Estimating nitrogen concentration with a portable field fluorometer

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Abstract
Field-portable active light fluorescence sensors have the potential to provide non-destructive estimates of canopy N, particularly for use in field experiments to supplement tissue analysis. In previous results, a handheld portable fluorometer was related to nitrogen concentration of leaves and stems in wheat, with a positive, linear relationship (R^2 = 0.69). In this paper we expand on the previous results, using data collected as part of four separate experiments run at the Australian Grains Free Air CO2 Environment (AGFACE) facility during 2014-2016. The data represent a variety of treatment factors including CO_2 (elevated and ambient), several wheat varieties, a range of N fertiliser applications, and differences in seasonal water inputs (rainfed and supplemental). Fluorometer readings were made on the fresh intact leaves, stems and heads from biomass cuts at GS65. Results from the pooled data indicated that plant components had different relationships between N concentration and fluorometer measurements. Linear models were fit for green leaves to relate leaf %N to fluorometer NBI_G. Three of the individual experiments resulted in similar fitted slope and intercept parameters to the overall dataset. Likewise, model parameters for measurements on cv Yitpi and Wyalkatchem were similar. R^2 values ranged from 0.65 (2014 all treatments) to 0.25 (Wyalkatchem all years). Values for root mean square deviations (RMSD) ranged from 0.28 to 0.42 (%N). The regression models form the basis of a calibration(s) for field measurements. The desired outcome is to establish a robust calibration across cultivars and N levels that would allow measurements made in the field from DC31 through DC65 to estimate canopy N.

Keywords
Proximal sensing, fluorescence, phenotyping.

Introduction
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). These field-portable sensors have the potential to provide non-destructive estimates of canopy N, particularly for use in field experiments to supplement tissue analysis. In previous results (Perry and Fitzgerald 2015), a handheld portable fluorometer was related to nitrogen concentration of leaves, stems and heads in wheat. The nitrogen index 'NBI_G' (Agati et al. 2013) was found to have the highest correlation to %N, with a positive, linear relationship with leaves and stems (R^2 = 0.69). These results were based on data acquired during one season, at GS65 (flowering). In this paper we discuss results from three years' of data in order to build on previous results

Methods
Data were collected as part of four separate experiments run at the Australian Grains Free Air CO_2 Environment (AGFACE) facility during 2014-2016. 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 wheat plots with a variety of treatment factors including CO_2 (elevated to 550 ppm and ambient, 400 ppm), several wheat varieties, a range of N fertiliser applications, and differences in seasonal water inputs (rainfed and supplemental irrigation plots). Fluorometer readings were made in the lab on the fresh intact flag leaves, stems, heads, and senescent leaves at GS65. The plant materials were then dried, processed, and sent to a commercial laboratory (CSBP, Bibra Lake, WA) to determine N concentration for the comp.

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 to make measurements on fresh leaves cut for biomass. All measurements were made using the 6cm aperture in front

of the sensor head. Two to three measurements were made for each of the plant components (green leaves, senescent leaves, heads and stems). The measurements were averaged to produce a single value per experimental plot and plant component. 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). Based on the previous results, and preliminary analysis of the expanded dataset, the index NBI_G was selected. 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.

Results and Discussion

Results based on 2014 AGFACE data (Perry and Fitzerald 2015) indicated that the fluorometer measurements of leaf and stem components could be combined to form a linear relationship over a broader range of %N values, compared with using only the green leaf measurements. When NBI_G is plotted against corresponding N concentration from tissue analysis for all data from 2014-2016 (Figure 1), the results from

