Title: Integration of leaf spectral reflectance variability facilitates identification of plant leaves at different taxonomic levels.

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Abstract

Plant identification is crucial to the conservation and management of natural areas. The shortwave spectral reflectance of leaves is a promising tool for rapidly identifying species at different taxonomic ranks. However, the spectral reflectance of leaves changes in response to biotic and abiotic conditions. This investigation asked how this variability affects the accuracy of methods used to predict plant taxonomies and what factors most influence the spectral signature of leaves. To answer these questions, we measured the reflectance of leaves of 62 woody species from the living collection at the NYBG twice in two pairwise samplings. We found that PLS-DA accuracy improved when we used a larger sample of natural variance in the classification model. Finally, to evaluate whether there was an influence of the species' relatedness or the growing environment on structural and biochemical traits predicted from the leaf reflectance, we ran a phylogenetic signal analysis and a series of mixed effects model analyses that showed no phylogenetic but an environmental influence. We found that the increase in temperature and relative humidity variability explained the increment of predicted carotene and the decrease of Nitrogen content for the first pairwise analysis. For the second pairwise analysis, we found that the reduction of relative humidity variability explained leaf water and Nitrogen content decrease, and relative humidity decrease combined with day length decrease explained a decline in LMA.

Key words: spectral reflectance, taxonomic classification, PLS-DA, PROSPECT-D RTM, PLSR, predicted structural and biochemical traits, phylogenetic signal, environmental influence.

1. INTRODUCTION

Plant identification is key to conservation (Trias-Blasi & Vorontsova, 2015) and management (Noss, 2001), and hence essential for human survival (Stuessy et al., 2014). Recent studies have shown that the shortwave spectral reflectance of leaves (0.3 to 2.5 microns), captured with proximal remote sensing instruments, is a promising tool for rapidly identifying species of plants into their different taxonomic ranks with high accuracy (Asner et al., 2014; Buitrago et al., 2018; Cavender-Bares et al., 2016, 2017; Cochrane, 2000; Durgante et al., 2013; Féret & Asner, 2011; Hesketh & Sánchez-Azofeifa, 2012; Lang et al., 2015, 2017; Meireles et al., 2020; Paiva et al., 2021; Price, 1994; Stasinski et al., 2021). It is expected that with the advent of new hyperspectral satellites (Qian, 2021), form the ground (e.g. in rapid floristic assessments; Higgins & Ruokolainen, 2004; Marcelo-Peña et al., 2015) the ability to identify plant species over large areas and through time with spectral methods will improve.

However, the spectral reflectance of leaves of any given species is excepted to change over time (Hesketh & Sánchez-Azofeifa, 2012). Because of this, before the addition of the spectra to the plethora of traits required for taxonomy or phylogenetics (Vale, 2019), we need to understand the effect of these variability on the spectral signature of leaves (Castro-Esau et al., 2006; Hesketh & Sánchez-Azofeifa, 2012).

The spectral signature or the spectral reflectance pattern of a leaf is the intensity of incident radiation reflected to the environment, measured across the visible (VIS), near infrared (NIR), and shortwave infrared (SWIR) portions of the electromagnetic spectrum, from 400 nm to 2400 nm. This reflectance pattern varies depending on the chemical and structural attributes of the

leaf, (e.g. photosynthetic pigments, water, cellulose, lignin; Brewer, 2012), and secondary metabolites concentrations (Fine et al., 2021). Because these components absorb, reflect, and transmit portions of the electromagnetic spectrum differentially (Campbell & Norman, 1998; Schweiger et al., 2017) the spectral signature is a phenotypic manifestation of leaves. Moreover, the environment and the evolutionary history (phylogenetic effect) of a plant promote variation in leaf phenotype (Díaz et al., 2016; Doyle, 2007; Fernando et al., 2007; Little et al., 2010) and the spectral signature is not an exception (Cavender-Bares et al., 2016; Meireles et al., 2020). Phylogenetic effects impact wavelengths differentially at higher taxonomic ranks, and differ among classes (Meireles et al., 2020). The spectral signatures of plant species, from dry and moist habitats in Panama, have shown to vary with the seasons of the year (Hesketh & Sánchez-Azofeifa, 2012). Spectral reflectance also differ across individuals of the same species when found in diffent habitas (Castro-Esau et al., 2006).

Most of the traits affecting the spectral signature of leaves are labile (Ball et al., 2015; Cotrozzi et al., 2017; De La Riva et al., 2016; Fyfe, 2003; Gamon et al., 2015; González-Rebeles et al., 2021; Li et al., 2018; Nguyen et al., 2017; Nunes et al., 2019; Paz-Kagan & Asner, 2017; Reich et al., 1991; Yang et al., 2016; Young, 1991). It is possible to predict many chemical and structural leaf traits from the spectral signature by using inversion of leaf level radiative transfer models (Fourty et al., 1996; Jacquemoud & Baret, 1990) regressions methods such as Partial Least Squares (PLSR; Serbin et al., 2014), and previous studies suggest that reflectance data closely tracks underlying changes in leaf biochemistry (e.g. Yang et al., 2016). Hence, we can use these traits to evaluate spectral responses to a fluctuating environment. Moreover, since the predicted values of these chemical and structural traits are a good approximation of empiric

measurements (Blackburn, 2007; Burnett, Anderson, et al., 2021; Ely et al., 2019; Féret & Asner, 2011; Fourty et al., 1996; Nakaji et al., 2019; Serbin et al., 2014; Shiklomanov et al., 2016), it is possible to make inference on what trait is contributing the most to the temporal variability of the spectra.

