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Remote estimation of chlorophyll on two wheat cultivars in two rainfed environments

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Abstract. For this study we hypothesise that the use of canopy chlorophyll content index (CCCI) and crop greenness will be useful in assessing crop nutritional status and provide a robust management tool by growth stage DC30 for fertiliser application across multiple sites without being confounded by soil and biomass differences. The objectives of this study were: (*i*) to study the robustness of the CCCI and greenness as a measure of crop N content at two different locations, and (*ii*) to validate the model developed for crop nitrogen (N) determination. Data were collected from two rainfed field sites cropped to wheat, one in Southern Italy (Foggia) and the other in the south-eastern wheat belt of Australia (Horsham). Data collection was conducted during the growing season in 2006–07 (December–June) for the Italian site and during the 2006 and 2007 (June–December) growing seasons for the Australian site. Measurements included crop biophysical properties (leaf area index (LAI), biomass, crop N concentration), hyperspectral remote sensing data, and SPAD (chlorophyll meter) determination. An independent dataset including SPAD, biomass, and remotely sensed data from Horsham (Australia) was used to test the validity of the model developed. Results showed that there is good correlation between SPAD and crop N content. The relationship between greenness (measured as LAI*SPAD) and CCCI was fitted with an exponential model and was not affected by biomass accumulation or soil reflectance ($r^2 = 0.85$; $y = 15.1e^{4.5424x}$; $P < 0.001$). When this model was tested on the independent dataset it yielded good results for the estimation of greenness ($y = 1.22x - 54.87$; $r^2 = 0.90$; $P < 0.001$; root mean square error 32.2; relative error 15%). In conclusion, SPAD measurements combined with LAI could be used as a crop nutritional management tool by DC30 for fertiliser application across multiple sites.

Additional keywords: canopy chlorophyll content index (CCCI), rainfed, remote sensing, SPAD, spectral.

Introduction

Nitrogen (N) is one of the most important nutrients for crop production, and N fertiliser is often applied to increase yields and maximise economic return. Several non-destructive techniques have been proposed and developed for assessing crop nutritional conditions and to adjust fertilisation accordingly. These techniques include popular hand-held chlorophyll meters, such as the Minolta SPAD-502 (Minolta Sensing Inc. 2003). Such instruments measure leaf red transmittance at 690 nm and near-infrared transmittance at 940 nm, which are found to be related to the chlorophyll level in leaves. The chlorophyll level in leaves is mainly influenced by N availability (Filella *et al*. 1995; Osborne *et al*. 2002); therefore, measurement of crop N with a chlorophyll meter can be a valuable tool in the diagnosis of N deficiency and in managing N fertiliser applications (Pinter *et al*. 1994; Blackmer and Schepers 1995; Bullock and Anderson 1998). Remote sensors used as a field-scale diagnostic method can also be used to detect N deficiency and have a management role in fertiliser application. However, they can potentially be used in an uncalibrated way to provide an absolute measure

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of crop nutritional status, thereby increasing their utility in the diagnosis of N deficiency and managing N fertiliser applications (Blackmer and White 1998).

Vegetation indices are mathematical combinations or ratios of various spectral bands that are designed to provide functional relationships of various crop characteristics. In agricultural crops, vegetation indices have been used to measure several crop parameters such as leaf area index (LAI), crop N, fraction of green cover, fraction of absorbed photosynthetically active radiation (fAPAR), and crop biomass (Asrar *et al*. 1984; Wiegand *et al*. 1990; Qi *et al*. 1994; Feng *et al*. 2008). Among the vegetation indices, the canopy chlorophyll content index (CCCI; Barnes *et al*. 2000) shows high potential to estimate crop N stress (El-Shikha *et al*. 2007). This is because it isolates the crop signal from soil reflectance as a function of changes in canopy cover and takes into consideration the relationship canopy cover *v*. canopy N (Rodriguez *et al*. 2005). The index was developed based on a two-dimensional approach with the use of the normalised difference vegetation index (NDVI; Rouse *et al*. 1973) as an estimate of canopy cover, and the normalised difference red edge (NDRE; Barnes *et al*. 2000) as a measure of crop N or chlorophyll content (Clarke *et al*. 2001). Recently, Fitzgerald *et al*. (2010) has shown that the CCCI was useful across a single experimental site over 3 years at an early stage of crop development (Zadoks DC30; Zadoks *et al*. 1974). They concluded that more research is required to: (*a*) validate the approach with independent locations and crop types, and (*b*) develop a robust method to estimate biomass non-destructively under chronic water stress and sparse canopy conditions using a combination of remote sensing and crop models.

