Reflectance spectroscopy allows rapid, accurate, and non-destructive estimates of functional traits from pressed leaves

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Abstract

More than ever, ecologists seek to employ herbarium collections as tools to estimate plant functional traits from the past or from places that are hard to sample. However, many functional trait measurements are destructive, which may preclude their use on valuable herbarium specimens. Reflectance spectroscopy is increasingly used to estimate traits rapidly from fresh or dried, ground leaves, as well as to differentiate and identify taxa. Here, we extend this body of work to pressed, intact leaves such as those in herbarium collections. Using a dataset with 619 plant samples belonging to 70 woody and herbaceous species, we used partial least-squares regression to build validated models linking pressed-leaf reflectance spectra to a broad suite of traits, including leaf mass per area (LMA), leaf dry matter content (LDMC), carbon (C_{mass}) and nitrogen (N_{mass}) concentrations, and carbon fractions such as cellulose and lignin. We compared the accuracy of these trait estimates to those from fresh- or ground-leaf spectra of the same samples. Our pressed-leaf models predicted these traits with moderate-to-high accuracy (R^2 = 0.586-0.924; %RMSE = 5.7-11.7%). For estimating chemical traits, pressed-leaf models performed better than fresh-leaf models but slightly worse than ground-leaf models. Pressed-leaf models did not perform as well as fresh-leaf models for estimating LMA and LDMC, but outperformed ground-leaf models for LMA. Accuracy was no worse for pressed leaves that underwent discoloration in storage. Finally, on a subset of common species in the dataset, we used partial least-squares discriminant analysis to classify specimens to species with near-perfect accuracy from pressed- (>97%), and ground-leaf (>96%) spectra and slightly lower accuracy from fresh-leaf spectra (>89%). The success of trait estimation and species classification from pressed-leaf spectra may owe to the fact that they combine advantages of fresh and ground leaves: like

fresh leaves, they retain some of the spectral expression of internal leaf structure; like ground leaves, they reveal minor absorption features of chemical constituents that would otherwise be masked by water. These results show that applying spectroscopy to pressed leaves is a promising way to estimate leaf functional traits non-destructively. Our study has far-reaching implications for capturing the wide range of functional, phenotypic, and taxonomic information in the world's preserved plant collections.

Introduction

The world's herbaria together contain more than 390 million specimens (Thiers 2020), which are collectively a rich source of information about global plant diversity. Herbarium specimens are collected for many reasons—for example, to document that a species is present in a certain location, or to serve as vouchers or sources for DNA sequences used to estimate phylogenies. But these specimens are often repurposed for new ends, unforeseen by the collectors (Heberling and Isaac 2017; Meineke et al. 2018; Heberling et al. 2019). More than ever, ecologists and evolutionary biologists are interested in using herbarium specimens to measure functional traits: for example, to evaluate long-term changes, often in response to human activity (e.g. Hietz et al. 2011; Willis et al. 2017; Koski et al. 2020; reviewed in Lang et al. 2018); to fill in gaps in sparse trait databases (Queenborough and Porras 2014; Perez et al. 2020); or to conduct comparative studies of clades whose species are hard to sample all at once (e.g. Jardine et al. 2020). Measuring functional traits on herbarium specimens carries the promise of letting us reach the inaccessible, including the past or distant parts of the world. Moreover, using herbarium specimens allows researchers to benefit from the taxonomic skill of collectors and curators and refer back to the same specimens for further use—for example, as prototypes for identification of taxa from new collections. By repurposing herbarium specimens towards new ends, researchers can address ecological and evolutionary questions that require merging functional, genetic, and distributional data at global scales.

Many functional trait measurements require destructive sampling—for example, by grinding up tissue for chemical analyses. Such methods include most techniques to determine the content or concentration of various elements, isotopes, macromolecules, or metabolites in a sample. Because of the high value of herbarium specimens—especially ones from historical collections—herbarium curators may hesitate to let samples be destroyed for ecological research, even in part. The development of non-destructive techniques to estimate leaf traits from herbarium specimens would make it more acceptable to use them in functional ecology.

Reflectance spectroscopy is a well-established technique used to estimate a variety of foliar functional traits non-destructively (Curran 1989). A reflectance spectrum is a measurement of the reflectance of light from a surface at a range of wavelengths. For leaf tissue, researchers tend to measure reflectance between about 350 and 2500 nm, a range that includes more than 98% of solar radiation reaching Earth's surface (American Society for Testing and Materials, 2006). Because the leaf's chemical and structural makeup determines how it absorbs, reflects, and transmits solar radiation, reflectance within this range carries information about many critical plant traits. These underlying chemical and structural traits are shaped by past evolutionary forces, raising the possibility that leaf reflectance spectra can also be used to distinguish lineages and study trait evolution (Meireles et al. 2020a).

