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1Quantifying spatio-temporal variation of leaf 2chlorophyll and nitrogen contents in vineyards

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14Abstract

15Precision viticulture requires the characterisation of the spatio-temporal variability of
16the vineyard status to design the appropriate management for each area. The goal of
17this work was to characterise the spatio-temporal variability of leaf chlorophyll (Chl) and
18nitrogen (N) content and their relationship with the vegetative growth in a three ha
19commercial vineyard (*Vitis vinifera* L.) using a geostatistical approach. Leaf Chl and N
20contents were assessed by two fluorescence indices provided by a hand-held
21fluorescence sensor. Fluorescence measurements were taken along five dates, from
22veraison to harvest, on 72 sampling points delineated on a regular grid across the
23vineyard. Shoot pruning weight (SPW) was measured for each sampling point as
24indicator of the grapevine vegetative growth. Geostatistical analysis was applied to
25model the spatial variability of leaf Chl and N content and SPW. The spread showed an
26increase of the variability of leaf Chl and N content during the ripening period, reaching

27maximum values prior to harvest. The variograms illustrated a similarity of the spatial
28variability structure of leaf Chl at all timings, unlike N which showed changing spatial
29variability structures along the ripening period. The Kappa index evidenced a slight
30intra-season stability for both Chl and N and showed that N could not be used alone as
31an indicator to delineate vigour management areas. The existence of spatio-temporal
32variability of key vegetative components was proved and its knowledge is crucial to
33implement precision viticulture approach such as variable rate application of fertilizers
34or water as needed.

35**Keywords:** proximal sensing, plant phenotyping, vegetative growth, geostatistical
36analysis, grapevine, chlorophyll fluorescence.

37**Nomenclature table**

38Chl: chlorophyll

39CmbI: Cambardella Index

40CV: coefficient of variation

41FRF_R: far red fluorescence evolved from red light emission

42FRF_UV: far red fluorescence evolved from UV light emission GPS: global positioning
43system

44ME: mean error

45MSE: mean square error.

46MSSE: mean square standardised error

47N: nitrogen

48NBI: nitrogen balance index

49RF_R: red fluorescence evolved from red light emission

50SFR: simple fluorescence ratio

51SPW: shoot pruning weight

52UV: ultraviolet

53**1. Introduction**

54Precision viticulture is the rational and differentiated agronomical management of
55vineyards based on the spatio-temporal variability of growth, yield and grape
56composition within the plot (Proffitt et al., 2006).

57Vineyard status has been proved to be spatially and temporally variable. Its spatial
58variability is primarily influenced by soil and mesoclimate. The interaction between root
59system and soil determines the availability of water and nutrients for the vine (Mullins,
60Bouquet, Williams, 1992). Under no restrictions, growth of main and lateral shoots and
61number of leaves increase. On the contrary, scarcity of nutrients or water supply
62negatively affects vegetative growth and in some cases, can also have an impact on
63yield. The mesoclimate, which is the climate at a parcel level, will vary according to the
64existence of differences in elevation, slope or aspect leading to differences in
65temperature, wind speed, solar radiation or humidity (van Leeuwen, 2010). This spatial
66variability also changes in time due to variations in climatic conditions through the
67years, known in viticulture as the vintage effect (van Leeuwen, 2010). The climate is
68the factor responsible for the yearly variations in vine performance and grape ripening,
69while soil type and plant material, with the exception of a disease infection, remain
70constant (van Leeuwen et al., 2004).

71The spatio-temporal variability of a number of vineyard characteristics has been
72moderately addressed in literature. Main grape composition parameters such as total
73soluble solids and titratable acidity revealed similar spatial variability pattern across
74vintages (Baluja et al., 2013; Tagarakis et al., 2014), while anthocyanins and total
75phenols have shown different spatial patterns between years (Baluja et al., 2013;
76Bramley, 2005; Tisseyre, Mazzoni, Fonta, 2008) and within the season (Baluja et al.,
772012b; Trought, Bramley, 2011). Yield displayed a temporally stable spatial pattern
78despite the differences in the average yield value of the vineyard among years
79(Bramley & Hamilton, 2004). In contrast, Reynolds et al. (2007) showed substantial
80changes in spatial variability of crop production over years. A significant temporal

81 stability was found for pruning weight, one of the most studied vigour variables (Taylor
 82 & Bates, 2013; Tisseyre, Mazzoni, Fonta, 2008). Canopy size, defined as the surface
 83 occupied by the canopy from a zenithal point of view, was also reported to be
 84 temporally stable (Tisseyre et al., 2008), while canopy density, described by the mean
 85 NDVI value of the pixels in the vine row has shown a changing spatial pattern within a
 86 given season (Hall et al., 2011). Davenport and Bramley (2007) assessed the spatio-
 87 temporal variability of nutrients like N, P, K, Ca, Mg, S, Cu, B, Zn, Fe and Na on a per
 88 vine basis. These components were analysed in petiole tissue at flowering and
 89 veraison, and in berry tissue at harvest. They found stability in the spatial variability of
 90 K and Mn, while other nutrients like N, P, S or Zn showed a less stable spatial pattern
 91 over time. Most of the scientific literature addressed either the spatial variability
 92 (Cerovic et al., 2009; Garcia et al., 2012; Zarco-Tejada et al., 2005; Zarco-Tejada et al.,
 93 2013) or the temporal variability of nutrient content, grapevine vegetative status, yield
 94 or grape composition (Kriedemann, Kliwer, Harris, 1970; Romero, García-Escudero,
 95 Martín, 2010; Schreiner, 2005), but few studies have evaluated both their spatial and
 96 temporal variability with a geostatistical approach. As indicated by Conradie (1991,
 97 2005), a perennial plant, such as grapevine, not only retrieves nutrients from the soil
 98 along a given growing season, but also allocates them from storage tissues, like wood
 99 and roots. Hence, the plant's nutrient uptake results from the combination of new and
 100 recycled nutrients. Therefore, it is important to understand the spatio-temporal
 101 variability of nutrient uptake, to supply the correct amount of nutrients in each vineyard
 102 subarea when the soil or the vine reserves may not provide enough.
 103 One of the most important nutrients involved in the photosynthesis is the N, which is
 104 also part of the Chl molecule. N is an essential nutrient as it is one of the most
 105 important elements for biomass production (Agati et al., 2013; Lemaire, Jeuffroy,
 106 Gastal, 2008) and grapevine metabolism, crucial for vine development and fruit yield
 107 (Guilpart, Metay, Gary, 2014). Furthermore, N is involved in various enzymatic proteins
 108 that catalyse and regulate plant-growth processes. Also N fertilization deeply influences

