Proximal sensing of vineyard soil and canopy vegetation for determining vineyard spatial variability in plant physiology and berry chemistry

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Abstract
Proximal sensing is used in vineyards to precisely monitor and manage spatial and temporal variability while reducing laborious and repetitive measurements. Soil electrical conductivity (EC) and canopy vegetation indexes are two frequently assessed variables with off-the-shelf sensors. In this study, the ecophysiological variability of a commercial vineyard comprising three varieties in three blocks, Cabernet-Sauvignon (CS), Cabernet franc (CF), and Petit Verdot (PV), was investigated. Random sampling was used to continuously assess spatial variability in plant physiology and berry composition. Soil EC and NDVI were also continuously monitored throughout the season. There was a noticeable spatial pattern in the normalised differential vegetation index (NDVI) in the vineyard and soil EC. The spatial pattern of NDVI can be partially elucidated by the season-long stem water potential (Ψstem), which was lowest in the CS block. However, leaf photosynthesis did not match this spatial pattern. The spatial distribution of NDVI and soil EC did not satisfactorily explain the spatial variations in yield components and berry chemistry. Principal component analyses (PCA) were performed resulting in a clear discrimination of each of the three cultivars. Soil EC showed a significant relationship with Ψstem integrals, total skin anthocyanins and tri- to di-hydroxylated flavonoids. In each cultivar block, soil EC showed some capability to be related to plant water status, and NDVI showed a relationship with yield. Overall, this study provided evidence of the spatial variability of grapevine physiology in a commercial vineyard with three cultivars. Also, it showed that the cultivar effect and uniform crop level management can diminish the efficiency of proximal sensing, thus weakening the relationship of soil and canopy indexes with plant physiology and berry chemistry. Nonetheless, our study showed that it is possible to apply temporal proximal sensing methods when assessing plant water status, primary metabolism, yield and berry secondary metabolism, which give an indication of the possibility of managing the spatial variability of both plant physiology and berry chemistry.

Keywords
soil apparent electrical conductivity, normalized difference vegetation index, spatial variability, grapevine, precision viticulture
INTRODUCTION

Proximal sensing is commonly utilised in wine grape vineyards to minimise the cost of the production resources (Bramley and Lamb, 2003), and to manage the site specific variability of grapevine physiology (Jiménez-Brenes et al., 2019), thus ensuring high quality and profitability (Bramley et al., 2003). Previous research has focused on the differential monitoring and management of spatial heterogeneity, which is a result of variable soil profiles or grapevine characteristics due to inconsistent cultural practices (Bramley et al., 2011a; Yu et al., 2020). Two proximal sensing methods are usually applied to capture spatial variability in vineyards: the normalised differential vegetation index (NDVI) for canopy assessment, and soil electrical conductivity (EC, or its inverse electrical resistivity) (Brillante et al., 2020). Soil EC sensing has revealed that soil moisture, salinity and texture are integrative and measurable parameters with good temporal and spatial resolution (Bittelli, 2011; Hardie and Doyle, 2012; Brillante et al., 2014). The results of previous studies have shown it is possible to spatially measure vineyard soil variability in terms of grapevine water uptake (Brillante et al., 2016), health (Bramley, 2001), productivity (Hall et al., 2002), berry soluble solid and organic acid content (Brillante et al., 2018), and berry skin flavonoid content (Yu and Kurtural, 2020).

It has been shown that NDVI can be used to assess grapevine leaf area index (Hall et al., 2008), biomass (Brillante et al., 2020), water status (Baluja et al., 2012), berry maturity (Anastasiou et al., 2018), yield (Carrillo et al., 2016), grape phenolics and colour (Gatti et al., 2017), and diseases (Di Gennaro et al., 2016). It has similar advantages over conventional ground-based measurements to proximal soil sensing, including non-destructibility and efficient data acquisition. In contrast to soil EC sensing, NDVI measures variables directly from the leaves, which can capture simultaneous responses from the canopy characteristics as the environmental factors vary. Although some effective management of the canopy can be achieved based on NDVI assessment, underlying environmental conditions, such as soil water content, are still the determining components of grapevine development, eventually exacerbating the vineyard spatial variability. Therefore, relying solely on NDVI for making management decisions might not be sufficient and any underlying environmental factors may also need to be investigated if all the dots are to be connected in the physiological development of a plant. In our previous study, it was reported that berry skin flavonol content is a reliable assessor for canopy architecture (Martínez-Lüscher et al., 2019). Flavonol content was negatively correlated to NDVI captured from the vineyard, and positively correlated to pruning mass. In this case, the fundamental relationship between the secondary metabolism of the grapevine and satellite sensed canopy reflectance (NDVI) was negative. Nevertheless, the spatial variability in this work stemmed from site topography, which in turn influenced plant water status, thus having a cascading effect on canopy architecture, and berry metabolism.

Most previous research has focused on the use of a single parameter for assessing spatial variability in plant physiology, potentially neglecting any concurrent significant influences that other unmeasured parameters may have had on grapevine development. At this point, multiple spatial layers may be considered and studied to determine the main driving factors of vineyard variability in the final yield and fruit composition. Ultimately, spatial variability can be harnessed to maintain grapevine productivity and quality when appropriate management practices are applied, thus optimising the oenological potential of vineyards.

