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# 1 **Fertilisation strategy and ground sensor measurements to optimise rice yield**

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9

## 10 **Abstract**

11 Nitrogen (N) fertilisation is the main agronomic practice that affects rice yield and quality; similarly,  
12 its mismanagement can affect both economic and environmental aspects of crop production.  
13 Therefore, it is highly important to direct N fertilisation during the critical growth stages of rice  
14 development using vegetation indices (VIs). To this end, a two-year experiment was conducted in  
15 2014 and 2015 in Castello d'Agogna (PV), northwest Italy. The study had three aims: i) establish the  
16 best N fertilisation management in temperate rice cropping systems, in terms of total N supply and  
17 splitting, to maximise crop yield and N apparent recovery (AR); ii) evaluate the capability of crop N  
18 status indicators (CNSIs) measured at panicle initiation stage (PI) to determine grain yield; iii) derive  
19  $N_{\text{fertiliser\_rate\_at\_PI}} = f(\text{CNSI})$  from a field trial to attain specific yield goals.

20 Results obtained for Centauro variety suggested that to maximise yield while avoiding AR reduction,  
21 a low dose of about 50 kg N ha<sup>-1</sup> should be supplied during early growth, then increased at PI. In  
22 addition, the final topdressing fertilisation can compensate for any previous stage supply deficiency

23 and can be determined from VI measurements. Findings also identified the normalised difference  
24 red edge (NDRE) index as the best VI to determine rice N status in specific agro-environmental  
25 conditions. SPAD and NDVI values measured with Rapid Scan can be used to determine N  
26 fertilisation at PI, although such measurements require correction through Sufficiency Indices (SIs)  
27 calculated as the ratio between VI measurements and VI values of a well-N fertilised plot. The trial  
28 also demonstrated that plots supplied with N amounts of 140 kg N ha<sup>-1</sup> (pre-sowing and tillering  
29 stages combined) can serve as reference plots for SI calculation that allows to consider the effect of  
30 weather and soil variability on VI measurements. A notable exception to this finding was NDVI  
31 measured with GreenSeeker, which showed limited ability to assess rice N status under study  
32 environmental conditions. Indeed, both VI and the derived SI were influenced by seasonal and soil  
33 fertility conditions.

34 Finally, a specific statistical method to derive calibration functions for variable rate application  
35 fertiliser spreaders from a suitable experiment was defined. These functions will establish the N  
36 amount to be supplied at PI related to the CNSI measure. For each CNSI, a specific slope of the  
37 calibration function is determined while the intercept is varied depending on the grain yield goal.  
38 The higher the acceptable reduction relative to the maximum obtainable yield, the lower the N  
39 supply required at PI.

40 **Keywords:** Crop yield estimation; Crop N status; Site-specific N management; Vegetation Indices;  
41 Precision Agriculture; Variable rate fertilisation.

42

43 **1. Introduction**

44 Rice (*Oryza sativa* L.) is one of the most important food crops in the world, being the staple food  
45 for three billion people (Barker *et al.*, 2007). On a world scale, rice is grown on an area of 157 million  
46 hectares (PROSPERA, 2012). In Italy, rice cultivation is mainly concentrated in the northwest, and it  
47 is cropped on about 227 000 ha (Ente Nazionale Risi, 2015).

48 Nitrogen (N) fertilisation is the main agronomic practice that affects yield and quality of rice crop.  
49 Mismanagement of N fertiliser may affect both the economic and environmental aspects of crop  
50 production (Tubaña *et al.*, 2011a). Nitrogen deficiency results in smaller leaf area, lower chlorophyll  
51 content, and biomass production, which lead to stunted crop growth and yield (Lin *et al.*, 2010).  
52 Excessive N input on the other hand, results in a dense canopy structure that facilitates pest and  
53 disease development, and leads to reduced plant resistance (Wu *et al.*, 2015, Hue *et al.*, 2016).  
54 Moreover, it can bring on lodging and extend growth periods and maturity achievement (Dong *et al.*,  
55 2015; Liu *et al.*, 2015). Excessive N fertilisation has also been reported to pollute the  
56 environment through N leaching and both N<sub>2</sub>O and NH<sub>3</sub> emission (Nguyen *et al.*, 2008).

57 Therefore, tools to calibrate the application of N fertilisers during critical growth stages are  
58 needed to improve both grain yield and nitrogen use efficiency (NUE) while avoiding N losses  
59 (Sathiya and Ramesh, 2009; Yoseftabar, 2013). In flooded systems, rice requires sufficient N input  
60 during the early and mid-tillering stages to maximise panicle number, and during the panicle  
61 initiation stage (PI) to optimise the number of spikelets per panicle and percentage of filled spikelets  
62 (Biloni and Bocchi, 2003, Bah *et al.*, 2009, Xue *et al.*, 2014). Nitrogen also increases sink size during  
63 late panicle formation (Manzoor *et al.*, 2006; Lee *et al.*, 2009; Tayefe *et al.*, 2014), which raises grain  
64 yield.

