

1 **Title:** Reflectance spectroscopy predicts leaf functional traits across wine grape varieties

3 **Running Title:** Spectral reflectance and leaf economics in wine grapes

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Summary

- Reflectance spectroscopy has emerged as a powerful tool for non-destructive and high-throughput phenotyping in plants. While the ability of reflectance spectroscopy to predict traits across diverse plant species and ecosystems has received considerable attention, whether or not this technique is able to quantify within species trait variation has not been extensively explored. Quantifying intraspecific variation in traits through reflectance spectroscopy is especially appealing in agroecology, where it may present an approach for better understanding crop performance, fitness, and trait-based responses to environmental conditions.
- We tested if reflectance spectroscopy coupled with Partial Least Square Regression (PLSR) predicts rates of photosynthetic carbon assimilation (A_{\max}), Rubisco carboxylation (V_{\max}), electron transport (J_{\max}), leaf mass per area (LMA), and leaf nitrogen (N), across six wine grape (*Vitis vinifera*) varieties (Cabernet Franc, Cabernet Sauvignon, Merlot, Pinot Noir, Viognier, Sauvignon Blanc).
- Our PLSR models showed strong capability in predicting intraspecific trait variation, explaining 55%, 58%, 62%, and 64% of the variation in observed J_{\max} , V_{\max} , leaf N, and LMA values, respectively. However, predictions of A_{\max} were less strong, with reflectance spectra explaining only 29% of the variation in this trait.
- Our results indicate that trait variation within species and crops is less well-predicted by reflectance spectroscopy, than trait variation that exists among species. However, our results indicate that reflectance spectroscopy still presents a viable technique for quantifying trait variation in agroecosystems.

Keywords: Agroecology, crop trait, functional trait, high-throughput phenotyping, reflectance spectroscopy.

Societal Impact Statement

High-throughput phenotyping (HTP) is a key aspect of plant and agricultural science, being applied in crop improvement, crop selection, and in devising farm management strategies. Our research tested the ability of one HTP technique, reflectance spectroscopy, to rapidly and reliably phenotype wine grape varieties. Reflectance spectroscopy predicts important wine grape

leaf traits including photosynthesis and biochemistry with high accuracy, but in a fraction of the time compared to traditional techniques. Reflectance spectroscopy can therefore rapidly characterize wine grape phenotypes, and in doing so, inform predictions of how vines, clones, and varieties will respond to environmental change.

Introduction

In 2022 grape vines were the primary crop on an estimated 6.73 million ha of agricultural land globally, ranking grapes—which includes wine, juice, table, and dried grapes—as the world’s 32nd most widespread agricultural crop species (Food & Agriculture Organization of the United, 2024). In this same year, the total economic production value for grapes was estimated at \$85.1 billion USD (Food & Agriculture Organization of the United, 2024), reflecting the high economic importance of this crop, particularly in the world’s wine-producing regions of the world (Keller, 2020). In Canada for example, where our research is situated, wine production includes an estimated 12,600 ha of land distributed across 1,700 producers, while contributing ~\$9-11.5 billion to the Canadian economy annually (Hewer & Gough, 2020). However, wine grape production is increasingly being challenged by environmental change including increases in growing season temperatures (e.g., Liles & Verdon-Kidd, 2020), increases in the number of extreme temperature events (e.g., White et al., 2006), reduced water availability (e.g., Santillán et al., 2019), among others (e.g., Wolkovich et al., 2017). Such changes have complex and interacting effects on wine grape physiology, phenology, and growth, and are therefore individually and cumulatively expected to impact the viability and productivity of vineyards globally (reviewed by van Leeuwen et al., 2024). Quantifying and predicting the impacts of global change on wine grape production is therefore of tremendous agronomic and economic importance.

There exist a multitude of approaches for quantifying wine grape responses to abiotic stressors at genetic- through to whole-plant levels of integration (Cramer, 2010; Gambetta et al., 2020), which cumulatively underpin vineyard-scale responses to environmental change (e.g., Bernardo et al., 2018). One approach for understanding and predicting plant responses to environmental change, is through quantification of plant functional traits: the morphological, physiological, or phenological characteristics of plants that are readily measurable at an organismal scale, and influence the performance and response of individuals to environmental

changes (Díaz & Cabido, 1997; Lavorel & Garnier, 2002; Westoby & Wright, 2006; Funk et al., 2017). Indeed, a considerable amount of effort has been directed towards understanding the extent, causes, and consequences of trait variation among plant species (Reich et al., 1999; Westoby et al., 2002; Diaz et al., 2004; Wright et al., 2004; Wright et al., 2005; Díaz et al., 2016). This body of literature has led to a deeper understanding of the key dimensions of functional trait variation that exist among the world's plant species (Díaz et al., 2016; Carmona et al., 2021), and how these traits predict plant environmental responses (Lavorel & Garnier, 2002).

Among the most well-studied dimensions of trait variation employed to describe and predict plant performance across environmental gradients, and the focus of our research here, is the “Leaf Economics Spectrum” (LES) (Reich et al., 1999; Wright et al., 2004; Wright et al., 2005). The LES is a suite of six core leaf traits that covary among plant species including maximum photosynthetic assimilation (A_{\max}), leaf dark respiration rate (R_d), leaf nitrogen (N) and phosphorus (P) concentrations, leaf mass per area (LMA), and leaf lifespan (LL). Taken together, LES trait expression defines how species vary across a continuum of life-history strategies, from fast-growing species characterized by rapid return on biomass investment, low structural investment, high leaf nutrient concentrations, and relatively short lifespans on one end, to resource-conserving species expressing the opposite suite of traits and by extension can be more resilient to resource limitation. Variation in LES traits largely owes to evolved trade-offs related to leaf biomechanics (Shipley et al., 2006; Blonder et al., 2011), as well as evolved or plastic variation in physiological and leaf structural traits including stomatal and mesophyll conductance (g_s and g_m , respectively), which in turn influence rates of maximum Rubisco carboxylation (V_{\max}), and electron transport (J_{\max}) (Onoda et al., 2017; Onoda & Wright, 2018; Xiong & Flexas, 2018): two traits that are explicitly important in wine grape responses to temperature change (Greer & Weedon, 2012).

Much of the seminal work on trait variation is based on interspecific comparisons, though recent research now focuses on quantifying the extent and ecological implications of intraspecific trait variation (Bolnick et al., 2011; Violle et al., 2012; Siefert et al., 2015; Anderegg et al., 2018; Westerland et al., 2021). Given the role that phenotypic plasticity and inheritable genetic variation play in governing plant ecophysiology and morphology, plant species can exhibit a high degree of intraspecific variation across a range of traits (Siefert et al.,

2015) and trait dimensions (Isaac et al., 2017; Anderegg et al., 2018). Quantifying intraspecific trait variation is especially critical in agroecosystems such as vineyards, where a relatively small number of plant species or varieties drive rates of ecosystem functioning on account of high abundances (Martin & Isaac, 2015; Milla et al., 2015). Indeed, considerable interest and efforts have been dedicated to quantifying the causes and consequences of intraspecific variation in the traits that are directly responsible for crop growth, survival, and reproduction.

Efforts to comprehensively assess intraspecific trait variation in a given species, especially crops, are often limited at the data collection phase of scientific enquiry (Cadle-Davidson et al., 2019). Traditionally, trait data are collected or derived from a combination of field and laboratory measurements, most of which can be laborious and time-consuming. This is especially true for “hard” traits (sensu Diaz et al., 2004) that are part of the LES such as A_{\max} and R_d which are generated through point sampling of gas exchange using portable infrared gas analyzers. Furthermore, in wine grapes specifically, V_{\max} and J_{\max} are key parameters in photosynthetic acclimation to increased and extreme temperatures (e.g., Greer & Weedon, 2012). However, these traits that contribute to the physiological basis of crop responses to environmental change rely on the execution and analysis of time-consuming photosynthetic CO_2 response curves ($A-C_i$) (reviewed by Saathoff & Welles, 2021). These methodological limitations often preclude trait collection across large numbers of vines, motivating the search for techniques capable of supporting high-throughput phenotyping in grapes and other crops (Cadle-Davidson et al., 2019).