The present work explores how the spectral signature of leaves and their temporal variability help us to identify tropical woody species from several regions of the world yet found in an artificial environment: the Enid A. Haup Conservatory (EAHC), at the New York Botanical Garden (NYBG). First, we ask how the variation of the spectral signature of leaves impacts the identification of species into their taxonomic levels (from family to the species) and how to better integrate the variation of the spectra into the classification models. We expect that the integration of all the temporal spectral variability will outperform the classification compared to the classification of species based on just one collecting season. Second, we ask what environmental factors within the EAHC (mean and standard deviation of temperature and relative humidity, and mean day length) have an effect over the predicted values of leaf photosynthetic pigments (total chlorophyl a & b content, carotene content), leaf water content, leaf nitrogen content, and leaf dry mass per area (LMA), where we expect variation of these traits as a response to the changing environmental conditions.

2. METHODS:

2.1. Data Collection

We collected leaves from a total of 62 woody species (Table 1S. One individual per species) belonging to the botanical families Acanthaceae, Ericaceae, Fabaceae, Myrtaceae, Rubiaceae, and Solanaceae from the Rainforest Pavilion at the EAHC. The initial collection was made

during the days of the year (DOY) 105 and 112 during the spring of 2019 where we collected five leaves from 51 species (1 individual per species). The second collection was during the summer of 2020 (DOY 251) where we collected 3 leaves from 24 species previously sampled and collected 18 new species (1 individual per species). The final collection took place during the winter of 2021 (DOY 57) where we also collected 3 leaves from 16 species previously collected and 3 new species (1 individual per species). All collections were made between 10 am and 3pm, the number of leaves were different during the second and third collection due to the COVID19 pandemic restrictions at the NYBG.

Two spectroradiometers were used in 2019: A Spectra Vista Corporation (SVC HR 1024i) and Spectral Evolution (SE PSR+) using the same type of foreoptic, an SVC LC-RP-Pro leaf clip with an internal, calibrated and full-spectrum light source. All leaf reflectance measurements were made inside the LC-RP-Pro leaf clips and were calibrated with an Spectralon® white reference. Despite using two different spectroradiometers, because they both covered the same spectral range, were radiometrically calibrated, and used the same Spectralon® references, the data could be combined together without needing any additional inter-calibration (e.g. Serbin et al., 2019). In 2020 and 2021 we used the SVC HR 1024i with the same leaf clip.

2.2. **Prediction of Leaf Structural and Chemical Traits.**

To estimate pigment (chlorophylls and carotenoids) and leaf water content, we used the Bayesian inversion of the PROSPECT-D RTM as implemented in the rrtm package (https://github.com/ashiklom/rrtm). In PROSPECT, the leaf is simulated as an *N* number of semi-translucid flat plates, where each plate has a specific wavelength-dependent transmissivity

(Feret et al., 2008). Moreover, the model transmissivity is based on the linear combination of specific spectral absorption measurements of the total photosynthetic pigment content (chlorophyll a + b and carotenoids) as well as water and dry matter content, which are multiplied by their respective quantities (Cab µg/cm^2, Car µg/cm^2, Cw g/cm^2) obtained from known datasets: LOPEX, ANGERS, HAWAII, and CALMIT. To evaluate the accuracy in the resulting parameter values, we used Shiklomanov et al., (2016) framework that provides the joint probability distribution of the PROSPECT parameters, with the residual's standard deviation as outputs.

To obtain leaf mass per area (LMA) and leaf Nitrogen content per area, we used the PLSR framework implemented in Burnett et al. (2021), which predicts these traits by using published coefficients (https://zenodo.org/badge/latestdoi/222699149). The output of PLSR modeling is a linear algorithm of regression coefficients by waveband that explains the contribution of each specific band to the prediction of a specific leaf functional trait (Burnett, Serbin, et al., 2021; Serbin et al., 2014)

2.3. Statistical Analysis

2.3.1. **Effects of temporal variation on the classification of leaf spectra at different taxonomic levels.** We investigated how temporal changes in leaf properties and resulting reflectance signatures would impact classification approaches. To do this, we used the Partial Least Squared Discriminant Analysis (PLS-DA) to classify leaves into their corresponding families and genera using samples from one season, and then apply to a different sampling date. At the family level the PLS-DA was ran with 43 species (one individual per species; 5 leaves per ind.) from the six botanical families

