Remote Sensing of Biomass and Yield of Winter Wheat under Different Nitrogen Supplies

Lydia Serrano,* Iolanda Filella, and Josep Peñuelas

ABSTRACT

Vegetation indices derived from reflectance data are related to canopy variables such as aboveground biomass, leaf area index (LAI), and the fraction of intercepted photosynthetically active radiation (fIPAR). However, under N stress the relationships between vegetation indices (VI) and these canopy variables might be confounded due to plant chlorosis. We studied the relationships between reflectance-based VI and canopy variables (aboveground biomass, LAI canopy chlorophyll A content [LAI × Chl A], and fIPAR) for a wheat (Triticum aestivum L.) crop growing under different N supplies. Nitrogen fertilization promoted significant increases in radiation interception (plant growth) and, to a lesser extent, in radiation use efficiency (RUE). The VI vs. LAI relationships varied significantly among treatments, rendering the VI-based equations unreliable to estimate LAI under contrasting N conditions. However, a single relationship emerged when LAI × Chl A was considered. Moreover, VI were robust indicators of fIPAR by green canopy components independently of N treatment and phenology. Aboveground biomass was poorly correlated with grain yield, whereas cumulative VI simple ratio (SR) was a good predictor of grain yield, probably because cumulative SR closely tracked the duration and intensity of the canopy photosynthetic capacity.

Estimation of cereal-crop production is considered a priority in most research programs (Steinmetz et al., 1990) due to the relevance of food grain to world agricultural production (Rudorff et al., 1996). Crop monitoring and yield forecasting can be done with models that range from statistical to mechanistic with a high number of input variables (Ridao et al., 1998). However, under nonoptimal growing conditions, estimates of crop growth and yield using crop growth models often are inaccurate (Clevens, 1997).

Under nonlimiting water supply, the N status of a crop is the major factor controlling the rate of biomass accumulation (Jensen et al., 1990), and, at any given time, there is a strong relationship between N and biomass. Thus, N availability is an important determinant of crop growth and productivity (van Keulen et al., 1989).

Numerous studies have assessed the effects of N availability on canopy spectral reflectance measurements. Nitrogen limitation is known to promote decreased chlorophyll content (Moorby and Besford, 1983; Peñuelas et al., 1994). When there is a severe N limitation, plants reflect more on the red spectral region as a consequence of this lower chlorophyll content. Thus, under N stress (chlorosis), VI confound the changes in soil cover and plant density with changes in vegetation color (Steven et al., 1990).

Since N content may promote changes in the VI vs. LAI relationships, the interpretation of VI as a function of fIPAR instead of LAI could be more appropriate. Furthermore, the interpretation of VI on a fIPAR basis allows a direct estimate of plant productivity by introducing a factor that represents the RUE. Thus, according to Monteith (1977), crop growth (biomass) could be determined as the following time integral:

\[
\text{Biomass} = \int \text{PAR} \times \text{fIPAR} \times \text{RUE} \times \text{\Delta}t \quad [1]
\]

where RUE is the radiation use efficiency (i.e., the efficiency of radiation conversion into dry biomass), fIPAR is the fraction of PAR intercepted by the foliage, PAR is the incident photosynthetic active radiation above the canopy, and \( \text{\Delta}t \) is time interval.

Because grain yield is closely linked to crop growth, and because reflectance-based VI respond to canopy variables such as LAI and fractional absorbed PAR (fAPAR) that largely determine crop growth, spectral data have been used in yield-predicting models (Das et al., 1993). Based on remote sensing data, Moran et al. (1997) recognized two main approaches for yield forecasting. In the simplest approach, single or multiple time-integrated VI, such as the normalized difference vegetation index (NDVI), can be used to predict crop yield by empirical regression equations. In the other approach, VI are used as estimators of fIPAR or LAI, which, in turn, are used as input data into crop growth and agrometeorological models (Clevers et al., 1994; Clevens, 1997).

In the context of a growing interest in remote sensing applied to agricultural studies (Moran et al., 1997; Clevens, 1997), we studied the effects of N fertilization on the relationships between remote sensing VI and several canopy variables. We aimed to characterize the canopy spectral response to different N supplies, to test the validity of the VI vs. LAI relationships, and, furthermore, to study the feasibility of VI to estimate biomass, fIPAR, and yield under varying N availabilities.

