SENSITIVITY OF VIS-NIR SPECTRAL INDICES TO DETECT NITROGEN DEFICIENCY AND CANOPY FUNCTION IN CV. BARBERA (*VITIS VINIFERA* L.) GRAPEVINES

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Abstract:
Context and purpose of the study - Precision nutrient management in viticulture can be addressed on the basis of a spatial characterization of within-vineyard vine nutritional status derived from proximal or remote spectral observations. However, a key challenge is the discrimination between mineral deficiencies and water stress related issues, often coexisting under low vigor conditions. In addition several mineral disorders are associated to a decrease in chlorophyll concentration in leaves resulting in a wide array of symptoms classified as chlorosis. Despite clearly associated to their origin, visible symptoms appear too late for supporting an efficient mineral management; thus, non-destructive early detection of either asymptomatic excess or deficient status become a challenging task of precision viticulture. This work evaluates the Vis-NIR reflectance spectra and the sensitivity of the derived-spectral indices to detect nitrogen deficiency in grapevines.
Material and methods - Well N-fertilized vs. unfertilized vines were compared over two seasons (2016 and 2017) on *Vitis vinifera* L. cv. Barbera potted vines. For each treatment, 24 leaves from eight representative vines were tagged in order to collect, at different phenological stages, contact Vis-NIR spectra and perform physiological measurements. The performance of several spectral vegetation indices sensitive to different biophysical (i.e. chlorophyll and carotenoids content, leaf area index) and physiological parameters (light use efficiency) was measured by means of a sensitivity (signal to noise ratio) analysis. Leaf greenness index was monitored with a handheld chlorophyll meter SPAD 502 whilst single-leaf gas exchanges were assessed by using a handheld analyzer. Multispectral analysis was associated to a rigorous ground-truthing as it concerns shoot growth, yield, fruit composition and pruning weight.
Results – In both years the differential fertilization increased leaf N concentration of N+ vines at veraison. Vine performance varied according to plant vigor and nutritional status. N+ increased canopy growth, vine productivity, and bunch compactness whilst N0 enhanced the proportion of shot berries and reduced titratable acidity and malate in juice. N deficiency resulted in lower SPAD readings and assimilation rates as compared to well N-fertilized vines. N0 vs N+ contact Vis-NIR spectra differed in Green and Red-edge regions with faster responses on basal leaves. Data were associated to a different sensitivity of Vis-NIR spectral indices specially when based on the Red-edge bands showing higher efficiency in detecting leaf N concentration since early stages of canopy growth.

Keywords: Mineral nutrition, Visual symptoms, Leaf age, Assimilation, Yield components, Phenotyping.

1. Introduction

Nitrogen is a key element affecting canopy growth, yield, fruit formation, and fruit composition (Keller, 2005). In the search for more efficient use of fertilizers, several methods have been developed in the past to assess plant nutritional status and to design suitable fertilization strategies (Bavaresco et al., 2010). However, addressing the parcel as a crop unit, these approaches do not consider within-field variability of soil properties and the consequent heterogeneous vigour, resulting in differential vine requirements. Since the end of the past century, precision viticulture has been trying to down-scale crop performance description and site-specific vineyard management (Bramley, 2010). Optical remote sensing (RS) provides multispectral characterization of grapevine properties at different spatial, spectral and temporal resolution. Most recently, a wide array of proximity sensors (PS), each based on different technologies, is allowing researchers to overcome typical limitations associated to RS in terms of field of view, canopy geometry and shading (Matese and Di Gennaro, 2015). With these tools, canopy reflectance readings are combined into vegetation indices for describing canopy properties. Specifically, this is done in the case of NDVI, the most widely used index combining reflectance in red and NIR bands for providing reliable estimation of plant vigour. However, little information is available about RS/PS methodologies, therefore allowing the discrimination between mineral deficiencies associated to a decrease in leaf chlorophyll concentration (Taskos et al., 2015). Hyperspectral assessment of leaf reflectance in vines subjected to different N-fertilisation is a preliminary approach to non-destructive early detection of either asymptomatic excess or deficiency. The study aims to describe the Vis-NIR reflectance spectra and the sensitivity of the derived-spectral indices to detect nitrogen deficiency in grapevines.