mention above. At the genus level, we ran the PLS-DA with 22 species (one individual per species) belonging to 9 genera. For both analyses, we trained the model with 70% of the data and tested them with 30% and set the cross-validated by using bootstrap cross-validation (Rodríguez-Pérez et al., 2018) by using the trainControl and train functions, respectively. The trainControl and train functions are part of the caret package (Kuhn & Johnson, 2013). Once we obtained the optimal number of components from the trained data and used them to run the PLS-DA with the plsda function also from the caret package (Kuhn & Johnson, 2013) in R. To test for the effect of spectral variability, we ran the PLS-DA using leaves sampled in 2019 for training and 2020 – 2021 leaves for testing and classify leaves at the family, genus, and species level. For the analyses at the species level, we used 43 individuals. After we ran another PLS-DA for all datasets together (2019, 2020, 2021) by creating a train/test datasets (70/30) with a stratified random data partition across species and collection dates with the same parameters stated for the first PLS-DA. To detect areas of high variability within the spectral signature, we calculated the coefficient of variation (CV) between the leaves of species sampled in 2019 and resampled in 2020 and 2021. We calculated the CV separately for each resampling season because the group of species sampled were different each time. To visualize these both the spectral signatures and the resulting CV differences, we plotted this information into heatmaps using the base function heatmap from R.

2.3.2. **Phylogenetic signal and principal component analyses:** To determine the contribution of the phylogeny into the spectral signature variability, we ran a phylogenetic signal analysis on the structural and chemical traits derived from

PROSPECT-D and PLSR models as proxies for directly measured functional traits. For that, we split the species based on date of resampling and calculated the interquartile rage to account for trait variation. We ran a phylogenetic signal analysis using the function physig from the phytools package (Revell, 2012) based on Pagel's lambda (Pagel, 1999) which accounts for polytomies (Münkemüller et al., 2012) within the *Macleania* clade (Hooker, 1837). We extracted the phylogenetic distances from the mega-tree GBOTB.extended.tre provided by the package V.Phylomaker in R (Jin & Qian, 2019) for each analysis. We then ran a principal component analysis (PCA) to determine trait contribution of each trait to the variation of species using the function prcomp from the stats package and the function fviz_pca_var from the package factoextra (Kassambara & Mundt, 2020).

2.3.3. **Effects of temperature, relative humidity, and length of the day:** To test for the effect of the environment over the derived traits, we first run a Pearson correlation to build the models, to avoid bias created by the strength of the correlation (Schielzeth et al., 2020). For that, we use the cor.test function from the package stats in R. After the strength of the correlation was established, we built the models to test for the effect of temperature (°C , mean/standard deviation), relative humidity (%, mean/standard deviation), and mean length of the day (hours) over the derived traits. The environmental data was downloaded from sensors inside EAHC, which are connected to a PRIVA® system that collects temperature and relative humidity every 5 minutes. To calculate the length of the day we used the function daylength from the insol package (Corripio, 2019). We calculated the mean and standard deviation of all environmental variables for 30 days prior and including the measurement day (Van

Goethem et al., 2013). We build seven mixed-effect models by first establishing the random effect by nesting species into collection date, and the fixed effects environmental variables as fixed effects. The first five models included mean temperature, mean relative humidity, and mean day length and the respective interactions. The sixth model included the standard deviation of temperature and the seventh the standard deviation of relative humidity. We use the function lmer from the lme4 package (Bates et al., 2015) in R and mixed from the afex package which calculates the p-value using the Satterthwaite's method (Henrik Singmann et al., 2021). We used the BoxCoxME function from the tramME package to transform heteroscedastic models with the error distribution set as Standard Gaussian and a Bernstein basis transformation (Tamási & Hothorn, 2021). Each mixed-effect models were run separately for individuals resampled in 2020 and 2021.

3. RESULSTS

The leaf classification, when using only samples from 2019, was very successful. The PLS-DA model was able to classify the leaves with an accuracy of 0.95 (Figure 1A, Table 2). At the genus level the accuracy level was 1 (Figure 1B). However, the inclusion of temporal variability into our classification models decreased its accuracy when the PLS-DA data was spited (training data with leaves from 2019; testing data with leaves from 2020 & 2021), but the accuracy increased tremendously when the PLS-DA model took the whole sample's variability into account. PLS-DA models using the 2019 dataset for training and the other two as testing datasets had a poorer performance (Figure 2: A, C, E) compared to the PLS-DA models containing all three datasets together and split into train/test datasets by using a stratified partition method (Figure 2: B, D, F). At the family level the classification accuracy improved from 0.39 to 0.94. At the genus level,

from 0.47 to 0.98. At the species level, from 0.21 to 0.96. All the models had an accuracy significantly different and substantially higher than the no information rate, and the kappa values for the stratified models were higher than 0.9 (Table 2).

Table 1. PLS-DA analysis for Split datasets, where 2019 leaves were used as training and 2020 and 2021 and Stratified datasets where all leaves were classified together and partitioned considering collection date. N of Comp is the number of components, 95% CI is the 95% confidence interval, and No Inf Rate is the no information rate.

