500	500
2	25	50		500	500
2	25	100		500	500
2	25	150		500	500
2	25	250		500	500
2	25	500		500	500
2	25	750		500	500
2	25	1000		500	500

In order to analyze data, organization, editing and data transformation was needed. This included specifying which data would be used for analysis. Some data were not included in analysis: (1) measurements taken for matching purposes or (2) measurements that were retaken due to non-equilibration of the LI-6400XT and finally, (3) entire data sets were removed when considered unsound. Specifically, certain data sets in the *A. pratensis* A- $C_i$  experiments were removed because the photosynthesis rate near zero  $C_i$  was quite high when it is expected to be near zero. So, after removing data sets, the final sample size for the *A. pratensis* experiments was 10 datasets for the A- $C_i$  experiment and 19 datasets for the A-PAR experiments. The *T. aestivum* experiments involved a sample size of five datasets for A- $C_i$  and three for A-PAR.

Using the LI-6400XT, certain experimental settings fluctuated away from their set values throughout experimentation – especially temperature and  $C_i$ . Other values, especially flow rate and irradiance, remained very near to the initial setting throughout. Therefore, during experiments regarding A- $C_i$ , there are a range of  $C_i$  levels at which photosynthesis is taken, though the goal was to achieve values at specific  $C_i$  levels. This is unlike the



experiments involving A-PAR, where the LI-6400XT achieves PAR levels within a few  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ . Comparatively,  $\text{CO}_2$  levels during experimentation may have been set at  $400 \mu\text{mol mol}^{-1}$  but actual  $\text{CO}_2$  levels varied sometimes well over  $50 \mu\text{mol mol}^{-1}$  at higher settings of  $\text{CO}_2$ . Therefore, to determine the mean experimental photosynthetic rate at a given  $C_i$  for statistical analysis reasons, data categorization was necessary.

Experimental  $C_i$  data were therefore categorized by the author. The experimental mean  $C_i$  value per group was calculated. And, within these groups, the experimental mean photosynthetic value was also calculated. The experiment is aimed to compare experimental and modeled photosynthetic response data, so it is important to accurately simulate the experimental environmental settings in the modeled environment, as much as possible. So, using these groups and averages allows for more precise comparison with model-generated A- $C_i$  data.

#### 4.4 - Data Analysis Methods

In order to compare experimental (LI-6400XT) data with modeled (A- $g_s$ ) data sets, units were changed to match one another. For temperature, the LI-6400XT records in Celsius and the A- $g_s$  model works with Kelvin. The units of irradiance used in the model are  $\text{W m}^{-2}$  PAR, while the LI-6400XT uses  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photons. The units for photosynthesis in the model are  $\text{mg CO}_2 \text{ m}^{-2} \text{s}^{-1}$ , while the LI-6400XT measures  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ .  $\text{CO}_2$  was recorded by the LI-6400XT in units of  $\mu\text{mol CO}_2 \text{ mol}^{-1}$ , while the model used  $\text{mg CO}_2 \text{ m}^{-3}$ .

The two experimental grass species (*T. aestivum* and *A. pratensis*) were analyzed for gas exchange under different environmental condition changes, i.e. light and  $\text{CO}_2$ . The experiments' different LI-6400XT settings for their gas exchange environments are shown in Tables 2 & 3.

Table 2. The table shows the exact conditions in the gas exchange experiments with *T. aestivum*. Units listed are those used in experimentation and recorded by the LI-6400 gas exchange device. These experiments were performed by Hugo de Boer.

<i>T. aestivum</i> Environmental Conditions	
PAR IN	$1500 \mu\text{mol m}^{-2} \text{s}^{-1}$
$\text{CO}_2$ Reference	$450 \mu\text{mol mol}^{-1} \text{CO}_2$
$T_{\text{block}}$	$20^\circ \text{C}$
Flow	$500 \mu\text{mol s}^{-1}$



Table 3. The table shows the exact conditions in the gas exchange experiment using *A. pratensis*. Units listed are those used in experimentation and recorded by the LI-6400 gas exchange device. These experiments were performed by the author of this research.

<i>A. pratensis</i> Environmental Conditions	
PAR IN	500 $\mu\text{mol m}^{-2} \text{s}^{-1}$
CO <sub>2</sub> Sample	400 $\mu\text{mol mol}^{-1} \text{CO}_2$
T <sub>leaf</sub>	25° C
Flow	500 $\mu\text{mol s}^{-1}$

Using Sublime Text (version 3.1.1) and Python Launcher (version 2.7.13), model equations were input with independent variable data resulting in the predicted dependent variable, CO<sub>2</sub> assimilation (in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  after unit conversion). So, using the mean C<sub>i</sub> value per group (e.g. C<sub>i</sub> set to 100, 250, 500  $\mu\text{mol mol}^{-1} \text{CO}_2$ ), or the mean PAR value per group, for that matter, the A-g<sub>s</sub> model could be run. Modeled data from the A-g<sub>s</sub> model was obtained through Python Launcher, with minor changes to the code. Changes include only extra commands (i.e. print) and variable changes so that the modeled environments best replicate the experimental environments. Therefore, the resulting model curves show the A-C<sub>i</sub> and A-PAR responses at conditions that mimic those at experimentation (Tables 2 & 3).

#### 4.5 - Statistical Analysis Methods

To test for the correspondence between modeled and measured gas exchange, one-sample t-tests were used. Modeled photosynthetic rate was considered, for statistical analysis, to be the population mean in the t-test. One-sample t-tests were run, utilizing a two-tailed test, for a more conservative estimate of the t-statistic and because tail preference was unknown.

Scatterplots and linear regression were used to compare the experimental and modeled data. Linear regression was used in this way by Jacobs (1994) for data analysis. Correlation was calculated using r<sup>2</sup> value and subsequently, Pearson's r was used to determine the significance of correlation. While the r<sup>2</sup> value can illustrate the linear relationship between modeled and measured data, it does not consider accuracy of the model. For this, the Root Mean Square Error (RMSE) was calculated to assess the deviation of the experimental versus modeled data compared to a 1:1 line. The RMSE was calculated three times, (1) for the entirety of the data (2) for the lower portion < 400  $\mu\text{mol mol}^{-1} \text{CO}_2$  or < 400  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$  and (3) for the upper portion, > 400  $\mu\text{mol mol}^{-1} \text{CO}_2$  or > 400  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ . This analysis scheme was carried out for all experiments.

## 5 – Results

Overall t-tests show the A- $g_s$  model output was generally significantly different from the experimental output (at highest significance value tested:  $\alpha = 0.001$ ). That is, p-values from t-tests overall, show experimental mean photosynthesis levels to be significantly different from modeled output in both plant species tested and both A-PAR and A- $C_i$  experiments. To give an overview of the following section, *T. aestivum* generated higher photosynthetic rates than *A. pratensis*, closer to modeled values. Though the modeled data and *T. aestivum* generated photosynthetic data which were near to each other, most were still significantly different. While all *A. pratensis* data was significantly different than modeled data, *T. aestivum* produced photosynthetic data, which in some rare cases was *not* significantly different than modeled data.

### 5.1 - Photosynthetic response to light: A- $g_s$ v. *T. aestivum*

While the model overestimates photosynthesis compared to these experiments, the curve generated by the model is very similar to the A-PAR experimental curve. The experimental grass just reaches a lower  $A_{max}$  than the model. The modeled and experimental photosynthesis were highly correlated, although the model tended to overestimate photosynthesis slightly at higher light levels (Figure 5). The greatest differences in photosynthetic rates occurs at 1200  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ , where photosynthesis varied around 5.5  $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ .

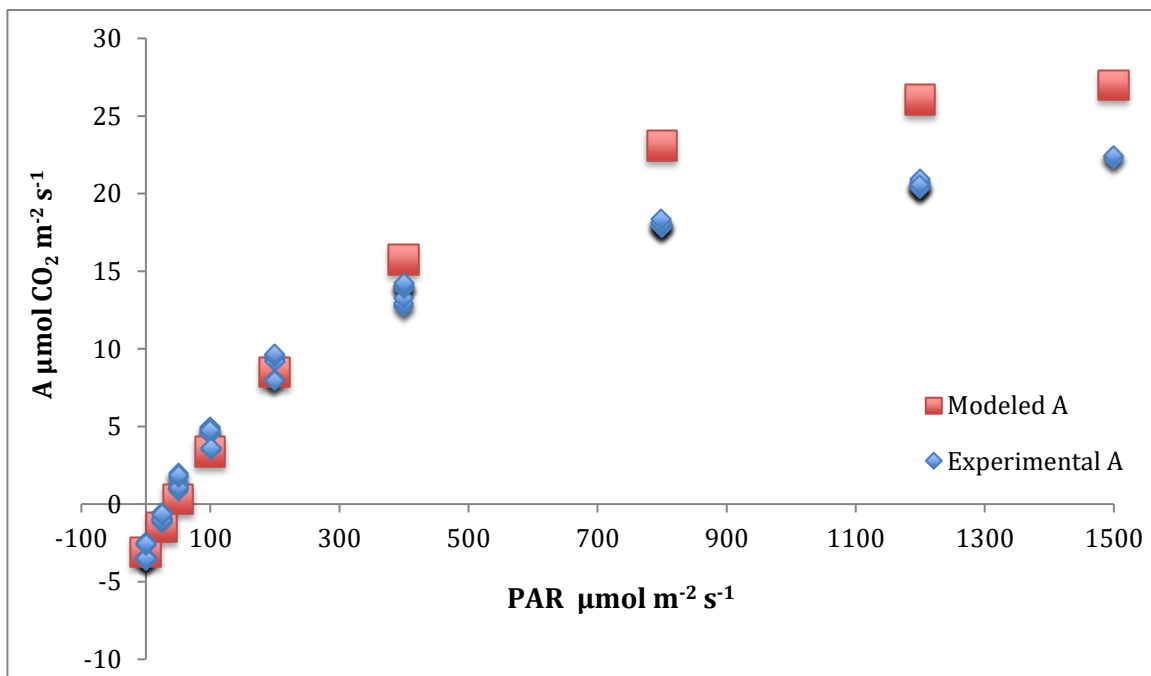


Figure 5. Photosynthetic response to change in PAR from experimental species, *T. aestivum*, versus modeled data from the A- $g_s$  model. The experimental data points shown include all collected data, shown as individual points.