The aim of this study was to assess the observed spatial variability of both soil EC and NDVI by carrying out continuous proximal sensing during the season. Furthermore, we investigated whether soil EC and NDVI could be related to berry primary and secondary metabolism in the wine grape cultivars Cabernet-Sauvignon (CS), Cabernet franc (CF), and Petit Verdot (PV) in a commercial vineyard in Napa Valley, CA, USA. Specifically, we aimed to understand the possibilities of using proximal sensing to temporally monitor the spatial variability of grapevine physiology and berry chemistry using multiple cultivars and rootstocks and applying relatively intense management.

MATERIALS AND METHODS

1. Plant materials, experimental site and weather

The study was conducted in 2017 in an experimental site comprising three commercial vineyards located in Oakville, CA, USA.
The cultivars/rootstocks were Petit Verdot/101-14 Mgt, Cabernet franc/101-14 Mgt, and Cabernet-Sauvignon/110R (Figure 1), hereafter referred to as PV, CF and CS respectively in all three vineyards, the grapevines were planted in 2008 with a spacing of 1.22 m × 1.83 m (vine × row) with a row orientation of SW-NE. All the grapevines were trained to a bi-lateral cordon and vertically shoot-positioned. During the dormant season, the grapevines were pruned to 2 bud-spurs, and approximately 8 buds per grapevine were retained. The vineyards were irrigated by a drip irrigation system at 50% crop evapotranspiration (ETc) replacement. Two emitters per grapevine distributed water once a week at a rate of 4 L water per hour. All three vineyards were closely monitored and managed by the growers; each vineyard was under contract to produce about 6 to 7 tonnes/ha. At Eichhorn–Lorenz (E-L) stage 17, the vineyard was uniformly shoot-thinned to retain one shoot per spur. At E-L stage 31, the vineyard was cluster-thinned to retain one cluster per shoot to meet production demands. The clusters were thinned again before E-L 35 to ensure that there were no more than 20 clusters per vine.

Weather information at the experiment site was obtained from the California Irrigation Management Information System (CIMIS) (station #77, Oakville, CA) to assess precipitation, air temperature and reference evapotranspiration (ETc). The CIMIS station was approximately 50 m from the experimental site, thus the weather data was relatively representative. Applied irrigation amounts were calculated as the product of ETc and crop coefficient (Kc) (Williams and Ayars, 2005). Irrigation was applied weekly at 50% ETc from bud break to harvest with a total amount of 68.9 mm applied for the season of 2017 per vineyard. Growing degree days (GDD) were calculated based on the equation:

\[
\text{GDD} \ (°C) = \sum \left[ \frac{(T_{\text{max}} + T_{\text{min}})}{2} - T_{\text{base}} \right] \]

where \( T_{\text{max}} \) is the maximum air temperature, \( T_{\text{min}} \) is the minimum air temperature, \( T_{\text{base}} \) is 10 °C; average air temperatures exceeding 10 °C are included in the calculation.

2. Experimental design

A stratified random sampling that contained 20 experimental units within each block and

![Figure 1](image.png)

**Figure 1.** USDA soil survey for the experimental site in a commercial vineyard with three cultivars in Oakville, CA, USA: 54.1% of Bale clay loam (104), 0.1% of Hambright-Rock outcrop complex (151), 6.4% of Perkins gravelly loam (168), and 39.4% of Yolo loam (182). Three blocks with each of the three cultivars are labelled and the geolocations of each experimental unit are marked by the black triangles.
A total of 60 experimental units were used for measurements and sampling. Each experimental unit consisted of 3 grapevines where on-site measurements were performed. Geolocations of each middle grapevine were recorded with a GNSS (Global Navigation Satellite System) unit (Yuma®, Trimble Inc., Sunnyvale, CA, USA) attached to a Trimble® Pro 6T DGNSS receiver (Trimble Inc., Sunnyvale, CA, USA).

3. Soil electrical conductivity assessment

Apparent soil electrical conductivity (EC) was spatially and temporally assessed by EM38-MKII (Geonics Ltd., Mississauga, ON, Canada) in the season on 25 July, 5 September and 18 September. In this study, the vertical dipole mode was utilised to measure soil depth to a depth of 1.5 m. The instrument was calibrated according to the manufacturer’s instructions. The device was placed on a PVC sled at approximately 15 cm from the ground surface and hauled by an all-terrain vehicle along the inter-rows. The sensor took measurements in one out of every three rows. The device was kept at a distance of about 1.0 m from the vehicle to avoid the metal interfering with the measurement. The sensor was connected to a GeoSCOUT X (Holland Scientific Inc., Lincoln, NE, USA) datalogger along with a GNSS sensor (Garmin® GNSS 18x PC, Garmin® International, Inc., Lenexa, KS, USA) to register the geolocations of each reading at Hz.

4. Assessment of the normalised difference vegetation index of the canopy

The canopy normalised difference vegetation index (NDVI) was assessed spatially and temporally with a Crop Circle ACS-430 Active Canopy Sensor (Holland Scientific Inc., Lincoln, NE, USA) on 30 May, 25 July and 5 September. NDVI readings and their geolocations were recorded by a GeoSCOUT X (Holland Scientific Inc., Lincoln, NE, USA) with a GNSS sensor (Garmin® GNSS 18x PC, Garmin® International, Inc., Lenexa, KS, USA) to register the geolocations of each reading at Hz.

