

# APPLICATION OF NON-NEGATIVE MATRIX FACTORIZATION FOR STUDYING SHORT-TERM PHYSIOLOGICAL CHANGES IN GRAPEVINE FROM CANOPY HYPERSPECTRAL REFLECTION

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## Abstract

We have applied non-negative matrix factorization to a database of leaf hyperspectral reflectance and DMSO chlorophyll extract absorption measurements from grapevine canopies at seven vineyards in Dalmatia, Croatia. Extracted spectral signatures show various monthly changes in grapevine production of chlorophyll. Our signatures represent maximal absorption values within a specific colour spectrum - blue, red, near infrared, green and yellow to orange. Association of signatures and chlorophyll indices vary through time. Signature S4 is the best chlorophyll proxy. Here we show that the same amount of chlorophyll can be produced by plants using multiple internal processes or absorption of different spectrums of light. Changes in these processes can be better understood by studying extracted reflection signatures instead of just chlorophyll concentration or vegetation indices. This study shows that non-negative matrix factorization applied to hyperspectral reflectance measurements can be a powerful tool in monitoring the short-term changes in physiology of plants thus could be applied in precision viticulture. We also tested this model on canopy hyperspectral reflectance measured at 0.5 m distance from the canopy. This study shows that NMF can be a powerful tool in monitoring the seasonal grapevine changes in physiology of plants thus could be applied in precision viticulture.

**Keywords:** non-negative matrix factorization, grapevine, physiology, chlorophyll, viticulture, vegetation indices, light absorption, short-term changes

## Introduction

Conversion of light energy into chemical energy in plants is a function of their pigment content (Gitelson *et al.* 2006). Foliar chlorophyll content is a key factor

affecting the performance of plant photosynthesis. Chlorophylls, Chl *a* and Chl *b*, facilitate the conversion so their content defines the photosynthetic functioning (Barry *et al.* 2009) and primary productivity of the plant (Danks *et al.* 1984; Lieth & Whittaker 1975). Evaluation of plant pigments using traditional field sampling methods is destructive, expensive and time consuming. This triggered the development of different tools and algorithms for rapid and non-destructive description of plant traits. Hyperspectral canopy reflectance is one of such new and promising tools being developed. The most popular type of algorithm in use is the one combining visible and near-infrared reflectance defining spectral vegetation indices. A model for calculation of pigment content from remotely sensed reflectance was developed and is in use as a non-destructive tool for estimating chlorophyll (Gitelson *et al.* 2003).

Instead of calculating chlorophyll density from canopy reflectance by calculating vegetation indices, we studied leaf reflections and absorptions of chlorophyll extracts, hypothesizing that this approach

might provide more reliable and diverse information about the physiology of the plant during its seasonal growth. Our hypothesis was that we can calculate chlorophyll from absorption of different colours not only one or two.

In this study we compared several spectral indices with our results: photoreflectance index (PRI) calculated according to Gamon *et al.* (1992), modified normalized difference vegetation index (mNDVI) calculated according to Sims and Gamon (2002), infrared percentage vegetation index (IPVI) formulated by Crippen (1990), ratio vegetation index (RVI; Pearson & Miller 1972), normalized difference vegetation index (NDVI) originating from Buschmann (1993) and difference vegetation index (Rouse *et al.* 1974).

We have applied non-negative matrix factorization (in further text NMF; Lee and Seung, 1999) to a database of leaf hyperspectral reflection measurements and DMSO chlorophyll extract absorption measurements. NMF has proven to be an important tool in human cancer research (Alexandrov *et al.* 2013) that deals with a huge amount of complex data.

Formulas for computing vegetation indices suffer from major disadvantages of not capturing chlorophyll patterns over time and focusing on single values of reflection curves. Hyperspectral reflectance data contain hundreds or thousands of contiguous spectral wavebands and complex information (Gómez-Casero *et al.* 2010). Reflectance patterns are inversely proportional to absorption curves. Therefore, we can obtain absorption patterns from reflection curves by applying NMF. Our idea was to factorize reflection curves in order to develop new signals (later in text signatures). These new signatures were then characterized with absorption curves and different vegetation indices. Signatures are thus extrapolated to captured absorption patterns. As plants seasonally exhibit different absorption preferences, using single reflection values for estimating pigment density will not be accurate as if we would take into consideration the entire reflection patterns. Moreover chlorophyll production is dependent on absorption of specific light spectrums which change during the seasons (Campbell 2019).

## Materials and Methods

### *Experimental sites*

Field measurements were conducted on seven vineyards on Pelješac peninsula and Konavle region in Dalmatia, Croatia (Table 1). The vineyards were selected as being economically important for the region and situated on different soil substrates characteristic of the region. Grapevine varieties on the Pelješac peninsula are dominated by Plavac mali (genetically close to popular Zinfandel; in translation Plavac = blue, mali = little), species well adapted to prolonged droughts and high mid-day temperatures. On the island of Mljet the old and almost forgotten autochthonous variety Mrkuša is being reintroduced in wine production. Similarly in Konavle (Dubrovnik) the old variety Dubrovačka malvasija considered autochthonous but DNA profiling confirmed it is the same variety as Malvasia di Lipari, Malvasia di Sardegna and Greco Bianco di Gerace, Italy, Malvasía de Sitges, Spain, Malvasia Cândida, Madeira - Crespan, (Crespan *et al.* 2006; Lopes *et al.* 2006) was recently reintroduced in commercial production in this region.

Dingač, the region on the southern exposures of Pelješac peninsula is most probably the most popular grapevine region in the country. Its main characteristic is steep vineyard hill (~45° inclination) facing direct south rising straight from the Adriatic Sea. It is important to note that the soil substrate in old Dingač vineyards is carbonate breccia, the remaining material from melted glaciers dating approximately 13,000 - 15,000 years before present. This material is semi porous rock, not fully solidified, and Plavac mali can protrude its roots up to 7-8 m in depth through this material, where accumulated water with nutrients can sustain the vines during prolonged droughts

which can last up to six months. New vineyards in this region are made by grinding the rocks to produce similar granulometry as ancient breccias and form such substrate up to 1 m thick. It is becoming more popular that the owners apply drop to drop irrigation to these vineyards to abate prolonged droughts. Another important characteristic of this region are barren limestone rock forming tops of the hills which heat significantly in July and August. During the days with almost no detectable wind, the hot air from the tops rises and “pumps” the cool air from the sea surface upwards through the vineyards thus cooling the vines during the hot summer. Wines are full bodied, characterized by high % alcohol by volume, bold tannins, cooked dark fruit flavors, stony minerality and medium to lower acidity.

Positions in the Pelješac field (Kuna, Pijavičino, Potojme and Prizdrina) occupying the central part of the peninsula are characterized by thick carbonate soil enriched with Mg as surrounded by patches of dolomites in the rock base. Niko Meštrović’s vineyard in Kuna was selected as being solely on dolomite white sand.

The two vineyards on the island of Mljet are characteristic as hosting an autochthonous rare variety Mrkuša. Both are located in karst fields with thick carbonate soil substrate. The Pomjenta field is located near the sea water lake (Big Lake, National Park Mljet) open to the south, while Ivanje Polje is completely surrounded by hills, also known as being cooler and more moist than any other position on the island. Both are located in National Park Mljet under the umbrella of legislations preventing the use of any synthetic chemicals. In both locations pesticides have never been used. The Konavle field is a completely different case. Soils are alluvial deposits with high content of clay and high moisture content.

### *Experimental design*

Data acquisition. Air and soil temperature were measured by Omega microprocessor thermometer model HH21 using type T thermocouple (Omega Engineering, Inc., Stamford, CT). Air temperature was measured in the shade of the vine canopy and soil temperature was measured next to the plant stem with a thermocouple placed 15 cm vertically in the soil. Temperature was recorded after the readings stabilized (3-5 min).

