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Detection of nitrogen deficiency in wheat from spectral reflectance indices and basic crop eco-physiological concepts

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Abstract. We tested the capacity of several published multispectral indices to estimate the nitrogen nutrition of wheat canopies grown under different levels of water supply and plant density and derived a simple canopy reflectance index that is greatly independent of those factors. Planar domain geometry was used to account for mixed signals from the canopy and soil when the ground cover was low. A nitrogen stress index was developed, which adjusts shoot %N for plant biomass and area, thereby accounting for environmental conditions that affect growth, such as crop water status. The canopy chlorophyll content index (CCCi) and the modified spectral ratio planar index (mSR_{Pi}) could explain 68 and 69% of the observed variability in the nitrogen nutrition of the crop as early as Zadoks 33, irrespective of water status or ground cover. The CCCi was derived from the combination of 3 wavebands 670, 720 and 790 nm, and the mSR_{Pi} from 445, 705 and 750 nm, together with broader bands in the NIR and RED. The potential for their spatial application over large fields/paddocks is discussed.

Additional keywords: water stress, plant density, remote sensing, nitrogen stress index.

Introduction

The successful adoption of in-season site-specific application of nitrogen (N) fertilisers depends on our capacity to identify areas in a field having differential responses to N. Rapid, reliable, and relatively inexpensive estimates of crop N status can be made from the analysis of stem juices (Follett *et al*. 1992) or from estimates of leaf chlorophyll content (Wood *et al*. 1993). However, these are point estimates that are of limited use when large paddocks need to be analysed.

Remotely sensed spectral indices derived from the reflectance of whole canopies in the green and near infrared have been successfully applied to reduce in-crop N inputs without reducing grain yield in irrigated maize (Bausch and Diker 2001). When sunlight reaches the crop, most of the irradiance is consumed by water transpiration and a small fraction is used in CO_2 assimilation. Part of the light energy is transmitted through the canopy, and part is reflected back towards the observer. Reflected light in the visible region of the electromagnetic spectrum is influenced by the presence of chlorophyll pigments in the leaf tissues, which have been found to relate to the concentration of leaf nitrogen (Thomas and Gausman 1977; Wessman 1990). There are 2 main absorption bands, one in the blue (450 nm) and another in the red (670 nm), which are due to the absorption of the 2 main leaf pigments, chlorophyll *a* and *b*. These account for about 65% of the total concentration of pigments in higher plants. Approximately 75% of the plant's total nitrogen is contained in the chloroplasts, mostly in Rubisco and chlorophyll binding proteins (Lawlor 1993). Therefore, remote sensing of chlorophyll content offers the possibility of rapidly estimating crop N status (Blackmer *et al*. 1986). However, as small amounts of chlorophyll are sufficient to saturate absorption in the 660–680 nm region, reducing the sensitivity of spectral indices, empirical models to predict chlorophyll content are usually based on reflectance far from pigment absorption maxima, e.g. in the 550 or 700 nm regions. On the other hand, to obtain maximal sensitivity of the pigment estimation, wavelengths have to be chosen as close as possible to the absorption bands. Another spectral area of considerable interest has been the region between the strong red light absorption by chlorophyll (680 nm) and the highly reflective near-infrared wavelengths (780 nm) (Barnes *et al*. 2000). This region of the spectrum has been referred to as the 'red edge', and several red edge indices have been described (Vogelmann *et al*. 1993; Filella and Peñuelas 1994; Barnes *et al*. 2000). In nitrogen deficient

 10.1071/AR05361 0004-9409/06/070781

crops, chlorophyll reduction causes light reflectance in the visible range (400–700 nm) to increase (Yoder and Pettigrew-Crosby 1995). Relative spectral indices of the N nutrition of wheat crops have been developed from comparisons with readings from well-fertilised strips (Raun *et al*. 2001) or calibration stamps (Raun *et al*. 2005). This avoids the need for calibrating the relationship between nitrogen content and crop reflectance, different growth stages or cultivars, and the need to convert data to surface reflectance factors (Pinter *et al*. 2003).

In field crops, the main source of variation is the simultaneous presence of water and N stress (Osborne *et al*. 2002). Moran *et al*. (1989) found that water-stressed canopies of alfalfa had a lower spectral reflectance in both the NIR and red wavebands due to changes in canopy architecture. They also found that the perpendicular vegetation index (PVI; Richardson and Wiegand 1977) decreased with stress-induced changes in the architecture of the crop, whereas the NIR/red ratio remained relatively constant. Bowman (1989) showed that the reflectance from cotton (*Gossypium hirsutum* L.) canopies in the near infrared and shortwave infrared regions (810, 1665, and 2210 nm) increased as the relative water content of the leaves decreased. Thus, the spectral signatures from nutrient deficiencies change when water deficiency is present and this change reduces, if not removes, the symptom-specific spectral characteristics. In addition, the spectral signature of water-stressed crops can be further confounded by the increasing proportions of bare soil and the reduced signals from smaller canopies.

In this work, we (*a*) tested whether changes in canopy structure and canopy properties, as induced by contrasting crop densities and water regimes, would affect our capacity to predict the nitrogen status of wheat canopies from ground-based multispectral canopy reflectance indices; and (*b*) developed a simple methodology to identify nitrogen-stressed wheat canopies of crops growing under contrasting plant densities and levels of water supply.

