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1 **Predicting leaf nitrogen content in olive trees using hyperspectral data**
2 **for precision agriculture**

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8

9 **Abstract**

10 Olive orchard is one of the main crops in the Mediterranean basin and, particularly, in
11 Spain, with 56% of European production. In semi-arid regions, nitrogen (N) is the main
12 limiting factor of olive trees after water and its quantification is essential to carry out
13 accurate fertilization planning. In the present study, N status of an olive orchard located
14 in Carmonita (southwest Spain) was analysed using hyperspectral data. Reflectance data
15 were recorded with a high precision spectro-radiometer through the full spectrum (350–
16 2500 nm). Different vegetation indices (VI), combining two or three wavelengths, and
17 partial least squares regression (PLSR) models were developed, and the prediction
18 capabilities were compared. Different pre-processing (smoothing, SM; standard normal
19 variate, SNV; first and second derivative) were applied to analyse the influence of the
20 noise generated by the spectro-radiometer measurements when computing the
21 determination coefficient between leaf N content (LNC) and spectra data. Results
22 showed that second derivative combined with SNV pre-processing produced the best
23 determination coefficients. The wavelengths most sensitive to N variation used to
24 perform VI were selected from the visible and the short-wave infrared spectrum regions,

25 which relate to chlorophyll a+b and N absorption features. DCNI and TCARI showed
26 the best fittings for the LNC prediction ($R^2=0.72$, $R^2_{cv}=0.71$; and $R^2=0.64$, $R^2_{cv}=0.63$,
27 respectively). PLSR models yielded higher accuracy than the models based on VI
28 ($R^2=0.98$, $R^2_{cv}=0.56$), although the large difference between calibration and cross-
29 validation showed more uncertainty in the PLSR models.

30

31 **Keywords:** Leaf nutritional status, Linear regression, Nitrogen indices, Olive orchards,
32 Partial Least Squares Regression, SWIR spectral region.

33

34 **Introduction**

35 Olive (*Olea europaea* L.) is one of the main crops in the Mediterranean basin. In 2016,
36 the area under olive trees accounted for about 5 Mha in the European Union, with a
37 production of 11.8 Mt of olives (61% of the worldwide yield; FAOSTAT 2017). 51% of
38 the total European olive orchard surface is concentrated in Spain, 23% in Italy, 17% in
39 Greece, 7% in Portugal and the 2% remaining in other countries (EUROSTAT 2017). In
40 Spain, in recent years, the oleic sector contributed between 8% and 13% of annual
41 agricultural production (FAOSTAT 2017).

42 Traditionally, the yield of most Spanish olive orchards was limited by water supply, and
43 soil management was mainly based on plough, disk and harrow tillage operations
44 (López-Granados et al. 2004). Currently, precision agriculture techniques are being
45 introduced in the sector and are targeted to reduce economic costs and adverse
46 environmental effects of agricultural inputs (Akdemir et al. 2018).

47 Nitrogen (N) is an essential element for plant growth and is the mineral nutrient most
48 commonly applied in agriculture and in olive orchards fertilization. Between 2002 and

49 2014, more than 1,200 Mt of N were consumed in agriculture worldwide, compared to
50 the 500 and 400 Mt of potassium (K) and phosphorus (P), respectively. During this
51 period, N consumption increased by 32% (FAOSTAT 2017). Over-fertilization has
52 negative economic and environmental effects. N loss by leaching and ground water can
53 result in ground and aquifer contamination (Puckett et al. 2011). This affects carbon
54 storage (Schulze et al. 1989) and produces a N deficiency that results in a reduction in
55 yields and, therefore, economic losses to the farmers (Haboudane et al. 2002). This
56 situation motivates the need to carry out efficient and sustainable management
57 programmes of agricultural fertilization.

58 Traditional techniques to estimate crop nutrient status are based on leaf sampling and
59 foliar analysis. However, these methods are destructive, time-consuming and expensive.
60 Moreover, traditional N estimates provide limited information, as sampling is based on
61 only a limited number of sites in a given field (Camino et al. 2018). Therefore, it is
62 necessary to consider efficient alternatives.

63 In recent years, remote sensing for characterizing biophysical parameters of vegetation
64 has had important potential implications for predicting chlorophyll (Chl) content as a
65 proxy of the plant N status (Clevers et al. 2017). Several studies have demonstrated
66 strong correlations between N content and Chl in crops, because of the large amount of
67 protein that complexes the photosynthetic pigment (Cilia et al. 2014; Clevers et al.
68 2017; Singh et al. 2017; Miphokasap and Wannasiri 2018).

69 Spectral sensing is a spectroscopic method commonly used for assessing N content
70 (Alchanatis et al. 2009). Hyperspectral data contain large amounts of redundant
71 information due to the relatively few parameters that really and effectively control the
72 spectral signatures of vegetation (Im and Jensen 2008). This relatively low number of
73 variables contrasts with the often more than 100 wavelengths available through imaging

74 spectroscopy and from commercially available spectro-radiometers (Atzberger et al.
75 2010). Therefore, in order to develop suitable indices, it is necessary to determine those
76 wavelengths that presented a better correlation with the measured biophysical
77 parameters.

78 Reflectance spectra in the short-wave infrared (SWIR) spectrum region (1100–2500
79 nm) have been found to be highly correlated with N content. Reflectance in this region
80 is directly related to N-hydrogen (H) stretch, first overtone and absorption features of
81 protein (Curran 1989). Several authors have shown improvements in N content
82 estimations using SWIR spectral region to determine vegetation indices (VI), sometimes
83 in combination with the visible and near-infrared (VNIR) region (350–1100 nm) (Cohen
84 and Alchanatis 2018). Serrano et al. (2002) showed that $NDNI_{1510_1680}$ (Normalized
85 Difference Nitrogen Index), using \log_{10} transformed reflectance, was sensitive to N
86 concentration in chaparral vegetation. Ferwerda (2005) recommended NRI (Normalized
87 Ratio Index) using the combination of reflectance at 1770 nm and 693 nm for the best
88 correlation with N content of different species: olive, willow, mopane, grass and shrubs.
89 Herrmann et al. (2010) obtained the best estimated N content in potatoes plants using
90 the NRI_{1510} and $MCARI_{1510}$ indices, which combined information from the 1510 nm and
91 660 nm wavelengths. Camino et al. (2018) reported that the use of the SWIR spectral
92 range to determine VI provided better quantification of N concentration in wheat plants
93 than when only the VNIR region was used. They found the highest agreement with N
94 concentration using $MCARI_{1510}$ and $NDI_{850,1510}$ (vegetation index estimated in a similar
95 way to NDVI) indices. Table 1 summarizes the VIs used by other authors to determine
96 the N status of several crops using wavelengths within the range 400-1800 nm.

97

98 Table 1. Vegetation indices (VI) used by other authors to determine the N status of
 99 crops, using wavelengths within the range 400–1800 nm (SR, Simple Ratio; NDI,
 100 Normalized Difference Index; MCARI, Modified Chlorophyll Absorption in
 101 Reflectance Index/ Optimized Soil Adjusted Vegetation Index; TCARI, Transformed
 102 Chlorophyll Absorption Ratio Index/ Optimized Soil Adjusted Vegetation Index; DCNI,
 103 Double-Peak Canopy Nitrogen Index).