2. Material and methods

Plant material – The trial was carried out at the Department of Sustainable Crop Production of the Università Cattolica del Sacro Cuore (45°02N, 9°43E, 54 m asl). A batch of 25 three-year old cv. Barbera vines grafted onto 110 Richter grown outside in 14L pots filled with loamy soil was used. Plants were head-trained and two 2-node spurs were kept at winter pruning. After budburst (BBCH 15), vines were thinned to four main shoots whilst the crop load was standardized to 4 bunches per vine by removing all distal inflorescences (BBCH 57). Vines were drip irrigated to pot capacity. N-fertilized (N+) vs. unfertilized (N0) vines were compared over three seasons (2016-2018). Phosphorous and potassium were provided to both N+ and N0 vines by using a 0-52-34 (N:P:K) fertilizer at the rate of 0.5 g per application. In parallel, differential fertilization was performed in N+ plants by using ammonium nitrate (34%) at the dose of 0.5 g per application. Fertilization was carried out manually by dissolving fertilizers in 1L of water per vine and by watering the top of the pot with the solution. Applications were performed three times per week between inflorescence swelling (BBCH 55) and beginning of ripening (BBCH 81) on DOY 144, DOY 118, and DOY 127 in 2016, 2017 and 2018, respectively.

Plant measurements – Measurements were performed on 8 vines per treatment randomly selected at the beginning of the trial in spring 2016; two shoots per vine (S1 and S2) were selected each season and three leaves were tagged on S1 to represent basal (4-5th node), median (8-9th node), and apical (11-12 node) shoot zones. On each leaf, the Greenness Index was determined by using a portable meter SPAD-502 (Konica Minolta, Osaka, Japan) and assimilation rates were assessed with the portable gas-exchange analyzer LCi-SD (ADC BioScientific Ltd, Hoddesdon, UK). All readings were taken in the morning hours (10:00–13:00) under clear sky and saturating light conditions. For each trial year, monitoring of the above-mentioned parameters started prior to fertilizations until pre-veraison. In 2016 and 2017, physiological measurements were associated to Vis-NIR spectra recorded on the same leaves with a spectroradiometer equipped with a contact probe (ASD FieldSpec® HandHeld 2 VNIR Spectroradiometer). Single-wavelength reflectance rates were measured and several vegetation indices calculated. Narrowband indices were derived from signals at 1nm spectral resolution whilst broadband indices reproduced the spectral configuration of Sentinel-2 satellite imagery (European Space Agency). Each year, between berry touch and beginning of ripening, basal, median, and apical leaves from the same vines used for physiological assessment were sampled and petioles removed. Leaf blades were washed in distilled water, dried in a forced air oven at 65 °C, ground and sent to an external laboratory for mineral ion analysis. At harvest occurring on DOY 256, 242, and 234 in 2016, 2017, and 2018, respectively, bunches from tagged shoots were individually picked, immediately weighed and taken to the laboratory. The remaining bunches were harvested together and their total number and mass recorded as well. Bunches from S1 and S2 were then destemmed, berries were counted and classified as a function of their size (normal-sized, chicken and shot berries). Accordingly, bunch compactness was evaluated as bunch mass-to-rachis length and the number of chicken berries was also recorded. A sub-sample of 10 intact berries per bunch was taken and stored at -18°C for subsequent determination of the berry mass and individual berry organs (skin, flesh and seeds). A second sub-sample of 50 healthy berries was stored at -18°C and then used for the determination of anthocyanins and total phenolic concentration according to Iland et al. (2011). Remaining berries were crushed and the juice immediately processed to determine TSS using a temperature compensated refractometer whilst pH and titratable acidity (TA) were measured by titration with 0.1 N NaOH to a pH 8.2 end -point and expressed as g/L of tartrate equivalents. The concentration of tartaric and malic acids was determined by HPLC. At the end of the season, immediately after leaf fall, the pruning weight of the one-year wood was measured by separating main and lateral canes, and the yield-to-pruning weight ratio calculated.