Material and methods

Crops, treatments, and experimental design

A field experiment was conducted at Horsham, Vic, Australia (36.65°S, 142.10°W) on a Horsham clay, a Grey Vertisol (Isbell 1966). At sowing, soil mineral nitrogen content was 180 kg N/ha (0–1 m), soil phosphorus content 30 mg/kg (0–0.2 m) (Colwell 1963), and soil pH_{H_2O} was 8.8 (0–1 m). Wheat (*Triticum aestivum* L. cv. Chara) was sown on 17 June 2004. At sowing a triple super phosphate fertiliser (46% P_2O_5) was applied to the entire experimental site at a rate of 61 kg fertiliser/ha. The crop emerged 9 days after sowing. Two seeding rates (52 and 102 kg seed/ha), 4 rates of nitrogen fertiliser (0, 16, 39, and 163 kg N/ha) applied as urea (46% N), and 2 water regimes (irrigated and rainfed), were combined in a factorial experiment with 3 replicates. Treatments were arranged in a split-plot design where nitrogen and density levels were randomised to subplots within the irrigation main plots. The irrigated plots were watered using an automatic sprinkler system every time the accumulated rainfall deficit reached 20–50 mm from the decile 9 of historical in-crop rainfall in Horsham (SILO station #79023, Polkemmet). Rainfall was 270 mm for the rainfed treatment, and rainfall plus irrigation was 390 mm for the irrigated treatment, corresponding to deciles 5.3 and 9.3 at Horsham, respectively. There were 6 irrigation events at 76, 93, 100, 113, 121 and 149 days after emergence (DAE). Weeds, pests, and diseases were frequently monitored and controlled as required. Further experimental details can be found in Rodriguez *et al*. (2005).

Remote sensing of canopy properties and crop measurements

Samplings took place at 51, 66, 90, 106, and 118 DAE, coinciding with 4 leaves unfolded (Zadoks 14), early tillering (Zadoks 30), mid tillering (Zadoks 33), booting (Zadoks 47), and anthesis (Zadoks 60), respectively. At each sampling date, canopy reflectance measurements were made using a portable spectroradiometer (FieldSpec Pro JR ASD, Co, USA) with a 25° field of view. The spectroradiometer has a range of 350–2500 nm with spectral resolution varying between 3.0 and 10 nm, depending on wavelength (ASD 2002). Readings were taken under clear-sky conditions in reflectance mode, after standardisation with a level 99% Spectralon panel (Spectralon, Labsphere Inc., North Sutton, NH). Data were collected at 40–50° solar zenith angle in a nadir orientation. The spectroradiometer was mounted on a 4-wheel drive motorbike, and the sensor optic head was fitted at the end of a 2-m-long boom and held at 3 m above the soil surface, yielding a spot size of 1.39 m^2. Two recordings, consisting of averages of 25 scans, were taken from each plot at each sampling. Immediately after completing the readings of canopy reflectance, samples of shoots were collected for measurement of leaf area, whole shoot dry weight (stem, sheath + laminae, and ears), and shoot nitrogen percent (shoot N%). Leaf areas were recorded using a leaf area meter (LI-Cor 3000, LI-Cor Inc., NE, USA) and total nitrogen was determined using a LECO CN2000 (LECO Cor., MI, USA). The sampled area consisted of 3 consecutive rows, 0.5-m-long spaced at 0.27 m, yielding ≈0.4 m^2 within the field of view of the spectroradiometer.

Data analysis

Several reflectance bands and published spectral reflectance indices (Table 1) were derived from the hyperspectral measurements of canopy reflectance taken with the FieldSpec spectroradiometer. Using shoot N% as the dependent variable in a partial least squares (PLS) regression modelling, important reflectance indices were identified from their loading weights (Wold *et al*. 1984). Loading weights are specific to PLS and express how the information in each independent variable relates to the variation in the dependent variable. Independent variables with large loading weight values are important for the prediction of the dependent variable. Calculations were carried out using Unscrambler *v*.7.5 (CAMO, ASA, Norway), a chemometric software package for multivariate data analysis.

When analysing mixed spectral signals from heterogeneous canopies composed of soil and vegetation elements within the field of view of the sensor, a methodology is required to derive meaningful indices representing the quality of the desired target, e.g. shoot N% in the vegetation. Planar domain indices were used to improve the prediction of shoot N% by minimising the interference from a changing proportion of bare soil (Clarke *et al*. 2001). Planar domain indices are created by measuring the proportion of the target component, i.e. vegetation, as its percentage in the whole, i.e. vegetation plus soil elements in the field of view of the instrument, and uses this variable to evaluate the quality of the signal. Hence, 2 indices are needed, one to measure the proportion of the element in the whole, and the other to define some intrinsic property of the element which is of interest (Clarke *et al*. 2001). In this work we used the normalised difference vegetation index (NDVI) to represent the proportion of crop in the field of view of the spectrometer, and 2 other spectral indices as indicators of the nitrogen status of the crop element.