65 The rate of N fertilisation and the growth stages critical to optimising N application vary with rice  
66 cultivar (Bah *et al.*, 2009). Several destructive and non-destructive methods have been developed

67 to establish optimum N fertilisation by monitoring crop N status (Tubaña *et al.*, 2011a). An ideal  
68 method to monitor N status has the following characteristics: non-destructive, fast, cost-effective,  
69 reliable, and obtains a value representative of the entire field (Xue *et al.*, 2004; Bajwa *et al.*, 2010).  
70 Optical properties of some leaf pigments, and in particular chlorophyll, have been shown to be  
71 reliable crop N status indicators that can be determined through vegetation indices (VIs) (Muñoz-  
72 Huerta *et al.*, 2013).

73 Different instruments are able to measure light transmission through leaf (chlorophyll meters, *e.g.*  
74 SPAD-502) or canopy reflectance (*e.g.* GreenSeeker and Rapid Scan). These optical measurements  
75 are normally affected by growth stage, cultivar, soil water availability, and non-N nutrient  
76 deficiencies (Muñoz-Huerta *et al.*, 2013), as well as by sun angle, soil roughness, and soil colour.

77 Vegetation indices are calculated from sensor data, based on certain waveband combinations  
78 (Bajwa *et al.*, 2010). The most frequently used are NDVI (Normalized Difference Vegetation Index)  
79 and NDRE (Normalized Difference Red Edge) indices (Rouse *et al.*, 1974; Barnes *et al.*, 2000). NDVI  
80 has been reported to have low sensitivity at high chlorophyll content or abundant aboveground  
81 biomass, that induce saturation (Li *et al.*, 2010; Kanke *et al.*, 2012; Shi *et al.*, 2015). In rice, the index  
82 becomes saturated when aboveground biomass is about 4000 kg ha<sup>-1</sup> and total N uptake reaches  
83 about 100kg ha<sup>-1</sup> (Yao *et al.*, 2014). Therefore, the anticipation of VI measurements must consider  
84 that, in flooded rice systems, reflectance is influenced by the presence of water, especially during  
85 the early growth stages when canopy cover is limited (Yao *et al.*, 2014). Measurements taken at PI  
86 to guide the last topdressing N application fit with the optimal time for data acquisition.

87 Under dense green biomass, NDRE is a more suitable N status measure, as it is less susceptible  
88 than NDVI to saturation (Barnes *et al.*, 2000; Kanke *et al.*, 2016). Red edge wavelength (730 nm),  
89 corresponding to the inflection point of the crop reflectance curve; combined with the NIR band has

90 shown to be the most effective measure for estimating rice plant N uptake before the heading stage  
91 (Yu *et al.*, 2013)

92 Previous studies have shown that these VIs can be used to estimate rice yield based on the  
93 relation between crop spectral measurements and N status that allows to quantify N requirements  
94 (Chang *et al.*, 2005; Tubaña *et al.*, 2011b). Needed now is a quantitative estimate of the VIs and  
95 grain yield relationship to develop a precise rice N fertilisation management that is part of today's  
96 move to precision agriculture. Such a tool has the potential to improve crop N management and to  
97 mitigate negative environmental impacts of intensive rice production (Zhang *et al.*, 2011).

98 This study pursued the following goals:

- 99 i) establishment of the best N fertilisation management, in terms of total N supply and  
100 splitting, to maximise crop yield and N apparent recovery (AR);
- 101 ii) evaluation of the capability of crop N status indicators (CNSIs) measured at PI to  
102 determine grain yield;
- 103 iii) derivation of  $N_{\text{fertiliser\_rate\_at\_PI}} = f(\text{CNSI})$  from a field trial to attain specific yield goals.

104 These tools will allow the development of a site-specific crop management strategy that can be  
105 adapted to different agro-environments reducing the effect of spatial variability, avoiding the  
106 negative impacts of N imbalances.

107

## 108 **2. Materials and methods**

### 109 *2.1 Site description and soil properties*

110 An experiment was designed to test a wide range of crop N statuses through different fertilisation  
111 managements expected to correlate with crop yield.

112 The study was carried out during two growing seasons (2014 and 2015) in an experimental field  
113 of the Rice Research Centre of Ente Nazionale Risi, located in Castello d'Agogna (PV) in northwest  
114 Italy (8° 41' 52'' E; 45° 14' 48'' N).

115 Climate of the area is temperate, with hot summers and two main rainy periods in spring and  
116 autumn. In Figure 1, the mean temperature and rainfall recorded during the experimental periods  
117 of March-October in 2014 and 2015 in Castello d'Agogna have been compared against 30-year (1984  
118 to 2013) values.