MATERIAL AND METHODS

Experimental Design

A field experiment was conducted with wheat (cv. Soissons) on an Aquic Xerochrept soil at the Experimental Station of Mas Badia, Girona, Spain (42°3′N, 3°4′E). Winter wheat was

Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; Chl, chlorophyll; DAS, days after sowing; FAPAR, fractional absorbed PAR; fIPAR, fractional intercepted PAR; LAI, leaf area index; LAI × Chl A, canopy chlorophyll A content; NDVI, normalized difference vegetation index; NIR, near-infrared; PAR, photosynthetic active radiation; RUE, radiation use efficiency; SEM, standard error of the mean; SR, simple ratio; VI, vegetation indices; ∑SR, cumulative simple ratio.
sown 12 Nov. 1992 in rows spaced 0.15 m apart at a population of 400 seeds m\(^{-2}\). Each plot was 4.5 by 1.2 m\(^2\). Plants were harvested 6 July 1993.

To ensure a high level of soil fertility, P and K fertilizers were applied at a rate of 60 kg P\(_{2}O_{5}\) ha\(^{-1}\) and 60 kg K\(_{2}O\) ha\(^{-1}\), respectively. Postemergence herbicides were used to control weeds. On 19 March (122 d after sowing [DAS]), five differential N treatments were started by applying NH\(_{4}\)NO\(_{3}\) at the following doses: 0, 50, 100, 150, and 200 kg N ha\(^{-1}\). Each treatment had three replicate plots arranged in a randomized block design. Irrigation was not provided due to the high rainfall (450.2 mm during the crop cycle) and the height of the water table.

Measurements were conducted 78, 96, 139, 158, 180 and 209 DAS, corresponding with the following developmental stages in the Zadoks decimal code (Zadoks et al., 1974): 2.4, 2.5, 3.1, 3.3, 5.5, and 6.5, respectively. These developmental stages correspond with tillering (2), stem elongation (3), heading (5), and postanthesis (6).

### Agronomic Measurements

Leaf area index and dry biomass were determined by destructive sampling the same days when reflectance measurements were taken. A 30-cm-diameter plastic ring was placed within the spectroradiometer field of view, and vegetation within the frame was clipped at the ground level. Aboveground biomass was separated into green and nongreen leaves (lamina only), stem (including leaf sheaths), and ears. Leaf area of green leaves was measured with a leaf area meter (Model LI-3000, LI-COR, Lincoln, NE). Leaf area index was computed as the ratio of green leaf area per sampled area. All plant components were dried at 60°C to constant weight and weighed. Canopy green fraction was estimated from the ratio of green leaf biomass to aboveground biomass.

A SPAD chlorophyll meter from Minolta (Model 502, Minolta, Ramsey, NJ) was used to measure leaf Chl A concentration (Monje and Bugbee, 1992). The relative units were previously calibrated measuring Chl A concentration after extraction of Chl A from fresh leaf disks in N,N’-dimethylformamide (Porra et al., 1989). Twenty leaves for each treatment, previously measured with the SPAD chlorophyll meter, were oven dried at 60°C until constant weight and analyzed for total N in a Carlo-Erba NA 1500 automatic analyzer (Carlo-Erba, Milan, Italy). Canopy Chl A contents were estimated by multiplying the chlorophyll per area foliar values of the LAI. All estimates of canopy structure and chemical content were expressed on the basis of ground area to allow comparison with spectral measurements.

### Radiometric Measurements

Canopy reflectance was measured over each plot with a narrow-bandwidth visible and near-infrared (NIR) spectroradiometer fitted with a 15° field of view foreoptics (model SE590 with detector CE390WB-R, Spectron Engineering, Denver, CO) and expressed as bidirectional reflectance after standardizing by the radiance of a leveled white standard (Spectralon, Labsphere, North Sutton, NH). The instrument measures 252 bands approximately evenly spaced between 390 and 1100 nm. Data were collected on cloudless days at solar zenith angles ranging between 40 and 50° in order to minimize disturbances from the atmosphere and changes in solar elevation. The radiometer was mounted on a tripod boom and held in a nadir orientation 2 m above the canopy. Four scans were averaged on each measurement.

The VI simple ratio (SR) and NDVI were calculated using narrow-band reflectance values (Penuelas and Filella, 1998) as follows:

\[
SR = \frac{R_{900}}{R_{680}} \quad [2]
\]

\[
NDVI = \frac{(R_{900} - R_{680})}{(R_{900} + R_{680})} \quad [3]
\]

where \(R\) indicates reflectance and the subindex indicates the wavelength (nm). In the absence of leaves and before emergence, NDVI and SR were formulated from the bare soil spectral signature.

