Reflectance and fluorescence measurements for wheat traits under elevated CO_2

Eileen M. Perry[1] and Glenn J. Fitzgerald[2]

[1] Agriculture Research, Department of Economic Development, Jobs, Transport and Resources (DEDJTR), Cnr. Midland Hwy and Taylor Street, Epsom VIC 3551, eileen.perry@ecodev.vic.gov.au
[2] Agriculture Research, Department of Economic Development, Jobs, Transport and Resources (DEDJTR), 402-404 Mair St, Ballarat VIC 3350

Abstract
The concentration of CO_2 continues to rise in the atmosphere and is expected to increase from 400 ppm currently to 550 ppm by 2050. Research has shown that this can increase yields due to the "fertilisation effect" of CO_2, but leaf nitrogen and grain protein levels in cereals reduce under elevated CO_2 (eCO_2). Despite results from Australian and international research showing a consistent decline in grain protein under eCO_2, there is currently no solution to slowing or reversing this decrease. Experiments during 2014 at the Australian Grains Free Air CO_2 Environment (AGFACE) facility tested lines of wheat with traits purported to improve nitrogen efficiencies and other characteristics that may contribute to resisting or reversing the decline in grain protein. As part of the research, non-destructive proximal measurements were assessed to detect trait differences between ambient and eCO_2 environments. Active optical sensing (Crop Circle 210) was used to assess differences in the canopy cover. A hand-held field fluorometer (FORCE-A Multiplex 3.6) was used to measure fluorescence excitation and emission ratios at the canopy level. NDVI time series from the active optical sensor responded to the differences between rainfed and well watered plots, but ANOVA results near anthesis were not significant for the CO_2 effect. The fluorometer index NBI_G was found to be positively, linearly related to leaf %N from analysis of plant samples ($R^2 = 0.69$) with a standard error of 0.32 %N. *In situ* fluorometer NBI_G measurements were significant for both water and CO_2 effects on one date following anthesis.

Key Words
Phenotyping, proximal sensing, elevated CO^2, fluorometer, nitrogen use efficiency

Introduction
Leaf nitrogen and grain protein levels in cereals have been shown to reduce under eCO_2 (Myers et al. 2014; Panozzo *et al*. 2014). Experiments at the Australian Grains Free Air CO_2 Environment (AGFACE) facility (Mollah *et al*. 2009) in 2014 tested lines of wheat with traits purported to improve nitrogen efficiencies and other characteristics that may contribute to resisting or reversing the decline in grain protein. In addition to plant sampling for dry matter and nitrogen concentration near anthesis, proximal sensors were used to monitor treatment differences. Active optical sensors measure the reflectance of canopy in the red and near infrared regions. While these measurements are related to chlorophyll concentration and biomass, the measurements most closely relate to fractional green cover (Perry *et al*. 2012), and can be used to track relative growth differences. Fluorescence measurements with active optical sensors (Agati *et al*. 2011) have been shown to be useful for measurements of leaf chlorophyll and flavonoids and as an indicator of leaf nitrogen (Agati *et al*. 2013). In this paper we describe the results from the non-destructive measurements with respect to testing the effectiveness of the technologies to detect trait differences.

Methods
Field site and sampling
The AGFACE facility is located near Horsham, VIC (142° 06' E longitude, 36° 44' S latitude). Details of the site, climate, and CO_2 dispersion mechanism can be found in (Mollah et al. 2009). In this paper we use the AGFACE 2014 wheat plots; treatment factors considered are CO_2 (elevated to 550 ppm and ambient, 400 ppm), four wheat varieties, and two differences in seasonal water inputs. The water input differences were rainfed and supplemental irrigation plots with 189 mm and 399 mm for the season, respectively. Wheat varieties Gladius and Wyalcatchem were selected for traits related to improved nitrogen use efficiency, and these were grown under rainfed conditions. The varieties Scout and Yitpi were planted in the well watered plots. Fluorometer readings were made in the lab on the fresh intact leaves for all treatments and reps (N = 72) prior to preparation for leaf N analysis.

Sensors

An active optical canopy sensor (CC210, Holland Scientific, Lincoln NE USA) was used to characterize the fraction of canopy cover. Weekly measurements were made over each plot. Sensor readings of NDVI were averaged for each plot and acquisition date. A handheld active light fluorometer (Multiplex 3.6, Force A, Orsay Cedex FRA) with four excitation bands (UV, blue, green, and red) and three detection bands (yellow, red and far red) was used both for field plots and fresh leaves cut for biomass. The fluorometer measurements resulted in a suite of indices from various combinations of the activation and detection wavelengths used (e.g., Gozlen *et al*. 2010). Four measurements per plot (*in situ*) were made on three dates; the four measurements were averaged for each plot. Measurements were also made on fresh stacked leaves from biomass cuts prior to processing for %N analysis.