VI	Equation	Reference
SR _{550,670}	R_{550}/R_{670}	Gómez-Casero et al. (2007)
SR _{780,550}	R_{780}/R_{550}	Gómez-Casero et al. (2007)
SR _{780,670}	R_{780}/R_{670}	Gómez-Casero et al. (2007)
NDI _{780,670}	$(R_{780} - R_{670})/(R_{780} + R_{670})$	Gómez-Casero et al. (2007)
DCNI	$[(R_{720} - R_{700})/(R_{700} - R_{670})]/(R_{720} - R_{670} + 0.03)$	Chen et al. (2010)
MCARI ₁₅₁₀	$[(R_{700} - R_{1510}) - 0.2(R_{700} - R_{550})](R_{700}/R_{1510})$	Herrmann et al. (2010)
TCARI ₁₅₁₀	$3[(R_{700} - R_{1510}) - 0.2(R_{700} - R_{550})(R_{700}/R_{1510})]$	Herrmann et al. (2010)
NDI _{1645,1715}	$(R_{1645} - R_{1715})/(R_{1645} + R_{1715})$	Pimstein et al. (2011)
NDI _{870,1450}	$(R_{870} - R_{1450})/(R_{870} + R_{1450})$	Pimstein et al. (2011)
NDI _{850,1450}	$(R_{850} - R_{1450})/(R_{850} + R_{1450})$	Camino et al. (2018)

104

105 The use of spectroscopy technology applied to olive yield has been mainly focused on
 106 the determination and identification of adulterants in olive oils (Muik et al. 2004). Few
 107 studies have been found about N content estimation and other nutrient deficiencies in
 108 olive orchards. Zarco-Tejada et al. (2004) used spectral data within the range 500–800
 109 nm to study the effects of scene components (soil, shadow and crown reflectance) on
 110 the estimation of canopy Chl content in olive orchards, using a digital airborne imaging
 111 spectrometer and reflective optics system imaging spectrometer images at 1 m spatial
 112 resolution. Ferwerda and Skidmore (2007) estimated the chemical composition (N, F,
 113 K, Ca, Na and Mg) of three tree species (willow, mopane and olive) and one shrub
 114 species (heather) using hyperspectral data (300–2500 nm) and different spectral pre-
 115 processing (reflectance spectral, derivative spectral data and band depth). Gómez-

116 Casero et al. (2007) used the VNIR spectral region (in this case, 400–900 nm) to
117 determine the hyperspectral reflectance curves of olive trees with different N or K leaf
118 content and to select the best estimating VI for N and K content. Rotbart et al. (2013)
119 analyzed the feasibility of determining N concentration in olive leaves using spectra
120 reflectance in the VNIR-SWIR (in this case, 450–1700 nm) range under laboratory
121 conditions and evaluated different types of spectrometers, different levels of sample
122 preparation and different types of mathematical pre-processing to generate spectral
123 models.

124 In conclusion, despite the wide literature on N estimation by spectral measurements,
125 very few studies are related to the approach of this work. Ferwerda and Skidmore
126 (2007) selected the bands with optimal fits to estimate leaf N content through a stepwise
127 regression routine, without taking into account the formulation of any VI, and requiring
128 four predictors. With respect to the study by Rotbart et al. (2013), its objective was not
129 to determine suitable bands to estimate leaf N content (LNC). They have showed the
130 power of using the whole spectra to estimate LNC in olive trees and five pre-processing
131 methods to develop PLSR models. Additionally, they have done that under laboratory
132 and not under field conditions.

133 Taking into account the importance of olive crop expansion, the yield in the
134 Mediterranean region and the few available studies about estimating N content in olive
135 trees using hyperspectral data, the present paper contributes to assess the usefulness of
136 olive leaf spectral features for the objective of estimating the nutritional status of this
137 crop. In this sense, the main aim of this study was to optimize the estimation of leaf N
138 content in olive trees. To do this the following sub-objectives have been addressed:

- 139 i) Analysing the potentiality of the entire spectral region (350–2500 nm) dataset to
140 estimate LNC in olive trees under field conditions.

- 141 ii) Testing eight different pre-processing methods (smoothing, standard normal
142 variate, first and second derivative, and different combinations among them) to
143 reduce the noise of the reflectance curves.
- 144 iii) Developing, implementing and applying a wavelength combination-based
145 method (using three predictors) to consider band combinations that produce the
146 optimal fit to the LNC.
- 147 iv) Comparing the predictive power of partial least squares regression (PLSR) and
148 VI estimations.

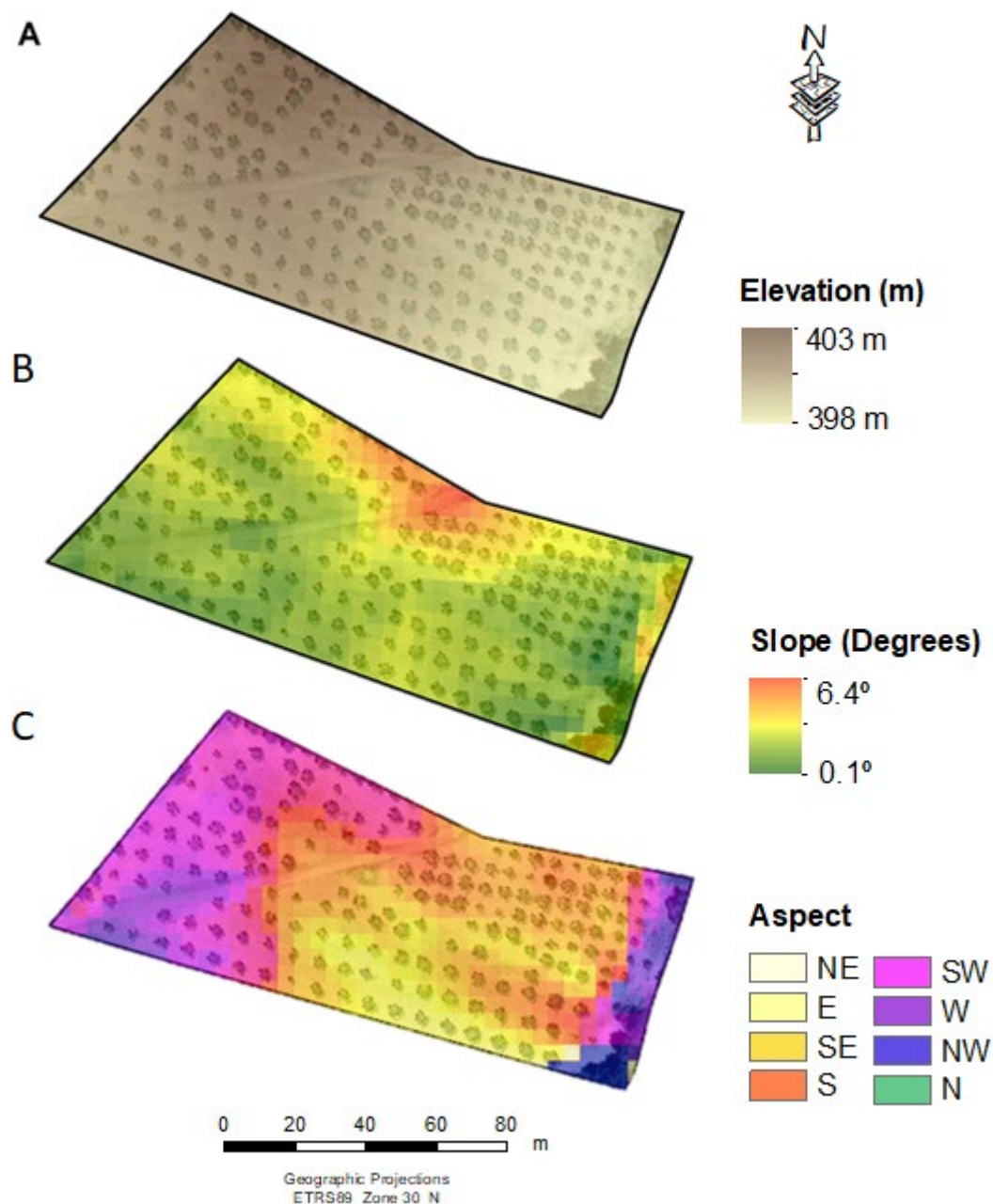
149

150 **Material and methods**

151 *Study area and field data collection*

152 The fieldwork was conducted during two consecutive days in July 2018 in an organic
153 olive orchard located in Carmonita (Badajoz) (39°09'04" N, 6°19'17" W), in southern
154 Spain.

155 The climate of the study area is semi-arid Mediterranean, with an average annual
156 temperature of 16 °C and mean annual rainfall amounts to 550 mm. The summers are
157 hot (24.5 °C) and dry (12.3 mm rainfall). Winters are warm (8.5 °C) and humid (79.1
158 mm rainfall) (Ninyerola et al. 2005). The study site has an area of 1.22 ha and a soil
159 depth about 0.60 m, with an average elevation of 400 m.a.s.l. (meters above sea level), a
160 medium slope gradient of 2.3°, and a predominantly south aspect (Figure 1). The olive
161 tree density is 150 trees/ha. The olive orchard is organic, and no fertilization treatments
162 were applied.



163

164 **Fig. 1** Topographic digital models (pixel size of 25 m²) of the study area: A) elevation,
 165 B) slope and C) aspect, generated using the cartography available at
 166 <http://centrodedescargas.cnig.es> (*Centro Nacional de Información Geográfica, Spain*).