After taking reflectance measurements, fIPAR was measured with a ceptometer (Model SF 80 Decagon Devices, Pullman, WA) and calculated as:

\[
fIPAR = 1 - \left(\frac{T}{S}\right) \quad [4]
\]

where \(T\) is the transmitted PAR through the canopy to the

### Table 1. Average leaf area index (LAI), canopy Chl A concentration (LAI × Chl A), and aboveground biomass for the different N treatments at different days after sowing (DAS).

<table>
<thead>
<tr>
<th>DAS</th>
<th>Treatment kg ha(^{-1}) N</th>
<th>LAI m(^2) m(^{-2})</th>
<th>LAI × Chl A mg m(^{-2})</th>
<th>Aboveground biomass g m(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>139</td>
<td>0</td>
<td>3.2 (0.69)(^{\dagger})</td>
<td>833 (230.5)</td>
<td>288 (52.0)(^{\ddagger})</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.6 (0.87)</td>
<td>1400 (290.6)</td>
<td>476 (65.3)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>8.3 (0.45)</td>
<td>1921 (280.0)</td>
<td>287 (36.8)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>4.4 (0.62)</td>
<td>1914 (258.0)</td>
<td>349 (46.5)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4.6 (0.60)</td>
<td>1874 (241.3)</td>
<td>362 (35.6)(^{\dagger})</td>
</tr>
<tr>
<td>158</td>
<td>0</td>
<td>5.6 (1.52)</td>
<td>1257 (442.2)</td>
<td>492 (288.1)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.6 (1.15)</td>
<td>1540 (316.1)</td>
<td>756 (68.7)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.5 (0.39)</td>
<td>2219 (116.8)</td>
<td>862 (63.8)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>6.8 (1.58)</td>
<td>2093 (511.0)</td>
<td>871 (135.4)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6.1 (2.29)</td>
<td>1772 (567.3)</td>
<td>1869 (0)(^{\dagger})</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>2.2 (0.50)</td>
<td>496 (169.7)(^{\dagger})</td>
<td>994 (269.4)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.1 (1.05)</td>
<td>1540 (316.1)(^{\dagger})</td>
<td>1227 (411.9)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.8 (0.19)</td>
<td>1010 (58.3)(^{\dagger})</td>
<td>1232 (269.5)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>5.0 (0.95)</td>
<td>1414 (185.9)(^{\dagger})</td>
<td>1242 (138.0)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6.0 (1.34)</td>
<td>1834 (427.3)(^{\dagger})</td>
<td>2089 (489.9)(^{\dagger})</td>
</tr>
<tr>
<td>209</td>
<td>0</td>
<td>0.2 (0.10)</td>
<td>9.4 (9.3)</td>
<td>1656 (154.8)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.0 (0.07)</td>
<td>0.4 (0.3)</td>
<td>1934 (318.6)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.0 (0)</td>
<td>0</td>
<td>2488 (262.5)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>0.0 (0)</td>
<td>0</td>
<td>2568 (88.4)(^{\dagger})</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.0 (0)</td>
<td>0</td>
<td>2807 (127.0)(^{\dagger})</td>
</tr>
</tbody>
</table>

\(^{\dagger}\) Values in parentheses indicate the standard error of the mean (\(n = 3\)).

\(^{\ddagger}\) For a given DAS, values followed by different letters are significantly different at the \(P < 0.05\) level (Fisher’s PLSD).
soil surface, and $S$ is the incoming PAR above the canopy. Ten readings were averaged within a 1-m$^2$ area.

**Data Analysis**

Spectral reflectance was analyzed using Igor (Wavemetrics, Lake Oswego, OR). Statistical analyses (ANOVA, Pearson correlation, regression, and covariance analyses) were carried out by using Statview 4.5 and Superanova (Abacus Concepts, Berkeley, CA).

**RESULTS**

**Biomass and Pigments**

Nitrogen treatments and great within-treatment variability, probably due to variable residual soil N content, promoted a wide range of responses in plant growth and pigment content. After N application (122 DAS), those treatments with higher N doses initiated a faster growth. The LAI values reached a maximum on 158 DAS, ranging from $5.6 \pm 1.52$ (standard error of the mean, SEM) to $7.5 \pm 0.42$ (SEM) among treatments (Table 1). From this date onwards LAI started to decrease due to leaf senescence.

