

1 Which Multispectral Indices Robustly Measure Canopy Nitrogen 2 across Seasons: Lessons from an Irrigated Pasture Crop

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11 Abstract

12 In precision farming, accurate estimation of canopy nitrogen concentration (CNC) is valuable
13 for effective crop growth monitoring and nitrogen (N) fertiliser management. To date, many
14 canopy multispectral indices have been proposed as indicators for CNC; however, many of
15 these indices have also shown sensitivity to biomass and their performance drops at high
16 biomass levels. Dependence on growth stage, season, or other environmental conditions limits
17 their efficacy as generalized CNC indices. The objectives of this study were to assess the
18 robustness of popular CNC indices across a wide range of biomass levels and fertiliser
19 application levels; and for two contrasting seasons – winter and summer. To achieve this, we
20 analysed the efficacy of seven canopy nitrogen indices, including canopy chlorophyll content
21 index (CCCI), together with eleven other commonly used spectral indices. We used canopy
22 level solar-induced hyperspectral reflectance data acquired using a hand-held optical
23 spectroradiometer across four growth stages in winter (May-June 2018) and four in summer
24 (January-February 2019) from an experimental field of irrigated perennial ryegrass with
25 variable N application in Victoria, Australia. The field contained 40 plots, each with one of

26 eight different N treatments. Almost all the indices exhibited similar correlation to CNC (%)
27 when applied to individual stages (days) in both winter and summer; however, relationships
28 between CNC and individual indices varied significantly between stages. We obtained similar
29 results for canopy biomass. When the data across the entire range of growth stages and
30 seasons were combined, the correlations between most canopy nitrogen indices and CNC
31 became weak ($R^2 < 0.25$, $0.9\% \leq RMSE \leq 1.0\%$). PRI exhibited the highest correlation with CNC
32 ($R^2 = 0.58$, $RMSE = 0.7\%$) for the combined data set. *Even so, PRI's association with CNC and*
33 *canopy biomass changed with the season. Most indices responded to both CNC and biomass*
34 *simultaneously, and this confounds the estimation of CNC due to strong but growth stage-*
35 *specific relationships between CNC and canopy biomass. This study shows that it is important*
36 *to consider a wide range of conditions when evaluating multispectral CNC indices.*

37 **Keywords:** Canopy nitrogen concentration; Vegetation index; N uptake; PRI; Canopy biomass

38 1. Introduction

39 The natural supply of nitrogen (N) from the soil is heavily supplemented by fertilisers
40 in modern intensive cropping systems. Soil mineral N and its uptake by crops depends on soil
41 characteristics, crop health and environmental variables that vary in space and time (Gupta et
42 al., 1997; Mamo et al., 2003). Nevertheless, N fertilisers are generally applied homogeneously
43 over a crop irrespective of spatial heterogeneity in crop N requirement and soil N status.
44 Optimal spatial application of N fertiliser can reduce the economic and environmental costs of
45 growing crops. Excess soil N induces emission of the greenhouse gas nitrous oxide (N_2O)
46 (Mosier et al., 1998; Thompson et al., 2019) and, when leached, contaminates surface and
47 groundwater systems (Wang and Li, 2019; Zhai et al., 2017).

48 The Nitrogen Nutrient Index (NNI), calculated as the ratio between actual Canopy
49 Nitrogen Concentration (CNC) and critical CNC, has been shown to provide an accurate

50 measure of canopy N status (Lemaire et al., 2008; Plénet and Lemaire, 1999). The minimum
51 CNC required to maintain the maximum growth is known as the critical CNC, which varies
52 over the growth period. NNI values >1 indicate luxurious N consumption, while values <1 are
53 linked to constrained N supply. The sensitivity of NNI to very small changes in crop N status
54 and its usefulness for N status diagnosis, have made NNI a reliable and popular indicator for
55 crop N status (Lemaire et al., 2008).

56 Unfortunately, field sampling and determining CNC in a laboratory from tissue
57 analysis is time-consuming, destructive, and labour-intensive; limiting its routine application
58 in crop management where high spatial and temporal resolution is required. In comparison,
59 remote sensing can map CNC in a non-invasive and rapid manner frequently and at high
60 spatial resolution, thereby aiding economic N fertiliser management. [Canopy N monitoring at
61 vegetative growth stages is important for site-specific fertilization \(Cammarano et al., 2014;
62 Fitzgerald et al., 2010\) and continuous monitoring of canopy N across growth stages would
63 yield high economic value \(Hank et al., 2019\).](#)

64 Vegetation remote sensing is closely associated with plant chlorophyll, biomass, and
65 water content, typically using contrasting response at different spectral reflectance bands to
66 measure these variables (Curran, 1989; Fourty et al., 1996; Haboudane et al., 2004). Many
67 authors have mentioned the close association between leaf optical reflectance and its
68 chlorophyll content (Baret and Fourty, 1997; Basso et al., 2016; Clevers and Gitelson, 2013;
69 Fourty et al., 1996; Hansen and Schjoerring, 2003; Sims and Gamon, 2002; Yoder and
70 Pettigrew-Crosby, 1995). While there is no direct linkage between leaf reflectance attributes
71 and N content in visible and near-infrared (NIR) (VNIR) bands, the correlation between leaf
72 chlorophyll and N provides the missing link between spectral response and N in leaves (Baret
73 and Fourty, 1997; Chen et al., 2010; Daughtry et al., 2000; Haboudane et al., 2002; Yoder and
74 Pettigrew-Crosby, 1995).

75 Both multispectral (MS) and hyperspectral models have been used for CNC estimation
76 and each has their own advantages. Here we concentrate on MS approaches. MS models use
77 only a few spectral bands and are particularly advantageous in terms of cost, ease of sensor
78 manufacture, tractability of data processing, ability to attain higher spatial resolution (for a
79 given budget) and scaling up to airborne and satellite monitoring.

80 Several MS indices have been developed to estimate CNC at canopy level.
81 Considering the relationship between chlorophyll and CNC, the response of chlorophyll in the
82 visible and red-edge (a region of rapid change in reflectance from red to NIR) parts of the
83 reflectance spectra provides one avenue to spectral indices for mapping CNC (Chen et al.,
84 2010; Flowers et al., 2003; Gabriel et al., 2017; Li et al., 2014a; Zhang et al., 2006). A
85 growing body of literature has examined red-edge based indices for mapping CNC (Chen et
86 al., 2010; Clevers and Kooistra, 2011; Guo et al., 2017; Li et al., 2014a; Schlemmer et al.,
87 2013; Sims and Gamon, 2002). Clevers and Gitelson (2013) presented red-edge band indices
88 that explained up to 80% of the variance in N content in grass. Similarly, Fitzgerald et al.
89 (2010) found a relationship between normalized difference red edge (NDRE) and CNC in
90 winter wheat ($R^2=0.55$).

91 In addition to the changes in CNC, many extraneous factors such as canopy structural
92 attributes (biomass, leaf area index (LAI) and leaf angle distribution (LAD)), view geometry,
93 background reflectance and environmental properties also affect the responses of MS indices
94 at canopy level (Cammarano et al., 2014; Haboudane et al., 2002; He et al., 2016; Van
95 Leeuwen and Huete, 1996). Low-temperature plants tend to have higher N compared to plants
96 grown at high temperature due to temperature directly affecting all physiological processes
97 within plants. Thus, temperature controls the accumulation of N in leaves (Reich and
98 Oleksyn, 2004). N application both induces changes in leaf CNC and modifies the canopy
99 structural attributes (e.g. biomass) (Plénet and Lemaire, 1999). Consequently, changes in

100 canopy MS indices are driven by a combination of many factors, rather than leaf pigments
101 alone (Asner, 1998; Chen et al., 2010; Daughtry et al., 2000; Haboudane et al., 2002;
102 Stroppiana et al., 2009; Viña et al., 2011); with biomass being the most dominant factor
103 (Stroppiana et al., 2009).

104 One important additional factor is LAI which can significantly affect the red-edge
105 feature of a reflectance spectra when obtained at canopy level (Lamb et al., 2002). LAI has
106 long been considered an important extraneous factor in designing spectral indices for pigment
107 detection at the canopy scale. Daughtry et al. (2000) found that >98% of the variance in
108 normalized difference spectral indices is associated with LAI. An example illustrating this
109 issue is the Photochemical Reflectance Index (PRI), which is a narrow-band spectral index
110 originally developed to track the xanthophyll cycle by Gamon et al. (1992). PRI can explain
111 significant variability in maize and soybean chlorophyll content ($R^2 \geq 0.93$); however, it is also
112 reported to be closely associated ($R^2 \geq 0.90$) with LAI (Gitelson et al., 2017). Barton and North
113 (2001) also found that canopy PRI is not only related to the xanthophyll cycle but also
114 influenced by LAI, LAD and background soil.

115 To fix one particular extraneous factor, background soil reflectance, which declines as
116 LAI increases, Barnes et al. (2000) and Clarke et al. (2001) adopted an innovative approach in
117 estimating cotton N concentration by using a two-dimensional index to compensate for
118 changing canopy cover/soil influence on canopy N indices. [The idea of this approach is that
119 one index compensates for canopy cover to correct the CNC signal from a second index, and
120 the result is referred to as the Canopy Chlorophyll Content Index \(CCCI\).](#)

121 CCCI yielded a strong association ($R^2=0.95$) with CNC in cotton (Barnes et al., 2000).
122 Encouraging results have also been obtained in mapping canopy N status using CCCI for a
123 range of crops including cotton (Barnes et al., 2000; El-Shikha et al., 2008), wheat (Basso et
124 al., 2016; Cammarano et al., 2014; Fitzgerald et al., 2010; Perry et al., 2012; Tilling et al.,

125 2007), broccoli (El-Shikha et al., 2007), and maize (Li et al., 2014a, 2014b). CCCI effectively
126 eliminated the canopy cover induced variance and can be used as a N status indicator for
127 canopy cover as low as 30% (El-Shikha et al., 2008).