Results

The active optical measurements made through the season form time series for the eight combinations of variety and CO_2 (Fig. 1). These time series show that while the variety Wyalcatchem initially had higher fractional green cover than the other varieties, the varieties in well watered plots (Scout and Yitpi) overtake the growth following GS30. For some of the growing season, the NDVI values for the eCO_2 plots appear to be slightly lower than the aCO_2 plots. In particular, the difference is observed for Scout and Yitpi (well watered plots) after 20 Aug. 2014 (GS34), and for Gladius (rainfed).

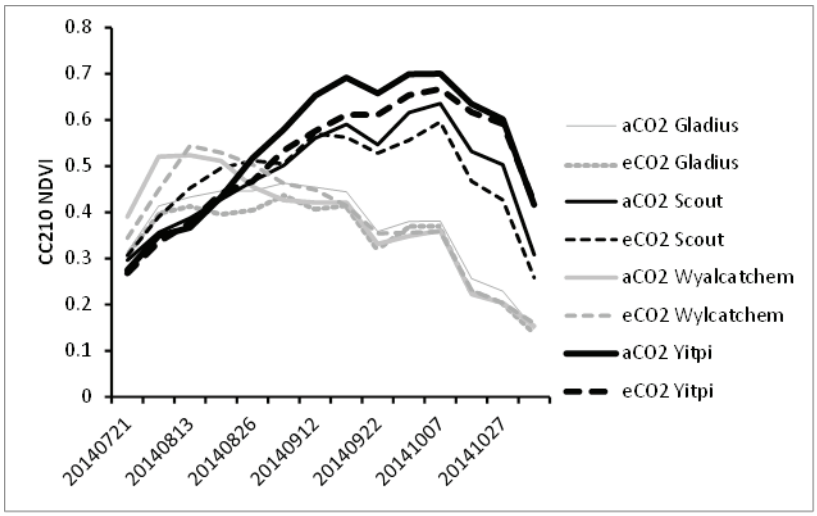

Fig. 1. NDVI values from the active optical sensor, tracking fractional green cover through time for the rainfed NUE plots (Gladius and Wyalcatchem) and the well watered plots (Scout and Yitpi).

A suite of the fluorometer indices was evaluated against measured leaf %N from the GS65 plant sampling. The nitrogen index 'NBI_G' (Agati *et al*. 2013) was found to have the highest correlation to leaf %N, with a positive, linear relationship ($R^2 = 0.69$) with a standard error of 0.32 %N. The index is computed by the instrument as the ratio of the infrared fluorescence excited with UV light, divided by the red fluorescence excited with green light. Based on these results, this index was selected for use to assess differences in plot canopy nitrogen. The GS65 sampling confirms lower leaf %N for values for elevated CO_2 plots relative to ambient plots, for both rainfed and well watered plots (Fig. 2a). The fluorometer measurements of NBI_G, taken on the fresh samples prior to processing, also indicate this trend (Fig. 2b). However, active optical measurements made on the plots just prior to sampling indicate differences between variety (and therefore water supply) but not CO2 effects (Fig. 2c). ANOVA results indicate that the difference in CO_2 are significant ($F_{pr} < 0.001$) for both the leaf %N and the fluorometer measurements made on fresh leaf samples, but not the active optical measurements (Table 1). The fluorometer measurements made in the field plots (*in situ*) for the three dates are shown in Fig. 2. Note that measurements made on 22 Sept 2014 (~GS50) do not show the same trends in leaf N (Fig. 2d). The differences in response with CO_2 is more evident in the 7 Oct measurements (Fig. 2e), and more obviously for the 15 Oct (Fig. 2f). The ANOVA results (Table 1) indicate the difference in CO_2 was not significant for the 7 Oct measurements, but was for the 15 Oct ($F_{pr} = 0.03$).