The N treatments resulted in different leaf N concentrations that increased from $3.71 \pm 0.17$ (SEM) percentage dry weight in 0 kg ha$^{-1}$ N treatment to $4.95 \pm 0.06$ (SEM) percentage dry weight in 200 kg ha$^{-1}$ N treatment. Leaf Chl A concentration differed among treatments (see Filella et al., 1995 for more details). Furthermore, when Chl A was expressed as canopy chlorophyll content (Table 1), resulting from multiplying LAI by leaf Chl A concentration (LAI $\times$ Chl A) (mg chlorophyll m$^{-2}$ soil), differences between treatments were emphasized (Table 1) ($P = 0.05$, ANOVA).

Biomass accumulation was strongly dependent on N application rates. As for LAI, aboveground biomass accumulated more rapidly with increasing N rates. Differences in aboveground biomass among treatments were significant ($P < 0.001$, ANOVA). They ranged from $1656 \pm 155$ (SEM) g m$^{-2}$ in the 0 kg ha$^{-1}$ N treatment to $2807 \pm 127$ (SEM) g m$^{-2}$ in 200 kg ha$^{-1}$ N treatment at the end of the growth cycle (Table 1). However, no significant differences were found in grain harvest. Average grain yield for all the treatments studied was $4956 \pm 158$ (SEM) kg ha$^{-1}$.

All N treatments experienced a rust infection (*Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.). On 180 DAS, the percentage of rust-affected area ranged between $5.2 \pm 2.2$ (SEM) % in the 0 kg ha$^{-1}$ N treatments to $10.0 \pm 0.8$% (SEM) in 200 kg ha$^{-1}$ N treatments.

**Spectral Signatures**

Changes in canopy structure and pigments throughout the developmental stages were translated into changes in the spectral signature (Fig. 1A). At the first growth stages (during tillering) the canopy was not well developed and the soil contribution to canopy reflectance was large. Although the spectral signature showed some characteristic features of green vegetation (lower reflectance in the red region), the average reflectance in the visible region was relatively high and the NIR reflectance was relatively low. As the canopy developed, reflectance in the visible region decreased and NIR reflectance increased up to maximum contrast on 158 DAS. On 209 DAS, vegetation was senescent and the spectral signature showed a steady linear increase across the red edge region (i.e., the transition zone between the red and NIR regions).

Spectral discrimination between treatments was greater during heading at the maximum LAI (158 DAS) (Fig. 1B). Nitrogen fertilization promoted a decrease in red reflectance and an increase in NIR reflectance. Conversely, N limitation increased red reflectance and decreased NIR reflectance.

**Time-Course of LAI, Fractional Intercepted PAR, and Vegetation Indices**

Changes in LAI throughout the growth cycle (Fig. 2A) were paralleled by changes in the VI. Thus, SR and NDVI reached a maximum on 158 DAS and started to decline from this date onwards (Fig. 2C and 2D, respectively). However, in the 200 kg ha$^{-1}$ N treatment, SR and NDVI diminished abruptly, while LAI was still...
high. On the other hand, fIPAR roughly represented the variations in LAI. While fIPAR increased along with increasing LAI, after the onset of senescence, when LAI substantially diminished, fIPAR slightly declined. Thus, on 209 DAS (when LAI < 0.05), fIPAR values were still higher than 60% (Fig. 2B).

**Vegetation Indices vs. Canopy Variables Relationships**

Whereas the relationship between NDVI and LAI was curvilinear (Fig. 3A), the relationship between SR and LAI was linear (Fig. 3B). The NDVI demonstrated to be a more sensitive index for low LAI canopies. However, the NDVI response saturated for LAI > 3, while SR still increased with increasing LAI. Neither SR nor NDVI showed significant correlation with aboveground biomass (Table 2). However, both VIs were significantly correlated with leaf Chl A concentration. Moreover, when expressing this canopy variable as LAI × Chl A, the degree of correlation increased substantially (Table 2).