Modeled and measured photosynthetic rates of *T. aestivum* show close agreement at low light ( $\leq 400 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ ), whereas modeled photosynthesis rates are higher than measured rates at higher light levels (Figure 5). Despite this deviation at higher light levels, the correlation between modeled and measured photosynthetic light response curves is very strong ( $r^2 = 0.99$ ) (Figure 6). Also, from the  $r^2$  value, Pearson's  $r$  was calculated. The P value of the linear regression line was  $<0.001$ , a significant correlation. Consistent with the  $r^2$  value, the RMSE demonstrates that the lower portion of the data show closer agreement than the upper portion of the data (Table 4).

Table 4. Photosynthetic rate data residuals and RMSE. There are three RMSE's shown: (1) The "RMSE Entire" shows the RMSE of the entirety of the data, (2) The "RMSE Lower" is the RMSE of the lower portion of the data, defined as  $< 400 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ . (3) The "RMSE Upper" shows the RMSE of the Upper portion of the data, defined then as photosynthetic rate at  $400 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ , or greater.

A-gs model vs. <i>T. aestivum</i> ; Root Mean Squared Error	0	25	50	100	200	400	800	1200	1500
PAR $\mu\text{mol m}^{-2} \text{ s}^{-1}$									
Residuals	-0.09	0.59	1.01	0.97	0.47	-2.11	-5.09	-5.52	-4.26
Squared Residuals	0.01	0.34	1.01	0.93	0.22	4.43	25.88	30.42	18.13
Root Mean Squared Error Entire	3.01								
Root Mean Squared Error Lower	0.71								
Root Mean Squared Error Upper	4.44								

A-PAR experiments with *T. aestivum* generated data which are very similar to modeled A-PAR. Yet, most experimental photosynthesis data were still found to be significantly different from modeled rates, and at the highest alpha ( $\alpha = 0.001$ ) (See Appendix Table 12 for P values and significance). This data set does, however, contain one measure which is not significantly different than modeled output: the first measure, at  $0 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ . Further, one measure ( $200 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ ) was significantly different from modeled output only at the  $\alpha = 0.05$  level.

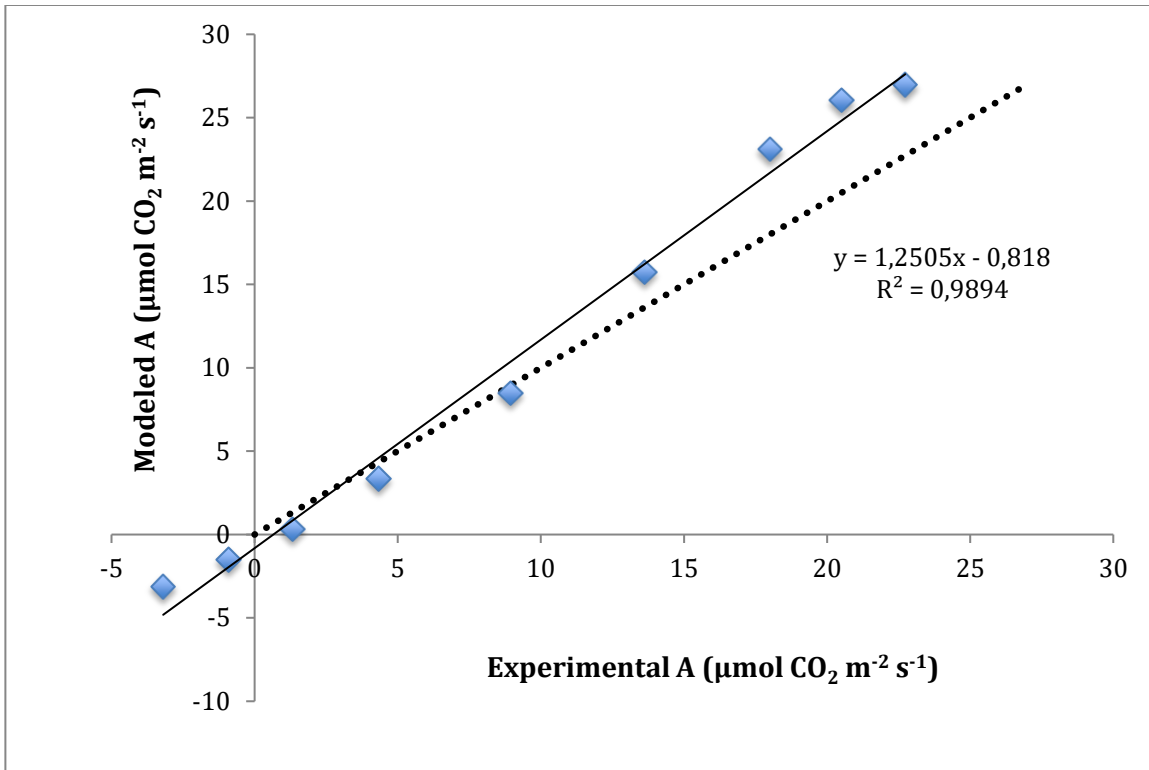


Figure 6. Photosynthetic response to change in PAR from experimental *T. aestivum* versus modeled data. Blue diamonds indicate mean photosynthesis data per light level. Linear regression was completed to determine the best-fit line to compare photosynthesis data, which is represented by the solid black line. The dashed line shows a 1:1 line which is useful for comparison against the best-fit linear regression.

Figure 6 shows the linear regression of mean photosynthetic rates, the best-fit line (shown in solid black). And compares this compares to a 1:1 line in Figure 6 (shown in a dashed line). The slope of the best-fit line illustrates slight overestimation by the model, overall. However, the similarity of these two lines demonstrates the nearly 1:1 nature in the comparison between modeled and experimental photosynthesis data.

## 5.2 - Photosynthetic response to light: *A-g<sub>s</sub>* v. *A. pratensis*

The model does not predict *A. pratensis* photosynthesis very closely in response to light. All measures taken experimentally were significantly different at the highest alpha ( $\alpha = 0.001$ ). Overall, the model highly overestimates the photosynthetic rate of *A. pratensis* in response to light (Figure 7).

The experimental response of *A. pratensis* to light is most similar to modeled response at lower light levels. Then, as light levels increase beyond 250  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ , the photosynthesis rates diverge until the modeled rates reach almost three times the experimental rate (at the highest light level: 1000  $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$ ) (Figure 7). While experimental photosynthesis levels appear to plateau at higher light levels, the modeled

photosynthesis does not. This leads to the vast overestimation of the photosynthetic values by the model in higher light levels. This large difference is also demonstrated by the highly sloped line resulting from linear regression (Figure 8).

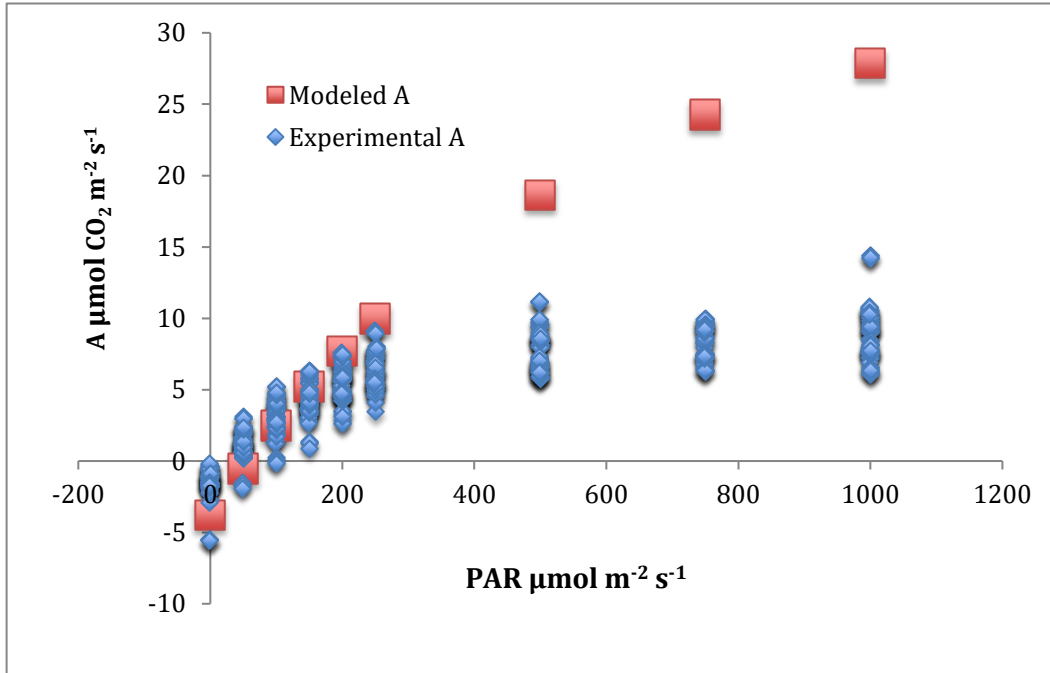


Figure 7. Scatterplot comparing the photosynthetic rate of experimental *A. pratensis* to the corresponding modeled rate, as produced by the A-g<sub>s</sub> model. This experiment was completed in response to changes in light level.