Table 1. List of acronyms, bands and reflectance indices, estimated biological parameters, and references analysed in this work

Index	Formulation	Estimated biological parameter	Reference
NDVI	$\frac{NIR-Red}{NIR+Red}$	Green biomass	Gamon *et al.* (1995)
GNDVI	$\frac{R_{780}-R_{550}}{R_{780}+R_{550}}$	Green biomass	Gitelson and Merzlyak (1994)
SAVI	$1.5\cdot\frac{R_{800}-R_{670}}{R_{800}+R_{670}+0.5}$	Green biomass	Huete (1988)
WI	$\frac{R_{900}}{R_{970}}$	Water content	Peñuelas *et al.* (1993)
Chl I	$\frac{R_{740}}{R_{720}}$	Chlorophyll	Vogelmann *et al.* (1993)
NDI1	$\frac{R_{750}-R_{705}}{R_{750}+R_{705}}$	Chlorophyll	Gitelson and Merzlyak (1994)
NDI2	$\frac{R_{780}-R_{710}}{R_{780}-R_{680}}$	Chlorophyll	Datt (1999)
NDI3	$\frac{R_{850}-R_{710}}{R_{850}-R_{680}}$	Chlorophyll	Datt (1999)
mND	$\frac{R_{750}-R_{705}}{R_{750}+R_{705}-2R_{445}}$	Chlorophyll	Sims and Gamon (2002)
mSR	$\frac{R_{750}-R_{445}}{R_{705}-R_{445}}$	Chlorophyll	Sims and Gamon (2002)
NDRE	$\frac{R_{790}-R_{720}}{R_{790}+R_{720}}$	Nitrogen status	Barnes *et al.* (2000)

NDVI, Normalised difference vegetation index; GNDVI, green normalised difference vegetation index; SAVI, soil adjusted vegetation index; WI, water index; Chl I, chlorophyll index; NDI 1–3, Normalised difference indices; mND, modified normalised index; mSR, modified simple ratio; NDRE, normalised difference red edge.

Results

Spectral signals

Figure 1 shows that most of the chlorophyll indices (mSR, Chl1, NDI1, NDI2 and mND) had high and positive loading weights, indicating that they were important and positively related to the observed variation in shoot N%. The normalised difference red edge (NDRE), normalised difference vegetation index (NDVI), the green NDVI and NDI3 indices had an intermediate value to describe N%, and the water stress index (WI) had a low positive loading weight.

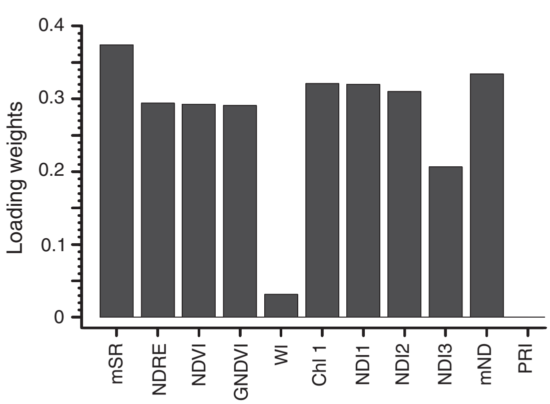

Fig. 1. Loading weights from the partial least square regression analysis between several published multispectral indices and shoot N%. These indices are defined in Table 1. Samples 1 and 2 were excluded in the analysis.

Plant growth, shoot N%, and spectral indices

The maximum values of leaf area index (LAI) were observed for the highest N levels (163 kg N/ha), i.e. 3.1 and 2.6 m^2/m^2 for the rainfed low and high densities, and 5.6 m^2/m^2 for both the irrigated low and high density treatments, respectively (Fig. 2*a–d*). High nitrogen supply and high plant densities had higher values of LAI starting early in the season, i.e. sampling 1 ($P = 0.013$ and $P = 0.005$, respectively) and at 51 DAE (Zadoks 14). Nitrogen supply significantly increased the values of LAI for samplings 3, 4 and 5 ($P < 0.001$). Irrigation significantly increased the values of LAI at sampling 4 ($P < 0.02$) and sampling 5 ($P < 0.001$). The effects of irrigation were more important at the highest levels of N supply, i.e. 2- and 2.9-fold increases in LAI for samplings 4 and 5, respectively (nitrogen × irrigation, $P < 0.001$). Shoot N% decreased with the time from emergence (Fig. 2*e–h*), and was affected by the treatments as early as sampling 2 (66 DAE, Zadoks 30). Shoot N% increased with N supply ($P < 0.001$) and was higher in the low plant density plots ($P < 0.001$), whereas the effect of plant density was more important at low levels of N supply (N × density $P = 0.005$). Irrigated plots had a higher shoot N% at sampling 4 ($P = 0.028$, 106 DAE, Zadoks 47). No significant interactions were observed with shoot N% among the studied factors.