5. Grapevine physiology assessments and yield components

The plant water status was assessed twice a week by midday stem water potential ($\Psi_{stem}$) measurements during the growing season before the vineyard was irrigated in the same week. The measurements were taken on 5 July, 21 July, 2 August, 22 August and 6 September. The measurements were conducted at solar noon from 11:00 to 15:00 on the measurement days. One leaf from the main shoot axis in the shade was selected from each vine; a total of three leaves was measured in each experimental unit. The leaves were concealed in pinch-sealed Mylar® bags for 2 h prior to the measurements. A pressure chamber (Model 615D, PMS Instrument Company, Albany, OR, USA) was used to take the readings.

Leaf gas exchange was assessed by using a portable infrared gas analyzer CIRAS-3 (PP Systems, Amesbury, MA, USA) twice a week before the vineyard was irrigated in the same week. The measurements were taken on 3 August, 22 August, 6 September, and 18 September. The measurements were conducted at solar noon from 11:00 to 15:00 on the measurement days. Three sun-exposed leaves were selected from the main shoot axis on three grapevines in each experimental unit, and three readings were taken from each leaf. The relative humidity was set at 40 %, and the CO₂ concentration was set at 400 μmol/mol, which is the standard environmental condition setting for CIRAS-3.

Yield per vine was recorded on a single harvest day (18 September 2017) in each vineyard. All the clusters were removed from grapevines by hand, then counted and weighed on a top-loading scale. The cluster mass was calculated by dividing yield per plant by the number of clusters harvested. Pruning weight was collected during the dormant season (5 February 2018). The plants were manually pruned; the wood was gathered and weighed on a top-loading scale.

6. Integral Calculations

To express the season-long response of plant physiological variables, including plant water status and leaf gas exchange variables, their integrals were calculated. The natural cubic splines for plant water status and gas exchange measurements were used to assess the cumulative values for these parameters for the whole experimental period during the growing season.
The δ13C values are reported in per-mille (‰) relative to the Vienna PeeDee Belemnite-CO₂ (VPDB-CO₂) international reference.

8. Berry skin flavonoid assessment
From the 20-berry subset, the skin tissues were manually removed with a scalpel. The skins were lyophilised with a freeze-drier (Triad Freeze-Dry System, Labconco, Kansas City, MO, USA) and ground with a mixing mill (MM400, Retsch, Mammelzen, Germany). To initiate the extraction for flavonoid analysis, 50 mg of dry skin powder from each sample was mixed with 1 mL of methanol/water/7 M hydrochloric acid (70:29:1) solution at 4 °C overnight. Extracts were centrifuged at 5,000 rpm for 10 mins. The supernatants were filtered using PTFE membrane filters (diameter: 13 mm, pore size: 0.45 μm, VWR, Seattle, WA, USA) and transferred into HPLC vials before injection.

Skin flavonoids were analysed by reversed-phase High Performance Liquid Chromatography (HPLC) (Agilent model 1260, Agilent Technologies, Santa Clara, CA, USA), which consisted of a vacuum degasser, an autosampler, a quaternary pump and a diode array detector with a column heater. A C18 reversed-phase column (LiChrosphere 100 RP-18, 4 × 520 mm², 5 μm particle size, Agilent Technologies, Santa Clara, CA, USA) was used following a protocol from a previous study (Martínez-Lüscher et al., 2019). The time lapse of the HPLC was 90 min, and the mobile phase flow rate was 0.5 mL/min. Two mobile phases were used comprising solvent A of 5.5 % aqueous formic acid (v/v) and solvent B of 5.5 % formic acid in acetonitrile (v/v). The detection of anthocyanins was carried out by the diode array detector at 520 nm. A computer workstation with Agilent OpenLAB (Chemstation edition, version A.02.10) was used for the chromatographic analysis.

All solvents used were of HPLC grade; these were: acetonitrile, methanol, hydrochloric acid and formic acid purchased from Fisher Scientific (Santa Clara, CA, USA). The standards used for compound identification were malvidin 3-O-glucoside purchased from Extrasynthese (Genay, France), and myricetin-3-O-glucuronide, myricetin-3-O-glucoside, queretin-3-O-glucuronide, queretin-3-O-galactoside, queretin-3-O-glucoside, kaempferol-3-O-glucoside,isorhamnetin-3-O-glucoside, and syringetin-3-O-glucoside purchased from Sigma-Aldrich (St. Louis, MO, USA).

9. Statistical analysis
The data obtained by proximal sensing were filtered according to Tukey’s rule to remove outliers either below the first quartile by 1.5 inter-quartile range or above the third quartile by 1.5 inter-quartile range. To further remove the outliers, the data were filtered by the speed that the vehicle drove at between the vineyard rows, which was between 3.2 km and 8 km/h. The best variogram model was assessed using the cross-validation routing of the automap package 1.0-14 (Hiemstra, 2013), and fitted to perform kriging.
Geostatistical analysis and kriging for soil EC and NDVI were performed in the R language using package gstat 1.1-6 (Pebesma, 2004), and the krigings were performed individually for each parameter. The specific soil EC and NDVI values were extracted from each experimental unit on each measurement date. The cumulative integrals were calculated as described above, and these values were further used to perform correlation analysis. An ordinary kriging was performed in ArcGIS (version 10.6, ESRI, Redlands, CA, USA), and the resulting maps were further processed in ArcGIS to be displayed as figures. The interpolation maps for the on-site measurements were all processed by using the automap package to perform the semi-variogram fitting and kriging.