The relatively high  $r^2$  value at  $r^2=0.87$ , indicating correlation, compares to the RMSE. The RMSE for the entire data set comparison was quite high, suggesting a departure from a 1:1 relationship. Yet, this was due only to influence of the upper portion on the whole (Table 5). Though the lower portion of the data appears similar, one-sample t-tests show that all light levels from experimentation produced photosynthetic rates which were significantly different from modeled data (at the highest significance level  $\alpha= 0.001$ ) (Appendix Table 13).

Table 5. Photosynthetic rate data residuals and RMSE. There are three RMSE's shown: (1) The "RMSE Entire" shows the RMSE of the entirety of the data, (2) The "RMSE Lower" is the RMSE of the lower portion of the data, defined as  $< 400 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ . (3) The "RMSE Upper" shows the RMSE of the Upper portion of the data, defined then as photosynthetic rate at  $400 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ , or greater.

A-g <sub>s</sub> model vs. A. pratensis	0	50	100	150	200	250	500	750	1000
PAR (μmol m <sup>-2</sup> s <sup>-1</sup> )									
Root Mean Squared Error									
Residuals	2.29	1.69	0.64	-0.74	-2.18	-3.63	-10.81	-16.00	-18.85
Squared Residuals	5.25	2.85	0.40	0.55	4.74	13.15	116.86	255.87	355.24
Root Mean Squared Error Entire	9.16								
Root Mean Squared Error Lower	2.12								
Root Mean Squared Error Upper	15.58								

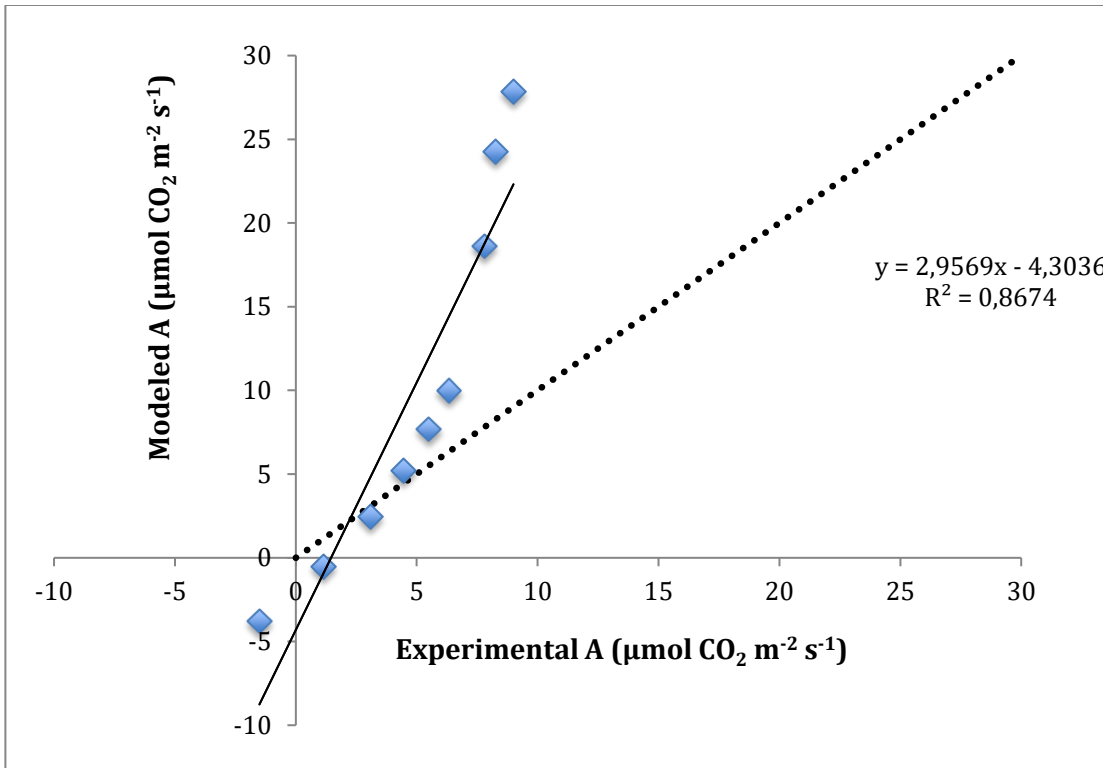


Figure 8. Photosynthetic response to change in PAR from experimental (*A. pratensis*) versus modeled data. Blue diamonds indicate mean photosynthesis data per light level. Linear regression was completed to determine the best-fit line to compare photosynthesis data, which is represented by the solid black line. The dashed line shows a 1:1 line which is useful for comparison against the best-fit linear regression.

The data comparison is illustrated by the shape of the data points as well as the best-fit line (Figure 8 solid black line). The goodness of fit of the best-fit line is represented by the  $r^2$  value, 0.87, a relatively strong correlation. From the  $r^2$  value in Pearson's  $r$  was calculated. From there, the P value of the linear regression line was calculated and the P value is  $< 0.001$ , and so the correlation is significant.

Though the  $r^2$  indicates strong correlation, compared to the 1:1 (Figure 8 dashed line), the best-fit linear regression line between experimental and modeled photosynthesis (Figure 8 solid black line) looks very far off. The slope of the best-fit line, being almost 3, shows that the model vastly overestimates photosynthesis, but only at higher light levels.

### 5.3 - Photosynthetic response to $C_i$ : *A-g\_s* v. *T. aestivum*

Comparing this data set to the others in this study, it is the only one where the modeled response data does not overestimate experimental response. The model both over- and underestimates experimental response.

Most  $C_i$  levels  $\leq 400 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$  produce photosynthetic rates which are significantly different, but at  $320 \mu\text{mol mol}^{-1}$ , the photosynthetic rate of *T. aestivum* is not significantly different than modeled data. All other measures from experimental data were found to be significantly different than those modeled.

Generally, there is little difference between modeled and experimental data sets (Figure 9). The linear regression comparing modeled and experimental data (Figure 10, solid line) shows a very high  $r^2$  (0.98), indicating a nearly linear relationship, or at least very high levels of correlation. From the  $r^2$  value, Pearson's  $r$  was calculated. From there, the P value of the linear regression line was  $< 0.001$ , and so the correlation is significant.

The RMSE (Table 6) shows the data is very similar in that both upper and lower RMSE are the same distance from modeled means. Figure 9 shows that the model first overestimates experimental data slightly. Then, at mid-range  $C_i$  ( $300\text{-}400 \mu\text{mol mol}^{-1}$ ), the model matches experimental data, while at high-range  $C_i$ , the model underestimates experimental data slightly.

Table 6. Photosynthetic rate data residuals and RMSE. There are three RMSE's shown: (1) The "RMSE Entire" shows the RMSE of the entirety of the data, (2) The "RMSE Lower" is the RMSE of the lower portion of the data, defined as  $< 400 \mu\text{mol CO}_2 \text{ mol}^{-1}$ . (3) The "RMSE Upper" shows the RMSE of the Upper portion of the data, defined then as photosynthetic rate at  $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$ , or greater.

A-gs@model@vs.@T.@aestivum								
$C_i$ @ $\mu\text{mol mol}^{-1}$ @CO2	44	111	176	246	320	438	608	971
Root@Mean@Squared@Error								
Residuals	-3.57	-4.57	-3.84	-2.08	-0.30	1.74	3.31	4.60
Squared@Residuals	12.77	20.88	14.74	4.31	0.09	3.02	10.96	21.12
Root@Mean@Squared@Error@Entire	3.31							
Root@Mean@Squared@Error@Lower	3.25							
Root@Mean@Squared@Error@Upper	3.42							