One limitation of the CCCI is that its application earlier than growth stage DC30 is hampered by significant soil background reflectance, which reduces its ability to directly estimate crop nutritional status (Rodriguez *et al*. 2006). Additionally, applying the CCCI much later than DC30, for example at DC65, results in a significantly reduced opportunity for farmers to intervene with fertiliser management tactics. Another index, LAI*SPAD, is commonly referred to as a measure of canopy greenness, and several studies have shown that it can be used along with classical remotely sensed vegetation indices to assess N levels in canopies and canopy response to N fertilisation (Ma *et al*. 1996; Daughtry *et al*. 2000; Eitel *et al*. 2010). Canopy N content and the changes in chlorophyll levels in leaves are closely related to the readings of the SPAD meter multiplied by LAI (Filella *et al*. 1995). A combination of indices that have greater sensitivity to chlorophyll changes (LAI*SPAD) with indices that have greater sensitivity to LAI and canopy cover changes (vegetation indices) improves the estimation of crop N content under different amounts of canopy cover (Daughtry *et al*. 2000). Results are often varied because of confounding effects of biomass on vegetation indices at different levels of canopy cover (Serrano *et al*. 2000).The limitation of using classical vegetation indices such as the NDVI is that they are influenced by soil reflectance early in the season at low canopy cover (Solari *et al*. 2008). Also, at LAI values >2, the NDVI is insensitive to further changes of LAI and biomass (Serrano *et al*. 2000).

For this study we hypothesise that the use of CCCI and crop greenness will be useful in assessing crop nutritional status and provide a robust management tool by growth stage DC30 for fertiliser application across multiple sites without confounding by soil and biomass differences. The objectives of this study were to study the robustness of the CCCI and canopy greenness as a measure of crop N content at two different locations, and to validate the model developed for crop N determination.

Materials and methods

Experiment description

Data were collected from two rain-fed field sites cropped to wheat, one in Southern Italy (Foggia) and the other in the south-eastern wheat belt of Australia (Horsham). Data collection was conducted during the growing season in 2006–07 (December–June) for the Italian site and during the 2006 and 2007 (June–December) growing seasons for the Australian site.

Foggia, Italy

The experiment was carried out at the Cereal Research Centre, Foggia, Italy (41°28′N, 15°32′E; elev. 75 m). Durum wheat (*Triticum durum* Desf. cv. Ofanto) was sown on 10 Dec 2006 (day of year (DOY) 344) in a north-west to south-east direction with a row spacing of 17 cm on a clay-loamy soil according to the USDA particle-size distribution limits. The experiment was part of a long-term monoculture wheat system, with two levels of N (0 and 90 kg N/ha). The experimental design consisted of two plots (6 by 65 m), separated from each other by 5 m of bare soil. One plot received 90 kg N/ha as a split application for 17 years: one application at sowing with 25 kg N/ha as diammonium phosphate, the other at tillering with 65 kg N/ha as urea. The second plot had received no N for 17 years. Each plot was divided into three subplots (each 6 by 20 m). In each subplot, measurements were collected at five different 1-m^2 areas. Two of those five areas were used for destructive determination of biomass content and crop N content on 15 March and 6 April 2007. Non-destructive measurements (LAI, SPAD, reflectance measurements) were collected in 2007 on 1 February (DOY 32), 12 February (DOY 43), 15 March (DOY 74), 6 April (DOY 96), 10 May (DOY 130), and 14 May (DOY 134); the subplots were harvested on 6 June (DOY 155).