Two main approaches exist to estimate traits from entire reflectance spectra. First, physics-based radiative transfer models like PROSPECT can be applied to fresh-leaf spectra to estimate traits like chlorophyll and water content and the number of mesophyll cell layers (Jacquemoud & Baret 1990; Féret et al. 2017). Second, statistical models, often created using machine learning techniques like partial least-squares regression (PLSR), can be used to estimate an even wider range of traits, albeit in a less mechanistic way (Serbin & Townsend 2020). Many ecologically important traits, like leaf nitrogen concentration (N_{mass}), lack a unique set of strong absorption features, complicating the development of physical models. For example, nitrogen is found alike in nucleic acids, chlorophyll, and proteins (e.g. RuBisCO), in ratios that vary from plant to plant. Moreover, absorption features of chemical bonds and compounds often overlap, especially after being broadened by multiple scattering (Curran 1989). A multivariate empirical approach based on statistical techniques like PLSR or stepwise regression gives researchers the flexibility needed to predict complex traits (Curran 1989). Likewise, multivariate classification techniques like partial least-squares discriminant analysis (PLS-DA) are often used to take advantage of the full spectrum in discriminating species, lineages, or other kinds of biological classes (Castro-Esau et al. 2006; Gold et al. 2020; Meireles et al. 2020b).

Empirical approaches like PLSR have been widely used by ecologists to estimate plant traits from spectroscopic data measured on fresh or ground leaves. These traits include N_{mass} and LMA (Doughty et al. 2011; Asner et al. 2011a; Serbin et al. 2014; Serbin et al. 2019; Streher et al. 2020), pigments (Asner et al. 2011a; Yang et al. 2016), condensed tannins and other defense compounds (Couture et al. 2016), non-structural carbohydrates (Ramirez et al. 2015; Ely et al. 2019), and even physiological rates like the maximum rate of RuBP carboxylation in photosynthesis (V_{cmax}; Serbin et al. 2012; Wu et al. 2019; Meacham-Hensold et al. 2019). Such leaf-level PLSR models have been used to address such varied ecological subjects as defense responses to herbivory (Kula et al. 2020), the role of biodiversity in ecosystem function (Schweiger et al. 2018), and functional trait responses to El Niño (Nunes et al. 2019). Although this multivariate statistical approach is flexible, it is sensitive to the kind of leaf tissue used for model development. For example, existing PLSR models have mostly been trained on spectra of fresh leaves (e.g. Serbin et al. 2019) or dried, ground leaves (e.g. Serbin et al. 2014). Such models cannot be expected to transfer to dried, intact leaves like herbarium specimens because both drying and grinding produce major changes in the features of the reflectance spectrum.

We tested whether we could use a PLSR modeling approach to estimate traits from the spectra of pressed leaves like herbarium specimens. We ask: Could models calibrated and validated on pressed leaves be as accurate as those from fresh or ground leaves? For most chemical traits, such as N_{mass} or leaf carbon fractions, we conjectured that ground-leaf spectral models would be the most accurate, as prior studies have found (Serbin et al. 2014; Couture et al. 2016; Wang et al. 2020). Both drying and grinding might be important in achieving this high accuracy. Drying may reveal minor absorption features of compounds in dry matter within the short-wave infrared range (SWIR) that, in fresh leaves, are obscured by the dominant effect of water absorption (Peterson et al. 1988; Ecarnot et al. 2013; Couture et al. 2016). Grinding homogenizes the leaf lamina, which otherwise varies in its structure and composition across its surface and along its cross-section, altering reflectance in complex ways due to internal scattering (Grant 1987). By evening out this variation, grinding may allow us to capture a more even and representative

sample of tissue. Since pressed leaves are dried but not ground, we predict their spectra would produce estimates of intermediate accuracy.

For structural traits like LMA, we instead expect fresh and pressed leaves to outperform ground leaves because grinding disrupts the leaf structure. Moreover, for water-related traits like leaf dry matter content (LDMC; dried mass divided by fresh mass), we expect fresh leaves to outperform both pressed and ground leaves because only they retain the water absorption features that allow direct, quantitative prediction of water content (Carter 1991; Rapoport et al. 2015). An additional consideration for herbarium specimens and other pressed-leaf tissue collections is that their optical properties may change in preparation or storage. We considered whether discoloration reduces the accuracy of trait estimates, which may indicate whether spectroscopy is useful on old or degraded specimens.