119 Site soil properties are summarised in Table 1. The soil texture was silty loam. Other soil  
120 characteristics included low organic matter content, slight acidity, and low available N. The C/N ratio  
121 was well balanced with normal organic matter mineralisation. The Cation Exchange Capacity (CEC)  
122 was low as expected with low organic matter and clay contents. P Olsen content was high, while  
123 exchangeable K availability was very low. Further details of the soil at the site can be found in  
124 Miniotti *et al.*, 2016.

## 125 *2.2 Experiment design and agronomic management*

126 The experiment compared four rates of N supplied as the sum of N amount supplied at the pre-  
127 sowing and tillering stages ( $N_{\text{PRE+TILL}}$ , 0-60-100-140 kg N ha<sup>-1</sup>) combined with four N rates supplied at  
128 PI ( $N_{\text{PI}}$ , 0-30-60-100 kg N ha<sup>-1</sup>). A plot supplied with a total N amount of 300 kg ha<sup>-1</sup> (200 + 100 kg N  
129 ha<sup>-1</sup>) was added as an over-fertilised plot. In all plots, dry granular urea was used as fertiliser.  
130 Treatments were laid out using a split plot design with  $N_{\text{PRE+TILL}}$  in the main plots and  $N_{\text{PI}}$  in the  
131 subplots. Each main plot measured 4.5\*26.4 m and was divided into subplots measuring 4.5\*6.6 m.  
132 Four replications for each treatment were established.

133 Both in 2014 and 2015 cultivar Centauro was planted, a round grain variety. The trial was  
134 ploughed in spring with conventional tillage equipment, after which it was laser levelled, and then  
135 harrowed. Phosphorus (56 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>) and potassium (112 kg ha<sup>-1</sup> of K<sub>2</sub>O) were uniformly

136 applied in all plots before harrowing, using a 0-14-28 fertiliser. Water seeding was carried out on  
137 May 19, 2014 and May 18, 2015. Water was managed with continuous flooding for most of the  
138 growing season. The only exceptions were a “pin-point” period of 4-6 days to allow for root  
139 extension, and two other 4-6 day periods of drainage for mid-season fertiliser and herbicide  
140 application during the second half of June and July, as is the traditional management of the area.  
141 Final draining occurred on September 8, 2014 and August 29, 2015. Adequate measures to control  
142 diseases were taken throughout plant growth. Weed control was performed with *oxadiazon*,  
143 *cyhalofop butyl*, *propanil*, *MCPA*, and *halosulfuron methyl*. The crop was harvested between  
144 October, 21 and 29 and between October 12 and 21 in 2014 and 2015, respectively. The date  
145 difference merely reflected when the crop matured, as determined by N supply.

### 146 *2.3 Field measurements*

147 SPAD, NDVI, and NDRE were determined using suitable instruments to establish crop N status at PI  
148 (July 15, 2014 and July 13, 2015). The Soil-Plant Analysis Development (SPAD) index was determined  
149 using SPAD-502 (Konica Minolta, Japan). Readings were acquired from the fully expanded top leaf  
150 of plants, approximately one-third of the distance away from the tip, as described in Lin *et al.*, 2010.  
151 To obtain a representative value for the entire plot, 20 readings for each plot were taken from 20  
152 leaves belonging to twenty different plants. For NDVI calculations, both GreenSeeker (Trimble©,  
153 Sunnyvale, California, USA) or Rapid Scan (Rapid Scan CS-45, Holland Scientific, USA) handheld active  
154 optical sensors were used. The first device detects canopy reflectance in the red (660 nm) and NIR  
155 spectral regions (770 nm), whereas the second incorporates three optical measurement channels  
156 (670, 730, and 780 nm), of which the first and third were used for NDVI calculations. The wavebands  
157 used to determine NDVI are different for the two instruments, so the two VIs are indicated as GS  
158 NDVI and RS NDVI for GreenSeeker- and Rapid Scan-read measurements, respectively. Finally, NDRE



159 was measured and calculated using only Rapid Scan, considering canopy reflectance at 730 and 780  
160 nm wavebands.

161 The measurements were collected by holding the instruments approximately 0.5 m above the  
162 rice canopy and walking at a constant speed along the entire length of the plot, as suggested in Xue  
163 *et al.*, 2014. Due to the technical characteristics of the instruments, sensor measurements width is  
164 approximately 0.3 m. Two measurements were taken from each of the length-wise sides of the plot  
165 in each treatment. The two values were then averaged to determine the mean value for each plot.

166 Biometric measures at PI are usually well correlated to final grain yield (Bajwa *et al.*, 2010). To  
167 confirm this relationship, aboveground biomass, total N concentration, and total N uptake were also  
168 determined. Aboveground biomass was collected from three 0.25 m<sup>2</sup> areas, oven-dried at 40°C to a  
169 constant weight, and then analysed by the Dumas method to establish N concentration. Then, total  
170 N uptake at PI was also calculated by multiplying plant N concentration and the sampled biomass  
171 (Zavattaro *et al.*, 2012).