From an ANOVA analysis, high plant density plots had significantly higher values ($P < 0.001$) for the NDRE, and mSR indices at samplings 1 and 2. Early in the season, at sampling 2, the values of NDI2 ($P < 0.01$), NDI3 ($P < 0.01$),

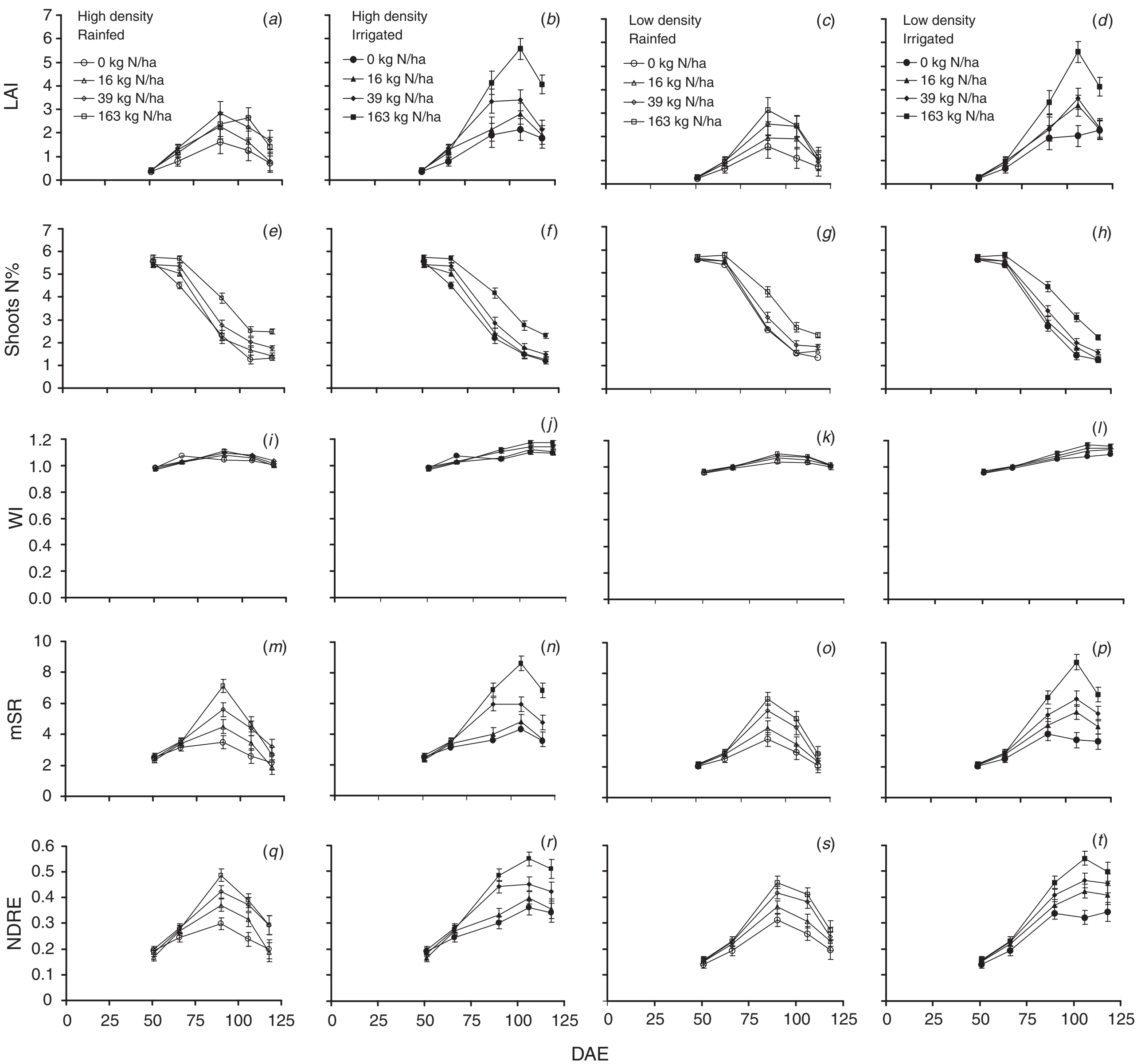

Fig. 2. Time course of leaf area index (LAI), shoot N%, and the spectral indices [water content (WI), modified spectral ratio (mSR), and normalised difference red edge (NDRE)], for the high (*a*, *b*, *e*, *f*, *i*, *j*, *m*, *n*, *q*, and *r*) and low plant densities (*c*, *d*, *g*, *h*, *k*, *l*, *o*, *p*, *s*, and *t*), and rainfed (open symbols) and irrigated (closed symbols) wheat crops growing under 4 levels of nitrogen supply (0, 16, 39, and 163 kg N/ha).

and NDRE ($P = 0.05$) increased with the application of N, whereas at samplings 3, 4 and 5 all spectral indices were significantly affected by N treatments ($P < 0.001$). Among all the spectral indices, the water stress index (WI) showed the smallest variation across the studied treatments (Fig. 2*i*–*l*). Water stress reduced the values of all the indices at samplings 4 and 5 ($P < 0.01$), although this reduction was particularly important at the highest levels of N supply ($N \times$ irrigation, $P < 0.01$), i.e. in those plots where the water stress was most intense. Despite several indices showing potential to discriminate between different levels of shoot N% (Fig. 2), in this manuscript we only present a more detailed analysis on a chlorophyll index (mSR) and a nitrogen content index (NDRE).