Statistical analysis – Plant growth, yield components, nutritional status, and fruit composition data were subjected to a two-way analysis of variance (ANOVA) assuming N-supply as main factor (N+ vs N0) and year as a random factor. Treatments were compared by Student-Newman-Keuls test at p=0.05. Treatment x Year interaction was partitioned only in case of F test significance, and mean values compared by standard error. Seasonal variation of Greenness Index and assimilation rates was displayed on line graphics with X-axis representing day of year (DOY); mean values were compared with standard error. Spectral data, narrow and broad-band vegetation indices depending on N-supply were compared by *t*-test at p=0.05.

3. Results and discussion

3.1. *Vegetative growth, yield components, and fruit composition*
Treatments induced significant variations in plant growth, yield components and fruit composition confirming the vital role of N on grapevine metabolism (Table 1). Data pooled over 3 years show that N+ increased N-concentration in leaf blades at veraison as compared to N0 (2.21 vs 1.60%). Values were within the intervals previously reported for other varieties (Bavaresco *et al.*, 2010); however, if leaf nitrogen in N+ was almost unchanged from year 1 to year 3, a drop (1.42%) was recorded in N0 during the driest and hottest 2017. Based on lower N availability, N0 limited plant growth and yield expression. Pruning weight was 26% lower in N0 than N+ (197 vs 267g/vine) whereas yield per vine dropped to 291 g under N-deficiency, i.e. 55% less than N+. The Ravaz Index was generally low as compared to literature; however, N-deficiency limited yield more than proportionally than vegetative growth leading to very low yield-to-pruning weight ratio (1.55 kg/kg). Bunches from N0 were significantly smaller than N+ (72 vs 155 g) with smaller berries (1.64 vs 2.08 g) and a higher proportion of shot berries (22.7 vs 8.4%) confirming the crucial role of N availability in driving several physiological processes such as inflorescence development and blooming (May, 2004). As a consequence, N-supply affected the fruit architecture, resulting in more compact bunches as compared to N0 (19.1 vs 9.8 g/cm, respectively). Moreover, the absolute skin weight was unchanged between treatments and the relative skin weight increased in N0 (8.97%) as compared to N+ (7.39%) confirming that N deficiency can modify the fruit-zone microclimate, resulting in a more open canopy with higher penetration of sunlight radiation (Poni et al., 2018). N-deficiency improved fruit ripening by varying both technological parameters and phenolic composition (Table 2). Must TSS were higher in N0 (25.8 Brix), whereas titrable acidity was the lowest (7.7 g/L). N-supply affected the ratio between tartaric and malic acids; accordingly, the former was higher under N-deficiency whilst N increased malate concentration. As expected, fruits from N0 vines were richer in both anthocyanins and total phenolics.

3.2. *Physiological assessment*
In 2016, treatments showed similar SPAD rates immediately after N application (Figure 1); significant differences occurred first for distal and median leaves (15d after the first application) followed by basal positions (28d later). Differences between treatments widened throughout the season and N0 leaves became pale green at pre-veraison with SPAD values ranging between 31 and 35. On the contrary, N+ leaves were darker irrespective to their position along the shoot, with SPAD values around 42 units on DOY 199. Similarly to the first season, in 2017 differences were initially more evident on median and apical positions, even if differences occurred faster than in 2016. In 2018, before N application, N0 leaves were already paler than N+ and the difference was maintained until the end of the season irrespective to their position. Results confirm that differential fertilization modified the plant N status, increasing greenness index and potentially affecting the spectral properties of the canopy (Hatfield et al. 2008). As a matter of fact, it is well known that a large amount of leaf N is allocated to Chl molecules (Evans 1989). Despite the marked drop in leaf Chl content observed in N0 vines as estimated by greenness index, plants subjected to N-deficiency showed a moderate decrease in assimilation rates that were slightly lower than well fertilized vines in both 2016 and 2017 (Figure 2); in 2018 treatments showed similar leaf photosynthetic capacity (Figure 2). Despite the positive relationship existing between N in xylem and leaf blades and photosynthetic rates (Evans, 1989; Poni et al., 1994a), it is also well known that under N-deficiency, leaves decrease their chlorophyll content to avoid oxidative stress induced by a more pronounced drop in rubisco activity as compared to the decrease in the electron transport capacity (Keller, 2005). Nevertheless, over the three years, our results show a long-term photosynthetic compensation confirming a weak relationship between Chl content and assimilation (Hunter and Visser, 1989, Poni et al. 1994a). In effect, photosynthesis increases according to leaf