Apogee SP-200 hyperspectral radiometer (measuring range 300-850 nm, sensitivity 0,5 nm) was used for all reflectance and absorption measurements. Measurements were conducted during the development of the plant and the grapes from May – August 2020 approximately at mid-day to minimize potential effects of light intensity on chloroplast activity. Leaves with distinctly different intensity of green colour were measured and sampled from randomly selected vine canopy at each vineyard and each month during the investigated period. A leaf disk was extracted from the exact same location as the reflectance measurement using Apogee reflectance wand. Leaf disks were immediately extracted using a 16 mm diameter custom made borer with an area of 63.6 mm<sup>2</sup> to replicate the area measured by the reflectance probe and placed in a vial containing 10 mL of DMSO (Parry *et al.* 2014). Vials were incubated in an oven at 65°C for 35 min. After incubation, a 3 mL aliquot was transferred to an optical-grade 10 mm analysis cell to measure light absorbance spectra using Apogee SP-200 spectroradiometer. Chlorophyll *a* and *b* (µmol m<sup>-2</sup>) concentrations were determined from spectral measurements using the equations developed by Wellburn (1994) for DMSO and for 0.1–0.5 nm spectral resolution.

We combined all reflectance measurements in a single data matrix. This matrix contains “information” about leaf reflection curves of all vineyards in a time period from May to August. This matrix was factorized to capture dominant signals, also called signatures (Fig. 1). We used Non-Negative Matrix Factorization (NMF) implementation in R programming language (Gaujoux &

Seoighe 2010). When characterizing what each signal represents, the enrichment of each signature per each field and each month was estimated. Then chlorophyll concentration was calculated for each vineyard. The entire experimental procedure is described in Fig. 2.

## Results

Reflectance and absorption measurements have shown opposite patterns, depicting the characteristic reflected green spectrum and absorbed blue, red and near-infrared (NIR) spectrums (Fig. 3)

During the investigated period (May-August) air temperature did not vary significantly between vineyards, however the soil temperature significantly varied between different positions (Fig. 4). TBPO vineyard is the warmest, while MH and PC vineyards are the coolest as related to both air and soil temperature. Low values of PAR in May are associated with dominantly cloudy weather at the end of May. Variations of Chlorophyll *a*, *b* and total Chlorophyll in different vineyards are shown in Fig. 5.

Initially we wanted to see how our chlorophyll measurements relate with NDVI and mNDVI indices, the popular chlorophyll proxies. The results indicate that both indices work according to the expected extent, however they do not capture chlorophyll patterns over time (Fig. 6).

Observing these data in more detail in different months, it is clear that chlorophyll is not always efficiently captured by NDVI and mNDVI (Fig. 6). Correlation is not statistically significant because we do not have enough information. The correlations would be better if we had a higher number of measurements. Even though, this is indicative that chlorophyll production depends on time (season) and light absorption characteristics. Therefore the association of chlorophyll and absorption patterns is not as simple as using a single formula for NDVI or mNDVI. For this purpose we developed a method which will map or estimate reflection curve to absorption pattern therefore estimating the chlorophyll concentration with higher precision. Our expectation is that we can delineate background processes involved in the process of photosynthesis, e.g. temperature of soil and air, air pressure, solar irradiation and maybe others.

NMF is a pattern extraction method but also dimensionality reduction method, both dependent on the amount of data (Alexandrov & Stratton 2014). During factorization, loss of information is inevitable, and it depends on the data therefore we compared residuals on observed data and randomized data. When comparing residuals for observed data as a function of *k* (number of signatures), residuals will decrease as *k* increases. The slope of change will depend on the dataset. Comparing both curves and finding the place they cross we can pinpoint a region which indicates optimal *k* (Fig. 7), where we do not lose a lot of information during the factorization and avoid modelling noise (Brunet *et al.* 2004).

Therefore, our *W* matrix consisted of five signatures where signature S1 represents green and intermediate NIR colours, signature S2 anti-green, yellow, red and low NIR, signature S3 blue and intermediate NIR, signature S4 low green and high NIR, and signature S5 green, intermediate NIR which is similar to S1 but slightly moved to the right (Fig. 8). Every signature represents patterns of reflection which are associated with different colour intensities.

The analysis of signatures and their association with different vegetation indices and our measurements of DMSO extracted chlorophyll (chl\_1 - group of younger leaves, chl\_2 - group of older leaves) shows that signature S1 is associated with DVI and PRI. Signature S2 has a small association to Chlorophyll *b*, signature S3 is associated with DVI and Chlorophyll *a*, signature S4 is associated with all of popular chlorophyll indices and also with chlorophyll itself, and signature S5 is associated

with PRI and Chlorophyll *b* (Fig. 9).

Signatures S1 and S2 can also be described as having “anticorrelation” with NDVI, indicating that the plant is in some way not healthy, not producing chlorophyll. Signature S4 is the best chlorophyll proxy. Signature S5 indicates that a portion of the near green spectrum is also reflected. It has more pronounced association with Chlorophyll *b* and higher PRI, depicting the background processes involved in chlorophyll production.

Association of signatures and chlorophyll indices vary through time, and signature S5 is consistently associated with PRI and captures the changes in mNDVI over time (Fig. 10). In May and June the correlation with mNDVI is negative, in July almost neutral and in August positive indicating that the change in chlorophyll production happened between June and July. Signature S4 is highly correlated with IPVI and mNDVI indices, also indicating strong positive correlation with PRI in August (Fig. 10).

Our signatures represent maximal absorption values within a specific colour spectrum - blue, red, near infrared, green and yellow to orange (Fig. 11). This heat map indicates which colour spectrum is absorbed the most by and associated with which signature. Signature S1 has small ambiguous associations and strong negative association to red colour. Signature S2 has small ambiguous associations. Signature S3 has small association with absorption of red and blue spectra. Signature S4 is associated with absorption of red and blue mostly. Signature S5 is associated with absorption of NIR.

If we compare our signatures to absorption patterns, they will indicate maximal absorption in a specific colour spectrum. Signature S4 seems to be a good estimate of chlorophyll. For example, if we look at signature S4 and the absorption pattern of one leaf measured in PC vineyard, as months pass, both signature S4 and red colour absorption peaks decrease (Fig. 12a) therefore, signature S4 is a good estimate of chlorophyll and/or mapping variability of red colour absorption over time.

If we use PM vineyard as an example and observe enrichment towards signature S5 as months pass by we can observe that enrichment pattern follows absorption of NIR spectrum (Fig. 12b).

Our results indicate that signature S5 is a good estimate of photochemical reflectance. Meaning that if PRI goes up, absorption should go down. If we observe signature S5 and absorption pattern of one leaf from MVDI vineyard as an example, we can see they are inversely correlated (Fig. 12c).

This is how we get extra information from one single measurement. The H matrix depicts enrichment of signatures for every observation (Fig. 13). Numbers above each group of bars represent chlorophyll content in  $\mu\text{mol}/\text{m}^2$ . The total sum of signature values at each vineyard and month is approximately 1. It is therefore possible to interpret the enrichment of signatures in percentages, too. This figure shows significant variability of signature enrichment in vineyards over time. Some sort of physiological change in all vineyards can be attributed to the time between June and July measurements. The TBPO vineyard has a unique pattern in May and June as compared to all others. Significant changes in July and August can be depicted especially in MH, PC and PM locations. Changes in signal enrichments in July and August are visible at other locations as well, however not in such significance. It is important to note here that each signature approximates different colour patterns, how the plants live and produce chlorophyll and modify reflection patterns and thereby how they produce the fruit.

Looking at the leaf chlorophyll concentration values shown in Fig. 13 we can see that there are quite a few similar numbers, however the enrichment towards signatures is very different. This indicates that the same amount of chlorophyll can be produced using multiple internal processes or absorption of different spectrums of light. Using TBPO leaf-1 as an example, we can see that chlorophyll absorption is quite similar for May and June (or July and August) however enrichment

towards signatures is rather different (Fig. 13). On the other hand, using DOL leaf-2 as an example we can see that signature enrichment does not vary that much. Using MH Leaf-1 as an example, we observed that chlorophyll values are similar for all four months however signature enrichment distribution varies a lot. As signature 4 decreases over time, signature 5 increases indicating that chlorophyll absorption switches dependency from red colour spectrum to near infrared (NIR) spectrum. We cannot get this type of precision from individual vegetation indices. For example NDVI cannot simply estimate chlorophyll with green colour reflectance. Knowing all extra information from signature characteristics we can estimate to which spectrum the chlorophyll production depends for a specific month.