Interactions between the N and irrigation treatments were also identified when shoot N% was plotted as a function of NDRE and mSR (Fig. 3*a*, *b*). No relationship was observed between shoot N% and the spectral indices for samplings 1 and 2 (points not shown). Further analysis was therefore limited to samplings 3 (Zadoks 33) to 5 (Zadoks 60).

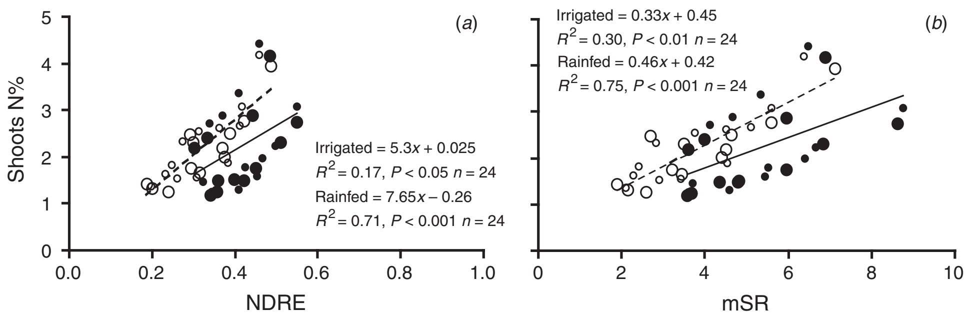

Fig. 3. Relationships between shoot N% and (*a*) the normalised difference red edge (NDRE), and (*b*) the modified spectral ratio (mSR), for the high (large symbols) and low (small symbols) plant densities, rainfed (open circles), and irrigated (closed circles) wheat crops growing under four levels of nitrogen supply (0, 16, 39 and 163 kg N/ha). Samples 1 and 2 were excluded.

Derived indices

Spectral indices were modified by applying planar domain geometry concepts (Clarke *et al*. 2001) (Fig. 4). Planar domain indices were empirically derived for the mSR (Fig. 5*a*) and the NDRE (Fig. 5*b*), converting them into the mSR_{Pi} and the CCCi (Barnes *et al*. 2000). The approach requires determining upper and lower limits of mSR and NDRE as a function of NDVI as shown in Figs 4 and 5*a*, *b* by fitting a line that just encompasses the data range. Figure 6*a* and *b* shows that normalising the spectral indices by an index of the level of ground cover (NDVI) did not help to account for the observed interactions between the nitrogen and irrigation treatments on the relationship between the mSR_{Pi} or CCCi and shoot N%.

The rate of N uptake of any crop is highly variable according to crop development, seasons, plant stands, and sites. However, under ample soil N availability, crop N accumulation is highly related to crop growth rate and to biomass accumulation (Greenwood *et al*. 1986), which may allow for comparisons among irrigation levels and plant densities as in our experiment. In Fig. 7, we show the derivation of a nitrogen stress index (NS_{index}) from the relationship between shoot N% and shoot dry weight.

The nitrogen stress index derived from scaling the crop property target, i.e. shoot N% by the accumulation of biomass was poorly described by the multispectral indices NDRE or mSR (Fig. 8*a*, *b*); however, the derived planar domain indices CCCi and mSR_{Pi}, explained 68 and 69% of the observed variation in the NS_{index}, irrespective of level of plant density

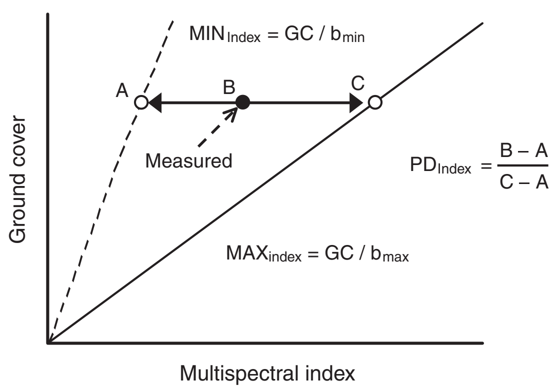

Fig. 4. General shape of a planar domain index (PD_{Index}) for soil/canopy mixed spectral information as in Clarke *et al*. (2001).

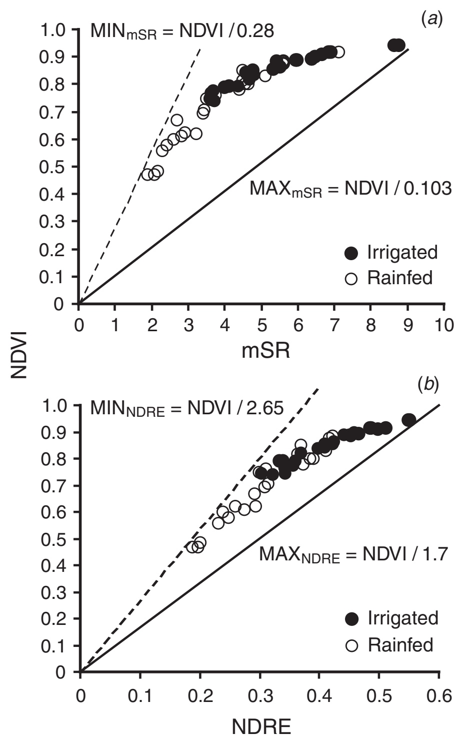

Fig. 5. Derivation of the planar domain indices, from the relationships between a measure of the proportion of the target (ground cover) in the field of view of the sensor (represented by NDVI) and the signal from the target property of interest (shoot N%) represented by the spectral indices (*a*) the modified spectral ratio (mSR), and (*b*) the normalised difference red edge (NDRE). Samples 1 and 2 were excluded.