**LAI Estimation from Simple Ratio Measurements**

Nitrogen fertilization promoted changes in the relationship between SR and LAI. Herein we discuss SR instead of NDVI because of its closer correlation with LAI. As N supply increased, the slope of the SR vs. LAI regression increased. Even though the relationship for all N treatments was highly significant ($r^2 = 0.89$, $P < 0.0001$) and the standard error of the estimate was small, analysis of covariance (ANCOVA) revealed significant differences between treatments in this relationship ($P < 0.01$, ANCOVA). Consequently, in order to derive LAI values from SR measurements, a separate linear equation for each treatment had to be considered (Table 3). The regression lines for treatments 0, 100, and 200 kg ha$^{-1}$ N treatments are depicted in Fig. 3B.

When considering the relationships between SR and LAI × Chl A, differences among treatments in the slope of the relationship were not significant. Consequently, a single linear equation was appropriate to derive LAI × Chl A values for the N treatments considered (Table 3). The relationship between SR and LAI × Chl A was linear and highly significant ($r^2 = 0.88$, $P < 0.0001$) (Fig. 3D). Thus, SR did not only respond to leaf area but to the canopy Chl A content.

**Deriving Intercepted PAR from Spectral Indices**

When considering the whole data set (e.g., before and after the onset of senescence), fIPAR was poorly correlated with SR ($r = 0.38$, $P < 0.01$) and the correlation with NDVI was not significant. However, if only the measurements taken before the onset of senescence were considered (Fig. 4, open symbols), NDVI and fIPAR showed a significant linear relationship ($r^2 = 0.81$, $P < 0.001$) (Fig. 4A). Similarly, SR and fIPAR were significantly related, although in an exponential manner ($r^2 = 0.84$, $P < 0.0001$) (Fig. 4B).

When considering the fIPAR by green vegetation (green fIPAR), calculated as fIPAR × (green leaf biomass/total biomass), significant relationships emerged, even when data points taken after the onset of senescence were considered. The relationships of green fIPAR with NDVI was curvilinear ($r^2 = 0.83$, $P < 0.0001$) and linear with the SR ($r^2 = 0.68$, $P < 0.001$) (Fig. 4C and 4D, respectively).
Nitrogen Effects on Radiation Use Efficiency

Since SR was a good indicator of green fIPAR, we calculated the corresponding cumulative vegetation index (ΣSR) by summing up the single values that resulted from multiplying the average SR value between two spectral measurements by the time interval (in days). Next, RUE was derived from the slope of the relationship between total aboveground biomass and ΣSR for each N treatment. Although the slope of these relationships increased with increasing N supply, the covariance analysis did not show any significant differences among N treatments. Similar results were obtained when RUE was estimated from the slope of the relationship between total aboveground biomass and the time integral of intercepted PAR. Thus, although not statistically significant, there was an increase in RUE from 3.15 ± 0.131 (SEM) g MJ⁻¹ in the 0 kg ha⁻¹ N treatment to 3.65 ± 0.158 (SEM) g MJ⁻¹ in 200 kg ha⁻¹ N treatment.

Predicting Crop Yield from Simple Ratio

We also studied the possibility of estimating crop growth and, ultimately, predicting grain yield from ΣSR. While grain yield and total biomass were significantly related with ΣSR ($r^2 = 0.74, P < 0.0001$; and $r^2 = 0.64, P < 0.001$, respectively) (Fig. 5A and 5B), total biomass and grain yield were poorly correlated ($r^2 = 0.35, P < 0.05$) (Fig. 5C).

DISCUSSION

Variations in the canopy LAI, aboveground biomass, and pigment concentration were translated into clear changes in the spectral signature. While increases in red reflectance were related to the decreases in chlorophyll content resulting from lower N supply (Filella et al., 1995), decreases in NIR reflectance mostly responded to decreases in LAI and green biomass, as has been widely reported for wheat crops (Asrar et al., 1984; Jensen et al., 1990; Fernández et al., 1994; Peñuelas et al., 1996).

The VI clearly indicated the canopy changes caused by developmental stages and N fertilization treatments. The SR increased linearly with increases in LAI, while NDVI showed a curvilinear response that saturated for LAI > 3, in agreement with the results obtained in previous wheat studies (Asrar et al., 1984; Wiegand et al., 1996).