167

168 To determine the real nutritional status of olive trees, a leaf analysis was carried out,
 169 collecting samples from 42 olive trees. To ensure that samples represent properly the
 170 study area, each sample was composed of 100 g of healthy leaves that were collected

171 from the middle portion of current-season shoots, about 1.5 m above the soil surface, at
172 the four cardinal points for every olive tree. Leaves from each sample were placed in a
173 pile on the soil, with direct incidence of sunlight (Ferwerda and Skidmore 2007) and
174 reflectance spectra were recorded using an Analytical Spectral Device (ASD)
175 FieldSpec® 4 spectro-radiometer (Malvern, United Kingdom). This instrument records
176 reflectance in the spectral range between 350 and 1000 nm, with a sampling interval of
177 1.4 nm, and between 1000 and 2500 nm, with a sampling interval of 1.1 nm. Spectral
178 data were interpolated to a spectral band width of 1 nm using the ASD software. In
179 order to calculate absolute reflectance, a reference spectrum was measured from a
180 Spectralon reference target between readings, at 15-minutes time intervals. For each
181 sample, 20 reflectance spectra were recorded and averaged, obtaining a single spectral
182 curve per olive tree. The spectral measurements were collected ± 2 h around solar noon,
183 under clear-sky conditions and in nadir orientation. Finally, leaves were placed in paper
184 bags and were taken to the laboratory following the protocol established by the
185 Agrifood Laboratory of the Junta de Extremadura (Cáceres, Spain).

186 The critical and sufficiency threshold of LNC of each olive tree were estimated at 1.4%
187 and 1.5%, respectively (Barranco et al. 1997; Fernández-Escobar et al. 1999).

188

189 *Spectral data pre-processing*

190 Spectral datasets were considered to predict N status of olive orchards and to identify
191 wavelengths directly related to LNC in olive trees. Spectra are often disturbed by
192 different interferences in the signal acquisition process due to the structure and physical
193 properties of the samples. Frequently, derivative transformations of the reflectance data
194 provide the best explanation of the variation, removing part of the noise (Hruschka
195 1987). Therefore, to minimize the undesired influence of physical attributes of samples

196 on measured spectra, mathematical pre-processing has become an important issue in
197 NIR spectral modelling (Bi et al. 2016).

198 In the present study, raw data were pre-processed using the following empirical
199 methods and mathematical operators: Savitzky-Golay Smoothing, which applies a
200 convolution method (SM; Savitzky and Golay 1964), Standard Normal Variate (SNV;
201 Barnes et al. 1989,1993), first derivative of the reflectance (D_1R), and second derivative
202 of the reflectance (D_2R) (Moros et al. 2010). In addition, the different methods and
203 operators were combined, obtaining a total of 8 spectral datasets: SM, SNV, D_1R ,
204 D_1R+SNV , $D_1R+SNV+SM$, D_2R , D_2R+SNV , $D_2R+SNV+SM$. Data processing was
carried out using Unscrambler

220 indices, different numbers of wavelengths (with a limit of three predictors) were
 221 systematically combined, following the formulations presented in Table 2, and the
 222 different pre-processing methods were tested.

223

224 Table 2. Formulations used to calculate systematic combinations of wavelengths, with a
 225 limit of three bands.

VI	Equation
SR	W_1/W_2
NDI	$(W_1 - W_2)/(W_1 + W_2)$
DCNI	$[(W_1 - W_2)/(W_2 - W_3)]/(W_1 - W_3 + 0.03)$
MCARI	$[(W_1 - W_2) - 0.2(W_1 - W_3)](W_1/W_2)$
TCARI	$3[(W_1 - W_2) - 0.2(W_1 - W_3)](W_1/W_2)$

226

227 All individual wavelengths were considered first, i.e. a total of 1,721 per each pre-
 228 processed dataset. Then, a total of 5,920,240 combinations using two wavelengths were
 229 computed for each pre-processed dataset. Finally, the same was performed with 3
 230 wavelengths and a total of 15,265,338,840 combinations were evaluated per each pre-
 231 processed dataset. Therefore, an exhaustive computation of all possible wavelength
 232 combinations for the considered indices and pre-processed datasets has been carried out.
 233 Third, wavelengths associated with known absorption features by the N and providing
 234 the largest determination coefficients (R^2) when correlating with LNC were selected to
 235 optimize the VI and considered for deeper analysis.

236 The simple linear regression model (between predicted and measured LNC) and the
 237 paired t-test were used. Results were considered statistically significant when p-values
 238 were lower than 0.05. Software R (Version 3.5.1) was used for calculations (R
 239 Development Core Team, 2008).

240

241 *Partial Least Squares Regression (PLSR)*

242 PLS is a bilinear calibration method using data compression by reducing the large
243 number of measured collinear spectral variables to non-correlated principal components
244 (PCs), which represent the relevant structural information that can be used to predict the
245 dependent variable. In this way, PLSR is a method often used for the retrieval of
246 vegetation biophysical parameters using spectral data because it is an efficient method
247 when predictors present multi-collinearity and when the number of wavelengths is
248 larger than the number of observations (Wold et al. 2001).

Spectral prediction models were constructed based on PLSR using Unscrambler

264 validation data for the considered model. This process is repeated 5 times, with each one
265 of the 5 subsets used exactly once as the validation data. Finally, the 5 measures are
266 averaged to produce an overall measure.

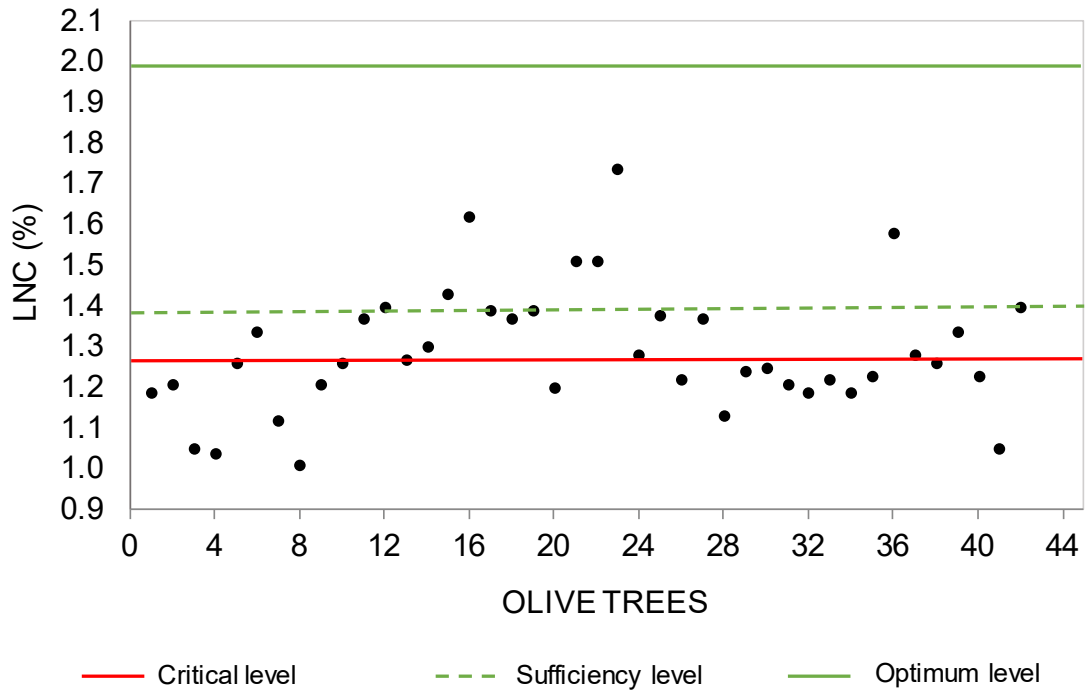
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268 **Results**