crop yield and biomass (Tremblay, Wang, Cerovic, 2012). In grapevines, excessive N can cause more damage than its deficiency because vines would be more prone to diseases and insect infestations due to an increase in the density of the canopy or clusters (Dordas 2009). Moreover, over-fertilization usually produces lower quality grapes (Keller 2010) and plants become more susceptible to flowering abortion and reduced fruit set (Vasconcelos et al., 2009). Therefore, an accurate estimation of N content at the time of potential application is crucial, especially in precision viticulture, where spatial variability is taken into account (Bramley 2010b). Moreover, Chl is a key compound in grapevine leaves as it is the pigment responsible for photosynthesis. Its content increases until grapevine leaves are fully expanded and starts to decrease afterwards, as soon as it attains its maximal value (Kriedemann et al., 1970). Leaf chlorophyll content is affected by environmental stress, nutrient deficiency or diseases (Hendry et al., 1987).

Chl and N are associated to grapevine vegetative growth-related variables such as the vine pruning weight (Lemaire et al., 2008), which is a measure of the plant's vegetative growth (Smart & Robinson, 1991). Therefore, the assessment of the spatio-temporal variability of leaf Chl and N content would be helpful in the frame of precision viticulture to delineate fertilization and canopy management strategies intended to improve the grapevine balance and fruit composition.

Leaf Chl and N contents are usually assessed using destructive wet chemistry procedures. Compared to these, optical methods provide much faster assessment, allowing the analysis of the whole plot. Among the wide variety of technologies used in proximal sensing, Chl fluorescence technique has been implemented in a commercial hand-held sensor, Multiplex™ (Ben Ghozlen et al., 2010a). This device has proved to be a powerful, rapid and efficient phenotyping tool to determine Chl and N contents in grapevine leaves (Rey-Caramés, 2015).

The goal of the present work was the characterisation of the spatio-temporal variability of grapevine leaf Chl and N contents and the study of their relationship with vegetative

137growth in a commercial vineyard (*Vitis vinifera* L.) using a geostatistical approach.

138**2. Materials and Methods**

139**2.1 Experimental site and layout**

140The study was conducted in a [three ha](#) commercial Tempranillo (*Vitis vinifera* L.)
141vineyard located in Navarra (42°38' N, 2°2' E, 518 m a.s.l.), Spain, during the 2011
142season. Grapevines were planted on a sandy-clay soil in 2004, at 2.4 x 1.6 m (inter-
143and intra-row) with north-south orientation. Vines were trained to a vertically shoot-
144positioned, spur-pruned cordon retaining 16 nodes per vine and uniformly irrigated
145twice across the season. Veraison occurred on the 17th of August and harvest was
146carried out on the 17th of October.

147A regular sampling grid was defined, consisting on 72 sampling points at 20 m
148intervals, following the sampling grid strategy described in [Baluja et al. \(2012a, 2012b\)](#).
149Each sampling point constituted of three adjacent vines, where the central one was
150georeferenced using a Leica Zeno 10 Global Positioning System (GPS) (Heerbrug, St.
151Gallen, Switzerland), with real time kinematic correction and working at <40 cm
152precision. The variables values obtained for each of the three vines per sampling point
153were averaged to have a unique averaged value per sampling point.

154**2.2 Assessment of leaf chlorophyll and nitrogen content**

155Measurements of ~~the~~ leaf Chl and N content in vine leaves were taken using the hand-
156held fluorescence-based sensor Multiplex3™ (Force-A, Orsay, France). This is a non-
157destructive active device that generates fluorescence in plant tissues by the excitation
158at four different wavelengths: UV_A (375 nm), blue (450 nm), green (530 nm) and red
159(630 nm). This proximal sensor includes three [detectors with specific](#) filters to record
160the fluorescence emission at three different bands: blue-green (447 nm) or yellow (590
161nm) depending whether blue excitation is used or not respectively, red (665 nm) and
162far-red (735 nm) ([Ben Ghazlen et al., 2010b](#)). [The sensor illuminates a surface of 8 cm-](#)
163[diameter, which is 50 cm² of measurement area, at a 10-cm distance from the source.](#)

Due to the reduced surface of measurement, it only captures information from the leaf with no background influence. Prior to field measurements, the operator must take some measurements pointing to the sky (not directly to the sun) and other measurements of a blue plastic-foil standard (Force-A, Orsay, France). These measurements allow to assure the correct functioning of the sensor, checking that they are within the limits indicated by the company, and to carry out the standardisation of the data recorded, in order to compare the data collected under other measuring conditions.