Table 2. Correlation coefficients and significance between the vegetation indices simple ratio (SR) and normalized difference vegetation index (NDVI) and several canopy variables ($n = 65$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>SR</th>
<th>NDVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAI</td>
<td>0.90***</td>
<td>0.85***</td>
</tr>
<tr>
<td>Leaf Chl A concentration</td>
<td>0.54***</td>
<td>0.68***</td>
</tr>
<tr>
<td>Chl A × LAI</td>
<td>0.93***</td>
<td>0.85***</td>
</tr>
<tr>
<td>Green leaf biomass</td>
<td>0.89***</td>
<td>0.87***</td>
</tr>
<tr>
<td>Aboveground biomass</td>
<td>0.12NS</td>
<td>0.01NS</td>
</tr>
</tbody>
</table>

*** Significant at the 0.0001 level of probability; NS is not significant.
† Leaf area index.

**Fig. 3.** Relationships of the vegetation indices normalized difference vegetation index (NDVI) and simple ratio (SR) with (A and B) leaf area index (LAI, m² m⁻²) and (C and D) canopy chlorophyll concentration (LAI × Chl A, mg Chl A m⁻² soil) for the five N treatments studied.
Table 3. Regression parameters for the linear relationships between Simple Ratio and leaf area index (LAI) and canopy chlorophyll A content (LAI × Chl A).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>$x$ intercept</th>
<th>Slope</th>
<th>$r^2$</th>
<th>$P$</th>
<th>$n$</th>
<th>SEE $\pm$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAI</td>
<td>N-0</td>
<td>1.968</td>
<td>2.166</td>
<td>0.86</td>
<td>$&lt;0.0001$</td>
<td>13</td>
<td>1.982</td>
</tr>
<tr>
<td></td>
<td>N-100</td>
<td>3.121</td>
<td>2.489</td>
<td>0.92</td>
<td>$&lt;0.0001$</td>
<td>13</td>
<td>2.259</td>
</tr>
<tr>
<td></td>
<td>N-200</td>
<td>3.428</td>
<td>2.805</td>
<td>0.89</td>
<td>$&lt;0.0001$</td>
<td>11</td>
<td>3.151</td>
</tr>
<tr>
<td>LAI × Chl A</td>
<td>All</td>
<td>2.892</td>
<td>0.009</td>
<td>0.89</td>
<td>$&lt;0.0001$</td>
<td>66</td>
<td>2.494</td>
</tr>
</tbody>
</table>

† SEE indicates standard error of the estimates.

al., 1992). This illustrates one of the problems in using NDVI for well-developed canopies: when LAI > 3, the addition of more leaf layers to the canopy does not entail large changes in NDVI (Sellers, 1987). On the other hand, NDVI was more sensitive for low LAI, which makes it more suitable for assessing crop growth at the initial stages. Non-green components contribute to the canopy spectral reflectance, and VI have been reported to vary due to the presence of non-green vegetation (Bartlett et al., 1990; van Leeuwen and Huete, 1996) and due to soil background (Huete, 1989). As a consequence, separate VI vs. LAI relationships before and after the onset of crop senescence have been considered (Wiegand et al., 1992). However, in our study, while significant differences in these relationships arose among treatments (see discussion below), a unique relationship was satisfactory for the different phenological stages.

Since there were differences in Chl A concentration among N treatments, we would expect changes in reflected radiation for a given leaf area. In fact, when we considered contrasting treatments (0, 100, and 200 kg ha$^{-1}$ N), the SR vs. LAI regression lines were significantly different. Similar results have been reported by Walburg et al. (1982) for corn (Zea mays L.) submitted to different N availabilities. In contrast, Fernández et al. (1994) reported a unique relationship between NDVI and LAI for wheat grown under different N and water supplies. However, these authors derived LAI values from fAPAR vs. NDVI relationships using Monsi’s Saeki equation. This implies that variations due to changes in Chl A concentration, and, thus, in leaf optical properties (if there were any) were already accounted for in fAPAR. This might explain the existence of a unique relationship independent of N treatment. Thus, if our ultimate goal is to estimate LAI from spectral measurements, specific relationships should be considered if Chl concentration were to vary. However, since both VI showed close correlation with LAI × Chl A regardless of N treatment, they could be good indicators of photosynthetic capacity.

