MODELLING RICE AND WHEAT RESPONSE TO RISING CARBON DIOXIDE CONCENTRATION

A Thesis submitted by

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Abstract

Better crop photosynthetic efficiency is important for enhancing field crop production. The improvement in the photosynthetic efficiency of a crop depends on its efficiency in the usage of resources, including CO_2, water, nitrogen (N) and radiation. However, prolonged exposure to elevated carbon dioxide concentration (e[CO_2]) and, a short supply of other resources may lead to a decline in photosynthesis – a process referred to as ‘acclimation.’ Studies have demonstrated photosynthetic acclimation at the flag leaf level in a variety of crops. However, progress is limited in addressing the gaps in knowledge about the link between leaf-level acclimation phenomena and canopy level performance, which is influenced by different growth and development processes and abiotic factors. Therefore, there is a need for crop models capable of accurately extrapolating the leaf-level response to canopy level, to understand the overall impact of changes in photosynthesis at the biochemical level and its consequence on crop growth, development and productivity. In this regard, the research described in this thesis is founded on the hypotheses, that i) primary plant responses, photosynthesis and stomatal conductance to e[CO_2] are regulated by the interaction of different environmental variables ii) photosynthesis acclimation, on prolonged exposure to e[CO_2], is associated with a change in the leaf ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) and N concentration and, iii) photosynthetic acclimation can be better captured when biochemical parameters are included in the crop models like APSIM which is based on the concepts of cross-scale modelling, facilitating crop growth and development.

A meta-analysis of the studies reported in the literature was conducted to evaluate the impact of e[CO_2] on two major physiological processes, photosynthesis and stomatal conductance in two primary functional groups of plants – C_3 and C_4. Within C_3 and C_4 crops, more specific groups including legumes, non-legumes, flowers, trees, shrubs and grasses were examined to evaluate their respective responses to e[CO_2] under different abiotic stresses. The abiotic factors like water, N and temperature were found to be critical in determining the photosynthetic efficiency and thus, the biomass of plants. Understanding the role of abiotic factors, particularly N, in the photosynthesis under continuous exposure to e[CO_2] is essential to predict the crop response to the possibility of an e[CO_2] in the earth's atmosphere, in the future. In this study, rice response to e[CO_2] was estimated using a system dynamics modelling tool, STELLA. An analytical modelling framework embedding leaf-level crop system including RuBisCO and N dynamics and crop growth processes are developed using the STELLA software. The secondary data on rice from a growth chamber experiment was utilised to validate the model. The simulated response strongly supported the occurrence of photosynthetic acclimation at both growth and biochemical levels, under different e[CO_2], at different levels of N supply.

Further, this study evaluated photosynthesis, in-depth, in determining e[CO_2]-induced acclimation and thus, growth. Two major parameters that were used for estimations are the maximum carboxylation capacity ($V_{c.max}$) and the electron transport capacity (J_{max}). Data from the Australian Grains Free-Air CO_2 Enrichment (AGFACE), Horsham, Victoria, Australia were analyzed and modelled to determine the changes in the photosynthetic response of another C_3 crop, wheat, to e[CO_2]. The Agriculture Production System Simulator coupled with the diurnal canopy photosynthesis-stomatal conductance model (hereafter referred to as $APSIM_{DCP}$) was used to validate the $APSIM_{DCP}$ model and evaluate the range of parameters associated with photosynthetic acclimation under e[CO_2]. It was established that $APSIM_{DCP}$ could adequately link the biochemical and crop level responses, to enable extending the leaf level model to the canopy level. Further, it successfully simulated the photosynthetic acclimation responses to e[CO_2] for different wheat cultivars which were characterized by reduction of $V_{c.max}$, J_{max} and leaf N concentration. However, all cultivars were not equally responsive to the e[CO_2], with some showing no response at all and, others showing responses of varying magnitude, illustrating genotypic variation in this trait. In summary, this study investigated the impact of e[CO_2] on variation in photosynthesis in rice and wheat at different physiological stages of growth to predict the biomass and yield responses accurately.

Certification of Thesis

This thesis is entirely the work of Mela Aryal except where otherwise acknowledged, with the majority of the authorship of the paper presented as a Thesis by Publication undertaken by the student. The work is original and has not previously been submitted for any other award, except where acknowledged.

Principal Supervisor: **Professor Saman Seneweera**

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Student and supervisors’ signatures of endorsement are held at the University.

Statement of Contribution

Chapter 2: Photosynthesis and stomatal conductance of C_3 and C_4 plants in response to elevated carbon dioxide under different abiotic stresses: A reassessment using meta-analysis. **Aryal M**, O’Leary G.J., Dassanayake B.K., Kouadio L, Seneweera S.

MA contributed 70% towards the concept, analysis of the data, literature collection, writing and revision of the manuscript. SS contributed 10% towards the concept and design, critical revision of the manuscript and final editorial input. LK, GO, and KD contributed 10% each towards a revision of the manuscript and final editorial input.

Chapter 3: Modelling photosynthetic acclimation to elevated carbon dioxide concentration in rice using key gas exchange and biochemical parameters. **Aryal M**, , Kouadio L, O’Leary G.J, Seneweera S.

MA contributed 60% towards the concept, analysis of the data, literature collection, writing and revision of the manuscript. GO contributed 20% towards the concept and design, critical revision of the manuscript and final editorial input. LK and SS contributed 10% each towards the concept, revision of the manuscript and final editorial input.

Chapter 4: Modelling wheat response to elevated carbon dioxide concentration incorporating parameters of photosynthetic acclimation under Free-Air CO_2 Enrichment (FACE). **Aryal M**, , O’Leary G.J, Kouadio L, Seneweera S.

MA contributed 60% towards the concept, analysis of the data, literature collection, writing and revision of the manuscript. LK and GO contributed 15% each towards the concept and design, critical revision of the manuscript and final editorial input. SS contributed 10% each towards the concept, revision of the manuscript and final editorial input.

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Abbreviations

a$[CO_2]$	Atmospheric carbon dioxide concentration
AGFACE	Australian Grains Free Air Carbon Dioxide Enrichment
APSIM	Agricultural Production Systems Simulator
$APSIM_{DCP}$	Agricultural Production Systems Simulator coupled with a diurnal canopy photosynthesis-stomatal conductance model
CH_4	Methane
CO_2	Carbon dioxide
$[CO_2]$	Carbon dioxide concentration
DC	Decimal Code
DCP	Diurnal canopy photosynthesis-stomatal conductance model
e$[CO_2]$	Elevated carbon dioxide concentration
FACE	Free Air Carbon Dioxide Enrichment
FAO	Food and Agriculture Organisation
F-gas	Fluorinated gas
GHG	Greenhouse gas
g_s	Stomatal conductance
HFC	Hydrofluorocarbons
J_{max}	Maximum electron transfer capacity
LAI	Leaf area index
LI	Light interception
N	Nitrogen
N_o	Leaf nitrogen content per area basis
N_b	Minimum value of nitrogen at or below which CO_2 assimilation rate is zero
N_2O	Nitrous oxide
PFC	Perfluorocarbons
RAD	Daily Radiation
RuBisCO	Ribulose-1,5-bisphosphate carboxylase oxygenase
RuBP	Ribulose-1,5-bisphosphate
RUE	Radiation use efficiency
SLN	Specific leaf nitrogen

STELLA	System Thinking, Experimental Learning Laboratory with Animation
TOS	Time of sowing
$V_{c.max}$	Maximum carboxylation efficiency of RuBisCO

Chapter 1: Literature Review

Introduction

The United Nations Food and Agriculture Organisation (FAO) has projected that feeding the predicted world human population of 9.1 billion in 2050, would require raising the current crop production by some 70%, under the prevailing environmental conditions (FAOSTAT, 2009). It is a challenging proposition, even under the present circumstances. However, with the accumulation of more convincing evidence suggesting that the prevailing conditions are likely to change drastically, due to climate change, makes the challenge even more daunting (IPCC, 2007). Climate change is expected to increasingly affect crop yields (Tubiello et al., 2007) and analyses of current global crop yield data indicate that it may already be happening (Lobell & Field, 2007).

In the context of agriculture and food production, the primary climate change elements that matter most, are rising atmospheric temperatures, elevated carbon dioxide levels, and precipitation changes (FAOSTAT, 2009). However, in addition to the direct effects on plant growth and productivity, the changing climate can impact crop production indirectly through its effects on several other components of the agricultural system, including hydrologic cycles, input supplies, and even the nutrient cycles (Adams et al., 1998). Thus, the impacts and consequences of climate change would exert additional pressures on global agricultural productivity and, likely to threaten future global food security (Ainsworth et al., 2008). The direct biophysical effects of climate change on crops can raise concerns on the sustainability of the current productivity levels of agricultural crops (Nelson et al., 2014).

Agriculture is an activity that depends heavily, on climate variables (Hansen, 2002). Therefore, assessing the impacts of a changing climate is necessary to address the future crop productivity and crop yield potential, and to develop appropriate strategies to correct any negative effects (Kant et al., 2012). The research described in this thesis has a specific focus on understanding the response to rising [CO_2], written henceforth as e[CO_2], in functionally different plant groups, represented by C_3 and C_4 plants.

Additionally, using the available secondary data, this study simulated the response of two major C_3 plants (rice and wheat) to e[CO_2] focusing on photosynthetic acclimation. Hence, this study would help to advance our understanding of the response to rising CO_2, particularly focusing on downregulation of photosynthesis, to enable developing strategies to mitigate the negative effects of climate change on agricultural production. The remainder of this chapter reviews the existing relevant literature.

1.1 Climate and agriculture

Agriculture in the 21st century has to meet the demands for increasing food production (FAOSTAT, 2009) under depleting water resources (Haddeland et al., 2014), increasing greenhouse gas emission and the alarming impact of climate change (Kulak et al., 2013). Globally, cereals including wheat, rice, barley, and maize, constitute the predominant food sources for the population (FAOSTAT, 2015). These cereals constitute the staple food of the majority, and consequently the primary calorie source for humans. As far as these crops are concerned, e[CO_2] in the atmosphere, by itself, has been found to be beneficial, because of the increase in the overall productivity, through increased photosynthesis. However, the combination of e[CO_2] with other climatic factors (for example, increased temperature) is shown to reduce both the yield and the quality of grains produced, including declines in protein, vitamins and mineral concentrations (Broberg et al., 2017; Fernando et al., 2015). These observations indicate the complex nature of the impact of various climatic elements on plant growth and productivity.

Globally, agricultural productivity is affected by a range of climate variables including precipitation, temperature and greenhouse gases (Brouder & Volenec, 2008). The current wave of changes in these critical climatic elements is attributed to various anthropogenic activities that generate greenhouse gases in excessive quantities (Nelson et al., 2014). Studies on the effects of atmospheric warming have produced varying results, with improved yields (O'Leary et al., 2015a; O’Leary et al., 2018), reduced yields (Ellis et al., 1995) and negligible effects (Lobell & Field, 2007; Pachauri et al., 2014). It is likely that geographic location and/or varying experimental conditions could explain part of the variation in outcomes of different studies. Hence, investigating the impact of climate change parameters that would directly affect agricultural production, under controlled experimental conditions, is needed. Such knowledge would help in developing strategies to mitigate the negative impact of climate change on agricultural productivity, in the future (Lobell & Field, 2007).

1.2 Carbon dioxide and agriculture

Climate change is driven by a variety of greenhouse gases. Table 1.1 presents the relative contribution by each of the gases known to affect the climate, along with their respective sources. Of these, the rising [CO_2] is documented as one of the crucial drivers to climate change, accounting for more than 70% of the global climate change (Solomon, 2007). Therefore, there has been greater attention on the impact of rising [CO_2] on the overall agricultural productivity (O'Leary et al., 2018; Roudier et al., 2011). Since the industrial revolution in 1859, there has been an exponential increase in fossil fuel burning as an energy source. This, together with the rapid deforestation has been identified as the causes of the steady increase in the a[CO_2] (IPCC, 2007; Qaderi & Reid, 2009). The concentration of a[CO_2] has risen from ~260 µmol CO_2 mol^{-1} approximately 150 years ago (Houghton et al., 2001) to the present level of 407 µmol CO_2 mol^{-1} (Ainsworth & Long, 2005). As shown in Figure 1.1 published fw the a[CO_2] has increased by about 25%, during 2012 -2017 period alone, and the trend is expected to continue (IPCC, 2014). The almost doubling of [CO_2], together with changes in other climatic elements, may have a significant effect on plant growth and development.

Concerns about the potentially drastic effects of climate change, resulting from increased atmospheric CO_2, rise in temperature and altered precipitation patterns have prompted global research to understand the plant responses to such climatic events. Such studies have provided valuable information on the impact of (e[CO_2]) on the growth and production properties in mixed populations of plants. However, there are suggestions that it is important to gain an understanding on how different plant functional groups respond to e[CO_2] which will shed light on the variation in response at a physiological level (Leakey et al., 2009; Long et al., 2004).

Table 1.1: Key drivers of greenhouse gas emissions and global shares of main sources. Source: EDGAR v5.0 for CO_2 (1970-2015); EDGAR v4.3.2 for CH_4 and N_2O (1970-2012); FT2017 all. (Jos & Jeroen, 2018)

Type of gas	**Share in GHG (%)**	**Source driver**	**Share in gas total (%)**
CO_2	73	Coal combustion	40
		Oil combustion	31
		Natural gas combustion	18
		Cement clink production	4
		Subtotal drivers of CO_2	92
CH_4	18	Cattle stock	21
		Rice production	10
		Natural gas production (including distribution)	13
		Oil production (including associated gas venting)	11
		Coal mining	11
		Landfill: municipal solid waste ~ food consumption	8
		Wastewater	8
		Subtotal drivers of CH_4	83
N_2O	6	Cattle stock (dropping on pasture, range, and paddock)*	21
		Synthetic fertilizers (N content)	18
		Animal manure applied to soils*	4
		Crops (share of N-fixing crops, crop residues, and histosols)	11
		Fossil fuel combustion	10
		Manure management (confined)	4
		Indirect: atmospheric deposition &leaching and run-off (NH_3)*	12
		Indirect: atmospheric deposition (NHx from fuel combustion)	7
		Subtotal drivers of N_2O, incl. other related drivers (*)	87
F-gases	3	HFC use (emission in CO_2 eq)	65
		HFC-23 from HCFC-22 production	19
		SF6 use	14
		PFC use and by-product (emission in CO_2 eq)	2
		Subtotal drivers of F-gases	100

1.3 Plant functional type

To overcome the problems arising from the vastly diverse array of plant types, Duckworth et al (2000) have recommended that it would be more convenient to classify them into smaller logical categories when modelling the response of plants to atmospheric changes. One such categorizing is on a functional basis, such as the plant's photosynthesis biochemistry or the physiology and biochemistry of plant nitrogen (N) assimilation. Classification of plants, by the plant functional type (PFT), is a system where different species that exhibit a similar response to a given abiotic condition and display analogous effects on the ecosystem, are grouped together (Díaz & Cabido, 1997). The PFT classification, based on function rather than on structure is a meaningful approach in studying plant responses to climate change. Information

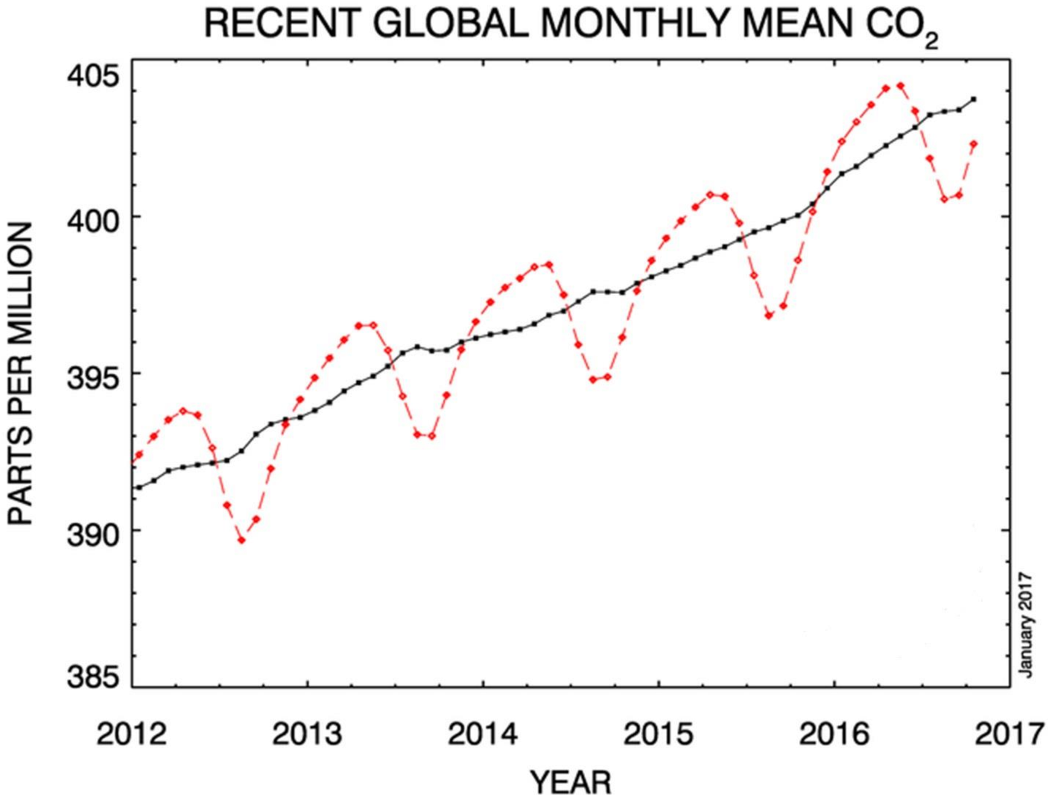

Figure 1.1: Monthly mean of CO_2 measured at Mauna Loa Observatory, Hawaii. The red dashed line represents the monthly mean values, centred on the middle of each month. The black line represents the same, after correction for the average seasonal cycle (NOAA, 2019).

obtained from PFT-based studies would provide a useful framework for modelling ecosystem response to the effects of climate change (Díaz & Cabido, 1997) and, therefore, be beneficial for the climate change research community (Liu & Cheng, 2011).

The PFT classification of plants may also be considered according to phylogenetic characteristics or life form characteristics or more specific properties (Pla et al., 2011). For instance, phylogenetic groups may include evolutionary development and diversification of species, life-form includes different morphological characters as a tree, shrubs, and herbs and more specific properties may include different photosynthetic pathways, plant size, leaf shape-size, seasonality, and root depth (Duckworth et al., 2000). Among these various PFTs, the research described in this thesis is on the plant classification based on two distinct photosynthetic biochemical pathways described as C_3 and C_4, with a specific focus on the two cereal crops: wheat and rice.

1.4 Photosynthetic pathways

Different plants use different mechanisms in fixing a[CO_2] to produce carbohydrate. These mechanisms belong to three distinct types of photosynthesis pathways: C_3 photosynthesis, C_4 photosynthesis and crassulacean acid metabolism (CAM). C_3 photosynthesis is the most common kind of photosynthetic pathway found in around 85% of all terrestrial plant species, including many varieties of cereals, vegetables, and fruit plants (Yamori et al., 2014). This mechanism produces 3-carbon product as the first product of photosynthesis (Liu & Cheng, 2011) whereas, in C_4 photosynthesis, 4-carbon product is the initial product. Only five per cent of plant species belong to the C_4 category (Yamori et al., 2014) which include advanced plant taxa including monocots, grasses, and sedges (Ehleringer & Cerling, 2002) such as sugarcane, maize, sorghum and millet. Plants that use the CAM photosynthesis pathway are rare (Ehleringer & Cerling, 2002), and include many epiphytes and succulents that grow in arid regions (Yamori et al., 2014). Table 1.2 provides a listing of some of the plant species, classified according to their photosynthetic pathway.

Table 1.2: List of C_3, C_4 and CAM species.

C_3 species	***Cereals:*** Rice, wheat, barley, rye, triticale, oat, teff, fonio, spelt ***Legumes:*** Dry bean, soybean, peanut, mung bean, faba bean, cowpea, common pea, chickpea, pigeon pea, lentil ***Fruits:*** Apple, banana, coconut, peach, cucumber, tomato, jackfruit, guava, lemon, mango ***Vegetables:*** Spinach, eggplants, potato, taro, yams, sweet potato, cassava, sugar beet, jackfruit, onion ***Oil crops:*** Sunflower, sesame, rapeseed, safflower ***Fibre crops:*** Cotton, jute, sisal ***Trees, shrubs and grasses:*** Lawn grasses like rye, fescue, kentucky bluegrass; evergreen trees and shrubs of the tropics, subtropics, the Mediterranean like English oak (*Quercus robur*), sycamore maple (*Acer pseudoplatanus*); temperate evergreen conifers like pine, deciduous trees, and shrubs of the temperate regions like European beech, weedy plants like water hyacinth, lambsquarters, bindweed, wild oats, eucalyptus; herbaceous plants like red campion (*Silene dioica*). All fern species (*Dryopteris affinis*), moss (*Sphagnum russowii*), conifer (*Pinus pinea*), clun mosses (*Huperzia phlegmaria*) and other non-flowering plants.
C_4 species	***Food crops:*** Maize, sorghum, sugarcane, millet ***Grasses:*** Crabgrass, amaranth, nutgrass, barnyard grass, four-winged saltbush, chenopods, elephant grass (*Miscanthus giganteus*), pampas grass (*Cortaderis selloana*), yellowtops (*Flaveria trinervia*)
CAM species	Cactus, euphorbia, pineapple, orchid, agave, spanish moss, some orchids, a family of Crassulacean members

Regardless of their photosynthetic pathways, plants of all species have the ability to sense any changes in the gaseous composition of the atmosphere (Hopkins & Huner, 2004). The photosynthetic organs including the guard cells of stomata and the mesophyll cells are very sensitive to changes in the a[CO_2] (Long et al., 2004). As a result, any change in a[CO_2] influences the rate of change in [CO_2] fixation which is directly correlated with the type of photosynthetic pathway (Yamori et al., 2014). The three photosynthetic pathways respond differently to changes in a[CO_2] (Ehleringer & Cerling, 2002) which is an important factor to note, in interpreting the findings from studies on climate impact on plants. In view of their abundance in the terrestrial ecosystem, research that involves C_3 and C_4 photosynthesis pathways would be more relevant to the understanding of plant productivity in a changing climate.

1.5 C_3 and C_4 Photosynthesis under e[CO_2]

The realisation that the a[CO_2] is increasing at an alarming rate has led to a greater research interest to study the effects of e[CO_2] on various agricultural crops. The major impact of e[CO_2] can be envisaged as being mainly on photosynthesis, which is the primary physiological process that drives plant growth and crop productivity, in addition to influencing many other plant processes (Yin & Struik, 2009). Research on the impact of e[CO_2] on plants, has been predominantly on C_3 species, due to both their abundance and agricultural importance. On the other hand, studies on C_4 have mostly involved advanced plant taxa, with a greater focus on monocots like grasses and sedges or on agriculturally important C_4 species like maize and sorghum (Ehleringer & Cerling, 2002).

In C_3 plants, CO_2 diffuses through stomata and the intercellular air spaces, eventually arriving at the chloroplast. In contrast, C_4 photosynthesis has a biochemical CO_2 concentrating mechanism that increases [CO_2], relative to the atmosphere, at the catalytic sites of RuBisCO in the bundle sheath (Yamori et al., 2014). Further description on the photosynthetic response including the mechanisms, is presented in section 2.6.1, under the photosynthetic response.

An increase in a[CO_2] stimulates net photosynthesis in plants with C_3 photosynthetic pathway, by increasing the [CO_2] gradient from the air to the leaf interior and by decreasing the photorespiration (Ainsworth & Long, 2005; Wang et al., 2012; Ziska et al., 1999). However, plants with the C_4 photosynthetic pathway possess an internal biochemical pump for concentrating CO_2 at the site of C_4 fixation and, hence, expect to show no response to rising a[CO_2] (George Bowes, 1996; Ghannoum et al., 2000).

1.6 Plant response to rising [CO_2]

Increase in a[CO_2] directly or indirectly affects the photosynthesis and stomatal conductance processes and, thus, the growth and development of plants (Seneweera &

Conroy, 2005). Plants sense and respond to rising [CO_2] through increased photosynthesis and decreased stomatal conductance (Ainsworth & Rogers, 2007; B. G. Drake et al., 1997).

1.6.1 Photosynthetic response

Growth and productivity of plants are directly driven by photosynthesis, which is a primary physiological process in plants (Yin & Struik, 2009). Many studies have documented that an increase in [CO_2] results in an increase in photosynthesis (B. G. Drake et al., 1997; Rosenthal & Tomeo, 2013) and, hence, increasing the overall crop productivity (Thompson, 2018). The photosynthetic rate of C_3 plants increases with an increase in [CO_2] (B. G. Drake et al., 1997; Ehleringer & Cerling, 2002; Yamori et al., 2014). When the [CO_2] is higher in the atmosphere, the ratio of CO_2 to O_2 increases at the site of carbon fixation. This increase in CO_2 to O_2 ratio stimulates the carboxylation efficiency of ribulose-1, 5-bisphosphate carboxylase-oxygenase (RuBisCO) increasing the overall photosynthesis (G Bowes, 1991) and at the same time suppress the photorespiration (Leakey et al., 2006). This has been well defined by the model of Farquhar et al. (1980). The model has proven the conserved properties of RuBisCO as a key to the photosynthetic response of C_3 crops to e[CO_2].

The RuBisCO is not CO_2 saturated at current a[CO_2]. However, C_3 plants are directly in contact with a[CO_2] via stomatal pores in the epidermis which directly connects the mesophyll cells containing RuBisCO with intercellular airspace. So, when the [CO_2] increases, RuBisCO tends to carboxylate and then increase the net photosynthesis (G Bowes, 1991; Seneweera & Conroy, 2005). Studies have demonstrated this response to an increased a[CO_2] in a variety of C_3 plants, including rice and wheat (Long et al., 2006).