Data were tested for normality by using Shapiro-Wilk’s test and subjected to mean separation between cultivars by using one-way analysis of variance (ANOVA) with the package stats 4.1.0. Specific data were extracted at each location and average values were calculated within each cultivar. Significant statistical differences were determined when \( p \) values were less than 0.05. A Tukey’s test was used to perform post hoc test by using the package “multcomp” (Hothorn et al., 2020). Means ± one standard deviation (SD) and their rankings were plotted in SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, USA). Pearson’s coefficient \( r \) values and \( p \) values were acquired to show the significance of the linear fittings.

Principal component analysis (PCA) was performed with the package “stats” to analyse the relationship between the physiological parameters and the different cultivars. The PCA for individuals and variables was visualised using package “factoextra” (Kassambara and Mundt, 2020). The PCAs were performed on the data from all three cultivars, as well as those from each cultivar to show the relationship with and without the cultivar/rootstock effects.

RESULTS

1. Experimental site soil and weather

In the vineyard, four main soil types had been identified by the United States Department of Agriculture (USDA) (Figure 1; Web Soil Survey, 2016). All the soils in the PV vineyard were classified as Bale clay loam, as were those in the northeastern half of CF block. The other half of the CF vineyard and its northeastern section comprised Yolo loam. The southwestern section of the CS vineyard had Perkins gravelly loam, and very small area of its southwestern section constituted Hambright-Rock outcrop complex. The Bale clay loam was characterised as poorly drained with slow run-off. However, Yolo loam had well-draining characteristics with slow to medium run-off. Similarly, Perkins gravelly loam and Hambright-Rock outcrop complex were defined as well-drained with faster run-off. In the spring of 2017, the experimental site received 900.2 mm of rainfall, which was considerably higher than the average precipitation in this region, but no precipitation occurred from June to September (Figure 2A). Air temperature was at its highest during this time; July was the hottest month during the growing season with an average air temperature of 20.5 °C and an average maximum air temperature of 31.3 °C, while the minimum air temperature reached its highest value of 12.2 °C and then started to decline (Figure 2B). The GDD accumulation started from March and reached 1220 °C at harvest in September. Temperature-wise, the weather at the experimental site in 2017 can be classified as “warm-summer Mediterranean climate”, noted as ‘Csb’ according to the Köppen classification (Peel et al., 2007). 2017 was also shown to be one of the hottest years in history with 22 days and 7 days exceeding 35 °C and 40 °C respectively during the growing season.

2. Soil EC assessment

The spatial soil EC patterns as shown on the interpolation maps in Figure 3 were more distinct per vineyard due to the lower values measured at the vineyard edges. In the PV vineyard, the northwestern section consistently showed higher soil EC (Figure 3: A1, A2, A3). As for CF and CS vineyards, the central section always had higher soil EC than at the edges. However, as from early September, the areas with the highest soil EC in both vineyards decreased in size (Figure 3: A2 and A3). In terms of the temporal variability of soil EC from July to September, soil EC increased slightly. Similar to the temporal pattern in the NDVI measurements, the differences between the highest and the lowest soil EC become more significant. The highest soil EC was always observed in the PV vineyard.

3. Canopy NDVI assessment

Out of the three vineyards, CS generally showed relatively lower NDVI. Early in the season at fruit-set, CF had the highest NDVI in
the central section of the vineyard (Figure 3: B1); these spatial patterns remained relatively consistent from May to September (Figure 3: B2 and B3). PV had the highest NDVI in the southwestern section of the vineyard, and the lowest in the northeastern part of the vineyard. However, CS had a significantly lower NDVI around fruit-set (Figure 3: B1); the difference between CS and the other two cultivars decreased as the growing season progressed, because the soil dried out as evaporative demand increased (Figure 3: B2 and B3). The edges on the southwestern and southern borders of the CS vineyard decreased in NDVI during the season. Meanwhile, the northwestern and eastern borders on the PV vineyard showed notably lower NDVI during the growing season compared to the rest of the block. In terms of the overall values from May to September, the ranges for NDVI decreased slightly over the season: from 30 May to 5 September, the highest and lowest values declined from 0.88 to 0.82 and from 0.84 to 0.74 respectively. This decline from early season (fruit-set) to harvest can be attributed to the remarkable decrease in NDVI measured in the PV vineyard.