128 Many N indices have shown joint sensitivity to CNC and biomass (Cammarano et al.,
129 2014; Jay et al., 2017; Stroppiana et al., 2009) or have been found to be sensitive to
130 biomass/LAI (Chen et al., 2010; Gitelson et al., 2017; Viña et al., 2011). Despite taking
131 canopy cover into account, even CCCI has shown a close association with LAI and biomass
132 (Cammarano et al., 2014; Li et al., 2014b). This raises an important question as to whether
133 existing multispectral indices are more closely associated with CNC or biomass/LAI and
134 implies that the performance of existing N indices as a predictor for CNC may rely on a
135 consistent relationship between CNC and biomass. Such consistency may not exist.

136 A variety of factors are known to influence the relationship between CNC and
137 biomass. For example, on one hand, increased N application tends to increase CNC and
138 biomass for a given growth stage (e.g. Plénet and Lemaire (1999)), inducing a positive
139 relationship with variable N application. On the other hand, considered over time, biomass
140 typically has a negative relationship with CNC as the crop grows (Plénet and Lemaire, 1999).
141 Together these two effects can lead to changing CNC-biomass relationship.

142 Taken together, the literature suggests that CNC indices should be assessed across a
143 wide range of conditions. Assessment of the indices within a limited range of conditions may
144 not reveal a) the confounding effects of CNC and biomass and b) the indices that are
145 universally applicable across the wide range of CNC and growth conditions. In many studies,
146 assessment of these indices was limited to one growth stage (e.g. Cammarano et al. (2014),
147 Fitzgerald et al. (2010) and Tilling et al. (2007)). A small number of studies have reported that
148 when assessed across the range of growth conditions, such as years, growth stages, and N
149 application rates, the correlation of indices degraded rapidly due to changing relationship

150 between CNC and the relevant spectral indices (Feng et al., 2016; Li et al., 2014a, 2010;
151 Stroppiana et al., 2009). The wide range of CNC-biomass relationships together with joint
152 sensitivity of MS indices to both CNC and biomass makes selecting robust sensing algorithm
153 challenging. Better understanding these interplays and how they impact different MS indices
154 have the potential to improve the interpretation of algorithms, and that is the focus of this
155 paper.

156 We examined the strength of relationship between each MS index and a) CNC and b)
157 biomass for a range of growth stages, seasons and N application for irrigated pasture. In
158 particular, we considered a wide range of MS indices that have been suggested for sensing
159 CNC/chlorophyll. We did this for eight separate occasions and with combined data for winter,
160 summer and the entire study. In addition, we characterized the changing relationship between
161 CNC and biomass and evaluated changes in the relationships between CNC, biomass and MS
162 indices.

163 **2. Materials and Methods**

164 *2.1. Study area and datasets*

165 The study site is located at Allansford, near Warrnambool (38°24'34.5"S,
166 142°38'13.0"E) in south-western Victoria, Australia. To investigate a wide range of growth
167 and canopy N conditions, observations from perennial ryegrass (*Lolium perenne*) with
168 different N treatments were analysed for several canopy conditions in each of two seasons,
169 May-June (winter) 2018 and January-February (summer) 2019 (refer to Table 1 for
170 measurement dates and pasture status (time since harvest)).

171 The field data collection was scheduled to cover four growth stages over a harvest-
172 growth-harvest cycle (32 days in winter, 19 days in summer) for each season. The ryegrass
173 was grown under the harvest-growth-harvest cycle in winter and summer seasons, and we

174 scheduled the first and last measurements to coincide with harvest events (first and the last
 175 harvest in a growth cycle). The remaining two measurements were performed sufficiently
 176 apart, depending on the biomass growth and logistics, the way through the growth cycle,
 177 resulting in four distinct measurements (Table 1). The last measurement dates, 18 June 2018
 178 and 19 February 2019 were a day or two before the last harvest of the growth cycle.

Table 1. Field data collection dates, season, indicative growth stage and time since previous harvest. The growth stage number increases in the order of biomass development during the season.

Year & season	Measurement date	Growth stage name	Days of growth following harvest
2018 winter	17 May	CC-4	0, immediately prior to harvest
	25 May	CC-1	7
	04 June	CC-2	17
	18 June	CC-3	31
2019 summer	30 January	CC-4	1 day prior to harvest (31 January)
	07 February	CC-1	6
	15 February	CC-2	14
	19 February	CC-3	18

The abbreviation CC stands for canopy condition.

179 The experimental area was 29 m × 23 m and included 45 equal-sized 3 m × 3 m plots
 180 (Fig. 1), each of which received one of eight N treatments or zero N (control). One of three
 181 types of N fertiliser were used for the N treatment of each plot: urea (U, 46% N), urine (from
 182 dairy), and enhanced efficiency fertiliser (EEF, green Urea NVTM). Five replicates of each
 183 application rate; 20, 40, 60, 80 kg/ha for U; 10, 20, 40 kg/ha for EEF, together with five
 184 control plots (no fertiliser, C) resulted in 40 plots with varying canopy N and biomass



Fig. 1. Field site layout showing irrigated ryegrass plots with different N treatments indicated (19 February 2019). U, EEF and C represent urea, enhanced effective fertiliser and control treatments, respectively, and numbers indicate application rates. There is one randomly located replicate for each treatment in each of five east-west rows. (Double column image, colour)

185 conditions. The five urine treated plots were not considered in the analysis since the
 186 distribution of the urine within the treatment plots was found to be non-uniform. [The variety](#)
 187 [of season, sampling time and N application ensures a wide range of growth conditions were](#)
 188 [incorporated to assess the robustness of the spectral indices.](#)

189 Each 3 m × 3 m plot was divided into 0.3 m × 0.3 m sampling sections. A randomly
 190 selected sampling section was then measured with an ASD spectroradiometer, followed by
 191 destructive sampling and laboratory analysis of the ryegrass canopy for CNC and canopy
 192 biomass determination; without overlap of sampling sections between successive sampling

193 dates.

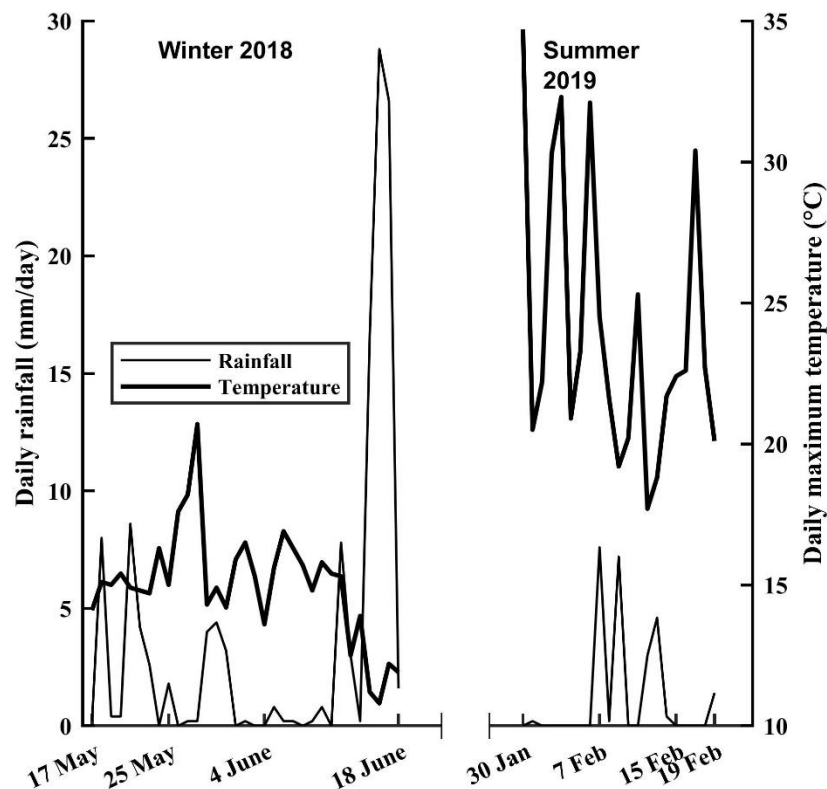


Fig. 2. Daily maximum temperature and rainfall during the winter 2018 and summer 2019 experimental periods. Data source is the Australian Bureau of Meteorology, station 90186 Warrnambool airport NDB station. (Single column image)

194 Fig. 2 shows the weather conditions (daily rainfall depth and maximum temperature)
195 during the data collection period in winter 2018 and summer 2019. The area has oceanic
196 weather with a temperate climate. Overall average temperatures of around 15°C and 24°C
197 were observed in winter 2018 and summer 2019 respectively during the data collection
198 period. Sufficient soil moisture was maintained to ensure it was not a limiting factor by using
199 a centre pivot irrigation system.

200 2.2. Spectroradiometer data collection

201 The canopy-level solar-induced reflectance data were collected over a 0.3 m × 0.3 m

202 sampling section within each plot. The canopy reflectance was measured using an Analytical
203 Spectral Devices (ASD) HandHeld-2 field spectroradiometer (Analytical Spectral Devices,
204 Inc., Boulder, CO, USA) between 12:00 and 14:35 local time. The device was warmed up for
205 approximately 20 minutes prior to sampling. To convert the spectral radiance to reflectance
206 and to compensate for changes in solar illumination, spectra were frequently captured from a
207 99% Spectralon (BaSO₄) panel (Labsphere, Inc., North Sutton, NH, USA) during the
208 sampling. Spectra collection was mostly under the partly cloudy to cloudy conditions due to
209 the coastal location.

210 The ASD HandHeld-2 spectroradiometer has a spectral measurement range of 325-
211 1075 nm, with a spectral resolution of 3.5 nm and a spectral sampling interval of 1.6 nm. An
212 inbuilt interpolation algorithm provided 1-nm interval spectral reflectance in the final output
213 spectra. Therefore, each measured spectrum consisted of a total of 751 reflectance points with
214 1-nm intervals ranging from 325-1075 nm.