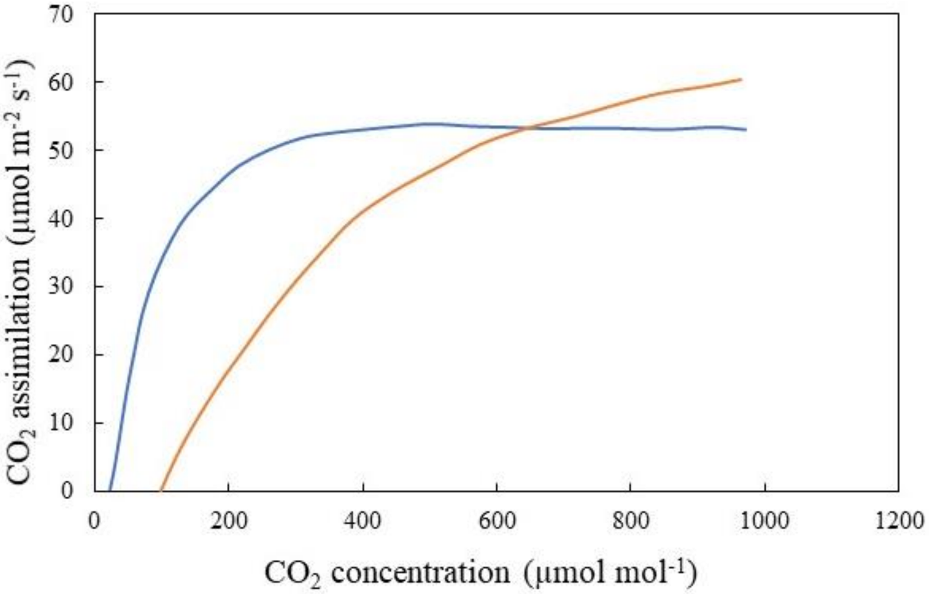

Figure 1.2: Changes in CO_2 assimilation for C_4 (blue colour) and C_3 (orange colour) plant species under different CO_2 concentrations (adapted from (Taiz & Zeiger, 2002).

The situation is different in C_4 plants as they are already saturated with CO_2 at the normal a[CO_2] (Yamori et al., 2014). In contrast to C_3, the RuBisCO in C_4 is saturated with CO_2 preventing further CO_2 uptake under e[CO_2] (G Bowes, 1991). Therefore, the response of C_4 plants to e[CO_2] is less predictable, compared to that of C_3 (Long et al., 2006). Nonetheless, there are reports of an increase in C_4 photosynthetic rates with an increase in a[CO_2] (Ainsworth & Long, 2005; S. Seneweera et al., 2001).

1.6.2 Photosynthesis acclimation

Increase in photosynthesis under e[CO_2] is a very well-established phenomenon. However, this increase does not always follow the same trend and stabilizes in the lower rate after extended exposure to e[CO_2] (Sage et al., 1989; S. Seneweera et al., 2011; Sharkey, 1985). This stabilization of the photosynthesis process on prolonged exposure to e[CO_2] is known as "photosynthesis acclimation" (G Bowes, 1991; Makino et al., 1983; Nowak et al., 2004; S. P. Seneweera et al., 2002). A number of mechanisms have been suggested to explain the photosynthetic acclimation, including suppression of nutrient supply (B. G. Drake et al., 1997; Nakano et al., 1997; S.

Seneweera et al., 2011); and increase in sink activities or the accumulation of non-structural carbohydrates (Nakano et al., 1997; Stitt & Krapp, 1999). Other explanations for the process of photosynthetic acclimation include lower N demand in leaves due to changes in N influx/efflux balance in growing tissues (S. Seneweera et al., 2011) and lower N in shoots due to suppression of NO_3^- photo-assimilation under e[CO_2] (Bloom et al., 2012). Thompson et al. (2017) in reviewing the findings in support of the various explanations suggest that photosynthetic acclimation is, likely, regulated by multiple processes each contributing to a different degree. Notably, differences have been observed between functional groups in the regulation of photosynthetic acclimation to e[CO_2]. In some functional groups, acclimation is driven by the suppression of synthesis of RuBisCO whereas in others, limited RuBP carboxylation regulate the photosynthetic acclimation to e[CO_2] (Chen et al., 2005).

1.6.3 Stomatal conductance

Stomatal conductance is related to the extent of stomatal aperture opening, a measure of CO_2 uptake and water loss through the stomata of a leaf (Pietragalla & Pask, 2012). The major role of stomata is to balance the photosynthetic CO_2 uptake against water loss from the leaves (Farquhar & Sharkey, 1982; Katul et al., 2003) and thus, stomatal conductance directly affects the photosynthetic productivity and water use efficiency of plants (P. L. Drake et al., 2013).

Several studies have reported a decline in stomatal conductance of plants under rising a[CO_2] (Ainsworth & Long, 2005; Ainsworth & Rogers, 2007; Farquhar & Sharkey, 1982; Medlyn et al., 2001). However, studies have reported the variable response of stomatal conductance under e[CO_2]. Some studies showed reduction ranging from 11 to 40% whereas in some no change was observed. Studies have even reported an increase in stomatal conductance under e[CO_2]. Medlyn et al. (2001) explained the reason behind the variability on the basis of the time of exposure to e[CO_2]. She elaborated that the sensitivity of plants towards e[CO_2] increases with an increase in the time of exposure. Further, the variability in the stomatal conductance is also driven by the ontogenetic stage stage and abiotic factors. According to Medlyn et al. (2001), the stomatal conductance of a leaf in its early growth stage is much higher than in a senescing leaf. Similarly, different abiotic factors including temperature, nutrient, water, and soil also play a vital role influencing stomatal conductance under e[CO_2] (Curtis & Wang, 1998; Maherali et al., 2003; Wullschleger et al., 2002).

1.6.4 Growth response to e[CO_2]

Plants response to e[CO_2] is highly variable. The stimulated growth of plants under e[CO_2] has been observed since 1890 which was verified again by different experiments showing an average of 33% increase in growth under e[CO_2]. The average plant growth was increases approximately by 26% across 400 experiments and the stimulation of growth was ranged as 58%, 35% and 41% for crop plnats, wild species and woody plants respectively (Poorter, 1993). However, all these growth responses to e[CO_2] directly depends on soil N effects, type of species, their developmental stage and period of exposure to [CO_2] (Cure & Acock, 1986; Poorter, 1993). Some of the studies have documented significant growth in biomass and shortening of the growth cycle particularly in wheat under e[CO_2] (S. Seneweera et al., 1994). Overall, the total above-ground biomass, leaf area, tiller number including relative growth rate, net assimilation rate, specific leaf area, and leaf area ratio are considered as crucial growth determinants under any environmental condition.

1.6.5 Wheat and rice response to [CO_2]

Both wheat and rice are widely cultivated cereal crops and important sources of carbohydrate as well as important grain protein for most of the human population. According to the Food and Agriculture Organisation (FAO), the world wheat and rice production forecast for 2019 is 766.4 million tonnes and 515 million tonnes, respectively. It is predicted that the human population will increase to 9.1 billion by 2050 and cereal production must be increased by 3 billion tonnes to feed the growing population (FAOSTAT, 2009). However, achieving the targets under changing climate including increasing [CO_2], high temperature and reduced rainfall has become a challenge.

Both wheat and rice fix CO_2 via C_3 photosynthetic pathway. Therefore, with increased CO_2 in the atmosphere, there is a parallel increase in photosynthesis in both the species, resulting in an increase in both the growth and grain yield (Tui and Roy, 2008; Gerstein and Otto, 2009). There are also reports of stimulation of tiller number and increase in above-ground biomass to be associated with increased grain yield at e[CO_2] in both wheat (Ziska et al., 2004) and rice (De Costa et al., 2007; Shimono et al., 2009). However, after an extended exposure to e[CO_2], the initial stimulation decreases, as a result of photosynthetic acclimation (Long et al., 2004; Seneweera et al., 2002, Seneweera et al., 2011). The mechanism of photosynthetic acclimation to e[CO_2] is different between wheat and rice. Both species acclimate to e[CO_2] but the physiological mechanism differs between them. As per S. P. Seneweera et al. (2002), in rice, photosynthesis was suppressed at e[CO_2] due to suppression of synthesis of RuBisCO whereas for wheat, photosynthesis acclimation was caused by limited RuBP regeneration (Zhang et al., 2009). However, reduction in both RuBisCO content and RuBP regeneration were also reported in rice (Chen et al., 2005).

1.7 Crop simulation

Improving field crop productivity by increasing resource (water and nutrient) use efficiency of crops is associated with crop photosynthetic efficiency (Long et al., 2015). Enhancing photosynthesis is directly linked with advancing crop biomass and yield (Long et al., 2015; Parry et al., 2010) as enhanced leaf photosynthesis leads to improved canopy photosynthesis (Wu et al., 2019). However, this connection between leaf-level photosynthesis and canopy crop performance is not straight forward as different factors including crop growth, development dynamics, and the dominant environment play a vital role. For instance, scaling up the leaf level photosynthetic efficiency to canopy level is difficult, because of the need to consider the canopy light interception effect (Wu et al., 2018). The relationship between carbon source and sink activity in the plant is yet another determinant of photosynthetic efficiency (Seneweera et al., 2002) and, therefore, needs consideration, when scaling up the effect to canopy level. The defoliation of plants dramatically increases the ratio of carbon sink to source activity increasing the photosynthetic efficiency at the whole canopy level which will decline during regrowth (Jeong et al., 2017; Rogers & Humphries, 2000). This change in photosynthetic efficiency due to the change in the relationship between carbon source and sink activity in the plant can also alter the occurrence of photosynthetic acclimation in plants under e[CO_2]. Rogers and Humphries (2000) found no acclimation after 89% defoliation of the canopy, which eventually increased under regrowth.

Most of the studies reported a change in photosynthesis efficiency or acclimation scenario at the leaf level (e.g. Seneweera et al., 2002). Either eighth leaf or flag leaf is used to represent different growth stages of crops before or after panicle initiation. There is a lack of connection to quantify the biochemical/leaf-level photosynthetic manipulation to the whole canopy level. Hence, there is a need to close this gap of knowledge between biochemical and crop levels which can be attempted through a modelling approach to help accelerate progress in photosynthetic enhancement for crop improvement. A simulation approach connecting these two levels along with relevant abiotic entities can represent the bigger picture addressing the gap. Simulation models can act as robust tools to extrapolate the changes in different parameters with changing climate and time, which would be impossible to test experimentally (O'Leary et al., 2015b). Simulation models have been used to model different crops under different [CO_2] to explore the resilience of crop production towards changes in [CO_2] and other abiotic factors (Amarasingha et al., 2015; Asseng et al., 2004; O'Leary et al., 2015b). A variety of simulation modelling approaches, ranging from simple to more complex, have been developed for climate change studies. With a view of providing predictions about food security status, addressing crop production in relation to climate, genotype, soil, and management factor, the Agricultural Production System Simulator (APSIM) was introduced (Keating et al., 2003).

Since canopy photosynthesis is a key driver of crop growth, it is a major consideration in crop modelling (El-Sharkawy, 2011; Wu et al., 2019; Wu et al., 2016). Crop models like APSIM and DSSAT (Decision Support System for Agrotechnology Transfer) Jones et al. (2003) utilize simple linear relationships between accumulated crop canopy biomass and radiation interception in modelling the canopy photosynthesis. On the other hand, the GECROS (Genotype by Environment Interaction on Crop Growth Simulator) crop model integrates photosynthesis of individual leaves in the canopy (Yin & Struik, 2009). These mechanical modelling approaches like in APSIM are useful. However, they lack biochemical approaches of photosynthesis in determining the canopy level responses (Wu et al., 2016). Some biochemical photosynthesis modelling approaches have been introduced (For example, by Farquhar et al. 1980).

Using them, Farquhar and von Caemmerer (1982) have successfully predicted responses of leaf photosynthesis which later have been subsequently upscaled to canopy level by De Pury and Farquhar (1997). However, these biochemical models lack the ability to combine the growth and development dynamics aspects of many crops (Wu et al., 2016). Hence, a cross-scale modelling approach connecting leaf-level photosynthesis to crop growth and development models via effective canopy modelling approaches is required (Wu et al., 2018; Wu et al., 2019).

Different modelling approaches have been used to gain insight into the biochemical process of photosynthesis. Yin and Struik (2009) pioneered a biochemical model of photosynthesis with GECROS crop model through upscaling to canopy photosynthesis with sunshade leaf modelling approach. Similarly, Zhu et al. (2004) and Long et al. (2006) also used canopy photosynthesis modelling approach developed through the upscaling of biochemical models to explore consequences of changing RuBisCO kinetic properties on daily canopy photosynthesis. However, these approaches were limited in their capacity to make daily predictions as the simulation model lacked the two-way connection between the biochemical models and crop growth and development dynamics.

1.8 Research Aims

There is a large volume of research evidence showing that plants, in their growth phase, if exposed to e[CO_2], exhibit changes in their growth properties leading to quantitative changes in biomass. The aim of this study was to understand the physiological and/or the biochemical basis of such a response to increased [CO_2], using C_3 and C_4 functionally distinct plants followed by an analysis of the specific features of C_3 crops of agricultural importance (rice and wheat), in greater detail.

A supplementary study, using a set of available secondary data, aims to model the species-specific differences in response to e[CO_2] using a crop simulation model. Hence, the two studies together should help to advance our understanding of the response to rising CO_2 by plants of different functional groups, using different simulation techniques and tools, with the following objectives.

1. To explore the extent of physiological changes in plants representing two functionally different groups (C_3 and C_4) under e[CO_2] using a meta-analysis approach (Chapter 2).

   Almost all the studies that have investigated the effect of e[CO_2] on photosynthesis and stomatal conductance, to date, have focused either on a particular functional group or on individual species. Apart from the meta-analysis by Ainsworth et al. (2007), none of the studies to my knowledge has explored the photosynthesis and stomatal conductance response to e[CO_2] under different abiotic factors (water, temperature, and N). Therefore, exploring the extent of physiological changes in plants belonging to different functional groups (C_3 and C_4) under e[CO_2] would be useful, for resolving the existing knowledge gap on the subject.

2. To analyse and model the photosynthetic acclimation in C_3 rice based on the changes in RuBisCO and leaf N content (Chapter 3).

   While it is established that photosynthesis stabilizes after extended exposure to e[CO_2], indicating photosynthetic acclimation, little is known about the mechanisms driving the photosynthetic acclimation process in different crops. This study focuses on the linkage between RuBisCO and N as crucial factors driving the process of photosynthetic acclimation. Simulating the response of photosynthesis, RuBisCO, and radiation use efficiency using an analytical modelling approach should assist in a closer investigation of the photosynthetic acclimation process in rice, to e[CO_2].

3. To explore and model photosynthetic acclimation response of wheat to [CO_2] under field experimental conditions (Chapter 4).

   It is proposed that prolonged exposure to e[CO_2] leads to photosynthetic acclimation, which could lower the potential biomass and yield of plants. However, the photosynthetic acclimatary response to e[CO_2] depends on the growth stage and the genetic make-up of the crop. Therefore, this study focuses on the photosynthetic acclimation of wheat at the crop level in field production systems which then simulate the acclimation pattern under e[CO_2] that is likely to develop as the climate warms.

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Chapter 2: Photosynthesis and stomatal conductance of C_3 and C_4 plants in response to elevated carbon dioxide under different abiotic stresses: A reassessment using meta-analysis

Abstract

An increase in atmospheric carbon dioxide concentration (a[CO_2]) affects plant growth and development through alterations in physiological processes, including photosynthesis and stomatal conductance. This meta-analysis summarizes the recent (2007 to 2019) literature on how two major physiological processes of the C_3 and C_4 functional groups of plants respond to changing [CO_2], with a further examination of the role of abiotic (water, temperature, and nitrogen (N) stresses in such responses. The review demonstrated that C_3 plants have a higher photosynthesis response to e[CO_2], with legumes being more sensitive but, both processes are not significantly affected in C_4 plants. The increase in photosynthesis among C_3 plants is a consequence of the relatively slower rate at a[CO_2] due to RuBisCO limitation, rather than RuBP limitation, promoting more carboxylation than photorespiration. When combined with abiotic stresses the impact differs depending on [CO_2], type of species, and degree of stress. Photosynthetic rate is more related to photosynthetic pathways than crop thermo-tolerance level. In the case of N, photosynthesis response under variable N rates is weak for legumes. A change of photosynthesis up to 35% can be found for grasses and non-legumes. Under water stress conditions, an increase in inter-cellular [CO_2] under e[CO_2] does not limit photosynthesis even under drought up to a certain level. However, a decrease in stomatal conductance is observed in all functional groups including C_4 under e[CO_2] and abiotic stresses.

2.1 Introduction

There is increasing evidence that climate change is with us already and, is likely to continue, challenging all forms of life on the planet (Pasqui & Guiseppe, 2019). All activities that support sustenance of life on earth, including food production, will be affected by climate variation, whether due to natural factors, such as changes of natural cycles of atmospheric and oceanic mechanisms or to anthropogenic activities, leading to increased greenhouse gas production. Among the different components of the climate, atmospheric carbon dioxide concentration (a[CO_2]) is projected to increase worldwide, impacting agriculture, in particular, because of its absolute dependence on the climate (O'Leary et al., 2018; Pachauri et al., 2014; Roudier et al., 2011). The atmospheric [CO_2] has risen from around 260 µmol CO_2 mol^{-1} approximately 150 years ago (Tans and Keeling, 2018) to the current level of around 407 µmol CO_2 mol^{-1} (NOAA, 2016) affecting overall plant growth, development, and yield of different species (Ainsworth et al., 2008; Seneweera & Conroy, 2005; Kant et al., 2012). A great deal is already known about how plants, in general, respond to changes in specific climatic elements. However, understanding how different plant species respond to e[CO_2] together with changes in other environmental factors, is crucial for developing adaptative strategies to climate change, particularly in the interest of maintaining optimum levels of crop production (Leakey et al., 2009).

The combined effect of e[CO_2] and abiotic stresses on photosynthesis and stomatal conductance response to e[CO_2] depends on the [CO_2] (Xu et al., 2013), plant species (Xu et al., 2013) and the severity of the abiotic stresses. Therefore, a fundamental understanding of the nature of the genetic and environmental factor interaction with CO_2 is essential to develop new adaptation strategies to climate change. There are arguments that due to the immense diversity of plant species in the ecosystem, restriction of high CO_2 research to studies on a few selected species would generate only limited information (Leakey et al., 2004; Rogers et al., 2009; Seneweera et al., 2005). This has generated a discussion on the relative merits of studies based on plant functional group diversity, instead of species diversity. Hence, categorizing them under small logical groups with species sharing morphological and physiological traits termed as a functional group of plants (Pokorny et al., 2005) would be more promising in efforts to understand the overall impact of abiotic stresses on plants. This study evaluated C_3 and C_4 as two major functional plant groups, based on their respective photosynthetic pathways.

In situations where there is a large volume of literature on a subject, emanating from studies that have employed varying experimental conditions, sampling and methodologies, with some extent of conflicting findings, a meta-analysis is a useful approach to combine and summarise the range of results and, to arrive at a consensus (White et al., 2011). A previous meta-analysis by Ainsworth (2007) documented the impact of e[CO_2] on the two major plant physiological processes i.e. photosynthesis and stomatal conductance based on the relevant data from the literature spanning the period from 1990-2007. However, a large volume of literature has accumulated since then on the subject, not only extending the scope of the research, but also there are studies involving innovative approaches, including modelling studies. Therefore, another meta-analysis was conducted, accumulating the most recently published data that document the response of diverse plant species to e[CO_2]. The analysis incorporated the literature on several plant groups including legumes, non-legumes, trees, shrubs, and grasses, of both C_3 and C_4 functional groups. The database that was created, was subsequently categorized according to different functional groups, where functionally similar species were grouped together, to predict their response to climatic change. The remainder of this chapter is organized as follows: data compilation, interpretation and analysis are outlined in Section 2 and then Sections 3 and 4 present and discuss the results, as well as suggestions for further research and conclusions. Specifically, this study aimed at:

- Assessing the response of photosynthesis and stomatal conductance of different plant functional groups to e[CO_2].
- Evaluating the response of photosynthesis and stomatal conductance of different plant functional groups to e[CO_2] under nutrient, water and temperature stressed condition.

2.2 Materials and methods

2.2.1 Database compilation

The present study is a meta-analysis of the relevant studies published from 2007 to 2019. A comprehensive literature search was conducted including publications during this period, that reported data on photosynthesis and stomatal conductance under e[CO_2]. In terms of the types of plants, the focus of the review was the two major functional groups i.e. C_3 and C_4. The broad C_3 and C_4 functional groups were classified into more specific groups including legumes, non-legumes, flowers, trees, shrubs and grasses.

The selection of publications was carried out by a search on the Google Scholar using keywords such as "elevated CO_2 impact on photosynthesis", "elevated CO_2 and stomatal conductance" "different functional groups under elevated CO_2", "C_3 and C_4 under elevated CO_2". The selection of publications was governed by the following inclusion criteria: limited to English language publications, from the beginning of 2007 to the end of 2019, studies with only C_3 or C_4 (excluding CAM plants) and, on experiments with photosynthesis and stomatal conductance measurements. Studies that generated data from all types of experiments (glasshouse, controlled environment chamber, open-top chamber, hydroponic, field, pot experiments and FACE) were included. The final database for the analysis comprised 180 studies.

Quantitative information on the responses to e[CO_2] from the selected literature was extracted and compiled into a database. The database included the name/s of the author/s, study location, provided facilities, month and year of the experiment, CO_2 concentration, temperature, species name with its cultivar as general information of the selected study. Also included were data on plant responses to various treatments such as N (low and high), water (drought and irrigated) and temperature (ambient and elevated) under e[CO_2] impacting the major variables of the study i.e. photosynthesis and stomatal conductance. The database covered studies conducted under different growth and environmental conditions including FACE (Free-Air CO_2 Enrichment), OTC (Open Top Chamber), ETC (Enclosed Top Chamber), CC (Controlled Chamber) and glasshouse facilities.

The average a[CO_2] was 400 µmol CO_2 mol^{-1} and the average e[CO_2] was 700 µmol CO_2 mol^{-1} in the reviewed primary literature. The a[CO_2] between 250 to 400 µmol CO_2 mol^{-1} was taken and was averaged as 400 µmol CO_2 mol-1. Similarly, e[CO_2] between 650 and 750 µmol CO_2 mol^{-1} was taken which was averaged as 700 µmol CO_2 mol^{-1}. Data that were extracted included the mean photosynthesis and stomatal conductance responses under elevated (x_e) and ambient (x_a) CO_2 concentrations. Data were extracted from the literature as numerical or graphical data. Online digitizing software "Web Plot Digitizer" (Rohatgi, 2018) was used to extract data from the graphs.

Experiments with higher than 800 µmol CO_2 mol^{-1} and lower than 300 µmol CO_2 mol^{-1} of CO_2 concentration, and experiemnts with O_3 were excluded for analysis and were identified as limitation of the study. However, these database were incorporated for discussion. Further, many reviwed literature have not mentioned about the previous crops and the amount of N supplied in the field during the previous cultivation which can effect the amount of N available in the field. Therefore, the database include only the amount of N that is supplied during the reviewed experiment which is also one of the limitations.

2.2.2 Data analysis and interpretation

The main consideration in the meta-analysis was the estimation of the treatment effect size including the magnitude of the response to an experimental treatment mean (x_e) to the control treatment mean (x_a) (Gurevitch et al., 2001). The effect of e[CO_2] was quantified by calculating the response ratio $R = x_e / x_a$. The response ratio was then transformed into a log response ratio because of potential non-normal distribution that is evident close to zero treatment mean (x_a), such as explained and used by Kimball (1983) in the first such meta-analysis of CO_2 effects on plants.

$$\ln(R) = \ln(R)$$

Unlike in the previous meta-analyses, where the effect size was weighted using the reciprocal of variance (Jablonski et al., 2002) or unweighted effect sizes (X. Wang, 2007), in the present analysis effect size was weighted by replication, using a function of the sample size ‘n’ for ambient (a) and elevated (e) CO_2 conditions.

$\text{Weight} = (n_a * n_e) / (n_a + n_e)$

The calculated values were analyzed using the statistical software METAWIN version 2.1 (Rosenberg et al., 2000) and graphs were prepared using GraphPad Prism (San Diego, CA, USA). Mean effect sizes with 95% bootstrapped confidence intervals (95% CI) were generated and reported as a percentage change. Treatment effect was considered significant if the 95% CI did not overlap zero.

2.3 Results

2.3.1 Overall response of photosynthesis to e[CO_2] for different plant functional groups

All plant species in the C_3 photosynthetic pathway functional group showed significant increases in the rate of photosynthesis under e[CO_2] (Figure 2.1). Among the different plant types, trees showed the largest positive response to e[CO_2], with a mean value around 50%, while the least (around 18%) stimulation of the photosynthesis rate by e[CO_2] was in legumes. The percentage increase in photosynthesis rate in non-legumes, shrubs and grasses were approximately 30%, 35%, and 40%, respectively, under e[CO_2]. However, in C_4 functional group plants, no significant change was evident in the rate of photosynthesis under e[CO_2] (Figure 2.1).

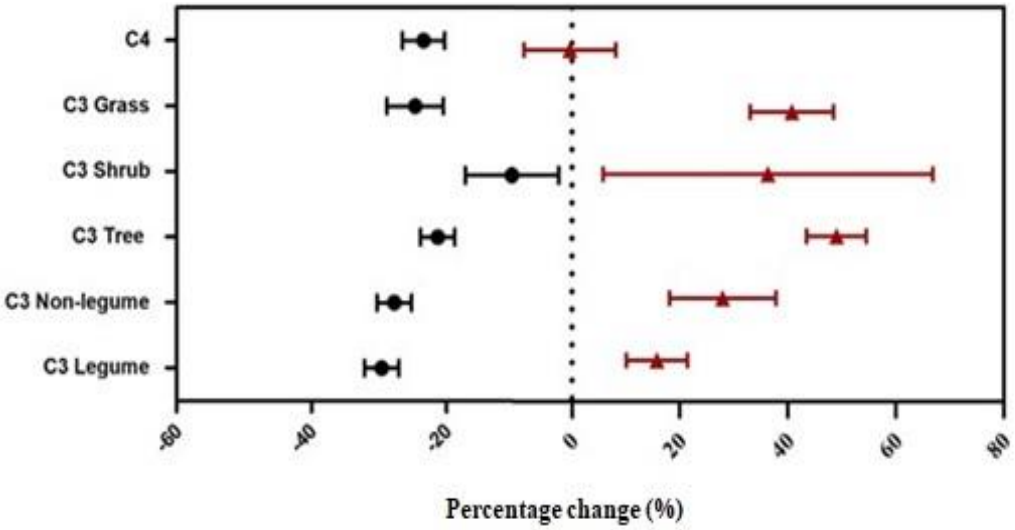

Figure 2.1. The response of photosynthesis (red triangle) and stomatal conductance (black circle) to elevated carbon dioxide concentration (e[CO_2]) for different plant functional groups. Average ambient and elevated [CO_2] for all studies are 400 and 700 µmol CO_2 mol^{-1}, respectively. The symbol represents the mean response ± 95% confidence interval.