Model	N of Comp	Accuracy	95% CI	No Inf Rate	p-value	Kappa
Family 2019	26	0.93	0.84, 0.97	0.2	< 0.001	0.91
Family Split	59	0.29	0.22, 0.36	0.24	< 0.001	0.14
Family Stratified	35	0.9	0.84, 0.94	0.22	< 0.001	0.9
Genus 2019	36	1	0.9,1	0.23	<0.001	1
Genus Split	50	0.34	0.24, 0.47	0.2	< 0.001	0.2
Genus Stratified	33	0.98	0.91, 0.99	0.22	< 0.001	0.98
Species Split	60	0.07	0.04,0.13	0.04	< 0.001	0.04
Species Stratified	60	0.97	0.91,0.99	0.06	< 0.001	0.97

We observed that the leaf spectral signatures measured at two different times of the year varied tremendously. The comparison between species resampled in 2020 (Figure 3C) shows that the areas of the spectrum with highest variability are in the visible (VIS 500-700 nm) and in the red-near infrared transition zone or red edge (~720 nm). For the same period, most species showed increased variability at the beginning of the first downward curve of the short-wave infrared known as SWIR I, as well as at 1900 nm which is where the second downward curve of SWIR starts (SWIR II).

We did not find a phylogenetic signal for the interquartile range of any of the derived traits. The phylogenetic signal analysis was not different from zero for any of the traits for both datasets (p-value > 0.05). The PCA indicates that the traits that contribute most of the variation between the species resampled in 2020 are LMA (PC1: 24.2%; PC2: 16.4%), total chlorophyll content/area (PC1: 21%; PC2: 25.6%), and carotene content/area (PC1: 19.9%; PC2: 25.6%). For this PCA, PC1 accounted for 62.1% of the variation and PC2 for 24.3%. For species resampled in 2021, the traits that contribute to most of the variation were nitrogen content/area (PC1: 13.6%; PC2: 47.1%), LMA (PC1: 23.8%; PC2: 15.4%), and total chlorophyll content/area (PC1: 27.3%; PC2: 3.8%). For this PCA, PC1 accounted for 61.1% of the variation and PC2 for 21.6% (Figure 4).

For the environmental variables the correlation between mean temperature, mean relative humidity, and mean daylength was weak (Fig. 2S). The correlation coefficients between the standard deviation of both temperature and relative humidity were high with all other variables and between themselves, except with mean relative humidity (Fig. 2S). Hence, we build seven mixed-effect models according to these results (Table 3).

Table 2. The seven mixed effect model equations. Where y is the trait in question; the β_s are the fixed-effects regression coefficients for the fixed effects T = temperature (℃); RH = relative humidity (%); DL = daylength (hours); u is the random complement to the β_s; (individual/year) is the nested random effects; ε is the residuals.

Model	Model Specification
M1	y = $\beta\ T_{mean}$ + u (individual/year) + ε
M2	y = $\beta_1\ T_{mean}$+ $\beta_2\ RH_{mean}$ + u (individual/year) + ε
M3	y = $\beta_1\ T_{mean}$+ $\beta_2\ RH_{mean}$ + $\beta_3\ DL_{mean}$+ u (individual/year) + ε
M4	y = $\beta_1\ T_{mean} *\ RH_{mean}$ + $\beta_3\ DL_{mean}$ + u (individual/year) + ε
M5	y = $\beta_1\ T_{mean} *\ RH_{mean} * \beta_3\ DL_{mean}$ + u (individual/year) + ε
M6	y = $\beta\ T_{sd}$ + u (individual/year) + ε
M7	y = $\beta\ RH_{sd}$ + u (individual/year) + ε

The results for the mixed-model analysis showed significant contribution of these environmental factors on the variation of the derived structural and chemical traits. The mixed effect models for species resampled in 2020 (Table 4) showed that the increased of daily temperature variation ($sdT_{2019} < sdT_{2020}$, Table 2S, Fig 2S), explained the increase of carotene content/area (p-value < 0.01; Fig 7C), and that the increase of daily relative humidity variation ($sdRH_{2019} < sdRH_{2020}$, Table 2S), explained the slight decrease in leaf nitrogen content/area (p-value < 0.01; Fig 7I). The mixed effect models for species resampled in 2021 (Table 5) showed that the decrease in the variation of relative humidity ($sdRH_{2019} > sdRH_{2021}$, Table 2S, Fig 2S) had a downward effect over nitrogen leaf content/area and the Box-Cox transformation of leaf water content/area, (p-value < 0.01), similar results are shown by untransformed model. Finally, the decrease in the average relative humidity, combined with a decrease of the mean daylength had a downward effect of LMA (p-values < 0.05; Fig 7H). All models presented were non heteroscedastic (Fligner test p-value > 0.05).

Table 3. Results for most parsimonious mixed effect models for species resampled in 2020.

Trait	Environmental Variable	Estimated value	Std. Error
Total Chlorophyll	Intercept	0.63356	0.03553
	Stand. Dev. Temperature (C)	0.03459	0.02356
Carotene	(Intercept)	0.164452	0.007807
	Stand. Dev. Temperature (C)	0.013512	0.004162
Leaf Mass per Area (transformed)	Stand. Dev. Relative Humidity (%)	-0.20381	0.15369
Nitrogen	(Intercept)	1.65447	0.10353
	Stand. Dev. Relative Humidity (%)	-0.14313	0.03536

Table 4. Results for most parsimonious mixed effect models for species resampled in 2021.