4. Plant water status and leaf photosynthesis

Plant water status and leaf photosynthesis were monitored throughout the growing season; the spatial interpolations for the season-long $\Psi_{stem}$, $g_s$, $A_n$, and WUE$_i$ integrals are shown in Figure 3. The highest $\Psi_{stem}$ was measured in the PV vineyard (Figure 4A), and the lowest $\Psi_{stem}$ in the CS vineyard. This loosely reflects what was observed in the soil EC maps, with the areas of high EC in the PV block having better plant water status. The PV vineyard did not show significantly lower $g_s$ compared to CS and CF vineyards as a whole (Figure 4B). On the other hand, CS showed the lowest overall lower $g_s$ compared to the other two blocks. The $A_n$ map partially agrees with the $g_s$ map for the CS vineyard, in which the highest overall $A_n$ values were observed (Figure 4C). The WUE$_i$ map was similar to the $g_s$ map, with the lowest the $g_s$ and the highest WUE$_i$ in the PV vineyard, and conversely the highest $g_s$ and the lowest WUE$_i$ in most of the CS vineyard (Figure 4D).
When comparing the three cultivars in terms of these variables on individual dates, most of them were found to be significantly different (Figure 5). Similar to the long-term $\Psi_{stem}$ observations, PV constantly had the highest $\Psi_{stem}$ (Figure 5A). Furthermore, the trend was similar for all three cultivars, whereby $\Psi_{stem}$ kept decreasing. CS had the highest $A_n$ in the early season and CF had the lowest (Figure 5B). However, closer to harvest, the $A_n$ of CF grapevines continuously increased. In PV, maximum $A_n$ was reached in early September, after which it declined. The development of $g_s$ was similar for all three cultivars, with the highest $g_s$ being reached in early September, after which it started to decline (Figure 5C). At the beginning of the season (fruit-set), PV had the highest $g_s$, but ended up as the lowest at harvest, although its $\Psi_{stem}$ was the highest at this time. Therefore, WUE$_i$ was the highest in PV towards harvest, but it was significantly lower than CS in the early season (Figure 5D).

To further validate the spatial interpolation of NDVI and soil EC, the three cultivars were compared for average values on individual measurement dates. The NDVI values kept decreasing while the EC values slightly increased approaching harvest (Figure 5E and 5F). Out of all three cultivars, CF constantly had the highest NDVI in particular (Figure 5E). CS started with the lowest NDVI early in the season, and NDVI did not decrease as much as PV did. Soil EC was similar in all three vineyards, except for a significantly higher soil EC in PV than CS on 5 September (Figure 5F).
5. Yield and berry primary and secondary metabolism

Due to the artificial dropping of fruits, there were no notable spatial yield patterns in the three vineyards. A few vines had the lowest yield in the PV and CF vineyards, and others were highest in the northeastern section of the CS vineyard (Figure 6A). On the other hand, there was an obvious difference in the pruning weight of these three cultivars, with the lowest pruning weight obtained for PV and the highest for CS; these differences are likely a result of cultivar rather than spatial variability in the soil (Figure 6B). CS pruning weight is reflected in the spatially-observed yield, with the highest values for both parameters in the northeastern section of the vineyard. The opposite pattern can be seen in the skin total anthocyanin map, with the highest values obtained by PV (Figure 6C). A similar pattern can be seen in the tri-hydroxylated to di-hydroxylated anthocyanin and flavonol ratio maps (Figure 6E and 6F) - with the highest tri- to di- hydroxylation ratio in PV and the lowest in CF, but it is not observed in the skin total flavonol map (Figure 6D).

6. Soil EC and NDVI relationship with plant physiology

A correlation matrix (Table 1) was used to show NDVI and EC relationships with plant physiology. The soil EC integrals showed a significantly positive relationship with $Ψ_{stem}$ integrals ($r = 0.29$), total skin anthocyanins ($r = 0.36$), and tri- to di-hydroxylation ratio for anthocyanins and flavonols ($r = 0.27$ and 0.29 respectively). Soil EC was also found to be significantly negatively related to pruning weight ($r = 0.29$), TSS ($r = -0.28$). The NDVI integrals showed a significant negative relationship with $δ^{13}C$ ($r = -0.40$), $g_\text{s}$ integrals ($r = -0.39$), TSS ($r = -0.44$), TA ($r = -0.65$), total skin flavonols ($r = -0.33$), and tri- to di-hydroxylation ratio for anthocyanins and flavonols ($r = -0.37$ and -0.39 respectively).
FIGURE 5. Progression of plant water status, leaf photosynthesis, NDVI and soil EC in 2017 in a commercial vineyard with three cultivars in Oakville, CA, USA. A) Stem water potential (Ψstem), B) net carbon assimilation (An), C) stomatal conductance (gs), D) intrinsic water use efficiency (WUEi), E) NDVI, and F) soil deep EC. Error bars represent one standard deviation from the mean, letters represent ranking. Significance codes: ‘***’: 0.001, ‘**’: 0.01, ‘*’: 0.5, ‘.’: 0.1. Cultivar denotation: PV = Petit Verdot, CF = Cabernet franc, CS = Cabernet-Sauvignon.
The NDVI integrals did not significantly correlate with yield ($r = 0.03$) or pruning weight ($r = -0.1$), but yield correlated significantly and positively with pruning weight ($r = 0.51$). As for the $\Psi_{stem}$ integrals and leaf gas exchange, $\Psi_{stem}$ did not correlate with $g_e, A_{net}$, or WUE$_i$, which was expected due to the unsynchronised development of photosynthesis observed in the cultivars (Figure 5). $\delta^{13}C$ was found to be weakly correlated with $\Psi_{stem}$ integrals ($r = -0.03$); however, a significant negative correlation with $A_{net}$ integrals ($r = -0.47$) and with $g_e$ integrals ($r = -0.56$) was found, as well as with yield ($r = -0.54$), pruning weight ($r = -0.35$), TSS ($r = -0.51$) and TA ($r = -0.60$).