Finally, we asked how accurately pressed-leaf spectra can be used to identify samples to species. Reflectance spectra often show phylogenetic signal, at least in particular wavelength ranges, because of phylogenetic conservatism in the evolution of their underlying traits (McManus et al. 2016; Diniz et al. 2020; Meireles et al. 2020a; Schweiger et al. 2021). This phylogenetic signal is what often makes it possible to classify species or higher-level taxa from fresh-leaf spectra (Cavender-Bares et al. 2016; Meireles et al. 2020b), but it may or may not be retained in pressed-leaf spectra. A few studies with tropical forest species have indeed demonstrated that absorbance spectra of pressed herbarium specimens can be used to classify species or higher-level taxa (Durgante et al. 2013; Lang et al. 2015; Lang et al. 2017; Prata et al. 2018). Here, we build on these studies by comparing the accuracy of classification from fresh-, pressed-, and ground-leaf spectra among the common species in our dataset. Because they preserve some of the spectral expression of leaf structure (unlike ground leaves) and reveal the distinctive SWIR absorption features of macromolecules and other compounds (unlike fresh leaves), pressed leaves may represent the best of both worlds for distinguishing species using spectroscopy.

Methods

Sampling

We compiled data from four projects, all part of the Canadian Airborne Biodiversity Observatory (CABO). Each project includes reflectance spectra and several structural and chemical leaf traits. The projects are:

1. Beauchamp-Rioux: Deciduous forest trees sampled throughout the growing season in 2018 at various sites in southern Québec and Ontario (n = 417).
2. Dessain: Forbs, grasses, shrubs, and broadleaf trees from forests and open areas sampled throughout the growing season in 2017 at four sites in southern Québec (n = 75).
3. Boucherville: Forbs, shrubs, and grasses sampled throughout the growing season in 2018 from the Parc national des Îles-de-Boucherville in southern Québec (n = 72).
4. Warren: A single broadleaf evergreen tree species (*Agonis flexuosa* (Willd.) Sweet) collected across a soil chronosequence in southwestern Australia (Turner et al. 2018; n = 67).

The full dataset includes 70 species, whose growth forms we classified following the Database of Vascular Plants of Canada (VASCAN; Desmet & Brouillet 2013). We classified *Agonis flexuosa* as a tree and manually disambiguated some species listed as adopting either shrub or three growth forms. The majority of samples were from trees (n = 518), with shrubs (n = 47) and forbs (n = 36) also moderately represented.

In general, we aimed to avoid leaves with noticeable damage from herbivores or pathogens. For each dataset, we measured reflectance spectra (350-2500 nm) of the leaves at three stages: (1) freshly sampled, (2) pressed and dried, and (3) ground into a fine powder. We also measured a suite of leaf functional traits on each of these samples. Further details on spectral and trait measurements are provided in later subsections, but we first lay out our sampling procedure.

For each sample, we collected a group of sunlit leaves (>3 h estimated sun exposure per day) at about the same vertical position of the same individual—the same branch or neighboring branches, for trees. After measuring fresh-leaf spectra and fresh mass in the field, we took leaves to the lab to measure leaf structural traits, such as leaf dry matter content (LDMC) and LMA. We then divided the leaves into two groups: (1) a portion to be pressed, and (2) a portion to be dried at 65 °C for three days, ground using a cyclone mill with 2 mm mesh, and stored for chemical analyses. We measured pressed-leaf spectra on the former portion prior to mounting, and ground-leaf spectra and chemical traits on the latter. Although these are distinct subsets of leaves, we treat them as identical in their trait values and spectra under the assumption that they are very close in canopy position and unlikely to differ systematically. A minimum of one representative pressed specimen of each species at each site and date is prepared as a specimen at the Marie-Victorin Herbarium in Montréal, Québec, Canada.

Spectral measurements

For the Beauchamp-Rioux, Boucherville, and Warren projects, we measured fresh-leaf directional-hemispherical reflectance spectra on each sample in 2018 using an HR-1024i spectroradiometer outfitted with a DC-R/T integrating sphere from Spectra Vista Corporation (SVC; Poughkeepsie, NY, USA). We measured spectra on the adaxial surface of six leaves or, for small or narrow leaves, single-layer leaf arrays following the protocol in Noda et al. (2013) as adapted for our instrument (Laliberté & Soffer 2018a; Laliberté and Soffer 2018b). For the Dessain project, we measured fresh-leaf reflectance spectra in 2017 using a FieldSpec 4 spectroradiometer equipped with an RTS-3ZC integrating sphere from Analytical Spectral Devices, Inc. (ASD; Boulder, CO, USA) following the leaf spectroscopy protocol from the Global Airborne Observatory (https://gao.asu.edu/spectranomics-protocols). We collected spectra from a variable number of leaves or arrays per sample—six in most cases, but as few as three or as many as eighteen from certain samples. For all projects, each reflectance measurement was calibrated against a white Spectralon 99% reflectance panel and corrected for stray light.