The fluorescence-based indices studied are defined as follows:

The simple fluorescence ratio (SFR)

$$SFR_{R_{AD}} = \frac{FRF_R}{RF_R} \quad (1)$$

The nitrogen balance index (NBI)

$$NBI_{C2-R_{AD}} = \frac{FRF_R / RF_R}{\log(FRF_R / FRF_{UV})} \quad (2)$$

where FRF_R and FRF_UV refer to the far red fluorescence evolved from red and UV excitation, respectively, and RF_R refers to red fluorescence excited by red light (Ben Ghazlen et al., 2010a).

The simple fluorescence ratio (SFR) index is a chlorophyll fluorescence emission ratio linked to the leaf Chl content of different species (Gitelson, Buschmann, Lichtenthaler, 1999; Tremblay, Wang, Cerovic, 2012). Recently, it has been calibrated for grapevine by Diago et al. (2016) against the leaf chlorophyll content of grapevine leaves assessed with an optical sensor. They showed a high correlation ($R^2 = 0.92$, $p < 0.001$) for the SFR index with the reference method for both the whole leaf (sum of adaxial and abaxial sides of the leaf) and the adaxial side of the leaf. It is a ratio of far-red emission (735 nm) divided by red emission (685 nm) under red excitation or green excitation. Due to the overlap of the Chl absorption and emission spectrum, re-absorption occurs at shorter wavelengths but not at longer wavelengths (Gitelson et al.,

1901999; Pedrós et al., 2010). Therefore, SFR increases with increasing sample Chl
191content.

192The Nitrogen Balance Index (NBI) is related to the N status of the plant and
193proportional to the chlorophyll-to-flavonols ratio proposed by Cartelat et al. (2005). It
194has recently been calibrated for the assessment of the grapevine N content by Rey-
195Caramés (2015). In this study, different ways of calculating the NBI were evaluated for
196the assessment of grapevine N content, among them the formula presented here. This
197particular NBI index showed high correlation with the reference method with a $R^2 =$
1980.90 ($p < 0.001$), proving its capability to estimate the N content of the grapevine.

199Measurements were carried out on three main leaves per vine, between the 8th and the
20010th node, along five dates during the season (17th of August, 2nd of September, 14th of
201September, 5th of October and 11th of October), from veraison until 6 days prior to
202harvest. This period of measurement was selected due to its interest to perform N
203application at veraison that has been proved important to attain a sufficient level of
204yeast assimilable N in grape juice at harvest. It has also been reported that the
205application of N at veraison leads to higher amounts of N in woody stems and leaves
206than the N application at berry set (Porro et al., 2006), also preventing excessive
207vigour, delayed maturity, and adverse changes in fruit properties that have sometimes
208been related with high applications of N earlier in the growing season (Hannam et al.,
2092014; Hannam et al., 2015). During the ripening process, N supply was proved to boost
210the levels of anthocyanins in the skins of Tempranillo berries, leading to increased must
211colour density (Delgado et al., 2004). Furthermore, an appropriate late-season
212grapevine N uptake and reserve accumulation (Bates, Dunst, Joy, 2002) are essential
213for the beginning of the next season since early season N demand cannot be achieved
214by root uptake (Cheng, Xia, 2004).

2152.3 Shoot pruning weight measurements

216The pruning weight of each vine was manually measured for each of the 72 sampling
217points using a hanging scale (20th of November 2011). The shoot pruning weight per

218vine (SPW) was calculated by dividing the pruning weight values per vine measured by
219the number of shoots per vine.

220**2.4 Soil characterization**

221In order to have a wider comprehension of the spatio-temporal variability of N and Chl
222contents, nine soil pits were carried out in different parts of the plot. Each soil profile
223was described and the first 30 cm sampled for the laboratory chemical and physical
224analyses (Klute 1986; Sparks 1996).

225**2.5 Statistical and geostatistical analysis**

226First, potential outliers were identified and removed by using box and whisker plots as
227samples with a value higher than two standard deviations. Once the database was
228refined, the measurements were averaged to obtain one mean value per sampling
229point, which involved a total of three vines. After that, descriptive statistics were
230calculated to have a first view of the temporal variation of each individual variable. In
231addition, the spread of the distributions was calculated as the ratio between the range
232and the median value (Bramley, 2005).

233A geostatistical analysis (Chiles & Delfiner, 2009) of the spatio-temporal variability of
234the experimental variables was performed. Variograms were computed for all the
235experimental variables at each date. The best model for the experimental variograms
236was selected based on a best visual fit for an omnidirectional variogram and taking into
237account the cross-validation results. All fitted variogram models (stationary models) are
238described by three parameters: nugget effect, sill and range. The nugget represents
239the variability at distances smaller than the sample spacing; the sill is the semivariance
240value at which the variogram reaches stationarity; and the range is the distance at
241which the variogram reaches the sill value and corresponds to the maximum
242autocorrelated distance. Based on these variograms and their descriptive parameters
243(nugget, sill and range), the Cambardella index (CmbI) related with data spatial
244structure was computed. The Cambardella index (Cambardella et al., 1994) is a ratio

245between the nugget variance C_0 (random variability) and total variance (C_0+C_1)
 246expressed as a percentage, which provides information on the spatial dependence of
 247the variable defining them as strong (less than 25%), moderate (between 25% and
 24875%) or weak (more than 75%).

$$249C_{mbi} = \left(\frac{C_0}{C_0+C_1} \right) \quad (3)$$

250where C_1 is the sill and C_0 is the nugget effect of the variogram.