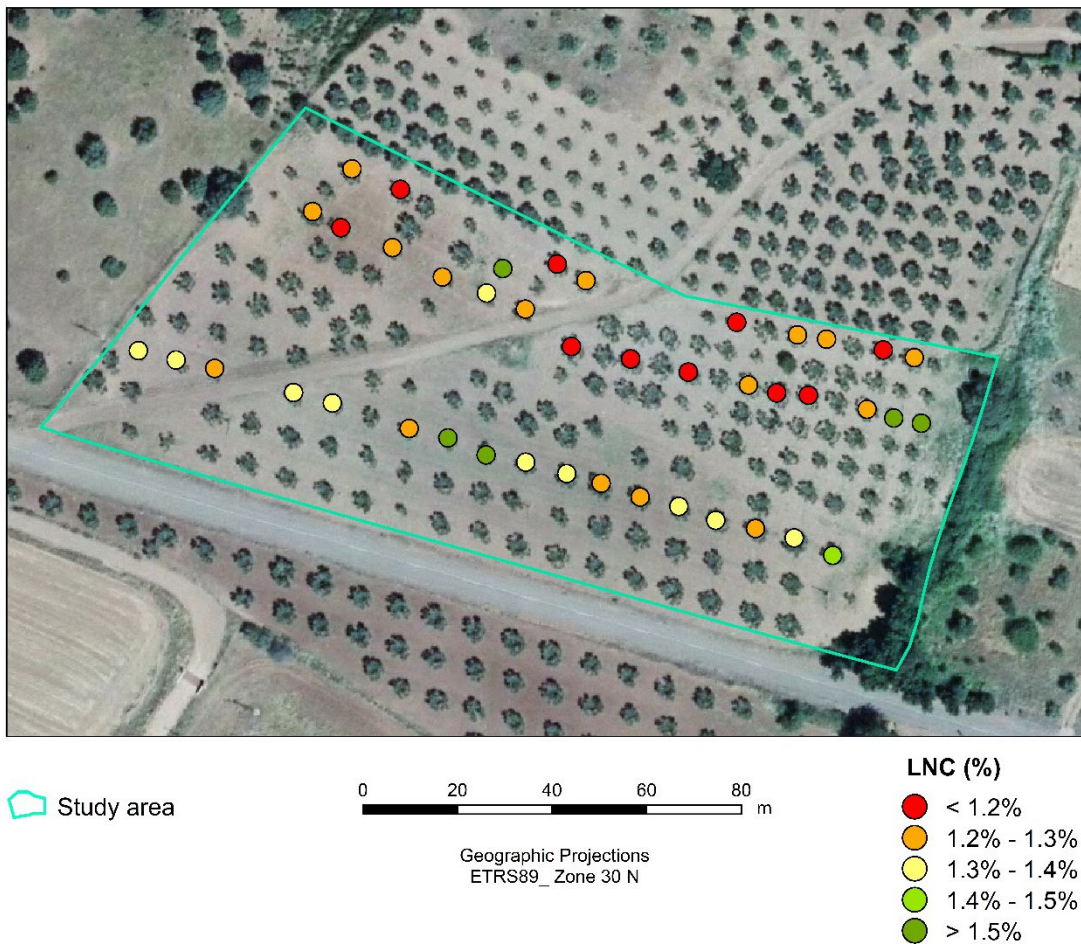
269 *Nutritional status of sampled and spectral data*

270 The foliar analyses ($n=42$) indicated that LNC varied between 1.01 and 1.74%, with an
271 average concentration of $1.3\pm 0.2\%$. Figure 2 represents the LNC in each sampled olive
272 tree and the levels of nutritional status. Most of the sampled olive trees (88.1%)
273 presented LNC below the sufficiency threshold of 1.5%, and only 11.9% of the olive
274 trees presented an appropriate LNC, within the range 1.5 – 2.0%. In addition, from the
275 olive trees with LNC below sufficiency threshold, 92% presented values below the
276 critical level of 1.4%. The spatial distribution of the sampled olive trees and nutritional
277 levels are presented in Figure 3.

278



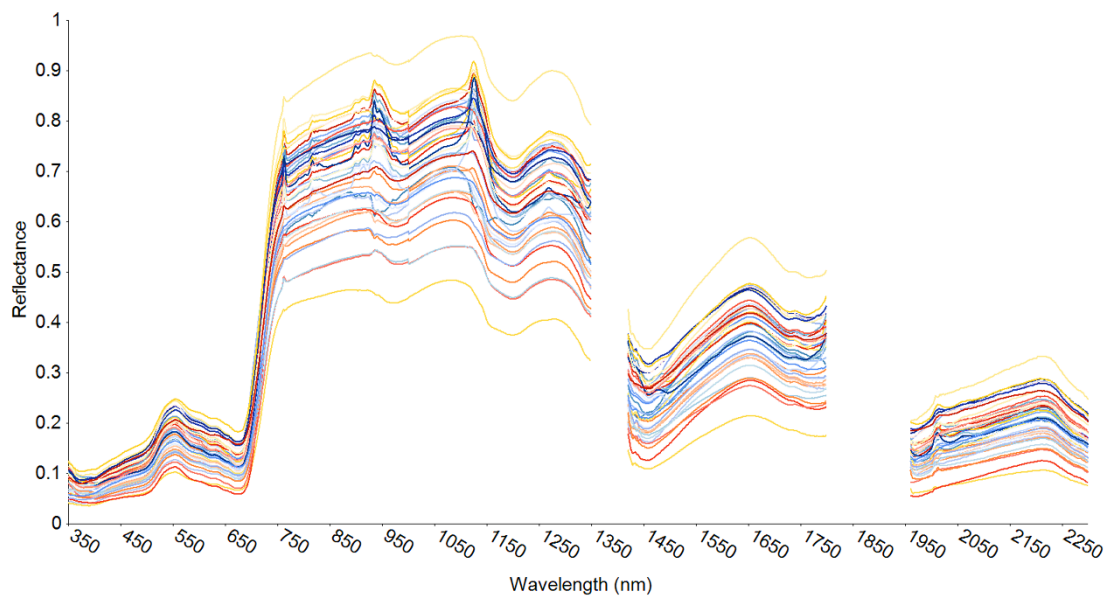
280 **Fig. 2** Leaf N content (LNC %) of sampled olive trees ($n=42$) and nutritional levels.



282 **Fig. 3** Spatial distribution of the leaf N content (LNC %) of sampled olive trees ($n=42$).

283

284 Reflectance curves of the sampled olive trees leaves are plotted in Figure 4. Overall
285 shapes were similar throughout the wavelengths measured, although the magnitude and
286 amplitude varied, specially in the NIR plateau (750–1100 nm). Reflectance curves
287 showed different reflectance peaks and absorption pits. In the VIS region, a reflectance
288 peak was centered at 554 nm (green region) and two absorption pits were centered at
289 390 nm and 680 nm (blue and red region, respectively). The NIR plateau presented a
290 higher reflectance than the VIS region, corresponding with a typical spectral signature
291 of green leaves (Liang 2005). In the SWIR region, three absorption pits, centered at
292 1200 nm, 1450 nm and 1720 nm, and three reflectance peaks, centered at 1280 nm,
293 1650 nm and 2200 nm were identified.



294

295 **Fig. 4** Reflectance curves of olive leaves within the ranges 350–1350 nm, 1421–1800
296 nm and 1961–2300 nm. Each colour line represents an olive tree reflectance curve.

297

299 Predicted LNC using VI formulated by other authors (see Table 1) presented very low
 300 determination coefficients ($R^2 < 0.21$) with measured values. No index presented
 301 statistically significant correlations with LNC (that is, all of them obtained p-
 302 values > 0.05), except for the indices that used reflectance at 1450 nm: $NDI_{870,1450}$ (p-
 303 value < 0.05 , $R^2 = 0.21$) and $NDI_{850,1450}$ (p-value < 0.05 , $R^2 = 0.12$), matching one of the
 304 absorption pits in the SWIR domain (see Figure 4). In spite of the significance, the
 305 determination coefficients were very low, and LNC variation was poorly explained by
 306 $NDI_{870,1450}$ and $NDI_{850,1450}$.

307 Results obtained from the systematic combinations of wavelengths, using the VIs in
 308 Table 2, indicated an increase of the determination coefficients as the number of
 309 combined wavelengths increased. For example, with raw data, $R^2 = 0.10$ for one
 310 wavelength, $R^2 = 0.36$ for two-wavelength VIs and $R^2 = 0.55$ for three-wavelength VIs
 311 (Table 3). In the same way, the application of pre-processing resulted in an
 312 improvement of the determination coefficients. Specifically, from $R^2 = 0.10$ to $R^2 = 0.46$
 313 for one wavelength, from $R^2 = 0.36$ to $R^2 = 0.56$ for two-wavelength VIs and from
 314 $R^2 = 0.55$ to $R^2 = 0.72$ for three-wavelength VIs (Table 3). Additionally, considering
 315 D_1R+SNV or D_2R+SNV , determination coefficients improved in almost all cases.