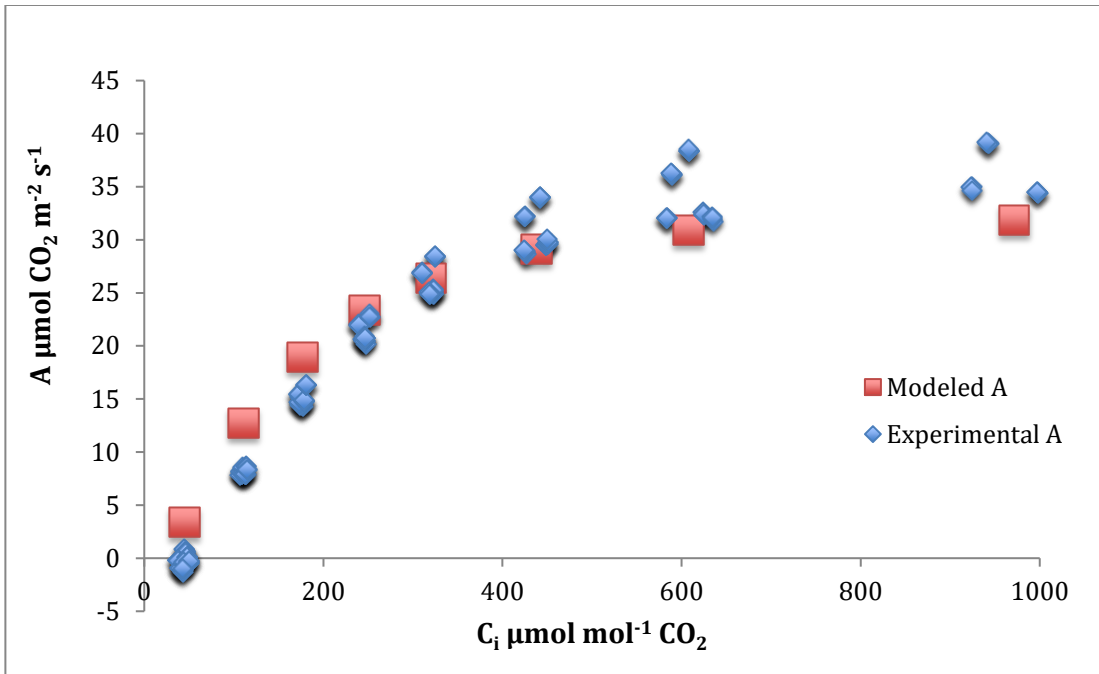


Figure 9. Photosynthetic response to change in C<sub>i</sub> from experimental *T. aestivum* versus modeled data. The model predicts values which are near to experimental values. Initially, the model overestimates, then underestimates photosynthesis of experimental *T. aestivum*.

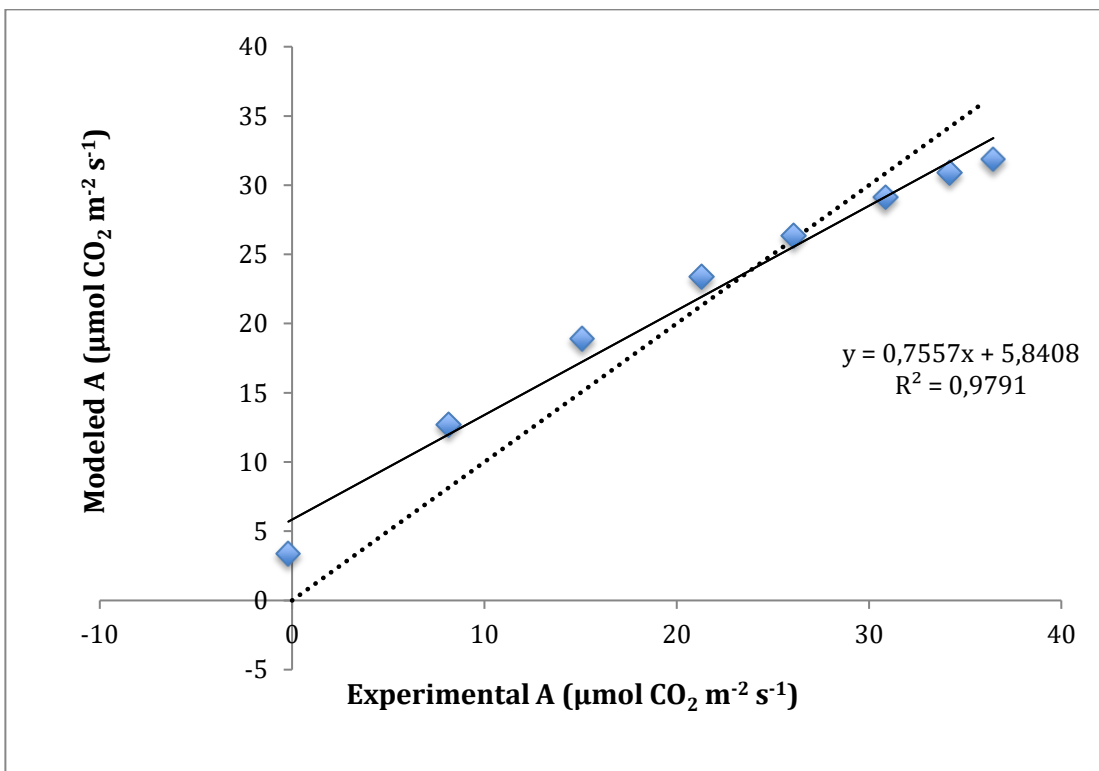


Figure 10. Photosynthetic response to change in C<sub>i</sub> from experimental *T. aestivum* versus modeled data. The data comparison is illustrated by the shape of the graph as well as the r<sup>2</sup> value, at 0.98. Blue diamonds indicate mean photosynthesis data per C<sub>i</sub> level. Linear regression was completed to determine the best-fit line to compare photosynthesis data, which is represented by the solid black line. The dashed line shows a 1:1 line which is useful for comparison against the best-fit linear regression.

One-sample t-tests were performed for each level of CO<sub>2</sub>. One measure of C<sub>i</sub> produced photosynthesis data which was not significantly different than modeled data. This occurred at 320 μmol mol<sup>-1</sup> CO<sub>2</sub>. All other C<sub>i</sub> values in the *T. aestivum* experiments produced a corresponding photosynthesis rate which was significantly different than the modeled value at the α= 0.001 level (Appendix Table 14).

### 5.4 - Photosynthetic response to C<sub>i</sub>: *A-g<sub>s</sub>* v. *A. pratensis*

The model does not predict *A. pratensis* photosynthesis very closely in response to C<sub>i</sub>, overestimating the experimental values. The RMSE shows that the model overestimates experimental data by about 8 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> (Table 7). This difference is consistent at lower and higher levels of C<sub>i</sub>.

Table 7. Photosynthetic rate data residuals and RMSE. There are three RMSE's shown: (1) The "RMSE Entire" shows the RMSE of the entirety of the data, (2) The "RMSE Lower" is the RMSE of the lower portion of the data, defined as < 400 μmol CO<sub>2</sub> mol<sup>-1</sup>. (3) The "RMSE Upper" shows the RMSE of the Upper portion of the data, defined then as photosynthetic rate at 400 μmol CO<sub>2</sub> mol<sup>-1</sup>, or greater.

A-g <sub>s</sub> model vs. A. pratensis	34	41	51	100	148	200	251	500	750	1001
C <sub>i</sub> μmol mol <sup>-1</sup> CO <sub>2</sub>										
Root Mean Squared Error										
Residuals	-1.50	-3.49	-5.29	-9.78	-10.90	-10.86	-10.92	-7.80	-7.77	-7.82
Squared Residuals	2.25	12.19	27.97	95.72	118.75	118.02	119.24	60.88	60.43	61.19
Root Mean Squared Error Entire	8.23									
Root Mean Squared Error Lower	8.40									
Root Mean Squared Error Upper	7.80									

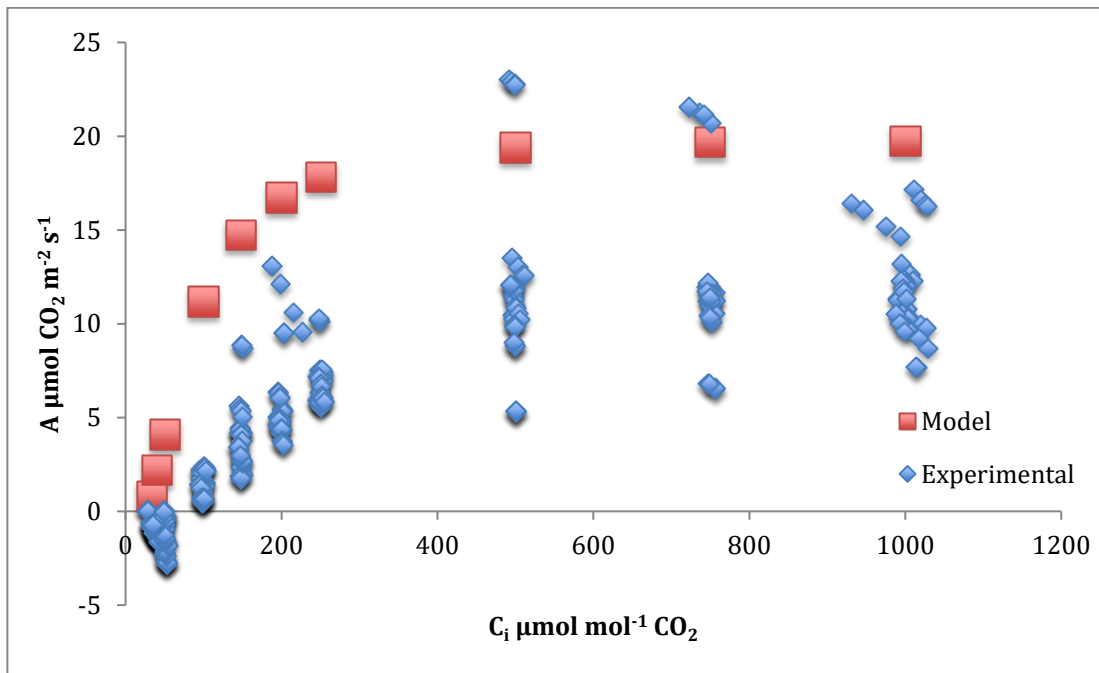


Figure 11. This figure compares the photosynthetic response to change in C<sub>i</sub> from experimental (*A. pratensis*) versus modeled data. Variation in the experimental data can be seen, yet modeled data clearly overestimates experimental mean photosynthesis rates.