In agreement with previous empirical wheat studies (Asrar et al., 1984; Steinmetz et al., 1990; Daughtry et al., 1992), NDVI and SR showed significant relationships with fIPAR before the onset of senescence. These results are consistent, since fIPAR is a good estimator of fAPAR as long as the canopy is green (Daughtry et al., 1992). As a consequence, the relationships between...
VI and fIPAR are usually studied as typically biphasic (Ridao et al., 1998). One equation is required to describe fIPAR for increasing LAI and another for decreasing LAI. However, Gallo et al. (1993) questioned the use of separate equations and suggested that the relationships between fAPAR and VI developed for increasing LAI may be used even during senescence when LAI is declining. Our results reinforce the use of this simplified approach. When we considered the whole data set and corrected for the empirical green fraction factors (Fig. 4C and 4D), the relationships between VI and green fIPAR were still highly significant, suggesting that VI are robust indicators of green fIPAR. Thus, fIPAR derived from spectral indices accurately characterized PAR interception by green, photosynthetically active vegetation, as has been previously shown in natural communities (Bartlett et al., 1990; Gamon et al., 1995). Contrasting results may be attributed to the inadequacy of the currently used optical ground methods to determine fIPAR. Since non-green canopy components substantially absorb in the visible spectrum (Serrano et al., 1997; Asner et al., 1998), measurements obtained with a ceptometer or a PAR sensor may not be reliable indicators of either fIPAR or fAPAR by photosynthetic (green) components (Serrano et al., 2000). Moreover, the relationship between SR and fIPAR was not affected by N treatments. Similarly, Joel et al. (1997) in sunflower (Helianthus annuus L.) indicated that the relationships between fIPAR and the spectral indices SR and NDVI were insensitive to N and water stress.

Cumulative SR was a good indicator of crop growth as has been previously shown for a variety of crops and growing conditions (see references in Moran et al., 1997). Nitrogen stress may impose limitations on the use of VI to determine crop growth due to changes in RUE. Increasing N supply progressively increased LAI and leaf Chl A concentration, and, hence, improved light interception, while differences in RUE were minor. In contrast with previous wheat studies (Green, 1987; Garcia et al., 1988; Steinmetz et al., 1990), differences in RUE (in terms of intercepted PAR) among N treatments were not statistically significant. However, our data fell in the range of previously reported RUE values for wheat (Green, 1987; Rudorff et al., 1996).

Several studies in winter wheat have shown a close correlation between total biomass at harvest and grain yield (Green, 1987; Rudorff and Batista, 1990). In our study, higher N fertilizer rates promoted larger canopy growth, yet the correlation of aboveground biomass with final grain yield was relatively weak. Nielsen and Halvorson (1991) suggested that water stress could impair the potential for greater grain yields resulting from increased N fertility. However, in a previous study, we showed that water availability was not a limiting factor in high N treatments (Peñuelas et al., 1996). Under our experimental conditions, poor correlation between aboveground biomass and grain yield could be partly attributed to the earlier senescence of the flag-leaf, and to the incidence of a rust infection (P. Graminis) that had a greater effect on those treatments with higher doses of N. This latter aspect is substantiated by the sharp decrease in SR and NDVI, while LAI was still high, which indicates a diminution in canopy greenness (see Fig. 2).

Since the spectral indices can respond to non-leaf photosynthetically active organs, such as heads and sheaths of cereals, canopy reflectance may be a more accurate measure of the crop photosynthetic capacity than LAI or aboveground biomass. On the other hand, crop losses resulting from the incidence of a rust infection (P. striiformis Westend) have been estimated accurately from VI (Hansen, 1991). Our results support these previous findings, particularly in the 200 kg ha

\[ \Sigma SR \] plots. Where the percentage of rust-affected leaf area was greater, SR and NDVI decreased without a concomitant decrease in LAI. Thus, in our study, \( \Sigma SR \) represented the intensity and duration of the photosynthetic activity of the crop better than aboveground biomass, providing an enhanced estimate of the final measured yield.
SUMMARY AND CONCLUSIONS

Under our experimental conditions, variations in crop growth and yield in response to N fertilization were mostly a result of changes in photosynthetic capacity (changes in leaf area and Chl content), while variations in RUE were minor. The relationships between VI and LAI were significantly affected by N supply. As a consequence, and under nonlimiting N supply, LAI would be overestimated when using equations obtained under N-deficient conditions. However, when considering LAI × Chl A, a single linear relationship independent of N treatment emerged. Thus, VI appear to be better indicators of canopy photosynthetic processes than LAI. Moreover, VI closely tracked PAR interception by green, photosynthetically active vegetation components independently of N treatment and phenology. This supported the use of a single equation to derive green fPAR from VI before and after the onset of crop senescence.