The LAI was measured non-destructively by using a portable LI-COR LAI 2000 (LI-COR Biosciences, Lincoln, NE, USA) on the same five points inside each subplot and at the dates mentioned above. Chlorophyll measurements were indirectly determined with the use of the SPAD chlorophyll meter (Minolta Sensing Inc. 2003). Readings were made on the fully expanded upper leaf for each date of sampling by measuring the light transmitted by leaves in the wavelengths at 650 nm and 940 nm. For each point of sampling, measurements were taken on the upper leaves of 10 plants inside 1 m^2. The measurements were taken in the field at two different points of the leaf, the mid-low and mid-high position, and then averaged to give one reading per leaf.

Aboveground biomass was determined by drying the fresh samples in an oven at 75°C for 24 h. Crop N concentration was measured with the Kjeldahl method, and N content was obtained by multiplying the N concentration by dry weight.

Horsham, Australia

The Horsham field experiment was at the Victorian Department of Primary Industries, Plant Breeding Centre (36°45′S, 142°06′E; elev. 120 m). The experimental facility 'Australian Grains Free Air Carbon Dioxide Enrichment (AGFACE)' and the experimental setup have been described in Mollah *et al*. 2009; Fitzgerald *et al*. 2010, respectively. In summary, in 2007 wheat (*Triticum aestivum* L. cv. Yitpi) was sown in a Latin square design with CO_2 concentration (550 ppm and ambient 380 ppm) and irrigation level (rainfed and supplemental) randomised at the ring level. Time of sowing was randomised at the half-ring level. Within each half-ring there were six subplots, each 1.7 by 4 m, randomised for cultivar (Yitpi and Janz) by N level (0 and 80 kg N/ha). Janz had only the 0 kg/ha level of N and was not sampled or considered for this study. The N was split with 30 kg N/ha as urea at sowing and 50 kg N/ha at tillering. Three of the six plots in each half-ring were used for destructive samplings, at DC30 and DC65, with the remaining three used for yield determination. Destructive measurements included aboveground biomass (kg/ha), total crop N content (g N/kg), and LAI (m^2/m^2).

The samples considered for this study were those obtained on 15 September and 29 October 2007, which corresponds to growth stages DC31 and DC65. Chlorophyll measurements were indirectly determined with the use of the SPAD chlorophyll meter (Minolta Sensing Inc. 2003). For each plot, 10 plants were randomly chosen and one leaf per plant was used for SPAD readings. On each leaf, two SPAD readings were collected. Canopy greenness was calculated as the product of the LAI and the SPAD readings.

Data were also obtained from a second nearby experiment in 2006 (Tilling *et al*. 2007). This design consisted of a single seeding rate and two N treatments (0 and 39 kg N/ha) with two irrigation treatments (142 and 275 mm) and was used as a validation dataset.

Remotely sensed data

For both sites, a hand-held hyperspectral radiometer, FieldSpec® Pro portable spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA; Analytical Spectral Devices 2002) was used to measure the reflected light from the canopy and the soil. The instrument's spectral range is 325–1075 nm with bandwidth 10 nm, and with sensor field of view of 25°. For both sites, all spectral readings were conducted under clear sky conditions and measurements were converted to reflectance by referencing a 99% Spectralon (Labsphere Inc., USA; Labsphere 1998) panel at various times during each sampling.

Index development

The CCCI was derived from wavelengths at 670, 720, and 790 nm that had bandwidths of 25, 10, and 25, respectively. Its derivation is shown in Fig. 1. The NDRE is used as surrogate for canopy N estimation, while the NDVI is used as a measure of groundcover, allowing quantification of canopy nutritional status at any groundcover. This is because it compensates for groundcover within the field of view of the instrument (Fitzgerald *et al*. 2006). Moreover, the spectral band at 720 nm has been found to further reduce the interference of soil reflectance on the overall