Trait	Environmental Variable	Estimated value	Std. Error
Total Chlorophyll	(Intercept)	0.67477	0.04248
	Mean Temperature (C)	0.04785	0.04172
Carotene	(Intercept)	0.169376	0.007304
	Mean Temperature (C)	0.008505	0.007184
Water (transformed)	Stand. Dev. Relative Humidity (%)	1.20086	0.20834
Leaf Mass per Area	(Intercept)	35.435	7.883
	Mean Temperature (C)	1.765	8.401
	Mean Relative Humidity (%)	8.208	12.501
	Mean Daylength (Hours)	10.865	8.917
	Mean Temperature (C) * Mean Relative Humidity (%)	-4.534	12.11
	Mean Temperature (C) * Mean Daylength (hours)	9.843	6.66
	Mean Relative Humidity (%) * Mean Daylength (hours)	19.229	7.123
	Mean Temperature (C) * Mean Relative Humidity (%) * Mean Daylength (hours)	-6.656	8.203
Nitrogen	(Intercept)	1.3465	0.10977
	Stand. Dev. Relative Humidity (%)	0.44352	0.08419

4. DISCUSSION

The identification of species at the taxonomic ranks of family, genus, and species were highly accurate when the variability of the spectral collection was taking into consideration (Figure 2 B, D, F, & Table 2). Kappa > 90% in all cases, which is indicative of good model performance (Landis & Koch, 1977; Schreier, 2012). It is therefore important to build spectral libraries for PLS-DA or other types of classification methods that consider the variability of leaf reflectance within and across species and though time to improve species identification. This is consistent with other studies exploring the ability to estimate traits with spectra (Schmitt et al., 2021). The errors made by the classification models in some cases could be an effect of plant relatedness at higher taxonomic levels, where spectral reflectance have shown phylogenetic signal (Meireles et al., 2020). For instance, for the classification at the family level in the 2019 dataset, the

misidentification of leave corresponding to the Rubiaceae family as belonging to the Acanthaceae family, could be linked to both belonging to from the Lamiids clade (Stevens, 2012). In the case of leaves misidentified with the stratified method, leaves belonging to the Solanaceae family were misidentified as Rubiaceae and vice versa, and that could be due to their shared relationship as part of Lamiid clade (Asterids) as well. At the genus level, Thunbergia was misidentified as Aphelandra, both genera belong to the Acanthaceae family (Lamiid/Asterids). At the species level, *Pterocarpus officinalis*, misclassified as *Tipuana tipu*, they both belong to the Fabaceae (Rosids) and *Thunbergia grandiflora* misidentified as *Aphelandra flava*, are both Acanthaceaes (Lamiids; Stevens, 2012).

When analyzing the variability of the spectra (Figure 3) we found high variability in the red edge zone (~720 nm), supporting Hesketh & Sánchez-Azofeifa's (2012) findings with other tropical species sampled in Panama at different times of the year. We also found that areas of high coefficient of variation are linked to other important absorption features for water, proteins, and cellulose. For instance, the individuals resampled in 2020 showed high variability in the visible and red – NIR transition zone (red-edge) where we can find absorption features of chlorophyl at 660 nm and at around 400 nm where carotenoids absorb radiant energy. For the same comparison, variability at the SWIR I range increased. In this spectral region we can find absorption features of water content, specifically at 1450 nm (Curran, 1989; Jacquemoud et al., 1996; Schweiger et al., 2018; Ustin et al., 2009).

The lack of phylogenetic signal in the interquartile range for each chemical and structural trait suggests that the evolutionary history is not impacting the plasticity of these traits. These results

could be indicative that the variability observed is most likely coming from the environment or other attributes of the leaf such as age (Chavana-Bryant et al., 2019).

LMA, total chlorophyl, and carotene contents/area contributed the most to the PC1, for species resampled in 2020. Nitrogen, LMA, and total chlorophyll where the major contributors were to PC1, for species resampled in 2021. Since these derived traits underlie leaf spectra (Meireles et al., 2020) we can expect that the areas of the spectra connected to these traits are varying the most, and subsequently that the variation of these traits are causing the variation of the spectra.

The growth environment measured as the standard deviation of temperature, and the standard deviation of relative humidity had statistically significant effect over carotene content/area and leaf nitrogen content/area for the species resampled in 2020. But, chlorophyll content/area nor LMA showed statistically significant results. Increases in carotenes are expected during the summer months when the incident radiation is higher and the photosystems need to be protected (Young, 1991). Nitrogen content/area showed a decrease (Fig. 1S) related to the increase in the variability of the EAHC relative humidity (RH_{sd}, Table 4). There is no direct evidence of how an increase in the variability of relative humidity affects photosynthetic (RuBisCo) and non-photosynthetic nitrogen content in leaves, but there is evidence that when relative humidity increases, water flux decreases (Kupper et al., 2011) which could affect nutrient uptake from the soil. At the same time both nitrogen content and LMA, are positively correlated (Onoda et al., 2017) and we can observe that LMA decreased as well for these species. For species resampled in 2021, the variation of relative humidity (M7; Table 3) had a statistically significant effect over water (box-cox transformed) and nitrogen content/area respectively. Additionally, the interaction

of mean humidity and length of daylight (M4; Table 3) had a statistically significant effect over LMA. The drier conditions within EAHC during the winter months could affect leaf water content, since watering was kept constant, and the relative amount of water in the air affects stomatal responses, and photosynthesis (Broughton et al., 2021). This second response may affect leaf nitrogen content as well. Nitrogen is an important component of photosynthetic pigments, such as chlorophyll, and proteins (such as RuBisCo), so, it is not surprising that reduced relative humidity and shorter days are impacting photosynthesis and protein production (Meletiou-Christou et al., 1994; Muller et al., 2011), as well as LMA.