215 To obtain a representative canopy level reflectance value for the ground truth
216 sampling area and to reduce measurement variability, 25-30 repeat scans were obtained at
217 approximate heights of 33 cm and 67 cm above the canopy in the nadir position. With a 25°
218 field of view, this configuration produced sampling footprints of approximately 0.15 m and
219 0.30 m in diameter, respectively. These were then averaged together.

220 *2.3. Laboratory processing of ryegrass samples*

221 Immediately after canopy reflectance measurement, the grazeable biomass was
222 harvested. Grazeable biomass comprised most of the above-ground biomass within the 0.3 m
223 × 0.3 m sampling sections was harvested. Sampling was done by cutting at approximate
224 grazing height (~5-7 cm above the soil level). The resulting samples contained the whole
225 canopy and left only stems for the regrowth. Ryegrass samples were preserved in a cold box
226 containing ice for transport to the University of Melbourne laboratory (4-5 hours after

227 sampling). The fresh biomass was then weighed, and samples were dried at a constant
228 temperature of 70°C until they reached a stable dry weight (72-96 hours). Then the dry
229 biomass as a measure of canopy biomass, was determined.

230 Following drying, samples were ground and processed for N analysis. The Dumas
231 Combustion – LECO Trumac method (Matejovic, 1995) was used to determine the CNC
232 (percent of dry weight) from the ground plant samples using a furnace temperature of 1350°C.
233 The N uptake (kg-N/ha) was calculated by multiplying the CNC (g-N/g-canopy biomass) with
234 canopy biomass (kg/ha).

235 *2.4. Multispectral reflectance indices*

236 Eighteen remote sensing indices commonly used for N status and/or chlorophyll
237 estimation at the canopy scale were selected to evaluate their robustness across the changing
238 growth stages and different seasons. Table 2 lists the spectral indices used in this study and
239 the reflectance bands used to calculate them. Based on the literature survey, NDRE, mSR,
240 DCNI, AIVI, WRNI and planar domain indices (CCCI and CCCI-mSR) are grouped as
241 canopy nitrogen indices. Most of the band-difference-ratio based indices in Table 2 were
242 calculated using 10-nm bandwidths centred at the indicated wavelength. An exception was
243 PRI, which was calculated using a 3-nm bandwidth (Trotter et al., 2002).

244 Planar domain indices designed to compensate for the canopy-cover changes in
245 estimating the canopy N status (Barnes et al., 2000; Clarke et al., 2001) were also used. The
246 variability in canopy cover mostly comes from the growth dependent LAI/leaf development
247 of the crop. *In a planar domain index, one of the dimensions uses an index sensitive to canopy
248 cover and the other uses an N sensitive index. NDVI is a routine choice for the canopy cover
249 index in the CCCI literature (Barnes et al., 2000; Cammarano et al., 2014; Fitzgerald et al.,
250 2010) and we have followed that practice here.* Two planar domain indices were evaluated –
251 CCCI and CCCI-mSR. These used NDRE and mSR as indices sensitive to canopy N,

Table 2. List of spectral indices used for CNC and/or chlorophyll status estimation in this study.

Index	Formula	Reference
<i>One dimensional index</i>		
NDVI (Normalized Difference Vegetation Index)	$NDVI = (R800 - R670) / (R800 + R670)$	Rouse Jr et al. (1973)
SAVI (Soil Adjusted Vegetation Index)	$SAVI = (1 + L) \times (R800 - R670) / (R800 + R670 + L)$; $L=0.5$	Huete (1988)
PRI (Photochemical Reflectance Index)	$PRI = (R531 - R570) / (R531 + R570)$	Gamon et al. (1992)
TCARI (Transformed Chlorophyll Absorption in Reflectance Index)	$TCARI = 3 \times [(R700 - R670) - 0.2 \times (R700 - R550) \times (R700 / R670)]$	Kim et al. (1994)
NPCI (Normalized Pigment Chlorophyll Ratio Index)	$NPCI = (R680 - R430) / (R680 + R430)$	Peñuelas et al. (1994)
SIPI (Structural Insensitive Pigment Index)	$SIPI = (R800 - R445) / (R800 - R680)$	Penuelas et al. (1995)
OSAVI (Optimised Soil-Adjusted Vegetation Index)	$OSAVI = (1 + 0.16) \times (R800 - R670) / (R800 + R670 + 0.16)$	Rondeaux et al. (1996)
NDRE (Normalized Difference Red Edge)	$NDRE = (R790 - R720) / (R790 + R720)$	Barnes et al. (2000)
CCII (Canopy Chlorophyll Inversion Index)	$CCII = TCARI / OSAVI$	Haboudane et al. (2002)
EVI (Enhanced Vegetation Index)	$EVI = 2.5 \times (R860 - R645) / (1 + R860 + 6 \times R645 - 7.5 \times R470)$	Huete et al. (2002)
mND705 (Modified Red-Edge Normalized Difference Vegetation Index)	$(mND705) = (R750 - R705) / (R750 + R705 - 2 \times R445)$	Sims and Gamon (2002)
mSR (Modified Simple Ratio)	$mSR = (R750 - R445) / (R705 - R445)$	Sims and Gamon (2002)
MTCI (MERIS Terrestrial Chlorophyll Index)	$MTCI = (R750 - R710) / (R710 - R680)$	Dash and Curran (2004)
DCNI (Double-peak Canopy Nitrogen Index)	$DCNI = (R720 - R700) / (R700 - R670) / (R720 - R670 + 0.03)$	Chen et al. (2010)
AIVI (Angular Insensitivity Vegetation Index)	$AIVI = [R445 \times (R720 + R735) - R573 \times (R720 - R735)] / [R720 \times (R573 + R445)]$	He et al. (2016)
WRNI (Water Resistance Nitrogen Index)	$WRNI = NDRE / fWBI^*$	Feng et al. (2016)

Planar domain index

CCCI (Canopy Chlorophyll Content Index)	$CCCI = (NDRE - NDRE_{min}) / (NDRE_{max} - NDRE_{min})$	Barnes et al. (2000)
CCCI-mSR	$CCCI-mSR = (mSR - mSR_{min}) / (mSR_{max} - mSR_{min})$	Rodriguez et al. (2006)

Note. RXXX represents the reflectance value at centre wavelength XXX nm. $fwBI^* = R900 / \min(R930-980)$ (Strachan et al., 2002).

252 respectively, and NDVI to represent canopy cover (Fitzgerald et al., 2010, 2006; Rodriguez et
 253 al., 2006). Fig. 3 illustrates the concept. Lower and upper bound lines of NDRE (or mSR) vs.
 254 canopy cover (NDVI) are determined, and the index is then calculated by scaling the

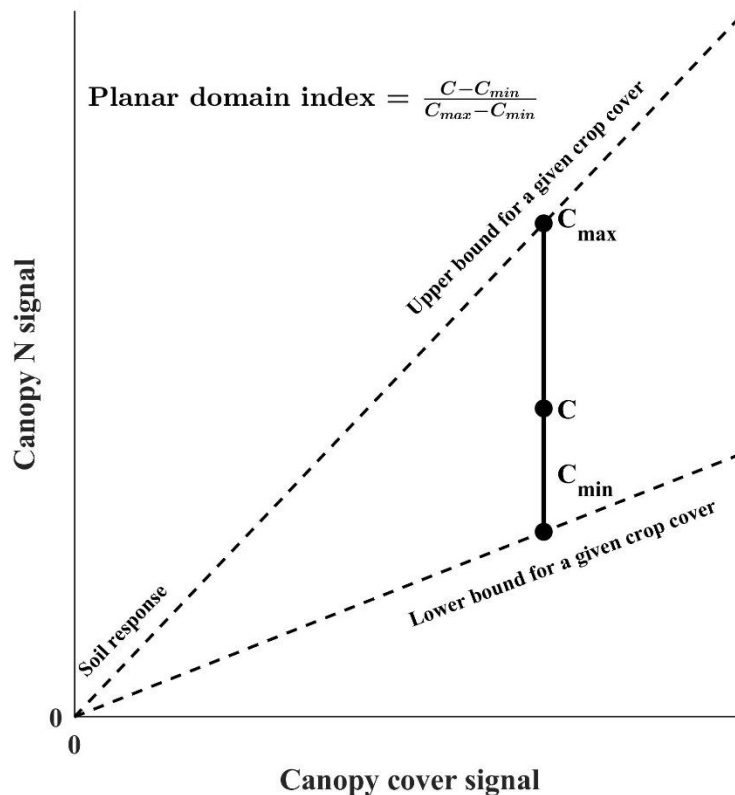


Fig. 3. Schematic illustration of the planar domain index calculation. (Single column image)

255 measured NDRE (or mSR) between the upper and lower bounds, which are determined from
 256 the measured NDVI using the bounding lines. The upper and lower bound relationships

257 between NDRE (or mSR) and NDVI, were defined using $1.1 \times$ maximum and $0.9 \times$ minimum
258 of ratio of NDRE (or mSR) to NDVI the dataset for the particular growth stage.

259 The intersection of the upper and lower bounds at the origin corresponds to the
260 hypothetical bare soil response. The planar domain indices have values between 0 and 1 for
261 all possible cases of canopy cover; however, values other than this range are also possible
262 when data lies outside the lower or upper bounds.