In our study, ΣSR was closely correlated with grain yield, even more than aboveground biomass, probably reflecting the contribution of photosynthetic organs other than leaves and the negative effects of a rust infection on photosynthesis. Our results indicate that, under stress conditions where crop growth and LAI are decoupled from final yield, ΣSR provides reliable information for yield forecasting.

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REFERENCES


Hard Red Spring Wheat Response Following the Intercropping of Legumes into Sunflower

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ABSTRACT

Intercropping legumes in sunflower (Helianthus annuus L.) may increase soil cover, reduce soil erosion, and increase soil C and N. Subsequent effects of this practice on hard red spring wheat (HRSW) [Triticum aestivum (L.) Emend. Theil.] yield and protein content were unknown. Our objective was to quantify effects of intercropping various legumes into sunflower on spring soil nitrate-nitrogen (NO$_3^-$–N) and grain yield and protein content of a subsequent HRSW crop. Field experiments were conducted near Carrington and Prosper, ND, from 1993 through 1995. Wheat was planted into non-legume plots and those previously intercropped with hairy vetch (Vicia villosa Roth), yellow-flowered sweetclover (Melilotus officinalis Lamin.), alfalfa (Medicago sativa L.), snail medic [Medicago scutellata (L.) Mill.], or black lentil (Lens culinaris Medik.). Soil NO$_3^-$–N (0–30 cm) plots previously intercropped with hairy vetch (41 kg ha$^{-1}$) was greater than control plots (26 kg ha$^{-1}$). Yield of HRSW was reduced at both Carrington and Prosper in 1993 when grown after a sweetclover intercrop. Yield of HRSW was reduced at Carrington in 1993 when grown after an alfalfa intercrop. Wheat grown after sweetclover intercropped in sunflower had higher protein content (142.0 g kg$^{-1}$) than HRSW grown after sunflower (140.6 g kg$^{-1}$) alone. Overall, intercropping hairy vetch at the V4 sunflower growth stage appears superior because it did not reduce sunflower yield, provided soil cover adding between 540 and 2400 kg ha$^{-1}$ above ground dry matter to the system, and increased NO$_3^-$–N levels at the beginning of the HRSW season in two environments.

Sunflower, when seeded in rows, can result in severe soil erosion during and after the growing season (Deibert, 1987). In a previous study, we evaluated effects of intercropping legumes into sunflower as a technique to increase surface residue cover (Kandel et al., 1997). We reported reduced sunflower yield when hairy vetch, sweetclover, alfalfa, and snail medic were seeded at the same time as sunflower. However, seeding these legumes at the V4 or V10 (Schneiter and Miller, 1981) sunflower growth stages did not reduce sunflower yield. Hairy vetch provided 1593 and 831 kg ha$^{-1}$ dry matter when seeded at the V4 or V10 sunflower growth stages, respectively (Kandel et al., 1997).

Some potential benefits to the farming system of intercropping a legume in sunflower are dinitrogen fixation, soil erosion control, and improvement of the soil structure and organic matter content (Biederbeck and Bouman, 1994). Intercropping may also improve snow trapping and green manure production during the year after legume establishment (Lilleboe, 1991). Shading by sunflower may decrease growth and dinitrogen fixing ability of the intercropped legumes (Morris and Garrity, 1993). Application of N to legume-based intercrops will usually favor the growth of the non-legume and further reduce dinitrogen fixation of the legume (Midmore, 1993; Davis and Woolley, 1993). Most legume-fixed dinitrogen will usually benefit only subsequent crops as opposed to the intercrop (Stern, 1993). For example, Jordan et al. (1993) reported that 8 wk after alfalfa was incorporated into the soil, corn (Zea mays L.) had recovered 8 to 10% of N fixed by alfalfa, the rest remained in the soil fraction.

Biederbeck et al. (1993) reported that under dry soil conditions legume growth and nodule number were reduced, limiting dinitrogen fixation. Brown et al. (1993) reported that hairy vetch intercropped into corn in August and chemically burned down the following spring, significantly increased soil NO$_3^-$–N in the top 15 cm, when measured 50 and 78 d after planting the subsequent crop. Soil NO$_3^-$–N tests measure the amount of plant available NO$_3^-$ but do not account for N in the unavailable organic form. Ladd et al. (1981) reported that wheat took up between 11 and 17% of labeled $^{15}$N from legume material that had been decomposing for 8 mo. They concluded that increased soil organic N was the main benefit derived from planting a legume.