PCA analyses were performed to investigate the proximal sensed NDVI and soil EC relationships with plant physiological parameters for all three cultivars, as well as for each single cultivar (Figure 7). Regarding the vineyard as whole with all three cultivars, the first two principal components (PC) explained 53.8% of the total variation in the dataset, and PC1 and PC2 accounted for 29.4% and 24.4% of the total variation respectively (Figure 7A). Each of the three cultivars was grouped separately, as can be seen in Figures B1-B3. The PV group comprises the highest $\Psi_{stem}$ total skin anthocyanins, tri- to di-hydroxylated anthocyanin and flavonol ratios and soil EC integrals.
### TABLE 1. Correlation matrix. Values are expressed in Pearson correlation coefficient ‘r’ in Oakville, CA, USA in 2017 a, b, c

<table>
<thead>
<tr>
<th>NDVI Int</th>
<th>EC Int</th>
<th>C13</th>
<th>SWP Int</th>
<th>An Int</th>
<th>gs Int</th>
<th>WUE Int</th>
<th>Yield</th>
<th>Pruning weight</th>
<th>TSS</th>
<th>TA</th>
<th>total anthocyanins</th>
<th>total flavonols</th>
<th>tri/di anthocyanin ratio</th>
<th>tri/di flavonol ratio</th>
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<td>SWP Int</td>
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<td>-0.16</td>
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<tr>
<td>An Int</td>
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<td>-0.47 **</td>
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<td>gs Int</td>
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<td>-0.56 ***</td>
<td>-0.16</td>
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<td>-0.34 **</td>
<td>-0.2</td>
<td>0.48 ***</td>
<td>0.18</td>
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<tr>
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<td>-0.35 **</td>
<td>-0.65 ***</td>
<td>0.31 *</td>
<td>0.5 ***</td>
<td>-0.02</td>
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<td>-0.28 *</td>
<td>-0.51 ***</td>
<td>-0.46 ***</td>
<td>0.2</td>
<td>0.53 ***</td>
<td>-0.15</td>
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<td>-0.60 ***</td>
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<td>0.60 ***</td>
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<td>0.05</td>
<td>0.60 ***</td>
<td>0.23</td>
<td>-0.05</td>
<td>0.1</td>
<td>-0.02</td>
<td>-0.65 ***</td>
<td>-0.34 **</td>
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<td>total flavonols</td>
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<td>0.1</td>
<td>-0.19</td>
<td>0.22</td>
<td>0.30 *</td>
<td>0.09</td>
<td>0.03</td>
<td>-0.02</td>
<td>-0.15</td>
<td>0.38 **</td>
<td>0.37 **</td>
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<tr>
<td>tri/di anthocyanin ratio</td>
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<td>0.27 *</td>
<td>-0.1</td>
<td>0.48 ***</td>
<td>0.23</td>
<td>0.05</td>
<td>0.09</td>
<td>0.12</td>
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<td>0.81 ***</td>
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<td>tri/di flavonol ratio</td>
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<td>-0.14</td>
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<td>0.30 *</td>
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<td>0.14</td>
<td>-0.41 ***</td>
<td>-0.06</td>
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<td>0.82 ***</td>
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a df = 59.
c Asterisks represents significant levels p: ***: p < 0.001, **, p < 0.01, *, p < 0.05.
FIGURE 7. Bi-plots from the principal component analyses (PCA) of all three cultivars and each cultivar separately within a commercial vineyard in Oakville, CA, USA, according to plant water status and leaf photosynthesis (SWP = stem water potential integrals, An = An integrals, gs = gs integrals, WUE = WUEi integrals), vineyard proximal sensing (NDVI integrals and soil deep EC integrals), yield components (yield and pruning weight), berry must primary metabolites (TSS = total soluble solids, TA: titratable acidity, C13 = δ13C), and berry skin flavonoids (t_anthocyanins = total skin anthocyanins, t_flavonols = total skin flavonols, tri_di_antho_ratio = anthocyanin tri- to di-hydroxylation ratio, tri_di_flav_ratio = flavonol tri- to di-hydroxylation ratio). A) PCA for all three cultivars, B) PCAs for each of three cultivars: B1) Petit Verdot, B2) Cabernet franc, and B3) Cabernet-Sauvignon.
The CF group shows the highest NDVI and δ¹³C and CS the highest $A_s$, $g_s$, yield, TSS, TA, pruning weight, and total skin flavonols. The first two PCs of the PV block explain 46.3% of the total variation in the dataset, and PC1 and PC2 accounted for 28.4% and 17.9% of the total variation respectively (Figure 7B1). NDVI integrals positively correlated with yield and TSS, and soil EC integrals positively correlated with TA, but negatively correlated with δ¹³C. $\Psi_{stem}$ integrals showed negative correlations with tri- to di-hydroxylated flavonoid ratios in berry skin. As regards the CF block, the first two PC explained 47.2% of the total variation in the dataset, and PC1 and PC2 accounted for 27.6% and 19.6% of the total variation respectively (Figure 7B2). NDVI integrals negatively correlated with TA, soil EC integrals positively correlated with TSS, $g_s$ integrals and $\Psi_{stem}$ integrals, but negatively correlated with WUE, integrals. δ¹³C negatively correlated with $\Psi_{stem}$ and $g_s$ integrals. The first two PCs of the CS block explained 46.7% of the total variation in the dataset, and PC1 and PC2 accounted for 26.7% and 20% of the total variation respectively (Figure 7B3). NDVI integrals positively correlated with $A_s$ integrals, berry skin total anthocyanins, WUE integrals and yield. Soil EC integrals positively correlated with pruning weight and tri- to di-hydroxylated flavonoid ratios and negatively correlated with berry skin total flavonols. However, it did not show any significant correlation with $\Psi_{stem}$ integrals. $\Psi_{stem}$ integrals positively correlated with berry skin total anthocyanins, TA and WUE integrals. There was no significant correlation between δ¹³C and $\Psi_{stem}$ integrals.