Reflectance spectroscopy has emerged as a central component of high-throughput phenotype assessments and related collection of physiological, chemical, and morphological trait data (Fahlgren et al., 2015). While multi- and hyperspectral sensors form a key component of remotely-sensed spectral diversity assessments at ecosystem scales (Asner et al., 2015; Singh et al., 2015; Asner et al., 2016; Chadwick et al., 2020), field-based reflectance spectroscopy offers an opportunity to rapidly amass species- or variety-scale data on leaf physiological, chemical, and morphological traits including those forming the LES (Serbin et al., 2012; Serbin et al., 2014; Kothari et al., 2023). Specifically, using Partial Least Square Regression (PLSR) models (Burnett et al., 2021a), studies have reported strong predictive relationships between reflectance spectra and LES traits including A_{\max} , leaf N, LMA, and related physiological parameters

including V_{cmax} and J_{max} (Serbin et al., 2014; Dechant et al., 2017; Doughty et al., 2017; Lamour et al., 2021).

Spectroscopy coupled with PLSR models has been successful in estimating plant traits, particularly when using multi-species datasets that present a wide range of trait values and spectral profiles (Serbin et al., 2014; Ely et al., 2019; Lamour et al., 2021). More recently, studies have begun employing these techniques to quantify and predict finer-scale intraspecific trait variation (Vasseur et al., 2022), including trait variation across individuals or genotypes of the same crop species (Heckmann et al., 2017; Yendrek et al., 2017; Silva-Perez et al., 2018; Meacham-Hensold et al., 2019; Fu et al., 2020; Burnett et al., 2021b). Analyses on intraspecific trait variation—where trait values and spectra are more constrained—are less common vs. studies analyzing trait values and spectral signatures from a number of species differing in life-history strategies (Serbin et al., 2014; Lamour et al., 2021) or agronomic profiles (Ely et al., 2019). Furthermore, studies using reflectance spectroscopy to detect intraspecific trait variation in crops, commonly screen plants from a range of managed environmental conditions, which further contributes a wider range of trait values (Yendrek et al., 2017). While these results are promising, there remains uncertainty regarding whether or not these techniques are able to differentiate LES traits across individuals or genotypes of the same species, in agroecosystems where environmental conditions are more homogeneous.

In grape and wine science spectroscopy has long been an important method for rapidly assessing berry composition and quality (Gishen et al., 2005). There also exists a small number of studies applying reflectance spectroscopy to quantifying leaf trait variation in wine grapes (e.g., De Bei et al., 2011; Diago et al., 2013). Though to our knowledge, studies evaluating whether or not this high-throughput technique is capable of quantifying the variation in leaf traits that exists among wine grape varieties, are absent from the literature. Our study therefore aims to contribute to the literature on high-throughput assessments of intraspecific trait variation, by evaluating the potential of reflectance spectroscopy to predict LES trait variations across multiple wine *V. vinifera* varieties. In this study we specifically ask: can reflectance spectroscopy reliably and rapidly estimate leaf functional traits in six of the world's most widely cultivated wine grape varieties?

Materials and Methods

Study site

We collected LES and related trait and spectral reflectance data for six of the most common wine grape varieties—Cabernet Franc, Cabernet Sauvignon, Merlot, Pinot Noir, Sauvignon Blanc, and Viognier—at the Niagara College Teaching Vineyard, Niagara-on-the-Lake, Ontario, Canada (43.1522° N, 79.1652° W). The site is an operational vineyard is situated in Canada’s largest wine-producing province (Hewer & Gough, 2020), characterized as non-irrigated, with imperfectly drained silty clays overlaying clay loam, till mixed with poorly drained lacustrine heavy clay, and uniformly tilled and sprayed (Macklin et al., 2022; Martin et al., 2022). All trait and reflectance data were collected during the fruit setting stage (at our site, from June 6-17, 2022) between 6:00-12:00. For each variety, we sampled 30 vines evenly distributed across three planting rows, which were roughly 10 meters apart from each other within one row, totalling $n=180$ individual vines. One leaf on each vine was selected from the uppermost segment of the individual for data collection, with all leaves being fully exposed, newly developed, fully expanded, and free of any damage (Perez-Harguindeguy et al., 2016).

Functional trait data collection

Trait data in our study included A_{\max} , V_{\max} , and J_{\max} , leaf N concentrations, and LMA. First, V_{\max} , J_{\max} , and A_{\max} data were collected in the field using a LI-6800 Portable Photosynthesis System (Licor Bioscience, Lincoln, Nebraska, USA). We first performed an $A-C_i$ curve on each leaf using the Dynamic Assimilation Technique (DAT) (Saathoff & Welles, 2021; Gregory et al., 2023; McClain & Sharkey, 2023) in order to estimate rates of V_{\max} and J_{\max} . For each curve, CO_2 assimilation rates on a per leaf area basis (A_{area} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) were logged every four seconds across continuously ramping CO_2 concentrations, with a ramp rate of $100 \mu\text{mol mol}^{-1} \text{ min}^{-1}$ (consistent with recommendations by Stinziano et al., 2019; McClain & Sharkey, 2023) beginning at $5 \mu\text{mol mol}^{-1} \text{ CO}_2$ and concluding at $1700 \mu\text{mol mol}^{-1} \text{ CO}_2$. Otherwise, conditions in the leaf chamber were set to a photosynthetic photon flux density (PPDF) of $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ of photosynthetically active radiation (PAR; 400-700 nm), 50% relative humidity, leaf vapour pressure deficits of 1.7 KPa, and leaf temperatures of 25°C. Furthermore, CO_2 and H_2O sensors were readjusted using the range match function after every five leaf measurements, and each DAT $A-C_i$ curve required approximately 10 minutes, including

a 60-120 second acclimation period (Saathoff & Welles, 2021). Following the completion of each $A-C_i$ curve, we then allowed leaves to acclimate to ambient conditions for ~10 minutes. Then, we collected steady-state A_{\max} values for each leaf at the same environmental conditions as mentioned above with a constant CO_2 concentration at 420 ppm which matched current ambient levels. We logged steady-state gas A_{\max} values after leaves were allowed to stabilize for 5-10 minutes.

Immediately following gas exchange measurements, we used an HR1024i full spectrum portal field spectroradiometer (Spectra Vista Corporation, Poughkeepsie NY, USA) to collect reflectance spectra for each leaf. This instrument is a full-range spectroradiometer (350-2500 nm) with a spectral resolution of ≤ 3.5 nm (350-1000 nm), ≤ 9.5 nm (1000-1800 nm), and ≤ 6.5 nm (1800-2500 nm), outfitted with an LC-RP Pro leaf clip that includes a calibrated internal light source. Reflectance spectra were collected at the same location on the adaxial side of each leaf from which $A-C_i$ and steady state gas exchange were performed, with integration times set to two seconds, and reference spectra taken on a white Spectralon standard prior to each measurement.

Once physiological and reflectance data were acquired, we collected and transported individual leaves to the University of Toronto Scarborough for quantification of LMA and leaf N concentrations. First, we removed all petioles, and the fresh area of all leaves was quantified using an LI-3100C leaf area meter (Licor Bioscience, Lincoln, Nebraska, USA), and then dried for 48 hours to constant mass. Dried leaves were then weighed and LMA was calculated as mass/area. Finally, dried leaves were ground to a fine and homogeneous powder using a MM400 Retsch ball mill (Retsch Ltd., Hann, Germany), and a LECO CN 628 elemental analyzer (LECO Instruments, Ontario, Canada) was used to determine leaf N concentrations on ~0.1 grams of powdered tissue.