We also applied this model to extract signatures from hyperspectral reflection measurements of grapevine canopies, measured from 0.5 m distance at an angle of  $\sim 45^\circ$  in relation to the incoming solar irradiation (Fig. 14). The principle behind NMF is to factorize an input matrix into two matrices, recovering non-linear patterns in the data. In our case W matrix represented the newly discovered signal (signatures) and H represented enrichment of each signature towards each observation (Lee & Seung 1999). Hence, fitting the model was done using Majorize-Minimize algorithm where W matrix was fixed and H matrix was iteratively modified until the residual sum of squares (RSS) has converged (Lee & Seung 2001). Our goal was to compare these measurements to reflectance measurements of individual leaves. Correlations between canopy and leaf reflectance signature enrichments is shown in Fig. 15. High values of Signature S2 in May and June (Fig. 14) indicate signatures originating from reflectance of the soil below the canopy, during the period when the canopies were small, not fully formed.

Can we use signatures to predict chlorophyll? The answer is yes. Since signatures reflect chlorophyll in five dimensions, it allows us to capture chlorophyll dynamics with more certainty. We experimented with different models but since the sample size we had was relatively low, we applied both linear and polynomial models. Observing  $R^2$ , residuals and addressing bias-variance trade off we decided to use a simpler model which does not use all of the signature enrichments. We randomly split the data in training and test subsets, using 80% and 20% of the total data, respectively. Using signatures and month with linear model we achieved  $R^2$  around 0.70. Using signatures S2, S4, S5 and month, with the same linear model we achieved  $R^2$  around 0.65. We know that signatures S4 and S5 are good proxies for chlorophyll and signature S2 is a good proxy for chlorophyll depletion. Therefore, we only need those parameters to achieve almost the same accuracy but the model is more robust (Fig. 16).

## Discussion

Grapes (*Vitis* spp.) are economically the most important fruit species not just in Croatia but in the whole world. They have been studied to great extent to better understand physiological, biochemical, and molecular aspects of grape berry maturation and the quality of wine (Conde *et al.* 2007). Besides regulating photosynthesis and growth of grapevine, sunlight provides radiant energy which heats plant surfaces. Fruit composition and development is influenced by both the direct and the indirect effects of sunlight exposure, primarily in terms of light quantity and quality and temperature. The influence of sunlight on grape berry composition and wine quality has been well documented during the past few decades (Reynolds *et al.* 1986; Dokoozlian & Kliewer 1996; Bergqvist *et al.* 2001). Additionally to sunlight irradiation and temperature, water availability is a crucial factor for vine growth and grape berry development (Delrot *et al.* 2001). Net assimilation by leaves decreases as water potential decreases (Chaves 1991; Lawlor 2002) as a consequence of stomatal closure which reduces the availability of  $\text{CO}_2$  in the leaf mesophyll (Chaves 1991; Flexas *et al.* 2002). As these factors change over time on small and large scales (diurnal and seasonal changes),

the time component must be also taken seriously into account when studying physiology of plants. For example, calculation of vegetation indexes such as NDVI and mNDVI does not take time into account.

Our goal was to focus on studying NMF signature enrichment over time rather than focusing only on chlorophyll content and vegetation indices, as these signatures incorporate these parameters plus many others. As depicted in Fig. 13, there is significant variability in NMF signatures over time and in respect of location. Signature enrichments indicate that MVPO and MVDI vineyards are the most stable investigated locations. The vines are mature and vineyards are maintained with a lot of care. Even though the MVDI vineyard receives the most of the sunlight irradiation due to its geomorphological characteristics, the constant breeze/wind from the sea maintains the air and soil temperature of not overheating as is the case with TBPO location which has almost the same orientation and slope inclination as MVDI. However it lacks the cool breeze/wind from the sea. TBPO signature enrichment indicate stressful growth in May-June with very pronounced enrichment in signature S4 and completely lacking signatures S2 and S3, characteristic only to this location. This most probably has to be also associated with the young age of the vines, planted in 2016. This makes it the youngest vineyard we investigated. Plants are obviously lacking resistance to environmental stress. All vineyards have increasing enrichment of signature S4 during the growth period indicating increasing chlorophyll density. Contrary to all other vineyards, TBPO has significant enrichment in June. Being a good chlorophyll density proxy, enrichment of signature S4 further indicates that grapevine canopy chlorophyll density is a governing factor of the photosynthetic capacity of plants (Taiz & Zeiger 2006; Ling *et al.* 2011).

Palliotti *et al.* (2009) showed that high levels of photosynthetic pigments might enhance light absorption thus increasing maximum quantum yield of photosystem II. We can see this in the behaviour of signature S5 through time, which exhibits general decrease therefore indicating increasing absorption of green and near infrared spectrums (Table 2).

Signatures S1 and S2 are “anti-signatures” of signatures S4 and S5 therefore should be indicators of the plant's struggle to resist stress. MH vineyard has the lowest enrichment in August as compared to other locations. This location is also the “coldest” vineyard so August temperatures plus the lack of water are not pronounced as in other locations. The peak of signature S4 actually indicates that the plants are doing well. Increased signature S1 and S2 enrichment in PC and PM vineyards can be associated with senescence of the plants in August, which can be confirmed with increasing numbers of yellow-green, yellow and yellow-brown leaves occurring throughout the vineyards, but not in others.

Signature S3 shows general increase over time in all vineyards with exceptions of MH and PC vineyards in which the enrichment oscillates which could possibly be associated with the thickness and composition of canopy leaves. At this point we do not know what are the causes of these oscillations. As this signature represents adsorption of a portion of red and blue spectrums it could indicate that the plants hypothetically slightly shift absorption wavelength preference within these two spectrums which is then reflected in signatures.

If we observe the correlation between individual leaf and canopy signature enrichment shown in Fig. 15, it does not show what might be described as expected. Canopy reflectance measurements also capture reflectance from the soil substrate. This implies that the canopy reflectance input should be somehow normalized so green colour and soil colour do not have such high importance. At this moment we do not have the solution to this problem.

This study shows that NMF can be a powerful tool in monitoring the seasonal grapevine changes in physiology of plants thus could be applied in precision viticulture.

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## APPENDICES: TABLES AND FIGURES

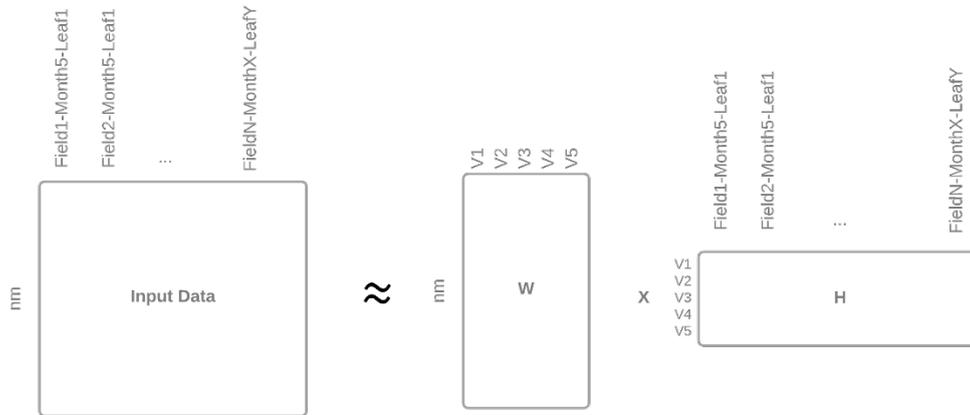
**Table 1.** Vineyards where field measurements were conducted in Dalmatia, Croatia.