**DISCUSSION**

1. **Soil EC and its relationships with plant physiology**

Soil EC sensing is often applied in precision viticulture research due to its relationship with soil water content (Brillante et al., 2014) and plant water status (Yu and Kurtural, 2020). Soil EC is an integrated soil parameter whose value is determined by different soil characteristics, such as soil water content, salinity, soil texture and temperature (Bittelli, 2011). Soil EC sensing has been shown to be a feasible approach to assessing the natural variability of soil (Rogiers et al., 2011), and it is a constant parameter over time. Previous research has also shown that soil water content is the main factor which determines the temporal variability of soil EC in non-saline soils (Brillante et al., 2014; Yu et al., 2021).

When water is uniformly distributed within the soil via irrigation, higher clay content will intensify water stress when water deficit irrigation is applied (Yu et al., 2021; Brillante et al., 2016). In accordance with the USDA soil survey, the Bale clay loam in PV block with poorer drainage showed the lowest $g_s$ and the better-draining Yolo loam and Perkins gravelly loam showed the highest $g_s$. However, the divergence in $\Psi_{stem}$ in terms of these relationships can be attributed to the effects of the cultivar or rootstock in question when environmental variations are not significant enough to alter this parameter. In our study, there was a positive relationship between soil EC and plant water status, which was confirmed by the results from each block. Therefore, a high soil EC likely indicates high water content, high clay content and low gravel content, which may help identify regions where grapevines will undergo less water stress when integrated over the season. Previous studies have observed similar positive relationships between soil EC and plant water status (Yu et al., 2020; Brillante et al., 2020), further confirming that soil water content is the factor that contributes most to soil bulk EC. However, in our study, TSS, total anthocyanins, tri- to di-hydroxylated anthocyanin and flavonol ratios had positive relationships with soil EC within the whole experimental site, although these relationships were not consistent in each block; this might be more due to the cultivar effect since berry chemistry profiles are directly related to cultivar (Cantos et al., 2002; Pomar et al., 2005).

Related to soil EC, soil water content will influence canopy development and canopy microclimate due to its significance in grapevine vegetative growth (Martinez-Lüscher et al., 2017; De Oliveira and Nieddu, 2013). When a canopy is managed based on NDVI at the veraison stage when the canopy stops growing, the variations originating from plant available water in the soil will still affect canopy architecture (Tramontini et al., 2013; Lavoie-Lamoureux et al., 2017). In our study, the three cultivars showed discrepant development in both leaf photosynthesis and berry chemistry. The canopy NDVI assessment early in the season did not illustrate the spatial patterns in yield components and berry chemistry; this can potentially be problematic when applying management strategies, since specific management can only be carried out once spatial variability has been accurately captured. Compared to NDVI sensing, soil EC sensing is relatively stable and constant, and there is even evidence that the assessment of soil EC at harvest can partially reveal season-long plant water status (Yu and Kurtural, 2020).
However, the most popular way of assessing soil EC is via electro-magnetic induction (EMI), which has been found to be sensitive to metal poles used to support the trellis systems (Lamb et al., 2005, Clark et al., 2007). Furthermore, as an integrated parameter, a ground-truthing procedure might need to be performed to scrutinise the soil profiles before directly using EMI for water management. These factors thus need to be addressed before using EMI to accurately assess soil EC data.