We can conclude that the areas of the spectrum influenced by photosynthetic pigments, water, nitrogen, and the constituents of LMA are driving the variability of the spectral signature, through time. This type of spectral variation affects species identification (Hesketh & Sánchez-Azofeifa, 2012) and trait predictions (Schmitt et al., 2021). The use of remote sensing instruments is gaining momentum in the study of biodiversity from space is a field that is gaining momentum because of the capacity instruments to cover large areas of the globe year-round (Cavender-Bares et al., 2022). Spectral variability affects the identification of species. We now know this variability is partly linked to the environment through its influence over specific leaf chemical and structural traits. The accuracy of the identification increases when the variability of the spectral signal is accounted for, and that should be a lesson for the development of more reliable spectral libraries. This will not only help the study of biodiversity at larger scales with the use of hyperspectral satellites, but it could be an excellent resource for rapid biodiversity assessments in the ground that become decision making tools for natural resources managers.

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Figures

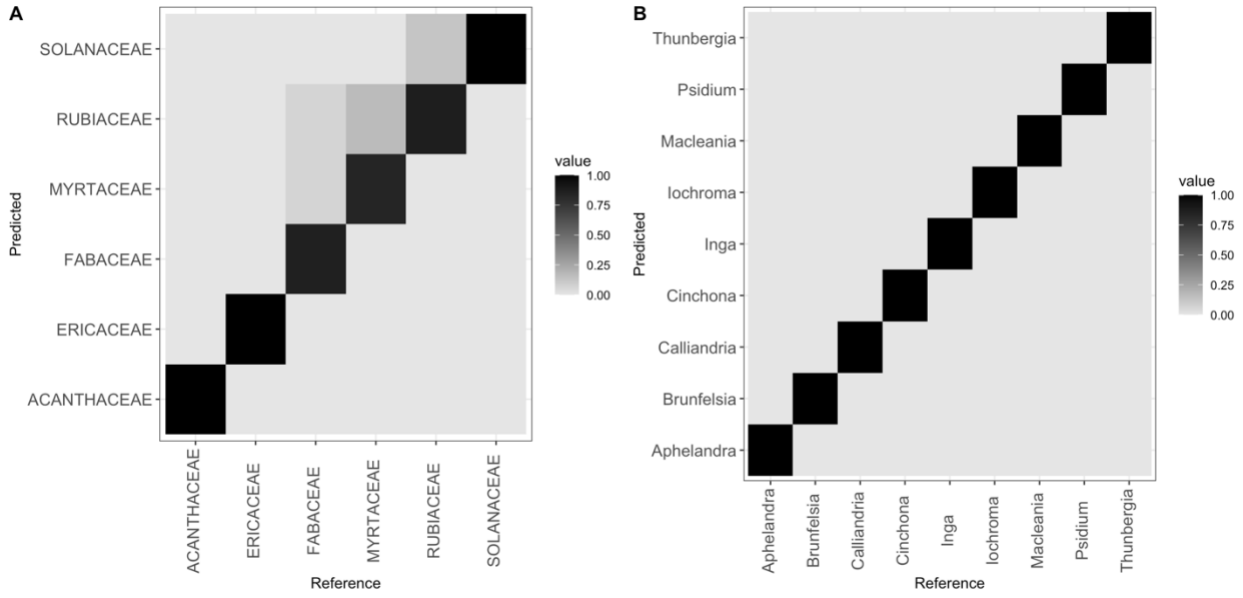

Figure 1. Confusion Matrix of the partial least square discriminant analysis at the family level (test dataset N =80 leaves) and genus level (test dataset N = 35). (A) The PLS-D analysis had an accuracy of 93% and specific accuracy ranges from 91% - 100% at the family level. (B) The PLS-D analysis had an accuracy of 100%. Specific accuracy of 100% at the genus level.