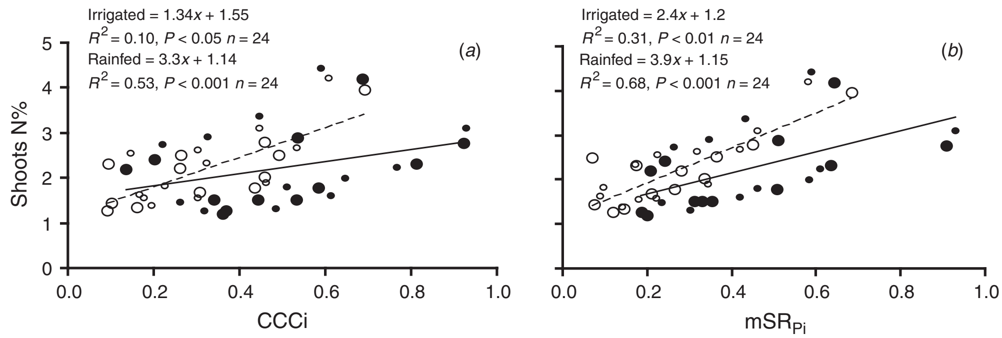

Fig. 6. Relationships between the shoot N% and (*a*) the planar domain indices derived from the normalised difference red edge, i.e. canopy chlorophyll content index (CCCi), and (*b*) the modified spectral ratio (mSR_{Pi}). The small and large symbols indicate low and high canopy densities, respectively, and the open and closed symbols show the rainfed and irrigated treatments, respectively. Samples 1 and 2 were excluded.

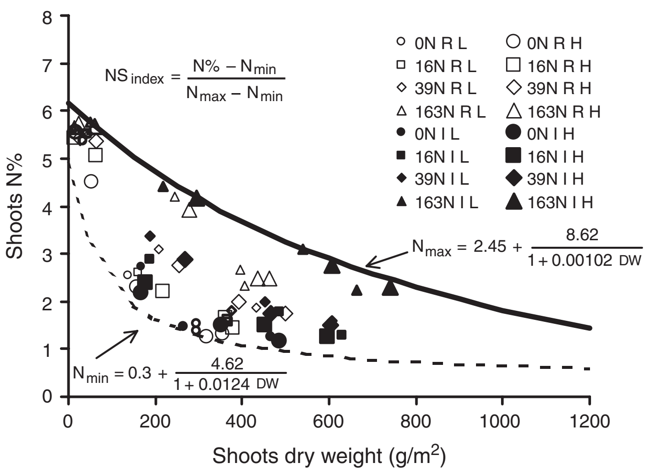

Fig. 7. Derivation of the nitrogen stress index (NS_{index}) from the relationship between shoot N% and shoot dry weights. The legend in the graph identifies the nitrogen levels (0, 16, 39 and 163 kg N/ha), irrigation treatments (R, rainfed; I, irrigated), and canopy density (L, low; H, high). N_{max} and N_{min} are the manually fitted upper and lower bounds of the relationship between shoot N% and shoot dry weight.

or irrigation (Fig. 8*c*, *d*). The slope and intercept of the regression lines in Fig. 8*c*, *d* did not differ from those of the separate irrigated and rainfed treatments (slopes and intercept in Fig. 8*c*, $P < 0.712$ and $P < 0.078$ $n = 48$; and in Fig. 8*d*, $P < 0.66$ and $P < 0.11$ $n = 48$, respectively).

Discussion

Although the data are only from a single growing season, the treatments applied created a wide range of canopy densities, structure, water status, and nitrogen level, commensurate with most typical growing conditions in south-eastern Australia. Maximum leaf area index ranged from 2.4 to 5.6 m^2/m^2 at Zadoks 47. Shoot N% ranged from 5.4 to 5.7% at Zadoks 1.4, from 5 to 5.7% at Zadoks 30, from 2.8 to 4.4% at Zadoks 33, from 1.25 to 3.1% at Zadoks 47, and from 1.2 to 2.5% at Zadoks 60. In a previous paper on this experiment, Rodriguez *et al.* (2005) also showed that the grain yields ranged from 525 to 4862 kg/ha, and that a canopy water stress index (CSI) determined at anthesis varied from 1.1 to 4.6°C/kPa among the treatments.