2.3.2 Overall response of stomatal conductance to e[CO_2] for different plant functional groups

The data on stomatal conductance of the various C_3 and C_4 plant functional groups categories showed a significant negative response to e[CO_2] (Figure 2.1). The highest decrease (-30%) in the stomatal conductance due to e[CO_2] was observed in C_3 legumes while C_3 shrubs had the least negative response (-10%). Other functional categories including C_3 non-legumes, trees, and grasses also showed a 20 to 30% decrease in stomatal conductance. Despite the lack of response to e[CO_2] in photosynthesis, stomatal conductance of C_4 plants was reduced by 20 to 30%, on average, at e[CO_2].

2.3.3 Overall response of photosynthesis and stomatal conductance to e[CO_2] under water stress

As documented by Markelz et al. (2011), photosynthesis of different plant species in the C_3 category, except legumes, showed an increasing trend under water-stressed conditions (Figure 2.2 A). An increase in photosynthesis of around 50% over the base level under a[CO_2], was observed for C_3 non-legumes, grasses, and trees under e[CO_2]. The same species also showed an increase in photosynthesis rates under adequately-watered condition but, the increase was about 40 to 45% lower, compared to that in the water-stressed condition.

The stomatal conductance of all species in both C_3 and C_4 categories showed a decreasing trend under both water-stress and adequately-watered conditions under e[CO_2] (Figure 2.2 B). No major difference in stomatal conductance was observed in the crops grown under adequately-watered and water-stressed condition. Particularly, C_3 grasses and C_4 species showed no significant effect in stomatal conductance under e[CO_2] and water stress.

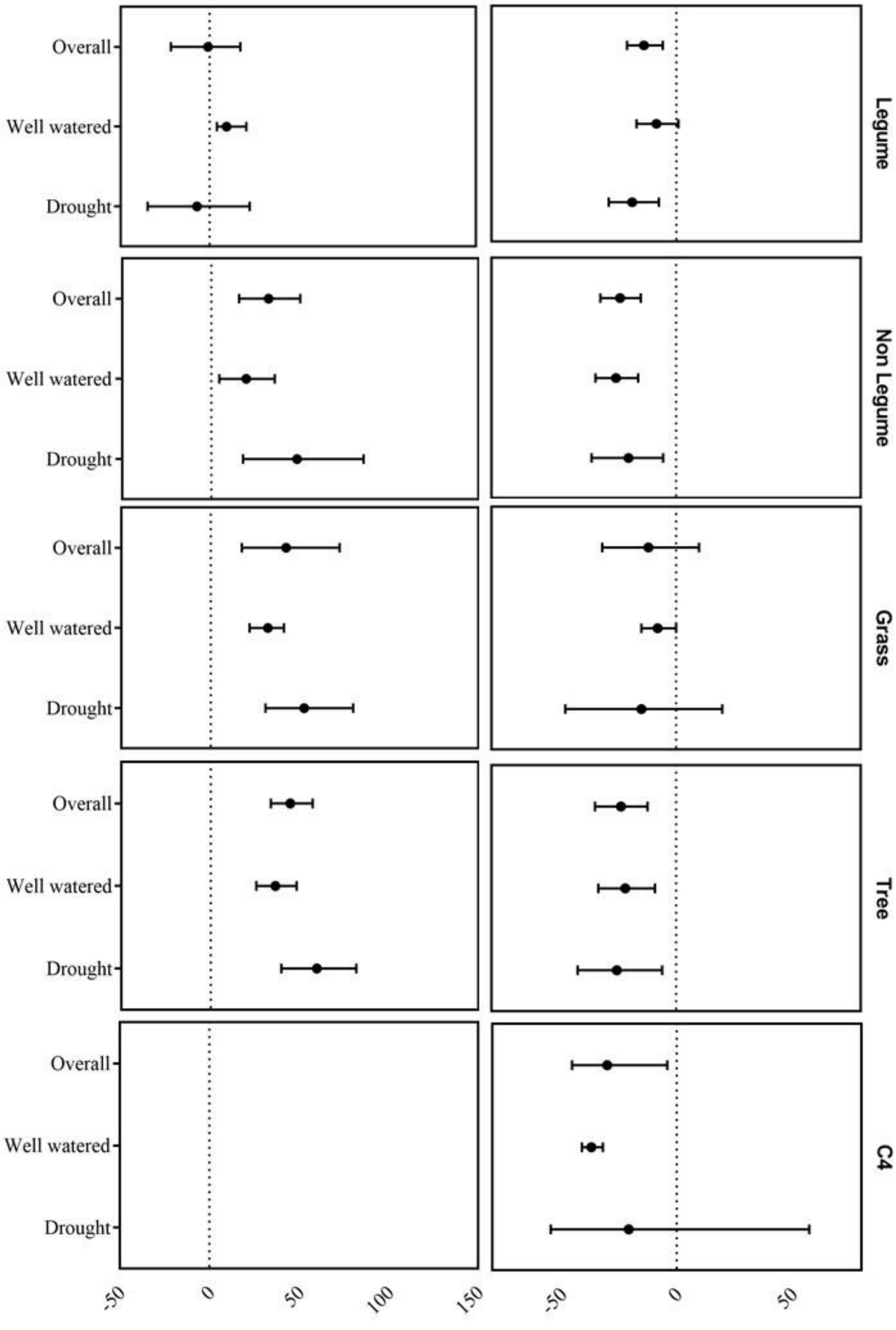

Figure 2.2. The response of photosynthesis (A) and stomatal conductance (B) to elevated carbon dioxide concentration (e[CO_2]) under drought and well-watered condition for different plant functional groups. Average ambient and elevated [CO_2] for all studies are 400 and 700 µmol CO_2 mol^{-1}, respectively. The symbol represents the mean response ± 95% confidence interval. (No data presented for C_4 in case of photosynthesis response.)

2.3.4 Overall response of photosynthesis and stomatal conductance to e[CO_2] under varying levels of N supply

Photosynthesis of plant species in the C_3 category showed an increasing photosynthetic rate under a combination of e[CO_2] and high N supply. Particularly, non-legumes showed a higher photosynthetic response to e[CO_2], compared to other plant types (Figure 2.3 A). In contrast, within the C_3 functional group, photosynthesis in legumes was least affected by e[CO_2] under both high or low N supply. Photosynthesis in C_4 crops was not significantly affected when exposed to e[CO_2] with an adequate supply of N (Figure 2.3 A). The stomatal conductance in all species of both C_3 and C_4, although showed a decreasing trend, was not significantly affected by either high or low N supply (Figure 2.3 B).

2.3.5 Overall response of photosynthesis and stomatal conductance to e[CO_2] under different temperatures

Among all plant types, photosynthesis rates were increased more prominently in trees than in others, at high temperature (Figure 2.4 A). The elevated temperature under e[CO_2] induced a 90% increase in photosynthesis rate in trees, while the increase was less than 50% at ambient temperature under e[CO_2]. However, there was a decrease in photosynthesis rate in C_4 plants at elevated temperature and e[CO_2] (Figure 2.4 A). The percentage change in photosynthesis at ambient temperature in the C_4 category was non-significant. For other plant types within the C_3 group, an increase in the photosynthetic rate of around 50 to 60% in legumes and non-legumes, respectively and, around 20% in grasses was observed both at elevated temperature and ambient temperature.

The stomatal conductance of C_3 legumes and non-legumes showed a higher reduction at ambient temperature than at elevated temperature under e[CO_2]. Despite the lack of change in the photosynthesis rate, C_4 crops showed a decrease in stomatal conductance similar to C_3 legumes and non-legumes (Figure 2.4 B).

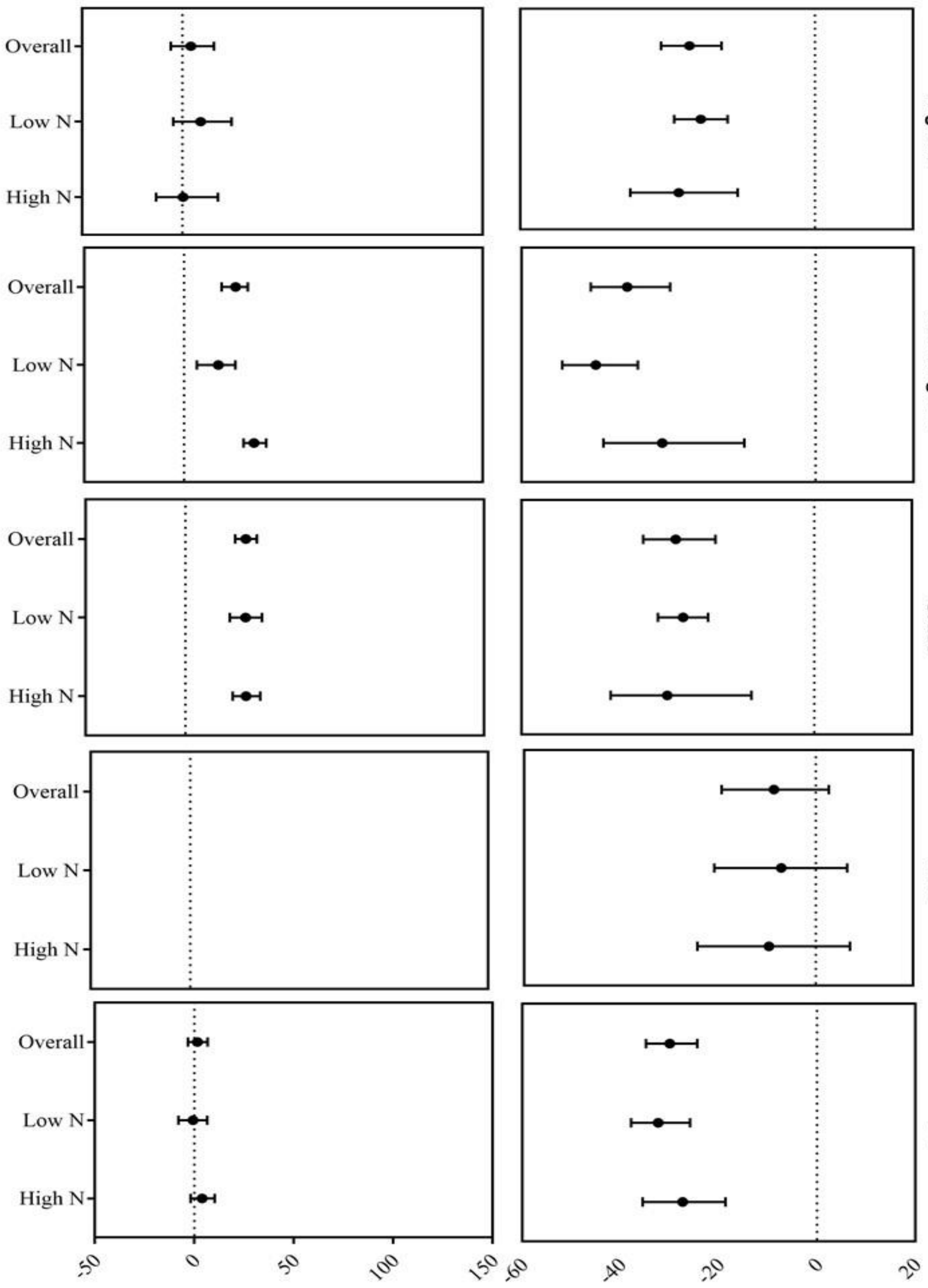

Figure 2.3. The response of photosynthesis (A) stomatal conductance (B) to elevated carbon dioxide concentration (e[CO_2]) under high and low concentration of N for different plant functional groups. Average ambient and elevated [CO_2] for all studies are 400 and 700 µmol CO_2 mol^{-1}, respectively. The symbol represents the mean response ± 95% confidence interval. (No data presented for Tree in case of photosynthesis response due to unavailability of enough data.)

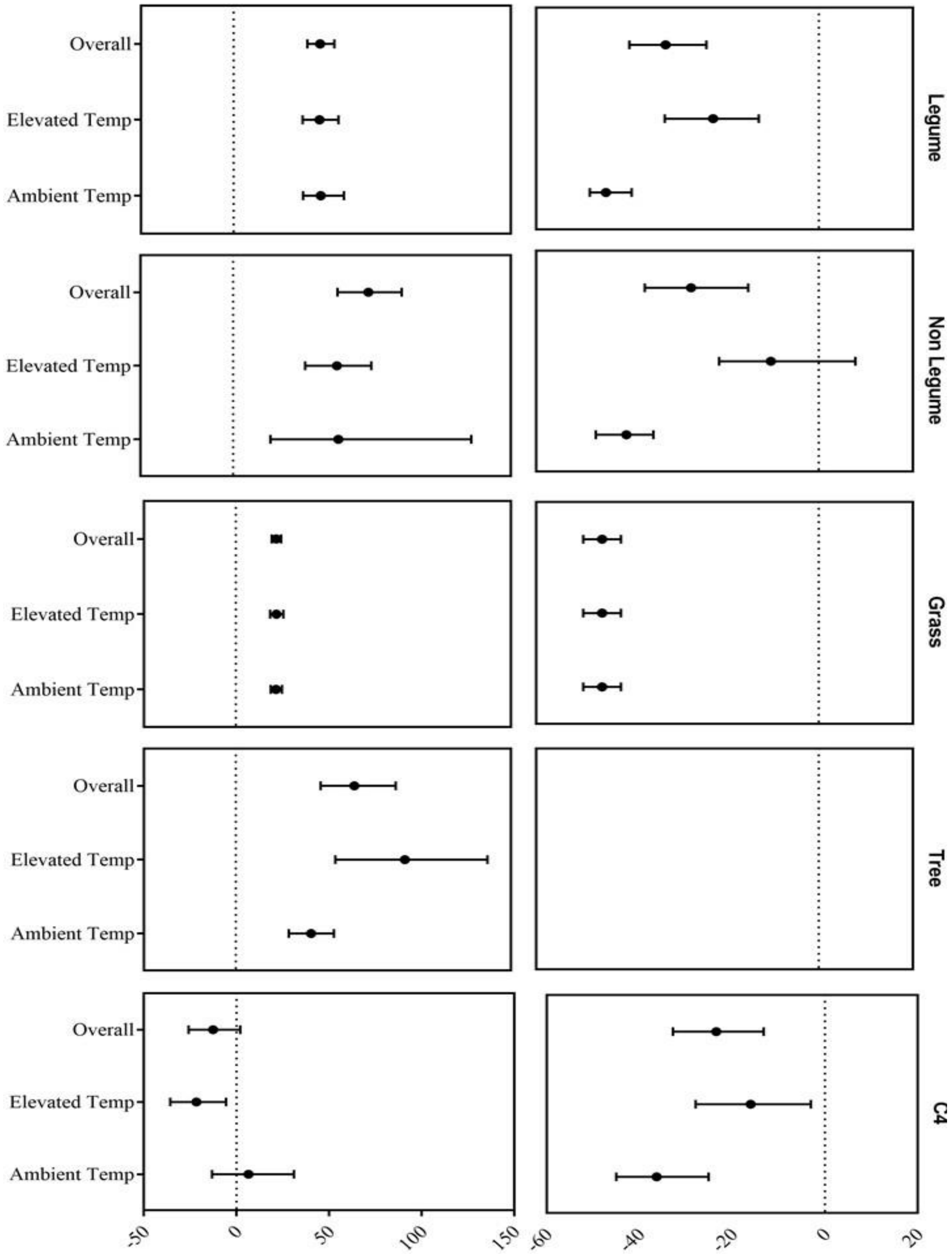

Figure 2.4. The response of photosynthesis (A) stomatal conductance (B) to elevated carbon dioxide concentration (e[CO_2]) under elevated and ambient temperatures for different plant functional groups. The average ambient and elevated [CO_2] for all studies are 400 and 700 µmol CO_2 mol^{-1}, respectively. The symbol represents the mean response ± 95% confidence interval. (No data presented for Tree in case of stomatal conductance response.)

2.4. Discussion

2.4.1 Overall

A finding that was common to most of the studies was that plants grown under e[CO_2] conditions, show increased growth resulting in increased biomass. The physiologic or biochemical basis of this overall effect is explained as the ability of plants to sense changes in [CO_2] in the environment and respond through increased photosynthesis and decreased stomatal conductance (Ainsworth & Rogers, 2007; Wang et al., 2012; Thilakarathne et al., 2013; Maseyk et al., 2018). This increased photosynthesis is consequent to the enhanced CO_2 fixation, under conditions of e[CO_2], which in turn enhances the rate of carboxylation reaction in RuBisCO, thereby increasing the rate of photosynthesis (Ainsworth & Rogers, 2007; Xu et al., 2013). On the other hand, the decrease in stomatal conductance is brought about by the reduction in water loss per unit of carbon gain under e[CO_2], with the net result of enhanced water use efficiency (Leakey et al., 2009; Swann et al., 2016). The changes in these physiologic phenomena could explain the changes in yield, biomass and overall productivity, that have been seen under experimental conditions of e[CO_2]. However, in the real world, e[CO_2] does not exert its effects in isolation but, in combination with several additional environmental factors (e.g. rainfall, plant nutrition and temperature), thereby having a collective influence on the overall growth and development of plants.

Photosynthesis is a RuBisCO-dependent process in all functional groups. Under normal atmospheric conditions the RuBisCO content is low and consequently, photosynthesis at a[CO_2], is a rather slow process (Ainsworth & Rogers, 2007; Lin et al., 2014). Increase in atmospheric [CO_2] leads to an enhancement of RuBisCO's carboxylation capacity, together with an increased CO_2 concentration at the site of CO_2 fixation, both phenomena contributing to an increase in the overall photosynthesis rate (Ainsworth & Rogers, 2007; Imai et al., 2007). However, the magnitude of the increase in photosynthesis, as a percentage of the base rate, is not the same for all species, even within the same functional group. Among different plant types, trees and grasses showed the highest photosynthesis stimulation under e[CO_2] (Ainsworth and Rogers, 2007). Variation in the kinetic properties and in the photosynthesis biochemistry of each RuBisCO may play a role in the variation in the response.

In C_4 plants, only a minor effect on photosynthesis is observed under e[CO_2], in most studies. This is, perhaps, due to the relatively higher basal rate of photosynthesis at a[CO_2] due to faster dicarboxylate cycle, around 10-20 times of that in C_3, in the bundle sheath cells. Furthermore, unlike C_3 plants, those of the C_4 group are near CO_2 saturation even under a[CO_2] (Reich et al., 2018; Sage, 2004). However, there are studies that showed deviations from this general trend. Some studies have documented none or little response to e[CO_2] (Taub, 2010; Ziska & Bunce, 1997), while others have demonstrated a significant response (Anderson et al., 2001; Wang et al., 2012). Despite the theoretical non-expectation, Ziska and Bunce (1997) have, indeed, demonstrated an increase in photosynthesis in C_4 plants under e[CO_2]. Such stimulation of photosynthesis in C_4 has been attributed to changes in plant-water dynamics (Leakey et al., 2009), C_3 like photosynthesis in immature C_4 leaves (Gowik and Wesrhoff, 2011) or lower stomatal conductance under e[CO_2] as a mechanism for conserving water resources and, promoting photosynthesis at later stages of plant growth (Taub, 2010; Ghannoum, 2009). As most of the C_4 plants are already CO_2 saturated, they avoid photorespiration (Sage and Kubien, 2012). This feature of C_4 plants enables them to perform better under e[CO_2], which is likely to give them a competitive advantage in a future CO_2 rich environment (Sage and Kubien, 2012).

An important revelation by some of the studies in the meta-analysis is that it is not just the concentration of CO_2 alone, which determines the overall change in photosynthesis and stomatal conductance. There are other environmental and abiotic factors that interact with the process. Among abiotic factors, water, nutrients, and temperature play a vital role in influencing the photosynthesis and stomatal conductance processes (Ainsworth & Rogers, 2007; Peñuelas et al., 2012; Ruiz-Vera et al., 2013). Water deficit (Xu et al., 2013; Zinta et al., 2014), high temperature (Xu et al., 2013; Zinta et al., 2014) and, insufficient nutrition (Adams et al., 2018; Seneweera et al., 2011) are considered critical stress factors controlling the plant growth response to e[CO_2]. Therefore, the magnitude of the plant response to e[CO_2] often depends on several other factors such as the temperature and the availability of other resources such as nutrients and water (Domec et al., 2016). This aspect is discussed in detail in the following sections:

2.4.2 Temperature

Environmental temperature is a critical variable, that is subject to change diurnally and seasonally and, expected to alter drastically, with climate change (Urban et al., 2017). It is one of the abiotic factors that have a regulatory effect on the plant growth process and hence, the overall productivity (Xu et al., 2016). Ambient temperature fluctuations affect several plant physiological processes, including photosynthesis, respiration and transpiration – a process regulated by the opening and closing of the stomata (Urban et al., 2017). As such, the impact of temperature on stomatal conductance indirectly influences the plant water use (Urban et al., 2017).

The meta-analysis showed a general consensus among studies, showing an increase in stimulation of photosynthesis with increasing temperature under e[CO_2] (Xu et al., 2016; Yamori et al., 2014). Extreme temperatures do affect some of the physiological processes of plants such as photorespiration and dark respiration (Dusenge et al., 2019; Walker et al., 2016). Although such stresses are experienced under a[CO_2], they are alleviated under e[CO_2] conditions (Long et al., 2004; Reddy et al., 2010). Therefore, e[CO_2] seems to moderate the adverse effects of high temperature resulting in enhanced net photosynthesis (Dusenge et al., 2019).

The meta-analysis also showed that most studies (for example, AbdElgawad et al., 2015) found an increase in stimulation of photosynthesis under e[CO_2] at higher temperatures, particularly in C_3 trees. However, two studies, in particular (Wang et al., 2008; Hamilton III et al., 2008) reported opposite effects in C_4 plants, under e[CO_2]. Therefore, the effect of temperature under e[CO_2] seems to be related to photosynthetic pathways, not to a crop's thermotolerance level.

In C_3 species, e[CO_2] typically increases the heat tolerance of photosynthesis, except for plants grown at supra-optimal growth temperature (Wang et al., 2008). However, the increasing photosynthetic rate in C_3 crops under e[CO_2], did not change, when the temperature was changed, especially in legumes, non-legumes, and grasses, indicating a thermal tolerance (Figure 4A). The relative benefit of e[CO_2] on photosynthesis thermo-tolerance was found to be significantly reduced in most of the C_3 species when grown at a supra-optimal pre-stress growth temperature (Wang et al., 2008). Other studies have also found a decrease in photosynthesis, above 35°C and little or slightly positive effects for plants grown between 25°C and 30°C (Hamilton III et al., 2008). Beyond 35-40°C, RuBisCO activity begins to reduce and thus reduce the photosynthetic rates in most of C_3 plants (Wang et al., 2008). But in C_4 plants, e[CO_2] frequently decreases photosynthesis at near-optimal growth temperature as well as at supra-optimal growth temperature (Hamilton III et al., 2008).

Moreover, some of the analysed studies have shown a negative relationship between temperature and stomatal conductance in some species, with increasing temperature resulting in decreased stomatal conductance. However, there are species, in which stomatal conductance increases with rising temperatures (Ameye et al., 2012). Hence, the response of stomatal conductance was different for different species under different temperatures (Ameye et al., 2012). For example, a decrease in stomatal conductance with rising temperature was reported in two oak species (Reynolds-Henne et al., 2010). An increase in stomatal conductance was reported in wheat, barley, and soybean (Bunce, 2000; Wilson & Bunce, 1997); no change in stomatal conductance was reported in two eucalyptus species (Ameye et al., 2012). Although e[CO_2] generally decreases stomatal conductance before and during heat stress, heat stress generally, either increases or has little effect on stomatal conductance (Hamilton III et al., 2008).

2.4.3 Nitrogen

Leaf N content has often been described as positively related to primary growth and productivity. Photosynthetic enzymes and pigments can account for up to 70% of the leaf N content (Imai et al., 2008). Increased leaf N usually supports increased photosynthetic capacity (Bassi et al., 2018). It has been documented that plants grown in e[CO_2] conditions showed a decrease in photosynthetic capacity, when the N supply was low (Gutiérrez et al., 2013). On the other hand, some non-legumes like wheat showed increased photosynthesis and stomatal conductance with increased N supply whereas other non-legume dicots showed reduced photosynthesis and stomatal conductance (Cabrera-Bosquet et al., 2009). Del Pozo et al. (2007), in their studies, found no change in photosynthesis and stomatal conductance under e[CO_2] and high N. However, the general consensus from the present meta-analysis pointed to an increase in photosynthesis particularly, in non-legumes and grasses, under e[CO_2] and high N.

Unlike non-legumes, most legumes, in the natural ecosystem have a reduced need for N fertilizer because of their symbiotic relationship with N-fixing diazotrophs (Adams et al., 2016). This symbiosis results in increased leaf nitrogen content which helps to directly mitigate leaf water loss (Adams et al., 2018). However, there is much variation among the reported results on photosynthesis and stomatal conductance responses in legumes, to changes in N supply under e[CO_2]. In legumes like soybean, both photosynthesis and stomatal conductance was unaffected (Moreira et al., 2015); in chickpea, photosynthesis increased but stomatal conductance decreased (Tak et al., 2010) and similarly for common beans, photosynthesis increased and stomatal conductance was invariant (Jifon & Wolfe, 2002). Studies have shown reduced leaf N content (Taub et al., 2008) and decreased stomatal conductance and transpiration under e[CO_2] (Li et al., 2017). But as legumes are N-fixing plants, the loss of leaf N is comparatively less (Ainsworth et al., 2004) which in turn favours soil water conservation by increasing water use efficiency more than photosynthesis (Adams et al., 2018; Gutiérrez et al., 2013). Hence, grain legumes appear to use N to increase water use efficiency more than to increase photosynthesis. Despite some of the variation, overall the findings lead to the conclusion that there is almost no effect of N fertilization, on the photosynthesis of legumes, consistent with the knowledge that legumes are less dependent on external N supply.

2.4.4 Water stress

Water stress or drought is a stage when the demand for water by a plant is not met. Drought is one of the main environmental factors limiting plant growth and the productivity of many crops (Nouman et al., 2018). However, plants are able to adapt to drought conditions either by shortening their growth cycle or by increasing their water uptake by augmenting root growth (Molnár et al., 2004). Markelz et al. (2011) have observed a significant reduction in stomatal conductance (up to 57%) and photosynthesis (up to 44%) during periods of soil drying (from near field capacity to near the permanent wilting point). But the scenario of decreasing photosynthesis and stomatal conductance under drought conditions might be different under e[CO_2].