Experimental data varies here more so than in other experiments. All data were found to be significantly different than modeled data at the highest alpha tested,  $\alpha = 0.001$  (Appendix Table 15). The modeled curve reaches its logarithmic limit, representing  $A_{\max}$ , quite early on, at a low  $C_i$  (Figure 11). This curve appears less hyperbolic in shape than other modeled or experimental curves. In relation to the experimental data, Figure 11 presents the many data sets used in this experiment, as well as the variation in data.

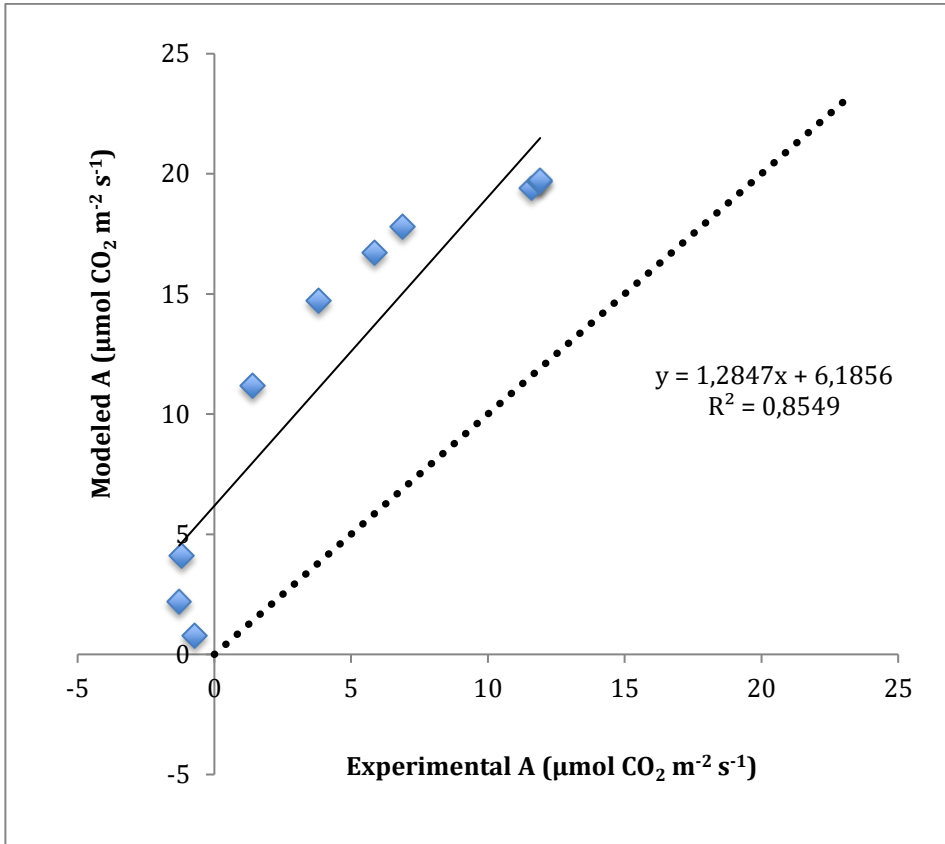


Figure 12. Photosynthetic response to change in  $C_i$  from experimental *A. pratensis* versus modeled data. The data comparison is illustrated by the shape of the graph as well as the  $r^2$  value, at 0.85. Blue diamonds indicate mean photosynthesis data per  $C_i$  level. Linear regression was completed to determine the best-fit line to compare photosynthesis data, which is represented by the solid black line. The dashed line shows a 1:1 line which is useful for comparison against the best-fit linear regression.

Looking at Figure 12, this  $r^2$  value suggests mid-levels of correlation between modeled and experimental *T. aestivum* data. The slope of the line being more than 1 shows that modeled photosynthesis response increases more with  $C_i$  than does experimental response. Further, the intercept of the best-fit line is 6.2, which is due to the model's overestimation of experimental results (Figure 12 solid black line).

The model greatly overestimates experimental photosynthesis values. However, the modeled and experimental  $A-C_i$  curves are similar in shape. This is represented in the

statistical analysis by the fact that the RMSE (Lower, Upper and Entire) was high for this experiment (around  $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) – suggesting distance from the 1:1 relationship, yet the  $r^2$  value was high – suggesting correlation between experimental and modeled data. Further, that the slope of the best-fit linear regression (Figure 12) is around 1.3 (and not higher) shows that the model and experimental curves are similar, only the experiments represent slightly less productive plants (in terms of net photosynthesis rate).

## 5.5 - Analysis of Results

Our results indicate that the generic relationships between light,  $\text{CO}_2$  and photosynthesis as measured on the species *T. aestivum* are reasonably well described by the A- $g_s$  model parameterized on *V. Vinifera* L. However, the model describes *A. pratensis* relationships less well. Notable differences were observed, especially in terms of the absolute magnitude of photosynthesis measured on *A. pratensis* under high light and  $\text{CO}_2$ , the model overestimating observations. *T. aestivum* in the A- $\text{C}_i$  experiments is first over-, then underestimated by the model.

The A-PAR response curve of *T. aestivum* was explained reasonably well by the model but not the curve of *A. pratensis*. The model-generated curve in the latter case does not reach  $A_{\text{max}}$  within the values measured. This compares to the experimental curve which does reach  $A_{\text{max}}$  within measured values.

For two experiments the RMSE is much greater in the upper portion than the lower: the A-PAR experiments on both *T. aestivum* and *A. pratensis*. This appears to indicate that the model is more accurate at predicting photosynthesis values at low light levels than high light levels in A-PAR experiments. Two experiments were analyzed where the RMSE for lower and upper measurements were very close. These were the A- $\text{C}_i$  experiments on both *T. aestivum* and *A. pratensis*, showing the model predicts at the same level of accuracy at low and high level  $\text{C}_i$ .

## 6 – Discussion

### 6.1 - Hypotheses considered

In regards to the A-PAR experiments, it was hypothesized in this research that at low light levels ( $\leq 400 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ ), the photosynthetic rate of experimental grasses (both *A. pratensis* and *T. aestivum*) would not be significantly different than the modeled photosynthetic rate. In fact, this hypothesis seems to be partially supported as most light levels  $\leq 400 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$  produce photosynthetic rates which are significantly different, but at  $0 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ , the photosynthetic rate of *T. aestivum* is not significantly different than modeled data. However, other than this exception, the data is significantly different and so the hypothesis is not fully supported, and is mainly refuted.

It was hypothesized that at high light, photosynthesis would reach a higher rate in experimental grasses than in the model. In literature review (Theoretical framework 3.3), grapevine photosynthesis is shown to reach  $\sim 16 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  while photosynthesis in the grasses reached  $\sim 23 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (Yu et al., 2004; Zufferey et al., 2000). This hypothesis is not supported by the results, rather the opposite, that the model at high light levels generated a higher photosynthetic rate compared with experimental grasses (both *A. pratensis* and *T. aestivum*).

In regards to the A- $C_i$  experiments, it was hypothesized that the model, parameterized with grapevine, would generate higher photosynthesis values at low  $C_i$  than experimental grasses, thought to be the case due to biological limitation of shrubs (e.g. grapevine) compared to herbaceous species (e.g. grasses) (Anderson, 2001; During, 1999; Jiang et al., 1999). Further, this was supported by literature review (During, 1991; Greer, 2012; Salazar-Parra et al., 2012). This hypothesis also is supported by the results of the present research, where in both experiments this is the case at low  $C_i$ ; and further, in the *T. aestivum* experiment, the model overestimates photosynthesis at low  $C_i$  but then underestimates it at higher  $C_i$ .

It was hypothesized that in A- $C_i$  experiments, maximum photosynthesis levels of modeled output and experimental response data would be similar, yet this hypothesis was due to contradicting evidence found in literature review. That is, literature review (3.2) showed C3 grasses to reach higher photosynthetic levels in response to saturating  $\text{CO}_2$  than grapevine (at high  $\text{CO}_2$  concentrations) (During, 1991; Greer, 2012; Morrison & Gifford, 1983; Salazar-Parra et al., 2012; Tabalenkova and Golovko, 2014; Yu et al., 2004). However, preliminary use of the A- $g_s$  model suggested that the model produced higher photosynthesis levels than seen in grapevine literature review. This was the basis for contradicting evidence for this particular hypothesis. In fact, the data show significant differences in these measures, supporting the contradictions found in evidence. The hypothesis that photosynthetic rates at high  $C_i$  would be similar, was not supported.

## 6.2 - Main Discussion

Certain discussion points are related to the effect of data organization and statistical procedures on the outcome of the results. Overall t-tests show the A-g<sub>s</sub> model output was generally significantly different from the experimental output (at the highest significance value tested:  $\alpha = 0.001$ ). However, it is the opinion of the author that the results of *T. aestivum* experiments are very close to the results of the A-g<sub>s</sub> model (in terms of net photosynthesis). The significant difference in experiments involving *T. aestivum* is thought to be due to limited sample size. While sample size in *A. pratensis* ranged from 10 to 19 data sets (for A-C<sub>i</sub> and A-PAR experiments, respectively), the *T. aestivum* experiments involved only five to three data sets (for A-C<sub>i</sub> and A-PAR, respectively). Therefore, changing the test for significance to favor difference when using a low sample size. While this is thought to be the case for *T. aestivum*, the significant difference between *A. pratensis* and the A-g<sub>s</sub> model (A-C<sub>i</sub> responses) is simply due to the overestimation by the model. The model, at the mid-level light intensity used for these experiments, results in a photosynthetic rate much higher than *A. pratensis* experimental data.