The main source of nitrogen for crops in soils of south-eastern Australia is from the breakdown of organic N accumulated under the original vegetation (Angus *et al.* 1998) and from N fixation during the legume phase of the rotation (McCallum *et al.* 2000). Simulated wheat yields under optimal management for the Wimmera region (O'Leary and Connor 1998) showed a maximum of 5.4 t/ha, with a long-term median of 4 t/ha, although yields of 8 t/ha are not uncommon under irrigation at the Plant Breeding Centre in Horsham, where this experiment took place (D. Pye, pers. comm.). Based on those yields, total N demand would then range from 121 to 242 kg N/ha, of which 67–195 kg N/ha can be present in the soil at sowing, and 43 to 99 kg N/ha could be mineralised during the cropping season (Angus *et al.* 1998). This indicates that under rainfed conditions, the margin for improving the nitrogen nutrition of the crop is small and that the most likely benefit of identifying zones in the field having a differential response to N would be the avoidance of losses in the non-responsive areas (Cook and Bramley 1998). Application of remote sensing techniques to identify zones of differential potential response to N at the paddock scale have been rare in Australia. One reason for this is the lack of affordable and simple methods capable of spatially identifying N status across large areas in the field that are independent of environmental and site conditions, i.e. ground cover and the simultaneous occurrence of water stress.

Remote sensing of nitrogen deficiency

In this paper, we used several published indices (Table 1) to evaluate their capacity to predict nitrogen concentration in shoots of wheat canopies growing under contrasting levels of nitrogen, density, and water supply. Simple ratios typically divide the reflectance at a reference wavelength

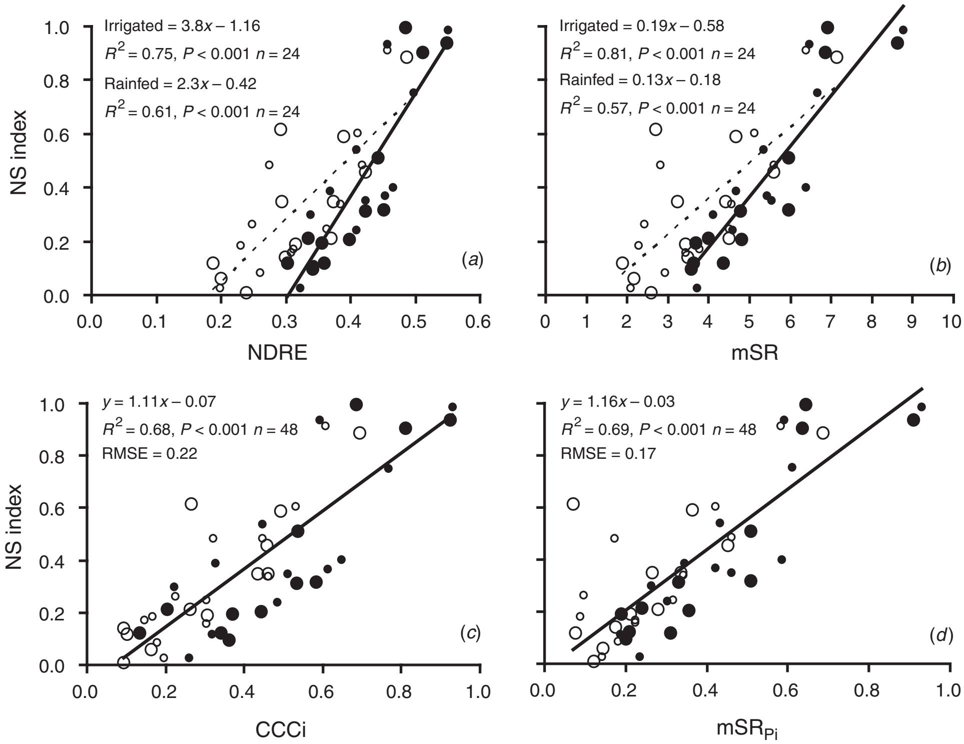

Fig. 8. Relationships between the nitrogen stress index (NS_{index}) and (*a*) the normalised difference red edge (NDRE), (*b*) the modified spectral ratio (mSR), (*c*) planar domain canopy chlorophyll content index (CCCi), and (*d*) modified spectral ratio (mSR_{Pi}). Open and closed symbols indicate the rainfed and irrigated treatments, and small and large symbols show the low and high canopy densities, respectively. The root mean squared errors (RMSE) are included in Fig. 8*c* and *d*. Samples 1 and 2 were excluded.

(typically between 750 and 900 nm) by an index wavelength (typically between 660 and 720 nm) (Sims and Gamon 2002). Reference wavelengths are independent of the stage of leaf development due to negligible chlorophyll absorption. Reflectance at these wavelengths is mostly driven by the light scattering properties of the leaves. Reflectance at 555 and 705 nm was found to be maximally sensitive to variations in chlorophyll and these wavelengths have been used as index wavelengths (Gitelson and Merzlyak 1994). To compensate for high leaf surface (specular) reflectance, which tends to increase reflectance across the whole visible spectrum, Sims and Gamon (2002) used the reflectance at 445 nm to define a modified simple ratio (mSR). Pigment absorption is minimal at this wavelength. Indices based on NDVI use similar wavelengths to the simple ratios but subtract rather than divide, the index from the reference, and the value is normalised through division by the sum of the reflectance at the same 2 wavelengths (Sims and Gamon 2002). In our work we used the NDRE, which uses a reference band in the edge band region (720 nm) in combination with a vegetation index.