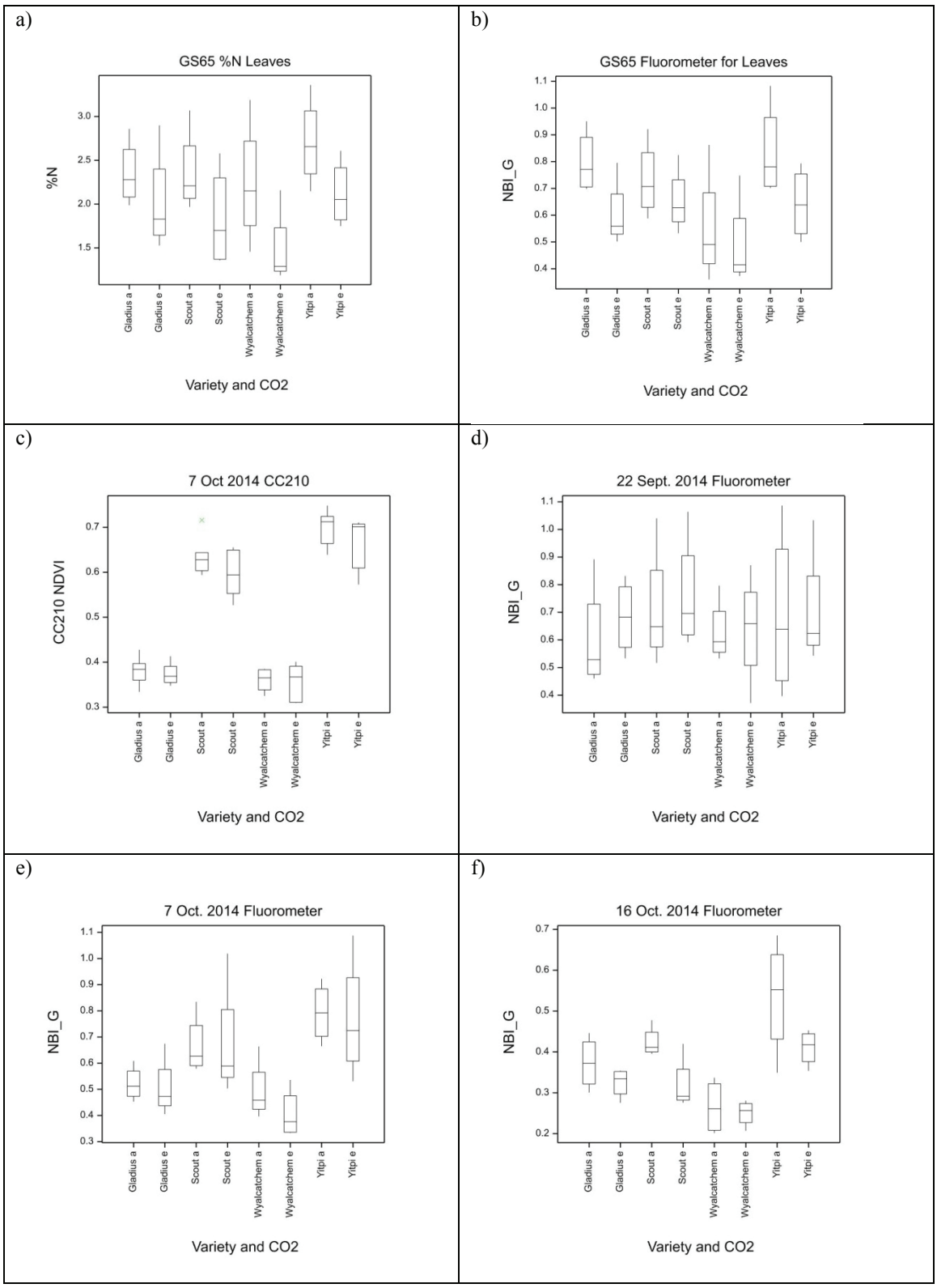

Fig. 2. Box plots showing the median, and +/- 25th percentile of various measurements by variety and CO_2. Gladius and Wyalcatchem were maintained on rainfed conditions (189 mm water for the season), while Scout and Yitpi were well irrigated (399 mm water for the season). CO_2 treatments are indicated by 'a' for ambient and 'e' for elevated (550 ppm). The measurements are a) leaf N at anthesis, b) fluorometer NBI on fresh cut leaves at anthesis, c) *in situ* active optical measurements at anthesis, and *in situ* fluorometer NBI measures made d) ~GS50, e) GS65, and f) following GS65.

Table 1. ANOVA results for %N of leaves, CC210 NDVI and Fluorometer NBI_G with respect to CO_2 and treatments (variety).

	d.f.	Sum of Sq.	Mean Sq.	F	F pr.
%N Leaf at GS65					
CO_2	1	2.42550	2.42550	54.40	0.005*
Variety	3	1.24666	0.41555	8.00	0.001*
Fluorometer NBI_G leaf samples					
CO_2	1	0.139868	0.139868	58.21	0.005*
Variety	3	0.229842	0.076614	14.84	<.001*
CC210 in situ (7 Oct 2014)					
CO_2	1	0.005156	0.005156	3.87	0.188
Variety	3	0.978132	0.326044	232.75	<.001*
CC210 in situ (20 Oct 2014)					
CO_2	1	0.002161	0.002161	2.90	0.187
Variety	3	1.018313	0.339438	116.17	<.001*
Fluorometer NBI_G in situ (22 Sept 2014)					
CO_2	1	0.01205	0.01205	0.26	0.645
Variety	3	0.05667	0.01889	1.13	0.363
Fluorometer NBI_G in situ (7 Oct 2014)					
CO_2	1	0.007351	0.007351	0.77	0.446
Variety	3	0.538688	0.179563	31.39	<.001*
Fluorometer NBI_G in situ (16 Oct 2014)					
CO_2	1	0.042566	0.042566	15.64	0.029*
Variety	3	0.186626	0.062209	18.90	<.001*
*Sig. at 0.05					

Conclusion

While active optical measurements (NDVI) are useful for tracking plant growth through the season, the fluorometer NBI_G measurements look promising as an estimate of canopy N. These measurements of canopy N are needed for assessing eCO_2 effects on wheat, and NBI could be used to estimate plant %N directly in the field and as part of scientific research. As this was the first season to utilize this instrument, future measurements will be used to validate the relationship between NBI (and other indices) and leaf and plant %N from lab analysis. Given the initial results, weekly to bi-weekly fluorometer measurements in situ are recommended to track differences through the season and could reduce the need for chemical leaf analyses.

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