316 Table 3. Maximum determination coefficients (R^2) for leaf N content and individual
 317 wavelengths (1W), VIs based on combinations of two wavelengths (2W, i.e. SR and
 318 NDI) and on combinations of three wavelengths (3W, i.e. DCNI, MCARI and TCARI)
 319 by considering several pre-processed datasets.

Pre-processing	1W	2W	3W
Raw data	0.10	0.36	0.55
SM	0.27	0.54	0.68

SNV	0.30	0.37	0.59
D ₁ R	0.27	0.54	0.67
D ₁ R+SNV	0.40	0.54	0.66
D ₁ R+SNV+SM	0.40	0.54	0.69
D ₂ R	0.31	0.56	0.69
D ₂ R+SNV	0.46	0.56	0.72
D ₂ R+SNV+SM	0.39	0.44	0.69

320

321 Table 4 summarizes the results obtained from the wavelength combination process to
 322 determine the most suitable index to estimate the LNC in olive trees, considering
 323 biophysical properties of the N reflectance curve. As can be observed, the best results
 324 were obtained by using the second derivative as pre-processing and, mainly, when the
 325 second derivative was combined with SNV.

326

327 Table 4. Maximum determination coefficients (R^2) between leaf N content and
 328 vegetation indices (VI). W considers only a wavelength and no index is defined. Pp
 329 indicates the pre-processing applied to spectra data, where A is D₁R, B is D₂R, and C is
 330 D₂R+SNV. *Selected W* are the wavelength combinations that presented maximum R^2 to
 331 each VI, considering biophysical properties of the nitrogen reflectance curve. *Index* is
 332 the formula of the specific VI in the present paper. The letters of the *Selected W*
 333 columns indicate the region of the spectrum where the wavelengths are located: b =
 334 blue, r = red and sw = SWIR region. * indicates repeated wavelengths in different
 335 indices.

VI	R^2	R^2_{cv}	Pp	Selected W (nm)	Index
W	0.46	-	C	646 ^r	R_{646}
MCARI	0.53	0.50	A	1659 ^{sw} , 1749 ^{sw} , 1128 ^{sw}	$((R_{1659}-R_{1749})-0.2(R_{1659}-R_{1128}))(R_{1659}/R_{1749})$
SR	0.56	0.53	B	1615 ^{sw} , 648 ^{r*}	R_{1615}/R_{648}
NDI	0.56	0.55	C	397 ^b , 648 ^{r*}	$(R_{397}-R_{648})/(R_{397}+R_{648})$

TCARI	0.64	0.63	C	1685 ^{sw} , 412 ^b , 2209 ^{sw}	$3((R_{1685}-R_{412})-0.2(R_{1685}-R_{2209}))(R_{1685}/R_{412})$
DCNI	0.72	0.71	C	395 ^b , 652 ^r , 1275 ^{sw}	$((R_{395}-R_{652})/(R_{652}-R_{1275}))/((R_{395}-R_{1275}+0.03))$

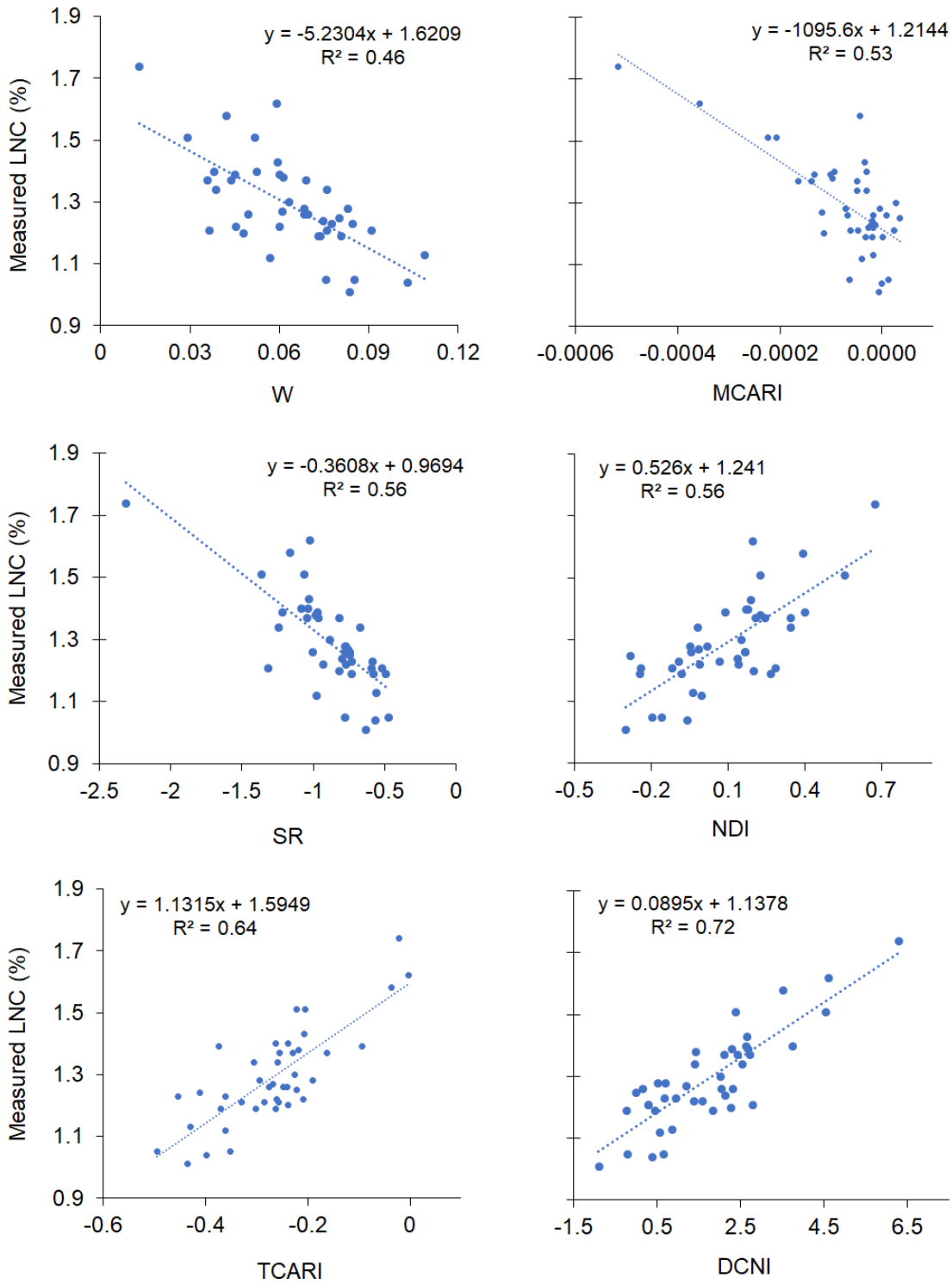
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337 In Figure 5, scatterplots for VI values and measured LNC with maximum determination
338 coefficients were plotted together with the regression line. DCNI yielded the best
339 goodness-of-fit with LNC ($R^2=0.72$; $R^2_{cv}=0.71$), combining the following wavelengths:
340 395 nm, matching with the absorption pit in the blue region; 652 nm, corresponding to
341 the absorption pit in the red region; and 1275 nm, matching with one of the reflectance
342 peaks in the SWIR region. The second best result was obtained with TCARI. It
343 predicted LNC with $R^2=0.64$ ($R^2_{cv}=0.63$), combining the wavelengths: 412 nm, the
344 absorption pit in the blue region; with reflectance peaks at 1685 nm and 2209 nm in the
345 SWIR region.

346 Determination coefficients obtained using MCARI, SR, and NDI indices were lower
347 than the ones obtained with TCARI and DCNI. SR predicted LNC with $R^2=0.56$
348 ($R^2_{cv}=0.53$) and the selected wavelengths were: 648 nm, the absorption pit in the red
349 region; and 1615 nm, a wavelength close to the reflectance peak at 1650 nm in the
350 SWIR domain. NDI estimated LNC with a similar determination coefficient ($R^2=0.56$)
351 but with a slightly greater $R^2_{cv}=0.55$. The combined wavelengths were: 397 nm, the
352 absorption pit in the blue region; and 648 nm, the absorption pit in the red domain.
353 MCARI predicted LNC with $R^2=0.53$ ($R^2_{cv}=0.50$), combining three bands in the SWIR
354 region: an absorbance band at 1749 nm with two reflectance bands at 1128 nm and 1659
355 nm.