172 Grain (normalised to a moisture content of 14%) and straw yield were determined at harvest. In  
173 order to have three sub-samples, three 0.25 m<sup>2</sup> areas were harvested by hand in each plot. Only a  
174 bulk of the three sub-samples of both grain and straw was analysed for N concentration using the  
175 above-mentioned method. Finally, N AR was determined according to Zavattaro *et al.* (2012):

$$176 \quad AR = \frac{N \text{ removal} - N \text{ removal}_{0N}}{N \text{ fertilizer}} \quad (1)$$

177 where *N removal* is the amount of N removed as yield, *N removal<sub>0N</sub>* represents the amount of N  
178 removed by the unfertilised plot, and *N fertilizer* is the total amount of N supplied with fertiliser  
179 application.

180

181 *2.4 Data analysis*

182 Statistical analysis was performed using R software, version 3.3.0 (R Development Core Team,  
183 2016).

184 General Linear Model (GLM) was used to explain yield and AR as a function of different N rates  
185 and splitting, year, block, and their interactions as follows:

$$186 \quad x = \beta_1 * N_{PRE+TILL} + \beta_2 * N_{PRE+TILL}^2 + \beta_3 * N_{PI} + \beta_4 * N_{PI}^2 + \beta_5 * N_{PRE+TILL} * N_{PI} + \beta_6 * \\ 187 \quad N_{PRE+TILL} * YEAR + \beta_7 * N_{PI} * YEAR + BLOCK + YEAR \quad (2)$$

188 where x represents yield and AR, while  $\beta_1$  to  $\beta_7$  represent the slopes of the covariates. YEAR is  
189 the year effect related to the agro-climatic conditions and BLOCK is the block effect.

190 With an aim to determine the  $N_{PI}$  that maximises grain yield and AR for each N rate supplied at  
191 pre-sowing and tillering stage, the first order partial derivative was calculated with respect to  $N_{PI}$   
192 and then set to zero.

193 The resulting equation is:

$$194 \quad N_{PI} = \frac{-\beta_3 - \beta_5 * N_{PRE+TILL}}{2 * \beta_4} \quad (3)$$

195 *Equation 3* can be expressed as  $N_{PI} = f(N_{PRE+TILL})$  only when  $N_{PI}$  and  $N_{PRE+TILL}$  show significant  
196 interaction. Otherwise,  $N_{PI}$  and  $N_{PRE+TILL}$  contribute both to increases in yield and AR. In such an  
197 instance, no compensative effect exists and the equation cannot be calculated.

198 A correlation analysis was also applied to investigate the capability of different indicators to  
199 determine crop N status. The different indicators of crop N status (CNSI) here considered were both  
200 VIs (SPAD, GS NDVI, RS NDVI, NDRE), and biometric measures (aboveground biomass, its N  
201 concentration and total N uptake) detected at PI.

202 Next, grain yield was determined through the same GLM as mentioned above, only CNSIs took the  
203 place of  $N_{PRE+TILL}$ . The goal was to determine the relations between  $N_{PI}$  and CNSIs, under the larger

204 aim of describing an equation to establish the best  $N_{PI}$  based on CNSIs. This statistical model was  
205 built as:

$$206 \quad Yield = \gamma_1 * N_{PI} + \gamma_2 * N_{PI}^2 + \gamma_3 * CNSI + \gamma_4 * CNSI^2 + \gamma_5 * CNSI * N_{PI} + \gamma_6 * CNSI * YEAR$$
$$207 \quad + \gamma_7 * YEAR * N_{PI} + BLOCK + YEAR \quad (4)$$

208 where  $\gamma_1$  to  $\gamma_7$  represent the slopes of the covariates.

209 To obtain good indicators of crop N status in a given season and location, Sufficiency Indices (SI)  
210 and Response Indices (RI) were also calculated and used as CNSIs. Several studies on different  
211 cropping systems (Holland and Shepers, 2013; Muñoz-Huerta *et al.*, 2013; Xue *et al.*, 2014)  
212 suggested that a well-fertilised plot serve as a reference plot to calculate SIs, defined as the ratio of  
213 vegetation index obtained from a to-be-evaluated crop to VI of a well-N fertilised plot, integrating  
214 the confounding effect provoked by factors other than crop N status (Hussain *et al.*, 2000; Holland  
215 and Shepers, 2013). Hussain *et al.* (2000) state that reference plot establishment is suitable in  
216 irrigated rice conditions. Indeed, continuous flooding, common in temperate rice cropping systems,  
217 avoids water stress onset and consequent influence on rice spectral response. Moreover, Tremblay  
218 and Belec (2006) put forth that the reference plot might be considered as an internal standard  
219 against which measurements taken in other plots can be compared. Consequently, in order to  
220 standardise VIs measurements considering site-specific conditions, a reference plot has to be  
221 established in each location. RI is defined as the ratio of the vegetation index measured on the to-  
222 be-evaluated crop to the vegetation index measured in an unfertilised plot (Mullen *et al.*, 2003).  
223 Last, an equation to optimise fertiliser application, as a function of a measured CNSI value was  
224 determined for CNSIs that have shown a negligible effect of year and soil variability, originally or  
225 after transformation in SI or RI.