development until 5-6 weeks after leaf unfolding and gradually declines towards senescence (Poni et al., 1994b). In addition, differences in leaf assimilation rates between our treatments could be smoothed because plants tend to translocate leaf N according to sunlight exposure in order to maximize their whole-canopy photosynthesis (Gastal and Lamaire, 2002). However, the importance of adequate N supply in vineyard is still crucial and our results (Tab. 1 and Fig. 2) confirm that N-deficiency impacts, with increasing magnitude, the following processes: photosynthesis, growth (-26%), and yield formation (-55%).

3.3. Hyperspectral assessment and vegetation indices

Leaf reflectance of vines subjected to different N-status varied along the season according to leaf position. In 2016, treatments differed on basal leaves in the green and red bands from DOY 180, whilst variations in the *red-edge* occurred earlier on DOY 172 (data not shown). Similarly, median and apical leaves differed on green, red and *red-edge* domains even if median leaves showed faster responses in green and *red-edge* from DOY 159 corresponding to 15 days after first N-application (Figure 3A). Under N-deficiency, basal leaves showed increased reflectance at 543 nm (green) over time, ranging between 0.098 and 0.154 on DOY 145 and 209, respectively; overall reflectance for N+ leaves was 0.104 (Figure 4). This pattern indicating higher absorption with higher N was consistent over leaf positions and seasons showing similar responses to hyperspectral characterisation of cotton leaves subjected to different N-supply (Muharam et al., 2018). Data acquired in 2017 confirmed major sensitivity of already mentioned wavelengths even if, according to SPAD readings (Figure 1), treatments were already different at the first acquisition campaign on DOY 129 (Figures 3B).

In 2016, broadband vegetation indices differed among treatments since DOY 159 (Figure 5) (15d after the first application) with differences occurring first on median leaves, followed by distal and basal positions. According to SPAD readings, median leaves were more reactive to differential fertilization. However, only *red-edge* based indices varied on DOY 159, whilst other indices showed lower sensitivity to N-deficiency allowing treatment differentiation from DOY 172. In 2017, all the considered vegetation indices were already different on DOY 129 before N-application, meaning that a carry-over effect occurred on season 2 resulting in paler leaves from early developmental stages of N0 vines (data not presented). Results allow classifying *red-edge* based vegetation indices such as RENDVI, OSAVI*re*, MTCI*re*, and CI*re* as the most reactive indicators of leaf N concentration. Contrariwise, SR and NDVI did not show sensitivity to leaf chlorophyll and N variations.

4. Conclusions

N-deficiency lowered leaf chlorophyll concentration in N0 vines. However, a physiological compensation was shown, resulting in a less than proportional decrease in assimilation rates and very similar leaf assimilation rates during the third year of the trial. Nonetheless, N-deficiency dramatically influenced yield components whilst vegetative growth of Barbera vines was slightly affected. The hyperspectral characterization of the leaf nutritional status showed increased reflectance in green and *red-edge* bands under N-deficiency, while *red-edge* based vegetation indices (CI*re*, RENDVI, OSAVI*re*, MTCI*re*) showed the higher efficiency in detecting leaf N concentration since early stages of canopy growth. Conversely, Vis-NIR indices such as GNDVI, GCI and CVI had a delayed response, while SR and NDVI did not show sensitivity to leaf chlorophyll and N variations.

5. Acknowledgments

This study was funded by Emilia Romagna Region (project Nutrivigna) within the POR-FESR 2014-2020 program.

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Table 1: Variation of leaf N content at veraison, pruning weight and yield components of Barbera vines subjected to different N-supply. (Data 2016-2018).

	N (%)	Pruning wt. (g/vine)	Yield (g/vine)	Ravaz Index	Bunch wt. (g)	Berry wt. (g)	Relative skin wt. (%)	Bunch compactness (g/cm)	Chicken berries (%)
N+	2.21 a	267 a	651 a	2.80 a	155 a	2.08 a	7.39 b	19.1 a	8.4 b
N0	1.60 b	197 b	291 b	1.55 b	72 b	1.64 b	8.97 a	9.8 b	22.7 a
Treatment (T)	**	**	**	**	**	**	**	**	**
T x Y	*	**	**	**	*	*ns*	*ns*	*ns*	*ns*

*,**, ns: Significant at p ≤ 0.05, 0.01, or not significant, respectively. Within columns, values followed by different letters are different at SNK test (p=0.05)

Table 2: Fruit composition at harvest of Barbera vines subjected to different N-supply. (Data 2016-2018).