Vineyard	Code	Grapevine varieties	Position
Niko Meštrović, Kuna Pelješac	DOL	Plavac mali	42°57'39" N 17°20'20" E
Mateo Vicelić, Potomje Pelješac	MVPO	Plavac mali	42°57'10" N 17°21'29" E
Mateo Vicelić, Dingač Pelješac	MVDI	Plavac mali	42°55'01" N 17°22'36" E
Mario Bartulović, Prizdrina Pelješac	TBPO	Plavac mali	42°57'17" N 17°19'59" E
Anka Hajdić, Pomjenta Mljet	PM	Mrkuša	42°46'35" N 17°21'54" E
Mario Hazdovac, Ivanje Polje Mljet	MH	Mrkuša	42°46'08" N 17°26'49" E
Petar Crvik, Konavosko polje Komaji	PC	Dubrovačka malvasija	42°32'47" N 18°18'57" E

**Table 2.** Summary of signature characteristics. Numbers in parentheses indicate correlation coefficients

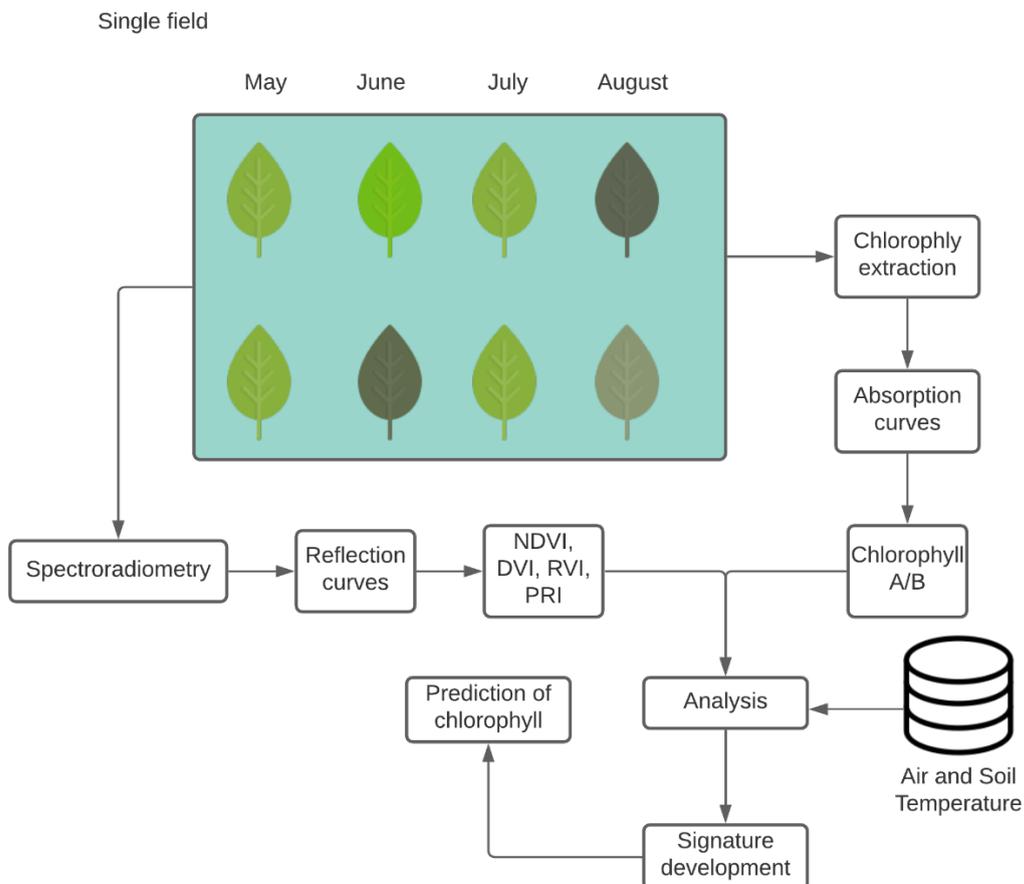
	<b>Absorption</b>	<b>Reflection Patterns</b>	<b>Chlorophyll indices</b>	<b>Chlorophyll</b>
S1	Red (-0.71), Blue (-0.63), Yellow-Orange (-0.29)	No Blue, High Green, Intermediate Yellow-Orange, Low NIR, No Red	PRI (-0.71), mNDVI (-0.78), IPVI (-0.59), NDVI (-0.59)	A (-0.30) B (-0.18)
S2	Red (-0.4) Blue (-0.45) Yellow-Orange (-0.26), Green (-0.29)	High Blue, No green, High Yellow-Orange, High Red, Intermediate NIR	NDVI (-0.95) IPVI (-0.95), mNDVI (-0.74), RVI (-0.65)	A (-0.28) B (-0.12)
S3	NIR (-0.22)	High Blue, Low Green, Low Yellow-Orange, Low Red, Intermediate NIR	DVI (0.37), RVI (-0.41)	A (0) B (-0.14)
S4	Red (0.49), Blue (0.55), Green (0.23), Yellow-Orange (0.22)	No Blue, Low Green, No Yellow-Orange, No Red, High NIR	RVI (0.69), mNDVI (0.86) NDVI (0.85) IPVI (0.85)  DVI (0.61), PRI (0.08)	A (0.25) B (-0.03)
S5	NIR (0.30) Yellow-Orange (0.1)	Intermediate Blue, High Green, Low Yellow-Orange, No Red, Intermediate NIR	RVI (-0.29), mNDVI (-0.26), DVI (0.15), PRI (0.64)	A (0.06) B (0.31)

Figure 1.



Non-negative factorization matrix where  $W$  = extracted signals (signatures), and  $H$  = Signature enrichment in single observation.

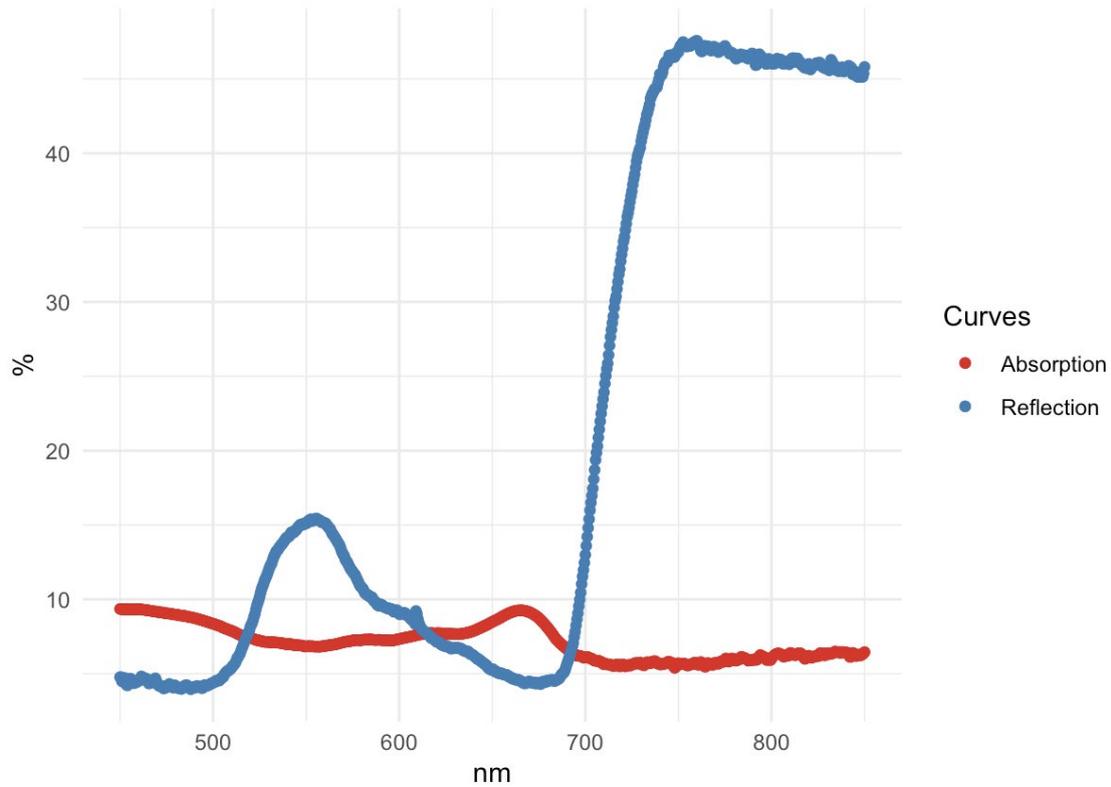
Figure 2.



Flow diagram of actions in data acquisition and analysis.