The loading weights derived from a PLS1 regression analysis (Fig. 1), indicated that several indices were highly related to the variation observed in shoot N%. Since temporal changes in LAI and shoot N% across the treatments were better related to the observed temporal variations in the mSR and NDRE indices (Fig. 2), we limited our analysis to these two. The NDRE index has been successfully used in cotton to identify nitrogen stress from other causes of reduced green biomass (Barnes *et al.* 2000; Clarke *et al.* 2001). The NDI3 and WI indices showed little sensitivity to the effects of the nitrogen treatments on shoot N%, and none of the indices was capable of explaining the nitrogen status of the crop early in the season at Zadoks 14 and 30 (Fig. 2). Our results showed that water supply and plant density were important confounding factors when trying to derive empirical predictive relationships between the spectral indices and shoot N%. In Fig. 3*a*, *b*, no single relationship could account for all the treatments. The capacity of the spectral indices to discriminate different levels of nitrogen was smaller in the water-stressed plots, and was particularly reduced when the intensity of the water stress was maximised in the high-N, high plant density plots, and for the anthesis sampling (Fig. 2). Confounding effects can originate from changes in canopy architecture, i.e. leaf rolling and leaf angle (Moran *et al.* 1989), reflectance coming from the bare soil (Bausch 1993; Clarke *et al.* 2001), and probably from changes in properties of leaf surfaces (Chaves *et al.* 2003). It has been suggested that measuring the proportion of the targetted component (i.e. crop) in the field of view of the sensor can be used to evaluate the quality of the signal and reduce unwanted spectral signals when the ground cover is incomplete (Clarke *et al*. 2001). We used planar domain geometry as in Clarke *et al*. (2001) to derive the CCCi (Barnes *et al*. 2000), and a new additional index mSR_{Pi} (Figs 4 and 5). The relationship between shoot N% and the planar domain indices, CCCi and mSR_{Pi}, was still affected by the water status of the crop (Fig. 6*a*, *b*). Variability in the accumulation of crop N across environments has been observed to be reduced when crop N content is related to crop biomass (Gastal and Lemaire 2002). The relationship between shoot N% and shoot biomass accumulation in crops (Fig. 7) relies on the inter-regulation of multiple crop physiological processes; among them, N uptake, crop C assimilation (and thus growth rate), and C and N allocation between organs, all processes influenced by the water status of the crop. In our experiment, water-stressed plants had lower biomass but similar shoot N% when compared with the well-irrigated treatments. This obviously modifies the amount of nitrogen detected in the crop when it is calculated or sensed per unit area. To overcome this problem, we devised a per area base nitrogen stress index (NS_{index}) that normalises shoot N% as a function of shoot biomass (Fig. 7). Despite important variations in the level of water stress and canopy density among treatments and sampling times, the planar domain indices, CCCi and mSR_{Pi}, were capable of explaining 68 and 69% of the observed variability of NS_{index} as early as Zadoks 33 (Fig. 8*c*, *d*).

Direct application of the results obtained in this paper can be achieved by customising airborne image acquisition systems, e.g. a 3-CCD digital camera, using the identified wave bands. The relationships developed in Fig. 8*c* and *d* can be used for variable rate applications of top-dressed fertilisers in wheat crops as early as Zadoks 33. In south-eastern Australia this corresponds to the months of July–August, a time of the year when farmers consider top-dressing wheat crops, and a time of year when the existing seasonal climate forecasting tools, such as the 5 SOI phase system (Stone and Auliciems 1992), start showing levels of forecasting skill higher than 70% consistent (Anwar *et al.* 2004) and economic value for in-season crop nitrogen management (Lythgoe *et al*. 2004).

Conclusions

With this paper we addressed the problem that even though remote sensing of the nitrogen status of the crop has been around for a long time, so far not many applications of the technology are available to the Australian farmers. We showed that the existence of confounding factors such as the level of ground cover and water status of the crop, and a lack of a physiological understanding regarding what is relevant as a prediction, can limit the value of the technology. To assist farmers with in-crop N management, we propose that we should not aim to predict N%, but indices indicative of potential for response to additional N additions. We also demonstrated how simple reflectance indices are affected by confounding factors, and how a simple methodology could be devised to make the derived indices more meaningful and relevant to the decision maker.

We tested several published multispectral indices for their capacity to estimate the nitrogen nutrition of wheat canopies grown under different levels of water supply and plant density and derived a new simple canopy reflectance index (mSR_{Pi}). This new index was derived from the combination of 3 narrow bands (445, 705, and 750 nm) together with broader bands in the NIR and RED (NDVI). The CCCi from Barnes *et al*. (2000), and the mSR_{Pi} indices can be used as spatial indicators of the nitrogen status of wheat canopies with great independence of the water status of the crop and plant density. The results in this paper highlight the importance of taking into consideration basic eco-physiological understanding when analysing remotely sensed spectral data from field-grown wheat canopies.

Acknowledgments

This work was fully funded by the Department of Primary Industries of Victoria, Australia. We greatly appreciate the excellent field work done by Russel Argall. We appreciate the comments made by Dr Graeme Wright and Tom Clarke on early versions of this manuscript. We also thank the personnel at the USA Water Conservation Laboratory for their hard work and dedication.

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Manuscript received 12 October 2005, accepted 17 February 2006