263 *2.5. Statistical analyses*

264 The relationship between remote sensing indices and crop variable (CNC and canopy
265 biomass) were quantified using standard regression analysis. The coefficient of determination
266 (R^2), root mean square error (RMSE) and normalized RMSE (NRMSE) were used as
267 performance metrics for assessing the degree of association in the relationships and their
268 precision. It should be noted that here these are measures of fit, not the predictive skill, as no
269 independent validation has been undertaken, given the aims of the study. The relationship
270 between each spectral index and crop variable was established using popular two-parameter
271 univariate regression models: linear (L), logarithmic-linear (LI), linear-logarithmic (IL),
272 exponential (E) and power (P) models (Table 3). Quadratic fit, a three-parameter model, was
273 also tested but produced minor changes and did not change the overall conclusion.
274 Relationships were developed for individual groups based on growth stage (CC-1, 2, 3 and 4)
275 and season (winter and summer), and to the entire data set separately. PRI was only fitted
276 with L, LI and E models due to the presence of negative values. The relationship chosen and
277 the statistics quoted in each case were based on the best performance according to RMSE.
278 RMSE and NRMSE were calculated using:

$$279 \quad RMSE = \sqrt{\frac{1}{N-p} \times \sum_{k=1}^N (Y_{predicted}^k - Y^k)^2}, \quad (1)$$

280
$$NRMSE = \frac{RMSE}{\bar{Y}} \times 100, \quad (2)$$

281 where Y , \bar{Y} , $Y_{predicted}$, N and p are the observed crop variable (CNC and canopy biomass),
 282 arithmetic mean of Y , predicted crop variable, number of observations and number of
 283 parameters in the regression equation (e.g. $p=2$ for two parameter models), respectively. The
 284 standard Spearman rank correlation coefficient (r) was used to quantify the strength and
 285 direction of monotonic relationship between CNC and canopy biomass.

Table 3. Model functional forms. a is a regression coefficient and b is an intercept term. X is a remote sensing index and Y is a crop variable (i.e. CNC or canopy biomass). The letters in bracket next to each model name give the abbreviation for that model.

Model	Functional formula
Linear (L)	$Y = a \cdot X + b$
Exponential (E)	$Y = a \cdot e^X + b$
Logarithmic-linear (LI)	$\ln Y = a \cdot X + b$
Linear-logarithmic (IL)	$Y = a \cdot \ln X + b$
Power (P)	$\ln Y = a \cdot \ln X + b$

286 **3. Results**

287 *3.1. Variability of canopy N and canopy biomass*

288 CNC, canopy biomass and N uptake derived from the ground biomass samples varied
 289 markedly with growth stage and season (Table 4). Fig. 4 shows the high variability in CNC
 290 and canopy biomass, which was due to the combination of variable N fertiliser application
 291 and different growth stages. [While overall CNC shows a declining trend with canopy biomass](#)
 292 [across growth stages in both seasons, CNC increases with canopy biomass at each growth](#)
 293 [stage \(Fig 4a,b\). Fig 4c,d shows the behaviour of some distinct N treatment groups \(U80,](#)

294 U40, U20 and C) across the growth stages. In general, higher N treatment groups, for
 295 example, U80 had high CNC and canopy biomass compared to lower N treatment groups
 296 (such as C) at a given growth stage, but CNC diluted as biomass increased for any given N
 297 treatment. Due to the combination of these opposing trends, CNC vs. canopy biomass from
 298 our experimental site features significantly wider scatter than conventional ‘dilution curves’
 299 constructed from uniformly fertilised fields.

Table 4. Canopy biomass, CNC and N uptake of ryegrass at four growth stages of winter and summer.

		Winter 2018				Summer 2019			
		CC-1	CC-2	CC-3	CC-4	CC-1	CC-2	CC-3	CC-4
Canopy biomass (kg/ha) ^a	mean	370	600	990	1540	560	880	1240	1450
	std	120	180	320	470	180	510	590	590
	CV	0.32	0.30	0.32	0.31	0.32	0.58	0.48	0.41
CNC (%)	mean	4.1	4.5	4.1	3.7	3.1	2.7	2.4	2.0
	std	0.5	0.5	0.5	0.5	0.8	0.5	0.5	0.4
	CV	0.12	0.11	0.12	0.14	0.26	0.19	0.21	0.20
N uptake (kg- N/ha) ^a	mean	15	28	42	58	18	27	33	30
	std	6	10	16	19	08	19	22	17
	CV	0.40	0.36	0.38	0.33	0.44	0.70	0.67	0.57

Note: std - standard deviation; CV- coefficient of variation (std/mean); ^a denotes area measurements are based on ground area. Sample size (*n*) is 40 for values in the table.

300 CNC varied over two quite different ranges in winter and summer (2.8% - 5.4% in
 301 winter, and 1.3% - 4.3% in summer). On average, CNC values were 58% higher in winter
 302 than summer. The observed decrease in summer season CNC is attributed to the influence of
 303 temperature on physiological processes within the plants leading to a decrease in leaf N in
 304 warm weather (Reich and Oleksyn, 2004). Response to different N treatments are more

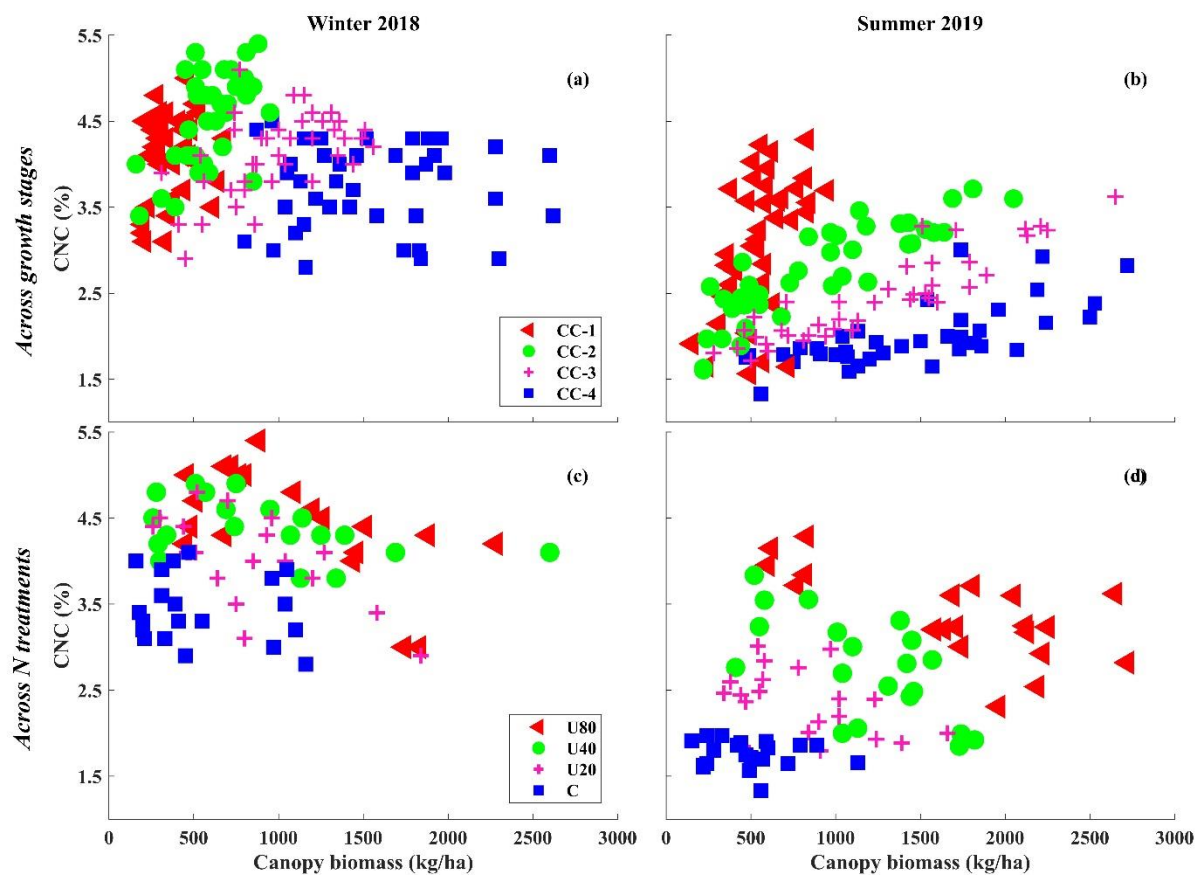


Fig. 4. Scatter plots of CNC (%) and canopy biomass (kg/ha). (a) and (b) show summer and winter results respectively, combined across all N application rates for each sampling occasion. (c) and (d) show summer and winter results respectively for selected N treatment groups; U80, U40, U20 and C. (Double column image, colour)

305 distinct in summer than in winter (Fig. 4c,d). CNC reduced as the sward grew in both seasons,
 306 with mean CNC declining from 4.1% to 3.7% (approximately 10%) in winter and 3.1% to
 307 2.0% (approximately 36%) in summer for CC-1 to CC-4, respectively. The variability, as
 308 measured by CV, remained relatively consistent within each season, compared with the
 309 difference in CV between seasons (Table 4). The overall variance in CNC with respect to both
 310 treatments and growth stages was almost double in summer compared with winter.

311 More rapid biomass growth occurred in summer than winter with average canopy

312 biomass being 17% higher and the harvest-growth-harvest cycle being 42% shorter in summer
313 than winter. Despite this difference, the range of biomass in both summer and winter
314 remained comparable (Fig. 4). Variation (standard deviation) in canopy biomass was also
315 higher in summer than winter after CC-1, increased with biomass. Biomass variability, as
316 measured by CV, was higher for summer growth stages (except CC-1), than winter, and
317 peaked for the middle growth stages in summer while remaining flat in winter (Table 4). On
318 average, summer growth stages exhibited higher variance in both CNC and canopy biomass
319 compared to winter (Table 4). In contrast to CNC, winter canopy biomass exhibited slightly
320 (around 7%) higher variance than summer.

321 In addition to CNC, N uptake is commonly used as a measure of canopy N content
322 when remote sensing is used to estimate crop N. Variance of N uptake followed a similar
323 pattern to canopy biomass, with summer growth stages exhibiting a higher CV (Table 4).
324 Generally, mean N uptake increased with canopy biomass. The exception was CC-4 in
325 summer when N uptake decline from its value at CC-3 due to the declining CNC (Table 4). N
326 uptake demonstrated a very high correlation with canopy biomass ($0.86 < r < 0.99$) on all
327 sampling occasions (Fig. 5), whereas the correlation between CNC and canopy biomass
328 changed with growth stage ($0.01 < r < 0.90$). These results suggest that N uptake is largely
329 determined by the canopy biomass with little information on CNC. Therefore, CNC will be
330 used in the subsequent sections to analyse canopy N vs. canopy biomass relationship and to
331 evaluate the association of the chosen MS indices. Both the correlation ($0.01 < r < 0.90$) and the
332 relationship between CNC and canopy biomass varied across a wide range depending on
333 individual growth stages (Fig. 5). The correlation increased from CC-1, peaked at CC-2
334 ($r=0.49$) in winter and CC-3 ($r=0.90$) in summer, and then declined. A risk in analysing
335 performance is that this high correlation could confound the distinct spectral attributes of
336 CNC at some growth stages.