356 The lowest coefficient was obtained from the relationship between LNC and the 646 nm
357 wavelength, close to the absorption pit in the red region, with an $R^2=0.46$. This case
358 considers only a wavelength and no index is defined.

359 Only the 648 nm (red region) wavelength correlated with LNC in two indices. The 395
360 nm, 397 nm and 412 nm wavelengths selected in DCNI, NDI and TCARI indices,
361 respectively, are located around of the chlorophyll_a absorption pit at 395 nm (Curran et
362 al. 2001). The wavelengths 646 nm (W) and 652 nm (DCNI) are very close to 648 nm
363 chlorophyll_b absorbance pit. Wavelengths from the SWIR region were selected to
364 calculate the SR, TCARI, MCARI and DCNI indices. No index combining wavelengths
365 from the NIR region presented a high determination coefficient. These results point to
366 combinations using blue, red and SWIR wavelengths as the most suitable to estimate
367 LNC in the studied olive trees using hyperspectral data.



368

369

Fig. 5 Scatterplots and regression line for the selected VI and measured leaf N content

370

(%).

371

372 Estimated N values obtained by using calibration and 5-fold cross-validation methods
 373 were considered to analyze the closeness of the estimations provided with those
 374 methods. The linear relationships between N predictions obtained with both methods
 375 are analyzed for all cases in Table 5, providing similar R^2 and R^2_{cv} in all these cases.
 376 The determination coefficients (R^2_{c-cv}) were close to 1.0 and the differences between
 377 LNC estimated with calibration and 5-fold cross-validation linear regressions were not
 378 statistically significant (p-values>0.05). RMSE and RMSE_{cv} were similar for each VI
 379 and the lowest value was produced by DCNI (Table 5).

380

381 Table 5. Comparison between calibration (R^2) and validation results (R^2_{cv}). R^2_{c-cv} is the
 382 determination coefficient between predicted LNC with calibration and predicted LNC
 383 with 5-fold cross-validation; *RMSE* is the root mean square error of calibration; RMSE_{cv}
 384 is the root mean square error of validation.

VI	R^2	R^2_{cv}	R^2_{c-cv}	RMSE	RMSE _{cv}	T-test (p-value)
MCARI	0.53	0.50	0.994	0.10	0.10	0.73
SR	0.56	0.53	0.995	0.10	0.10	0.81
NDI	0.56	0.55	0.997	0.10	0.10	0.74
TCARI	0.64	0.63	0.997	0.09	0.09	0.39
DCNI	0.72	0.71	0.997	0.08	0.08	0.44

385

386 *Multi-dimensional approach based on PLSR*

387 PLSR searches the sensitive information from spectra data and then uses the 5-fold
 388 cross-validation procedure to calculate the calibration PLSR model. Different PLSR
 389 models were developed using raw and pre-processed data (Table 6).

390

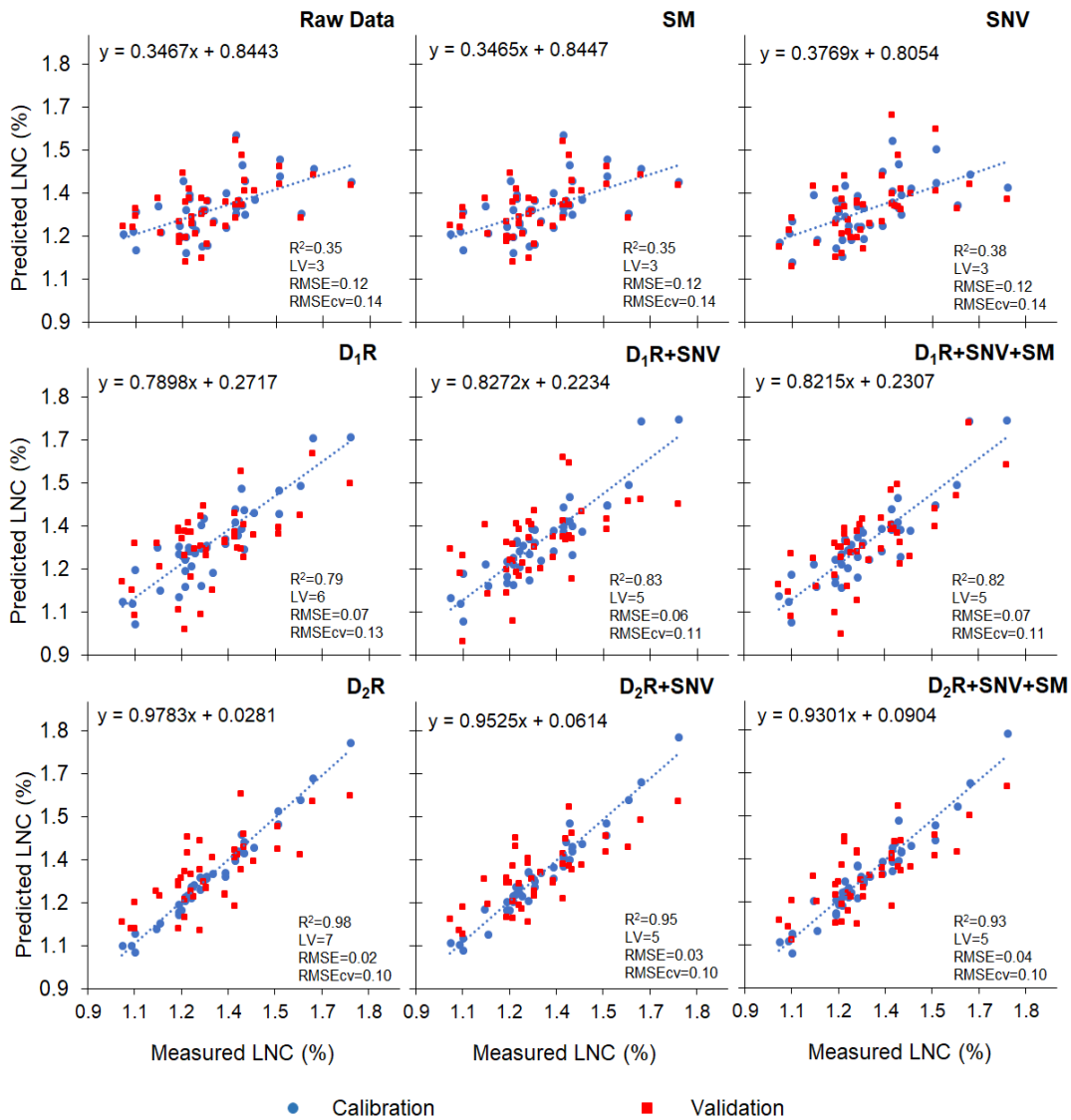
391 Table 6. PLSR results for each pre-processed dataset. CV and CV_{cv} are the variation
 392 coefficients of calibration and cross-validation models, respectively.

Model/Pre-processing	R^2	R^2_{cv}	LV	RMSE	$RMSE_{cv}$	CV	CV_{cv}	T-test (p-value)
Raw data	0.35	0.23	3	0.12	0.14	0.07	0.07	0.35
SM	0.35	0.23	3	0.12	0.14	0.07	0.07	0.35
SNV	0.38	0.23	3	0.12	0.14	0.07	0.08	0.49
D ₁ R	0.79	0.33	6	0.07	0.13	0.11	0.10	0.55
D ₁ R+SNV	0.83	0.52	5	0.06	0.11	0.11	0.10	0.72
D ₁ R+SNV+SM	0.82	0.52	5	0.07	0.11	0.11	0.11	0.41
D ₂ R	0.98	0.56	7	0.02	0.10	0.12	0.09	0.60
D ₂ R+SNV	0.95	0.58	5	0.03	0.10	0.12	0.09	0.87
D ₂ R+SNV+SM	0.93	0.57	5	0.04	0.10	0.12	0.09	0.99

393

394 Analysing the results presented in Table 6, determination coefficients ranged between
 395 0.35 and 0.98 for the calibration models and between 0.23 and 0.58 for the cross-
 396 validation models. D₂R model presented the largest $R^2=0.98$, followed by D₂R+SNV
 397 model ($R^2=0.95$) and by D₂R+SNV+SM model ($R^2=0.93$) (see Figure 6). The
 398 calibration and validation RMSE were relatively small in those cases (≤ 0.04 N% in the
 399 calibration and 0.10 N% in the validation) and the differences between both were lower
 400 than 20% (0.20), indicating that the number of LV selected for each pre-processing was
 401 suitable, without overfitting the models (Shao et al. 2007). The difference between R^2
 402 and R^2_{cv} was large: 0.42, 0.37 and 0.36, respectively. However, the differences between
 403 average LNC estimated with calibration and average LNC estimated with 5-fold cross-
 404 validation obtained with PLSR were not statistically significant (p-values >0.05) and the
 405 differences between the variation coefficients (CV) were ≤ 0.03 in all cases. These
 406 results indicated that, despite the difference between the determination coefficients of
 407 the calibrated and validated models, the LNC estimates were similar.