Data categorization of C<sub>i</sub> to determine the experimental A-C<sub>i</sub> curves was necessary for statistical analysis and the best way to get accurate results, in this case (see Methods, Data Analysis, 4.4). However, the data categorization to determine experimental means of C<sub>i</sub> and their corresponding photosynthesis rates may give the A-C<sub>i</sub> results slightly less validity, at least in the linear regressions and t-tests using these experimental means.

Our results indicate that the A-g<sub>s</sub> model can closely describe the photosynthetic response of C3 grass species, *T. aestivum*. However, the model describes the similar *A. pratensis* responses less well. Returning to the research of Prentice et al. (2015), it seems that species-specific parameterization may be necessary in this case, because *A. pratensis* was less accurately predicted by the model than *T. aestivum*. Alternatively, these results may point to changes needed to the A-g<sub>s</sub> model to be more or less sensitive to environmental conditions (especially light).

In relation to the A-PAR experiment with *A. pratensis*: the model first slightly underestimates photosynthesis and then at higher light levels, triples it. This suggests that the model is more sensitive to light than were experimental *A. pratensis* (Figures 7 & 8 Results). There is more evidence that the A-g<sub>s</sub> model appears to be overly sensitive to light levels in regards to photosynthesis rate. This case relates to A-C<sub>i</sub> curves, and particularly at which C<sub>i</sub> A<sub>max</sub> occurs.

In the A-C<sub>i</sub> experiments hypotheses were supported: that the model, parameterized with grapevine, would generate higher photosynthesis values at low C<sub>i</sub> than experimental grasses, due to biological limitation of shrubs compared with grasses (During, 1999; Jiang et al., 1999). The modeled response at low C<sub>i</sub>, like grapevine, is stronger than experimental response (slightly stronger than *T. aestivum* and much stronger than *A. pratensis*) (Figures 9 & 11). This result may point to the concern that the model, in its current parameterization, is suited for grapevine and possibly similar shrubs, but not

herbaceous grass species. However, parameterization may not be the problem- the problem may also lie in the sensitivity of the A-g<sub>s</sub> model.

Data suggests that the A-g<sub>s</sub> model is overly sensitive to increases in C<sub>i</sub> at low C<sub>i</sub> levels when light is non-saturating, as in the *A. pratensis* model simulation. The evidence for this is the comparative shape of the modeled A-C<sub>i</sub> curves at the two light intensities. Remember, the experiments on *A. pratensis* and *T. aestivum* use considerably different light intensities in their C<sub>i</sub> experiments (i.e., 500 μmol mol<sup>-1</sup> for the former and 1500 μmol mol<sup>-1</sup> for the latter, Tables 2 & 3). Saturating/high light levels used in the *T. aestivum* model simulation resulted in an A-C<sub>i</sub> curve which continued to grow until the final measurement (Figure 9 red squares). Here, A<sub>max</sub> is reached at levels close to observations from *T. aestivum* experimental data (Figure 9 blue diamonds). Further, the curve resembles A-C<sub>i</sub> curves from the literature in shape, in that A<sub>max</sub> is reached more gradually (During, 1991; Greer et al., 2012; Morrison & Gifford, 1983). So, while the model predicted a realistic curve for *T. aestivum* simulation, the *A. pratensis* model simulation curve was less realistic (in shape). At low light levels like those used in the *A. pratensis* model simulation, the resulting curve began very steeply, and reached A<sub>max</sub> at a low C<sub>i</sub> (approximately 400 μmol CO<sub>2</sub> mol<sup>-1</sup>) (Figure 11 red squares). By comparison, in experiments with *A. pratensis* (utilizing the same low light levels), photosynthesis continued to grow with increasing C<sub>i</sub> until approximately 750 μmol CO<sub>2</sub> mol<sup>-1</sup> (Figure 11 blue diamonds & Experimental Mean, Appendix Table 15 for details). Therefore, the resulting question becomes: Does the model do better at predicting photosynthesis of grasses studied at saturating light than it does at mid-level light, where it appears to overestimate photosynthesis? If this is true, this could be a factor in why the *T. aestivum* A-C<sub>i</sub> experiment is closer to modeled data than *A. pratensis*.

Parameterization using grapevine generated a model that was suited for this species and more, the individual plants from Central Spain. This can be illustrated, for example, by the high temperature at which the photosynthetic optimum occurs in the model (Jacobs, 1994). A model suited to this species and warm climates may not accurately describe cold-weather grasses, such as German-grown *T. aestivum* and Dutch-grown *A. pratensis*. However, these grasses are very similar and so one would expect the parameterization to generate accurate predictions or not, for both grass species: after all, the grass species are in the same PFT (with the same photosynthetic pathway, C3). In the experiment, this is not the case, as the model describes *T. aestivum* reasonably well and *A. pratensis* less well. These two grasses being similar species, and their modeled comparison being dissimilar, again, points to problems with model sensitivity to environmental conditions rather than parameterization. This is thought to be the case because it is recommended by current researchers that LSMs are parameterized using parameterization specific to the PFT, rather than necessarily as specific as species (Kala et al., 2016, Prentice et al., 2015), and so the family-level of species specificity (C3 Poaceae) should suffice in model comparison (rather than species-specific comparison). Thereby, the sensitivity of the model to light is of possible concern.



### 6.3 - Limitations and Further Research

The effect of photosynthetic response is known to depend on growth conditions as well as the moment that measurements are taken (Brown & Trlica, 1974, Long & Drake, 2013). In this research, measurements were taken by different people and with plants in different growth conditions and different stages of development. As for experimentation of *A. pratensis* by the author, many samples were taken and therefore, the gas exchange by the plants were measured across months and at different times of day. Over the months, photosynthetic response will likely vary due to seasonal changes and plant age (Hager et al., 2016; Salazar-Parra et al., 2012). Further, the time of day will cause variance (Brown & Trlica, 1974; During, 1991; Wang et al., 2013). However, given that *A. pratensis* experiments were performed by one person, there were inherent constraints on efficiency. It would be ideal to collect data within the same hours each day and only for a few weeks. Introducing fewer confounding variables in this way would benefit further research.

Nextly, due to the significant difference of *T. aestivum* to A-g<sub>s</sub> model results, the author recommends another experiment with more samples (see Discussion 6.2). This way, it would be possible to see if a higher sample size would change the statistical equation, so to say, and cause similar results to those in this study to produce results which are not statistically different than modeled data.

It is recommended by the author to do further gas exchange experiments to determine full A-C<sub>i</sub> curves at various light intensities. To explain the recommended experiment: collect response data on *A. pratensis* at high light levels and *T. aestivum* at low light levels (opposite of the beforehand research). If the model accurately predicts the photosynthetic response to C<sub>i</sub> in both species, yet only at high light, then this supports the claim that the model contains issues with light sensitivity (see Discussion 6.2). If, in these additional experiments, the model accurately predicts results for only *T. aestivum* and not *A. pratensis*, then this supports the need for species-specific parameterization. Therefore, the beforehand research will remain somewhat inconclusive until further experimentation can be completed.

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# Appendix

## LI-6400XT List of Symbols

Table 8. This table shows the symbols used for equations of the LI-6400, gas exchange measurement system (LI-COR, Inc., 1999). The description of the symbol is written, followed by the symbol's units.

Summary of Symbols
a = net assimilation rate, mol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> ,
A = net assimilation rate, μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>
c <sub>i</sub> = incoming CO <sub>2</sub> concentration, mol CO <sub>2</sub> mol air <sup>-1</sup> .
c <sub>o</sub> = outgoing CO <sub>2</sub> concentration, mol CO <sub>2</sub> mol air <sup>-1</sup> .
C <sub>s</sub> = mole fraction of CO <sub>2</sub> in the sample IRGA, μmol CO <sub>2</sub> mol <sup>-1</sup> air
C <sub>r</sub> = mole fraction of CO <sub>2</sub> in the reference IRGA, μmol CO <sub>2</sub> mol <sup>-1</sup> air
C <sub>i</sub> = intercellular CO <sub>2</sub> concentration, μmol CO <sub>2</sub> mol air <sup>-1</sup>
E = transpiration, mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>
F = molar flow rate of air entering the leaf chamber, μmol s <sup>-1</sup>
gbw = boundary layer conductance to water vapor, mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>
gsw = stomatal conductance to water vapor, mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>
g <sub>tc</sub> = total conductance to CO <sub>2</sub> , mol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup>
g <sub>tw</sub> = total conductance to water vapor, mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>
kf = (K <sub>2</sub> + 1)/(K + 1) <sup>2</sup> ,
K = stomatal ratio (dimensionless); estimate of the ratio of stomatal conductances
of one side of the leaf to the other
s = leaf area, m <sup>2</sup>
S = leaf area, cm <sup>2</sup>
u <sub>i</sub> = incoming flow rate, mol air s <sup>-1</sup> .
u <sub>o</sub> = outgoing flow rate, mol air s <sup>-1</sup> .
w <sub>i</sub> = incoming H <sub>2</sub> O mole fraction, mol H <sub>2</sub> O mol air <sup>-1</sup> .
w <sub>o</sub> = outgoing H <sub>2</sub> O mole fraction, mol H <sub>2</sub> O mol air <sup>-1</sup> .
W <sub>s</sub> = sample IRGA mole fraction of water vapor, mmol H <sub>2</sub> O mol air <sup>-1</sup> .
W <sub>r</sub> = reference IRGA mole fraction of water vapor, mmol H <sub>2</sub> O mol air <sup>-1</sup> .
W <sub>l</sub> = mole fraction of water vapor within the leaf, mmol H <sub>2</sub> O mol air <sup>-1</sup> .