For all projects, we measured reflectance spectra on the adaxial surface of pressed, intact leaf samples using a PSR+ 3500 spectroradiometer with a leaf clip foreoptic (Spectral Evolution, Haverhill, MA) between six months and three years after collection. We calibrated reflectance against a white Spectralon reflectance panel and measured 3-7 spectra per sample. Although all samples were pressed in a plant press, some nevertheless dried in a way that created an uneven leaf surface. We noted any visible discoloration that samples underwent during drying or storage, including browning or blackening. In general, we aimed to avoid uneven or discolored areas when measuring spectra, although it was sometimes not entirely possible when discoloration was pervasive.

For all projects, we also measured ground-leaf spectra using the PSR+ 3500 spectroradiometer with a benchtop reflectance probe foreoptic that pressed loose powder into a smooth, even pellet. For each sample, we added at least 0.6 g of leaf powder into a sample tray; in preliminary tests, adding additional material did not change the spectra, suggesting that transmittance was close to zero. We calibrated reflectance against a white Spectralon reflectance panel placed in an identical sample tray. We measured three spectra per sample on the same pellet, turning the sample tray 120° between the first two spectra, and loosening and mixing the powder before reshaping the pellet between the second and third spectra. A small number (<15) of specimens could not be measured because there was not enough ground leaf material.

Our spectral processing pipeline varied by the type of data. Although the actual spectral resolution was more variable and coarser, the ASD Field Spec 4 and Spectral Evolution PSR+ 3500 spectrometers' software automatically resampled the spectra to 1 nm resolution and interpolated over the overlap region between sensors (Schweiger & Laliberté 2020). For data from the SVC HR-1024i spectrometer, we performed these steps using linear interpolation. For pressed-leaf spectra only, we detected jumps in reflectance at the sensor overlap region and removed them using the function *match_sensors()* in *R* package *spectrolab v. 0.0.10* (Meireles et al. 2017). Next, we averaged all spectra for a given sample and

tissue type (fresh, pressed, or ground). Spectra measured with integrating spheres tend to have greater noise, especially at the ends. To reduce noise in fresh-leaf spectra, we applied a Savitzky-Golay filter using *R* package *signal 0.7.6* (Signal Developers, 2013), with varying order and length: order 3 and length 21 from 350-715 nm, order 3 and length 35 from 715-1390 nm, order 3 and length 75 from 1390-1880 nm, and order 5 and length 175 from 1880-2500 nm. Finally, we trimmed all spectra to 400-2400 nm, removing the extremes where sensors show greater noise. We did all spectral processing in *R v. 3.6.1* (R Core Team 2020) using package *spectrolab v. 0.0.10* (Meireles et al. 2017).

Trait measurements

We performed all trait measurements excluding petioles, but including the rachis for compound leaves, since the rachis is functionally analogous to the midrib of a simple leaf. We measured the following leaf structural and chemical traits: Leaf mass per area (LMA; kg m^{-2}), leaf dry matter content (LDMC; mg g^{-1}), leaf nitrogen per unit mass (N_{mass}), leaf carbon per unit mass (C_{mass}), and carbon fractions (soluble cell contents, hemicellulose, cellulose, and lignin) on a mass basis.

We weighed six leaves per sample for fresh mass shortly after collection. We then rehydrated them in a plastic bag with a damp paper towel for at least 12 h and scanned and weighed them for rehydrated mass and area. Lastly, we dried them for 72 h in a drying oven at 65°C before reweighing for dry mass. We calculated LDMC as total dry mass divided by total fresh mass and LMA as total dry mass divided by total rehydrated area (Laliberté 2018).