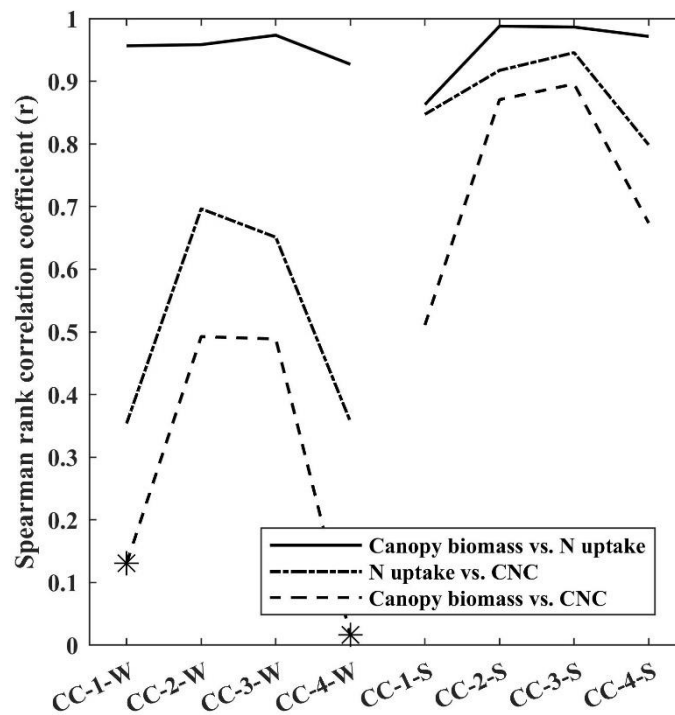


Fig. 5. Spearman rank correlation (r) between CNC (%), canopy biomass (kg/ha) and N uptake (g-N/m^2) over the growth stages in winter 2018 and summer 2019 ($n=40$). W and S indicate winter and summer seasons, respectively. * indicates correlation not statistically significant ($p>0.05$). (Single column image)

337 3.2. Relationship between spectral indices and crop variables (CNC and canopy biomass) at
 338 individual growth stages

339 The most important criteria for the selection of a remote sensing index are a high
 340 correlation with the target variable (CNC in this case) and low sensitivity to the other
 341 confounding factors (Daughtry et al., 2000). Fig. 6 and Fig. 7 show R^2 and NRMSE for the
 342 CNC and canopy biomass estimated by 18 remote sensing indices at each growth stage (see
 343 corresponding tables, Table S1 and S2 in the supplementary material). While there was
 344 contrasting performance between stages, most of the indices exhibited similar performance at
 345 individual growth stages, except for TCARI, CCII and DCNI. The best index varied between

346 stages, but the difference between indices in explaining either CNC or canopy biomass
 347 variation was small within the growth stage. For instance, PRI ($R^2=0.46$, $RMSE=0.4\%$) was
 348 best for CC-2 during winter while OSAVI ($R^2=0.84$, $RMSE=0.2\%$) was best for CC-2

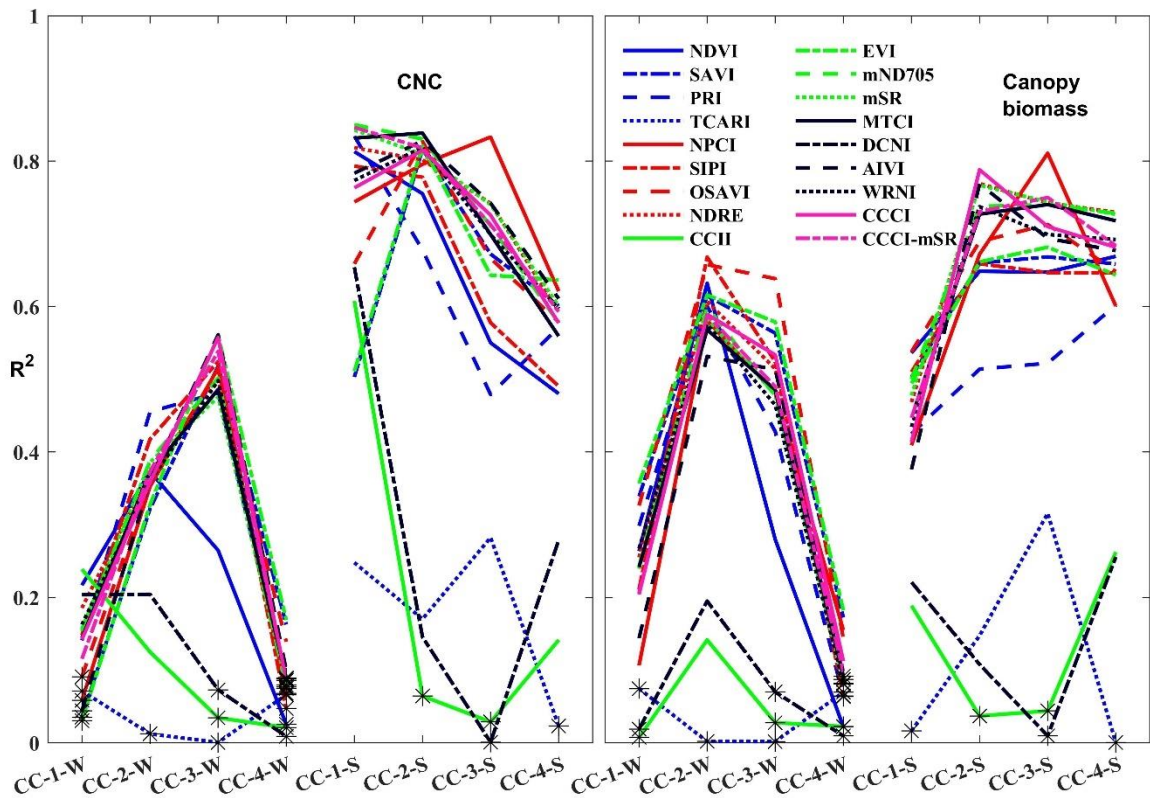


Fig. 6. Coefficient of determination (R^2) for CNC (left panel) and canopy biomass (right panel) at individual growth stages in winter 2018 and summer 2019 ($n=40$). * indicates that the CNC/biomass model did not show statistically significant skills ($p > 0.05$). W and S indicate winter and summer seasons, respectively. The best functional form among L, E, LI, IL and P relation between spectral indices and crop variables is used to construct the plots. (Double column image, colour)

349 summer CNC assessment. CNC estimation was best for CC-3 in winter and CC-2 in summer
 350 (Fig. 7). Compared with CC-3, in winter, the mean NRMSE of spectral indices (except
 351 TCARI, CCI and DCNI) was around 27%, 14% and 38% higher for CC-1, 2 and 4

352 respectively. Similarly, CC-2 in summer exhibited the lowest NRMSE in CNC estimation,
 353 with NRMSE in CC-1, 3 and 4 being 27%, 24% and 22% higher, respectively.

354 There was a large difference in terms of R^2 and NRMSE in CNC assessment between
 355 summer and winter (Fig. 6 and Fig. 7). CNC was much more variable in summer and this
 356 primarily led to the higher R^2 values. While raw RMSE was slightly lower in summer, the
 357 higher mean CNC in winter (Table 4) led to the lower NRMSE in winter than summer.

358 Although the planar domain indices (CCCI and CCCI-mSR) aim to control for changes in
 359 canopy cover when measuring CNC, their association with CNC was similar to the other well-
 360 performing indices such as mSR and MTCI (Fig. 6 & Fig. 7).

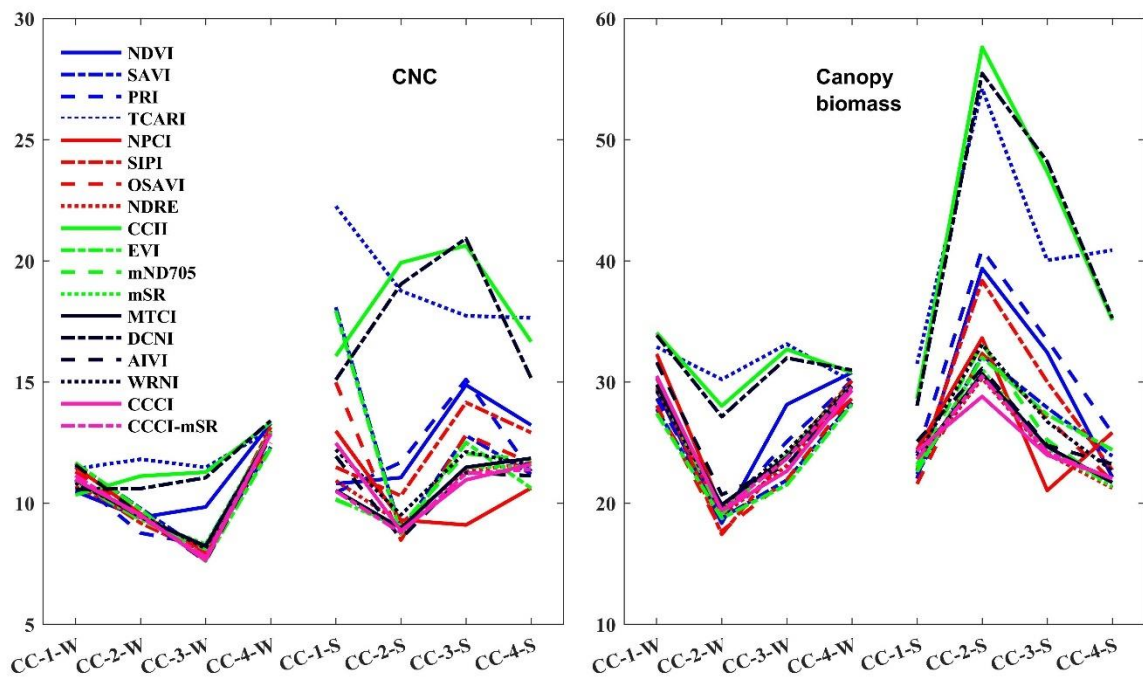


Fig. 7. Corresponding to Fig. 6, normalized RMSE (NRMSE) plots of the remote sensing indices' regression against CNC and canopy biomass in all the individual growth stages in winter 2018 and summer 2019. (Double column image, colour)

361

362 Compared with CNC, the best canopy biomass estimate was shifted towards earlier
363 growth stages – CC-2 (mean NRMSE=21%) in winter and CC-1 (mean NRMSE=25%) in
364 summer (Fig. 7). The NRMSE for canopy biomass showed opposite seasonal patterns;
365 peaking at CC-2 in summer but with the minimum at CC-2 in winter (Fig. 7). As with CNC,
366 the planar domain indices performances in estimating canopy biomass were similar to the
367 conventional one-dimensional indices (e.g. MTCI and AIVI).