Data analysis

We used R statistical software v. 4.2.0 (R Foundations for Statistical Computing, Vienna, Austria) for all data analysis. First, we fit the Farquhar, von Caemmerer and Berry (FvCB) model to each individual $A-C_i$ curve, using the 'fitaci' function in the 'plantecophys' R package (Duursma, 2015), in order to estimate rates of V_{\max} and J_{\max} , along with their standard errors. These FvCB models were fit using non-linear least square regression (Duursma, 2015), such that V_{\max} and J_{\max} were corrected to 25°C, and V_{\max} and J_{\max} are considered apparent as mesophyll

conductance was assumed to be infinite. These data were merged with other traits, and the distribution of each individual trait was assessed using the ‘fitdist’ function in the ‘fitdistrplus’ R package (Delignette-Muller & Dutang, 2015). Traits were determined to be either normally or log-normally distributed (as per the highest log-likelihood value) and transformed data was employed in further analyses in accordance with these results. We then performed an analysis of variance (ANOVA) to test for significant trait differences across varieties.

We then followed the methods described by Burnett et al. (2021a) to evaluate how reflectance spectra predicted trait values across our dataset, using a PLSR modelling approach. All PLSR models included reflectance spectral data from the 500-2400 nm wavelength range and aimed to predict either non-transformed or log-transformed trait data, as informed by our distribution fitting procedure. For each PLSR model, the spectra-trait dataset was split into a calibration dataset (which included 80% of all data points) and a validation dataset (comprised of the remaining 20% of data). Since we were explicitly interested in testing the ability of reflectance spectra to quantify variation in leaf traits across grapes broadly, and the ability to differentiate varieties, we performed and analyzed two data splits. First, datasets were split into calibration vs. validation according to variety identity, such that both the validation and calibration datasets had approximately equal proportions of trait and spectra data from all varieties. Second, we used a completely randomized data split, whereby the proportion of data across varieties was allowed to vary randomly.

Using the calibration datasets, we then used the ‘find_optimal_components’ function in the ‘spectratrait’ R package (Lamour & Serbin, 2023) to determine the optimal number of components used in the final PLSR model, based on the minimization of the prediction residual sum of squares (PRESS) statistic. For each trait, a PLSR model was fitted from the calibration dataset using the leave-one-out cross-validation (LOO) procedure, specified with the ‘pls’ function in the ‘pls’ R package (Liland et al., 2022). Model performance was then assessed with the validation datasets as an external validation, in which the predicted values and the observed values in the validation dataset were compared. For the final models, we used the validation coefficient of determination (r^2), root mean squared error of prediction (RMSE), and percent root mean squared error of prediction (%RMSE) as metrics to illustrate model fits.

To further evaluate the model performance, we used the model coefficients and variable influences on projection (VIP) values to explore the effect of different areas of the spectra on

predicting the trait variable. Following this, we performed a jackknife permutation analysis to assess model uncertainty, using the jackknife argument of the 'plsr' function in the 'pls' R package (Liland et al., 2022). The resulting jackknife coefficients were then compared to that of the full model. And finally, using the full model and jackknife permutation outputs, the mean, and 95% confidence and prediction intervals were calculated for each predicted trait value from the validation dataset.

Results

Reflectance spectroscopy for predicting within-variety leaf traits

Leaf traits measured here all varied significantly as a function of variety identity ($p < 0.001$ in all cases). Specifically, across the entire dataset, physiological traits were most variable, with A_{\max} ranging from 3.8-29.0 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (CV=34.8), V_{\max} from 28.9-131.7 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (CV=27.5), and J_{\max} from 60.3-253.1 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (CV=25.8). In comparison, LMA and leaf N also varied significantly across varieties, though these traits were less variable with LMA ranging from 52.8-101.8 g m^{-2} (CV=12.8) and leaf N from 2.04-4.39% (CV=13.7). All reflectance spectra presented generally the same shape, with a few Cab. Franc individuals situated closer to the lower range, Merlot and Pinot Noir closer to the upper range, and others in and around the 95% confidence interval (Figure 1).

When calibration vs. validation data were evenly split across varieties (i.e., 80% of each variety allocated to each dataset), reflectance spectra and PLSR models explained between 18-64% of the variation in wine grape traits (Table 1, Figure 2). Specifically, physiological traits including A_{\max} , J_{\max} , and V_{\max} were predicted by 4-5 spectral components which cumulatively explained 18%, 44%, and 30% of the variation in these traits, respectively. In these cases, model %RMSE values ranged from 21.6% in A_{\max} models, 24.1% in V_{\max} models, and 18.9% in J_{\max} models. Comparatively, reflectance spectra and PLSR models expressed stronger predictive ability towards log-LMA and leaf N, with models (r^2) explaining 64% (%RMSE=14.3) and 62% (%RMSE=15.2%) of the variation, respectively (Table 1, Figure 2).

The predictive power of PLSR models was sensitive to the configuration of calibration and validation datasets, though general trends were nuanced. When calibration and validation datasets were comprised of varieties in random proportions, physiological traits were better predicted than in datasets where variety proportions were equal. Specifically, in randomized data

splits, A_{\max} model $r^2=0.29$, V_{\max} $r^2=0.58$, and J_{\max} $r^2=0.55$, all of which were higher vs. the same models in variety-weighted data splits. Alternatively, PLSR models for log-LMA and leaf N had lower predictive power when calibration and validation datasets were randomly created, with r^2 values of 0.53 and 0.5, respectively (Table 1, Figure 2). In all cases, the number of spectral components retained in the final PLSR models also differed depending on the nature of calibration and validation dataset construction.

The impact of the data splitting method is also observed in the model regression coefficient trends, which reflect the contribution of certain wavelengths to trait prediction. For physiological traits, the shapes of regression coefficient trends are similar within the same splitting method, but distinctly different between splitting methods (Figure 3). Here we ignore the random split model of A_{\max} from this comparison, due to its limited number of model components. On the other hand, splitting data randomly or proportionally across varieties did not influence the regression coefficient distributions of log-LMA or leaf N (Figure 3). VIP scores of the models suggest similar wavelength regions of importance for model prediction across different traits, regardless of data splitting methods (Figure 4).

Discussion

Our findings contribute to the growing literature that reflectance spectroscopy is well-equipped to detect trait variation within and among plant species (Serbin et al., 2014; Ely et al., 2019; Serbin et al., 2019; Burnett et al., 2021b; Lamour et al., 2021; Vasseur et al., 2022; Kothari et al., 2023). A considerable proportion of earlier work in this area focused on quantifying the interspecific trait variation that exists among plants of different functional types, that differ widely their evolutionary histories and trait diversity (e.g., Serbin et al., 2014; Kothari et al., 2023). To this end, previous studies have indicated that reflectance spectroscopy is better equipped to explain trait variation, in situations where trait values within calibration and validation datasets vary more widely (Lamour et al., 2021). This tendency positions these techniques for rapid trait estimation in natural ecosystems (Asner et al., 2016), with many such studies reporting a high predictive ability of PLSR models in quantifying interspecific trait variation. Though a recent renewed focus on the importance of intraspecific trait variation in driving ecosystem functioning (Siefert et al., 2015; Westerband et al., 2021), along with

applications of these techniques in agroecology, necessitates quantifying and disentangling the drivers of finer-scale trait variation that generally exists within species (Martin & Isaac, 2015).