Many studies have documented a decrease in plant transpiration rate under water stress (particularly under drought), with e[CO_2], which is an adaptive mechanism to ameliorate the adverse effects of drought stress (Ainsworth & Rogers, 2007; Kadam et al., 2014; Tausz ‐ Posch et al., 2013). Zhang et al. (2018) found that water stress leads to altered stomatal function to reduce water losses at e[CO_2]. Under e[CO_2], drought-induced inhibition of photosynthesis and, consequently of growth, yield, and net productivity have been reported (Leakey et al., 2009; Leakey et al., 2006) for C_3 non-legumes, C_3 grasses and C_3 trees. The drought-induced reduction in soil water or, soil drying, is generally slower in e[CO_2] treatments, particularly in the middle and bottom soil layers (van der Kooi et al., 2016). This slower drying at e[CO_2] is associated with a decrease in stomatal conductance due to the depolarization of the membrane potential of guard cells (Shelke et al., 2019) which lessens a plant's dependence on soil water (Markelz et al., 2011). Thus, it enables a plant at e[CO_2] to continue to photosynthesize and grow more days into drought cycle than plants at a[CO_2].

However, the impact of drought under e[CO_2] also depends on the stage of growth of the plant and the duration of drought. The meta-analysis shows almost no effect or a reduction in photosynthesis under drought (Figure 2.2). The impact of drought on photosynthesis or stomatal conductance is more pronounced when drought conditions occur for a prolonged period. Our analysis also showed a decrease in photosynthesis in legumes that were exposed to prolonged or severe drought condition.

It has been suggested that e[CO_2] might increase the tolerance to drought by lowering osmotic potential and thereby maintaining high plant water potential (Miranda-Apodaca et al., 2018). Plants grown in e[CO_2] may utilize less water, use it more efficiently and be able to tolerate drought better under some situations Nouman et al., 2018). Hence, soil water depletion in the root zone might occur at a low rate for plants growing under e[CO_2] (van der Kooi et al., 2016). According to Robredo et al. (2007), under conditions of adequate water supply, the relative effects of CO_2 enrichment on photosynthesis remained remarkably stable during the assay period. In contrast, under water shortage, photosynthesis was significantly higher under e[CO_2]compared to under ambient [$CO_{2]}$. However, over the extended assay period, the drought caused a dramatic reduction in photosynthesis.

Even during drought, plants grown under e[CO_2] exhibit reduced stomatal conductance, as a consequence of partial stomatal closure (Robredo et al., 2007) – an effect of the increased intercellular [CO_2]. It is well-known that stomatal conductance reduces with increasing [CO_2] and, also it is known that stomata close in response to decreasing soil moisture. Hence, it is not surprising that the plants grown under e[CO_2] in drying soil exhibit greater stomatal closure. Similarly, e[CO_2] lower the stomatal conductance resulting in reduced water use. When the soil water content is low due to drought, e[CO_2] tends to delay the reduction in stomatal conductance and photosynthesis, by increasing water use efficiency.

2.5 Conclusion

Despite some degree of variation in the findings evident in the large volume of literature, the overall consensus from the analysed studies is that there is an increase in photosynthesis and a decrease in stomatal conductance in response to e[CO_2], particularly in the C_3 functional group. However, the effect was not the same in C_4 plants. Even within the C_3 functional groups, different crop categories showed different percentages of change in photosynthesis and stomatal conductance under e[CO_2].

In the long run, the initial stimulation of photosynthesis under e[CO_2] often does not persist and begins to decline as photosynthesis acclimation sets-in (Ainsworth and Long, 2005; Warren et al., 2014). This acclimation of photosynthesis under e[CO_2] is also found to be affected by different abiotic stresses. The reduction in leaf N content has been documented as one of the major reasons for the downregulation of photosynthesis (Leakey et al., 2009; Yin et al., 2019). Similarly, under drought stress, studies have shown a substantial reduction in both the capacity of RuBP regeneration and the carboxylation efficiency of RuBisCO (Perdomo et al., 2017). Hence, the role of abiotic factors might be even more crucial under prolonged exposure to e[CO_2] that lead to photosynthetic acclimation. Therefore, the findings from this meta-analysis open up potential areas of research to understand the effects of e[CO_2] on plants of different functional groups, based on photosynthetic acclimation.

In view of the finding that abiotic stresses directly impact the photosynthetic response of different plant functional groups under e[CO_2], we propose some future studies to assess how these functional groups will react on prolonged exposure. From this study, we can connect the dots of photosynthetic acclimation in relation to different abiotic variables like N. Studies have documented the stabilization of photosynthesis in plant acclimation, as the [CO_2] increases. Further studies are needed to assess how different species (rice and wheat) react under different [CO_2] and if they are approaching photosynthetic acclimation. The studies reported in the next two chapters assess the photosynthetic acclimation to e[CO_2], focusing on C_3 crops where N availability appears to play a controlling factor.

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Chapter 3: Modelling photosynthetic acclimation to elevated carbon dioxide concentration in rice using key gas exchange and biochemical parameters

Abstract

The studies described in this chapter are founded on the hypothesis that photosynthetic acclimation to elevated carbon dioxide concentration (e[CO_2]) is directly associated with leaf RuBisCO content and N supply to the leaf blades. An analytical modelling framework, that applies leaf to canopy-level rice crop system using RuBisCO and N dynamics and crop growth processes, was developed by the application of a set of secondary data on rice from a growth chamber experiment. The rate of N uptake by the plants is one of the most important factors determining the extent of RuBisCO synthesis and, consequently, the overall process of photosynthetic acclimation in rice. The photosynthetic results strongly support an acclimation at both morphological and biochemical levels under different N supply levels, under e[CO_2]. The biochemical and gas exchange variation together with modeling data fully explain the ontogentic variation photosynthetic acclimation in rice.

3.1 Introduction

The increasing atmospheric CO_2 concentration (a[CO_2]) is a major determinant of photosynthesis of C_3 plants and thus final productivity (Bagley et al., 2015). Many studies, including those in the meta-analysis, presented in chapter II, have clearly demonstrated an enhancement of photosynthesis in C_3 crops under elevated e[CO_2] conditions (Drake et al., 1997; Kimball et al., 2002; Seneweera et al., 2002). However, the initial stimulation is known to diminish over time and, get stabilized at a rate lower than even the pre-stimulation level, indicating a down-regulation – a phenomenon described as acclimation of photosynthesis (Ainsworth et al., 2003; Pérez et al., 2011; Seneweera et al., 2002).

The underlying mechanism of photosynthesis acclimation is not well understood. The hypotheses put forward to explain this phenomenon include reduced N supply to the leaf blade (Bloom et al., 2010) or accelerated leaf senescence (Ludewig et al., 2000). The established positive correlation between photosynthesis and leaf N content in higher plants (Makino, 2011) and, in rice plants exposed to e[CO_2] for extended period of time (Seneweera et al., 2011) lend support to the N supply hypothesis. For example, more than 70% of the N is invested in the above-ground part is in the leaf blades (Imai et al., 2008). It must be, however, important to note that alternate hypotheses, such as downregulation have been proposed as a possible mechanism (Reviewed by Thompson et al., 2017).

Leaf N concentration decreases substantially, in many species of plants when grown at e[CO_2] for an extended period under various conditions. It has been suggested that lower leaf N concentration at e[CO_2] leads to altered source-sink balance, particularly, the inability to make new sink organs such as tillers, grains and leaves (Bloom et al., 2010; Taub and Wang 2008). There are several hypotheses to explain the reduction in leaf N concentration at e[CO_2], including dilution of leaf N due to increasing growth (Leakey et al., 2009) or inhibition of nitrogen uptake due to the closing of stomata under e[CO_2] (Ward et al., 2013). However, the knowledge about how plants acclimate to e[CO2] and, its impact on plant growth remains incomplete.

RuBisCO is the rate-limiting enzyme for photosynthesis at the current atmospheric level of CO_2 (Makino et al., 1994). The concentration of RuBisCO in leaf blades is determined by the balance between protein synthesis and degradation which varies with the growth stage of leaves (Imai et al., 2008, Seneweera et al., 2011). RuBisCO synthesis is rapid during leaf expansion but declines to a very low rate, after full expansion (Seneweera et al., 2002). It is hypothesised that N partitioning into the leaf blade is strongly related to RuBisCO synthesis and, thus, to maintain the photosynthetic rates of the leaf blades.

Despite the extensive literature that provides an insight to the short-term responses in photosynthesis to e[CO_2] under experimental conditions, much less is known about the response of crops in field conditions to prolonged exposure to e[CO_2]. Such unclear and unresolved knowledge may be advanced by systems thinking that allows extrapolating known responses to new untested conditions. A variety of mathematical models have been developed to investigate the impact of rising [CO_2] on crops which have improved our understanding of the crop's response to e[CO_2]. However, very few models have been developed to simulate the impact of e[CO_2] on the biochemical level of plants particularly focusing on photosynthesis acclimation using RuBisCO and N dynamics parameters. Therefore, an accurate model needs to be developed to better understand the acclimation process and subsequent growth at e[CO_2].

This chapter describes a study in which the dynamics of leaf N content, RuBisCO content and photosynthesis rates were used to model plant growth response to e[CO_2]. The studies explored the links between leaf N and RuBisCO on photosynthetic acclimation to e[CO_2] using the Structural Thinking, Experiential Learning Laboratory with Animation (STELLA) model (ISEE Systems, 2006). In the modelling process, overall photosynthetic acclimation under different [CO_2] with changing RuBisCO and N concentration was developed. Such acclimation, theoretically, can occur at any CO_2 concentration, but it is proposed to be largely driven by leaf RuBisCO and N relationship. The specific aims of the studies were,

- To assess the impact of photosynthesis acclimation to e[CO_2] in association with reduced RuBisCO content.
- To explore the changes in RuBisCO content considering the reduced N uptake by plant or N content in the soil.

3.2 Material and methods

3.2.1 Description of the data source

Data from a study by Seneweera et al. (2011) and Seneweera et al. (unpublished) which involved growing rice plants in a hydroponic experiment conducted in a controlled environment, were used and analysed. The details of the controlled environment are summarized in Table 3.1. The hydroponic solution used in the experiment was prepared according to Makino et al. (1988). The concentration of NH_4NO_3 in the basal solution was of 1 mM. Half of the respective plants were supplied with three fold concentration of nitrogen (3 mM NH_4NO_3) after the emergence of flag leaf. The basic plant and meteorological measurements from the experiment included photosynthesis, RuBisCO content, N flow including other major abiotic parameters like [CO_2], radiation, light interception, and radiation use efficiency (RUE). For calculating the photosynthesis and RuBisCO content, gas exchange measurement and other biochemical assays were carried out on fully expanded leaves of 70 to 80 day- old plants. Data collection and growth conditions are detailed in Seneweera et al. (2011).

Table 3.1: Details of controlled growth chamber settings (Seneweera et al., 2011).

Rice *(O. Sativa L. cv. Notohikari)*			
CO_2 concentration	Photoperiod	Temperature	Relative humidity
390/1000 µmol CO_2 mol^{-1}	14-hour	25/20°C day and night	60%

3.2.2 Model concept

The schematic diagram (Figure 3.1) summarises the expected changes in photosynthesis and other components in response to changing CO_2 concentration. Considering a baseline CO_2 level of 390 µmol CO_2 mol^{-1}, the study then compared the respective relative responses under e[CO_2] with different levels of N supply. Nine different processes are considered (light green box) showing the rates of change and resultant accumulation of critical mass variables. Apart from photorespiration, all other processes were upregulated as they grow under e[CO_2] with sufficient soil N supply (as indicated by red arrows). However, the rate of increment slows down (as indicated by blue arrows) even under e[CO_2] as the N supply was reduced.

As documented in the literature, the initial stimulation of photosynthetic rates under e[CO_2] is not maintained over a longer period (Seneweera et al., 2002; Seneweera et al., 2011; Sharkey et al., 1985). The downregulation of photosynthesis on exposure to e[CO_2] for an extended period, is attributed either to the available soil N or to the amount of N supplied to the plant. Figure 3.1 also illustrates the photosynthetic acclimation that occurs under reduced soil N supply. If the N supply to the growing leaf blades declines, the rate of photosynthesis will decline substantially, thereby initiating photosynthetic acclimation (Drake et al., 1997; Nakano et al., 1997; Seneweera et al., 2011). The unavailability of sufficient sinks to accommodate the increasing photo-assimilate under e[CO_2] could further exacerbate the process of photosynthetic acclimation (Ainsworth et al., 2008). Therefore, the CO_2 fertilization effect on plant growth will be progressively constrained by the N availability in the soil over time.

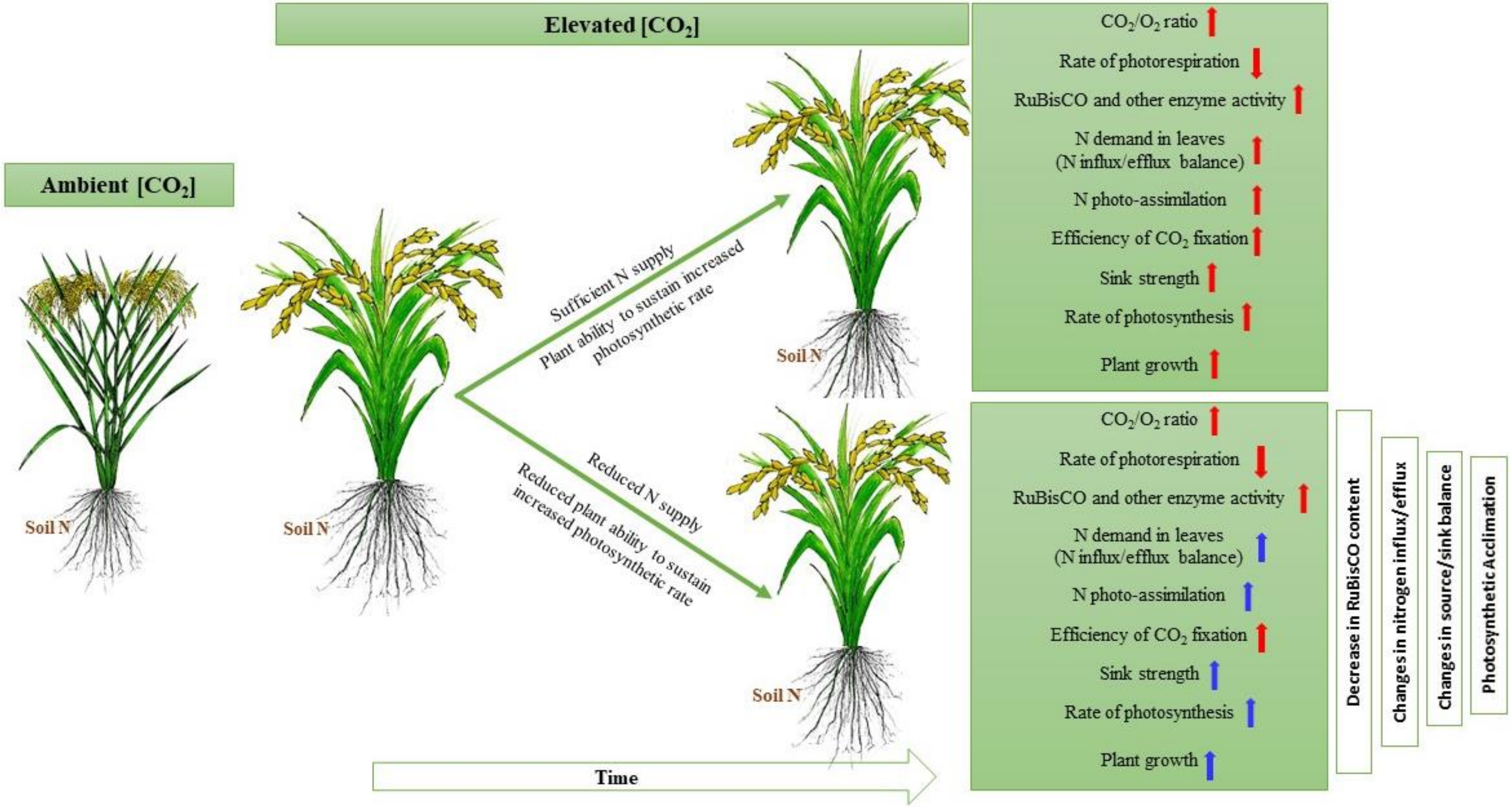

Figure 3.1: A schematic diagram showing the change in photosynthesis along with other different components as affected by changing [CO$_2$] and N supply. Red arrows indicate an increase (up) or a decrease (down) in the activity. Blue arrows indicate an increase in the activity under e[CO$_2$] but at lower rate with insufficient N supply and sink strength to accommodate increasing photo assimilates under e[CO$_2$] leading to photosynthetic acclimation.

3.2.3 Model system and structure

The diagrammatic representation created from a STELLA model during this research (Figure 3.2) includes all eight major parameters described in Figure 3.1 and hence represents the theoretical model. Further, the model includes different abiotic factors including CO_2, radiation, RUE, LI, N, and temperature directly affecting the different stages of crop growth. For simplicity, the radiation value was kept constant throughout the simulation.

The first step in the modelling process involved the development of basic structures to capture the overall process as described in the above equations. The model was then assigned with initial values for stocks as well as equations and input values for flows and converters. The flow, stocks, converters, and connectors link the overall flow in a loop which eventually delivers the change in required variables (for instance RuBisCO and N) with a change in [CO_2]. The diagrammatic representation created from a STELLA model (Figure 3.2) embeds dynamics of crop growth and development, leaf-level crop system including RuBisCO and N dynamics. The detail of the abbreviations used in the model including their units and input values are as listed in Table 3.2.

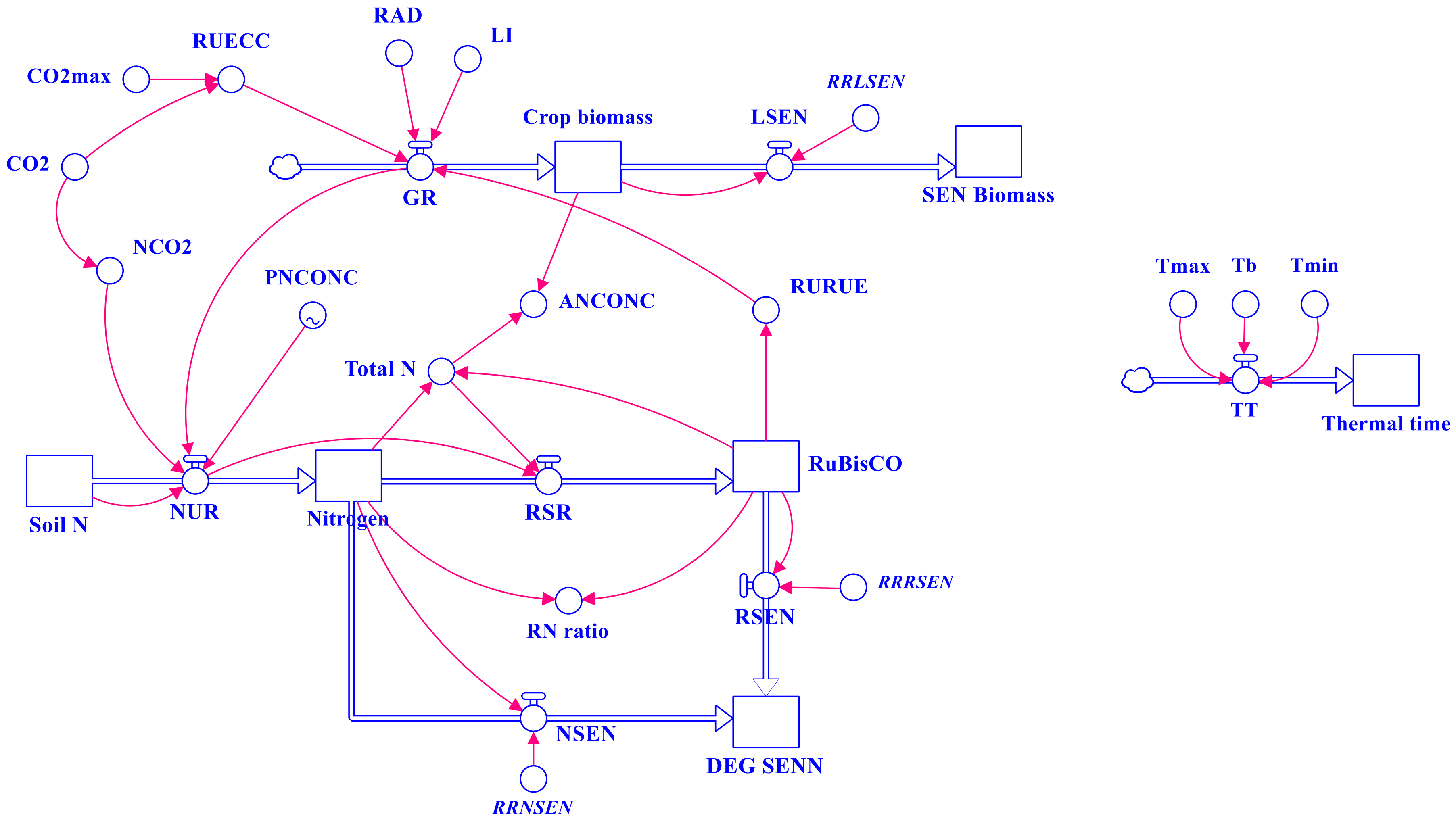

Figure 3.2: Diagrammatic representation of photosynthetic acclimation as driven by a change in RuBisCO, N and CO_2 concentration. Table 3.2 describes the State, Rate, Weather and Intermediate variables depicted by the various symbols in this daily-time step model.

3.2.4 Variables used in the model

State variables

The model consists of seven different variables which define the current state or a condition of those variables. Represented by the rectangular boxes in Figure 3.2, these include leaf biomass, senesced biomass, soil mineral N, plant N, RuBisCO N, senesced N and thermal time. Details of the state variables are explained with all abbreviations, explanations, units and formulas in Table 3.2.

Rate variables

The change in the state variables over time is calculated per daily time step from the seven rate variables (valve symbols in Figure 3.2). These are positive when the direction of the arrow flows into the state variable and negative when it flows away from the state variable. The defined rate variables are leaf growth rate, leaf senescence rate, mineral N uptake rate, RuBisCO synthesis rate, RuBisCO degradation rate, N efflux rate and daily thermal time. Details of the rate variables are explained further in Table 3.2.

Weather variables

The model also includes different weather variables which affect the overall system in many ways. The major weather variables incorporated in the model directly impacting the overall system includes CO_2 and temperature. For this non-water stressed study, only four weather variables (atmospheric CO_2 levels, maximum and minimum daily temperature and daily solar radiation) were considered.

Intermediate endogenous variables and parameters

Numerous intermediate endogenous variables (circles in Figure 3.2) provide a stable system with designed feedbacks and feed-forward processes. These define the variables such as light interception, growth response to CO_2, radiation use efficiency, thermal base temperatures and N concentration (Table 3.2).

Table 3.2: Model input parameters including acronyms, explanations, units, and values as per Seneweera et al., (2011) and theoretical calculations.

Description	Symbol	Units	Initial input values
STATE VARIABLES			
Leaf Biomass	Accumulated leaf biomass	g m^{-2}	159
SEN Biomass	Accumulated senescent biomass	g m^{-2}	
Soil N	Soil mineral N mass	G N m^{-2}	250
Nitrogen	Accumulated N mass	g N m^{-2}	
RuBisCO	Accumulated RuBisCO N mass	g N m^{-2}	
DEG SENN	Degraded senescence N mass	g N m^{-2}	
Thermal time	Accumulated thermal time	^{0}C days	
RATE VARIABLES			
GR	Crop growth rate	g m^{-2} day^{-1}	LI*RAD*RUECC*RURUE
LSEN	Leaf senescence rate	g m^{-2} day^{-1}	Leaf Biomass* RRLSEN
NUR	N uptake rate	g m^{-2} day^{-1}	NCO$_2$*PNCONC*GR*Soil N/150
RSR	RuBisCO synthesis rate	g g^{-1} day^{-1}	MIN (0.15*NUR, 0.15*Total N)
RSEN	RuBisCO senescence rate	g g^{-1} day^{-1}	RuBisCO * RRRSEN
NSEN	N senescence rate	g m^{-2} day^{-1}	N*RRNSEN
TT	Thermal time rate	^{0}C day^{-1}	[{(Tmax+Tmin)/2}-Tb]
WEATHER VARIABLES			
RAD	Daily radiation	MJ m^{-2}	25
CO$_2$	Atmospheric CO$_2$ concentration	μmol CO$_2$ mol^{-1}	350/450/550/650/750/850/1000
Tmin	Daily minimum temperature	^{0}C	20
Tmax	Daily maximum temperature	^{0}C	25
INTERMEDIATE VARIABLES			
RUECC	Radiation use efficiency as affected by CO$_2$	g MJ^{-1}	(-CO$_2$_max/(350*(1-CO$_2$_max)) *CO$_2$*CO$_2$_max) / (-CO$_2$_max/(350*(1-CO$_2$_max)) *CO$_2$ + CO$_2$_max)
RURUE	Radiation use efficiency as affected by RuBisCO	g MJ^{-1}	MIN (2, MAX (0.75, RuBisCO * 0.25))
NCO$_2$	N uptake rate as affected by CO$_2$	g m^{-2} day^{-1}	MAX (0.85, MIN (2.447086/CO$_2$^0.153630, 1))
RRRSEN	Relative rate of RuBisCO senescence	g m^{-2} day^{-1}	0.006
RRNSEN	Relative rate of N senescence	g m^{-2} day^{-1}	0.002
RRLSEN	Relative rate of leaf senescence	g m^{-2} day^{-1}	0.003
PNCONC	Potential N concentration	g g^{-1}	Graphical
ANCONC	Actual N concentration	g g^{-1}	Total N / Leaf Biomass
Total N	Accumulated total N mass in crop	g m^{-2}	N in plant + RuBisCO N
RNratio	RuBisCO to N ratio	g g^{-1}	RuBisCO/N
LI	Light interception	MJ m^{-2}	0.8
CO$_{2max}$	Maximum CO$_2$ growth response	(g m^{-2})/(g m^{-2})	1.2
Tb	Base temperature	^{0}C	8

3.3 Results

3.3.1 Photosynthesis at different developmental stages

The experimental photosynthesis rate measurements (Seneweera et al., unpublished) in the flag leaf from initiation to the senescence stage showed that the response was similar at different development stages, within each CO_2 concentration. Photosynthesis rate reached a peak of around 20 µmol m^{-2} s^{-1} at 12th to 15th day after sowing. However, from then, photosynthesis rate continued to decline until the senescence stage, to a stabilized rate of 5 µmol m^{-2} s^{-1}, which was lower than the initial rate (Figure 3.3.A). This decreasing trend showed that suppression of photosynthesis is much greater after the full expansion of the leaf blade. The comparison between the ambient and elevated CO_2 showed greater suppression of photosynthesis, under e[CO_2] than under a[CO_2] (Figure 3.3).