368 *3.3. Evaluation of relationship between spectral indices and crop variables across wide range* 369 *of conditions*

370 For the intermediate growth stages, remote sensing indices exhibit moderate to very
371 high performance in estimating CNC and canopy biomass, as shown in Fig. 6. What is not
372 evident from R^2 in Fig. 6 is that the relationships move about over time. This is illustrated
373 using PRI and NPCI in Fig. 8 and Fig. 9, which are typical examples. Plots for the other
374 indices are included in the supplementary information. Thus, the relationship between indices
375 and crop variables established at one growth stage can't be successfully transferred to another
376 growth stage (typical example Fig. 9).

377 To examine whether some indices might be more transferrable between stages, Table
378 5 provides regression summary statistics for the relationship between each spectral index and
379 each of CNC and the perturbing factor, canopy biomass, for pooled data. When all the growth
380 stages were pooled, performance reduced markedly, with only PRI ($R^2=0.58$) having an R^2
381 greater than 0.26 for CNC. Correlation with biomass also declined markedly, with the best R^2
382 being 0.39 for NPCI.

383 Table 5 also summarises fits to all the data from each season separately. Generally, the
384 RMSE for CNC was similar for summer and winter but the R^2 values for winter are much
385 lower than those for summer as the observed CNC had a lower variance in winter ($CV=0.14$)
386 (Table 5). In summer, most of the indices were associated with both CNC and canopy

Table 5. Regression statistics between remote sensing indices and crop variables (CNC and canopy biomass) across the seasons (winter 2018 (W), summer 2019 (S) and all seasons together (W+S)). The best out of linear (L), exponential (E), logarithmic-linear (LI), linear-logarithmic (IL) and power (P) model's RMSE and corresponding coefficient of determination (R^2) values have been listed here.

Index	CNC (%)						Canopy biomass (kg/ha)					
	R^2			RMSE			R^2			RMSE		
	W	S	W+S	W	S	W+S	W	S	W+S	W	S	W+S
NDVI	0.04 ^E	0.29 ^E	0.26 ^E	0.6	0.6	0.9	0.28 ^E	0.50 ^{IL}	0.26 ^E	460	460	500
SAVI	<u>0.00</u> ^{IL}	0.28 ^{IL}	0.24 ^{IL}	0.6	0.6	0.9	0.72 ^{LI}	0.41 ^E	0.34 ^E	340	460	470
PRI	<u>0.02</u> ^L	0.77 ^{LI}	0.58 ^L	0.6	0.3	0.7	0.41 ^E	<u>0.01</u> ^L	0.03 ^E	420	600	570
TCARI	0.07 ^{IL}	<u>0.00</u> ^E	0.04 ^{IL}	0.5	0.7	1.0	0.16 ^E	0.07 ^E	0.07 ^E	500	580	550
NPCI	<u>0.00</u> ^E	0.24 ^E	0.13 ^E	0.6	0.6	0.9	0.53 ^{IL}	0.43 ^{IL}	0.39 ^{IL}	370	450	450
SIPI	<u>0.02</u> ^E	0.26 ^{IL}	0.16 ^{IL}	0.6	0.6	0.9	0.53 ^P	0.51 ^P	0.43 ^P	430	450	470
OSAVI	<u>0.01</u> ^{IL}	0.30 ^{IL}	0.26 ^{IL}	0.6	0.6	0.9	0.68 ^{LI}	0.43 ^E	0.33 ^E	370	450	470
NDRE	0.03 ^{IL}	0.40 ^{IL}	0.17 ^{IL}	0.6	0.5	0.9	0.40 ^E	0.47 ^E	0.36 ^L	420	440	460
CCII	0.10 ^E	0.08 ^{IL}	<u>0.01</u> ^E	0.5	0.7	1.0	<u>0.00</u> ^{IL}	<u>0.01</u> ^{IL}	<u>0.01</u> ^{IL}	540	600	570
EVI	<u>0.00</u> ^{IL}	0.32 ^{IL}	0.24 ^{IL}	0.6	0.6	0.9	0.60 ^E	0.38 ^E	0.34 ^E	340	470	470
mND705	0.03 ^{IL}	0.45 ^E	0.24 ^E	0.6	0.5	0.9	0.38 ^E	0.38 ^E	0.29 ^E	430	480	490
mSR	0.03 ^{IL}	0.45 ^{IL}	0.24 ^{IL}	0.6	0.5	0.9	0.38 ^{IL}	0.39 ^L	0.29 ^{IL}	430	470	490
MTCI	0.03 ^{IL}	0.49 ^{IL}	0.24 ^{IL}	0.6	0.5	0.9	0.38 ^{IL}	0.36 ^L	0.28 ^{IL}	430	480	490
DCNI	0.10 ^{IL}	0.21 ^L	0.05 ^L	0.5	0.6	1.0	<u>0.01</u> ^L	<u>0.00</u> ^{IL}	<u>0.01</u> ^{IL}	540	600	570
AIVI	<u>0.01</u> ^{IL}	0.50 ^{IL}	0.18 ^E	0.6	0.5	0.9	0.41 ^{IL}	0.33 ^E	0.29 ^{IL}	420	490	480
WRNI	0.04 ^{IL}	0.44 ^L	0.18 ^E	0.6	0.5	0.9	0.32 ^{IL}	0.37 ^E	0.29 ^{IL}	450	480	480
CCCI	0.03 ^{IL}	0.42 ^{IL}	0.13 ^E	0.6	0.5	0.9	0.41 ^L	0.45 ^E	0.37 ^L	420	440	460
CCCI-mSR	0.03 ^{IL}	0.54 ^P	0.22 ^L	0.6	0.5	0.9	0.38 ^L	0.36 ^E	0.28 ^{IL}	430	480	490

Sample size (n) is 160, 160 and 320 for W, S and W+S respectively. The bolded numbers indicate the best performing index's R^2 with crop variables for the given dataset. Underlined R^2 values indicate $p > 0.05$ for the significance of regression slope.

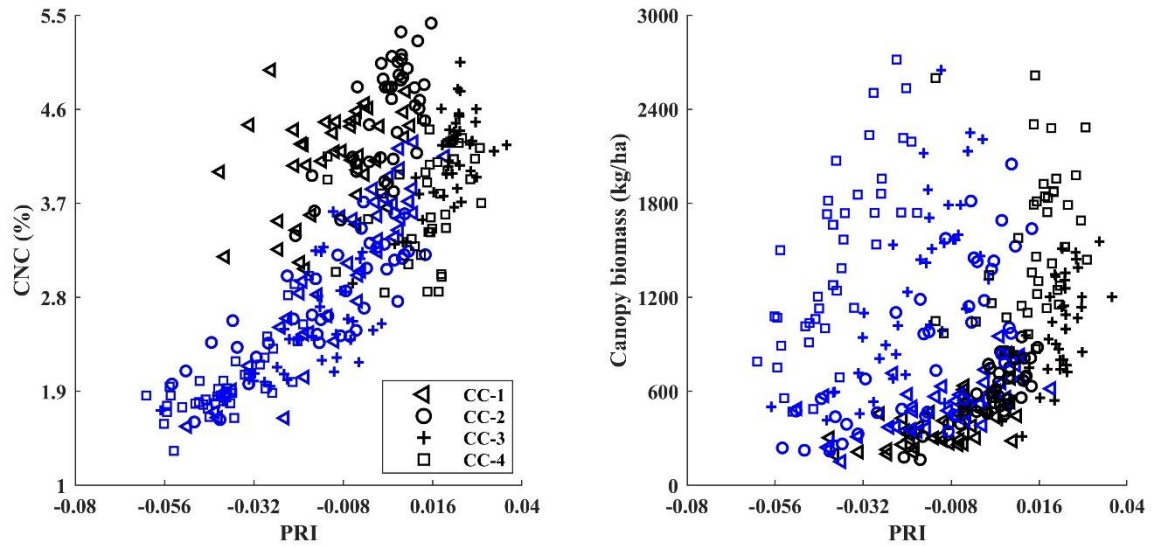


Fig. 8. Relationship between PRI and CNC and canopy biomass in winter (black) and summer (blue) at each growth stage. (Double column fitting image, colour)