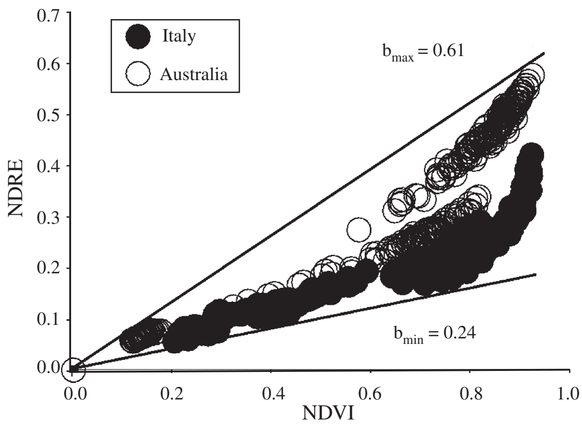

Fig. 1. Canopy chlorophyll content index (CCCI) development from the relationship between normalised difference red edge index (NDRE) and normalised difference vegetation index (NDVI) for the Italian and Australian sites at growth stages DC30 and DC65, for the 2006–07 growing season for the Italian site and the 2007 growing season for the Australian site.

reflectance (Daughtry *et al*. 2000). Two boundaries, NDREmax and NDREmin, are the maximum and minimum slopes with zero intercept that enclose all the points of the NDRE *v.* NDVI relationship. Their derivation is explained in detail in Fitzgerald *et al*. (2006, 2010) but is summarised below:

$$\mathrm{NDVI} = (790\,\mathrm{nm} - 670\,\mathrm{nm})/(790\,\mathrm{nm} + 670\,\mathrm{nm}) \quad (1)$$

$$\mathrm{NDRE} = (790\,\mathrm{nm} - 720\,\mathrm{nm})/(790\,\mathrm{nm} + 720\,\mathrm{nm}) \quad (2)$$

$$\mathrm{CCCI} = (\mathrm{NDRE} - \mathrm{NDRE_{min}})/(\mathrm{NDRE_{max}} - \mathrm{NDRE_{min}}) \quad (3)$$

For this study, the upper ($NDRE_{max}$) and lower ($NDRE_{min}$) boundaries for the CCCI calculation for the growing season 2006–07 for both Australia and Italy were 0.62 and 0.24, respectively. The upper and lower limits for the 2006 study were the ones developed by Fitzgerald *et al*. (2010) and were 0.61 and 0.34, respectively.

Statistical analyses

For both Italian and Australian sites, the experimental designs were analysed using ANOVA. For each site and each growth stage, a correlation matrix between the parameters studied was obtained using the Pearson product moment correlation. Regression analyses for the CCCI *v.* greenness relationship were made for both experimental sites at DC30, and the best fit was obtained with the curve estimator procedure in GENSTAT. All statistical analyses were performed using GENSTAT 10th edition (Lawes Agricultural Trust 2007).

Data from the AGFACE experiment were used for this study because its aim is to understand the feasibility of using the CCCI and greenness as a measure of crop nutritional status. CO_2 and irrigation treatments did not significantly affect crop growth and nutritional content by DC30 and they were therefore adequate for testing our hypothesis (Smith 2007). The validation of the greenness estimation was evaluated by using absolute and relative measures of error, the root mean square error (RMSE) and relative error (RE), respectively.

Results

Biomass values at the Australian site were in the range 48–66 g/m^2 by DC30 and 525–823 g/m^2 by DC65. At the Italian site, values were in the range 104.5–191.22 g/m^2 by DC30 and 287.6–486.8 g/m^2 by DC65. Crop N concentration (%) at the Australian site was in the range 2.3–5.07% by DC30 and 2.58–3.42% by DC65. The Italian site showed values in the range 2.71–3.01% by DC30 and 1.84–2.37% by DC65 (Tables 1 and 2). LAI values were higher for the Italian site than the Australian site at both growth stages, with an average of 1 for 0 N and 1.7 for 90 kg N/ha by DC30, and 2.7 for 0 N and 3.1 for 90 kg N/ha by DC65. The Australian site, for the growing season 2007, showed LAI values of 0.5–0.6 for both 0 and 90 kg N/ha by DC30 and 1.1–1.8 for 0 N and 1.1–2.4 for 90 kg N/ha by DC65 (Table 2). SPAD values were higher for the Italian site and for 90 kg N/ha, whereas 0 N SPAD readings were lower for Italian site than the Australian site (Tables 1 and 2).