3.3.2 Change in RuBisCO content

The observed RuBisCO content increased under e[CO_2], during the first few days to reach a maximum of around 2.5 mg per leaf blade, which then declined to around 0.2 mg per leaf blade during senescence. The trend was similar at all developmental stages, from initiation to senescence of the flag leaf blade (Figure 3.3.B). Although there was an initial increase in RuBisCO during early stages of growth, even under a[CO_2], the peak content was comparatively lower (1.6 mg per leaf blade) than that under e[CO_2]. However, under a[CO_2] too, there was a decrease to reach 0.18 mg per leaf blade during senescence.

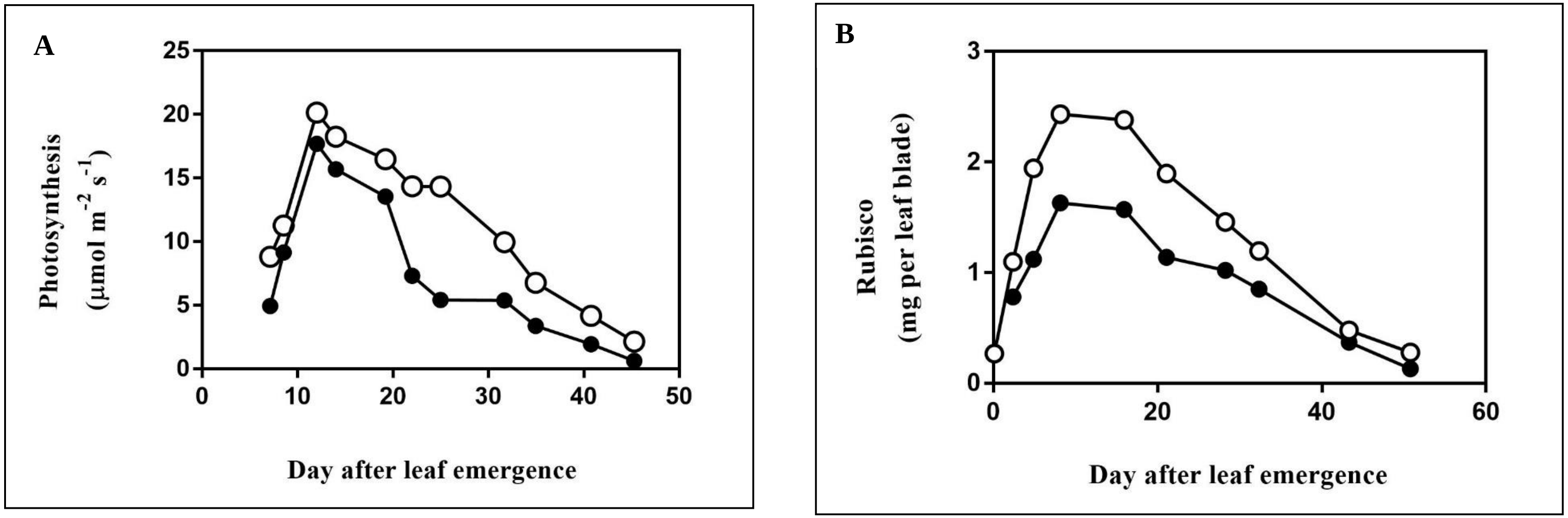

Figure 3.3: Observed response of (A) photosynthesis (µmol m^{-2} s^{-1}) and (B) RuBisCO content (mg per leaf blade) during flag leaf development of rice grown at either a[CO_2] of 390 µmol CO_2 mol^{-1} (open circle) or e[CO_2] of 1000 (closed circle) µmol CO_2 mol^{-1}. Values are means of four replicates for each CO_2 treatments (Seneweera et al., (unpublished)).

3.3.3 The relationship between photosynthesis and RuBisCO at e[CO_2]

Therefore, the noteworthy observation is the similarity in the pattern of changes in photosynthesis and RuBisCO from leaf emergence (Figure 3.3) of an initial rise reaching a peak value between the 10th and 15th day, followed by a fall under both a[CO_2] and e[CO_2]. Thus, as illustrated in Figure 3.3 there is a strong correlation between the photosynthesis and leaf RuBisCO content during leaf development. However, quantitively, there was greater suppression under e[CO_2] than under a[CO_2].

3.3.4 Simulation performance

Simulation performance - Photosynthesis

The simulated canopy photosynthesis using the STELLA model (Figure 3.4.A) showed an increase from around 12 g $m^{-2}day^{-1}$ to around 16.5 g $m^{-2}day^{-1}$ which then decreased to 14 g $m^{-2}day^{-1}$ towards the maturity of the plant under a[CO_2]. Similarly, under e[CO_2], the photosynthesis rate increased from around 13.5 g $m^{-2}day^{-1}$ to around 17.5 g $m^{-2}day^{-1}$ and then decreased to 14.5 g $m^{-2}day^{-1}$ towards maturity. This simulated canopy photosynthesis of rice showed a trend of the initially elevated photosynthesis rate declining towards the rate under a[CO_2], as the plant approaches maturity. As depicted in figure 3.4.A, the photosynthesis continues to decrease after around 50 days of leaf emergence heading towards photosynthetic acclimation.

Simulation performance – RuBisCO

The simulated RuBisCO content, after an initial increase, showed a decreasing trend after flowering, closely following the trend in photosynthesis (Figure 3.4.B). With an initial value of 1.1 g m^{-2}, the RuBisCO content increased to 2.4 g m^{-2} and 2.2 g m^{-2} under a[CO_2] and e[CO_2] respectively. This was followed by a decrease in RuBisCO content, similar to photosynthesis, to 2 g m^{-2} and 1.8 g m^{-2} under a[CO_2] and e[CO_2] respectively.

Simulated response – RUE

The radiation use efficiency (RUE) for the rice canopy was calculated as a direct linear function of RuBisCO (from Figure 3.4) with a minimum (0.75 g MJ^{-1}) and maximum (2.0 g MJ^{-1}) defined (Table 3.2). The simulated RUE via the RuBisCO control (Figure 3.4.C) also depicted the acclimation trend towards maturity showing an increase from 0.75 g MJ^{-1} to 1 g MJ^{-1} and 0.93 g MJ^{-1} respectively under a[CO_2] and e[CO_2] at around 50 days after leaf emergence. The value then decreased exponentially as the leaf grew towards senescence reaching 0.85 g MJ^{-1} and 0.8 g MJ^{-1} under a[CO_2] and e[CO_2] respectively.

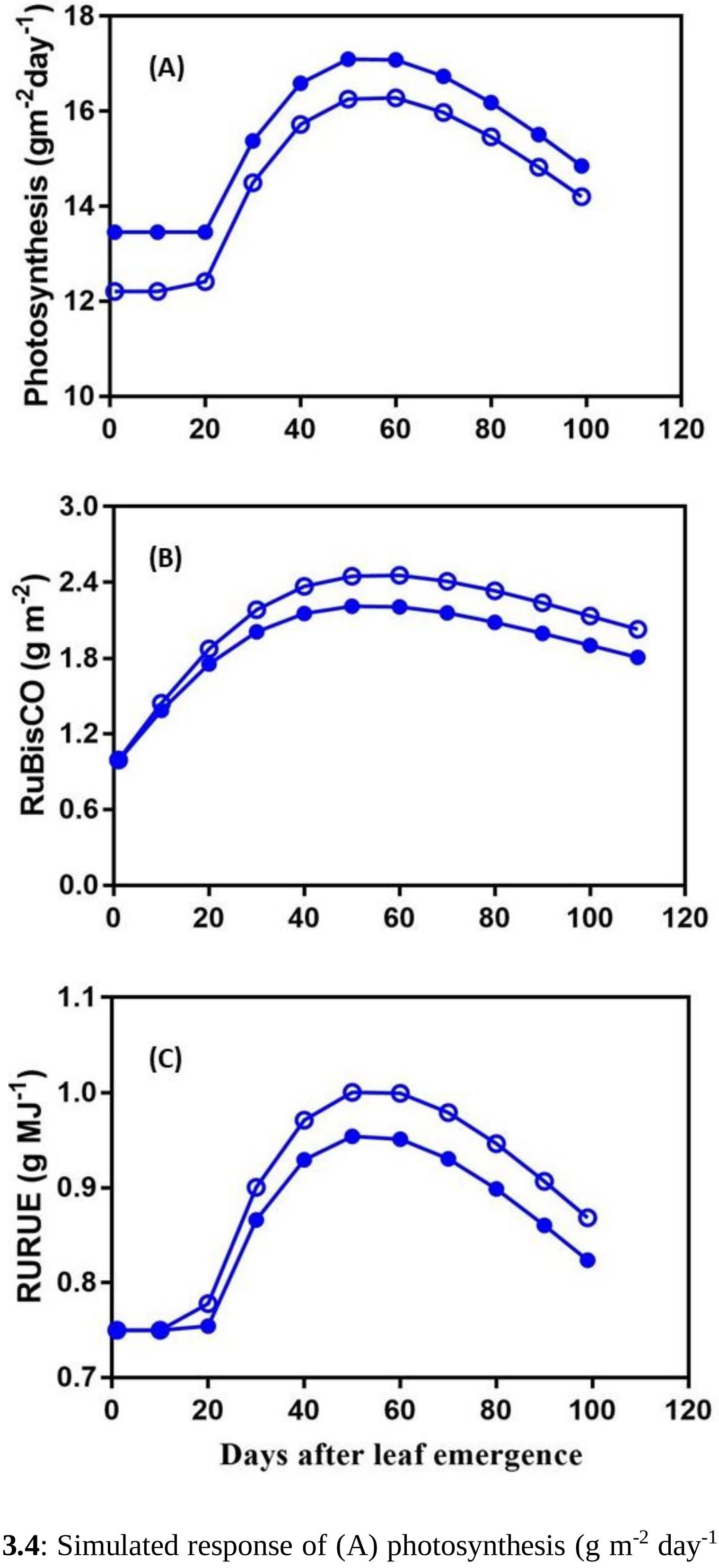

Figure 3.4: Simulated response of (A) photosynthesis (g m^{-2} day^{-1}), (B) RuBisCO (g m^{-2}) and (C) RUE as affected by RuBisCO (RURUE) (g MJ^{-1}) at a[CO_2] of 390 µmol CO_2 mol^{-1} (open circles) and e[CO_2] of 1000 µmol CO_2 mol^{-1} (closed circles).

3.3.5 Simulation performance under different [CO_2]

The leaf biomass, RuBisCO, and RUE via the RuBisCO control were simulated under different [CO_2] with an increase of 100 µmol CO_2 mol^{-1} instalments, starting from 350 µmol CO_2 mol^{-1} (Figure 3.5).

Simulated leaf biomass showed a similar trend to the theoretical framework in Figure 3.1. Comparison of modelled growth over different [CO_2] showed a linear increase from 350 to 700 µmol CO_2 mol^{-1} which stabilized thereafter. The biomass value reached a peak of 1.46 g m^{-2} at around 700 µmol CO_2 mol^{-1} which was 1.36 g m^{-2} at 350 µmol CO_2 mol^{-1}.

Similarly, simulated RuBisCO response under different [CO_2] demonstrated a similar trend as hypothesized in Figure 3.1 and as observed from experimental data in Figure 3.3.B. RuBisCO content continue to decrease from 1.96 g m^{-2} at 400 µmol CO_2 mol^{-1} to 1.73 g m^{-2} at 1000 µmol CO_2 mol^{-1}.

Further the simulated RUE response under different [CO_2] via the RuBisCO control also demonstrated a declining trend under e[CO_2]. After reaching a maximum value of 0.867 g MJ^{-1}, the RURUE then declined substantially with increasing [CO_2] maintaining its lowest value at 0.823 g MJ^{-1} at 1000 µmol CO_2 mol^{-1}.

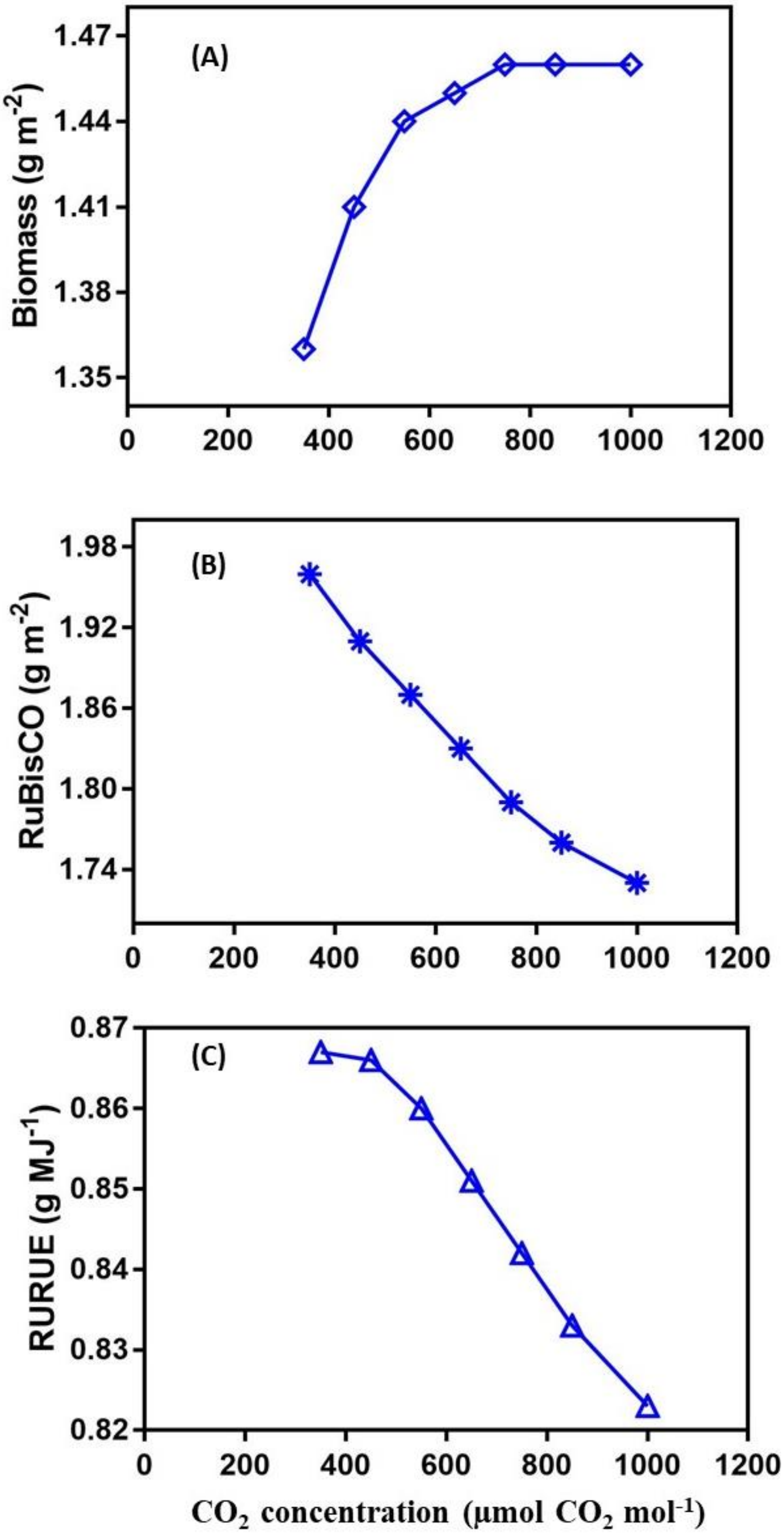

Figure 3.5: Simulated (A) leaf biomass (g m^{-2}), (B) RuBisCO (g m^{-2}), (C) RUE as affected by RuBisCO (RURUE) (g MJ^{-1}) response under different CO_2 concentration from 390 µmol CO_2 mol^{-1} to 1000 µmol CO_2 mol^{-1}.

3.4 Discussion

This study investigated the change in photosynthesis and RuBisCO under e[CO_2] in association with changes in leaf N, leading to photosynthetic acclimation in rice, through a conceptual modelling approach using State and Rate variable. The observed overall reduction in RuBisCO content, despite an initial increase, on exposure to e[CO_2] in the present study, under both experimental and simulated conditions is, indeed, a commonly observed feature associated with photosynthetic acclimation to e[CO_2] (Imai et al., 2008; Seneweera et al., 2011; Zhang et al., 2009). It has been suggested that the reduction in RuBisCO content is due to the combined effect of its accelerated degradation and the lower protein synthesis (Donnison et al., 2007; Makino and Sage, 2007; Suzuki et al., 2012). RuBisCO is not saturated at a[CO_2] as the concentration of CO_2 is very low at the site of fixation. Under e[CO_2] that limitation is overcome, leading to enhanced net photosynthesis in C_3 plants (Drake et al., 1997). These authors showed that photosynthesis in C_3 plants approximately doubles when exposed to e[CO_2] of around 700 µmol CO_2 mol^{-1}. However, the long term photosynthesis response to e[CO_2] is highly unpredictable, as it depends on several environmental conditions and abiotic factors, including nutrient availability.

Nitrogen is one of the critical nutrients for plant growth. A major proportion (~80%) of leaf N is allocated to chloroplast and most of the N in the chloroplast is invested in photosynthetic proteins, with a significant proportion (15-35%) in RuBisCO synthesis (Evans, 1989). The amount of RuBisCO in the leaf blade was linearly related to the total N in the leaf blade under both a[CO_2] and e[CO_2] (Seneweera et al., unpublish; Figure 3.6). These emerging data indicate a strong correlation ($R^2 = 0.97$) between leaf N content and RuBisCO content, confirming that the N availability is an important determinant of the RuBisCO content. Hence, this shows a strong evidence that the supply of N to total RuBisCO is the primary cause of acclimation in C_3 plants like rice.

Further, researchers have documented the relationship between photosynthesis, leaf N and RuBisCO where photosynthesis was linearly correlated with leaf N content under a wide range of conditions (Imai et al., 2008; Seneweera et al., 2011). On the other hand, the documented decrease in RuBisCO activity in C_3 crops under e[CO_2] perhaps indicates a reduced demand for N for synthesis of RuBisCO (Makino, 2003; Leakey et al., 2009). However, this relationship between leaf N and photosynthesis relationship substantially varies among plant species (Adams et al., 2016; Evans and Clarke, 2019). The reduction in RuBisCO content together with leaf N is generally associated with the photosynthetic acclimation response (Long et al., 2006; Yin et al., 2019). The present results also showed that in the rice leaf, there was a reduction in both the photosynthesis and RuBisCO after pre-anthesis stage (Figure 3.3) suggesting lower RuBisCO activity or lower RuBisCO concentration at e[CO_2].

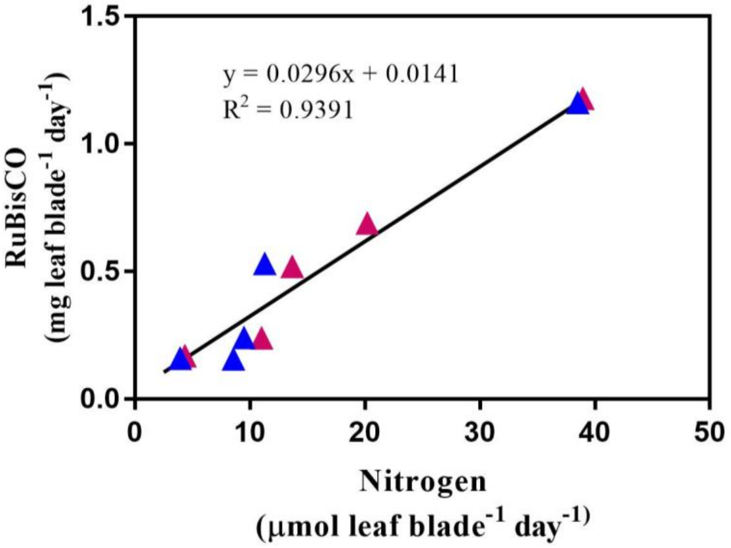

Figure 3.6: The observed relationship between total RuBisCO and total nitrogen in the flag leaf blades of rice at a[CO_2] of 390 µmol CO_2 mol^{-1} (blue triangles) and e[CO_2] of 1000 µmol CO_2 mol^{-1} (pink triangles) (Seneweera et al., (unpublished)).

The experimental evidence for changes in RuBisCO and photosynthesis in response to e[CO_2] was supported by the findings of the simulation study using the STELLA modelling approach. The crop model used in the study simulated the response under canopy level as canopy photosynthesis is a key driver of crop growth (Wu et al., 2016). Different crop models have incorporated the dynamics of canopy development using different modelling platforms like APSIM (Wu et al., 2018; Zheng et al., 2019), DSSAT (Bezuidenhout et al., 2003; Liu et al., 2011), and O'LEARY-CONNOR (O'Leary et al., 2015). However, the relationship between leaf level and canopy level photosynthesis is not always straightforward, as it is dependent on the effects of various environmental factors such as light interception, wind speed, and temperature, as well as other crop growth and developmental dynamics. By using a similar concept of up-scaling the leaf level photosynthetic response to canopy level by a single-layer sunshade leaf approach (de Pury and Farquhar, 1997), the STELLA conceptual model satisfactorily simulated the evolution of photosynthetic acclimation by considering RuBisCO and N content. The simulated response of both the photosynthesis and RuBisCO followed a similar trend as experimental observations, showing a decrease in both photosynthesis and RuBisCO as the leaf grows towards maturity.

Enhanced leaf photosynthesis would lead to improved canopy photosynthesis or *vice versa* (Wu et al., 2019). For extrapolating the photosynthesis from leaf level to canopy level, canopy approximations through Radiation Use Efficiency (RUE) are considered as acceptable in photosynthesis modelling (Asseng et al., 2019; Huang et al., 2016; Wu et al., 2016). Several researchers have suggested that improved leaf photosynthetic traits including RuBisCO content would contribute to higher RUE and consequently to higher biomass and yield (Huang et al., 2016; Mitchell and Sheehy, 2006; Zhang et al., 2009). However, there is little understanding of the photosynthetic acclimation to RUE under field conditions. Therefore, this study simulated the response of RUE from the day of leaf emergence and found a decreasing trend from flowering towards maturity, in parallel with the downregulation of photosynthesis and RuBisCO content. Therefore, the present results may be explained in terms of the improved photosynthesis up to pre-anthesis stage being responsible for the higher RUE initially and the subsequent decline to be associated with the decrease in photosynthesis. Despite the report by Huang et al. (2016) showing a higher photosynthetic rate in rice, even in the later stages of growth, the overwhelming evidence supports a decline leading to photosynthetic acclimation. This is supported further by the present finding of a high correlation between RUE value with both photosynthesis rate and RuBisCO content, indicating a close association among them.

The photosynthetic acclimation in rice depends on RUE and light intensity, particularly the enhancing or diminishing of the intensity of sunlight by the canopy (Murchie et al., 2002). Other factors that matter include the position of the leaf in the canopy, the canopy structure, and the maturity status of the leaves. Further, it can depend on the amounts of photosynthetic components and total leaf N. The supply and demand status of N can be one of the major drivers of the overall process towards or away from acclimation when considering the canopy response. During the pre-anthesis stage, biomass production is accelerated through increased tiller production and leaf area. This rapid growth requires a higher supply of N for protein synthesis with the demand under e[CO_2] being even higher (Seneweera et al., 2011; Bloom, 2015).

The increasing demand for N in the early growth phase is met by continuous N uptake and remobilization of N from lower leaves. In the later growth stages, as the mature leaves senesence, the protein in the senescing leaves is degraded enabling the remobilization and transportation of N to the young leaves for the synthesis of new RuBisCO (Seneweera et al., 2002). Thus, the leaves acting as a sink during the pre-anthesis stage now act as a source in the post-anthesis stage. In this latter stage, the panicles act as a major sink and other leaves including the flag leaves act as a major source. This change in source-sink demand and supply hence decreases the N uptake dramatically dropping down the overall RuBisCO synthesis limiting the rate of photosynthesis (Seneweera et al., 2002; Tegeder and Masclaux, 2018). The simulated results reflect the same trend of reduced RuBisCO and reduced photosynthesis along with the reduction of RUE via RuBisCO control towards the post-anthesis stage. This also explains the N uptake/content decreases as the leaf grows towards senescence promoting N efflux.

Further, our simulations studies under different [CO_2] also demonstrated the occurrence of photosynthetic acclimation after 550 µmol CO_2 mol^{-1} at the canopy scale (Figure 3.5). Following the observed photosynthetic acclimation phenomenon in lower leaf blades (Seneweera et al., 2011), some studies have explored the mechanism of photosynthetic acclimation to e[CO_2] using the whole canopy (Drake et al., 2016; Vico et al., 2019), They found that the phenomenon does occur at the canopy level too. A predominantly expressed explanation is that greater self-shading by larger leaf area prohibits the entry of light into the lower canopy and causes photosynthetic acclimation (Casal, 2013; Kurepin and Pheris, 2014; Mathur et al., 2018). Our simulated results also showed the exponential decrease in both RuBisCO and RUE after 550 µmol CO_2 mol^{-1} demonstrating photosynthetic acclimation response (Long et al., 2006; Murchie et al., 2002). Further, increased photosynthesis under e[CO_2] leads to an increase in biomass accumulation and subsequent changes in plant morphology and developmental traits (Masle 2000; Seneweera and Conroy, 2005). In agreement with such findings, our study clearly indicated an increase in biomass up to

550 µmol CO_2 mol^{-1} which then plateaus with higher [CO_2] suggesting the occurrence of photosynthetic acclimation. It has been suggested that photosynthesis acclimation may be modulated by environmental conditions and the developmental stage of the plant (Seneweera et al., 2002). However, there are also reports that when plants are exposed to 1000 µmol CO_2 mol^{-1}, acclimation of photosynthesis occur at all stages of leaf development (Baker and Aleen, 2005) indicating the key role of the amount of CO_2 to which plants are exposed in the induction of photosynthetic acclimation.

Temperature stresses can also influence the process of photosynthetic acclimation (Chapter II). Temperature-related photosynthesis acclimation is seen to follow a non-linear bell-shaped relationship, with photosynthesis rate being highest at intermediate temperature but, lower at higher and lower temperatures extremes (Yamori et al., 2005; Yamaguchi et al., 2019). Generally, optimum temperature increases the leaf mass per area leading to a higher N area (Yamaguchi et al., 2019). As a large fraction of leaf N is allocated to RuBisCO, the plant will then have a large amount of RuBisCO per leaf area leading to higher photosynthetic rates. However, both the higher and lower extremes of temperature decrease the rate of photosynthesis introducing photosynthesis acclimation leading to decreased RuBisCO content and $V_{c.max}$ (Yamaguchi et al., 2019). The STELLA model used here did not include acclimation patterns under increased temperatures. This can be investigated in future studies to explore other traits (e.g. root growth) and trait combinations (temperature and water stress) that are known to affect biomass partitioning, growth and yield.