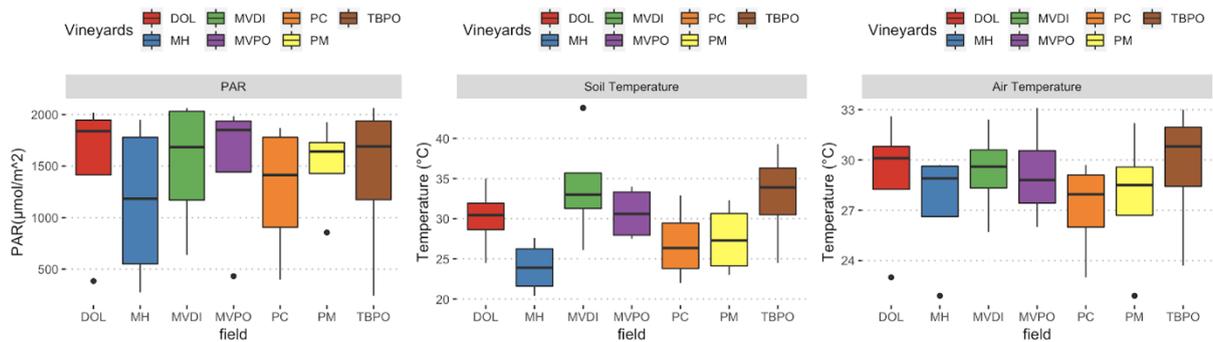


Figure 3.



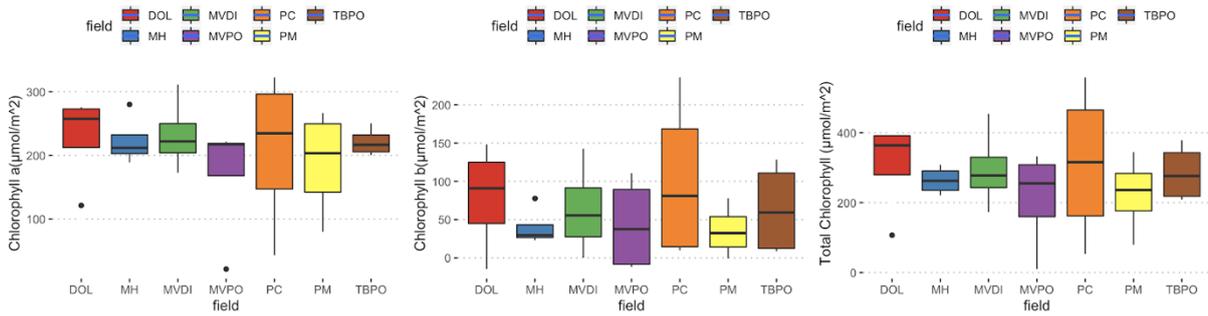
Example of reflectance and absorbance curves showing opposite patterns. Reflection curves nicely indicate that green spectrum is reflected, and absorbance curves show the patterns of blue and red spectrums absorbed by the plant.

Figure 4.



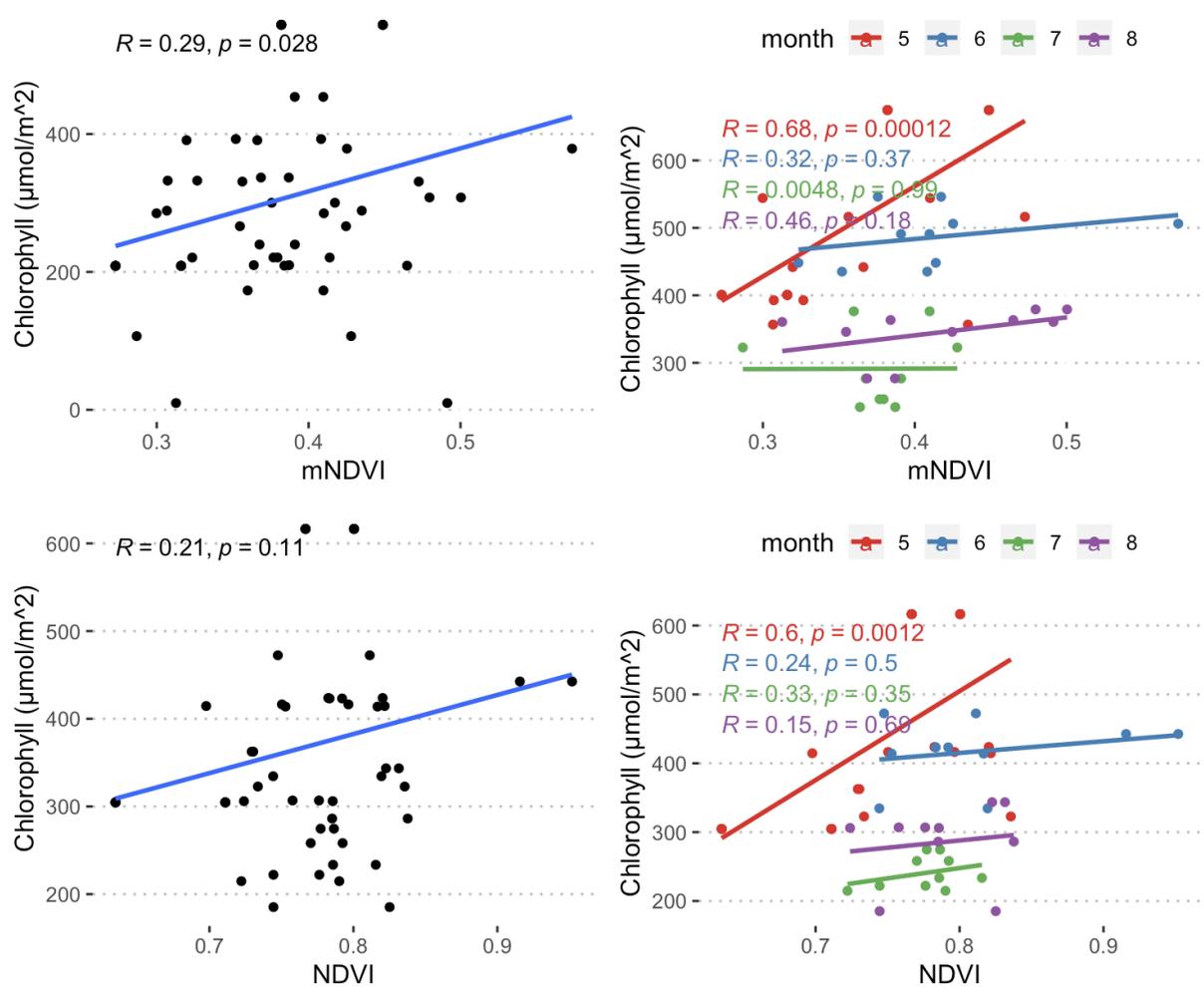
Variation of air temperature, soil temperature and photosynthetic active radiation (PAR) between different vineyards during the investigated period.

Figure 5.



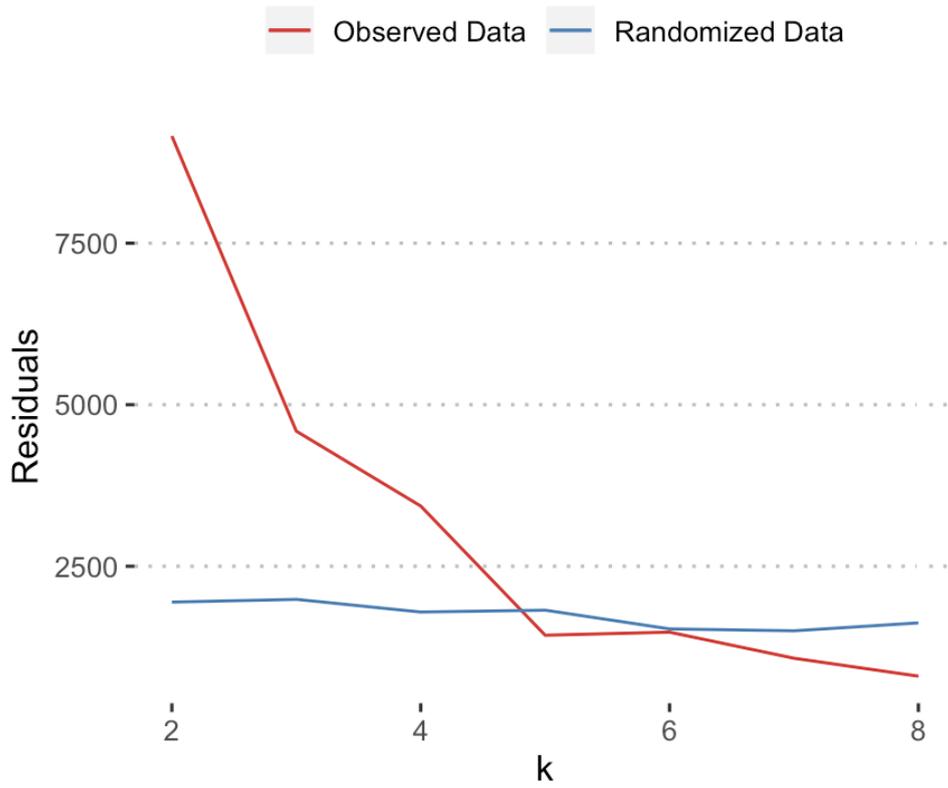
Variations of Chlorophyll a, b and total Chlorophyll in different vineyards.