	TSS (°Brix)	pH	TA (g/L)	Tartrate (g/L)	Malate (g/L)	Anthocyanins (mg/g)	Phenolics (mg/g)
N+	23.6 b	3.42	11.8 a	7.57 b	5.89 a	0.58 b	1.87 b
N0	25.8 a	3.39	7.7 b	8.58 a	2.65 b	1.05 a	2.94 a
Treatment (T)	**	*ns*	**	*	**	**	**
T x Y	**	*ns*	*ns*	**	**	**	*ns*

*,**, ns: Significant at p ≤ 0.05, 0.01, or not significant, respectively. Within columns, values followed by different letters are different at SNK test (p=0.05)

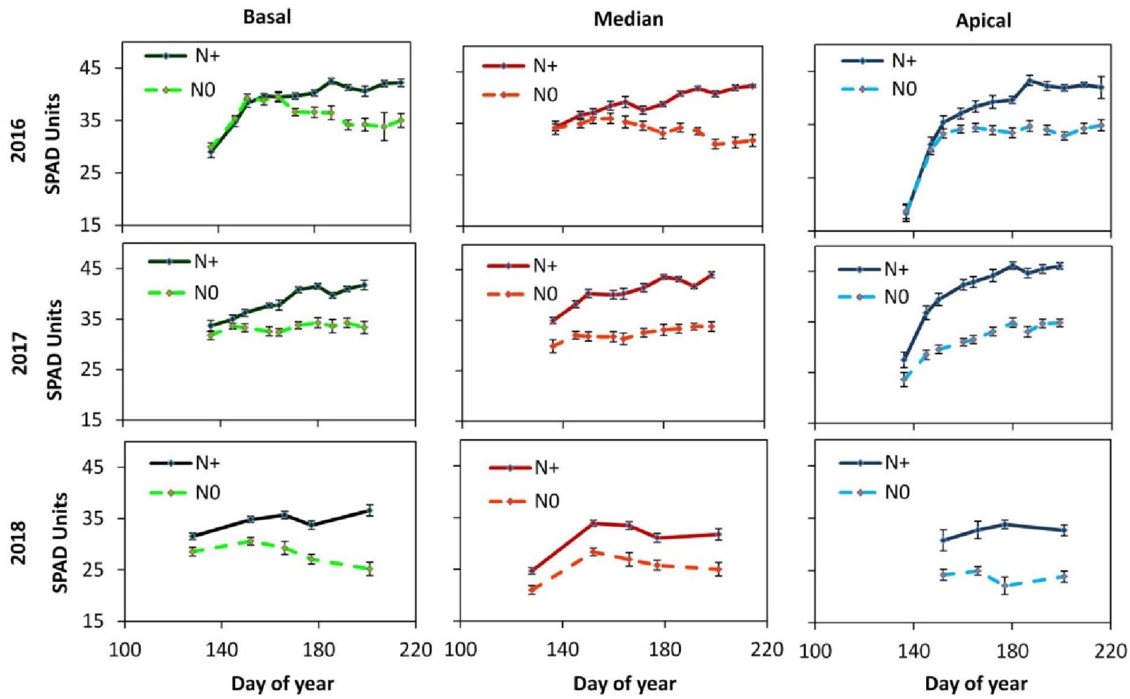

Figure 1: Seasonal variation of the leaf Greenness Index (SPAD Units) of Barbera vines subjected to different N-supply. For each season of the 3-year trial (2016-2018), data are presented as a function of leaf position (Basal, Median, and Apical). Per each point, mean values are compared with standard error (n = 8). N-supply started on DOY 144, DOY 118, and DOY 127 in 2016, 2007 and 2018, respectively.