Pearson product moment correlations between the crop parameters for the two sites are shown in Tables 3 and 4. For both sites the correlation between LAI and biomass was highly significant by DC30 and DC65 (pseudo-stem elongation and anthesis; Zadoks *et al.* 1974); the same trend was observed between LAI and crop N content (g N/m^2). For the Australian site, crop N% did not show significant correlations with SPAD at both growth stages (Table 3). At the Italian site, most of the correlations are higher than the Australian counterpart, especially for N% and SPAD readings (Table 4). Overall, there was a strong correlation between greenness and crop N content (g N/m^2) for the Australian and Italian sites at both growth stages (Tables 3 and 4). Plant N concentration (N%) was significantly correlated with SPAD readings for the Italian site at both growth stages, but weak correlations were obtained at the Australian site (Tables 3 and 4).

The relationship between NDVI and crop biomass is shown in Fig. 2*a*. The index levels out at biomass values >100 g/m^2, in contrast to the relationship shown by CCCI *v.* biomass (Fig. 2*b*). The CCCI does not show any saturation at high values of crop biomass, with the Italian data (closed circle) lying in the same data space as the Australian data (open circle). The use of CCCI and crop greenness is shown in Fig. 3*b*. The CCCI reaches a saturation level at greenness values of ~175. The r^2 of the model was 0.85, with the following equation: $\text{greenness} = 15.1e^{4.5424\text{CCCI}}$ ($P < 0.001$). This model was then successfully validated on an independent dataset taken at Horsham for the 2006 growing season ($y = 1.22x - 54.8$; $r^2 = 0.90$, RMSE 32.2, RE 15%; Fig. 4). There was a poor relationship between SPAD and for the Italian and Australian data at DC 30 (Fig. 3*a*).

Table 1. Mean wheat biophysical variables for Foggia (Italy), for the growing season 2006–07 at growing stages DC30 and DC65 for cv. Ofanto

LAI, Leaf area index. Values are means with standard error of mean in parentheses ($n = 12$)

N rate (kg/ha)	Growth stage	Biomass (g/m^2)	LAI (m^2/m^2)	Crop N (%)	Crop N (g N/m^2)	SPAD (unitless)
0	DC30	104.5 (3.14)	1.06 (0.04)	3.01 (0.24)	3.1 (0.08)	42.5 (1.2)
0	DC65	287 (11.2)	1.7 (0.11)	1.8 (0.07)	5.3 (0.3)	27.3 (0.67)
90	DC30	191.2 (5.41)	2.70 (0.17)	2.70 (0.018)	5.2 (0.13)	48.2 (0.9)
90	DC65	487 (4.0)	3.1 (0.14)	2.3 (0.12)	11.5 (0.5)	36.4 (1.08)

Table 2. Mean wheat biophysical variables for Horsham (Australia), for the growing season 2007 at growing stages DC30 and DC65 for cv. Yitpi

At DC30 none of the treatments had a significant effect on biomass, leaf area index (LAI), and crop N. Values are means with standard error of mean in parentheses ($n = 4$)