3.5 Conclusion

A modelling approach for investigating RuBisCO and N dynamics under e[CO_2] and its impacts on growth and development in rice was developed using a conceptual State and Rate framework. The framework model presented in this study satisfactorily explains a model of rice leaf photosynthesis with growth, physiological changes and different abiotic drivers to the canopy scale. The results from the STELLA framework clearly showed a decrease in photosynthesis, RuBisCO content and RUE after the pre-anthesis stage as shown by the field data.

A similar type of modelling framework can be adapted to other C_3 crops such as wheat to explore the impacts e[CO_2] on crop plants. However, the parameters used in this framework would vary depending on experimental conditions and species. Such variability is assumed to be minor compared to the variability of the two parameters determining photosynthetic capacity: particularly, capturing maximum carboxylation capacity ($V_{c.max}$) and the electron transport capacity (J_{max}). Studies have reported the reduction in $V_{c.max}$ and J_{max} as common features of photosynthesis acclimation (Rogers and Humphries, 2000; Seneweera et al., 2002). However, this study did not consider the biochemical components ($V_{c.max}$ and J_{max}) and thus had a limitation in assessing the potential capacity for photosynthesis at a given developmental stage. In that regard, the next chapter (Chapter IV) examines these biochemical parameters, $V_{c.max}$ and J_{max}, to widen the study of photosynthetic acclimation in another important C_3 crop, wheat, using a different cross-scale modelling approach.

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Chapter 4: Modelling the wheat response to elevated carbon dioxide concentration incorporating parameters of photosynthetic acclimation under Free-Air CO_2 Enrichment (FACE)

Abstract

The extent and mechanisms of biomass and yield stimulation by e[CO_2] under various agricultural conditions (e.g. nitrogen fertilizer and water availability) has to be fully investigated to provide more realistic predictions of future food productivity. In this chapter, the extent of photosynthetic acclimation in wheat at the canopy level in field production systems under elevated carbon dioxide concentration e[CO_2] was assessed. The response of wheat growth and yield to e[CO_2] was modelled using two different versions of the APSIM -Wheat model. The first was the basic APSIM-Wheat model (V:7.10) and the second was a modified version ($APSIM_{DCP}$; that coupled a diurnal canopy photosynthesis-stomatal conductance model). Data obtained from the use of these models were compared with the published data from the Australian Grains Free-Air CO_2 Enrichment (AGFACE) experiment, which involved biochemical and morphological studies on wheat grown under different environmental conditions. Further, the genotypic variation of photosynthetic acclimation was also investigated by comparing five wheat cultivars. The two key input parameters modified in $APSIM_{DCP}$ were the maximum carboxylation rate of RuBisCO ($V_{c.max}$) and the electron transport capacity (J_{max}). Overall, the performance of $APSIM_{DCP}$ in simulating a wheat response to e[CO_2] (550 µmol CO_2 mol^{-1}) was satisfactory and better than that of the standard version of APSIM (version 7.10), namely for biomass at maturity and grain yield.

4.1 Introduction

Elevated carbon dioxide (e[CO_2]) alters the metabolic process of plants through increased photosynthetic rates and a reduction of stomatal conductance, which could result in higher biomass and grain yield (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007; Ahmed et al., 2019; Kruijt et al., 2008; Varga et al., 2015). However, it has also been shown that prolonged exposure of plants to e[CO_2] reverses the initial gain by a photosynthesis down-regulation, also known as photosynthetic acclimation. Lower N supply to the growing leaf blade and reduced RuBisCO content are associated with the photosynthetic acclimation in rice (Chapter III). Advancing field crop productivity can be achieved through improved crop photosynthetic efficiency, particularly fine-tuning the photosynthesis biochemistry (Long et al., 2015; Wu et al., 2018). Further, the extent and the mechanisms of biomass and yield stimulation by e[CO_2] under various agricultural conditions (e.g. adequate N fertilizer and water availability) are yet to be fully investigated to gain the necessary knowledge, to enable more accurate predictions of future global food production.

Process-based crop simulation models help to simplify the complex and dynamic soil-plant-environment inter-relationships. They are useful tools to represent important individual processes of crop growth and development (e.g. photosynthesis, respiration, phenology, assimilate partitioning, etc.) within the soil-plant-environment nexus, which would be practically impossible to test experimentally (O'Leary et al., 2015). Such models have been used to investigate crop behaviour under different climatic scenarios (Asseng et al., 2004; Asseng et al., 2013; Amarasingha et al., 2015; Christy et al., 2018). The connection between leaf-level photosynthetic enhancement under changing environmental conditions (i.e. e[CO_2]) and crop performance is not straight forward as it is influenced by genetic factors, leaf-level biochemical processes, crop growth and development dynamics, and environmental conditions. Integrating biochemical-based canopy photosynthesis into crop models could potentially help in addressing the existing knowledge gaps.

Mechanistic biochemical photosynthesis models; as introduced by de Pury and Farquhar (1997), Farquhar et al. (1980) and Farquhar and von Caemmerer (1982) have been incorporated into many vegetation growth models to explore the biological functionality of crop models (Humphries and Long, 1995; Long et al., 2006; Yin and van Laar, 2005; Zhu et al., 2004). Crop models that incorporate both source- and sink-limited crop growth provide an effective framework for examining the relationship of photosynthesis with crop growth, development and yield simulation (Wu et al., 2016; Wu et al., 2018). The Agricultural Production System Simulator (APSIM) crop model (Holzworth et al., 2014; Keating et al., 2003) is one such example.

APSIM is a farming systems simulation framework that has been designed to allow field- and farm-scale decision-making in the face of climatic risk, climate change or changes in policy (Holzworth et al., 2014; Keating et al., 2003; O'Leary et al., 2015). The APSIM crop models simulate crop growth, development, and yield on a daily time-line basis, using different crop management and biophysical modules, all driven by meteorological data (rainfall, solar radiation, maximum and minimum temperatures). The APSIM models have been extensively tested at the experimental crop and farm level under various environmental conditions (e.g. Carberry et al., 2013; Gaydon et al., 2017; Hochman et al., 2009; Holzworth et al., 2011; Zhang et al., 2012). The response of crop growth and resource use to e[CO_2] in APSIM is simulated through changes to radiation use efficiency (RUE), transpiration efficiency (TE) and leaf critical N concentration (CNC) for crop growth (Reyenga et al., 1999). The dynamic response of RUE to varying e[CO_2] is a non-linear relationship expressed by the ratio of light-limited photosynthetic response at the elevated CO_2 to that at 350 µmol CO_2 mol^{-1}. The responses of TE and leaf CNC to increased CO_2 are assumed to be linear models (Holzworth et al., 2014; Keating et al., 2003).

The capability of APSIM coupled to an hourly diurnal canopy photosynthesis simulation model (i.e. the diurnal canopy photosynthesis-stomatal conductance model) for simulating canopy CO_2 assimilation and biomass accumulation of wheat was tested by Wu et al. (2019) using a cross-scale modelling approach. Wu et al. (2019) simulated three photosynthetic manipulation targets related to leaf CO_2 capture and light-energy efficiencies [maximum carboxylation rate of RuBisCO ($V_{c.max}$), electron transport capacity (J_{max}) and mesophyll conductance (g_m) for CO_2] and the response of crop growth and yield to various levels of water availability in a typical Australian production system.

The cross-scale model (hereinafter referred to as $APSIM_{DCP}$) has been shown to represent the known responses of varying levels of $V_{c.max}$, J_{max} and g_m, and to predict satisfactorily, the responses of biomass and yield across a wide range of water and N treatments under ambient CO_2 (i.e. 370 µmol CO_2 mol^{-1}) compared to the standard version of APSIM (Wu et al., 2019), making it a preferable model for use in further studies of the biochemical photosynthetic changes and impacts on crop yield in field production systems, especially under e[CO_2]. Thus, the main objective of this study was to assess the extent of photosynthetic acclimation in wheat at the crop level against observed field crops in a semi-arid environment using Free-Air CO_2 Enrichment (FACE) experimental data. Specifically, this study aimed at:

- Parameterising of $APSIM_{DCP}$ with photosynthetic measurements obtained from the AGFACE experiment.
- Evaluating the performance of $APSIM_{DCP}$ in simulating the response of wheat growth and yield to e[CO_2].
- Assessing any photosynthetic acclimation pattern under e[CO_2] in wheat using $APSIM_{DCP}$.
- Exploring any genotypic variation of photosynthetic acclimation pattern under e[CO_2] among five wheat cultivars using $APSIM_{DCP}$.

4.2 Materials and methods

4.2.1 Australian Grain Free-Air CO_2 Enrichment (AGFACE) data

Growth and morphological data for wheat grown under different environmental conditions from the AGFACE experiment at Horsham, Australia (36°45'07", 142°06'52", 128 m above sea level) (Mollah et al., 2009; Fitzgerald et al. 2016) were used in this study. The FACE [CO_2] was maintained by injecting pure CO_2 into the air from the octagonal FACE ring (Mollah et al., 2009). Morphological and growth data included maximum tillering/initiation of stem elongation (DC: Decimal code; DC31), anthesis (DC65) and maturity (DC90) under ambient CO_2 (a[CO_2]; 365 µmol CO_2 mol^{-1}) and e[CO_2] (550 µmol CO_2 mol^{-1}) during the 2007-2009 period. These data were used for investigating the responses of wheat biomass and yield under a[CO_2] and e[CO_2] and for validating $APSIM_{DCP}$ in the AGFACE experimental conditions. Details of the AGFACE experimental settings is provided in O'Leary et al., (2015). Wheat was planted at two different sowing dates each year during the 2007-2009 period under different irrigation and N treatments (Tables 4.1 and 4.2). The agronomic design comprised a complete randomized block experimental design of four replicates. Data were extracted for wheat cultivar “Yitpi” grown during three consecutive cropping years (2007-2009).

For the biochemical analysis, gas exchange data measured in the AGFACE conducted in the year 2010 were used (Seneweera et al., (unpublished)). Leaf gas exchange measurements were carried out in AGFACE for seven different wheat cultivars, out of which data from five cultivars (H45, Hartog, Drysdale, and Silverstar and Yitpi) were used. The measurements were done at 365, 550 and 700 µmol CO_2 mol^{-1} deriving the linear slopes of photosynthesis versus intercellular [CO_2] (Ci) which were then used to estimate apparent maximum carboxylation efficiency of Ribulose 1.5-bisphosphate carboxylase/oxygenase (RuBisCO) ($V_{c.max}$) as described by Farquhar et al., 1980. The maximum electron transfer capacity (J_{max}) was estimated from the same A/Ci response measurements as described by von Caemmerer and Farquhar 1981. The Michaelis-Menten constant used for CO_2 and O_2 were K_c = 335 µbar and K_o = 304 mbar respectively, and the CO_2 compensation point, Γ^*= 31 µbar.

Table 4.1: Details of experimental settings including plant density, row spacing, sowing depth, sowing rate and surface residue type in the AGFACE experiment during the 2007-2009 period. TOS1 and TOS2 refer to times of sowing 1 and 2, respectively.

	TOS 1			**TOS 2**		
	2007	**2008**	**2009**	**2007**	**2008**	**2009**
Sowing date	18th June	4th June	23rd June	23rd August	5th August	19th August
Plant density (plant/m^2)	123	141	103	90	143	93
Row spacing (mm)	214	214	195	214	214	195
Sowing depth (mm)	30	30	50	30	30	50
Sowing rate (kg/ha)	75	75	75	75	75	75
Surface residue type	Millet	Canola	Canola	Millet	Canola	Canola

Table 4.2: Total amounts of irrigation and nitrogen (N) as applied in the AGFACE experiment during the 2007-2009 period. Different growth settings were involved: ambient [CO_2] (365 µmol CO_2 mol^{-1}) and elevated [CO_2] (550 µmol CO_2 mol^{-1}) (A/E), sowing dates (T1 and T2), with N (+N), without N (-N), with irrigation (+I), and without irrigation (-I). The detail of the amount of irrigation and N applied as per date is stated in a Supplementary Table 3.

Settings	**Irrigation (mm)**	**N (kg N/ha)**	**Irrigation (mm)**	**N (kg N/ha)**	**Irrigation (mm)**	**N (kg N/ha)**
	2007		**2008**		**2009**	
A/E_T1-N-I	48	0	10	0	0	0
A/E_T1+N-I	48	138	10	53	0	53
A/E_TI-N+I	96	0	40	0	70	0
A/E_T1+N+I	96	138	40	53	70	53
A/E_T2-N-I	48	0	25	0	0	0
A/E_T2+N-I	48	138	25	53	0	53
A/E_T2-N+I	96	0	80	0	60	0
A/E_T2+N+I	96	138	80	53	60	53

4.2.2 The APSIM$_{DCP}$ model

The APSIM$_{\mathrm{DCP}}$ model is a cross-scale model which connects the hourly diurnal canopy photosynthesis-stomatal conductance (DCP) module to the standard daily APSIM-wheat model (Wu et al., 2019). Canopy-level photosynthesis and transpiration are simulated within DCP using a single-layer sunlit and shade leaf modelling approach (Hammer and Wright, 1994; de Pury and Farquhar 1997). The APSIM$_{\mathrm{DCP}}$ modelling approach involves converting daily environmental variables (solar radiation, temperature and air vapour pressure deficit) to hourly values over the daylight period (from sunrise to sunset), summing hourly values to obtain daily canopy CO_2 assimilation, partitioning canopy leaf area (which is simulated using the crop model) into sunlit and shaded leaf fractions on the basis of solar geometry and canopy architecture, and calculating potential daily (24 h) biomass increment and transpiration demand for a crop under defined management practices (Wu et al. 2018; Wu et al., 2019). Daily canopy-leaf N status is predicted with the APSIM-wheat model and used to determine the photosynthetic physiology of the leaf fractions. For a given level of absorbed light, canopy leaf N status and plant water availability, canopy photosynthesis and transpiration are calculated for each leaf fraction on an hourly basis; and then integrated to the daily time step to drive subsequent canopy growth and changes in soil water. A comprehensive description and list of the DCP model equations and parameters can be found in Wu et al. 2018 and Wu et al., 2019.

4.2.3 Parameterisation of APSIM$_{DCP}$

The AGFACE database included leaf gas exchange and biochemical measurements at different crop stages under ambient and elevated [CO_2]. Given the number of parameters in DCP (Table 4.3) and the availability of AGFACE experimental data to proceed with any new parameterisation, relevant parameters related to $V_{c.max}$ and J_{max} in APSIM$_{\mathrm{DCP}}$ were modified using AGFACE data for 550 µmol CO_2 mol^{-1}and 700. µmol CO_2 mol^{-1}. Reduction in $V_{c.max}$ and J_{max} have been shown as common features of photosynthetic acclimation to e[CO_2] (Ainsworth and Long 2005; Rogers and Humphries 2000; Seneweera et al., 2002;). $V_{c.max}$ and J_{max} values were calculated using the following equations (de Pury and Farquhar, 1997):

$$PsiV_c = \frac{V_{c.max}}{N_0 - N_b} \qquad (4.1)$$

$$PsiJ = \frac{J_{max}}{N_0 - N_b} \qquad (4.2)$$

where $PsiV_c$ is the slope of the linear relationship between $V_{c.max}$ per leaf area at 25°C and specific leaf N (N_0); and $PsiJ$ is the slope of the linear relationship between J_{max} per leaf area at 25°C and N_0. N_b represents the minimum value of N at or below which CO_2 assimilation rate is zero. The Michaelis-Menton constant for CO_2 and O_2 were taken as K_c= 335 µbar, K_o = 304,000 µbar respectively. N_b value was taken as 25 mmol N m^{-2} (de Pury and Farquhar, 1997; Wu et al., 2018). Values for $V_{c.max}$, J_{max} and N_o used in the equations are presented in Table 4.4.

Table 4.3: Description, symbols, units and values of different parameters in default APSIM$_{DCP}$ under ambient CO$_2$ (370 µmol CO$_2$ mol^{-1}) (Wu et al., 2019).

Symbol	**Description**	**Units**	**APSIM$_{DCP}$**
Kc P25	Michaelis Menten constant of RuBisCO carboxylation at 25°C	µbar	273.42
Kc Ea	Michaelis Menten constant of RuBisCO carboxylation at 25°C fitted constant	µbar	93720
Ko	Michaelis Menten constant of RuBisCO oxygenation at 25°C	µbar	165820
Ko Ea	Michaelis Menten constant of RuBisCO oxygenation at 25°C fitted constant	µbar	33600
Ci	Intercellular CO$_2$	µbar	259
Ca	Ambient CO$_2$ partial pressure	µbar	370
Ci/Ca	The ratio of Ci to Ca		0.7
Oc	Oxygen partial pressure	µbar	210000
B	Biomass conversion coefficient	g biomass (g CO$_2$)$^{-1}$	0.41
V$_{c.max}$ Ea	V$_{c.max}$ fitted constant	µmol CO$_2$ m^{-1} s^{-1}	65330
V$_{c.max}$/V$_{o.max}$	Ratio of V$_{c.max}$ to V$_{o.max}$	-	4.59
V$_{c.max}$/V$_{o.max}$ Ea	V$_{c.max}$/V$_{o.max}$ fitted constant	-	35713.2
JTMin	Minimum temperature of J$_{max}$	µmol CO$_2$ m^{-1} s^{-1}	0
JTOpt	The optimum temperature of J$_{max}$	µmol CO$_2$ m^{-1} s^{-1}	30
JTMax	Maximum temperature of J$_{max}$	µmol CO$_2$ m^{-1} s^{-1}	45
gm P25	Mesophyll conductance at 25˚C	molCO$_2$ m^{-2} s^{-1} bar^{-1}	0.55
gmTMin	Minimum temperature of gm	molCO$_2$ m^{-2} s^{-1} bar^{-1}	0
gmTOpt	Optimum temperature of gm	molCO$_2$ m^{-2} s^{-1} bar^{-1}	29.24
gm TMax	Maximum temperature of gm	molCO$_2$ m^{-2} s^{-1} bar^{-1}	42
Rd Ea	Leaf day respiration fitted constant		46390
Psi Vc	The slope of the linear relationship between V$_{c.max}$ per leaf area at 25°C and specific leaf nitrogen	mmol CO$_2$ mol^{-1} N s^{-1}	1.1
Psi J	The slope of the linear relationship between J$_{max}$ per leaf area at 25°C and specific leaf nitrogen	mmol CO$_2$ mol^{-1} N s^{-1}	1.85
Psi gm	The slope of the linear relationship between gm per leaf area at 25°C and specific leaf nitrogen	mol CO$_2$ s^{-1} bar^{-1} mmol^{-1} N	0.005296
SLN ratio top	The ratio of the specific leaf nitrogen at the top of the canopy to that of the canopy average	-	1.3
Canopy UO	Canopy wind speed	m s^{-1}	1.5

Table 4.4: Maximum carboxylation efficiency of ribulose 1,5-bisphosphate carboxylase (RuBisCO) ($V_{c.max}$), maximum electron transfer capacity (J_{max}), and leaf N content on an area basis (N_o; mmol N m^{-2}) of wheat cultivar Yitpi grown under e[CO_2] (550 and 700 µmol CO_2 mol^{-1}). Data are means of n = 4 replicates (Seneweera et al., unpublish).

[CO_2] (µmol CO_2 mol^{-1}**)**	**$V_{c.max}$** (µmol CO_2 m^{-2} s^{-1})	**J_{max}** (µmol CO_2 m^{-2} s^{-1})	**N_o** (mmol N m^{-2})
550	61	262	128.52
700	53	235	128.52

4.2.4 Simulation configurations

In this study, $APSIM_{DCP}$ (used with APSIM-wheat v.7.10) was parameterised using wheat field experimental data from the AGFACE and then analysed for its performance in predicting biomass and yield responses to different [CO_2]. Weather data (maximum and minimum temperatures, solar radiation, and rainfall) for 2007-2009 were recorded on-site or nearby (O'Leary et al. 2015). Similarly, soil data were also extracted from the AGFACE database as measured on the site. The values of soil parameters are listed in Supplementary Table 4. Large soil mineral N content was measured at the site during 2007-2009. They were also considered in the model configuration (Supplementary Table 1, 2 and 3)

4.2.5 Assessing the photosynthetic acclimation in wheat

The responses of biomass at DC31, DC65 and DC90, and final grain yield to e[CO_2] using $APSIM_s$ and $APSIM_{DCP}$ were first compared against the observed data and corresponding results for six crop models (APSIM-Wheat, APSIM-N wheat, CAT-Wheat, CROPSYST, OLEARY-CONNER, SALUS) as reported in O’Leary et al. (2015).

To assess the impact of increasing [CO_2] beyond 550 µmol CO_2 mol^{-1} and examine for any occurrence of photosynthetic acclimation, the simulations were also carried out under 700 µmol CO_2 mol^{-1}. $APSIM_{DCP}$ was parameterised using AGFACE biochemical measurements for 700 µmol CO_2 mol^{-1}. The responses of biomass at DC31, DC65 and DC90, and final grain yield to e[CO_2] were then assessed.

Given the availability of biochemical measurements under [CO_2] of 550 and 700 µmol CO_2 mol^{-1} for four additional wheat cultivars (H45, Drysdale, Hartog, and Silverstar) from the 2010 AGFACE experiment, we investigated the photosynthetic acclimation response across this genetic variability. Similar to Yitpi cultivar, both $APSIM_s$ and $APSIM_{DCP}$ were parameterised based on the AGFACE biochemical measurements for 550 and 700 µmol CO_2 mol^{-1} for all the selected four cultivars. Table 4.5 lists $V_{c.max}$ and J_{max} values of four different cultivars under different e[CO_2] (550, and 700 µmol CO_2 mol^{-1}).

Table 4.5: Maximum carboxylation efficiency of ribulose 1,5-bisphosphate carboxylase (RuBisCO) ($V_{c.max}$), maximum electron transfer capacity (J_{max}) for four different wheat cultivars grown under a[CO_2] (384 µmol CO_2 mol^{-1}) and e[CO_2] (550 and 700 µmol CO_2 mol^{-1}). Data are means of n = 4 replicates (Seneweera et al., unpublish).

Cultivars	[CO_2] (µmol CO_2 mol^{-1})	$V_{c.max}$ (µmol CO_2 m^{-2} s^{-1})	J_{max} (µmol CO_2 m^{-2} s^{-1})
H45	550	58	248
	700	55	251
Drysdale	550	66	315
	700	63	294
Hartog	550	67	290
	700	64	311
Silverstar	550	62	295
	700	62	298

4.3 Results

The values of *PsiV*$_c$ and *PsiJ* under 365, 550 and 700 µmol CO_2 mol^{-1}, along with Ci/Ca values are presented in Table 4.5. From 365 to 700 µmol CO_2 mol^{-1} *PsiV*$_c$ decreased, whereas Ci/Ca increased. Similar to the latter, *PsiJ* increased with an increase in CO_2, from 365 to 550 µmol CO_2 mol^{-1}.

Table 4.6: List of parameters used for the parameterisation of wheat crop under APSIM$_{DCP}$. Both *PsiV*$_c$ and *PsiJ* values under 550 and 700 µmol CO_2 mol^{-1} were calculated as per the AGFACE database. For Ci/Ca values Onoda et al., (2005) and Inomata et al., (2018).

Symbol	Description	Units	CO_2 concentration		
			365	550	700
PsiVc	Slope of linear relationship between $V_{c.max}$ per leaf area at 25^{0}C and specific leaf nitrogen	mmol CO_2 mol^{-1} N s^{-1}	1.1	0.59	0.52
PsiJ	The slope of the linear relationship between J_{max} per leaf area at 25^{0}C and specific leaf nitrogen	mmol CO_2 mol^{-1} N s^{-1}	1.85	2.54	2.28
Ci/Ca	Ratio of Ci to Ca	-	0.7	0.74	0.83

4.3.1 Biomass and yield responses to e[CO_2]

Multi-year crop simulations were conducted for wheat to assess the likely impact on biomass at different growth stages. Both APSIMs and APSIM$_{DCP}$ tended to over simulate the biomass at DC31 but DC65 and DC90 are considered satisfactory (Table 4.7; Figure 4.1). However, both models simulated well the response to e[CO_2] with similar slopes to the observed data (Figures 4.1 and 4.2). The observed increase in wheat biomass at DC31, DC65, and DC90 to e[CO_2] (550 µmol CO_2 mol^{-1}) was 21%, 23%, and 28%, respectively (Figure 4.1). In comparison, the simulated response to e[CO_2] using APSIM$_S$ revealed an increase of 25%, 21%, and 20% at DC31, DC65, and DC90, respectively and when using APSIM$_{DCP}$ the corresponding simulated responses were 19%, 27% and 26%, respectively (Figure 4.1).

For grain yield response to e[CO_2], $APSIM_{DCP}$ showed slightly better performance compared to $APSIM_s$. The simulated responses were 18% and 28% using $APSIM_s$ and $APSIM_{DCP}$, respectively, where the observed response was 25% (Figure 4.2). $APSIM_s$ slightly under simulated the yield whereas $APSIM_{DCP}$ was closer to the observed response of the yield. The RMSE between observed and simulated yield ranged between 185 to 250 kg ha^{-1} across three different $APSIM_s$ and $APSIM_{DCP}$ settings (Table 4.6). As in the biomass, $APSIM_{DCP}$ showed the highest R^2 value with the lowest RMSE under both a[CO_2] and e[CO_2].

Table 4.7: Statistics for biomass at DC31, DC65, DC90, (DC: Decimal code) and grain yield of wheat. The Coefficient of Determination (R^2), root mean square error (RMSE, kg ha^{-1}) and mean absolute error (MAE, kg ha^{-1}) calculations were conducted for different APSIM settings including APSIM$_s$ and APSIM$_{DCP}$.