2. NDVI and its relationship with plant physiology

NDVI is being increasingly utilised in vineyards to monitor grapevine response to the environment (Pena-Barragan et al., 2011; Ferrer et al., 2020). Anastasiou et al. (2018) found that there is a relationship between NDVI and grapevine yield and berry diameter. The majority of studies on the link between NDVI and plant physiology have shown that NDVI can be used to indicate plant vigour and biomass (Stamatiadis et al., 2010; Brillante et al., 2020). However, these relationships were not constantly observed in our study, in which NDVI showed almost no correlation with yield or pruning weight of all three cultivars together. However, there was evidence of NDVI being positively correlated with yield in the PV and CS blocks. Previous research has revealed a positive relationship between NDVI and leaf stomatal conductance (Marino et al., 2014), possibly due to the changes in spectral reflectance arising from variations in plant water status. Significant correlations have also been found between NDVI and leaf water content and leaf water potential in other plants (Stimson et al., 2005 ). However, the opposite was observed with the three cultivars in this study, and there was no relationship between the two parameters for a single cultivar. This may be due to a combination of the water deficit irrigation applied in this study and the cultivar effect; specific cultivar grapevines with higher NDVI can have greater vigour and water status (i.e., gs) (Ferrer et al., 2020). This is likely to have caused the discrepancy between NDVI and plant water status observed in previous studies. Other studies have found that higher NDVI/vigour lowered the TSS accumulation or enhanced the acidity because of a potential delay in phenology with higher vigour grapevines (Koundouras et al., 2006; Tagarakis et al., 2013). However, this was not true for the PV block, for which NDVI was found to positively correlated with TSS accumulation; this might be because this specific cultivar has a well-known slow phenological development, resulting in a different relationship to other cultivars between vegetative growth (i.e., canopy growth) and reproductive growth (i.e., berry sugar accumulation). In a previous study, the flavonol derivative kaempferol was found to act as a reliable indicator for canopy porosity, leaf area index (LAI), and berry exposure to solar radiation, and NDVI was related to kaempferol composition (Martínez-Lüscher et al., 2019). Flavonols are sensitive to solar radiation due to the close relationship between the transcript abundance of a flavonol-related gene and light regulation (Koyama et al., 2012). Negative correlations were observed between NDVI and the ratios of tri- to di-hydroxylated anthocyanins and flavonols of all three cultivars in this study; this can be attributed to the clusters with higher NDVI receiving heavy shade from the canopy, and thus reducing the accumulation of the tri-hydroxylated flavonoids (Cortell and Kennedy, 2006). The latter can in turn be explained by a decrease in the transcription of VvF3’5’H, which determines the tri-hydroxylation of flavonoids due to increased grapevine canopy shade (Koyama and Goto-Yamamoto, 2008). However, in terms of a single cultivar, the relationship between NDVI and total skin flavonols was not significant. PV and CS showed positive correlations between NDVI and berry skin anthocyanins, which is in agreement with previous observations of the greater cluster exposure of grapevine with smaller canopy in hot climates accelerating anthocyanin degradation (Torres et al., 2020).

NDVI assessment in current agricultural research is mostly performed by satellites, unmanned aerial vehicle (UAV) or planes (Jackson et al., 2004; Wahab et al., 2018). When the assessment is conducted by these overhead methods, the ground interference with or without cover crop and shadows in closely spaced rows can all affect reflectance (Pettorelli et al., 2005). Even though the imagery can be sharpened by data processing, the assessable canopy would still only be a fraction of the top canopy, which would potentially give unrepresentative results. In a previous study, NDVI did not show any close relationships with either plant water status or leaf photosynthesis, but only with pruning weight (Brillante et al., 2020). Another study found that the stability in NDVI values tended to decrease when cultural practices were carried out in the vineyard (Kazmierski et al., 2011), which might explain the
alteration in the spatial NDVI pattern from May to July in our study. Some studies have used NDVI in the vineyard to help delineate different zones for management purposes (McClymont et al., 2012; Tagarakis et al., 2013). However, in the present study, the results showed a significant cultivar effect on NDVI and berry development, with CF constantly showing high NDVI. When managing a vineyard consisting of multiple cultivars, there would thus be limitations to using NDVI for assessing spatial variability, since different cultivars develop at different paces in terms of canopy growth and phenological development in general. In addition, the existing variability in grapevine physiology would still be apparent due to the variations in environmental conditions (Edwards et al., 2011; Tramontini et al., 2013). Therefore, the management of such vineyards would require additional spatial variability information to complement what is already being expressed in plant physiology.

3. The potential of combining NDVI and soil EC to understand temporal and spatial variability in grapevine physiology.

Our study revealed that, due to inconsistent correlations between soil EC and NDVI for all three cultivars and in each single cultivar block, a data fusion method is not appropriate for refining variability interpolation. However, previous studies have proved that the information provided by stacking spatial layers could help manage variability in plant physiology and berry chemistry (Priori et al., 2013; Bonfante et al., 2015). Moreover, several studies have pointed out that $\delta^{13}$C could potentially provide good estimations of plant water status and leaf photosynthesis, and thus be used as a biomarker for the quick assessment of long-term plant responses in terms of these parameters (Brillante et al., 2020, Yu et al., 2020). In the present study, the severity of management applied in the vineyard to control the canopy size or crop load might have masked the spatial variability of vineyard production from the natural conditions, whereby higher soil EC might have translated into higher yield or canopy size. The crop load was heavily managed to sustain the low yield goal from growers, the spatial variability in soil EC would not be related to the plant physiological and chemical profiles as anticipated. This yield manipulation approach could minimise the existing spatial variability, but its practicability would be limited by its cost. With agricultural industries generally facing a labour shortage, the cost of labour has been continuously increasing in California, and the financial return is often not enough to compensate for this increase (Lapsely, 2010). However, variable rate management strategies are being developed to be integrated into production; these are based on correctly capturing spatial variability by proximal sensing (Matese and Di Gennaro, 2015).

CONCLUSIONS

In this study, the spatial variability in deep soil EC and NDVI was assessed by proximal sensing in a vineyard of three different cultivars. Proximal sensing revealed that there was not a strong link between physiological development and spatial variability due to the cultivar effect and heavy canopy and crop management during the growing season. Nonetheless, when the vineyard as a whole and the single cultivars were considered, it was possible to link berry chemistry - including primary and secondary metabolism - and yield to NDVI, and season-long plant water status to soil EC. Soil EC correlated with berry chemistry because of its role in the plant water status. $\delta^{13}$C proved to be a good indicator of leaf gas exchange, yield and berry primary metabolism. Overall, this study has provided evidence that the integration of multiple sensing technologies into vineyard management could help determine the vineyard spatial variability of plant physiology and berry chemistry, with further potential for minimising the spatial variability of a commercial vineyard with multiple cultivars.

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