Treatment	Growth stage	Biomass (g/m^2)	LAI (m^2/m^2)	Crop N (%)	Crop N (g N/m^2)	SPAD (unitless)
No CO_2, irrigation, 0 N	DC30	50.25 (8.34)	0.61 (0.13)	5.04 (0.05)	2.54 (0.43)	50.87 (1.42)
No CO_2, irrigation, 0 N	DC65	573 (49.34)	1.76 (0.04)	1.6 (0.27)	9.40 (1.45)	55.72 (1.48)
No CO_2, irrigation + N	DC30	45.50 (10.9)	0.49 (0.13)	5.06 (0.06)	2.29 (0.53)	50.83 (2.65)
No CO_2, irrigation + N	DC65	721 (56.56)	2.07 (0.08)	1.9 (0.09)	13.7 (1.5)	54.78 (1.65)
CO_2, irrigation, 0 N	DC30	49.50 (5.54)	0.52 (0.087)	2.30 (0.33)	4.61 (0.18)	48.72 (1.25)
CO_2, irrigation, 0 N	DC65	781 (49.42)	1.83 (0.11)	1.7 (0.15)	13.08 (1.30)	54.08 (2.63)
CO_2, irrigation + N	DC30	53.50 (5.95)	0.57 (0.097)	4.67 (0.11)	2.51 (0.33)	49.62 (2.68)
CO_2, irrigation + N	DC65	823 (61.04)	2.40 (0.49)	1.9 (0.15)	15.33 (2.60)	57.42 (0.93)
CO_2, no irrigation, 0 N	DC30	51.75 (5.51)	0.54 (0.068)	4.37 (0.21)	2.28 (0.32)	49.38 (1.46)
CO_2, no irrigation, 0 N	DC65	602.2 (18.44)	1.12 (0.14)	1.6 (0.08)	9.70 (0.33)	56.36 (1.61)
CO_2, no irrigation + N	DC30	58.50 (8.74)	0.64 (0.089)	4.80 (0.10)	2.82 (0.46)	52.58 (1.41)
CO_2, no irrigation + N	DC65	759.5 (119.1)	1.33 (0.31)	1.7 (0.18)	13.40 (3.70)	57.45 (0.80)
No CO_2, no irrigation, 0 N	DC30	56.25 (10)	0.62 (0.10)	4.95 (0.10)	2.79 (0.51)	49.54 (1.68)
No CO_2, no irrigation, 0 N	DC65	631.5 (110.8)	1.40 (0.27)	2.2 (0.38)	14.21 (4.11)	57.70 (0.47)
No CO_2, no irrigation + N	DC30	53.00 (5)	0.58 (0.075)	5.07 (0.13)	2.70 (0.30)	49.26 (0.68)
No CO_2, no irrigation + N	DC65	566.2 (12.4)	1.15 (0.10)	2.06 (0.19)	11.74 (1.20)	56.59 (1.36)

Table 3. Correlation matrix between canopy biophysical variables at Horsham (Australia) for the growing season 2007 at growing stages DC30 and DC65

LAI, Leaf area index; SPAD, chlorophyll meter. $^{**}P<0.01$; $^{***}P<0.005$; n.s., not significant

	Biomass	LAI	Crop N (%)	Crop N (g N/m^2)	SPAD	Greenness
			DC30			
Biomass	1					
LAI	0.95***	1				
Crop N (%)	0.22n.s.	0.32n.s.	1			
Crop N (g N/m^2)	0.98***	0.94***	0.41**	1		
SPAD	–0.37**	–0.41**	0.21n.s.	–0.36**	1	
Greenness	0.94***	0.98***	0.32n.s.	0.94***	–0.23n.s.	1
			DC65			
Biomass	1					
LAI	0.73***	1				
Crop N (%)	0.17n.s.	0.25	1			
Crop N (g N/m^2)	0.82***	0.69***	0.68***	1		
SPAD	–0.10	–0.32	–0.75	–0.18	1	
Greenness	0.74***	0.99***	0.25	0.68***	–0.18	1

Table 4. Correlation matrix between canopy biophysical variables at Foggia (Italy) for the growing season 2006–07 at growing stages DC30 and DC65

LAI, Leaf area index; SPAD, chlorophyll meter. $^{**}P<0.01$; $^{***}P<0.005$; n.s., not significant

	Biomass	LAI	Crop N (%)	Crop N (g N/m^2)	SPAD	Greenness
			DC30			
Biomass	1					
LAI	0.99***	1				
Crop N (%)	–0.92***	–0.90***	1			
Crop N (g N/m^2)	0.98***	0.97***	–0.88***	1		
SPAD	0.67***	0.68***	–0.43	0.69***	1	
Greenness	0.98***	0.99***	–0.88***	0.98***	0.74***	1
			DC65			
Biomass	1					
LAI	0.94***	1				
Crop N (%)	0.60***	0.43**	1			
Crop N (g N/m^2)	0.90***	0.81***	0.85***	1		
SPAD	0.79***	0.70***	0.53**	0.80***	1	
Greenness	0.96***	0.96***	0.50**	0.85***	0.84***	1

Discussion

The CCCI and canopy greenness index appear robust measures of crop N content regardless of the geographical location used in this study. Greenness is correlated with the amount of canopy N content (g N/m^2) at early stages in both sites (Tables 3 and 4), and this result agrees with findings of Filella *et al*. (1995). The canopy greenness index achieves its utility from LAI. SPAD meter readings are single-point measurements made somewhere on a leaf; they do not necessarily match the hand-held remote sensing measurements that are made on a canopy over an area. It is, therefore, not unexpected that SPAD and CCCI were not related because they are measuring different things (Fig. 3*a*), but when SPAD is combined with LAI, then greenness and CCCI show robust predictability across two diverse dryland sites (Fig. 3*b*).