251These variograms were also used for applying the spatial interpolation method of
 252ordinary kriging. This method was used to estimate a continuous surface of the indices
 253and the vegetative variable (Z). For every variable, the estimation model was validated
 254applying the cross-validation technique and studying the mean error (ME), the mean
 255square error (MSE) and the mean square standardised error (MSSE), defined as
 256follows:

$$257 \begin{aligned} & \sum_{i=1}^n [k(x_i) - Z(x_i)] \\ ME &= \frac{1}{n} \sum_{i=1}^n \end{aligned} \quad (4)$$

$$258 \begin{aligned} & \sum_{i=1}^n [k(x_i) - Z(x_i)]^2 \\ MSE &= \frac{1}{n} \sum_{i=1}^n \end{aligned} \quad (5)$$

$$259 MSSE = \frac{1}{n} \sum_{i=1}^n [(Z_k(x_i) - Z(x_i)) / \sigma_k]^2 \quad (6)$$

260Where n is the size of the population, $Z_k(x_i)$ corresponds to the ordinary krigged datum
 261of the experimental value ($Z(x_i)$) and σ_k is the standard deviation of the kriging
 262estimation.

Interpolated maps provide a better understanding of the spatial pattern at each date, that is, the values of the studied variables and the way they are distributed along the vineyard. This must not be mistaken with the spatial structure of each variable that is analysed by the variograms, which indicate whether the variable is spatially dependent and how this spatial dependency works.

Maps of the SFR and NBI indices obtained for the 5 dates from veraison to harvest, as well as the map of SPW, were classified in three zones corresponding to low, medium and high values applying an iso-cluster unsupervised classification. Cross tabulation of the classified maps was carried out among the five dates of measurement for each variable and also among SPW and each date of the fluorescence indices, SFR and NBI. Kappa index was applied to measure the temporal stability across the season in the spatial pattern of the same variable and the agreement between shoot pruning weight and each date of SFR and NBI indices by using the equation proposed by Hudson and Ramm (1987) and those values were interpreted following the classification proposed by Landis and Koch (1977). This index is traditionally used in remote sensing studies to assess the land cover changes by measuring the concordance between two classified maps (Chuvieco, 2010). The statistical and spatial analysis were carried out using Microsoft Office Excel 2013 (Microsoft Corporation, Washington, USA), Statistica 9.0 (Stat Soft, Inc., Tulsa, USA) and ArcGIS Desktop 10.3 (ESRI, Redlands, CA, USA).

3. Results

3.1 Spatio-temporal variability of leaf chlorophyll and nitrogen content

The descriptive statistics of the vegetative variables, comprising the SFR index, indicator of the leaf Chl content, the NBI index, indicator of the N content and SPW are summarised in Table 1. Leaf Chl content (expressed by the fluorescence index SFR_{RAD}) showed its maximum on the 2nd of September. Afterwards, its mean values diminished but its spatial dispersion increased, as shown by its CV and spread. On the

other hand, leaf N content (expressed by the fluorescence index NBI_{C2_RAD}) showed its maximum value at veraison (17th of August), and decreased steadily until harvest. However, concerning the spatial variability of **NBI index**, the CV, showed its maximum on the 14th of September, while the spread increased until the 5th of October and stabilised thereafter.

SPW showed higher values of CV and Spread (CV = 26.08%, Spread = 142.17 %) than those observed for **the SFR** (mean CV = 8.91 %; mean Spread = 43.17 %) and **NBI indices** (mean CV = 12.30 %; mean Spread = 61.32 %).

The temporal variation of **the SFR and NBI indices** was illustrated by box plots (Figure 1). As shown previously by the CV and the Spread, **SFR index values** increased until the 2nd of September, when it started to decrease until harvest. On the other hand, the **NBI** steadily diminished from veraison to harvest.

Table 1 also shows the range of the variogram and the Cambardella Index (CmbI) of each variable as a measure of the spatial autocorrelation. The range of the variogram is the measure of the autocorrelation distance of the variable. At distances higher than the range, the variable has no spatial correlation. The **SFR index** showed an increasing range from 85 m to 115 m as harvest was approaching. However, the range for **NBI** was variable and did not display any trend during the season. Its minimum values were found close to harvest (range of 35 m and 30 m on the 5th of October and the 11th of October, respectively), while the highest range for N occurred in the middle of the season (range of 140 m on the 14th of September). The range of the SPW was 115 m, the same as that of **the SFR index** at harvest (Table 1).

The CmbI (C_0/C_0+C) represents the percentage of the non-structured spatial variability. This corresponds to the variability at a scale lower than the sampling grid, with respect to the global variability of the vineyard. The latter corresponds to the variance of the sample, which in theory is the same as the denominator (C_0+C). The **SFR index** fluctuated from strong (CmbI < 25) to moderate (25 < CmbI < 75) spatial correlation (CmbI between 11.76 % and 30 %). As for the **NBI**, the CmbI showed higher values than for

the **SFR index** (CmbI between 23.08 % and 39.29 %), even though they also varied from strong to moderate spatial correlation. On the other hand, SPW showed a moderate spatial correlation (CmbI = 46.76 %).

The spatio-temporal behaviour of the **SFR and NBI indices** was studied by variographic analysis. Experimental variograms were **calculated and fitted** using spherical variogram models (Figure 2). The **SFR index** showed similar values of nugget and range and alike structure along the five dates of measurement (Figure 2A-E). Regarding the **NBI** (Figure 2F-J), the experimental variograms of the 5 dates of measurements showed a different behaviour between dates, but nearly all of them had a short range of 50 m, approximately. The **NBI** measured on the 14th of September showed the best structure of spatial correlation of all measurement dates.