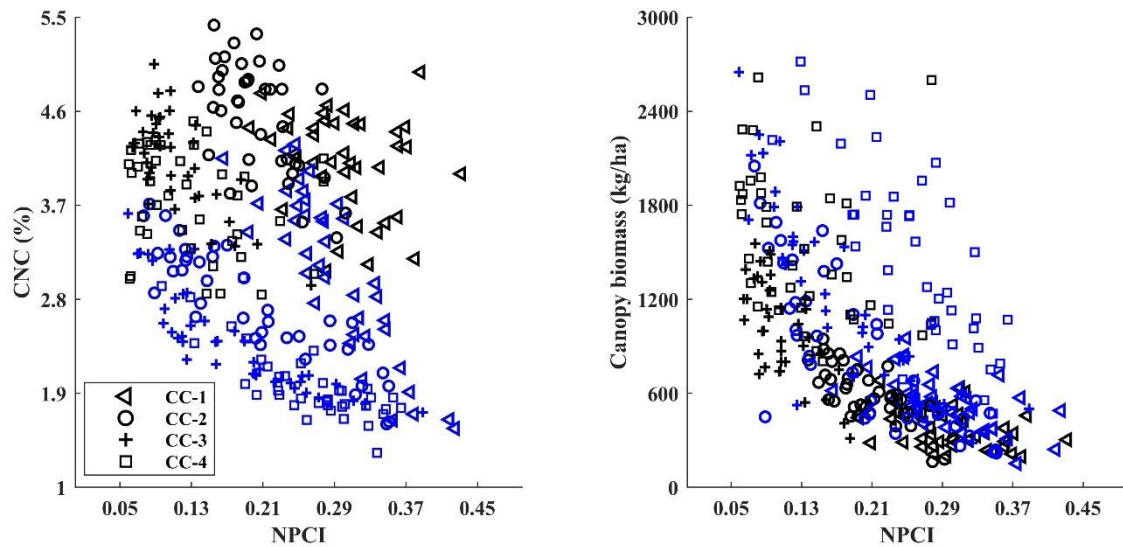


Fig. 9. Relationship between NPCI and CNC and canopy biomass in winter (black) and summer (blue) at each growth stage. (Double column image, colour)

387 biomass; the exceptions being PRI, DCNI, CCII and TCARI. In contrast to CNC, association
 388 with biomass were generally better in winter than summer, both in terms of R^2 and RMSE.

389 PRI is best correlated with CNC, with $R^2=0.77$ and $RMSE=0.3\%$ in summer 2019, and

390 $R^2=0.58$ and $RMSE=0.7\%$ overall, with effectively no correlation with canopy biomass
391 ($R^2=0.01$). In winter, PRI had a poor relationship with CNC but an R^2 of 0.41 for canopy
392 biomass (Table 5). Fig. 8 shows the relationship of PRI with CNC and canopy biomass across
393 all the data with growth stages and seasons identified. There is wide scatter at individual
394 growth stages (particularly at CC-1 and 2 in winter), but a consistent relationship when all
395 stages are combined. PRI's relationship with canopy biomass shifted with growth stage and
396 season, leading to particularly high RMSE and significant scatter in summer (Table 5 and Fig.
397 8).

398 Despite nominally controlling for changes in canopy cover confounding canopy N
399 signals, the planar domain indices were poor at representing CNC (Table 5). Furthermore,
400 overall, they were actually more sensitive (higher R^2) to canopy biomass than CNC (Table 5).
401 CCCI-mSR's performance was slightly better than CCCI in CNC estimation.

402 Unlike CNC estimation, no single standout spectral index was found for estimating
403 canopy biomass. There were many candidate indices with relatively better sensitivity towards
404 canopy biomass (e.g. EVI, SAVI, OSAVI, NDRE, CCCI, NPCI showed similar performance)
405 in winter, summer and pooled data. RMSE increased for all spectral indices, especially SAVI,
406 PRI, NPCI, OSAVI and EVI, when estimating canopy biomass during summer compared
407 with winter (Table 5). EVI and NDRE exhibited better performance in estimating canopy
408 biomass in winter and summer, respectively (Table 5). However, when a wide range of
409 conditions were pooled together, NPCI was the most robust and accurate. Most of the spectral
410 indices (except for TCARI, CCII and DCNI) were only slightly to moderately correlated with
411 canopy biomass in the pooled dataset. Wider scatter was observed at CC-4 in both winter and
412 summer for the relationship between NPCI, CNC and canopy biomass (Fig. 9). This high
413 scatter was also evident with other spectral indices at CC-4 in both seasons (see
414 supplementary information for plots).

415 Saturation was observed for spectral indices such as NDVI and SIPI for both CNC and
416 canopy biomass (see supplementary information for plots). As with the results for individual
417 days (season and growth stage), TCARI, CCII and DCNI did not respond to either CNC or
418 canopy biomass. Moreover, fWBI (used in estimating WRNI in this study) (Strachan et al.,
419 2002) had the highest and most consistent correlation with canopy biomass in both seasons
420 and collectively ($R^2= 0.64, 0.66, 0.55$; RMSE= 360, 320, 390 kg/ha, respectively for winter,
421 summer and pooled data).

422 **4. Discussion**

423 This study aimed to evaluate the performance of selected spectral indices for sensing
424 CNC across a wide range of growth and environmental conditions, as well as to evaluate the
425 influence of canopy biomass on CNC estimation. The most important finding that emerges
426 from the results is that the relationship between various spectral indices and CNC changes
427 between growth stages and seasons (Fig. 6 and a typical example Fig. 9). *Therefore, the*
428 *correlation between CNC and individual indices varied significantly across growth stages and*
429 *seasons. This finding is consistent with those of Feng et al. (2016) (wheat), Gitelson et al.*
430 *(2017) (soybean and maize) and Li et al. (2014a) (maize) who found different relationships*
431 *between the indices they considered and canopy properties (N and chlorophyll) at different*
432 *growth stages. As a consequence of this changing relationship, the correlation between*
433 *individual indices and CNC declines markedly when evaluated against the pooled data (Table*
434 *5) for nearly all indices.* Moreover, many of the indices previously reported as CNC indicators
435 responded to both CNC and canopy biomass (Table 5 and Fig. 6). Fig. 6 shows that most of
436 the indices have similar sensitivity to changes in CNC and canopy biomass. Spectral indices
437 are influenced by many factors including biomass, soil background, canopy structure and
438 environmental variables (Cammarano et al., 2014; Govaerts et al., 1999; Hansen and
439 Schjoerring, 2003), with biomass being the most significant (Stroppiana et al., 2009). Thus,

440 designing an index sensitive to only one target variable (e.g. CNC here) has been challenging
441 and a prime focus of the remote sensing community. In most previous CNC sensing studies,
442 indices were evaluated or developed with data from a limited range of growth stages and
443 seasons. An implication of our results is that future research on developing indices less
444 influenced by biomass and showing consistent sensitivity to CNC should focus on
445 incorporating a comprehensive range of growth and environmental conditions.

446 In addition, caution should be used when designing/modifying indices for CNC
447 mapping at the canopy scale. As can be seen from Fig. 5, certain growth stages have a high
448 correlation between ground CNC and canopy biomass (as high as 0.90). This high correlation
449 may confound the retrieval of canopy scale CNC signals that are distinct from the biomass
450 effects. The susceptibility to biomass attributes of canopy level indices further adds
451 complexity in retrieving distinct CNC signals (Daughtry et al., 2000; Haboudane et al., 2002;
452 Stroppiana et al., 2009; Yoder and Pettigrew-Crosby, 1995) and the sensitivity of some
453 indices at certain growth stages to CNC may be only applicable to the extent that CNC and
454 canopy biomass are correlated. Moreover, incorporating a wide range of N application
455 treatments brings more scatter to the CNC-biomass relation (Fig 4c,d). One possible
456 implication is that under relatively homogenous N treatments or certain soil-plant interactions,
457 CNC-biomass may be negatively correlated across growth stages (due to the CNC dilution).
458 Therefore, indices significantly influenced by biomass may perform better for CNC sensing to
459 the extent this correlation holds. Thus, it could be argued that even across growth stages,
460 performance of spectral indices may not be a decisive test of its sensitivity to CNC.

461 While we found poor performance from many indices when confronted with a range
462 of growth stages, PRI performed quite well. PRI was originally designed with the aim of
463 sensing the xanthophyll cycle and has been related with many canopy properties such as
464 photosynthetic light use efficiency (LUE) (Barton and North, 2001; Gamon et al., 1992;

465 Goerner et al., 2009; Jia et al., 2018), chlorophyll : carotenoid ratio (Gamon et al., 2015) and
466 LAI (Gitelson et al., 2017). Depending on the temporal scale of measurements, the stronger
467 relationship switches between LUE and the chlorophyll : carotenoids ratio (Gamon et al.,
468 2015). Nevertheless, among the 18 spectral indices evaluated, PRI showed the best and most
469 consistent performance in monitoring CNC (Table 5). PRI has not been explicitly recognized
470 as a CNC indicator in the literature, but some recent studies have also reported moderate to
471 high association of PRI with CNC for wheat (He et al., 2016; Ranjan et al., 2012), rice (Tian
472 et al., 2011) and barley (Xu et al., 2014). PRI's positive relation with chlorophyll :
473 carotenoids ratio and chlorophyll content at the seasonal timescale (Gamon et al., 2015;
474 Gitelson et al., 2017), together with the functional relation between chlorophyll and CNC
475 likely explains the robust performance of canopy level PRI for CNC here. While PRI showed
476 the greatest consistency with CNC, the relationship deviated at the lowest canopy covering
477 growth stage (CC-1 winter), which is consistent with the findings of soil influence by Barton
478 and North (2001) (Fig. 8).