226 A statistical model was then built to determine yield from PI N supply and CNSI values as follow:

$$227 \quad Yield = \gamma_8 * N_{PI} + \gamma_9 * N_{PI}^2 + \gamma_{10} * CNSI + \gamma_{11} * CNSI^2 + \gamma_{12} * N_{PI} * CNSI \quad (5)$$

228 where  $\gamma_8$  to  $\gamma_{12}$  represent the slopes of the covariates.

229 Year, block, and their interactions were not included in the statistical model, as after the results  
230 analysis, these parameters were shown not to be significant in determining yield.

231 To derive the most appropriate N fertilisation as a function of measured CNSI, the Maximum  
232 Grain Yield Approach was followed. Therefore, a first order partial derivative with respect to  $N_{PI}$  was  
233 calculated for *Equation 5* and then set to zero to determine the N amount that has to be supplied  
234 at PI to maximise rice grain yield.

235 First order partial derivative with respect to  $N_{PI}$  can be expressed as:

$$236 \text{Yield}' = \gamma_8 + 2\gamma_9 * N_{PI} + \gamma_{12} * CNSI \quad (6)$$

237 After rearranging the equation and setting the partial derivative to zero, N supply at PI can be  
238 determined as:

$$239 N_{PI} = \frac{-\gamma_8 - \gamma_{12} * CNSI}{2\gamma_9} \quad (7)$$

240 Again, *Equation 7* can be expressed as  $N_{PI} = f(CNSI)$  only when  $N_{PI}$  and CNSI show significant  
241 interaction. Otherwise,  $N_{PI}$  and CNSI both contribute to increase yield and AR. In such an instance,  
242 no compensative effect exists and the equation cannot be calculated.

243 Results analysis highlighted that a high N amount has to be supplied at PI to achieve the highest  
244 grain yield, as the function that describes maximum grain yield shows a smooth curvature close to  
245 the peak. Worthy of note is the considerable reduction in N rates that can be obtained with just a  
246 slight reduction in maximum grain yield. So, a method to determine  $N_{PI}$  to achieve a reduced yield  
247 was studied, with the assumption that the reduced yield could be considered as a percentage of  
248 maximum grain yield (*Equation 8*).

$$249 \text{Reduced yield} = R * \text{Maximum Yield} \quad (8)$$

250 where R is the reduction coefficient, and assumed to be 0.90, 0.95, 0.99, 0.995, and 0.999 to analyse  
251 different reductions in maximum grain yield and the consequent reductions in N fertiliser applied.

252 Moreover, this approach allows identification of the CNSI threshold over which no further N  
253 fertiliser must be added, depending on the grain yield goals.

## 254 **3 Results**

### 255 *3.1 Climate*

256 The two cropping seasons exhibited different climatic conditions. Rainfall was plentiful in the  
257 summer of 2014. Conversely, the 2015 summer saw reduced rainfall (704 mm) and higher  
258 temperature (13°C) compared to the 30-year means.

259

### 260 *3.2 Response of rice yield to different N rates*

261 Rice grain yield showed a parabolic increase with rising total N supply ( $N_{\text{PRE+TILL}}$  plus  $N_{\text{PI}}$ ) in both  
262 years (Figure 2). Maximum grain yield (11.1 Mg ha<sup>-1</sup>) was achieved in 2014 when a total of 200 kg N  
263 ha<sup>-1</sup> was applied, while in 2015 the maximum grain yield (11.2 Mg ha<sup>-1</sup>) was reached with 120 kg N  
264 ha<sup>-1</sup>. Additional N increases resulted in either a constant yield or yield reduction in both years.

265 Total N uptake (grain + straw) behaved differently than did grain yield. The trend was almost  
266 linear across all explored N fertilisation levels in both years (Figure 3), despite different N uptake  
267 value ranges in each year (2014: 127 to 325 kg ha<sup>-1</sup> and 2015: 129 to 223 kg ha<sup>-1</sup>). Consequently, AR  
268 values were different between the two growing seasons, even though N uptake in the unfertilised  
269 treatments was quite similar in both years (Figure 4). In both years AR decreased when N supplied  
270 increased, but with a slope more pronounced in 2014. In 2014, at lower N levels, mineral fertilisation  
271 promoted higher crop exploitation of soil N resources than observed in the unfertilised plot, and  
272 produced AR values above 1.0. On the contrary in 2015, AR values were lower and remained almost  
273 constant with rising N amount.