In order to improve the understanding of spatial and temporal dynamics of ChI and N along the vineyard, interpolated surfaces were built for each variable and date. Figure 3 shows the interpolated surfaces of **SFR and NBI indices** at the five measurement dates. The higher values of both variables were obtained at veraison, decreasing until harvest. The maximum values were located in the west part of the plot at veraison and the minimum values started to appear in the centre and East part of the plot as harvest approached. The **NBI** spatial behaviour in the plot showed a large dispersion in accordance with the spread (Table 1) illustrated in the box-plots (Figure 1B). Despite this dispersion, the maps showed a continuous decreasing temporal trend through the ripening season.

Figure 3 also depicts the spatial variability of SPW, which showed its lowest values (37.9 g - 47.3 g) at the centre and south parts of the vineyard, while the highest values appeared mainly at the north part (54.2 g – 68.7 g).

3.2 Intra-seasonal stability of the spatial pattern of leaf chlorophyll and nitrogen contents.

The assessment of the intra-seasonal stability of the spatial pattern of leaf ChI and N contents was carried out by **clustering every variable into three** zones of high, medium

346and low values (figure 4). As the ripening season progressed, the areas with lowest
347and highest values of the SFR index changed. The lowest SFR values were observed
348at the centre-north part of the plot on the 17th of August and 14th of September and at
349the centre-South part of the vineyard on the 2nd of September and 5th of October. Close
350to harvest, the lowest SFR values content areas were identified at the centre and west
351area of the vineyard. With regard to N content, the location and distribution of low and
352high values of NBI also changed across the season. The lowest values started to
353appear at veraison at the upper right part of the plot and, as ripening progressed, their
354distribution changed across the vineyard until nearly encompassing the whole plot at
355harvest.

356Concerning SPW, the lowest values were located at the centre and south area of the
357vineyard, while the highest values were found at the north of the plot and two small
358areas at the east-south and west-south of the vineyard.

359The stability of the spatial patterns of the fluorescence indices indicators of leaf Chl and
360N contents across the ripening season was quantitatively assessed by executing a
361cross-tabulation and the computation of the Kappa index from each variable 3-cluster-
362maps (table 2).

363The Kappa index for the 5 dates of the SFR index yielded values ranging from -0.06 to
3640.50, that is, from poor to moderate agreement. The highest Kappa index value for the
365SFR index (0.50) was yielded between the 2nd of September and the 5th of October,
366while the lowest and the highest values were located at the centre-down and at the
367upper and left areas of the vineyard, respectively (figure 4). Regarding NBI, the Kappa
368index between dates yielded values lower than those for SFR, ranging from poor (-
3690.15) to fair (0.29) agreement. As was also observed for the SFR index, the two dates
370with a better concordance of the NBI spatial pattern were the 2nd of September and the
3715th of October.

372This work also allowed to analyse the concordance between SPW and both
373fluorescence indices for every measurement date (table 2). The values of the Kappa

index varied from poor agreement (-0.06) to fair agreement (0.31). With respect to the correspondence between the clustered maps of SPW and the **SFR index**, the best agreement was found with the **SFR index** measured on the 5th of October (0.22, fair agreement). Slightly better results were observed with the **NBI**. Likewise, two dates of measurement of **NBI**, 2nd of September and 5th of October, revealed fair agreement with the SPW zonification (0.24 and 0.31 respectively).

3.3 Soil characterization

Table 3 shows the values of the physical and chemical properties (texture, soil moisture, organic matter content, soil pH and cation exchange capacity) of the soil profiles from the nine pits carried out in the vineyard. The existence of high variability within the vineyard for all the characteristics studied was evidenced. Both organic matter content and cation exchange capacity were found to be higher in the north part of the vineyard, and denoted a richer soil, which would favour the vegetative development of the grapevines. Regarding the presence of coarse elements, the distribution did not show differences between north and south, but it has shown a longitudinal area in the centre of the plot with values much higher in those pits (D, F and H) than in the others. The sand or the clay fraction evidenced high variability among pits, which indicated a changing soil texture along the vineyard. The same was observed for the pH and the moisture. This variability of soil properties could also influence the variability of the vegetative development of the vines.

4. Discussion

4.1 Spatio-temporal variability of leaf chlorophyll content.

The spatio-temporal dynamics of the **SFR index**, indicator of the leaf Chl content in grapevines, was investigated during the ripening period, from veraison to harvest. While Chl accumulates in the leaves until these are fully expanded, its concentration starts to decrease immediately after reaching its maximum (Kriedemann et al., 1970), leading to senescence and leaf falling (Palliotti, Silvestroni, Petoumenou, 2010). **Chl**