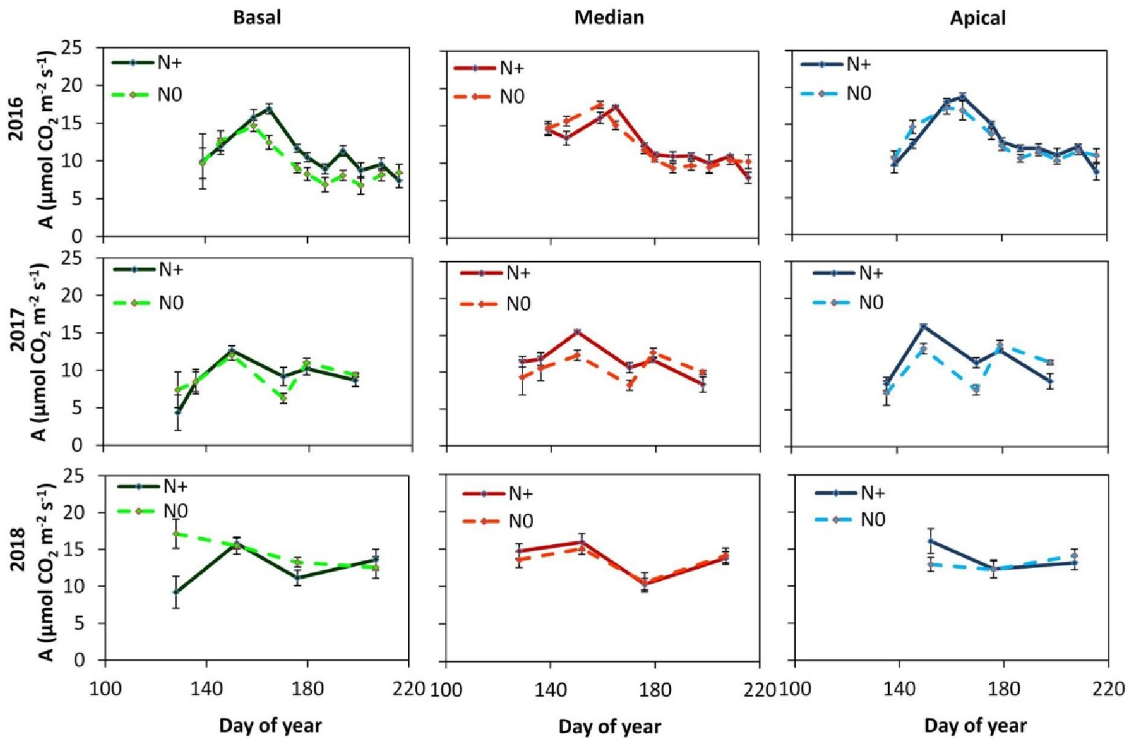

Figure 2: Seasonal variation of the leaf assimilation rates of Barbera vines subjected to different N-supply. For each season of the 3-year trial (2016-2018), data are presented as a function of leaf position (Basal, Median, and Apical). Per each point, mean values are compared with standard error (n = 8). N-supply started on DOY 144, DOY 118, and DOY 127 in 2016, 2007 and 2018, respectively.

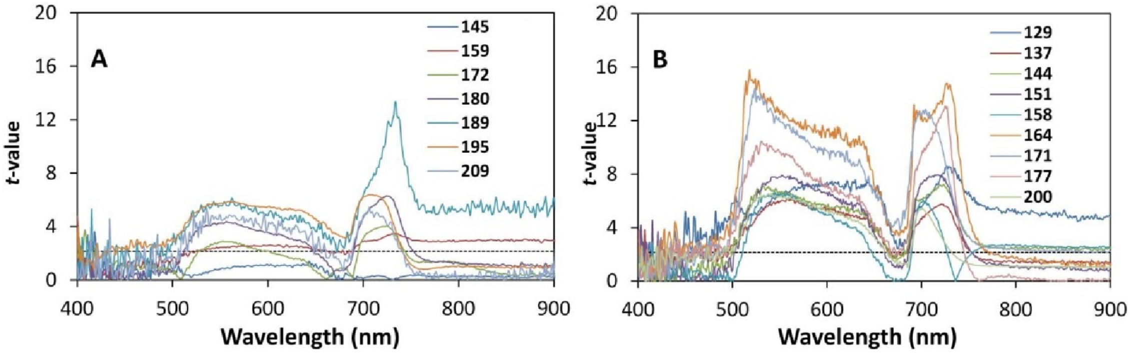

Figure 3: Seasonal variation of the *t*-values resulting from the comparison of the median-leaf reflectance rates assumed by N+ vs N0 vines in the Vis-NIR electromagnetic spectrum in 2016 **(A)** and 2017 **(B)**. Dotted line corresponding to critical value at p = 0.05 (n = 8); coloured lines corresponding to DOY (day of year).