274 The GLM based on *Equation 2* was applied. Results are reported in Table 2.

275 Grain yield and total biomass production were influenced by N applied at the pre-sowing plus  
276 tillering stages, N supplied at PI, and their interaction. Total N concentration and total N uptake  
277 were instead influenced by N fertilisation at the sum of pre-sowing and tillering, and PI stages, but  
278 not by their interaction. Differences among years were significant for yield, total N concentration,  
279 and AR. Interaction between year and N supply at pre-sowing and tillering stages or at PI was  
280 significant for most of the variables considered.

281

### 282 *3.3 Crop yield maximisation and consequences on N apparent recovery*

283 The N amount that must be supplied at PI to maximise yield as a function of  $N_{\text{PRE+TILL}}$ , can be  
284 calculated using *Equation 3* (represented in Figure 5). Maximum grain yield ( $11.1 \text{ Mg ha}^{-1}$ ) was  
285 achieved when about  $42 \text{ kg N ha}^{-1}$  was applied during pre-sowing and tillering; maximum AR (59.8%)  
286 was reached with a  $N_{\text{PRE+TILL}}$  of about  $53 \text{ kg N ha}^{-1}$ . When  $42 \text{ kg N ha}^{-1}$  was applied at the pre-sowing  
287 and tillering stages, AR fell slightly (59.7%). As Figure 5 shows,  $150 \text{ kg N ha}^{-1}$  must be supplied at PI  
288 to maximise yield, while just  $140 \text{ kg N ha}^{-1}$  is enough to maximise AR. Nonetheless, some  
289 uncertainties are associated with these early stage and PI fertilisation values.

290

### 291 *3.4 Capability of different VIs to determine N status at PI stage*

292 The capability of different VIs to determine rice N status at PI was verified by correlation analysis.  
293 Results are reported in Table 3.

294 Analysed VIs correlated highly with one another as their coefficients were above 0.8. They also  
295 correlated highly with biometric measures, with very high coefficients for NDRE and falling  
296 progressively for SPAD, RS NDVI, and GS NDVI. Moreover, VIs correlated better with crop  
297 aboveground biomass than with N uptake, except for SPAD. Alternatively, N concentration

298 determination was the poorest. Differences recorded between GS and RS NDVI in the various  
299 correlations related to the differing wavelengths used by GreenSeeker and Rapid Scan.

300

### 301 *3.5 Capability of different CNSIs to determine grain yield*

302 The capability of different CNSIs, including both biometric measures and VIs measured at PI, to  
303 determine yield was investigated using GLM according to *Equation 4*. Results are shown in Table 4.  
304 All CNSIs and most of their squares detected at PI were significant on determining crop yield.  
305 Interaction of the different CNSIs with N supply at PI was also found to be significant for all  
306 considered CNSIs.

307 According to Table 4, the biometric measures evaluated at PI were good at determining grain  
308 yield as demonstrated by high  $R^2$  values; biomass was best, followed by N uptake, and N  
309 concentration alone led to a poorer estimate. All VIs performed better than N concentration. The  
310 NDRE  $R^2$  was even better than that obtained by biomass. The two NDVIs were shown to be a little  
311 less able to determine grain yield; SPAD was the poorest. Moreover, VIs are essentially proxy  
312 measures of biometric variables as the correlation analysis demonstrated and can be extensively  
313 measured.

314 Except for NDRE, year or block effect was significant for all VIs. This makes quantification of N  
315 fertiliser needs based on VI measurements difficult because of the wide variation in agro-climatic  
316 and soil conditions. Consequently, Sufficiency Indices (SIs) and Response Indices (RIs) were  
317 calculated. Plots that received 60, 100, 140, or 200 kg N ha<sup>-1</sup> as N<sub>PRE+TILL</sub>, or 0 kg N ha<sup>-1</sup> were  
318 considered reference plots to determine the SIs or RIs, respectively. Then, the *Equation 4* was  
319 applied to determine rice grain yield at the best using SIs and RIs as CNSIs.

320 SIs and RIs calculated for each VI were also found to determine yield significantly (Table 5).

321 Interaction between SI or RI and N supplied at PI was always significant too. Maximum  $R^2$  values

322 were obtained for all SIs except GS NDVI using as a reference plot those receiving 60 kg N ha<sup>-1</sup> at  
323 pre-sowing and tillering. With these SIs, block effect was negligible, while year effect was significant  
324 for SIs calculated from SPAD and GS NDVI. If plots considered as reference received 140 kg N ha<sup>-1</sup> at  
325 the pre-sowing and tillering stages, R<sup>2</sup> values were slightly lower (except for GS NDVI), but year and  
326 its interaction effects were not significant (except again for GS NDVI). As previously shown, year and  
327 block effects were originally negligible only for NDRE. SI calculation considering reference plots as  
328 those receiving N<sub>PRE+TILL</sub> = 140 kg N ha<sup>-1</sup> allowed their effect to be eliminated from SPAD and RS NDVI  
329 measurements. Consequently, calibration functions were calculated for NDRE, SPAD SI, and RS NDVI  
330 SI only, as they were more suitable to assess rice N status in specific agro-environmental conditions.