We analyzed ground bulk tissue samples for N_{mass} and C_{mass} using a Vario MICRO Cube combustion analyzer (Elementar, Langenselbold, Germany; Ayotte et al. 2019). We analyzed the same samples for carbon fractions using an ANKOM 2000 Fiber Analyzer (Ankom Technology, Macedon, New York, USA) to perform a sequence of digestions (Ayotte & Laliberté 2019). The first digestion in a heated neutral detergent washes off soluble cell contents. The second digestion in a heated acidic detergent

removes hemicellulose and bound proteins—which, for simplicity, we just refer to as hemicellulose. The third digestion in 70% sulfuric acid removes cellulose, leaving behind lignin and recalcitrant compounds. We ashed all samples and used ash content as a correction factor to calculate the ash-free lignin fraction.

Scoring discoloration

We inspected each pressed specimen visually to note signs of discoloration that could have occurred in preparation or storage. Typical forms of discoloration include blackening or browning or the development of a silvery or whitish finish on the leaf surface. Based on our descriptive notes, we scored each leaf on a discrete scale from 0 to 4. A score of 0 indicates no noticeable discoloration. Scores 1 through 4 indicate increasing discoloration, from 1 (either <5% blackening/browning or development of a silvery finish to the leaf) to 4 (>80% blackening/browning).

PLSR modeling for trait estimation

We used a PLSR modeling framework to link the fresh-, pressed-, and ground-leaf spectra to the traits we measured. PLSR is well-suited to handle leaf spectral datasets, which have large numbers of collinear predictors, because it projects the spectral matrix to a smaller number of orthogonal latent components in a way that maximizes the ability to predict the response variable. Moreover, it does not require assuming that the variables predicted are measured without error. We implemented PLSR modeling using *R* package *pls v. 2.7.1* (Mevik et al. 2019). We conducted analyses separately for fresh-, pressed-, and ground-leaf spectra, using the full spectrum (400-2400 nm) to predict each trait. Some previous studies have restricted the ranges of wavelengths used in prediction to those known or assumed to contain features relevant to a given trait (e.g. Serbin et al. 2014). We avoided this approach because the features important for prediction may vary depending on whether the tissue is fresh, pressed, or ground. In preliminary analyses, restricting the range of wavelengths, using first or second derivatives of reflectance, or calculating pseudoabsorbance of ground tissue (Norris et al. 1976) made little difference for predictive accuracy.

Our methods for model calibration and validation largely follow Serbin et al. (2014). First, we divided the data into calibration (80%) and validation (20%) datasets, stratified by project. We began by fitting a model for each trait on the full calibration dataset, using 10-fold cross-validation to select the optimum number of components. For each trait, we selected the smallest number of components for which the cross-validation root mean squared error of prediction (RMSEP) was within one standard deviation of the global minimum RMSEP at any number of components. This number of components—a different number for each trait (10-27)—was used in further analyses that aimed to predict traits in the independent validation dataset. We used the variable importance in projection (VIP) metric calculated for calibration models to see which parts of the spectrum were most important for predicting each trait (Wold et al. 1994).

To test how well we could predict traits from the independent validation dataset, we developed a new set of models for each trait. We did a jackknife analysis by repeatedly (100×) dividing the 80% calibration data further into random 70% training and 30% testing subsets. For each trait, we trained models on the 70% using the previously determined optimal number of components and predicted the remaining 30% to get a distribution of model performance statistics (R^2, RMSE). The distribution of model predictions reveals how sensitive model performance is to varying sets of training and testing data.

Next, we applied the 100 jackknife models for each trait to the 20% independent validation dataset. For each sample in the validation dataset, we generated a distribution of predictions from the 100 models. After averaging predictions of each validation sample's traits from the 100 models, we quantified model performance using R^2 and root mean squared error (RMSE) using OLS regression. In all cases, we calculated RMSE relative to the 1:1 line rather than the observed-predicted regression line, as recommended by Piñeiro et al. (2008) to account for bias. We also report the RMSE as a percentage of the sample data range (%RMSE), following Feilhauer et al. (2010). For each trait, we also tested whether

the magnitude of residuals (observed minus predicted) in the validation dataset varied among leaves scored with different amounts of discoloration. We performed all statistical analyses in *R* v. 3.6.1.

PLS-DA modeling for species classification

We tested the ability to use fresh-, pressed-, and ground-leaf spectra in species classification using partial least-squares discriminant analysis (PLS-DA; Barker and Rayens 2003). We took spectra from the ten most common species in our dataset: *Acer rubrum* L., *A. saccharinum* L., *A. saccharum* Marshall, *Agonis flexuosa*, *Betula papyrifera* Marshall, *B. populifolia* Marshall, *Fagus grandifolia* Ehrh., *Populus grandidentata* Michx., *Populus tremuloides* Michx., and *Quercus rubra* L. All are deciduous trees except *A. flexuosa*, which is evergreen. Each of these species was represented by at least 20 specimens (~480 total).