decline occurs mainly during ripening, as evidenced by the interpolated maps at the
 five dates of study, in which a decreasing temporal trend of the SFR index was shown
 across the vineyard. The spatial component of the present work revealed that SFR
 index values reduction was not regular across the vineyard plot. The Kappa index
 indicated that there were also changes in the spatial pattern during ripening.
 The different evolution within the vineyard regarding the SFR index has been
 evidenced, as the SFR values started to decrease at the centre-North of the plot before
 it spread throughout the rest of the vineyard. As the monitored leaves were selected to
 be fully expanded leaves of the mid-upper part of the shoot (nodes 6 to 12), this
 different development may be related with soil features, such as the amount of organic
 matter (Nguyen, Fuentes, Marschner, 2013). The enhancement of soil organic matter
 reduces the risk of Fe chlorosis due to the Fe chelating ability of the humic and fulvic
 substances and the stimulation of soil microbial activities by organic components
 (Tagliavini & Rombolà, 2001). Therefore, a different soil organic matter content through
 the vineyard might lead to different leaf Chl content and evolution among vines. This
 was supported by the different organic matter content showed in the soil profiles, with
 less content of organic matter in the south area than in the north part of the vineyard.
 The topography might also influence the leaf Chl content. In this regard Figure 5 shows
 the different orientations of the vineyard, changing from east to northeast, north and
 northwest. Changes in the total radiation influence leaf Chl content (Bertamini &
 Nedunchezian, 2004), as a higher sunlight exposure might lead to an increase in leaf
 Chl content on a surface basis (Agati et al., 2007), so the different sun exposure of the
 different areas of the vineyard might also influence the leaf Chl content and prompt to a
 spatial variability of the Chl content of the leaves. This changing spatial pattern through
 time has also been found for spectral indices such as NDVI by Hall et al. (2011), who
 showed how the spatial distribution of the NDVI values changed through the season. It
 might also be influenced by the soil and the mesoclimate, as mentioned for the SFR
 index, leading to different spatial development within a vineyard. Moreover, the soil

characteristic that showed the highest contrast among pits was the presence of coarse elements. The area with clearly larger proportion of coarse elements corresponded to a longitudinal band in the centre of the plot, which overall matched the lowest values of Chl through the ripening period. This high concentration of coarse elements results in less water-holding capacity of the soil, favouring episodes of water deficiency, which may have advanced the senescence process in those areas. This agrees with the finding of Van Leeuwen et al. (2004) who reported an early defoliation, after veraison, on gravelly soil due to a lower water-holding capacity.

The variograms of the 5-dates SFR index showed a consistent spatial structure throughout the ripening period. The similarity of the variograms evidenced the proportional effect in the spatio-temporal variation structure of the SFR index. The type of the variogram models and the maximum spatial autocorrelation distance were very similar among dates, but increasing sills, reflected the increasing statistical dispersion of the SFR index as ripening season progressed, as expected in a senescence process. Therefore, it could be concluded that there was only one pattern of the spatio-temporal variability of the SFR index, indicator of the grapevine leaf Chl content in the studied vineyard. As harvest was approaching, a proportional effect of variation due to the variance of each date data dispersion could be observed. It will be interesting, for future studies, to analyse the possibility of building a global prediction model for leaf Chl content any date between these two phenological stages (veraison and harvest). This leaf Chl content model could be useful to optimize the data sampling and also could help to apply selection criteria on the sampling process of future seasons.

14.2 Spatio-temporal variability of the leaf nitrogen content.

NBI values, as indicator of leaf N content steadily diminished from veraison to harvest, in agreement with the results obtained by Prieto et al. (2012) who reported a decrease in the N content in grapevine leaves during ripening. This trend agrees with the relocation of nutrients within the vine plant that starts after flowering (Vivin, Castelana-Estrada, Gaudillere, 2015). The interpolated maps (Figure 3) illustrated the spatial

457 pattern of this negative temporal trend, likewise [SFR index](#). Similarly to [SFR index](#)
 458 spatio-temporal dynamics, there was an asynchronous [trending NBI](#) among vineyard
 459 zones, those located at the centre-North of the vineyard pioneering the decrease of
 460 [NBI values](#). The results yielded by the Kappa index indicated that there was a low
 461 consistency in the [NBI](#) spatial pattern through ripening period. Furthermore, the maps
 462 showed some areas where the values of [NBI](#) did not decrease or even subtly
 463 increased.

464 Unlike [SFR index](#), which had a very similar spatial structure, [the NBI index](#) presented a
 465 heterogeneous [structure](#) among dates, attested by the different structures of the
 466 variograms throughout the ripening period, especially as harvest approached. The
 467 Cambardella Index of the second and forth dates of measurements showed values of
 468 moderate correlation. As this index represents the percentage of the non-structured
 469 spatial variability, that is, the variability at a scale lower than the sampling grid, it
 470 suggested that it would be appropriate to reduce the sampling distance to attain a more
 471 accurate description of the spatial [structure of the NBI index](#). In addition to the effects
 472 of leaf senescence in N content, its different spatial behaviour along time could also be
 473 related to the leaf flavonol content along the vineyard, as this is inversely related with
 474 the N content of the plant (Cartelat et al., 2005). Furthermore, it could be explained by
 475 some events of weak mineralization of soil N in some areas of the vineyard, which
 476 would prevent it from being available for plant nutrition, as postulated by Garcia et al.
 477 (2012), especially in dry and hot seasons. In this regard, the weather conditions during
 478 season 2011 at the experimental vineyard can be described as very dry in comparison
 479 to historical data (total rainfall from July to September was 30.9% lesser on the
 480 average for historical rainfall during this period), accompanied by warm temperatures
 481 (the average for the month mean temperatures exceeded between 1.5 and 3.2 °C the
 482 value of the average for the historical series mean temperatures; data not shown).
 483 Despite the two irrigation events imposed during the season, these weather conditions
 484 may have induced a strong water deficit in specific subzones of the plot, contributing to

the appearance of weak mineralization events. Furthermore, important differences were found in the content of coarse elements among pits. The highest values were located at a longitudinal band in the centre of the plot. They generally were coincident with the areas of lowest N content. As previously mentioned, these areas with coarse texture together with the dry conditions of that season may have led to water deficiency, resulting in restrictions on N uptake (White, et al., 2007). Different values of soil moisture and organic matter content were found in the soils profiles distributed along the vineyard (Table 3). Moreover, the soil analyses have shown a pH higher than 8, which has been related with reduced availability of N (Davidson, 1991). The spatial differences in soil characteristics such as coarse elements, moisture, organic matter content and pH, along with the differences in texture and high temperatures of 2011 season, might explain the diverse spatial pattern found for the NBI index, as these factors are crucial in N mineralization process (Wang et al. 2004). From a practical point of view, it is important to assess the N content through the season within the vineyard, in order to implement the correct fertilization management for each area and diminish the environmental impact of nitrate leaching or volatilization.