331

### 332 *3.6 Calibration functions aimed at reaching specific yield goals*

333 Figure 6 shows the calibration functions obtained when considering different grain yield goals.  
334 The solid black line represents the first estimation of the calibration function that has to be used to  
335 achieve the maximum grain yield for the three different CNSIs values. The dashed black lines  
336 represent the calibration functions obtained when a reduction coefficient (R) is used with respect  
337 to maximum grain yield. R was assumed equal to 0.999, 0.995, 0.99, 0.95, and 0.90 in the various  
338 scenarios, respectively. As expected, all calibration functions recommended lower N amounts when  
339 vigour increases. When NDRE, SPAD SI, and RS NDVI SI reached 0.5, 124, and 110, respectively, no  
340 additional N supply at PI was required to maximise yield. For each CNSI, the slope of the function  
341 remains almost constant, while the intercept varies depending on the R value being proportional to  
342 it. Therefore, yield reduction limits the N amount that has to be supplied at PI.



## 343 4 Discussion

### 344 4.1 Crop yield maximisation and consequences on N apparent recovery

345 In each year of the experiment, a similar (about 11.2 Mg ha<sup>-1</sup>) maximum grain yield was reached,  
346 but it was accomplished with very different total N amounts associated with different crop densities  
347 in the two years. Maximum grain yield was reached with about 200 kg N ha<sup>-1</sup> in 2014 and 120 kg N  
348 ha<sup>-1</sup> in 2015. Rice grain yield stayed constant or decreased with additional N fertiliser, showing a  
349 parabolic trend. The absent or negative effect of further N doses after the peak yield confirmed the  
350 results of previous studies, in which yield declines were linked to lodging or disease (Dong *et al.*,  
351 2015; Liu *et al.*, 2015).

352 Grain yield was affected by N fertilisation at the pre-sowing + tillering and at PI stages and by their  
353 interaction, which revealed the potential to compensate with an N topdressing fertilisation at PI for  
354 deficient N supplies during the initial stages. These results align with reports of previous studies by  
355 Manzoor *et al.* (2006) and Lee *et al.* (2009). Rice responded well to increased N supply at PI,  
356 especially when low N amounts had been applied during the early growth stages.

357 In general, increases in N supply reduced apparent recovery (AR) (Xue and Yang, 2008; Yesuf and  
358 Balcha, 2014). Differences between the two cropping seasons were evident. Even though N uptake  
359 of the unfertilised plots was similar in both seasons, 2015 AR values were lower than 2014. In 2014,  
360 reaching maximum grain yield resulted in an AR of nearly 0.8 *versus* the 2015 value of just below  
361 0.5. The lower 2015 AR value is a consequence of reduced N uptake mainly from a lower N  
362 concentration in the aboveground biomass of rice at harvest (2.26 % and 0.95 % in 2014 and 2015,  
363 respectively). Indeed, the 2014 crop was denser than in 2015. In 2015, rice compensated for a low  
364 panicle density with an increase in spikelets per panicle and in 1000-grain weight, which produced  
365 the same grain yield. Consequently, AR values were very different, despite similar N uptake in the  
366 unfertilised treatment in both years. *Equation 3* established the N supply required at PI for each

367  $N_{\text{PRE+TILL}}$  maximising grain yield or AR. The relationship between grain yield or AR and  $N_{\text{PRE+TILL}}$  showed  
368 a clear parabolic trend. Maximum grain yield and maximum AR were obtained separately and they  
369 approximated the same total N amount (about 195 kg ha<sup>-1</sup>), with about 50 kg N ha<sup>-1</sup> supplied at the  
370 first two applications and the remaining dose at PI. The amount of N at the pre-sowing and tillering  
371 stages needed to maximise AR was slightly lower than that required to maximise grain yield.

372

#### 373 *4.2 Capability of different CNSIs to determine grain yield*

374 Correlation analysis showed that VIs (SPAD, GS NDVI, RS NDVI, and NDRE) were highly correlated  
375 with biometric measures at PI. In particular, NDRE had the highest correlation coefficients. All VIs  
376 correlated most with total aboveground biomass, except as expected for SPAD, which correlated  
377 better with total N uptake. NDVI, measured with both GreenSeeker and Rapid Scan instruments at  
378 PI, correlated less well with crop N status than other VIs did.

379 This result may arise from the presence of an abundant biomass at which saturation starts to  
380 reduce index sensitivity (Kanke *et al.*, 2012; Muñoz-Huerta *et al.*, 2013; Novotna *et al.*, 2013; Cao *et*  
381 *al.*, 2016).

382 In this study, NDVI saturated at a biomass production of about 7600 kg ha<sup>-1</sup>, or an N uptake of  
383 180 kg ha<sup>-1</sup>.