Figure 6.



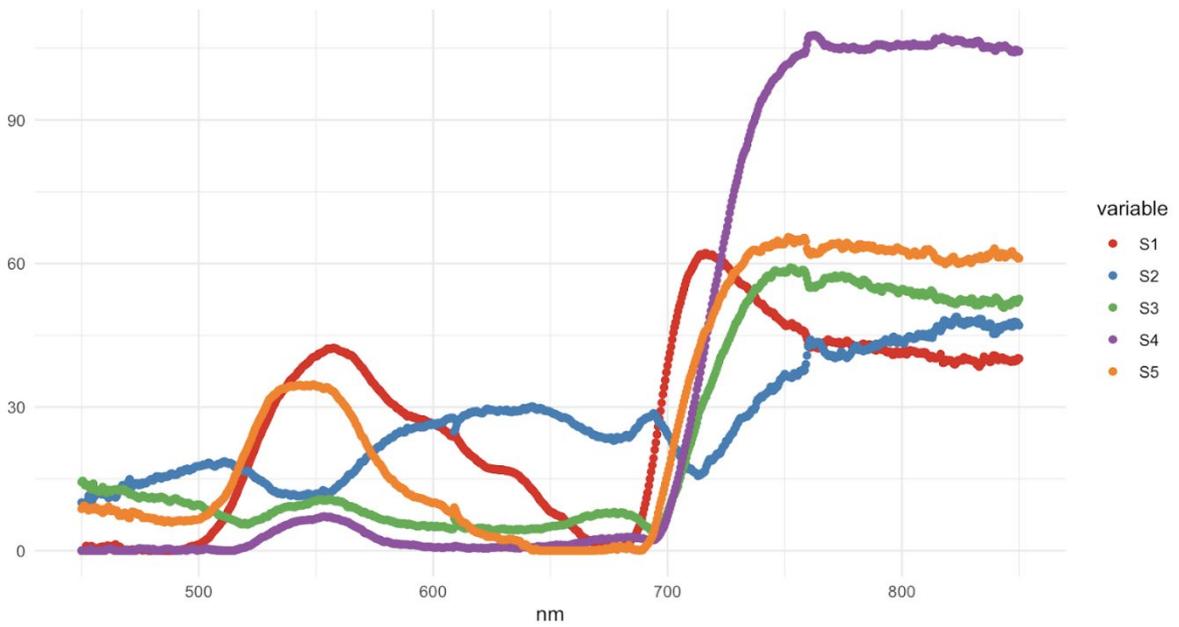
NDVI and mNDVI association with Chlorophyll.

Figure 7.



Comparison of residuals with observed data and randomized data.

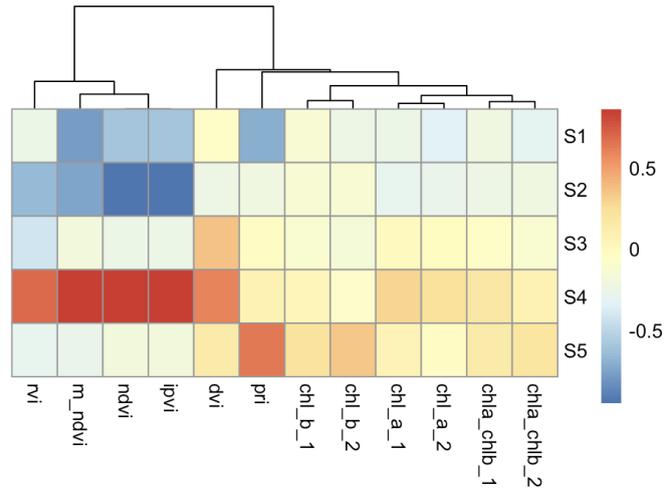
Figure 8.



Representation of reflection signatures.

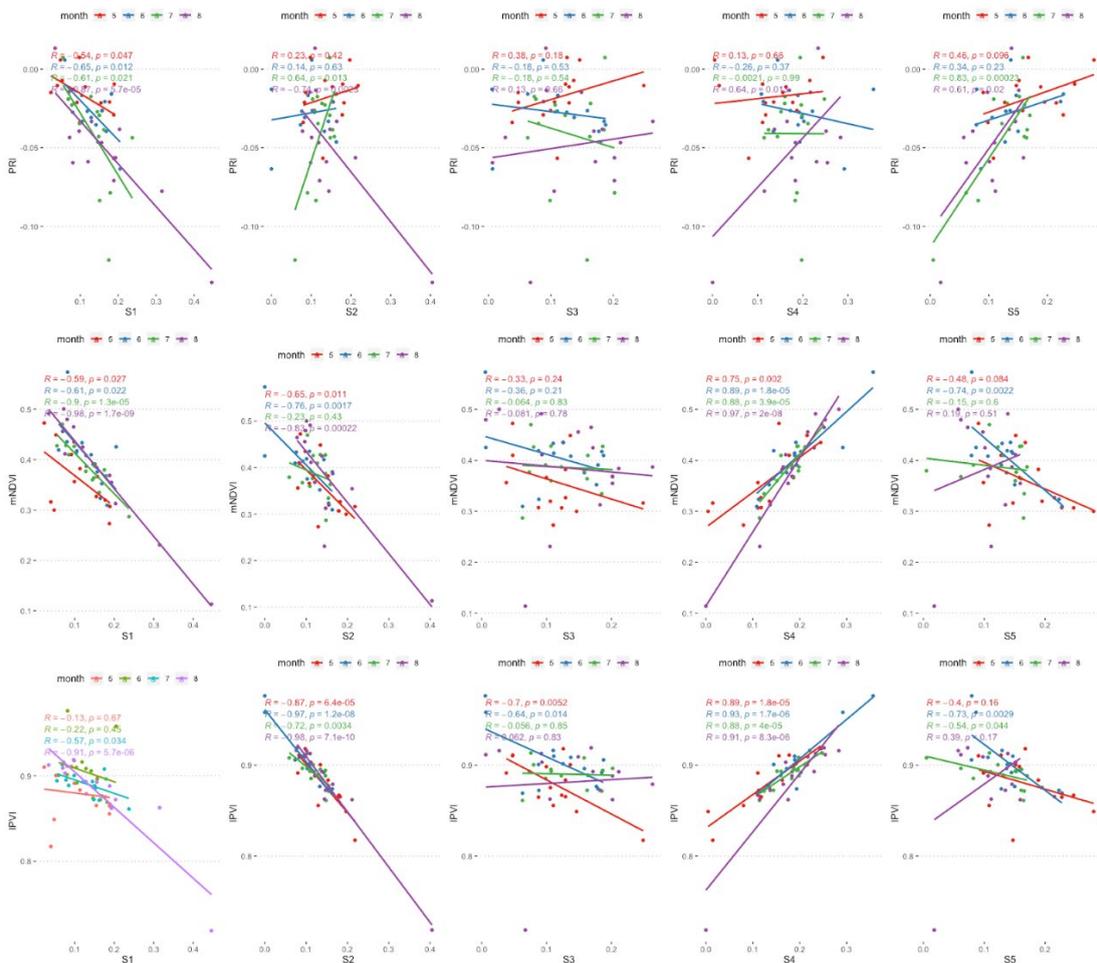


Figure 9.