	Biomass at DC31			Biomass at DC65			Biomass at DC90			Yield		
	R^2	RMSE	MAE	R^2	RMSE	MAE	R^2	RMSE	MAE	R^2	RMSE	MAE
a[CO_2] 365 µmol CO_2 mol^{-1}												
APSIM$_s$	0.31	311	1471	0.83	348	1508	0.77	560	2558	0.59	221	949
APSIM$_{DCP}$	0.46	269	1276	0.80	310	1320	0.82	472	2124	0.58	186	782
e[CO_2] 550 µmol CO_2 mol^{-1}												
APSIM$_s$	0.28	406	1918	0.77	361	1501	0.71	610	2620	0.51	247	999
APSIM$_{DCP}$	0.50	319	1502	0.78	396	1677	0.77	630	2687	0.53	259	1072

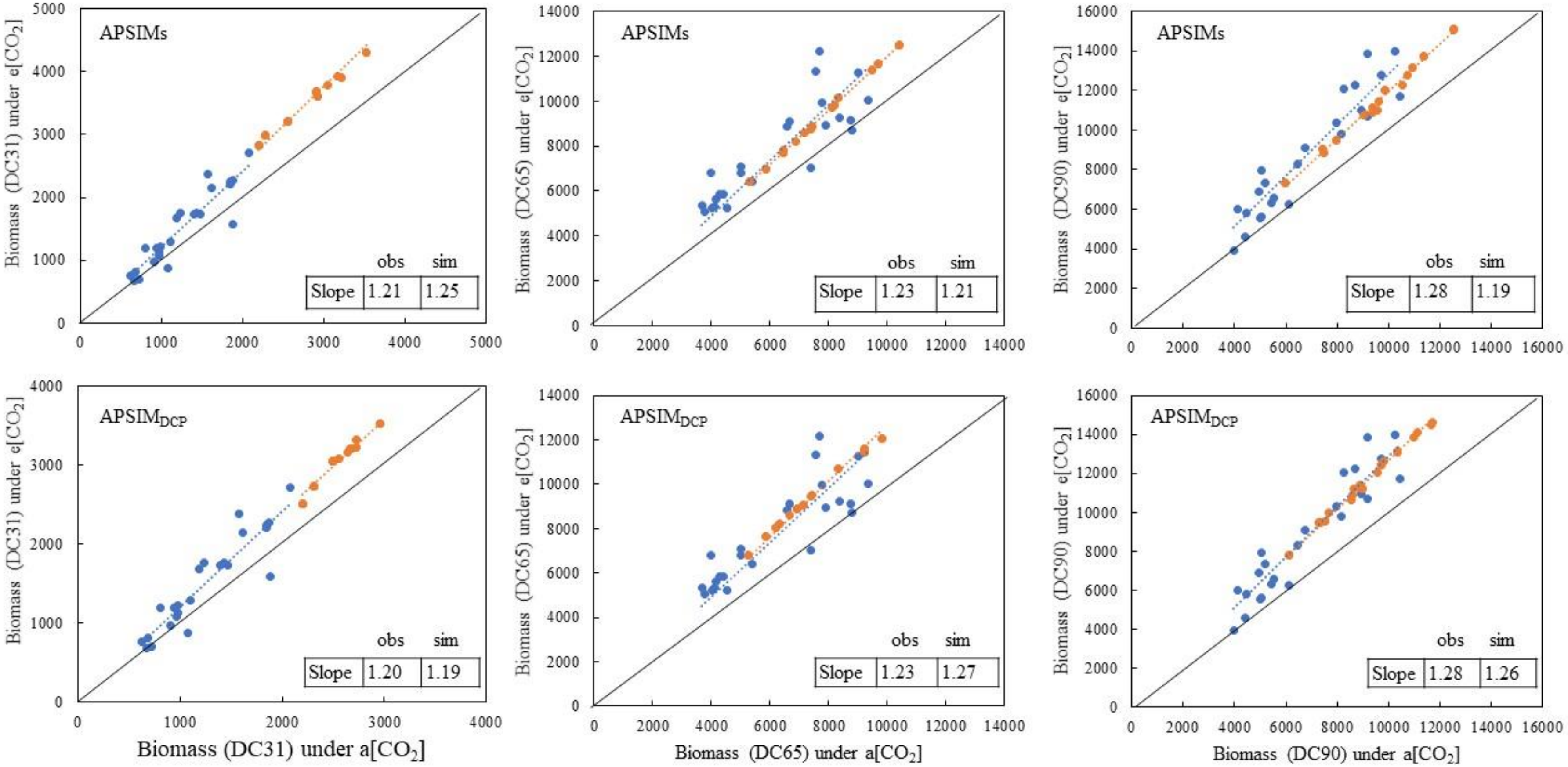

Figure 4.1: Response of wheat (kg ha^{-1}) biomass at DC31, DC65, and DC90 under e[CO$_2$] (550 µmol CO$_2$ mol^{-1}) using APSIM$_s$ and APSIM$_{DCP}$. The simulated response to e[CO$_2$] (orange dots and orange fitted lines) compared to the observed response to e[CO$_2$] (blue dots and blue fitted lines). The 1:1 line (dashed line) is the line of zero response to e[CO$_2$]. a[CO$_2$] = 365 µmol CO$_2$ mol^{-1}.

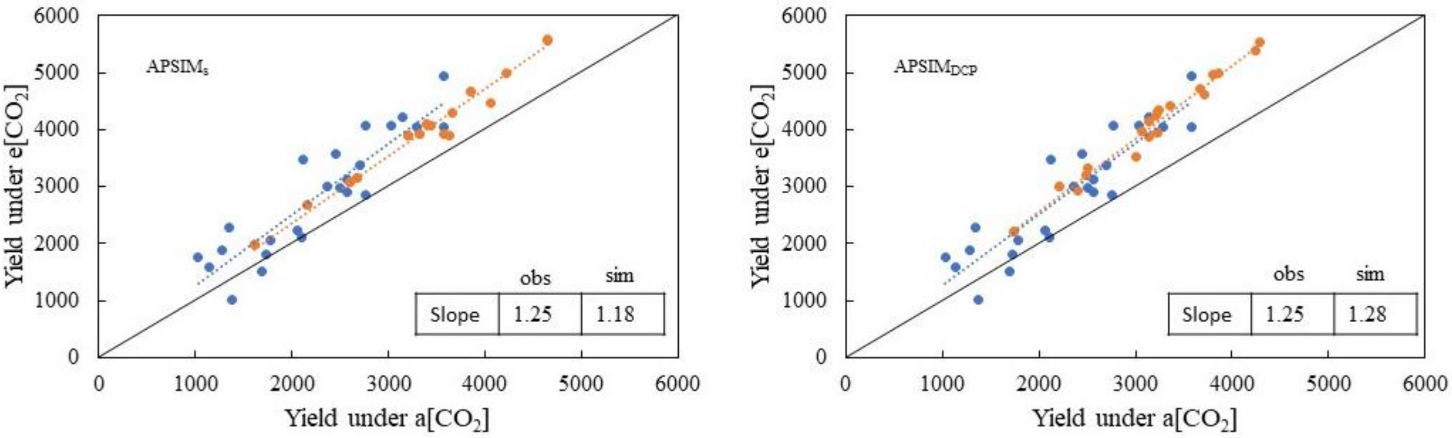

Figure 4.2: Response of yield (kg ha^{-1}) to e[CO$_2$] (550 µmol CO$_2$ mol^{-1}) using APSIM$_s$ and APSIM$_{DCP}$. The simulated response to e[CO$_2$] (orange dots and orange fitted lines) compared to the observed response to e[CO$_2$] (blue dots and blue fitted lines). The 1:1 line (dashed line) is the line of zero response to e[CO$_2$]. a[CO$_2$] = 365 µmol CO$_2$ mol^{-1}.

4.3.2 Comparison of APSIM$_{DCP}$ performance to reported results

The performance of APSIM$_{DCP}$ was compared against reported results for six different crop models. For the biomass at DC31, the APSIM$_s$ simulated value was close enough to APSIM-Wheat 7.4 and was the same as the model CAT-Wheat. The APSIMs was found simulating around the observed range whereas other models were either under or over simulating. However, the APSIM$_{DCP}$ simulated the response quite close to the slope of the observed data. For biomass at DC65, all the models as used in O'Leary et al. (2015) along with APSIM$_s$ was found simulating the values around the same range. However, only of the models among the six models used, CROPSYST and the APSIM$_{DCP}$ was found over simulating the response.

Further, for the yield value, APSIM$_{DCP}$ simulated the value close enough to the observed response whereas APSIM$_s$ under simulated the response. All other models reported simulated within the range of observed value except the APSIM-Wheat 7.4 which under simulated as APSIM$_s$.

Table 4.8: Comparison of slope values of biomass DC31, Biomass DC65, and LAI DC65 for different crop models under e[CO$_2$]: 550 µmol CO$_2$ mol^{-1}.

	Biomass	Biomass	Yield	Source
	DC31	DC65		
Observed	1.21	1.23	1.26	O'Leary et al. (2015)
APSIM-Wheat (v7.4)	1.29	1.22	1.19	O'Leary et al. (2015)
APSIM-N wheat	1.18	1.21	1.28	O'Leary et al. (2015)
CAT-Wheat	1.25	1.20	1.20	O'Leary et al. (2015)
CROPSYST	1.40	1.28	1.27	O'Leary et al. (2015)
OLEARY-CONNER	1.45	1.24	1.21	O'Leary et al. (2015)
SALUS	1.09	1.16	1.25	O'Leary et al. (2015)
APSIM$_s$ (v7.10)	1.25	1.20	1.18	This study
APSIM$_{DCP}$	1.19	1.27	1.28	This study

4.3.3 Assessment of photosynthetic acclimation patterns

In agreement with O'Leary et al. (2015), the photosynthetic response of wheat biomass and yield were found to be linear to an increase in [CO_2] from 365 to 550 µmol CO_2 mol^{-1}. However, modelling the response to e[CO_2] under a 700 µmol CO_2 mol^{-1} revealed variable results in terms of photosynthetic acclimation.

4.3.4 Simulation performance: Biomass

The biomass at different growth stages showed increases under 550 µmol CO_2 mol^{-1} as explained above (Figure 4.1). However, the increasing trend was substantially reduced as it moved from 550 to 700 µmol CO_2 mol^{-1} showing an increase of only 9% to 11% with $APSIM_s$ and less than 1% to 5% with $APSIM_{DCP}$ (Table 4.9). The rate of increase in biomass was reduced by around 56%, 55%, and 52% at DC31, DC65, and DC90 under $APSIM_s$ as [CO_2] increased from 550 to 700 µmol CO_2 mol^{-1}. Further, the rate of increase in biomass was substantially reduced by 95%, 93%, and 81% at DC31, DC65, and DC90 under $APSIM_{DCP}$ as [CO_2] increased from 550 to 700 µmol CO_2 mol^{-1}.

4.3.5 Simulation performance: Yield

Similarly, the yield response also decreased as compared to the increment under 550 µmol CO_2 mol^{-1} (as in Table 4.9). The increment under 700 µmol CO_2 mol^{-1} was only 9% and 11% which was 50% and 61% lower than the increment under 550 µmol CO_2 mol^{-1} under $APSIM_s$ and $APSIM_{DCP}$ respectively.

4.3.6 Simulation performance: Slope comparison

Both $APSIM_s$ and $APSIM_{DCP}$ simulated the responses within the observed value range up to 550 µmol CO_2 mol^{-1} (Table 4.9; Figure 4.3). After the increment of [CO_2] from 550 to 700 µmol CO_2 mol^{-1}, the response of both biomass and yield under $APSIM_s$ continue to rise linearly. But the incorporation of DCP within $APSIM_s$ i.e. $APSIM_{DCP}$ showed a fall of biomass particularly at DC90 and yield value as [CO_2] increases from 550 to 700 µmol CO_2 mol^{-1}, pointing to acclimation.

Table 4.9: Comparison of response to CO_2 slopes of observed (AGFACE database) and simulated data under $APSIM_S$ and $APSIM_{DCP}$ under different CO_2 concentration compared to ambient levels.

		550/365	**700/550**
Biomass at DC31	Observed	1.20	NA[1]
	$APSIM_S$	1.25	1.11
	$APSIM_{DCP}$	1.19	0.99
Biomass at DC65	Observed	1.23	NA
	$APSIM_S$	1.20	1.09
	$APSIM_{DCP}$	1.27	0.98
Biomass at DC90	Observed	1.28	NA
	$APSIM_S$	1.19	1.09
	$APSIM_{DCP}$	1.27	0.95
Yield	Observed	1.25	NA
	$APSIM_S$	1.18	1.09
	$APSIM_{DCP}$	1.28	0.89

[1] NA: not applicable.

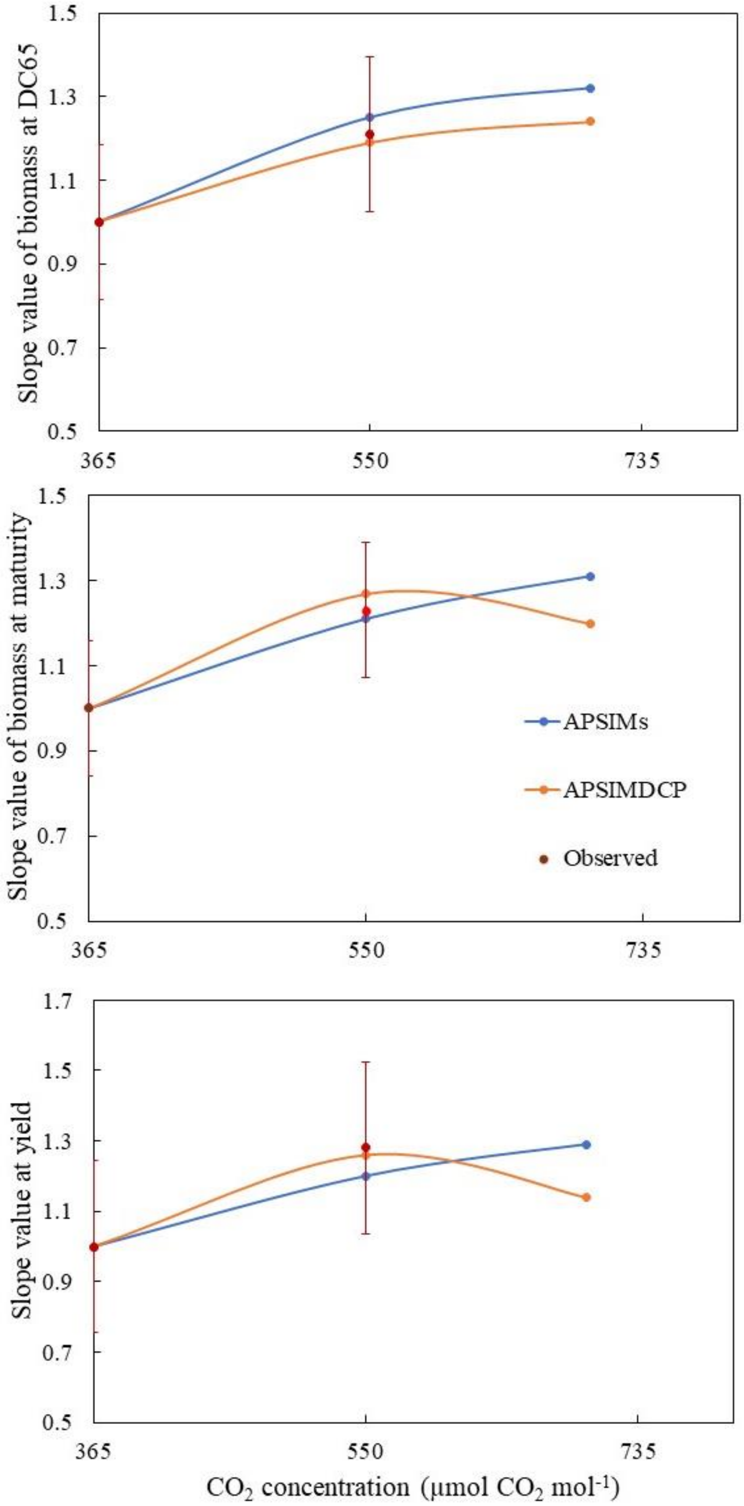

Figure 4.3: Comparisons of the responses of biomass and yield at different e[CO_2] (550 and 700 µmol CO_2 mol^{-1}) using $APSIM_S$ and $APSIM_{DCP}$. The slope values represent e[CO_2] values against a[CO_2] values. Observed values were from the AGFACE experiment with two CO_2 concentration (350 µmol CO_2 mol^{-1} (ambient) and 500 µmol CO_2 mol^{-1} (elevated).

4.3.7 Simulations using different genotypes

Genotypic variation of photosynthetic acclimation to e[CO_2] was assessed only with biomass at DC90 and with yield response (Figure 4.4 and 4.5) as the acclimation response was predominant at maturity with Yitpi. The response of APSIMs to the increasing [CO_2] was linear in case of all genotypes similar to Yitpi except Silverstar. No photosynthetic acclimation was observed without the integration of $V_{c.max}$ and J_{max} values even under increasing [CO_2] with APSIMs.

With the incorporation of biochemical parameters, the $APSIM_{DCP}$ successfully traced photosynthetic acclimation responses under e[CO_2] after 550 µmol CO_2 mol^{-1} in all the cultivars. However, the range of rise and fall in both the biomass and yield in different cultivars were found to be different. Among all the cultivars Yitpi showed a decrease in biomass by almost 6% as [CO_2] continue to rise after 550 µmol CO_2 mol^{-1}. The cultivars Drysdale and Hartog almost showed a similar trend of rise and fall in biomass as [CO_2] increases. Both the cultivars showed a decrease in biomass value by 0.8% as [CO_2] increased above 550 µmol CO_2 mol^{-1}. Similarly, for H45 the biomass decreased by around 1.7% with increasing [CO_2]. However, the cultivar Silverstar was the only one showing increased biomass by around 1.6% as [CO_2] rose above 550 µmol CO_2 mol^{-1}. The increment is, however, substantially low compared to the increase in biomass as [CO_2] rises from 365 to 550 µmol CO_2 mol^{-1}.

A similar photosynthetic acclimation pattern was observed for all the cultivars when yield response is considered. Yitpi showed a substantial decrease in yield by around 11% as [CO_2] increases from 550 to 700 µmol CO_2 mol^{-1} showing the highest decrease among the cultivars. This was then followed by cultivar Hartog with a decrease in yield by 10% as [CO_2] increased from 550 to 700 µmol CO_2 mol^{-1}. Furthermore, the cultivar H45 showed only a decrease of around 0.85% and for Drysdale and Silverstar showed a linear increase up to 550 µmol CO_2 mol^{-1} which stabilizes thereafter.

However, all other cultivars showed an acclimation response but at different rates. Yitpi showed the highest acclimation response in both biomass and yield whereas the Silverstar showed the least.

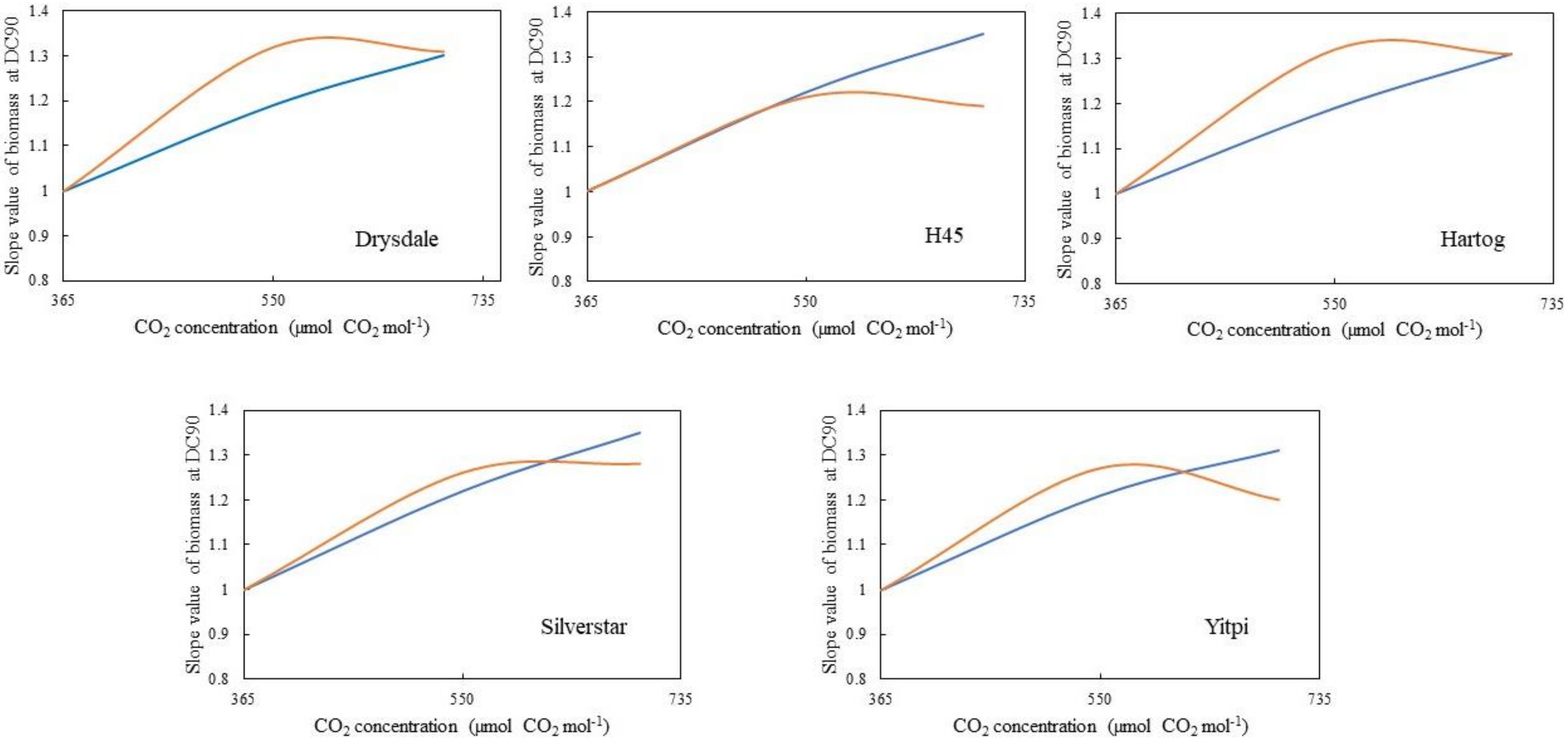

Figure 4.4: Comparisons of the responses of biomass (DC90) at different [CO_2] (365, 550 and 700 µmol CO_2 mol^{-1}) for five different wheat cultivars (Drysdale, H45, Hartog, Silverstar, and Yitpi) using $APSIM_S$ (blue line) and $APSIM_{DCP}$ (orange line). The slope value represent e[CO_2] values against a[CO_2] values.

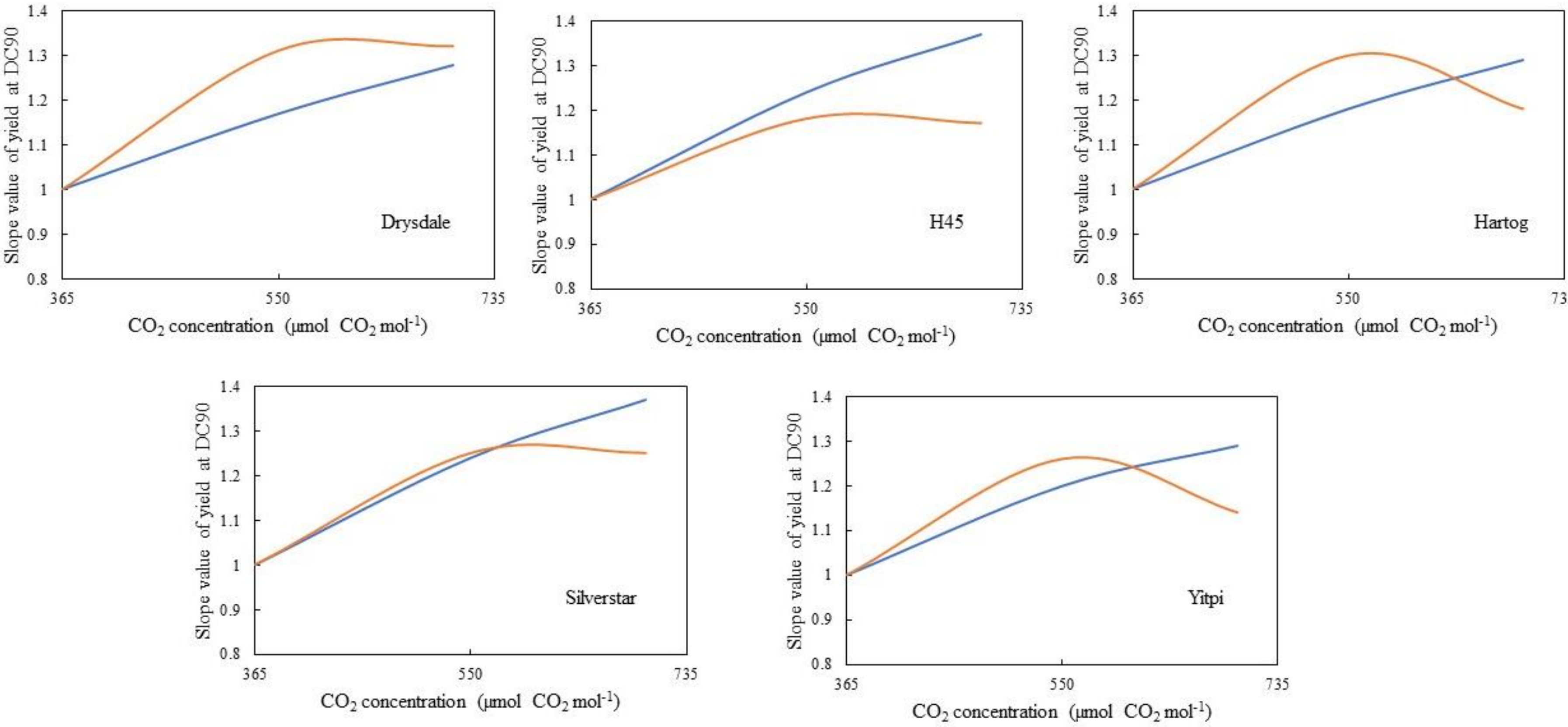

Figure 4.5: Comparisons of the responses of yield at different $[CO_2]$ (365, 550 and 700 µmol CO_2 mol^{-1}) for five different wheat cultivars (Drysdale, H45, Hartog, Silverstar, and Yitpi) using $APSIM_s$ (blue line) and $APSIM_{DCP}$ (orange line). The slope value represents $e[CO_2]$ values to $a[CO_2]$ values.

4.4 Discussion

The observed response of wheat as per AGFACE to e[CO_2] over three years, in biomass and yield, was robust and consistent. The response was considered as substantial (O'Leary et al., 2015), but it was not indicative of a photosynthetic acclimation scenario until the [CO_2] reached 550 µmol CO_2 mol^{-1} and passes early growth phases (DC31 and DC65). The simulated response using either $APSIM_s$ (version 7.10) or APSIM-Wheat (version 7.4) was similar (Table 4.8; O'Leary et al., 2015). The response to e[CO_2] (550 µmol CO_2 mol^{-1}) of around 18-25% in biomass and yield using $APSIM_s$ was either under- or over-simulating as compared to 19-28% when $APSIM_s$ was upgraded to $APSIM_{DCP}$. The value under $APSIM_{DCP}$ was found to be closer to the observed response, than the response under $APSIM_s$ (Figure 4.1, 4.2).