4.3 Non-destructive and early assessment of vigour status.

SPW is the most informative indicator of vine vigour but classical manual methods only allow to assess this variable in late autumn or winter (Smart & Robinson, 1991). This prevents its application for canopy management correction until next season. It is usually assessed manually by counting and weighting at winter pruning. Recently, other fast and non-destructive methods such as laser scanning, have been successfully applied to estimate pruning weight (Tagarakis et al., 2013). From a practical point of view, it would be helpful to estimate the SPW not only in a fast and non-destructive way but also at earlier dates to carry out a differentiated and adequate vegetative management in the vineyard. In this regard, the use of vegetation indices to estimate SPW was successfully explored (Dobrowski, Ustin and Wolpert, 2003). However, cross-tabulation analysis and the Kappa index performed in this work indicated that the

NBI index itself could not provide a satisfactory explanation of the spatial variation of shoot pruning weight.

These key parameters could also be used as indicators of different variables related with the vegetative status, yield or the berry composition of the grapevine. For instance, Baluja et al. (2012a) assessed the leaf chlorophyll content through the SFR index and the SPAD finding very similar and significant correlations with vegetative variables such as main shoot length ($r = 0.58$ for SFR and 0.64 for SPAD), with yield ($r = 0.80$ for SFR and 0.77 for SPAD) and even with grape anthocyanin content at harvest ($r = -0.65$ for SFR and -0.65 for SPAD). Garcia et al. (2012) also found a high correlation between the CNN index, a combination of NDVI and NBI, with yield (R^2 of 0.84 in 2011 and 0.75 in 2011) for two years and three different grapevine cultivars.

Nevertheless, characterizing the variability of important parameters of the vineyard vegetative growth, such as leaf Chl and N contents, provides useful information to support decision taking regarding fertilization and canopy management practices, such as defoliation, shoot thinning, hedging and cluster thinning, oriented to improve vine balance and fruit quality for subsequent seasons.

5. Conclusions

The present work focused on the study of the spatio-temporal variability throughout the ripening period of two important parameters for vegetative growth of grapevines: leaf Chl and N contents assessed by the fluorescence indices $SFR_{R_{AD}}$ and $NBI_{C2_{R_{AD}}}$ provided by the hand-held fluorescence sensor Multiplex™.

The existence of spatio-temporal variability of leaf Chl and N contents between veraison and harvest within a vineyard was demonstrated. It is important to note that leaf Chl and N content spatial variability within the vineyard increased as the season advanced, until stabilising prior to harvest. Both, leaf Chl and N content, were found to have slight stability of the spatial pattern through the ripening season. However, in the case of the leaf Chl content, the variographic analysis showed a similar spatial

structure along the ripening period, which provides the opportunity of developing a spatio-temporal model for the leaf Chl content from veraison to harvest in future works. Furthermore, the results suggested that the leaf N content alone could not be used, as a suitable indicator to describe the shoot pruning weight variability within the plot and therefore it could be useful to delineate vigour and vegetative growth management zones within the vineyard before the data could be assessed by direct and destructive measurements.

Overall, the assessment of the spatio-temporal variability of key vegetative components such as leaf Chl and N contents is of great importance to carry out a well-founded and differentiated vegetative management of the vineyard in the frame of precision viticulture.

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735 Figures

736 Figure 1 – Temporal variation of leaf chlorophyll and nitrogen content.

737 Temporal variation of leaf Chl content –SFR_{AD}- (A) and N content –NBI_{C2}-R_{AD}- (B)

738 measured along five dates from veraison to harvest, for all the 72 sampling points in

739the vineyard. Black dots represent the mean values, the boxes represent the standard
740deviation for each date, and whiskers represent twice the standard deviation.

741**Figure 2 – Variographic analysis of leaf chlorophyll and nitrogen content.**

742Experimental variograms of the 5 dates for leaf Chl content, SFR_{RAD} (A-E) and N
743content, $NBI_{C2_{RAD}}$ (G-K) fitted to spherical models (solid red line).

744**Figure 3 – Interpolated surfaces of leaf chlorophyll and nitrogen contents from
745veraison to harvest and shoot pruning weight.**

746Leaf Chl content (SFR_{RAD}) kriged surfaces, leaf N content ($NBI_{C2_{RAD}}$) kriged surfaces
747and shoot pruning weight (SPW) kriged surface obtained from the 72 sampling points
748in a Tempranillo vineyard from veraison to harvest. Maps were represented by terciles.

749**Figure 4 – Clustering maps of leaf chlorophyll and nitrogen content and shoot
750pruning weight.**

751Maps of the 3-zones clusters of leaf Chl content (SFR_{RAD}), N content ($NBI_{C2_{RAD}}$) and
752shoot pruning weight.

753**Figure 5 – Map of the aspect and solar exposure of the vineyard.**

754Map describing changes in aspect and solar exposure of the studied vineyard. It was
755elaborated from the digital elevation model with 5 m resolution of the Spanish National
756Center of Geographic Information (CNIG).