Heatmap where each cell indicates correlation coefficient between popular chlorophyll indices and signatures.

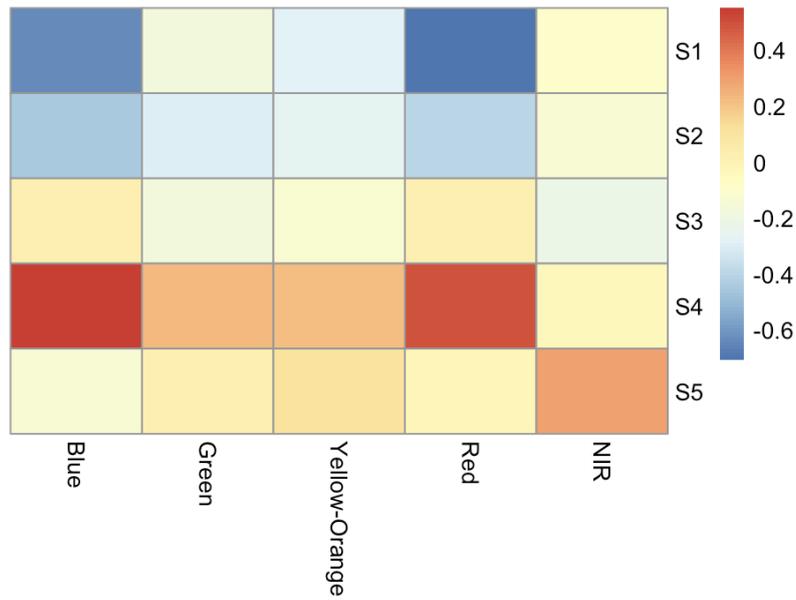
Figure 10.



Association between developed signatures and reflectance indices.

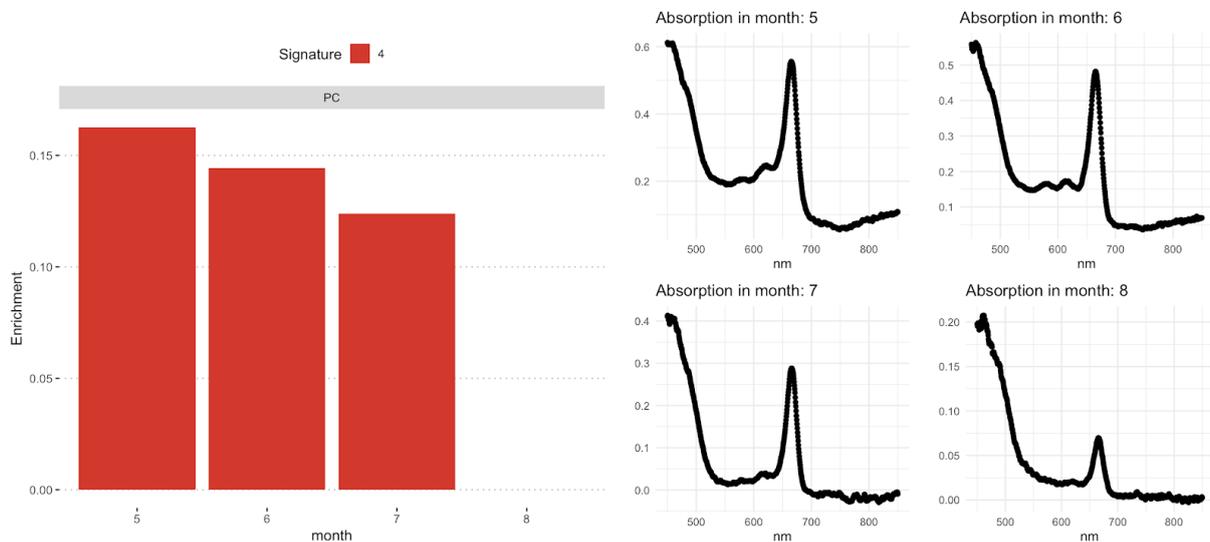


Figure 11.



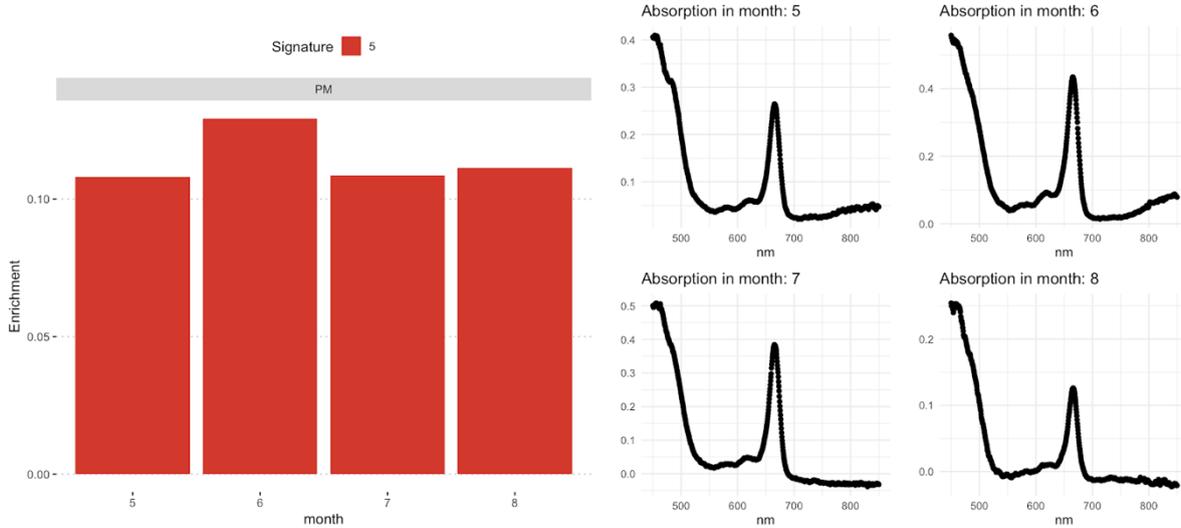
Heatmap where each cell indicates correlation coefficient between max value of absorption at specific color spectrum and developed signatures.

Figure 12a.



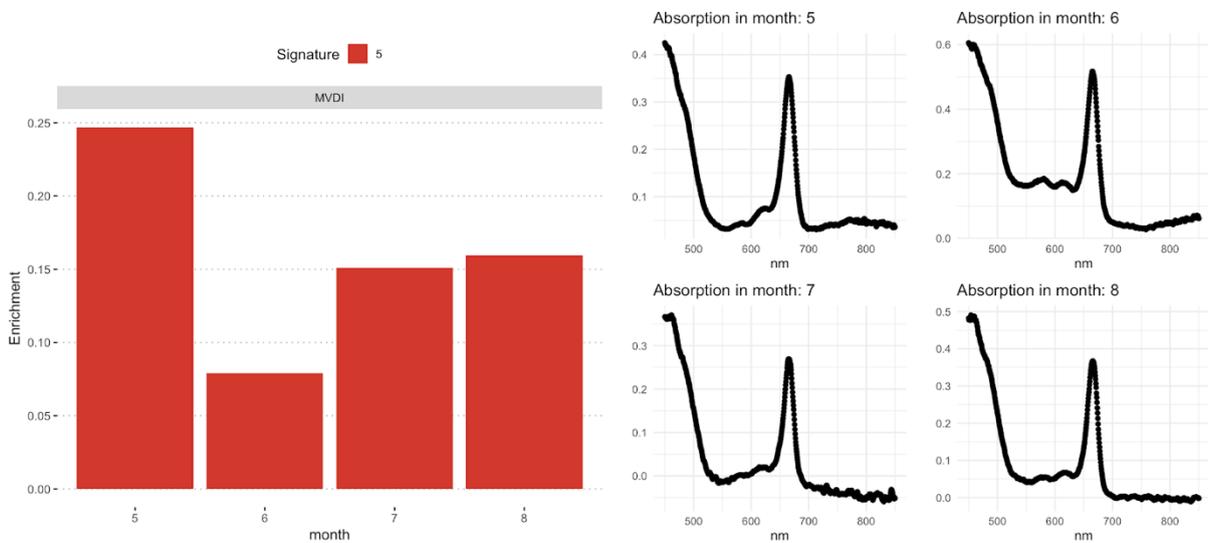
Using PC vineyard and signature S4 as example we observed same downward patterns comparing signature S4 and absorption of red spectrum.