In this regard, our results show the strong predictive power of PLSR models to capture between 50-64% of the within-species trait variation in wine grapes, for key LES and related traits including V_{cmax} , J_{max} , log-LMA, and leaf N (Table 1, Figure 2). Previous studies that examined within-species trait variation using PLSR approaches have yielded broadly similar results. For example, Meacham-Hensold et al. (2019) reported PLSR models that explained 60%, 59%, and 83% of the variation in V_{cmax} , J_{max} , and leaf N, respectively, across six tobacco (*Nicotiana tabacum*) genotypes, though when three additional genotypes and larger sample sizes were included in analyses, these PLSR model r^2 values increased to 0.61 for V_{cmax} , and 0.62 for J_{max} in the validation dataset. Similarly, Fu et al. (Fu et al., 2020) modelled photosynthetic traits of six tobacco genotypes using PLSR methods, and reported similar r^2 values (0.60 and 0.56) for V_{cmax} and J_{max} , respectively.

Other single-species studies that applied reflectance spectroscopy and PLSR models to predict leaf traits across experimental treatments or environmental gradients have also presented similar results. For example, Yendrek et al. (2017) found reflectance spectra were strong predictors of leaf N ($r^2=0.92-0.96$) and V_{cmax} ($r^2=0.56-0.65$) of maize (*Zea mays*) genotypes grown across gradients of ozone and soil N availability. Finally, in an analysis that screened over 200 genotypes of wheat (*Triticum aestivum*, *T. turgidum*, and triticale germplasm) from six sets of experiments, Silva-Perez et al. (2018) included detected high predictive power of PLSR models, with r^2 values ranging from 0.70-0.89 for leaf N, LMA, V_{cmax} , and J_{max} . Though in this same experiment, consistent with our results, CO_2 assimilation rates were relatively poorly captured by PLSR models: in our analysis, the r^2 for models for A_{max} were 0.18-0.29, vs. r^2 values of 0.49 in Silva-Perez et al. (2018).

In addition to model diagnostics alone, in our analysis PLSR models generally support the same inferences surrounding the comparative trait biology of wine grape varieties (relative to observed trait data). Specifically, our previous analysis of LES trait variation—with trait data observed in the field using traditional gas exchange and analytical chemistry techniques—found that white grape varieties Sauvignon Blanc and Viognier occupy the “resource-acquiring” end of an intraspecific LES in wine grapes (characterized by high rates of A_{max} , V_{cmax} , J_{max} , leaf N, and low LMA), while red varieties (Cabernet Franc, Cabernet Sauvignon, Merlot) define the

“resource-conserving” end of the wine grape LES (characterized by low A_{\max} , V_{\max} , J_{\max} , leaf N, and high LMA). Our PLSR models support this same general trend (Figure 1), with white varieties expressing predictions that indicate resource-acquiring trait values.

Quantifying functional trait variation across the wide diversity of wine grapes is challenging, with estimates suggesting there exist anywhere between 6,000-10,000 varieties *Vitis vinifera* subsp. *vinifera* worldwide. In turn, ~1,100 of these varieties are cultivated solely for wine production, though recent data suggests the genetic diversity being planted on vineyards is narrowing considerably, with ~12 “international varieties” now comprising up to 70-80% of vineyards in prominent wine-growing nations (Wolkovich et al., 2018). Reflectance spectroscopy therefore representing one potential avenue for quantifying this wide phenotypic diversity, and in turn, meeting a pressing need for high-throughput techniques able to quantify the traits that underpin wine grape responses to environmental change (Cadle-Davidson et al., 2019).

To this end, our analysis contributes evidence that reflectance spectroscopy and PLSR modelling approaches can be used to 1) directly predict intraspecific trait variation with a relatively high degree of accuracy in wine grapes, and 2) differentiate intraspecific variation in life-history strategies in across grape varieties. Though our analysis here is based on a small subset of the 1,000s of wine grape varieties that exist globally (Wolkovich et al., 2018), expanding this work to include a greater number of wine grape genotypes and traits such as those associated with drought tolerance (De Bei et al., 2011; Silva-Perez et al., 2018), presents a viable opportunity to more rapidly screen trait expression in one of the world’s most economically important crops.

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Author Contributions

B.C. and A.R.M. planned and designed the research; A.R.M., B.C., and R.O.M. performed field and lab analyses; B.C. performed statistical analyses; B.C. and A.R.M. wrote the draft manuscript; R.O.M., G.R., and K.C. edited the manuscript; K.C, G.R., and A.R.M. secured funding related to this research.

Data Availability Statement

Functional trait data employed in this analysis are available through the TRY Functional Trait Database. Furthermore, raw spectral reflectance data, PLSR models, and functional trait datasets are also available upon request to the corresponding author.

Conflict of Interest Statement

The authors declare no conflicts of interest.

References

- Anderegg LD, Berner LT, Badgley G, Sethi ML, Law BE, HilleRisLambers J. (2018). Within-species patterns challenge our understanding of the leaf economics spectrum. *Ecology Letters* **21**(5): 734-744.
- Asner GP, Knapp DE, Anderson CB, Martin RE, Vaughn N. (2016). Large-scale climatic and geophysical controls on the leaf economics spectrum. *Proceedings of the National Academy of Sciences* **113**(28): E4043-E4051.
- Asner GP, Martin RE, Anderson CB, Knapp DE. (2015). Quantifying forest canopy traits: Imaging spectroscopy versus field survey. *Remote Sensing of Environment* **158**: 15-27.
- Bernardo S, Dinis L-T, Machado N, Moutinho-Pereira J. (2018). Grapevine abiotic stress assessment and search for sustainable adaptation strategies in Mediterranean-like climates. A review. *Agronomy for Sustainable Development* **38**(6): 66.
- Blonder B, Violle C, Bentley LP, Enquist BJ. (2011). Venation networks and the origin of the leaf economics spectrum. *Ecology Letters* **14**(2): 91-100.
- Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VH, Schreiber SJ, Urban MC, Vasseur DA. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution* **26**(4): 183-192.
- Burnett AC, Anderson J, Davidson KJ, Ely KS, Lamour J, Li Q, Morrison BD, Yang D, Rogers A, Serbin SP. (2021a). A best-practice guide to predicting plant traits from leaf-level hyperspectral data using partial least squares regression. *Journal of Experimental Botany* **72**(18): 6175-6189.
- Burnett AC, Serbin SP, Rogers A. (2021b). Source: sink imbalance detected with leaf-and canopy-level spectroscopy in a field-grown crop. *Plant, Cell & Environment* **44**(8): 2466-2479.