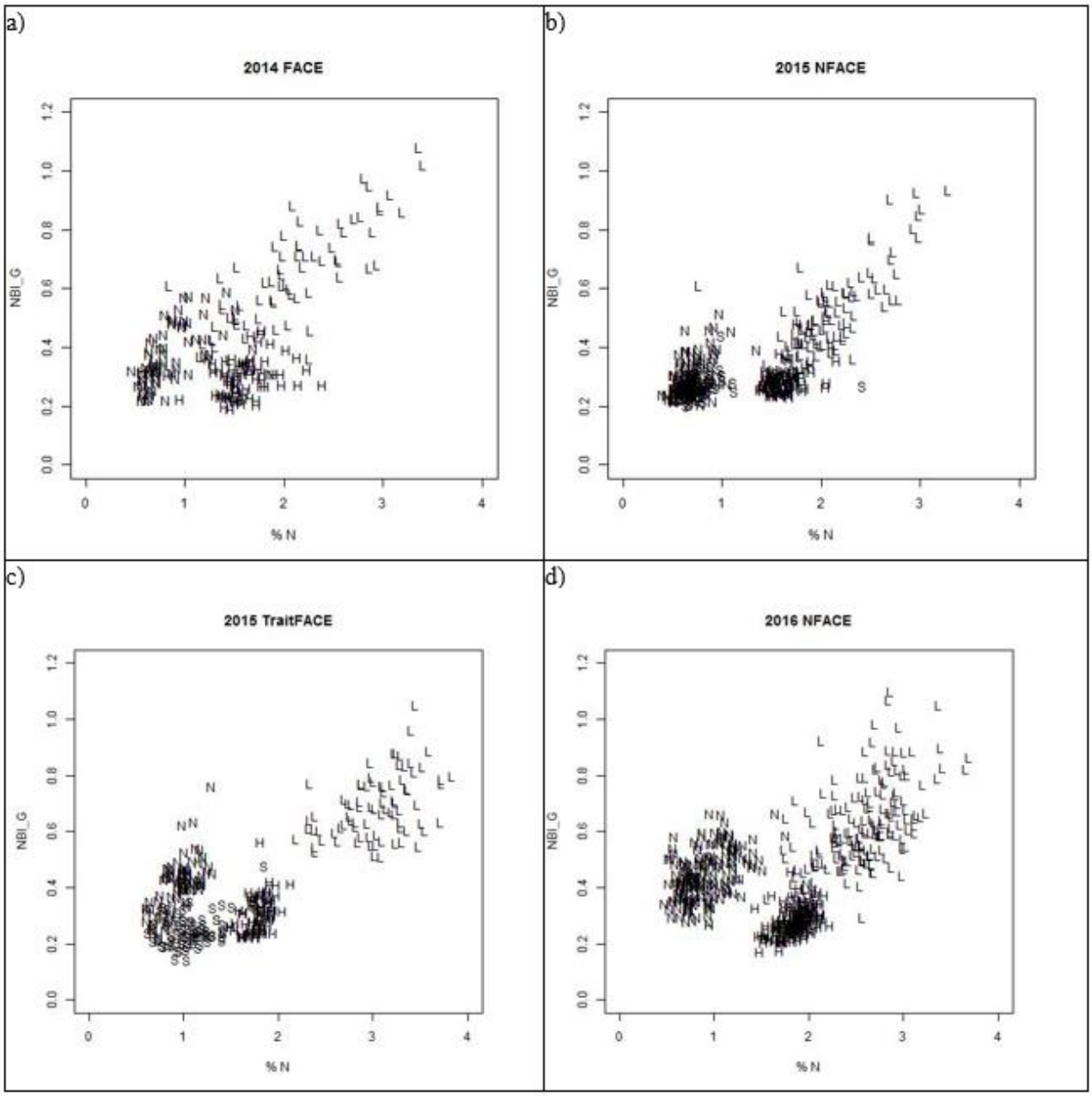

Figure 1. Fluorometer index 'NBI_G' plotted for corresponding tissue N concentration for a) 2014 AgFACE, b) 2015 NFACE, c) 2015 TraitFACE, and d) 2016 NFACE. The plotting symbols H, L, N, and S represent heads, leaves, stems, and senescent leaves respectively.

the additional data indicate different relationships between fluorometer measurements of the plant components and %N. The fluorometer measurements for the wheat heads are similar over the three seasons and four experiments, representing different varieties and treatments. This would indicate that the index value NBI_G is stable through time. While the senescent leaves and stems have similar N concentrations, the NBI_G values for the stems are higher. The index values for green leaves appear to have the most variability (scatter).

Given the apparent differences in relationships between the fluorometer measurements and N concentration by plant component, regression analysis was performed using only the green leaf data for the four experiments. Table 1 lists the linear regression results for leaf %N on the index NBI_G. These results indicate some differences among the datasets in model fitted values and R^2. The 2014 FACE experiment resulted in the highest R^2 (0.65), while the 2016 NFACE trial has the lowest ($R^2 = 0.26$). The 2016 data variability in the sensor response to treatment cannot be explained at this time. Selecting only the measurements on the cultivar 'Yitpi' (R^2 of 0.57) improved the relationship over the entire dataset (R^2 of 0.40). Overall, the fitted model parameters are similar for entire dataset, Yitpi and Wyalkatchem and have RMSD values between 0.33 and 0.42 (%N).

Table 1. Regression results for leaf %N on fluorometer index NBI_G

Leaf %N on NBI_R	N	Intercept (s.e.)	Slope (s.e.)	R^2	RMSD (%N)
All Years	383	1.12 (0.08)	2.10 (0.08)	0.40***	0.40
Yitpi Only	300	1.02 (0.10)	2.28 (0.14)	0.57***	0.33
Wyalkatchem Only	78	1.14 (0.23)	2.24 (0.43)	0.25***	0.42
2014 FACE	72	0.28 (0.16)	2.77 (0.241)	0.65***	0.35
2015 TraitFACE	32	1.24 (0.32)	2.37 (0.45)	0.46***	0.28
2015 NFACE	100	1.03 (0.11)	2.10 (0.20)	0.53***	0.27
2016 NFACE	179	1.75 (0.11)	1.35 (0.17)	0.26***	0.33

*Pr<0.05; **Pr<0.01; ***Pr<0.001

Conclusions

The regression models for leaf %N on the fluorometer index NBI_G form the basis of a calibration(s) for field measurements. The desired outcome is to establish a robust calibration across cultivars and N levels that would allow measurements made in the field from DC31 through DC65 to estimate canopy N. To achieve this, the next steps in the research include:

- Incorporate measurements made at DC31 with the DC65 data. This would effectively extend the range of %N values upwards.
- Compare the fluorometer measurements made in the field with the corresponding (same date) counterparts made in the lab to evaluate any differences.
- Determine how specific (e.g., by cultivar, and/or water treatment) the calibration relationships need to be.

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