The relationship between greenness and a vegetation index must not be influenced by the amount of biomass accumulated by the crop and the percentage of soil background, because it diminishes the sensitivity in detecting changes in canopy N early in the season (Filella *et al*. 1995). Using CCCI instead of the NDVI improves the estimation of canopy N content (Fig. 3*b*) because at such early stage (DC30), biomass differences did not influence the CCCI response at either site. The index is based on the NDVI and the NDRE. The former is used as a surrogate for canopy cover but our results show that it saturates at wheat crop biomass levels ~100 g/m^2. This is because the NDVI is influenced by two factors: the effects of plant growth, which causes scattering of the near infrared reflectance within the leaves as further vegetation layers are added (Asrar *et al*. 1984; Carlson and Ripley 1997; Serrano *et al*. 2000), and the simultaneous effects of chronic and acute water stress, which influence the ratio of near infrared and visible light reflected by the crop (Abuzar *et al*. 2009). The latter component (NDRE) is a normalised difference index in which the electromagnetic band at 720 nm is used as a reference point, because at this wavelength, soil and

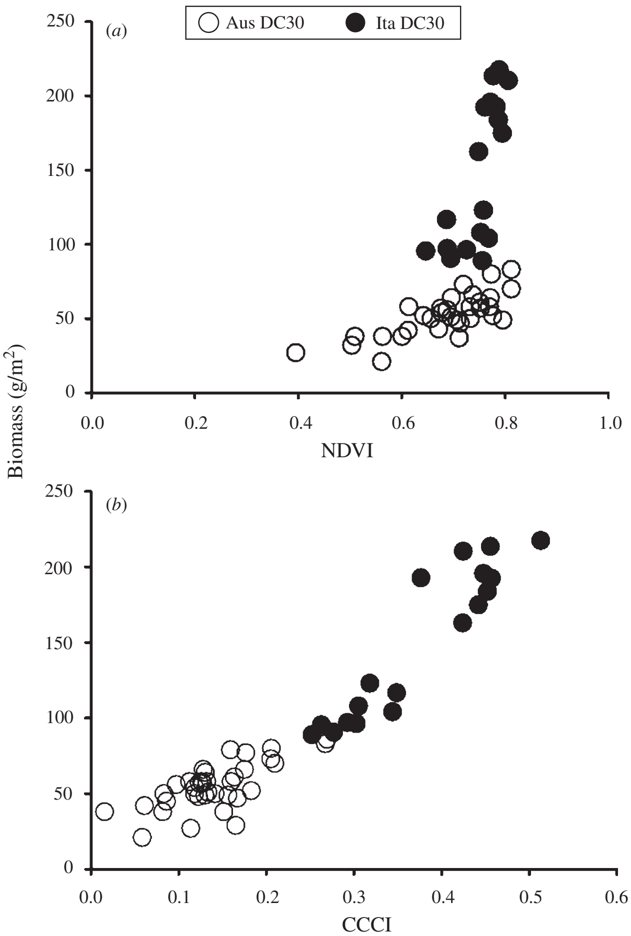

Fig. 2. Relationship between (*a*) normalised difference vegetation index (NDVI) and crop biomass, and (*b*) canopy chlorophyll content index (CCCI) and crop biomass, for the Australian dataset (○) and Italian dataset (●) at growing stage DC30 (stem elongation).