408 Predicted LNC using the calibrated and validated PLSR models are plotted in Figure 6
 409 for each dataset, raw and pre-processed data. It can be observed that second derivative
 410 pre-processing presented better fitting between measured and predicted LNC than the
 411 other pre-processing.



413 **Fig. 6** Measured vs. predicted leaf N content (*LNC* %) using PLSR and different pre-
 414 processing methods.

415
 416

417 **Discussion**

418 VI and PLSR models were used to relate LNC and reflectance data over the full
419 spectrum (350–2500 nm) using a high precision spectro-radiometer. It has been shown
420 that these methods produced high coefficients of determination, particularly when the
421 reflectance spectra were expressed as the second derivative and combined with SNV or
422 SM pre-processing. The wavelengths most sensitive to N variation used to calculate VI
423 were selected from the VIS and SWIR spectral regions, which relate to Chl and N
424 features. PLSR models yielded a higher accuracy (R^2) than VI, although the
425 uncertainties associated with the noise of the hyperspectral data were higher.

426 Ferwerda and Skidmore (2007) and Rotbart et al. (2013) focused on a similar topic. The
427 novelty of this paper with respect to these studies is the method used to determine the
428 band combinations more suitable to estimate LNC in olive trees. Ferwerda and
429 Skidmore (2007) selected the bands with optimal fits to the N content through a
430 stepwise regression routine, without taking into account the formulation of any VI, and
431 requiring four predictors. Besides, they only analyzed two pre-processing methods
432 (Savitzky–Golay smoothing and continuum-removed spectra). In the present paper, the
433 wavelengths of all the spectral regions are combined, taking into account their
434 biophysical basis and the formulation of the VIs more suitable to estimate LNC
435 according to other authors: SR, NDI, DCNI, MCARI and TCARI (see Tables 1 and 2).
436 The number of predictors is set to three, simplifying the model respect to the one
437 developed by Ferwerda and Skidmore (2007), that used four, at the same time that
438 better results are obtained in this work. Moreover, this paper analyzes eight pre-
439 processing methods, instead of only two. Furthermore, in this paper, the results are
440 compared with those obtained by other authors and those obtained with the PLSR
441 method.

442 With respect to the study by Rotbart et al. (2013), its objective was not to determine
443 suitable bands to estimate LNC. Besides, Rotbart et al. (2013) only used five pre-
444 processing methods to develop the PLSR models, instead of the eight analyzed in this
445 paper. Even more, Rotbart et al. (2013) used a lower spectral range (450 nm–1000 nm
446 and 1100–1700 nm) due to the use of three different spectrometers (USB-2000, LIGA
447 and Luminar-5030), whereas in this paper all the spectral range (350 nm–2500 nm) has
448 been used. Finally, Rotbart et al. (2013) determined N concentration in olive leaves by
449 using spectral reflectance of the VIS-NIR range under laboratory conditions and not in
450 the field such as is the case of this paper.

451

452 *Nutritional status of olive trees and spectral data*

453 N status of sampled olive trees was deficient considering a critical and sufficiency
454 threshold of LNC of 1.4% and 1.5%, respectively (Barranco et al. 1997; Fernández-
455 Escobar et al. 1999). Fernández-Escobar et al. (2009) studied the long-term effect of N
456 fertilization on olive trees. The results indicated that yield and growth were maintained
457 in trees non-fertilized and with LNC within the range 1.4% and 2.0% during several
458 consecutive years. In this sense, Fernández-Escobar et al. (2009) recommended that the
459 leaf critical threshold of LNC in olive trees must be revised and is likely to lie between
460 1.2% and 1.3%. Regarding results presented in the current study, if 1.3 % or 1.2% are
461 considered as critical limits of LNC, then, 59.5% or 21.4%, respectively, of the sampled
462 trees had a deficient N status, instead of the 81.0% obtained with a threshold of 1.4%.
463 All these reports suggest that fertilization management depends on the LNC critical
464 limit established and, therefore, spatial and temporal analyses are necessary to carry out
465 appropriate fertilization planning to improve olive orchards production. According to
466 López-Granados et al. (2004), Gómez-Casero et al. (2007) and Fernández-Escobar et al.

467 (2009), N fertilization should be applied only to olive trees with an N value that does
468 not exceed the considered threshold, because they reported that, in the case of olive
469 orchards, annual maintenance application of N is not necessary to improve yield and
470 tree growth.

471 Reflectance curves of the leaves of the sampled olive trees presented a similar shape
472 throughout the spectral range. The reflectance curves obtained within the range 400
473 nm–900 nm were similar to that obtained by Zarco-Tejada et al. (2004) and Gómez-
474 Casero et al. (2007) for olive orchards without fertilization treatments, presenting
475 absorption pits and reflectance peaks at the same wavelength ranges. Absorption pits
476 were identified at 500 ± 10 nm and 680 ± 10 nm, whereas reflectance peaks were
477 identified at 550 ± 10 nm and 760 ± 20 nm. Wavelength absorption at 680 nm could be
478 related to the absorption feature of Chl_a at 660 nm (Curran 1989). Chl is the main
479 pigment responsible for the properties of the reflectance and transmittance of radiation
480 in the VIS region and has a close correlation with the N content in the leaves of plants
481 (Haboudane et al. 2002). N shortage will reduce leaf Chl content and consequently the
482 reflectance in the VIS region will increase (Daughtry et al. 2000). Therefore, the
483 reflectance peak at 550 nm could indicate a low Chl content and consequently a low
484 LNC, matching the results obtained considering 1.4% as a critical threshold.

485

486 *Systematic wavelength combinations and pre-processing*

487 The systematic combination of wavelengths from all spectral ranges showed that the
488 determination coefficients increased as the number of combined wavelengths increased.
489 The application of pre-processing decreased considerably the noise generated by the
490 measurements obtained from the spectro-radiometer, also improving the results. As in
491 the case of the PLSR models, second derivative combined with SNV and/or SM

492 produced the best determination coefficients. These results agree with the ones from
493 other publications which demonstrated the significant and positive effects of using the
494 whole spectral range and pre-processing to estimate LNC in olive trees. Ferwerda and
495 Skidmore (2007) carried out a stepwise regression, with a limit of four predictors,
496 between leaf chemical composition (N, K, Na, P, Ca and Mg) of four woody plant
497 species (olive tree, mopane, heather and willow) using different datasets: raw
498 reflectance spectra, derivative spectra and band depth. The model based on derivative
499 spectra offered the highest prediction accuracy. Rotbart et al. (2013) obtained the most
500 robust prediction models applying the first derivative to the logarithm of the reflectance
501 reciprocal [$D_1(\log(1/R))$] with the PLSR method.

502

503 *VI to estimate LNC*

504 Reflectance-based indices formulated using wavelengths associated with known
505 absorption features showed lower determination coefficients with LNC than indices
506 derived from the systematic wavelength combinations carried out in this study. The
507 wavelengths combined in the VIs with higher determination coefficients were located
508 mainly in the VIS region (350 nm–750 nm) and in the SWIR region (1100 nm–2500
509 nm).

510 In the present study, wavelengths located in the blue range (395 nm, 397 nm and 412
511 nm) were selected. They could be directly related to the absorption pits of the Chl_a at
512 430 nm (Curran 1989) and indirectly related to the N content (Haboudane et al. 2002).
513 Organic components (e.g., cellulose, lignin, proteins, oil, sugar or starch) absorb
514 radiation strongly in the blue spectral domain as a result of stretching and bending
515 vibrations of the strong molecular bonds between hydrogen atoms and the atoms of
516 carbon, nitrogen and oxygen (Osborne 2000). Other studies showed that blue bands

517 were sensitive to estimate LNC at later growing stage of the plant (Cohen and
518 Alchanatis 2018). Therefore, 395 nm, 397 nm and 412 nm wavelengths also could be
519 related to the absorption region of these organic components in the blue region and their
520 reactions with the N content. This could justify bio-physically why these wavelengths
521 were selected to optimize the VIs.