479 When considering each season separately, PRI yielded a moderate correlation with
480 canopy biomass in winter and no relationship in summer, and vice versa for CNC (Table 5).
481 PRI has multiple drivers at canopy level, one of which is LAI (Barton and North, 2001;
482 Gitelson et al., 2017). Together with low photoprotection (xanthophyll cycle) activity during
483 winter, this might explain the enhanced correlation between PRI and canopy biomass that
484 appeared only in winter. This seasonal association of PRI with biomass affects its ability to
485 estimate CNC across the seasons. Thus, the coefficient of determination (R^2) dropped from
486 0.77 in summer to 0.58 in the pooled data (Table 5). Considering the conflicting conclusions
487 by Gamon et al. (2015), Gitelson et al. (2017) and Sims and Gamon (2002) together with a
488 lack of ground LUE or chlorophyll : carotenoid ratio data, it remains unclear why PRI was
489 related to CNC in summer but to canopy biomass in winter. Further studies investigating

490 PRI's changing relationship with canopy biomass and CNC over the different seasons are
491 needed.

492 Another key issue in this study is that the spectral indices designed for
493 CNC/chlorophyll actually performed better for sensing canopy biomass. The performance of
494 most of the indices was also more consistent for canopy biomass than CNC, even in the
495 pooled dataset (Table 5), suggesting that a significant amount of the variability in the canopy
496 level indices comes from canopy biomass. Daughtry et al. (2000) also reported a very
497 significant proportion (>90%) of variability in the spectral indices attributed to LAI. Like
498 Viña et al. (2011) for maize and soybean, we found that EVI and MTCI more consistently
499 represent canopy biomass. Zarate-Valdez et al. (2012) also reported the same for EVI from
500 the almond crop. NDVI and SIPI showed (results in supplementary material) early saturation,
501 compared with other indices. We also found that NPCI, SAVI, OSAVI, EVI, NDRE are more
502 consistently related to canopy biomass (Table 5). In accordance with our results, previous
503 studies have also demonstrated saturation of NDVI at high biomass level (Stroppiana et al.,
504 2009) in rice and Viña et al. (2011) in maize and soybean). Contrary to the results of He et al.
505 (2016) on AIVI (developed on wheat), we found it more consistent with canopy biomass than
506 CNC in ryegrass (Table 5).

507 The relationship between spectral indices and canopy biomass exhibits most scatter at
508 the highest biomass stages, in contrast to CNC where the early growth stages (lower biomass)
509 show more scatter (Fig. 8 and Fig. 9). This is likely due to a) saturation for high biomass and
510 b) the influence of visible soil at low biomass. Saturation at high biomass is common in
511 canopy remote sensing (Haboudane et al., 2004; Serrano et al., 2000; Strachan et al., 2002;
512 Schlemmer et al., 2013; Viña et al., 2011), while the significant influence of soil background
513 on the inference of CNC from spectral indices was discussed by Bausch (1993), Daughtry et
514 al. (2000) and Huete (1988). Bausch (1993) (corn) and Flowers et al. (2003) (wheat)

515 demonstrated that NDVI was significantly affected by the soil background reflectance.
516 Furthermore, Flowers et al. (2003) found that adequate above-ground biomass is required
517 when mapping wheat N using spectral indices.

518 Recognizing the impact of canopy cover on CNC detection, Barnes et al. (2000)
519 proposed CCCI. We found a weaker association between planar domain indices (CCCI and
520 CCCI-mSR) and CNC than between some other indices such as PRI and CNC, both in
521 seasonal and pooled data (Table 5). This contradicts CCCI results reported by Fitzgerald et al.
522 (2010), Li et al. (2014a, 2014b), Rodriguez et al. (2006) and Tilling et al. (2007) in wheat;
523 Barnes et al. (2000), El-Shikha et al. (2008) in cotton; Li et al. (2014a) in maize; and Varco et
524 al. (2013) in cotton and maize. Each of those studies was limited to certain growth stages
525 and/or less diverse N fertiliser application conditions, which likely explains this. Our results
526 support findings from Cammarano et al. (2014), El-Shikha et al. (2008), Li et al. (2014a) and
527 Tilling et al. (2007) who found that CCCI performance decreased when evaluated across
528 growth stages. Finally, while planar domain indices have been found to improve CNC
529 detection at low canopy cover compared with other indices (El-Shikha et al., 2008), this was
530 not the case at CC-1 in this study (Fig. 6).

531 The relationship between CCCI and biomass is also interesting to consider. CCCI
532 nominally controls for canopy cover, which is related to biomass; however, we found a
533 stronger relationship between CCCI and biomass than CCCI and CNC. It is likely that the
534 correlation between CNC and canopy biomass (Fig. 5) is driving the stage specific
535 relationship between CCCI and CNC. The relationship between CCCI, LAI and biomass
536 observed by Cammarano et al. (2014) and Li et al. (2014b) corroborate our findings of a more
537 consistent association between CCCI and canopy biomass than CNC, and CNC-CCCI
538 relationship may be applicable to the limit that biomass-CNC are correlated.

539 In applying remote sensing to assess canopy N status, both CNC and N uptake have

540 been used. Many previous remote sensing studies have used N uptake as a N status diagnosis
541 tool (Cammarano et al., 2014; Clevers and Gitelson, 2013; Clevers and Kooistra, 2011;
542 Fitzgerald et al., 2010; Guo et al., 2017; Hansen and Schjoerring, 2003; Li et al., 2014b;
543 Schlemmer et al., 2013); however, there is often a strong correlation between canopy biomass
544 and N uptake ($0.86 < r < 0.99$ here), indicating that N uptake and canopy biomass measurements
545 are not independent of each other (Fig. 5). Moreover, there is some value to this in an
546 environment where N application is uniform. Then N uptake might reflect (negatively
547 correlate with) remaining N availability. This implicitly assumes other N loss processes are
548 either minor or spatially uniform, which is unlikely in many landscapes. Reinforcing the
549 potential issues of relying on N uptake, Chen et al. (2010) reported that NNI calculation from
550 CNC is more accurate than using N uptake. The remote sensing issue here is that any spectral
551 index sensitive to canopy biomass is also likely to be sensitive to canopy N uptake but may
552 have the limited skill in predicting CNC.

553 Another important issue is the transferability of spectral indices or models based on
554 specific spectral indices across crop types. Some indices developed on different crops are
555 performing poorly here in ryegrass. [Useful relationships have been found with CNC for CCII](#)
556 [\(Haboudane et al., 2002\) in corn, DCNI \(Chen et al., 2010\), TCARI \(Haboudane et al., 2008\)](#)
557 [in corn and wheat, NDRE \(Barnes et al., 2000\) in cotton, NPCI \(Peñuelas et al., 1994\) in](#)
558 [sunflower leaves and AIVI \(He et al., 2016\) in wheat; but we did not find any agreement](#)
559 [between these indices and CNC.](#) This is probably due to idiosyncrasies of spectral responses
560 for different crop types. It is likely that the difference in the position of highly correlated
561 bands of CNC between crop types have lowered the sensitivity of these indices (Shi et al.,
562 2015). Haboudane et al. (2008) have even shown clear differences in the prediction power of
563 indices in wheat and corn with the same chlorophyll content range.

564

565 Another source of uncertainty in index performance is optical remote sensing's
566 operational linkage to CNC, which derives from its correlation with chlorophyll. This
567 correlation is not consistent and robust across the species (Berger et al., 2020; Hallik et al.,
568 2009; Homolova et al., 2013). N taken up by the plants is not only insignificantly invested in
569 chlorophyll compounds but distributed among other nonphotochemical proteins, which may
570 alter the relationship between chlorophyll and CNC and, ultimately, the predictive
571 performance of spectral indices from one species to another (Berger et al., 2020).

572 The fact that most indices are sensitive to both CNC and canopy biomass (Fig. 6 and
573 Table 5) raises an important question of finding a band combination that is best suited to
574 estimating CNC at canopy level with minimal interference from other biophysical and
575 environmental factors. [One possible way to identify spectral indices that are sensitive to CNC
576 and robust across a contrasting range of canopy biomass and seasonal conditions is to couple
577 hyperspectral reflectance data with a data mining approach. This could consist of an
578 exhaustive search of all band combinations in some general spectral index formula \(e.g. Yao
579 et al. \(2014\)\). In such future investigations, it is important to consider the CNC-biomass
580 correlation.](#)

581 Despite using contrasting growth stages and seasons, it should be noted that the above
582 findings are drawn from irrigated ryegrass pasture, and therefore the fidelity and
583 generalizability of this work need further investigation using more diverse crop types and
584 agroecological environments. CNC and chlorophyll relations are likely to be species
585 dependent. Currently, it is not clear to what extent a uniform approach across species is
586 practical, and there needs to be more cross-species comparisons to investigate this. The
587 confounding effects of multiple stress factors such as water stress and a range of other macro-
588 and micro-nutrients may also alter the plant function and consequently, the relationship
589 between spectral indices and CNC.

590 **5. Conclusion**

591 CNC information from remote sensing platforms with suitable spatial and temporal
592 resolutions can potentially improve the efficiency of N fertilisation and the economic output
593 of farms and reduce environmental impacts of excess N fertiliser application. For the remotely
594 sensed CNC to be adopted in practice, the spectral index used should have sufficient
595 sensitivity to CNC across a variety of environmental conditions and be independent of
596 confounding factors such as biomass. This paper uses field data for ryegrass under a range of
597 N treatments and a variety of growth stages and seasons to examine the performance of
598 eighteen existing spectral indices for sensing CNC and canopy biomass. It particularly
599 considers the interaction between biomass and CNC.

600 The following conclusions can be drawn from this study.

601 1. For most of the indices considered, the relationship of MS indices with CNC varied
602 between individual growth stages. Therefore, their performance reduced greatly when applied
603 to pooled data. Furthermore, some growth stages have a high correlation between CNC and
604 canopy biomass. This can lead to biomass sensitive indices being a good CNC indicator at
605 those times but to inconsistent performance between growth stages. Therefore, the selection
606 of remote sensing indices should be based on a wide range of conditions, not just individual
607 growth stages or seasons. Using a wide range of conditions can lead to a more reliable
608 selection of a spectral index for CNC sensing applicable to a variety of conditions.

609 2. PRI showed the best correlation with CNC when assessed across a contrasting range of
610 conditions. Nevertheless, environmental variables can shift the relationship of PRI with CNC
611 and canopy biomass.

612 3. Planar domain indices did not exhibit superior skill in CNC estimation compared with
613 conventional spectral indices, including at the lowest canopy cover stages (CC-1).

614 4. Most of the indices showed correlation with CNC, though the correlation and relationship
615 were dependent on specific growth conditions.

616 5. Given that the eighteen indices considered responded to both CNC and canopy biomass,
617 further studies separating CNC and biomass signals are needed to develop more broadly
618 applicable CNC models.

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628 **Conflicts of Interest**

629 The authors declare no conflict of interest.

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