- Cadle-Davidson L, Londo J, Martinez D, Sapkota S, Gutierrez B. (2019). From phenotyping to phenomics: present and future approaches in grape trait analysis to inform grape gene function. *The Grape Genome*: 199-222.
- Carmona CP, Bueno CG, Toussaint A, Träger S, Díaz S, Moora M, Munson AD, Pärtel M, Zobel M, Tamme R. (2021). Fine-root traits in the global spectrum of plant form and function. *Nature* **597**(7878): 683-687.
- Chadwick KD, Brodrick PG, Grant K, Goulden T, Henderson A, Falco N, Wainwright H, Williams KH, Bill M, Breckheimer I. (2020). Integrating airborne remote sensing and field campaigns for ecology and Earth system science. *Methods in Ecology and Evolution* **11**(11): 1492-1508.
- Cramer GR. (2010). Abiotic stress and plant responses from the whole vine to the genes. *Australian Journal of Grape and Wine Research* **16**: 86-93.
- De Bei R, Cozzolino D, Sullivan W, Cynkar W, Fuentes S, Dambergers R, Pech J, Tyerman S. (2011). Non-destructive measurement of grapevine water potential using near infrared spectroscopy. *Australian Journal of Grape and Wine Research* **17**(1): 62-71.
- Dechant B, Cuntz M, Vohland M, Schulz E, Doktor D. (2017). Estimation of photosynthesis traits from leaf reflectance spectra: Correlation to nitrogen content as the dominant mechanism. *Remote Sensing of Environment* **196**: 279-292.
- Delignette-Muller ML, Dutang C. (2015). fitdistrplus: An R package for fitting distributions. *Journal of Statistical Software* **64**: 1-34.
- Diago MP, Fernandes AM, Millan B, Tardáguila J, Melo-Pinto P. (2013). Identification of grapevine varieties using leaf spectroscopy and partial least squares. *Computers and Electronics in Agriculture* **99**: 7-13.
- Díaz S, Cabido M. (1997). Plant functional types and ecosystem function in relation to global change. *Journal of Vegetation Science* **8**(4): 463-474.
- Díaz S, Hodgson J, Thompson K, Cabido M, Cornelissen JH, Jalili A, Monserrat-Martí G, Grime J, Zarrinkamar F, Asri Y. (2004). The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science* **15**(3): 295-304.
- Díaz S, Kattge J, Cornelissen JH, Wright IJ, Lavorel S, Dray S, Reu B, Kleyer M, Wirth C, Colin Prentice I. (2016). The global spectrum of plant form and function. *Nature* **529**(7585): 167-171.
- Doughty CE, Santos-Andrade P, Goldsmith G, Blonder B, Shenkin A, Bentley L, Chavana-Bryant C, Huaraca-Huasco W, Díaz S, Salinas N. (2017). Can leaf spectroscopy predict leaf and forest traits along a Peruvian tropical forest elevation gradient? *Journal of Geophysical Research: Biogeosciences* **122**(11): 2952-2965.
- Duursma RA. (2015). Plantecophys-an R package for analysing and modelling leaf gas exchange data. *PLoS One* **10**(11): e0143346.
- Ely KS, Burnett AC, Lieberman-Cribbin W, Serbin SP, Rogers A. (2019). Spectroscopy can predict key leaf traits associated with source-sink balance and carbon-nitrogen status. *Journal of Experimental Botany* **70**(6): 1789-1799.
- Fahlgren N, Gehan MA, Baxter I. (2015). Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. *Current Opinion in Plant Biology* **24**: 93-99.
- Food and Agriculture Organization of the United Nations (2024). FAOSTAT statistical database: [Rome] : FAO, c1997-.

- Fu P, Meacham-Hensold K, Guan K, Wu J, Bernacchi C. (2020). Estimating photosynthetic traits from reflectance spectra: a synthesis of spectral indices, numerical inversion, and partial least square regression. *Plant, Cell & Environment* **43**(5): 1241-1258.
- Funk JL, Larson JE, Ames GM, Butterfield BJ, Cavender-Bares J, Firn J, Laughlin DC, Sutton-Grier AE, Williams L, Wright J. (2017). Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews* **92**(2): 1156-1173.
- Gambetta GA, Herrera JC, Dayer S, Feng Q, Hochberg U, Castellarin SD. (2020). The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. *Journal of Experimental Botany* **71**(16): 4658-4676.
- Gishen M, Damberg R, Cozzolino D. (2005). Grape and wine analysis-enhancing the power of spectroscopy with chemometrics. A review of some applications in the Australian wine industry. *Australian Journal of Grape and Wine Research* **11**(3): 296-305.
- Greer DH, Weedon MM. (2012). Modelling photosynthetic responses to temperature of grapevine (*Vitis vinifera* cv. Semillon) leaves on vines grown in a hot climate. *Plant, Cell & Environment* **35**(6): 1050-1064.
- Gregory LM, Roze LV, Walker BJ. (2023). Increased activity of core photorespiratory enzymes and CO₂ transfer conductances are associated with higher and more optimal photosynthetic rates under elevated temperatures in the extremophile *Rhazya stricta*. *Plant, Cell & Environment* **46**(12): 3704-3720.
- Heckmann D, Schlüter U, Weber AP. (2017). Machine learning techniques for predicting crop photosynthetic capacity from leaf reflectance spectra. *Molecular plant* **10**(6): 878-890.
- Hewer MJ, Gough WA. (2020). Assessing the impact of projected climate change on the future of grape growth and wine production in the Niagara Peninsula (Canada). *Journal of Wine Research* **31**(1): 6-34.
- Isaac ME, Martin AR, de Melo Virginio Filho E, Rapidel B, Roupsard O, Van den Meersche K. (2017). Intraspecific trait variation and coordination: Root and leaf economics spectra in coffee across environmental gradients. *Frontiers in Plant Science* **8**: 1196.
- Keller M. (2020). *The science of grapevines*: Academic Press.
- Kothari S, Beauchamp-Rioux R, Blanchard F, Crofts AL, Girard A, Guilbeault-Mayers X, Hacker PW, Pardo J, Schweiger AK, Demers-Thibeault S. (2023). Predicting leaf traits across functional groups using reflectance spectroscopy. *New Phytologist* **238**(2): 549-566.
- Lamour J, Davidson KJ, Ely KS, Anderson JA, Rogers A, Wu J, Serbin SP. (2021). Rapid estimation of photosynthetic leaf traits of tropical plants in diverse environmental conditions using reflectance spectroscopy. *PLoS One* **16**(10): e0258791.
- Lamour J, Serbin S (2023). spectratrait: A simple add-on package to aid in the fitting of leaf-level spectra-trait PLSR models.
- Lavorel S, Garnier E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology* **16**(5): 545-556.
- Liland K, Mevik B, Wehrens R (2022). pls: Partial Least Squares and Principal Component Regression.
- Liles C, Verdon-Kidd D. (2020). Refining the growing season temperature parameter for use in winegrape suitability analysis. *Australian Journal of Grape and Wine Research* **26**(4): 343-357.

- Macklin SC, Mariani RO, Young EN, Kish R, Cathline KA, Robertson G, Martin AR. (2022). Intraspecific leaf trait variation across and within five common wine grape varieties. *Plants* **11**(20): 2792.
- Martin AR, Isaac ME. (2015). Plant functional traits in agroecosystems: a blueprint for research. *Journal of Applied Ecology* **52**(6): 1425-1435.
- Martin AR, Mariani RO, Cathline KA, Duncan M, Paroshy NJ, Robertson G. (2022). Soil compaction drives an intra-genotype leaf economics spectrum in wine grapes. *Agriculture* **12**(10): 1675.
- McClain AM, Sharkey TD. (2023). Rapid CO₂ changes cause oscillations in photosynthesis that implicate PSI acceptor-side limitations. *Journal of Experimental Botany* **74**(10): 3163-3173.
- Meacham-Hensold K, Montes CM, Wu J, Guan K, Fu P, Ainsworth EA, Pederson T, Moore CE, Brown KL, Raines C. (2019). High-throughput field phenotyping using hyperspectral reflectance and partial least squares regression (PLSR) reveals genetic modifications to photosynthetic capacity. *Remote Sensing of Environment* **231**: 111176.
- Milla R, Osborne CP, Turcotte MM, Violle C. (2015). Plant domestication through an ecological lens. *Trends in Ecology & Evolution* **30**(8): 463-469.
- Onoda Y, Wright IJ. (2018). The leaf economics spectrum and its underlying physiological and anatomical principles. *The Leaf: A Platform for Performing Photosynthesis*: 451-471.
- Onoda Y, Wright IJ, Evans JR, Hikosaka K, Kitajima K, Niinemets Ü, Poorter H, Tosens T, Westoby M. (2017). Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytologist* **214**(4): 1447-1463.
- Perez-Harguindeguy N, Diaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, Bret-Harte MS, Cornwell WK, Craine JM, Gurvich DE. (2016). Corrigendum to: New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of botany* **64**(8): 715-716.
- Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD. (1999). Generality of leaf trait relationships: a test across six biomes. *Ecology* **80**(6): 1955-1969.
- Saathoff AJ, Welles J. (2021). Gas exchange measurements in the unsteady state. *Plant, Cell & Environment* **44**(11): 3509-3523.
- Santillán D, Iglesias A, La Jeunesse I, Garrote L, Sotes V. (2019). Vineyards in transition: A global assessment of the adaptation needs of grape producing regions under climate change. *Science of the Total Environment* **657**: 839-852.
- Serbin SP, Dillaway DN, Kruger EL, Townsend PA. (2012). Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. *Journal of Experimental Botany* **63**(1): 489-502.
- Serbin SP, Singh A, McNeil BE, Kingdon CC, Townsend PA. (2014). Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecological Applications* **24**(7): 1651-1669.
- Serbin SP, Wu J, Ely KS, Kruger EL, Townsend PA, Meng R, Wolfe BT, Chlus A, Wang Z, Rogers A. (2019). From the Arctic to the tropics: multibiome prediction of leaf mass per area using leaf reflectance. *New Phytologist* **224**(4): 1557-1568.
- Shipley B, Lechowicz MJ, Wright I, Reich PB. (2006). Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology* **87**(3): 535-541.