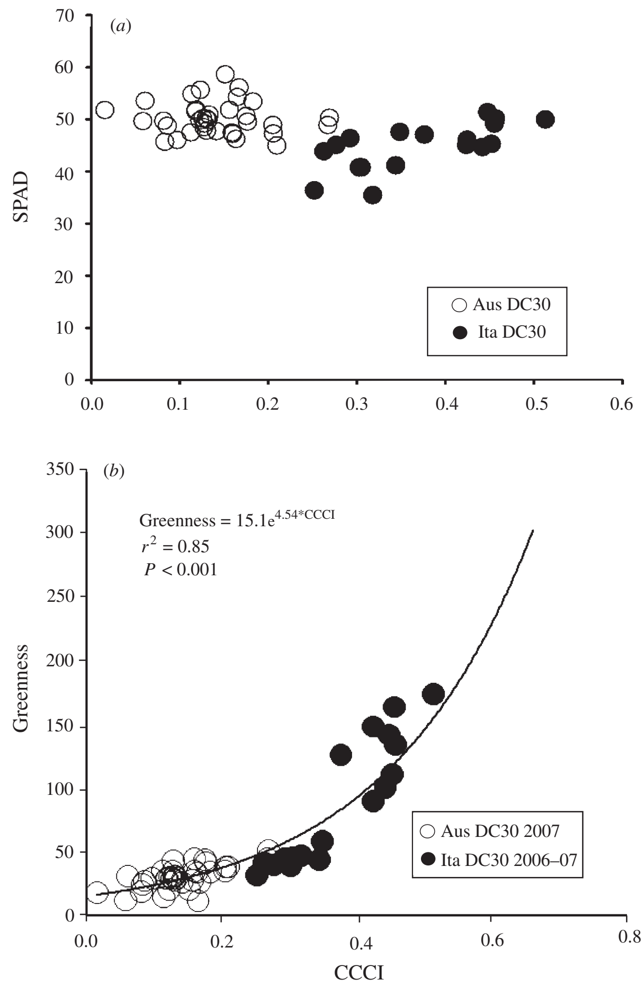

Fig. 3. Relationship between (*a*) the canopy chlorophyll content index (CCCI) and the SPAD readings, and (*b*) CCCI and greenness, for the Australian dataset (○) and Italian dataset (●) at growing stage DC30 (stem elongation).

plant reflectance values are similar. Therefore, it is used to minimise the soil reflectance effects on the plant signal and compensates somewhat for saturation shown by the NDVI at high biomass.

In summary, the relationship between CCCI and greenness provides an estimate of canopy nutritional status in rainfed environments using remote sensing. Further research is needed to validate the relationship with other locations, over several years, and to study the effects of chronic water stress on the linear behaviour of CCCI *v.* biomass. This is the first step for possible applications of the CCCI for detecting canopy nutritional status early in the season. Moreover, crop biomass and canopy N content can be measured using three wavelengths (670, 720, and 790 nm, which are the wavelengths used to determine the NDVI and the NDRE) in a 3-CCD digital camera whose images use three separate charge-coupled devices (CCDs). Each one of the CCD takes separate measurements of red, green, or blue light, achieving better precision than single-CCD cameras.

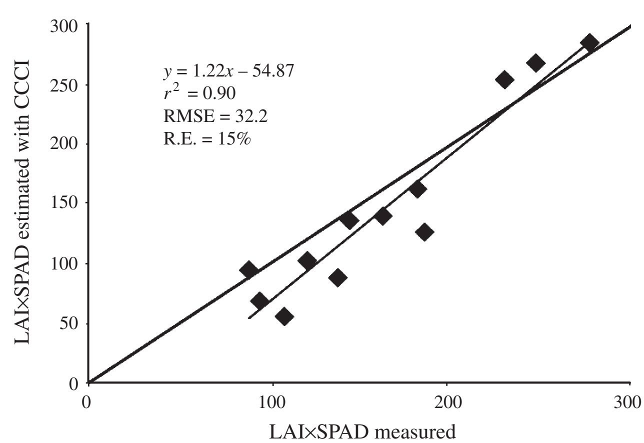

Fig. 4. Validation of the model for estimating greenness using the equation developed from the relationship in Fig. 3*b*, from an independent dataset (Horsham, Australia, 2006). The model performance resulted in an r^2 of 0.90, a root mean square error 32.2, and relative error of 15%.

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