522 Wavelengths at the red domain of the VIS region (e.g., 646 nm, 648 nm and 652 nm)
523 appeared in several of the proposed VIs. These wavelengths could be related to the
524 absorption pits of the Chl_b and Chl_a at 640 nm and 660 nm (Curran 1989). This link is
525 related to the strong influence of N on Chl production and functioning. Therefore, N
526 could be indirectly estimated using wavelengths within the red domain (Haboudane et
527 al. 2002). The 750 nm wavelength is in the limit between red (VIS) and red edge (NIR)
528 domain. Several authors selected this wavelength to estimate Chl_{ab} content from
529 hyperspectral reflectance (Zarco-Tejada et al. 2001; Wu et al. 2008). Therefore, it also
530 could be related to N content.

531 The 1128 nm, 1275 nm, 1615 nm, 1659 nm, 1685 nm, 1749 nm and 2209 nm
532 wavelengths centered at SWIR region and selected to optimize VIs could be related to
533 N, protein, oil, lignin, sugar, cellulose, starch and water content (Curran 1989). The
534 spectral range 1500 nm–1600 nm is a region dominated by absorption features due to
535 N-H bond stretching located at 1510 nm (Curran 1989) and this bond is related to the
536 amount of N present in the protein (Ferwerda 2005). However, the reflectance within
537 the SWIR region is sensitive to foliar water content. Ceccato et al. (2002) and Ferwerda
538 (2005) identified that the indices which included the 1770 nm absorption feature were
539 least affected by foliar water content.

540 Authors like Zarco-Tejada et al. (2004), Ferwerda (2005), Ferwerda and Skidmore
541 (2007) or Gómez-Casero et al. (2007) selected similar wavelengths to the ones

542 considered in the present study to estimate N content in olive trees. Zarco-Tejada et al.
543 (2004) combined 750 nm and 710 nm wavelengths in a simple ratio formulation and
544 used the 550 nm, 670 nm and 700 nm wavelengths to calculate MCARI (Daughtry et al.
545 2000) and TCARI (Haboudane et al. 2002) indices. Ferwerda (2005) obtained their best
546 determination coefficients combining wavelengths from the VIS (627 nm - 640 nm and
547 690 nm - 700 nm) and from the SWIR (1516 nm - 1580 nm, 1770 nm, 1803 nm and
548 2196 nm) regions in the NDI formulation. Ferwerda and Skidmore (2007) obtained their
549 best determination coefficients combining the 733 nm, 1203 nm, 1792 nm and 955 nm
550 wavelengths through a stepwise regression routine and limiting the number of predictors
551 to four. Gómez-Casero et al. (2007) selected different wavelengths within the VIS-NIR
552 region depending on the type of fertilization applied. For soil application, selected
553 wavelengths were within the blue (400 nm, 420 nm and 470 nm), red (610 nm, 630 nm
554 and 720 nm) and NIR domain (750 nm–900 nm). For soil and foliar application,
555 selected wavelengths were within the blue (420 nm and 490 nm), green (510 nm), red
556 (690 nm and 710 nm) and NIR domain (890 nm).

557 In the present study, the best determination coefficient and the lowest RMSE/RMSE_{cv}
558 were obtained by using the DCNI index formulation ($R^2=0.72$). Camino et al. (2018)
559 estimated LNC for durum and bread wheat using this index with the formulation
560 developed by Chen et al. (2010), and reported a determination coefficient of $R^2=0.56$,
561 with wavelengths within the red domain (670 nm, 700 nm and 720 nm). In this work,
562 TCARI and MCARI indices reported $R^2=0.64$ and $R^2=0.53$, respectively. Zarco-Tejada
563 et al. (2004) applied these indices to estimate LNC in olive trees using wavelengths
564 within the VIS region (550 nm, 670 nm and 700 nm), according to the formulation of
565 Daughtry et al. (2000) and Haboudane et al. (2002). The determination coefficients
566 were lower in the case of the TCARI index ($R^2= 0.60$) and higher in the case of MCARI

567 index ($R^2=0.64$). SR and NDI indices also presented similar determination coefficients
568 ($R^2=0.56$ in both cases), although the selected wavelengths were different. SR index
569 combined VIS and SWIR regions (648 nm and 1615 nm) and NDI combined
570 wavelengths at the blue and red domain (397 nm and 648 nm). Therefore, based on the
571 whole previous discussion and indices, and in agreement with Herrmann et al. (2010),
572 the SWIR region is more sensitive to N content than the VNIR region. Additionally,
573 indices based on combination between SWIR and VIS wavelengths are better predictors
574 of LNC in olive trees than the ones that only use the VIS region.

575

576 *PLSR models*

577 The largest determination coefficient was obtained with D₂R dataset and 7 LV
578 ($R^2=0.98$). D₂R+SNV+SM produced lower determination coefficient ($R^2=0.93$), but the
579 difference between calibration and validation coefficients, as well as the number of LV,
580 was lower. These results indicated that this model is more robust, which presented a
581 RMSE of 0.04% and a RMSE_{cv} of 0.10%. Rotbart et al. (2013) estimated LNC in olive
582 trees using the whole spectral range and PLSR models, applying different pre-
583 processing. The results were similar to those reported in this paper, i.e. the largest
584 determination coefficient was obtained with pre-processing data ($R^2=0.91$), with a
585 number of 7 LV, and RMSE and RMSE_{cv} of 0.05% and 0.07%, respectively.

586 In agreement with other authors that reported that PLSR methods have greater potential
587 than spectral indices for deriving N content in crops such as winter wheat (Hansen and
588 Schjoerring 2003; Li et al. 2014) or maize (Quemada et al. 2014), in this study PLSR
589 models (D₁R, D₁R+SNV, D₁R+SNV+SM, D₂R, D₂R+SNV and D₂R+SNV+SM)
590 produced higher determination coefficients (R^2) than VI. However, the results obtained
591 with the PLSR method show higher difference between R^2 and R^2_{cv} than the VI method,

592 indicating a possible overfitting of the models. But the differences between RMSE and
593 $RMSE_{cv}$ were lower than 20% (0.20) in all cases. Therefore, according to Shao et al.
594 (2007), this result indicates a suitable number of LV selected for each pre-processing
595 and, accordingly, the models did not present overfitting.

596 Taking into account the uncertainties presented by both methods, it is difficult to
597 determine which method yielded better fits between the estimation and the LNC
598 measured. R^2_{cv} is an indicator of the prediction capacity of the model. Therefore, since
599 DCNI and TCARI vegetation indices presented higher R^2_{cv} and lower $RMSE_{cv}$ than
600 PLSR models in all pre-processed datasets, in the case of this study, VI are more
601 suitable than PLSR models to estimate LNC in olive trees without fertilization
602 treatments.

603

604 **Conclusions**

605 The present study demonstrated that hyperspectral data are useful to estimate leaf N
606 content in olive trees without fertilization treatments. The VI and PLSR models
607 considered by using the full spectrum (350–2500 nm) produced larger determination
608 coefficients than the ones from spectral indices formulated using wavelengths
609 associated with known absorption features. Applying pre-processing to the spectral
610 data, the noise generated by the measurement of the spectro-radiometer was reduced
611 and the correlations between leaf N content and reflectance data were improved,
612 particularly when the reflectance spectra were expressed as the second derivative and
613 combined with SM and/or SNV pre-processing. VIs that produced the largest
614 determination coefficients were DCNI and TCARI. The wavelengths most sensitive to
615 N variation used to define VIs were selected from the VIS and SWIR spectral regions,

616 which relate to chlorophyll a+b and N absorption features. Therefore, implementing the
617 SWIR region to estimate leaf N content in olive trees improved predictions. PLSR
618 models yielded higher accuracy than VI, although the uncertainties associated with the
619 noise of the hyperspectral data were higher. These methods would allow accurate
620 fertilization plans depending on the olive tree requirements. Further research is
621 necessary to gain knowledge about the temporal variation of the leaf N content in olive
622 trees to offer to the farmers accurate information about the nutritional status of the
623 plants at each phenological stage.

624

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633

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