- Siefert A, Violle C, Chalmandrier L, Albert CH, Taudiere A, Fajardo A, Aarssen LW, Baraloto C, Carlucci MB, Cianciaruso MV. (2015). A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecology Letters* **18**(12): 1406-1419.
- Silva-Perez V, Molero G, Serbin SP, Condon AG, Reynolds MP, Furbank RT, Evans JR. (2018). Hyperspectral reflectance as a tool to measure biochemical and physiological traits in wheat. *Journal of Experimental Botany* **69**(3): 483-496.
- Singh A, Serbin SP, McNeil BE, Kingdon CC, Townsend PA. (2015). Imaging spectroscopy algorithms for mapping canopy foliar chemical and morphological traits and their uncertainties. *Ecological Applications* **25**(8): 2180-2197.
- Stinziano JR, McDermitt DK, Lynch DJ, Saathoff AJ, Morgan PB, Hanson DT. (2019). The rapid A/C_i response. *New Phytologist* **221**(2): 625-627.
- van Leeuwen C, Sgubin G, Bois B, Ollat N, Swingedouw D, Zito S, Gambetta GA. (2024). Climate change impacts and adaptations of wine production. *Nature Reviews Earth & Environment*: 1-18.
- Vasseur F, Cornet D, Beurrier G, Messier J, Rouan L, Bresson J, Ecartot M, Stahl M, Heumos S, Gérard M. (2022). A perspective on plant phenomics: coupling deep learning and near-infrared spectroscopy. *Frontiers in Plant Science* **13**: 836488.
- Violle C, Enquist BJ, McGill BJ, Jiang L, Albert CH, Hulshof C, Jung V, Messier J. (2012). The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution* **27**(4): 244-252.
- Westerband A, Funk J, Barton K. (2021). Intraspecific trait variation in plants: a renewed focus on its role in ecological processes. *Annals of Botany* **127**(4): 397-410.
- Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ. (2002). Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* **33**(1): 125-159.
- Westoby M, Wright IJ. (2006). Land-plant ecology on the basis of functional traits. *Trends in Ecology & Evolution* **21**(5): 261-268.
- White MA, Diffenbaugh N, Jones GV, Pal JS, Giorgi F. (2006). Extreme heat reduces and shifts United States premium wine production in the 21st century. *Proceedings of the National Academy of Sciences* **103**(30): 11217-11222.
- Wolkovich E, Burge D, Walker MA, Nicholas K. (2017). Phenological diversity provides opportunities for climate change adaptation in winegrapes. *Journal of Ecology* **105**(4): 905-912.
- Wolkovich E, García de Cortázar-Atauri I, Morales-Castilla I, Nicholas K, Lacombe T. (2018). From Pinot to Xinomavro in the world's future wine-growing regions. *Nature Climate Change* **8**(1): 29-37.
- Wright IJ, Reich PB, Cornelissen JH, Falster DS, Garnier E, Hikosaka K, Lamont BB, Lee W, Oleksyn J, Osada N. (2005). Assessing the generality of global leaf trait relationships. *New Phytologist* **166**(2): 485-496.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M. (2004). The worldwide leaf economics spectrum. *Nature* **428**(6985): 821-827.
- Xiong D, Flexas J. (2018). Leaf economics spectrum in rice: leaf anatomical, biochemical, and physiological trait trade-offs. *Journal of Experimental Botany* **69**(22): 5599-5609.

598 Yendrek CR, Tomaz T, Montes CM, Cao Y, Morse AM, Brown PJ, McIntyre LM, Leakey AD,
599 Ainsworth EA. (2017). High-throughput phenotyping of maize leaf physiological and
600 biochemical traits using hyperspectral reflectance. *Plant Physiology* **173**(1): 614-626.

601

Tables and Figures

Table 1. Partial least squares regression model fits evaluating the ability of reflectance spectra to explain variation in leaf traits measured on six wine grape varieties. Presented here are results from two different modelling approaches which divide our total sample into calibration (80% of our data) vs. validation (20% of our data) datasets. In the results associated with the “Variety” approach, calibration and validation data both included approximately the same proportions of observations from all varieties, while the “Random” approach made this division randomly. Here, n_{obs} refers to the total observations in our dataset for a given trait, which entails a corresponding sample size in the validation dataset (n_{val}). For each model, we present the number of components derived from reflectance spectra that were included in the final predictive model (n_{comp}), along with the root mean square error (RSME), r^2 value, and %RMSE for the final predictive model. All models were based on Trait acronyms as follows: light saturated photosynthetic rate (A_{max}), maximum velocity of Ribulose 1,5-bisphosphate (RuBP) carboxylation (V_{cmax}), maximum rate of electron transport (J_{max}), leaf mass per area (LMA), leaf nitrogen (N) concentration.

| Data split | Trait | n_{obs} | n_{val} | n_{comp} | RMSE | r^2 | %RMSE |
|------------|-------------------|------------------|------------------|-------------------|-------|-------|-------|
| Variety | A_{max} | 178 | 36 | 4 | 3.9 | 0.18 | 21.63 |
| | V_{cmax} | 177 | 36 | 5 | 14.62 | 0.3 | 24.1 |
| | J_{max} | 177 | 36 | 4 | 24.22 | 0.44 | 18.88 |
| | log-LMA | 178 | 36 | 10 | 0.08 | 0.64 | 14.27 |
| | Leaf N | 176 | 36 | 9 | 0.25 | 0.62 | 15.16 |
| Random | A_{max} | 178 | 36 | 1 | 4.42 | 0.29 | 19.25 |
| | V_{cmax} | 177 | 36 | 9 | 14.47 | 0.58 | 15.6 |
| | J_{max} | 177 | 36 | 8 | 28.35 | 0.55 | 15.6 |
| | log-LMA | 178 | 36 | 13 | 0.08 | 0.53 | 16.44 |
| | Leaf N | 176 | 36 | 11 | 0.33 | 0.5 | 15.92 |