We divided the full dataset into 60% calibration and 40% validation datasets. In the *R* library *caret v. 6.0.84* (Kuhn 2020), we trained models on the calibration dataset using 10-fold cross-validation. Imbalanced groups within the calibration dataset can bias classification algorithms (Sun et al. 2009; Lindström et al. 2011), so prior to the cross-validation procedure, we upsampled within less-represented species classes at random with replacement to the point that each species had representation equal to the best-represented species within the calibration dataset. We chose the number of PLS components during cross-validation by maximizing Cohen's kappa (κ). This statistic describes the agreement between the true and predicted species identities while accounting for the probability of agreement by chance. To reduce the chance of overfitting due to our upsampling procedure, we capped the number of components at the optimal number we would have selected by running the same procedure, but instead downsampling classes to the size of the smallest class. (For example, if cross-validation with downsampling showed maximum κ at *n* components, we would allow the cross-validation with upsampling to select a model with no more than *n* components.) We then applied the cross-validated model (with upsampling) to the validation dataset and summarized PLS-DA model performance using raw classification accuracy and κ.

Results

Patterns in traits and reflectance spectra

We saw large variation among samples in each of our target traits, ranging from 1.4-fold variation in C_{mass} to 23-fold variation in lignin (Table 1). The range of most traits in our dataset is generally narrower than in the global TRY dataset (Kattge et al. 2020), excluding the lower part of the range for soluble carbon, the upper part for remaining carbon fractions, and both extremes for remaining traits. Trees tended to have higher LDMC, C_{mass}, and lignin than other growth forms, consistent with prior research (Ma et al. 2018); grasses had particularly high hemicellulose and cellulose and low lignin content. Forbs generally had high N_{mass}. Some of the trait variation was driven by projects that occupied particular portions of trait space; for example, *A. flexuosa* in the WCS project tended to have particularly high LMA and C_{mass} and low N_{mass}.

The coefficient of variation (CV) of fresh-leaf reflectance was usually largest where reflectance itself was lowest—namely, in the visible range (400-700 nm) and the water absorption features (the largest of which are centered around 1450 and 1930 nm; Fig. 1A). Within the visible range, this pattern did not always hold. The CV remained high along the green reflectance hump (~530-570 nm) even as reflectance increased; it also has a local minimum at 682 nm and a local maximum along the red edge at 697 nm. These patterns may be a consequence of chlorophyll's absorbance spectrum. Where chlorophyll absorbs most, as at 660-685 nm, leaf absorptance may be nearly saturated regardless of chlorophyll content. Where chlorophyll absorbs less strongly, as along the green hump and red edge, leaf absorptance is much more sensitive to chlorophyll content (Curran 1989; Gitelson et al. 1996; Gitelson & Merzlyak 1997).

Median reflectance across the entire spectrum was higher for pressed leaves than fresh leaves, as expected based on changes in water content and leaf structure (Carter 1991; Fig. 1B). Indeed, water absorption features largely disappeared in pressed leaves. The red edge became less sharp, such that the global

maximum of median reflectance was at a longer wavelength (954 nm vs 872 nm for fresh-leaf spectra). Mean reflectance again drove most variation in the CV, and a small number of specimens, primarily the grasses *Phragmites australis* and *Phalaris arundinacea*, had much higher visible reflectance than all other specimens. The CV of pressed-leaf reflectance once again increased into the short-wave infrared (SWIR) range as mean reflectance declined, although this increase was less pronounced than for fresh leaves due to the near-absence of water absorption.

Ground-leaf reflectance was higher than pressed-leaf reflectance across nearly the entire spectrum (except 400-425 nm) because transmittance was nearly zero (Fig. 1C). The red edge was even more strongly blunted than for pressed leaves, with the global maximum of median reflectance occurring at an even longer wavelength (1313 nm). The CV again was inversely related to mean reflectance, although the rise in the SWIR was less pronounced than among pressed-leaf spectra in part because the standard deviation of reflectance was low.

PLSR modeling for trait estimation

In general, our models predicted traits in the independent validation dataset with moderate-to-high accuracy (Tables 1, 2, 3; Figs. 2 and 3). The optimal number of components selected to predict each trait was between 10 and 27, but was always higher for a given trait in ground-leaf models than the others. Fresh-leaf models were best for predicting structural or water-related traits, including LMA and LDMC. On the other hand, ground-leaf models were best for predicting chemical traits, including soluble cell contents, hemicellulose, cellulose, lignin, N_{mass}, and C_{mass} (validation R^2 = 0.736-0.954, compared to 0.513-0.708 for fresh leaves and 0.586-0.821 for pressed leaves). Statistics from jackknife analyses show that model performance was most variable and dependent on the training/testing datasets for traits that were predicted less accurately (Figs. S1, S2, and S3).