384 The correlation between NDRE and biometric measures at PI was also strong under dense  
385 biomass. Indeed, the red-edge wavelengths utilised by Rapid Scan are more sensitive at higher levels  
386 of chlorophyll content, as is illustrated by its strongest correlation with crop N concentration at PI,  
387 and consequently with rice N uptake. In this study, NDRE saturation effects were first noted at  
388 biomass production levels of about 8000 kg ha<sup>-1</sup> or 190 kg N ha<sup>-1</sup> of total N uptake.

389 When CNSIs replaced  $N_{\text{PRE+TILL}}$  in the statistical model (*i.e.*, using *Equation 4* instead of *Equation*  
390 *2*), all CNSIs and most of their squares resulted significant on determining grain yield. Moreover,

391 interaction with N rate supplied at PI was also significant on determining yield for all CNSIs  
392 considered. This confirms that topdressing N fertilisation at PI can compensate for low CNSI values,  
393 showing again that N supplied at PI can balance low  $N_{\text{PRE+TILL}}$  amounts. All CNSIs demonstrated  
394 themselves to be good at determining grain yield, with  $R^2$  values near or above 0.60. NDRE was the  
395 best.

396 Nonetheless, as year or block differences were detected for all VIs except NDRE, SIs were  
397 calculated from other corresponding VIs to overcome the influence of year and block on VIs values.  
398 Year and block can be assumed to represent the effect due to climate and soil variability in the  
399 direction of blocks. Sufficiency Indices calculated using reference plots that received  $140 \text{ kg N ha}^{-1}$   
400 supplied during pre-sowing and tillering combined removed agro-climatic and soil variability effects  
401 best. In addition, statistical models applied to SIs improved yield determination, as  $R^2$  reached values  
402 higher than 70%. Consequently, the results of this study not only confirmed the benefit of  
403 establishing a well-fertilised reference plot to obtain better indicators of in-season rice N status  
404 (Hussain *et al.*, 2000), but also estimated that plots receiving  $140 \text{ kg N ha}^{-1}$  as the sum of the pre-  
405 sowing and tillering stage N supplies, can serve as a reference plot for Centauro variety. Of course,  
406 the reference plot must be relocated each year to avoid long term effects of differentiated N  
407 fertilisation (Holland and Shepers, 2013).

#### 408 *4.3 Calibration functions aimed at reaching specific yield goals*

409 Calibration functions are improved when obtained from CNSIs not influenced by agro-climatic  
410 conditions and soil variability. In this study, NDRE alone demonstrated these features. However,  
411 through SI calculations, block and year effects were also made negligible for SPAD and RS NDVI,  
412 which made it possible to determine the calibration functions for these CNSIs in addition. It is  
413 recommended that NDRE be determined at PI, but SPAD and RS NDVI can work, so long as SIs  
414 referred to these VIs are calculated from a well-N fertilised reference plot.

415        Obtained calibrations associate required N supplies at PI to field-measured CNSIs, and the  
416 calibration equation relates to a specified grain yield goal. The highest yield goal requires the highest  
417 supply of N at PI. Alternatively, a lower yield goal permits a reduced  $N_{PI}$  amount, as the slope of the  
418 function remains almost constant while the intercept is proportional to the grain yield goal.  
419 One method to determine an acceptable maximum yield reduction is selection of a CNSI threshold  
420 over which N fertilisation at PI is ineffective. Suitability of the threshold should be based on the field  
421 and potential yield in specific situations.

## 422 **5 Conclusions**

423        Results reported in this study suggest that yield and apparent recovery maximisation are not  
424 conflicting goals. The statistical models developed here indicate that Centauro variety grain yield is  
425 optimised most effectively when N fertiliser supply is reduced in the early growth stages and  
426 concentrated at PI (about 70% of total N). Moreover, a topdressing N fertilisation amount can be  
427 determined from measured CNSI values to avoid N imbalances. This study confirmed that VIs  
428 measured at PI act as biometric proxy measures, and help avoiding time-consuming and destructive  
429 analyses. NDRE was demonstrated to be the best at determining grain yield variability specific agro-  
430 environmental situations. It can be used to determine Centauro variety calibration functions that  
431 improve N fertilisation.

432        Sufficiency index (SI) calculations that consider as reference those plots receiving  $140 \text{ kg N ha}^{-1}$   
433 N supply (sum of the pre-sowing and tillering stages) can correct SPAD and RS NDVI measurements,  
434 making possible to calculate the calibration function for these CNSIs as well. NDVI measured with  
435 GreenSeeker was less suitable for making N fertilisation determinations at PI for Centauro variety  
436 in the environmental conditions presented in this study, as both the index and derived SI were  
437 influenced by ago-climatic conditions and soil variability.

438 The determined calibration functions allow a site-specific rice N fertilisation management that  
439 accounts for year and spatial variability, and avoids consequent negative environmental impacts. It  
440 must be noted that the calibration functions were derived only for Centauro variety under the  
441 specific environmental conditions presented. Therefore, extension to other rice varieties and  
442 environments can be obtained following the same method presented in this work.

443

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