17 August

2 September

14 September

5 October

11 October

17 August

2 September

14 September

5 October

11 October

**Leaf chlorophyll
content ($\text{SFR}_{\text{R}_{\text{AD}}}$)**

1.21 - 1.60

1.61 - 1.80

1.81 - 2.16

17 August

2 September

14 September

5 October

11 October

**Leaf nitrogen
content ($\text{NBI}_{\text{C2}_{\text{R}_{\text{AD}}}}$)**

1.13 - 1.43

1.44 - 1.55

1.56 - 1.97

**Shoot pruning
weight (g)**

37.86 - 47.31

47.32 - 54.22

54.23 - 68.65

0 25 50 100 150 200 m

Leaf chlorophyll content (SFR_{AD})

17th August

2nd September

14th September

5th October

11th October

Leaf nitrogen content (NBI_{C2-AD})

17th August

2nd September

14th September

5th October

11th October

Shoot pruning weight

Tables

Table 1 – Descriptive and spatial statistics of fluorescence-based indices of leaf chlorophyll and nitrogen contents and shoot pruning weight.

Descriptive and spatial statistics for fluorescence-based indices of leaf chlorophyll content (SFR_R_{AD}), leaf nitrogen content (NBI_{C2}_R_{AD}) and shoot pruning weight (SPW) in Tempranillo grapevine leaves at five dates from veraison to harvest.

Variable	Date	Mean	Standard deviation	Coefficient of variation (%)	Spread (%)	Range (m)	Cambardella Index
Leaf chlorophyll content (SFR_R_{AD})	17 August	1.84	0.117	6.39	24.10	85	25.64
	2 September	1.90	0.121	6.39	32.66	90	11.76
	14 September	1.70	0.137	8.10	38.73	95	20.00
	5 October	1.54	0.183	11.87	57.70	95	30.00
	11 October	1.49	0.176	11.78	62.66	115	22.73
Leaf nitrogen content (NBI_{C2}_R_{AD})	17 August	1.61	0.18	11.14	52.23	65	27.03
	2 September	1.55	0.16	10.08	53.11	45	25.81
	14 September	1.48	0.22	14.87	62.06	140	23.08
	5 October	1.46	0.19	13.07	71.79	35	39.29
	11 October	1.42	0.17	12.32	67.97	30	24.32
Shoot pruning weight (g)	20 November	51.44	13.42	26.08	142.17	115	46.76

_AD: Spectral indices denoted with this subscript were determined on the adaxial sides of the leaves.

Table 2 – Kappa index

Cross-tabulation outputs and the Kappa index obtained among the 5-dates-classified maps of leaf chlorophyll content, expressed by the fluorescence index (SFR_R_{AD}), the 5-dates-classified maps of leaf nitrogen content, expressed by the fluorescence nitrogen balance index (NBI_{C2}_R_{AD}) and between shoot pruning weight with leaf chlorophyll content (SFR_R_{AD}) and leaf nitrogen content (NBI_{C2}_R_{AD}) at 5 dates from veraison to harvest in a Tempranillo (*Vitis vinifera* L.) vineyard.

		Leaf chlorophyll content (SFR-R _{AD})					Leaf nitrogen content (NBI _{C2} -R _{AD})				
		17 Aug.	2 Sep.	14 Sep.	5 Oct.	11 Oct.	17 Aug.	2 Sep.	14 Sep.	5 Oct.	11 Oct.
Leaf chlorophyll content (SFR-R _{AD})	17 Aug.	1									
	2 Sep.	0.36	1								
	14 Sep.	-0.02	-0.06	1							
	5 Oct.	0.24	0.50	-0.05	1						
	11 Oct.	0.31	0.11	0.42	0.15	1					
Leaf nitrogen content (NBI _{C2} -R _{AD})	17 Aug.						1				
	2 Sep.						0.18	1			
	14 Sep.						0.02	-0.15	1		
	5 Oct.						0.27	0.29	0.03	1	
	11 Oct.						0.25	0.12	0.18	0.16	1
Shoot pruning weight		-0.04	0.15	0.15	0.22	-0.06	-0.02	0.24	0.06	0.31	0.09

_AD: Spectral indices denoted with this subscript were determined on the adaxial sides of the leaves.

Table 3. Soil profiles.

Description of chemical and physical characteristics of the soil profiles and map of the spatial distribution of these profiles through a Tempranillo (*Vitis vinifera* L.) vineyard.

Soil profiles spatial distribution	Soil characteristics	A	B	C	D	E	F	G	H	I
Texture classification	Clay loam	Loam	Loam	Loam	Loam	Loam	Loam	Loam	Loam
	Field capacity moisture (%)	22.7	22.7	20.3	18.8	21.8	19.4	19.3	18.1	21.4
	Coarse elements > 2 mm (g/100g)	0.0	6.0	16.7	41.74	0.0	50.5	4.3	24.76	5.4
	Sand fraction 2.00-0.05mm (g/100g)	36.6	35.7	42.5	48.8	37.8	46.7	46.5	47.1	38.7
	Silt fraction 0.05-0.002mm (g/100g)	36.6	38.0	34.5	29.8	37.4	31.0	31.8	34.1	37.1
	Clay fraction <0.002mm (g/100g)	26.8	26.3	23.0	21.4	24.8	22.3	21.7	18.8	24.3
	Organic matter content (%)	1.89	1.99	2.05	2.03	1.56	1.65	1.53	1.27	1.58
	pH	8.3	8.2	8.2	8.21	8.3	8.4	8.3	8.46	8.4
	Cation exchange capacity (Cmol (+)/Kg)	14.15	14.20	13.10	16.83	12.67	11.94	11.47	13.76	12.51