We tested for relationships between discoloration and the magnitude (absolute value) of the residuals for each trait. Our independent validation dataset contained only one specimen scored at a 4 for discoloration. After removing this specimen as a potential outlier with undue influence on the slope estimates, there was no correlation between the magnitude of residuals and discoloration for any trait ($p > 0.05$).

For all traits except LMA, the VIP metric for fresh-leaf spectra showed a global maximum between 710 and 720 nm (Fig. 4A)—wavelengths slightly longer than the typical inflection point of the red edge between the visible and near-infrared (NIR) regions (Gitelson et al. 1996). Many traits, especially LDMC, N_{mass}, and C_{mass}, show high VIP also across the wavelengths ~530-570 nm, corresponding to the green hump. Bands in the NIR range were less important for predicting most traits than much of the visible range. The SWIR range was generally important for predicting LMA, and many other traits showed several local peaks of importance, most prominently at about 1880 nm, but also near 1460 and 1720 nm.

For predicting traits from pressed-leaf spectra, the general trend held that visible reflectance and several band ranges in the SWIR were important for predicting most traits, while NIR reflectance was less important (Fig. 4B). In fresh-leaf models, LMA had particularly low VIP in the visible range and high VIP and in the SWIR, but in pressed-leaf models, it fell much more in line with other traits. The red edge peak of importance for most traits was near 705 nm, and another prominent local maximum of importance lay close to 1920 nm. We saw broadly similar patterns in VIP from ground-leaf spectra (Fig. 4C).

PLS-DA modeling for species classification

Our PLS-DA analyses showed that models using pressed- and ground-leaf spectra showed near-perfect performance at classifying species, with misclassifications only among species within the same genus (Fig. 5). Models using fresh-leaf spectra were slightly worse but still showed excellent performance. The optimal fresh-leaf model, which had 29 PLS components, correctly predicted the taxonomic identity of 170 of the 189 samples in the training dataset ($\kappa = 0.884$; $p < 0.0001$; Fig. 5). The best pressed-leaf

model, which had 29 PLS components, correctly predicted 184 of the 188 samples ($\kappa = 0.975$; $p < 0.0001$).The best ground-leaf model, which had 30 PLS components, correctly predicted 183 out of 189 samples ($\kappa = 0.963$; $p < 0.0001$). In all cases, a majority of misclassifications were between congenerics.

Discussion

We show that we could accurately estimate a wide range of leaf functional traits for 70 woody and herbaceous species from reflectance spectra of fresh leaves, pressed leaves, and ground leaf material (Tables 1 and 2; Figs. 2 and 3). Importantly, using pressed-leaf spectra may provide an accurate, non-destructive method for estimating the traits of herbarium specimens. Ground-leaf estimates were most accurate for chemical traits—consistent with the success of such models in prior research (Serbin et al. 2014)—and fresh-leaf estimates were most accurate for structural or water-related traits. Pressed-leaf estimates may represent a good compromise in allowing both classes of traits to be estimated with intermediate but quite high accuracy. These results show that pressed-leaf spectra can provide an integrative measure of leaf phenotypes, much like fresh-leaf spectra (Cavender-Bares et al. 2017). We also showed that pressed-leaf spectra could be used to classify species as well as ground-leaf spectra and better than fresh-leaf spectra.

Our findings about which kind of tissue was best for predicting each trait supported our predictions. For estimating chemical traits, ground-leaf spectra showed strong performance, likely resulting from the way that grinding homogenizes the lamina and removes the potentially confounding influence of leaf structure. Pressed-leaf spectra showed intermediate performance in estimating such traits because, like ground leaves, they lack the major water absorption features that mask the smaller absorption features of other compounds in the SWIR range (Peterson et al. 1988; Ecarnot et al. 2013). On the other hand, these same reasons may explain why pressed- and (especially for LMA) ground-leaf spectra performed worse at predicting LMA and LDMC. Indeed, it may seem perplexing that we could succeed at all in predicting LMA from ground-leaf spectra, or LDMC from pressed- or ground-leaf spectra. The ability to estimate

these traits must not be a result of optical features driven by the traits themselves. Instead, we suggest that we sense these traits via their correlations with traits whose optical signatures are not destroyed by pressing or grinding. This kind of effect—a "constellation effect" (*sensu* Chadwick and Asner 2016; Nunes et al. 2017)—has been invoked to explain the ability to estimate other traits like leaf micronutrients (Nunes et al. 2017) and $\delta^{15}N$ (Serbin et al. 2014) that do not have strong absorption features in the measured range of wavelengths. However, models that rely on such constellation effects would be less robust in situations where such trait covariance breaks down.