## LI-6400XT List of Collected Experimental Data

Table 9. This table shows the measurements taken by the LI-6400XT, gas exchange measurement system. This list contains all measurements relevant to this study (LICOR, Inc., 1999).

LI-COR Code	Description
CO2R_μml	Reference cell CO2 (μmol CO2 mol <sup>-1</sup> )
CO2S_μml	Sample cell CO2 (μmol CO2 mol <sup>-1</sup> )
H2OR_mml	Reference cell H2O (mmol H2O mol <sup>-1</sup> )
H2OS_mml	Sample cell H2O (mmol H2O mol <sup>-1</sup> )
ΔCO2_μml	CO2 delta (sample - reference) (μmol CO2 mol <sup>-1</sup> )
ΔH2O_mml	H2O delta (sample - reference) (mmol H2O mol <sup>-1</sup> )
Flow_μml	Flow rate to the sample cell (μmol s <sup>-1</sup> )
RH_S_%	Relative humidity in the sample cell (%)
Photo	Photosynthetic rate (μmol CO2 m <sup>-2</sup> s <sup>-1</sup> )
Cond	Conductance to H2O (mol H2O m <sup>-2</sup> s <sup>-1</sup> )
Ci	Intercellular CO2 concentration (μmol CO2 mol <sup>-1</sup> )
Trmmol	Transpiration rate (mmol H2O m <sup>-2</sup> s <sup>-1</sup> )
Ci/Ca	Intercellular CO2 / Ambient CO2
VpdL	Vapor pressure deficit based on Leaf temp (kPa)
VpdA	Vapor pressure deficit based on Air temp (kPa)
totalCV%	Sum of ΔCO2CV_%, ΔH2OCV_%, and Flow_CV%
ΔCO2CV_%	Coefficient of variation of ΔCO2_μml (%)
ΔH2OCV_%	Coefficient of variation of ΔH2O_mml (%)
Flow_CV%	Coefficient of variation of Flow_μml (%)
RH_R_%	Relative humidity in the reference cell (%)
RH_S_%	Relative humidity in the sample cell (%)
Prss_kPa	Atmospheric pressure (kPa)
ParIn_μm	In-chamber quantum sensor (μmol m <sup>-2</sup> s <sup>-1</sup> )
ParOut_μm	External quantum sensor (μmol m <sup>-2</sup> s <sup>-1</sup> )
Tblock;C	Temperature of cooler block (C)
Tair_;C	Temperature in sample cell (C)
Tleaf;C	Temperature of leaf thermocouple (C)



## A-g<sub>s</sub> Model Variables and Values

Table 10. The following table gives information on the values which comprise the photosynthesis/stomatal conductance portion of the CLASS model, the A-g<sub>s</sub> model. The default values for all variables and parameters are shown, as well as the values actually used to run the model (which are occasionally different than default values) (van den Dries et al., 2013, de Arellano et al., 2015).

Option	Name python	Default value	Description	Unit	Value
ad		0.07	Regression coefficient to calculate Cfrac	kPa <sup>-1</sup>	0.07
Ag			CO <sub>2</sub> gross primary productivity at leaf	mg m <sup>-2</sup> <sub>leaf</sub> S <sup>-1</sup>	
Ag,c			CO <sub>2</sub> gross primary productivity at canopy	mg m <sup>-2</sup> <sub>ground</sub> S <sup>-1</sup>	
Alpha			light use efficiency	mg J <sup>-1</sup>	
alpha0		0.017	Initial low light conditions	mg J <sup>-1</sup>	0.017
Am			CO <sub>2</sub> primary productivity	mg m <sup>-2</sup> <sub>leaf</sub> S <sup>-1</sup>	
Am,max			CO <sub>2</sub> maximal primary productivity	mg m <sup>-2</sup> <sub>leaf</sub> S <sup>-1</sup>	
Am,max298		2.2	maximal primary productivity	mg m <sup>-2</sup> <sub>leaf</sub> S <sup>-1</sup>	2.2
An			Net flow CO <sub>2</sub> into the plant	mg m <sup>-2</sup> <sub>leaf</sub> S <sup>-1</sup>	
cfrac			fraction of the concentration (C <sub>i</sub> - Γ)/(C <sub>s</sub> - Γ)	-	
Ci			CO <sub>2</sub> concentration in the interior of the plant	mg m <sup>-3</sup>	
CO2comp298		68.5	CO <sub>2</sub> compensation concentration	mg m <sup>-3</sup>	45
Cs			CO <sub>2</sub> concentration at leaf level; we assume C <sub>s</sub> = <CO <sub>2</sub> >	mg m <sup>-3</sup>	
do			water vapor pressure deficit	kPa	
ds			water vapor pressure deficit	kPa	
f0		0.89	Maximum value Cfrac (regression coefficient)	-	0.89
fmin			minimum value Cfrac		
gc			CO <sub>2</sub> conductance	mm s <sup>-1</sup>	
gm298		7	Mesophyll conductance at 298K	mm s <sup>-1</sup>	7
gmin		2.5 E -4	Cuticular (minimum) conductance	m s <sup>-1</sup>	2.5 E -4
PAR			Photosynthetically active radiation inside the canopy	W m <sup>-2</sup> <sub>leaf</sub>	
PAR t			Photosynthetically active radiation at the canopy top	W m <sup>-2</sup> <sub>ground</sub>	

Option (Cont.)	Name python	Default value	Description	Unit	Value
Q10Am		2	Parameter to calculate maximal primary productivity	-	2
Q10CO2		1.5	Parameter to calculate CO2 compensation concentration	-	1.5
Q10gm		2	Parameter to calculate mesophyll conductance	-	2
Rd			CO2 dark respiration	mg m <sup>-2</sup> leaf S <sup>-1</sup>	
T1Am	T1Am,max	281	Reference temperature to calculate maximal primary productivity	K	281
T1gm		278	Reference temperature to calculate mesophyll conductance	K	278
T2Am	T2Am,max	311	Reference temperature to calculate maximal primary productivity	K	311
T2gm		301	Reference temperature to calculate mesophyll conductance	K	301
w2		0.42	Volumetric water content deeper soil layer	m <sup>3</sup> m <sup>-3</sup>	0.6
wfc		0.491	Volumetric water content field capacity	m <sup>3</sup> m <sup>-3</sup>	0.491
wwilt		0.314	Wilting point	m <sup>3</sup> m <sup>-3</sup>	0.314
Γ			CO2 compensation point	ppmv	
	co2abs				400
	e				701.19
	eps_input				0.017
	es				1297.48
	rho				1.2

## Full code of A-g<sub>s</sub> Model Equations

Table 11. Shown is the code that comprises the photosynthesis/stomatal conductance portion of the CLASS model, called the A-g<sub>s</sub> model (de Arellano et al., 2015). Descriptions of equations are provided by the author of this research.

Equation used by Model	Description
$CO2comp = CO2comp298 * Q10CO2^{(0.1*(T-298.))} * rho$	The CO <sub>2</sub> compensation point (Γ) is calculated. The Γ is dependent on the temperature difference from 298 K (24.85°C), at which the Γ is a constant in the model.
$gm = gm298 * Q10gm^{(0.1*(T-298.))} / ((1.+exp(0.3*(T1gm-T)))*(1.+exp(0.3*(T-T2gm))))$	The mesophyll conductance (gm) is calculated. Gm is dependent on the temperature difference from 298 K (24.85°C), at which Gm is a constant in the model.
$gm = gm / 1000.$	
$rm = 1. / gm$	The mesophyll resistance is calculated by finding the inverse of mesophyll conductance.
$fmin0 = gmin/1.6 - gm*(1./9.)$	Fmin0 is dependent on the mesophyll conductance and the minimum cuticular conductance (gmin).
$fmin = (-fmin0 + sqrt(fmin0^{**2} + 4*(gmin/1.6)*gm)) / 2*gm$	fmin is the minimum value of Cfrac and is dependent on the mesophyll conductance
$Ammax = Ammax298 * Q10Am^{(0.1*(T-298.))} / ((1.+exp(0.3*(T1Ammax - T)) * (1.+exp(0.3*(T-T2Ammax))))$	A <sub>mmax</sub> is calculated based on a difference in temperature from temperature producing maximum primary productivity (298 K).
$Am = Ammax*(1.-exp(-gm*(ci-CO2comp) / Ammax))$	A <sub>m</sub> is determined by the A <sub>mmax</sub> and the difference in C <sub>i</sub> from the CO <sub>2</sub> compensation point. Further, A <sub>m</sub> is also determined by the mesophyll conductance.
if any(Am<0.):	
Am[[Am<0.]] = nan	
$Rd = Am / 9.$	