Figure 12b.



Using PM vineyard and signature S5 as example we observed similar similar patterns between signature S5 enrichment and NIR absorption.

Figure 12c.



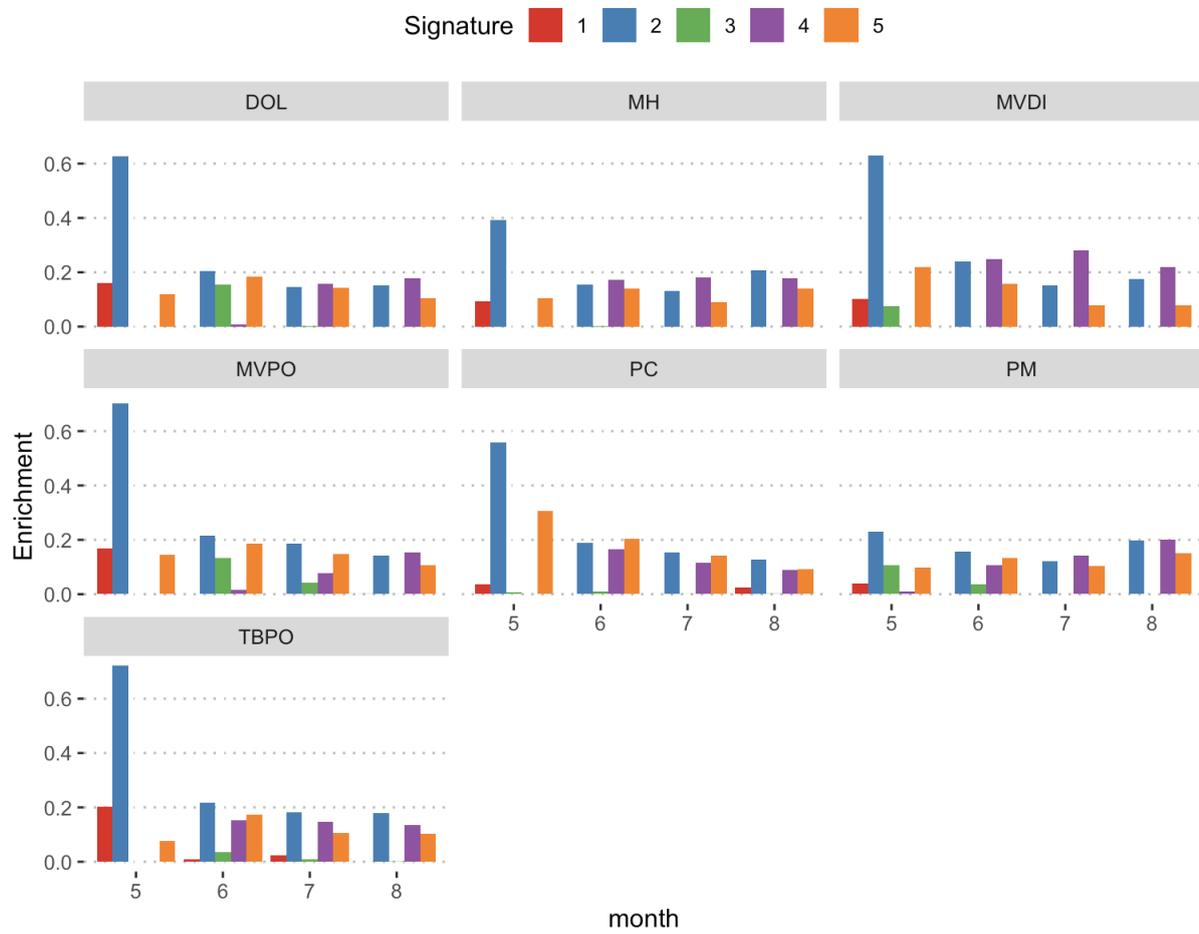
Using MVDI vineyard and signature S5 as example we observed inverse correlation between photochemical reflectance (PRI) and absorption of green spectrum.

**Figure 13.**



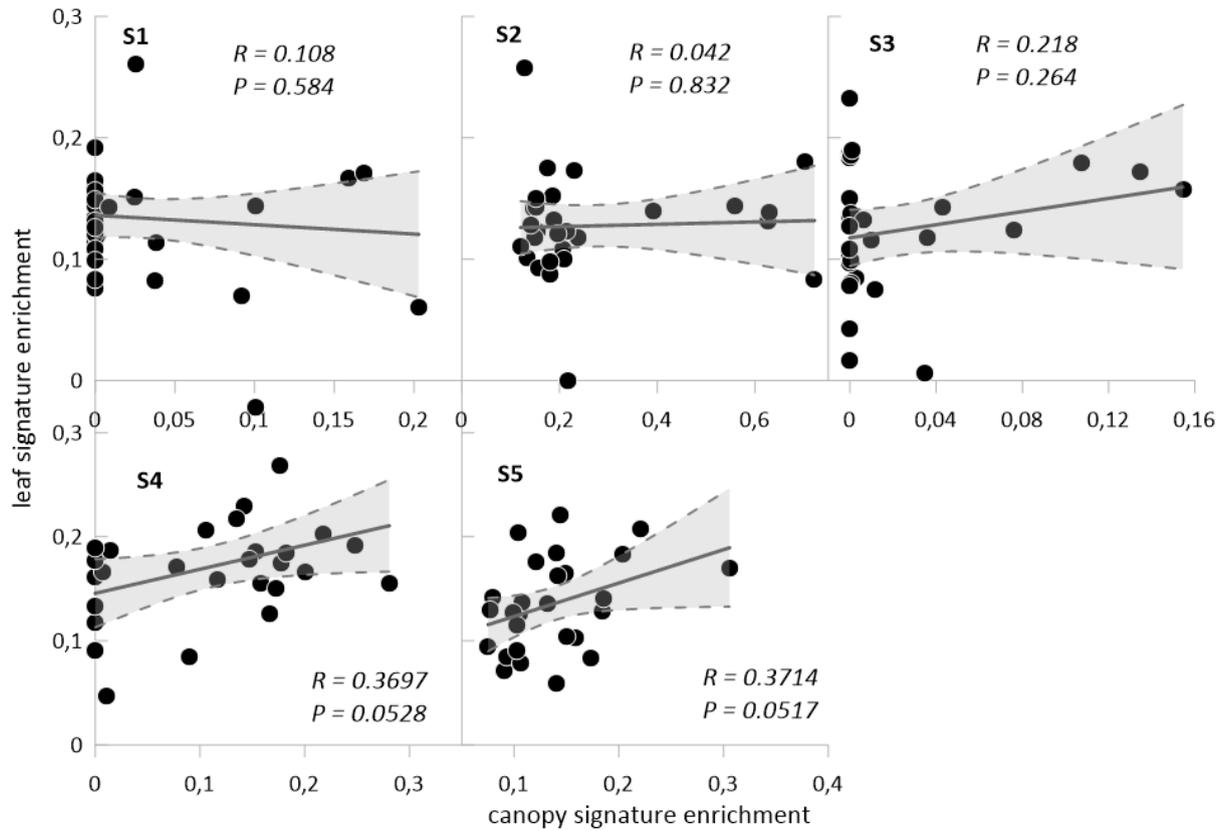
Upper panel indicates enrichment of signatures in every vineyard in a time range of four months for the first leaf and in lower panel for the second leaf. Numbers above each group of bars represent the chlorophyll absorption in  $\mu\text{mol}/\text{m}^2$ .

Figure 14.



Signature enrichments in every vineyard in a time range of four months for the canopy hyperspectral reflectance measured 0.5 m distance from the grapevine canopy.

Figure 15.



Correlations between canopy and leaf reflectance signature enrichments in all vineyards between May and August 2020.