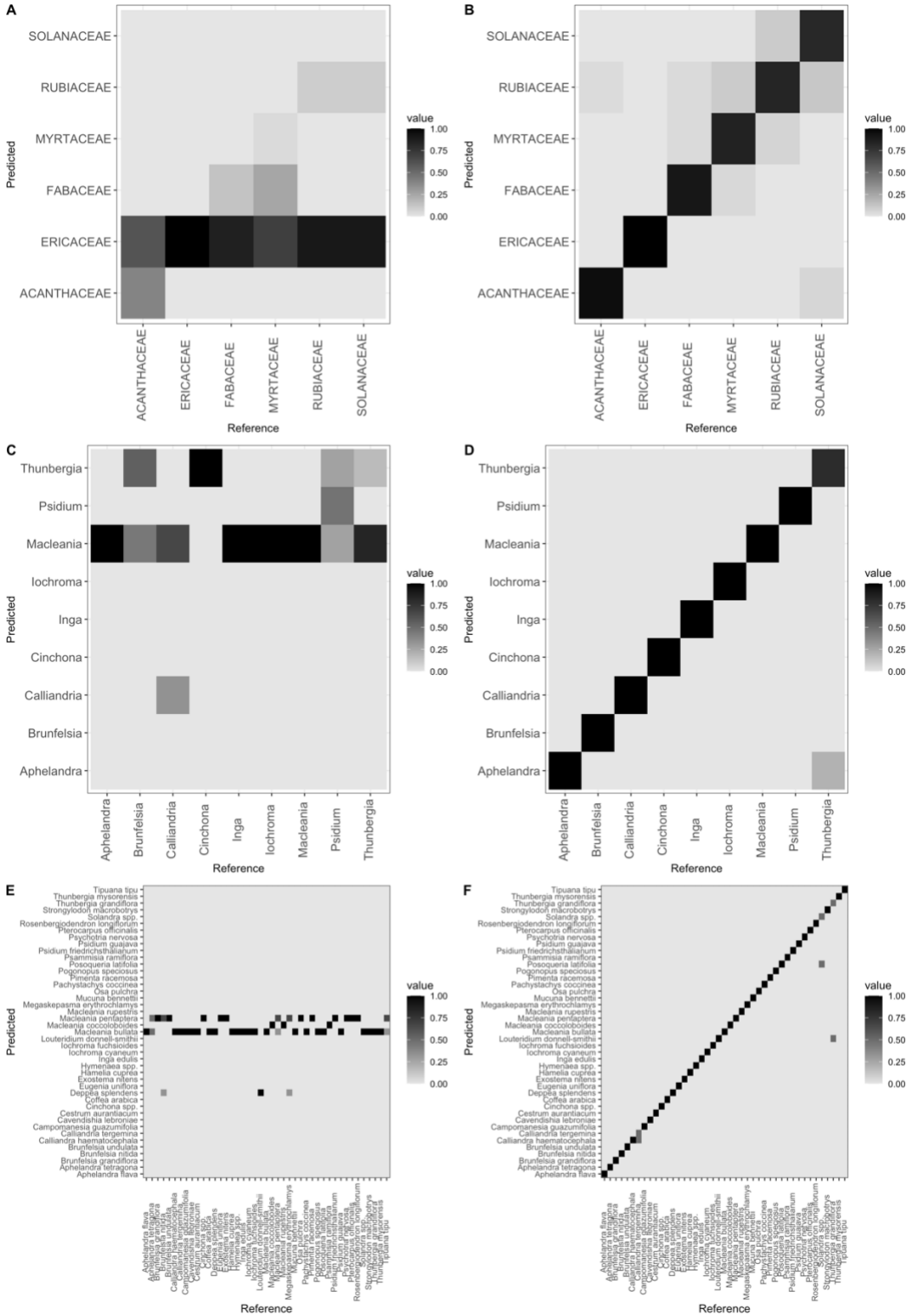

Figure 2. PLS-D analysis with 2019 as the training data set and 2020 and 2021 as test datasets (A, C, E), and all datasets together with stratified partition (B, D, F). At the Family level the accuracy, split pls-da had an accuracy of 59%

and the stratified analysis had an accuracy of 90%. The specific accuracy varied 50% to 69% for the split pls-da, and from 89% to 100% for the stratified pls-da.