Several studies have shown strong evidence of photosynthesis acclimation when grown under e[CO_2] for an extended period (e.g Drake et al., 1997; Leakey et al., 2009; Nakano et al., 1997; Sage et al., 1989; von Caemmerer et al., 2001). In the present study, $APSIM_s$ did not simulate the acclimation response of wheat even at [CO_2] of more than 500 µmol CO_2 mol^{-1}. However, with $APSIM_{DCP}$, increase in [CO_2] from 550 to 700 µmol CO_2 mol^{-1} showed a decreasing trend in both biomass at maturity and yield in the cultivar Yitpi, indicating photosynthetic acclimation to e[CO_2] (700 µmol CO_2 mol^{-1}). Also, the present results clearly showed a genetic variability in photosynthetic acclimation to e[CO_2] (700 µmol CO_2 mol^{-1}), with a greater acclimation response in cultivar Yitpi, compared to cultivar Silverstar which showed the least response. Further, the photosynthetic acclimation to e[CO_2] was found predominantly at a later stage of the development which was characterised by lower $V_{c.max}$ and J_{max} value. Therefore, the simulation studies using $APSIM_{DCP}$ provided evidence to conclude that the magnitude of photosynthetic acclimation is dependent on both the genotype and the phenological stage of the plant. Therefore, the present findings offer a potentially useful approach, namely, either the use of cultivars that resist photosynthetic acclimation or breeding for that characteristic, to meet the challenge of sustaining current productivity levels of agricultural crops.

The $APSIM_{DCP}$ model consists of various parameters, incorporating biochemical components of wheat (Wu et al., 2019). Because the present study was based on measured data from AGFACE, the lack of various parameters within the default DCP script was a major limitation of the study. Hence, the focus only on a few major parameters including specific leaf N (SLN), $V_{c.max}$, J_{max}, Ci/Ca, and [CO_2] to modify the default DCP as $APSIM_{DCP}$. $V_{c.max}$ and J_{max}, considered as a crucial parameter for photosynthesis model (Farquhar et al., 1980; von Caemmerer et al., 2000) utilizes SLN as an input (de Pury and Farquhar, 1997). Thus, SLN is recognised as one of the major parameters in the default DCP script. In that regard, the study selected SLN, $V_{c.max}$, and J_{max} as the major input parameters for modifying the default DCP as per the AGFACE database. SLN acts as a key driver of both crop-level RUE and leaf-level photosynthesis (Evans, 1989; Sinclair and Horie, 1989; Thilakarathne et al., 2015).

One approach to modelling leaf-level photosynthesis that incorporates SLN is to associate some key photosynthetic parameters with SLN. This approach is applicable for driving the biochemical photosynthesis models with crop physiological attributes, which can be done by establishing relationships between biochemical photosynthesis model parameters and SLN. This approach allows SLN, which is often related to canopy-level RUE, to be linked to leaf-level models, and thus facilitates effective links across these scales. To incorporate the effects of SLN on photosynthetic physiology, this model assumed that at the reference temperature of 25°C, the $V_{c.max}$ and J_{max} were all zero below a minimum SLN and increased linearly with a slope of $PsiV_c$ and $PsiJ$ respectively. The respective $PsiV_c$ and $PsiJ$ value were calculated, based on equations as described by Pury and Farquhar, 1997.

For Ci/Ca value, the literature suggests that the ratio would be stable with Ca between 100 to 400 µmol CO_2 mol^{-1} (Wu et al., 2018). Further, Ainsworth and Long (2005) reported that Ci/Ca does not appear to change under elevated Ca. However, other studies reported the value of Ci/Ca to vary in the range from 0.6 to 0.9 for C_3 crops under different [CO_2] (Tan et al., 2017). In this study, we adopted the Ci/Ca ratios of 0.7, 0.74 and 0.83 for 365, 550, and 700 µmol CO_2 mol^{-1} respectively, which are similar to those adopted by Inomata et al. (2018), Onada et al. (2005) and Wu et al. (2019), in their studies. After modifying these parameters, $APSIM_{DCP}$ simulated biomass and yield for the diverse validation set of AGFACE database for wheat and showed some difference from the values simulated by $\mathrm{APSIM_S}$. There was a slight loss in precision and predictive capability for both biomass (DC31 and DC90) and yield and a little increment in the bias of prediction of biomass (DC65) when simulated under $\mathrm{APSIM_{DCP}}$. The overall response between observed and simulated values of biomass and yield across a range of N and water supply for wheat indicated a robust predictive capability of the $\mathrm{APSIM_{DCP}}$ model.

The photosynthesis response to temperature variation by different C_3 or C_4 crops is mostly simulated effectively (Bernacchi et al., 2002; Wu et al., 2018; Wu et al., 2019). Parameters like K_c, K_o, $V_{omax}/V_{c.max}$ are usually assumed to be similar among the C_3 species, in simulating the temperature response (von Caemmerer et al., 2013). Wu et al., (2019) also adopted these values from *N tabacum* (Bernacchi et al., 2002) considering it to be similar among C_3 species. Though this study's focus was on the [CO_2] response to photosynthesis, rather than to temperature, we adopted the same default value for running the simulation except for the available measured data at the AGFACE site. The major aim of the study was to validate the default DCP model to different [CO_2] as no studies were known to date to simulate the response of different [CO_2] to leaf photosynthesis, particularly by using models like $\mathrm{APSIM_{DCP}}$.

Variability in photosynthetic rate has been recognised, both within and among the plant functional groups (Evans et al., 2002). Such variability may account for part of the photosynthesis response to e[CO_2] and, may also contribute to variability in growth and yield responses. Thus, the study continued simulating the impact of four other wheat cultivars (H45, Hartog, Silverstar and Drysdale) to evaluate the genetic variability in photosynthesis acclimation to e[CO_2]. While all four cultivars, when tested at e[CO_2] (700 µmol CO_2 mol^{-1}), showed a photosynthetic acclimation pattern in the post-anthesis stage, albeit with variation in magnitude, among the cultivars.

It has been demonstrated that reduction of $V_{c.max}$ and J_{max} as a common feature of photosynthesis acclimation to e[CO_2] (Ainsworth and Long 2005; Rogers and Humphries 2000; Seneweera et al., 2002). Other studies also verified this by showing a reduction in $V_{c.max}$ and J_{max} by 13% and 17% respectively under e[CO_2] (Turnbull et al., 1998). In the same study, RuBisCO content was reduced by 40%. Thus, the findings of the present study also suggest that the acclimation of photosynthesis to

$e[CO_2]$ mainly occurs as a result of the rapid degradation of key enzyme RuBisCO (chapter III). The reduction of RuBisCO content together with a reduction in leaf N and $V_{c.max}$ is generally associated with photosynthesis acclimation responses (Bowes et al. 1996; Long et al., 2006; Moore et al., 1999; Nakano et al., 1997). As per the AGFACE database, four out of five cultivars showed a reduction in $V_{c.max}$ and N_o suggesting lower RuBisCO activity or lower RuBisCO concentration per given N content at $e[CO_2]$.

Studies have suggested that resource availability regulates the effect of $e[CO_2]$ on total biomass production and yield. Availability of N is a critical determinant of the overall growth and production mechanism under $e[CO_2]$. Particularly, in C_3 plants photosynthetic acclimation is more pronounced at low N supply as the available N in the soil declines overtime at $e[CO_2]$ in comparison to $a[CO_2]$ as explained by progressive N limitation phenomenon (Luo et al., 2004).

4.5 Conclusion

This study investigated the extent of photosynthetic acclimation in wheat at the crop level and field production systems under $e[CO_2]$. The results presented in this chapter are driven by the $APSIM_{DCP}$ model output which was adequately tested against AGFACE data, including a variety of morphological and biochemical parameters. The study showed that both $APSIM_s$ and $APSIM_{DCP}$ were useful models for studying the response of wheat. However, several limitations exist in the analysis, due to the unavailability of some measurements for all the parameters required by the DCP model. Focusing on the major component for determining the photosynthetic acclimation, $V_{c.max}$ and J_{max}, the study explored the acclimation responses with measured SLN. All the cultivars showed different responses to increase in $[CO_2]$ as reported by the decreasing value of $V_{c.max}$ and J_{max} under $e[CO_2]$. Therefore, the study successfully traced the photosynthetic acclimation response of five different wheat cultivars at different growth stages and further demonstrated that better predictive capacity of $APSIM_{DCP}$ compared to $APSIM_s$.

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Supplementary Table 1: List of dates including the amount of irrigation (mm) and N (kg N ha^{-1}) applied for different settings under sowing date 1 (Time of sowing: TOS1) as per AGFACE database (2007-2009).

AT1-N-I		AT1+N-I		AT1-N+I		AT1+N+I	
Date of application	Quantity	Date of application	Quantity	Date of application	Quantity	Date of application	Quantity
Irrigation (mm)							
17-Sep-07	10	17-Sep-07	10	17-Sep-07	10	17-Sep-07	10
8-Oct-07	10	8-Oct-07	10	24-Sep-07	10	24-Sep-07	10
16-Oct-07	8	16-Oct-07	8	2-Oct-07	10	2-Oct-07	10
17-Oct-07	10	17-Oct-07	10	8-Oct-07	10	8-Oct-07	10
18-Oct-07	10	18-Oct-07	10	16-Oct-07	8	16-Oct-07	8
3-Oct-08	10	3-Oct-08	10	17-Oct-07	10	17-Oct-07	10
2009	0	2009	0	18-Oct-07	10	18-Oct-07	10
				14-Nov-07	8	14-Nov-07	8
				15-Nov-07	10	15-Nov-07	10
				16-Nov-07	10	16-Nov-07	10
				8-Sep-08	5	8-Sep-08	5
				9-Sep-08	5	9-Sep-08	5
				10-Sep-08	5	10-Sep-08	5
				11-Sep-08	5	11-Sep-08	5
				25-Sep-08	20	25-Sep-08	20
				6-Oct-09	5	6-Oct-09	5
				7-Oct-09	5	7-Oct-09	5
				22-Oct-09	15	22-Oct-09	15
				23-Oct-09	15	23-Oct-09	15
				3-Nov-09	15	3-Nov-09	15
				4-Nov-09	15	4-Nov-09	15
Nitrogen (kgN/ha)							
		31-Jul-07	46			31-Jul-07	46
		5-Oct-07	46			5-Oct-07	46
		5-Nov-07	46			5-Nov-07	46
		22-Jul-08	53			22-Jul-08	53
		21-Aug-09	53			21-Aug-09	53

Supplementary Table 2: List of dates including the amount of irrigation (mm) and N (kg N ha^{-1}) applied for different settings under sowing date 2 (TOS2) as per AGFACE database (2007-2009).

AT2-N-I		AT2+N-I		AT2-N+I		AT2+N+I	
Date of application	Quantity	Date of application	Quantity	Date of application	Quantity	Date of application	Quantity
Irrigation (mm)							
17-Sep-07	10	17-Sep-07	10	17-Sep-07	10	17-Sep-07	10
8-Oct-07	10	8-Oct-07	10	24-Sep-07	10	24-Sep-07	10
16-Oct-07	8	16-Oct-07	8	2-Oct-07	10	2-Oct-07	10
17-Oct-07	10	17-Oct-07	10	8-Oct-07	10	8-Oct-07	10
18-Oct-07	10	18-Oct-07	10	16-Oct-07	8	16-Oct-07	8
16-Oct-08	7	16-Oct-08	7	17-Oct-07	10	17-Oct-07	10
17-Oct-08	8	17-Oct-08	8	18-Oct-07	10	18-Oct-07	10
26-Oct-08	10	26-Oct-08	10	14-Nov-07	8	14-Nov-07	8
2009	0	2009	0	15-Nov-07	10	15-Nov-07	10
				16-Nov-07	10	16-Nov-07	10
				4-Dec-07	10	4-Dec-07	10
				8-Sep-08	10	8-Sep-08	10
				24-Sep-08	20	24-Sep-08	20
				16-Oct-08	15	16-Oct-08	15
				17-Oct-08	15	17-Oct-08	15
				25-Oct-08	20	25-Oct-08	20
				22-Oct-09	15	22-Oct-09	15
				23-Oct-09	15	23-Oct-09	15
				3-Nov-09	15	3-Nov-09	15
				4-Nov-09	15	4-Nov-09	15
Nitrogen (kgN/ha)							
		5-Oct-07	46			5-Oct-07	46
		9-Nov-07	46			9-Nov-07	46
		4-Dec-07	46			4-Dec-07	46
		30-Sep-08	53			30-Sep-08	53
		30-Sep-09	53			30-Sep-09	53

Supplementary Table 3: List of total amounts of irrigation (mm) and N (kg N ha^{-1}) applied under different setting in three consecutive years (2007-2009) as per AGFACE database (2007-2009).

Observed	Irrigation	N	Irrigation	N	Irrigation	N
	2007		2008		2009	
AT1-N-I	48.00	0.00	10.00	0.00	0.00	0.00
AT1+N-I	48.00	138.00	10.00	53.00	0.00	53.00
AT1-N+I	96.00	0.00	40.00	0.00	70.00	0.00
AT1+N+I	96.00	138.00	40.00	53.00	70.00	53.00
AT2-N-I	48.00	0.00	25.00	0.00	0.00	0.00
AT2+N-I	48.00	138.00	25.00	53.00	0.00	53.00
AT2-N+I	96.00	0.00	80.00	0.00	60.00	0.00
AT2+N+I	96.00	138.00	80.00	53.00	60.00	53.00

Supplementary Table 4: List of values of soil parameters used in APSIM as per AGFACE setting in Horsham from O'Leary et al. (2015).

LL: Lower limit; DUL: Drain upper limit; SAT: Saturated soil; BD: Bulk Density; APSIM KL: APSIM rate constant ; APSIM XF: APSIM exploration factor

Depth (cm)	**Air dry (g/cm3)**	**Crop LL (g/cm3)**	**DUL (g/cm3)**	**SAT (g/cm3)**	**BD (g/cm3)**	**pH**	**APSIM KL**	**APSIM XF**
10	0.15	0.20	0.39	0.46	1.14	8.4	0.06	1.00
20	0.18	0.23	0.40	0.47	1.30	8.4	0.06	1.00
40	0.25	0.27	0.42	0.48	1.37	8.9	0.04	1.00
60	0.27	0.30	0.43	0.47	1.40	9.0	0.02	0.80
80	0.28	0.33	0.45	0.47	1.40	9.0	0.02	0.80
100	0.30	0.35	0.45	0.47	1.40	9.0	0.02	0.60
120	0.32	0.36	0.45	0.47	1.40	9.0	0.02	0.60
140	0.33	0.37	0.45	0.47	1.40	9.1	0.02	0.20
160	0.34	0.37	0.45	0.47	1.40	9.1	0.02	0.20
180	0.34	0.37	0.45	0.47	1.40	9.1	0.02	0.20

Chapter 5: General discussion and conclusions

5.1 General discussion

In the interest of sustaining food security for the increasing human population, in a situation of drastic climate change forecasts, the effects of increasing [CO_2] and temperature on plant growth and productivity is a major concern. Elevated [CO_2] (e[CO_2]) causes increased photosynthesis and plant growth, which leads to greater production of carbohydrates and biomass (Ainsworth et al., 2008; Leakey et al., 2009). Photosynthesis is recognised as a major determinant for overall crop productivity as it is the entry point for carbon assimilation. Therefore, assessment of the impact of exposure to increasing [CO_2] on photosynthesis is significant in understanding the impact of e[CO_2] on crop growth and development (Bagley et al., 2015). A notable feature of the increased photosynthesis response to e[CO_2] is its short-lived nature and the rapid return to a photosynthesis decline phase, which is being attributed to several processes (Thompson et al., 2017), perhaps as a physiological adaptive mechanism. The phenomenon of the decline in photosynthesis over time is referred to as photosynthetic acclimation (Ainsworth et al., 2007; Long et al., 2004; Rodrigues et al., 2016; Seneweera et al., 2011). The present research was an effort to gain an insight to the physiology and the biochemistry of the process of photosynthetic acclimation in agricultural crops exposed to e[CO_2].

While acknowledging the complex nature of the regulation of photosynthesis, this study focused on a few selected aspects, namely N and water use, temperature effects, as well as the relationship between single leaf productivity and canopy productivity. Knowledge about the efficiency of N and water usage by crops is imperative for a better understanding of photosynthesis regulation (Long et al., 2006; Zhu et al., 2010). Indeed, the critical role of the availability of these resources and, the impact of temperature, in determining the overall productivity and growth of a plant, are well documented, including Chapter 2 of this thesis. Another complex aspect of the subject is the relationship between single-leaf and the whole crop because the rate of photosynthesis at the leaf-level, by itself, may not necessarily correlate with the overall productivity as it may be subjected to modulation by many factors (Hammer et al., 2006; Long et al., 2006; Sinclair et al., 2004). Hence, bridging the knowledge gap on the link between the leaf and crop level production is needed to help accelerate the progress in photosynthesis enhancement for crop improvement in a future CO_2-rich environment. The use of modelling tools has been recognized as an excellent approach for this purpose (Wu et al., 2019).

The study incorporated three different modelling techniques including system dynamics model comprising state and rate variables, STELLA, and a cross-scale modelling approach like $APSIM_{DCP}$ that connects leaf and crop level dynamics along with major abiotic factors involved. Using different secondary data from published experiments, this study re-investigated the change in photosynthesis of different functional group species under e[CO_2] followed by a further assessment that provokes the process of photosynthesis to move towards acclimation. Further, the study assessed the change in RuBisCO content and change in N concentration throughout the growth stage of the leaf which was upscaled to canopy level through simulation. The key findings from each study to investigate a few selected aspects of physiological response to e[CO_2] are discussed below, followed by the conclusion and some suggestions for future research.

Chapter II: The exploration of the extent of physiological changes in two functional groups of plants (C_3 and C_4), under e[CO_2], using a meta-analysis of recent studies.

Increasing [CO_2] has significant implications on the productivity of agricultural crops (Ainsworth et al., 2008; Drake et al., 1997). Plants respond to rising [CO_2] through increased photosynthesis and decreased stomatal conductance, eventually increasing the overall productivity of the crops (Ainsworth et al., 2007). The change in these physiological parameters has been well explained in regard to changing [CO_2] focusing particularly on certain species (Long et al., 2006; Rogers et al., 2004). However, the impact of e[CO_2] on major functional groups including both C_3 and C_4 is very limited and not up to date. Therefore, this chapter compared the change in physiological parameters, photosynthesis and stomatal conductance, among and within the C_3 and the C_4 crops under e[CO_2].

Many studies have documented an increase in photosynthesis and a decrease in stomatal conductance particularly in C_3 crops (Ainsworth et al., 2007; Long et al., 2006; Rogers et al., 2004). However, an interspecific variation within a functional group was observed in response to e[CO_2]. Within the C_3 group, trees and grasses showed a higher increase in photosynthesis as e[CO_2] increases the carboxylation and reduces the photorespiration (Ainsworth et al., 2007). In contrast, other groups within C_3 showed comparatively lower stimulation as photosynthesis become limited by the capacity of RuBP regeneration, and a further increase in photosynthesis under e[CO_2] results only from the repression of photorespiration (Long et al., 2004). However, no change in photosynthesis was found for C_4 species under e[CO_2], yet stomatal conductance decreased at e[CO_2]. .

Further, the study investigated the change in physiological processes under different abiotic stresses including water, temperature, and N. The unavailability or sometimes, the excess availability of these abiotic stresses was found affecting the affinity of RuBisCO for $CO_{2,}$ hence obstructing the physiological process. Thus, the meta-analysis depicted that the change in photosynthesis and stomatal conductance under e[CO_2] is directly attributed to the abiotic stresses. The optimal availability of the abiotic factors was found necessary along with e[CO_2] to promote the growth and development of crops. Under the unavailability of required abiotic factors, the major physiological process may follow the decline or may stabilize introducing the‘photosynthetic acclimation’ phenomena.

Chapter III: To model the photosynthetic acclimation in rice based on biochemical processes.

N is an essential macronutrient for plant growth affecting the overall crop productivity (Imai et al., 2008). Particularly, in the rice plant, about 70% of N is allocated in the leaf blades supporting photosynthesis (Mae and Ohira, 1982). The most abundant protein, RuBisCO, constitutes 12-35% of total leaf N participating actively in the photosynthesis process (Kumar et al., 2002; Makino et al., 2003). In this chapter, the links between nitrogen (N) and RuBisCO are explored by hypothesizing that the photosynthesis acclimation to elevated [CO_2] (e[CO_2]) is associated with reduced RuBisCO contents that are directly related to the N supply into the leaf blades. An analytical modelling framework applying leaf to a canopy-level rice crop system using RuBisCO and N dynamics and crop growth processes were developed using the

STELLA software and secondary data of rice from a growth chamber experiment. The N influx is one of the most important factors determining the extent of RuBisCO synthesis and eventually the overall process of photosynthetic acclimation in rice. The change in photosynthesis, RuBisCO, and radiation use efficiency (RUE) were assessed over the growth period from transplantation to maturity. The results strongly support an acclimation response on both morphological and biochemical levels under different N rates and [CO_2]. Such an analytical approach should guide the incorporation of nutrient limitation under e[CO_2], and resulting impact on crop growth, into more complex crop models.

Chapter IV: To simulate photosynthetic acclimation in wheat to e[CO_2] under filed experimental conditions.

Photosynthesis is central for progressing field crop productivity. However, the occurrence of photosynthetic acclimation under e[CO_2] continues to drag the productivity behind under different environmental and growth conditions. This chapter continued exploring photosynthetic acclimation response with another C_3 crop, wheat, by developing a cross-scale modelling approach connecting the leaf-level biochemical parameters with a canopy level dynamic. The response of wheat growth and yield to e[CO_2] was modelled using two different versions of the APSIM -Wheat model. The first was the basic daily time step APSIM-Wheat model (V:7.10) and the second was a modified version ($APSIM_{DCP}$; that coupled an hourly daytime diurnal canopy photosynthesis-stomatal conductance model). These models were compared to data published from the Australian Grains Free-Air CO_2 Enrichment (AGFACE) experiment data which involved biochemical and morphological data for wheat grown under different environmental conditions. This chapter simulated major photosynthetic manipulation targets including $V_{c.max}$, J_{max} and their responses under variable [CO_2] (365, 550, 700 µmol CO_2 mol^{-1}).

Additionally, the genotypic variation of photosynthetic acclimation pattern under e[CO_2] in five wheat cultivars was investigated. The two key input parameters modified in $APSIM_{DCP}$ were the maximum carboxylation rate of RuBisCO ($V_{c.max}$) and the electron transport capacity (J_{max}). Overall, the performance of $APSIM_{DCP}$ in simulating a wheat response to e[CO_2] (550 µmol CO_2 mol^{-1}) was satisfactory and better than that of the standard version of APSIM (version 7.10), namely for biomass at maturity and grain yield. Simulations under increased CO_2 showed a clear picture of photosynthetic acclimation for the majority of wheat cultivars assessed under the AGFACE experimental conditions. The down-regulation of photosynthesis in wheat above 550 μmol CO_2 mol^{-1} was found to be driven by the reduction in RuBisCO content and/or total activity supporting the primary thesis of this study that the supply and flux of N is a primary determinant of acclimation to e[CO_2] in wheat and rice C_3 crops. The study by Gesch et al. 2003 revealed that rice cultivars have different capacity of carboxylation to e[CO_2]. In that regard, the different magnitude of $V_{c.max}$ and J_{max} values suggest different capacities of carboxylation and electron transport in the cultivars assessed. This paves the way for genetic selection in these variables.

5.2 Conclusions and Future directions

- This study was intended to understand the effect of e[CO_2] on photosynthesis, which is a major physiological process in plants, through a meta-analysis of relevant studies reported in the 2007 to 2019 period and, using modelling approaches. The consensus that could be arrived at, from the meta-analysis, is that plants respond to e[CO_2], with an increase in photosynthesis and decreased stomatal conductance, particularly in the C_3 functional group. Photosynthesis and stomatal conductance response are not the same in C_4 functional group plants. Also, within the C_3 functional groups, different crop categories showed different percentages of change in photosynthesis and stomatal conductance under e[CO_2]. Furthermore, the meta-analysis revealed the role of abiotic factors as critical determinants of photosynthesis and stomatal conductance under e[CO_2].
- The study found that different modelling approaches (STELLA state and rate modelling and $APSIM_{DCP}$) can assist in understanding crop response to variations in the key environmental factors (CO_2, RUE, N). The capacity of these models to capture the photosynthetic attributes makes them a valuable tool, which when combined with reliable climate predictions can also evaluate photosynthetic responses of plants, in future climatic conditions.

- The study analysed several data sets generated from field and laboratory experimental observations, with the major focus on C_3 rice and wheat. It would be useful, in future studies, to build on the present findings, by evaluating the acclimation trends among different functional groups and, among different species within functional groups. That would require complex experimentation which is beyond the scope of the present research. The analysis of the effect of e[CO_2] on different crops including cereals, legume crops, and oil crops will give insight into the inter-species variation in the response to rising [CO_2].

- The study also illustrated the important role of different abiotic factors in determining the rate of photosynthesis under increasing [CO_2]. Hence, it is highly recommended that studies are conducted, either lab-based or FACE experiments, with different abiotic stress factors along with e[CO_2] to observe the real-life impact on the photosynthesis and, consequently, on the overall productivity of crops.

- A positive correlation between the rate of photosynthesis and N content in the leaves of a plant was noted. RuBisCO, accounting for 12-35% of total leaf N, was reported to attribute the photosynthetic capacity of the plants which may vary as per the N availability. However, the study has not included the details on the role of eight small subunits (SSUs) and eight large subunits (LSUs) of RuBisCO which are the products of the nuclear *rbcS* genes and the chloroplast *rbcL* genes respectively. It is further recommended to extend the knowledge on the effect of the level of N supply on the relationships between the levels of *rbcS* and *rbcL* mRNAs and the amount of RuBisCO synthesized during leaf development.

- Studies have identified the role of N and RuBisCO in the overall photosynthesis process. However, the synthesis and degradation of RuBisCO along with the influx and efflux of N play an important role when quantifying the photosynthetic acclimation response in detail. Hence, it is highly recommended to explore the RuBisCO synthesis and degradation and N influx and efflux dynamics in future studies.

- The study incorporated data from different FACE and glasshouse experiments regarding photosynthesis, RuBisCO content, and N. The collected database mostly includes a single or just two N concentrations. It is highly recommended to conduct studies that cause greater variation in N concentration so that a more robust impact of N under e[CO_2] can be observed.

- This study incorporated different crop modelling tools including STELLA, $APSIM_S$, and $APSIM_{DCP}$ simulating the impact under different CO_2 and N concentration on growth and yield. It observed the efficiency of the models in representing the photosynthetic acclimation scenarios using different variables directly correlated with the photosynthetic capacity of crops. In the future, other approaches should also be considered, such as the Structural Equation Modelling (SEM) approachto investigate the relationship between different variables. The SEM modelling approach has been used in a wide range of areas including ecological to medical but its use on physiological variables are very limited. This is an area worthy of future exploration.

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