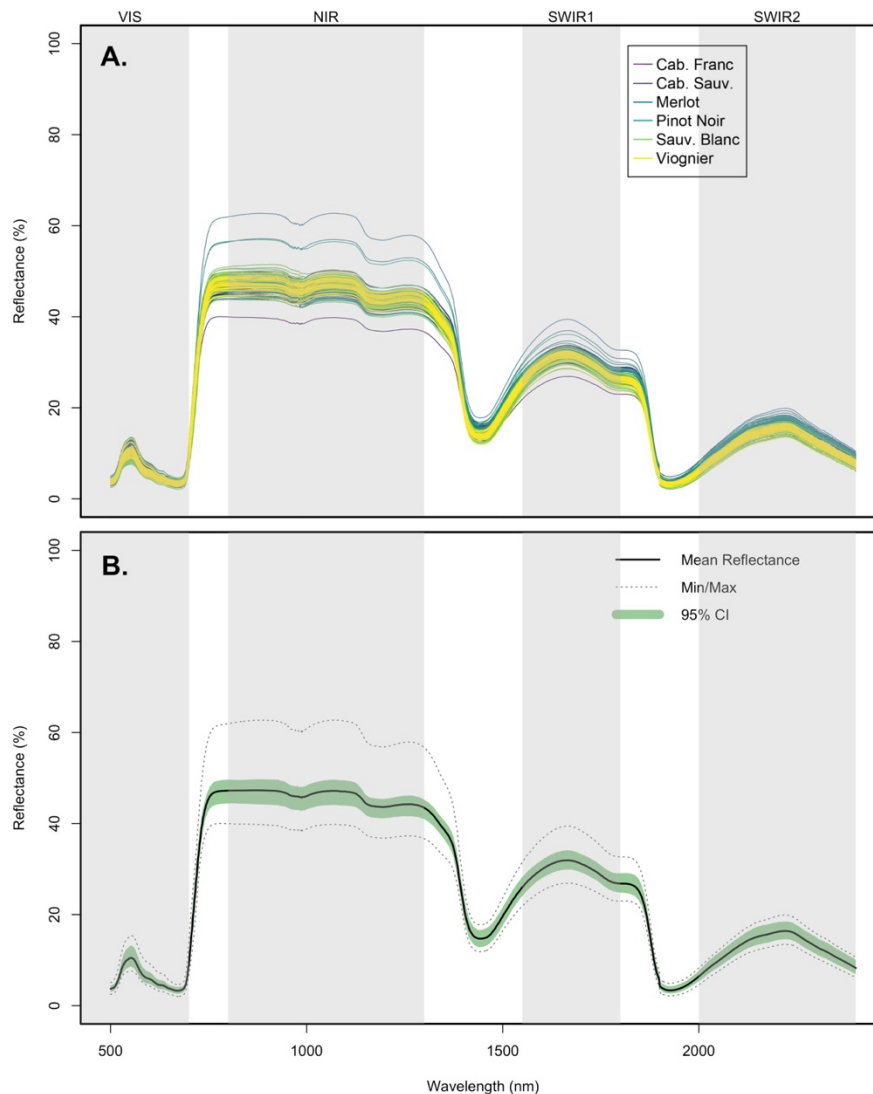


Figure 1. Reflectance spectra of 179 wine grape leaves plotted A) individually with six wine grape varieties specified, and B) all together with mean, range, and 95% confidence interval estimates. All spectral data were trimmed to the 500-2400 nm range where the PLSR models were built from. The grey shaded areas indicate different spectral regions: Visible Spectrum (VIS), Near Infrared (NIR), Short Wave Infrared 1 (SWIR1), and Short Wave Infrared 2 (SWIR2).