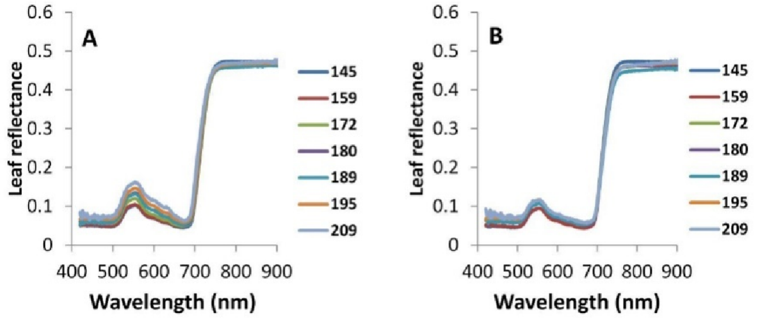

Figure 4: Seasonal variation of Vis-NIR leaf reflectance assessed on basal nodes of N0 **(A)** and N+ **(B)** vines in 2016. Coloured lines corresponding to DOY (day of year).

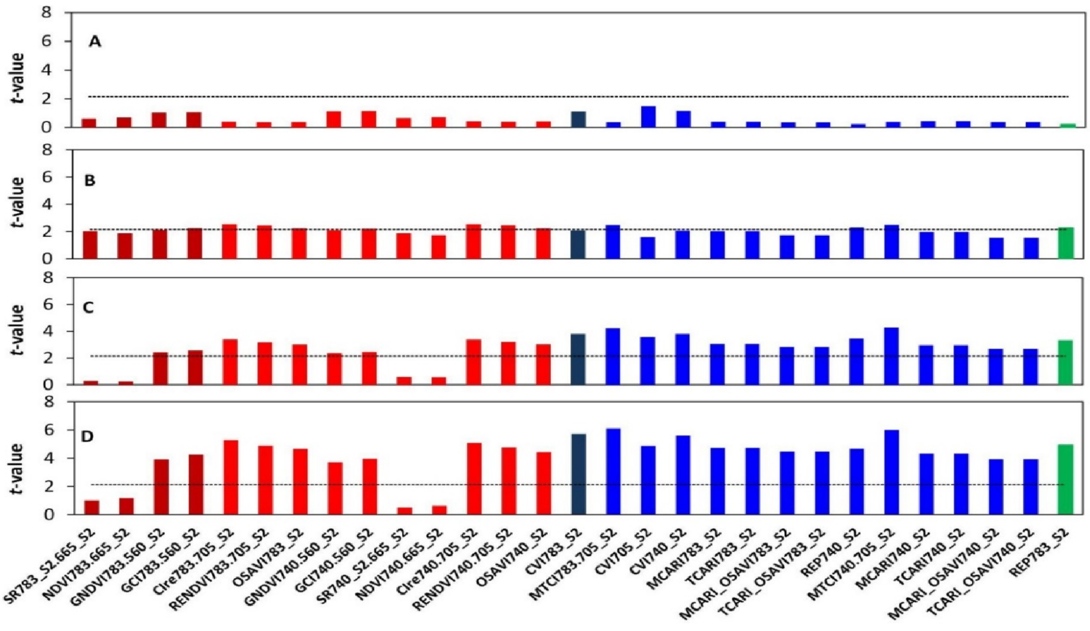

Figure 5:*t*-values resulting from the comparison of the broadband vegetation indices of median leaves depending on different N-supply (N+ vs N0) in 2016. Data referred to different stress conditions: (A) DOY 145 = before fertilization; (B) DOY 159; (C) DOY 172; (D) DOY 180 = maximum stress. Dotted line corresponding to critical value at p = 0.05 (n = 8).