Equation used by Model (Cont).	Description
$\alpha = \alpha_0 * (C_s - CO2_{comp}) / (C_s + 2 * CO2_{comp})$	<p>alpha is the light use efficiency and is calculated using initial low light conditions (alpha_0). Further, alpha is determined by the difference in atmospheric CO2 to the CO2 compensation point.</p>
$A_g = (A_m + R_d) * (1 - \exp((-alpha * PAR) / (A_m + R_d)))$	<p>ag is the CO2 gross primary productivity and is determined by the sum of Am and Rd as a function of light use efficiency and irradiance.</p>
$\epsilon_s = \epsilon_{input} * ((C_s - CO2_{comp}) / (C_s + 2 * CO2_{comp}))$	<p>eps is determined by the difference in atmospheric CO2 to the CO2 compensation point.</p>
$A_n = (A_m + R_d) * (1 - \exp((-eps * PAR) / (A_m + R_d))) - R_d$	<p>The A<sub>n</sub> is calculated based on Am and the difference between the atmospheric CO<sub>2</sub> and the CO<sub>2</sub> compensation point. Further, the A<sub>n</sub> is determined by the light level and eps.</p>
$AGSa1 = 1.0 / (1 - f_0)$	
$D_0 = (f_0 - f_{min}) / a_d$	<p>D0 is the water vapor pressure deficit</p>
$D_{star} = D_0 / (AGSa1 * (f_0 - f_{min}))$	
$\# D_s = (e_s - e) / 10.0$	
$\beta_{etaw} = (w_2 - w_{wilt}) / (w_{fc} - w_{wilt})$	<p>betaw is determined by the difference of Volumetric water content at deep soil levels and the wilting point. This value is divided by the difference between Volumetric water content field capacity and the wilting point.</p>
$f_{str} = \beta_{etaw}$	<p>Fstr is equal to betaw.</p>
$g_c = g_{min} + AGSa1 * f_{str} * A_n / ((co2_{abs} - CO2_{comp}) * (1 + D_s / D_{star}))$	<p>CO2 conductance is determined by cuticular minimum conductance</p>
$\# g_c = g_c * 1000. \#(\text{to convert to mm})$	
$g_c = 1./g_c$	
$\text{return } A_n, g_c, g_m, r_m, A_m, R_d$	
$\text{print 'Output in order: } A_n, g_c, g_m, r_m, A_m, R_d'$	

## Photosynthetic response to light: A-g<sub>s</sub> model compared with *T. aestivum*

Table 12: This table shows the P value of a one-sample t-test, which compares the photosynthetic rate of two data sets of photosynthetic rate at different light values. One data set comes from experimental data from *T. aestivum* and the other data set is model-generated from the A-g<sub>s</sub> model. It can be seen that the first light level response was not significantly different than modeled response, but all other light levels produced significantly different responses from the model. All values produce photosynthetic rates which are significantly different at the  $\alpha=0.001$  level, except at 200  $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$  which is significantly different at the  $\alpha=0.01$  level.

A-g <sub>s</sub> model vs. <i>T. aestivum</i>	0	25	50	100	200	400	800	1200	1500
PAR ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )									
Pop. Mean (Model) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	-3.10	-1.50	0.32	3.36	8.48	15.74	23.10	26.04	26.99
Count	12	12	12	12	12	12	12	12	8
Degrees of Freedom	11	11	11	11	11	11	11	11	7
Sample Mean (Experimental) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	-3.19	-0.91	1.32	4.33	8.95	13.63	18.01	20.52	22.73
Difference in Means	-0.09	0.59	1.01	0.97	0.47	-2.11	-5.09	-5.52	-4.26
Standard Deviation	0.47	0.22	0.41	0.59	0.73	0.56	0.16	0.18	0.41
Standard Error	0.13	0.06	0.12	0.17	0.21	0.16	0.05	0.05	0.14
t statistic	-0.67	9.08	8.49	5.70	2.26	-13.10	-108.05	-106.85	-29.39
Absolute Value t statistic	0.67	9.08	8.49	5.70	2.26	13.10	108.05	106.85	29.39
P value	0.51926493	1.92021E-06	3.69971E-06	0.00013749	0.0451595	4.7146E-08	5.332E-18	6.0285E-18	1.3582E-08

P value	Legend
P < 0.001	###
P < 0.01	###
P < 0.05	###
P > 0.05	###

## Photosynthetic response to light: A-g<sub>s</sub> model compared with *A. pratensis*

Table 13: This table shows the P value of a one-sample t-test, which compares the photosynthetic rate of two data sets of photosynthetic rate at different light values. One data set comes from experimental data from *A. pratensis* and the other data set is model-generated from the A-g<sub>s</sub> model. All values produce photosynthetic rates which are significantly different at the  $\alpha=0.001$  level.

A-g <sub>s</sub> model vs. <i>A. pratensis</i> PAR (μmol m <sup>-2</sup> s <sup>-1</sup> )	0	50	100	150	200	250	500	750	1000
Pop. Mean (Model) (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	-3.80	-0.53	2.46	5.19	7.69	9.97	18.61	24.25	27.85
Count	76	76	76	76	76	76	76	48	75
Degrees of Freedom	75	75	75	75	75	75	75	47	74
Sample Mean (Experimental) (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	-1.51	1.16	3.10	4.45	5.51	6.34	7.80	8.26	9.00
Difference in Means	2.29	1.69	0.64	-0.74	-2.18	-3.63	-10.81	-16.00	-18.85
Standard Deviation	1.11	1.01	1.22	1.27	1.26	1.24	1.44	1.20	1.85
Standard Error	0.13	0.12	0.14	0.15	0.14	0.14	0.17	0.17	0.21
t Statistic	18.05	14.54	4.53	-5.07	-15.07	-25.47	-65.45	-92.06	-88.15
Absolute Value of t Statistic	18.05	14.54	4.53	5.07	15.07	25.47	65.45	92.06	88.15
P Value	5.1862E-29	1.6057E-23	2.1921E-05	2.779E-06	2.1778E-24	1.1668E-38	6.411E-68	9.788E-55	1.0768E-76

P value	Legend
P < 0.001	###
P < 0.01	###
P < 0.05	###
P > 0.05	###

## Photosynthetic response to $C_i$ : A- $g_s$ model compared with *T. aestivum*

Table 14: This table shows the P value of a one-sample t-test, which compares the photosynthetic rate of two data sets of photosynthetic rate at different  $C_i$  values. One data set comes from experimental data from *T. aestivum* and the other data set is model-generated from the A- $g_s$  model. One  $C_i$  value produces a mean photosynthetic rate which is not significantly different than modeled output. This occurs at 320  $\mu\text{mol mol}^{-1}$ , where modeled data intersects with experimental data. All other mean photosynthetic values are significantly different than modeled values at the  $\alpha=0.001$  level.

A- $g_s$ model vs. <i>T. aestivum</i>								
$C_i$ ( $\mu\text{mol mol}^{-1} \text{CO}_2$ )	44	111	176	246	320	438	608	971
Pop. Mean (Model) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	3.38	12.72	18.93	23.37	26.38	29.12	30.89	31.87
Count	36	20	20	20	20	20	20	20
Degrees of Freedom	35	19	19	19	19	19	19	19
Sample Mean (Experimental) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	-0.19	8.15	15.09	21.29	26.08	30.86	34.20	36.47
Difference in Means	-3.57	-4.57	-3.84	-2.08	-0.30	1.74	3.31	4.60
Standard Deviation	0.61	0.27	0.71	0.95	1.40	1.98	2.69	2.95
Standard Error	0.10	0.06	0.16	0.21	0.31	0.44	0.60	0.66
t Statistic	-34.95	-75.60	-24.20	-9.73	-0.96	3.93	5.50	6.97
Absolute Value t Statistic	34.95	75.60	24.20	9.73	0.96	3.93	5.50	6.97
P Value	8.321E-29	5.007E-25	9.6889E-16	8.2228E-09	0.34958	0.00089	2.632E-05	1.2182E-06

P value	Legend
P < 0.001	###
P < 0.01	###
P < 0.05	###
P > 0.05	###



## Photosynthetic response to $C_i$ : A- $g_s$ model compared with *A. pratensis*

Table 15: This table shows the P value of a one-sample t-test, which compares the photosynthetic rate of two data sets of photosynthetic rate at different intercellular CO<sub>2</sub> values. One data set comes from experimental data from *A. pratensis* and the other data set is model-generated from the A- $g_s$  model.

A- $g_s$ model vs. <i>A. pratensis</i>	$C_i$	34	41	51	100	148	200	251	500	750	1001
Pop. Mean (Model) $\mu$ (umol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )		0.78	2.21	4.10	11.17	14.72	16.72	17.81	19.41	19.67	19.74
Count		27	32	54	40	39	34	35	40	34	38
Degrees of Freedom		26	31	53	39	38	33	34	39	33	37
Sample Mean (Experimental) $\mu$ (umol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )		-0.72	-1.29	-1.19	1.39	3.82	5.86	6.89	11.60	11.90	11.91
Difference in Means		-1.50	-3.49	-5.29	-9.78	-10.90	-10.86	-10.92	-7.80	-7.77	-7.82
Standard Deviation		0.34	0.22	0.91	0.66	1.80	2.46	1.22	4.32	3.78	2.55
Standard Error		0.07	0.04	0.12	0.10	0.29	0.42	0.21	0.68	0.65	0.41
t Statistic		-22.96	-89.18	-42.77	-94.24	-37.71	-25.72	-53.04	-11.43	-11.99	-18.91
Absolute Value Statistic		22.96	89.18	42.77	94.24	37.71	25.72	53.04	11.43	11.99	18.91
P Value		2.99431E-19	6.213E-40	2.4781E-43	1.3556E-48	2.6584E-32	7.2465E-24	4.9631E-35	3.5444E-14	9.2097E-14	6.241E-21

P value	Legend
P < 0.001	###
P < 0.01	###
P < 0.05	###
P > 0.05	###