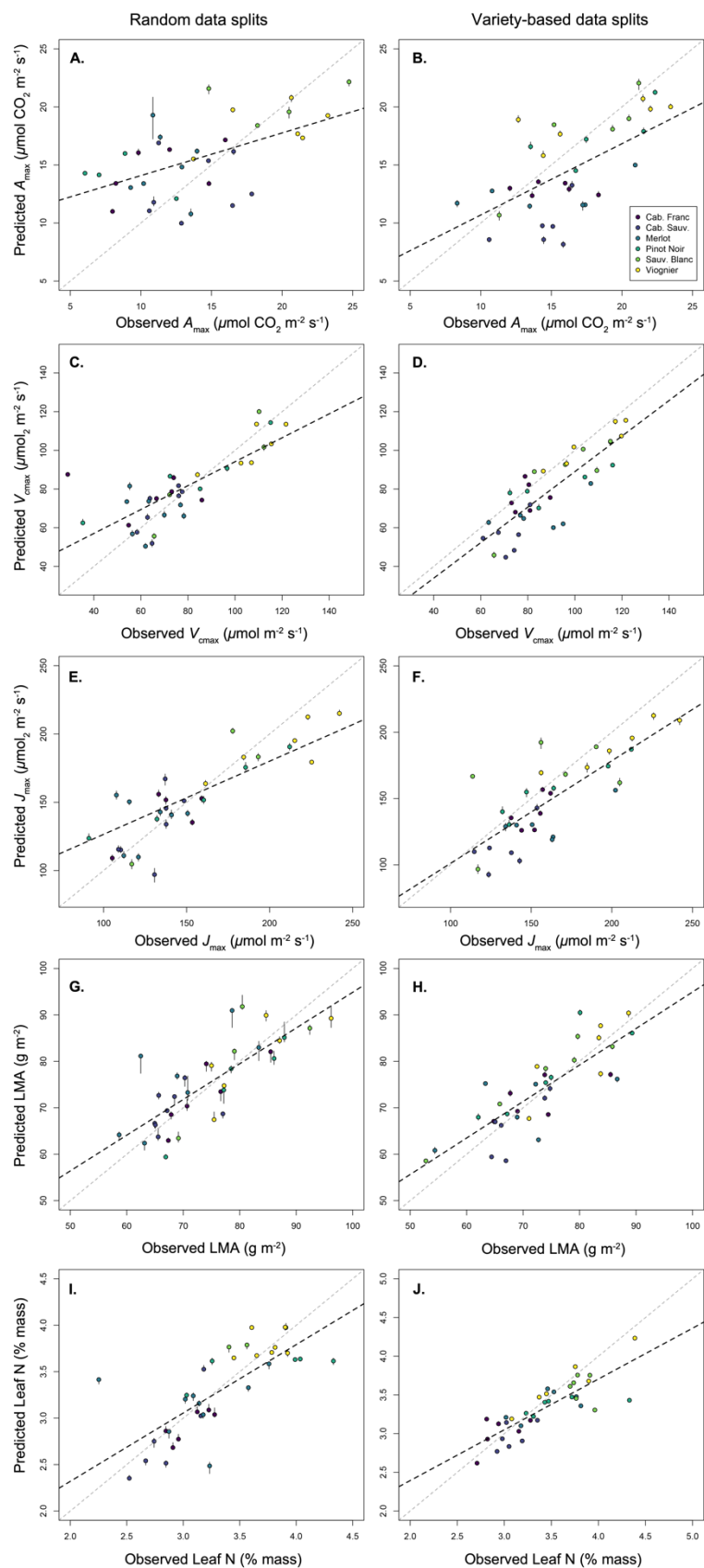
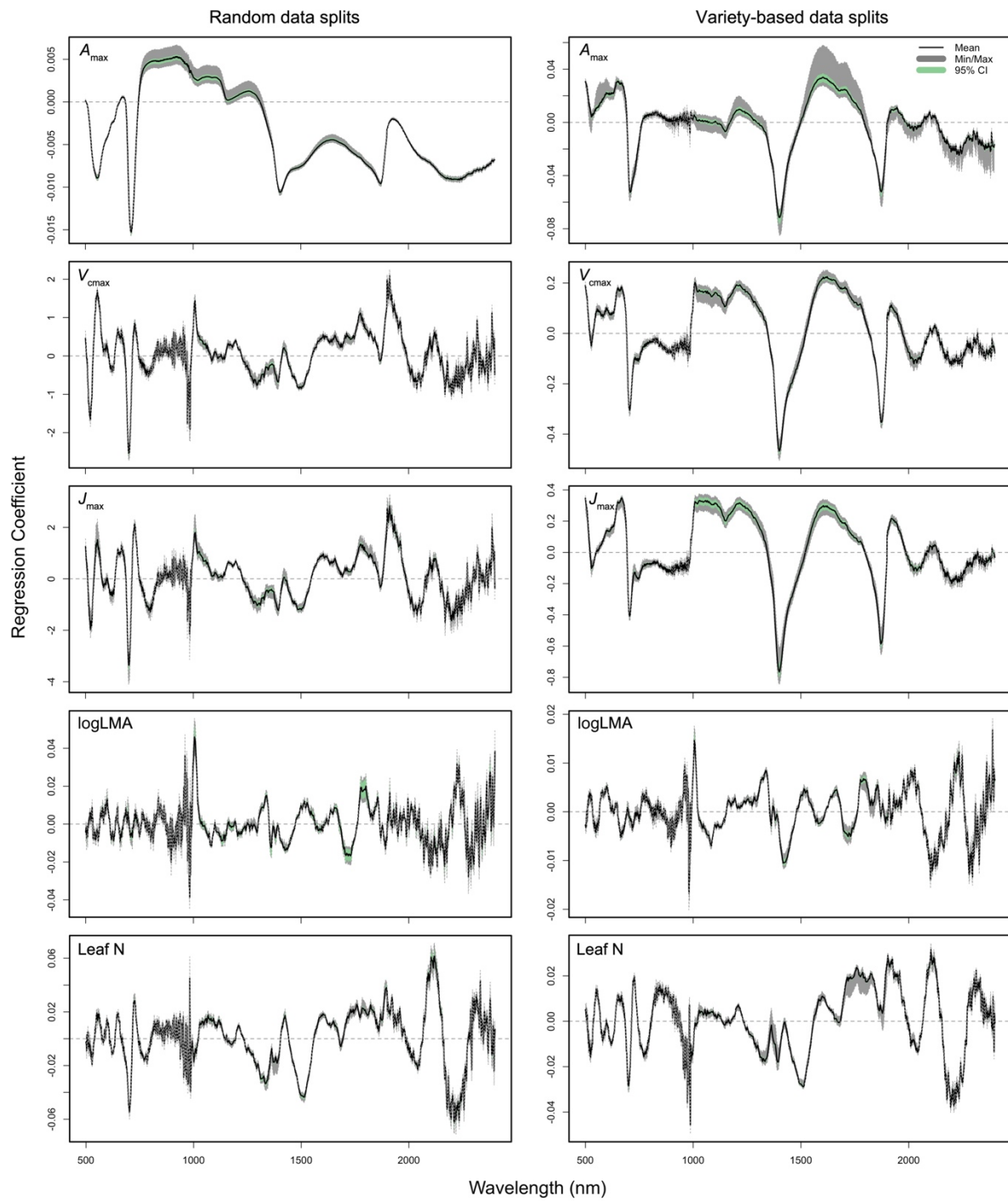
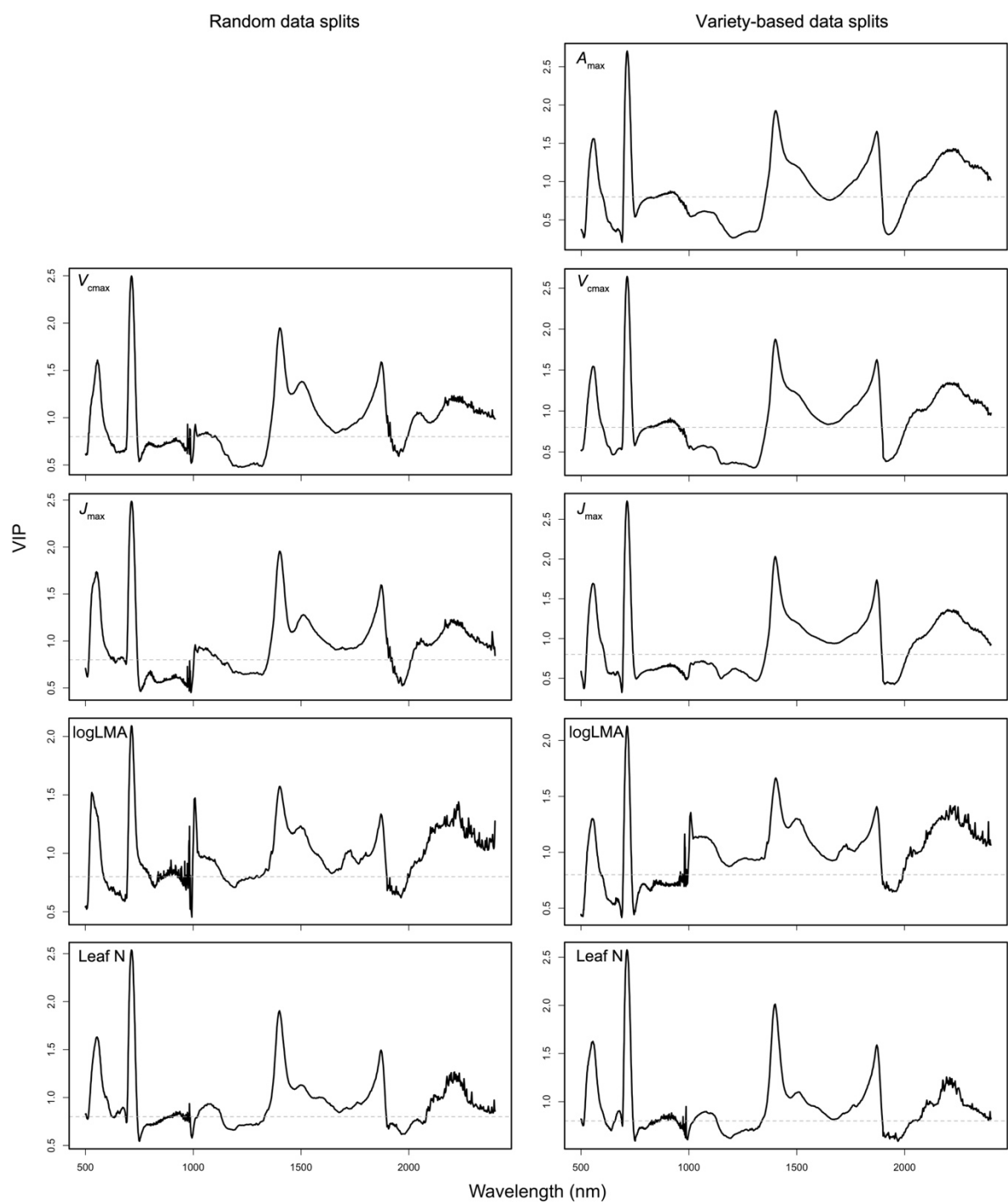


Figure 2. Results of partial least squares regression (PLSR) models predicting leaf physiological, chemical, and morphological traits in six wine grape varieties. Shown here are the data points used to validate the models ($n=36$ in all cases) fitted to a set of calibration data points ($n=140-142$; see Table 1). Calibration and validation datasets were selected on the basis of a fully randomized data split (left panels), and a data split where all six varieties were equally represented in the calibration datasets (right panels). Dashed black lines represent linear model fits between observed vs. expected trait values, while the dotted gray lines represent a 1:1 relationship.



637 **Figure 3.** Jackknife regression coefficients of the PLSR models of A_{\max} , V_{\max} , J_{\max} , log-LMA,
638 and leaf N, based on the calibration data. The dashed horizontal line in each panel indicates
639 where the coefficient is zero. The black curve represents the mean, the grey area represents the
640 range, and the green area represents the 95% confidence interval.



642 **Figure 4.** Variable influences on projection (VIP) scores of the final PLSR models of A_{\max} ,
643 V_{\max} , J_{\max} , log-LMA, and leaf N. The dashed horizontal line in each panel indicates where the
644 VIP score is 0.8. The A_{\max} model using random data split method had one component and
645 therefore did not generate valid VIP scores.