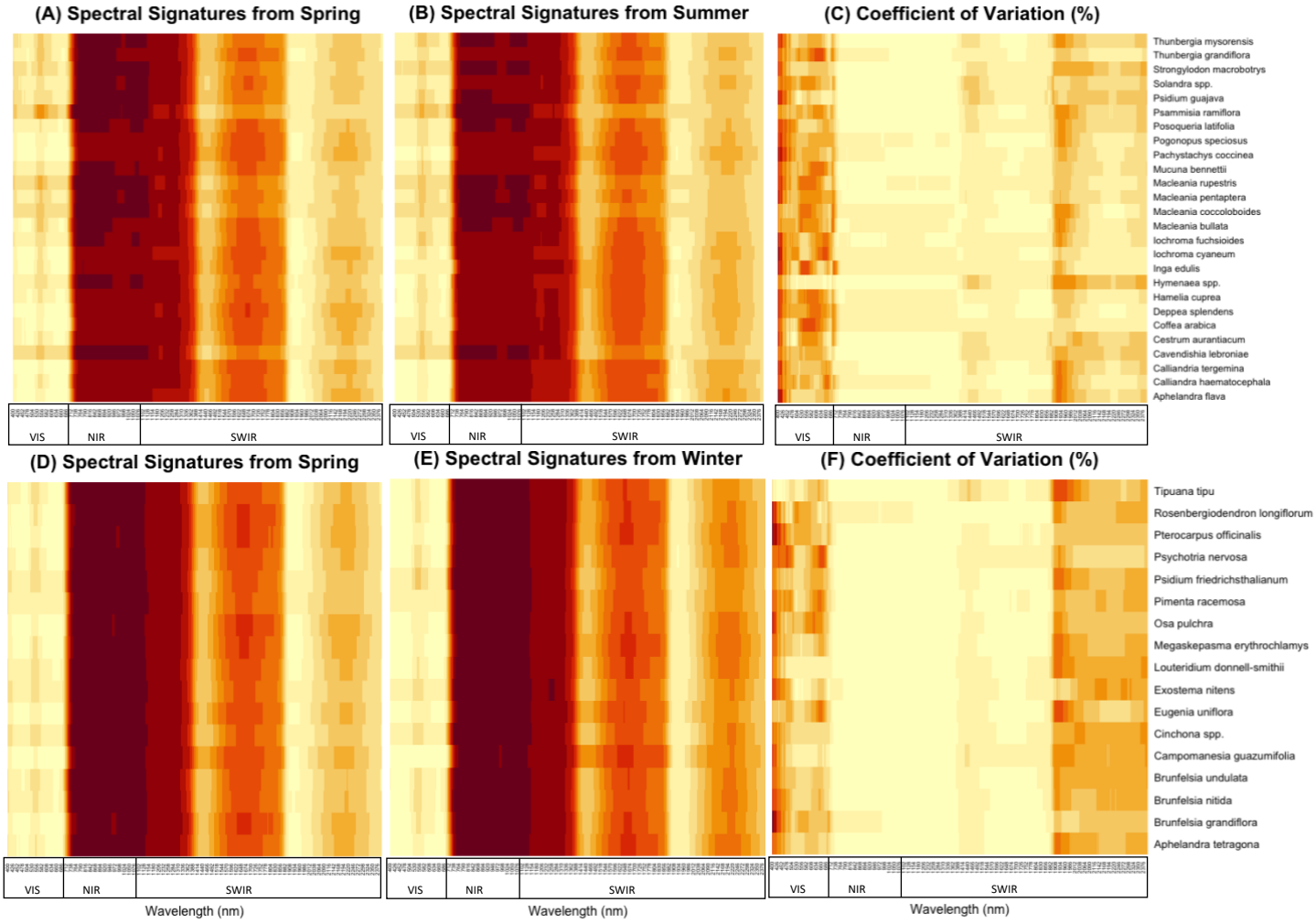

Figure 3. Spectral signature from mean reflectance (%) for species sampled in the spring of 2019 (A & D), resampled in the summer of 2020 (B) and the winter of 2021 (E). Coefficient of variation (%) calculated from leaves collected in 2019 and resampled in 2020 (C), and from leaves collected in 2019 and resampled in 2021(F). Spectral reflectance was measured from 400 nm to 2400 nm and ranges from 0.72 – 61% (yellow to dark red) and coefficient of variation 0.01- 0.66% (yellow to dark read).

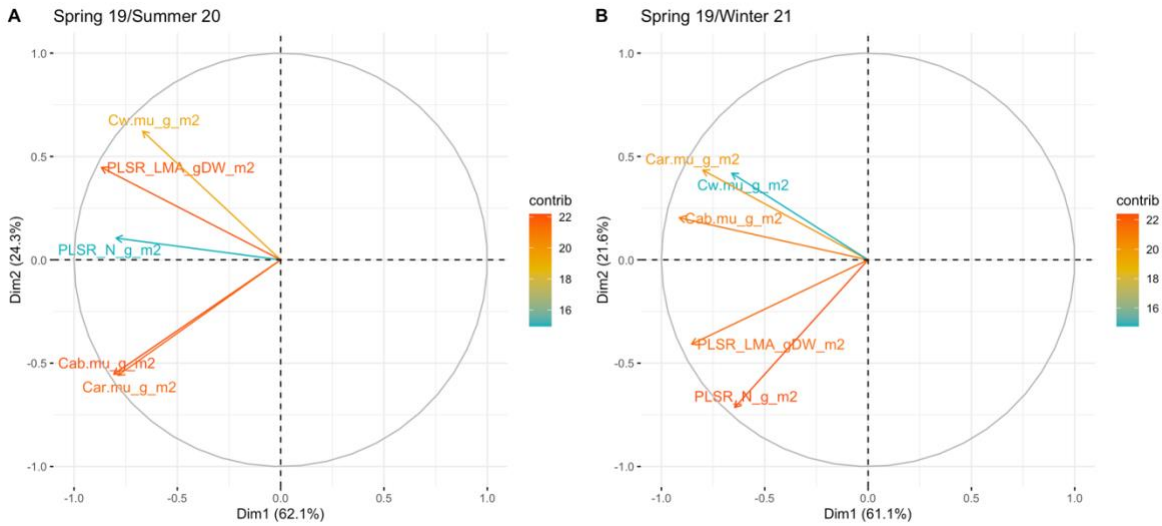

Figure 4. Contribution of traits to the variance explain by PC1 (or Dim 1) and PC2 (or Dim2) for A) species resampled in 2020, and B) species resampled in 2021.

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