The performance of pressed-leaf models in this study was comparable to the successes obtained here and elsewhere using fresh- and ground-leaf spectra. For example, the ground-leaf models in Serbin et al. (2014) had a validation RMSE of 1.4 and 2.4 for cellulose and lignin. In comparison, our pressed-leaf models achieved an RMSE of 1.42 and 2.16 for these traits (Table 2). On the other hand, our pressed-leaf model for N_{mass} performed worse than that in Serbin et al. (2014; validation RMSE = 0.13 vs 0.247), but better than the fresh-leaf models in Doughty et al. (2011; RMSE = 0.45). Our models for LDMC from pressed leaves had a lower RMSE (33.8 mg g^{-1}) than Streher et al. (2020) obtained from fresh leaves (53 mg g^{-1}). Our models for LMA also had an RMSE (0.010 kg m^{-2}) lower than full-range fresh-leaf models from the literature, including Asner et al. (2011b; 0.015 kg m^{-2}), Doughty et al. (2011; 0.019 kg m^{-2}), Serbin et al. (2019; 0.015 kg m^{-2}), and Streher et al. (2020; 0.051 kg m^{-2}). Although these studies sampled from different kinds of vegetation with different ranges of trait values, such comparisons establish that pressed-leaf models are not categorically worse than ground- or fresh-leaf models, and in fact are often better. It may depend on the research question whether the error obtained from these models is acceptable, but we suggest that it would be for a wide range of questions concerning large-scale ecological or evolutionary patterns that encompass a wide range of trait variation.

The VIP metric helps us understand what features allow these models to work, but interpreting its patterns can be a challenge because reflectance at a given band usually cannot be understood in terms of a single

chemical or structural trait. Fresh-leaf VIP for most traits had peaks in the visible and SWIR ranges, with a global maximum at 705 nm. Streher et al. (2020) found a similar peak for LMA and LDMC, among others scattered primarily in the SWIR (Fig. 4A). The importance of red edge reflectance (particularly 700-725 nm) may owe to this region's sensitivity to both chlorophyll content and leaf structure (Gitelson et al. 1996; Richardson et al. 2002). Much of the visible range was proportionally even more important for pressed- and ground-leaf models, and the NIR was less important (except for LMA; Fig. 4B-C). There are multiple small peaks in the SWIR, most notably one at 1920 nm that appears similar to a VIP feature in Serbin et al. (2014). Reflectance at 1920 nm is often associated with absorption by water in fresh tissue, but any causal link to water content is unlikely because the pressed and ground leaves were dried in preparation; whatever water might remain comes only from the air. Bands near 1920 nm include broad absorption features for many components of dry matter, including protein, cellulose, lignin, and starch, any or all of which may contribute to the importance of this region (Curran et al. 1989; Fourty et al. 1996).

With some exceptions, the VIP metric showed that the same bands are often important for predicting different traits (Fig. 2). This might be taken as an artifact of trait covariance, but surprisingly, across the whole dataset, many traits covaried only weakly. While soluble cell contents, hemicellulose, and cellulose all showed correlations, positive or negative (R^2 = 0.463-0.791), the others often did not. For example, while N_{mass}, C_{mass}, LMA, and LDMC all showed broadly similar patterns of pressed-leaf VIP across the spectrum, including peaks at 705 and 1920 nm, they covaried weakly with each other (R^2 = 0.029-0.380). The fact that some of the same regions of the spectrum were important for predicting traits may not be purely as an artifact of trait covariance. The VIP statistic is just a heuristic, and does not show the direction in which a band's reflectance alters trait estimates; the same bands may matter for different traits in different ways, like the red edge, which is affected by both chlorophyll and leaf structure.

PLS-DA models showed that fresh-, pressed-, and ground-leaf spectra alike could be used to classify species with near-perfect accuracy (>96%) for pressed or ground leaves, and excellent (>89%) accuracy for fresh leaves, much as in work on tropical flora (Durgante et al. 2013; Lang et al. 2015; Lang et al. 2017; Prata et al. 2018). In contrast to prior work that deliberately selected many congenerics, our most common species were not all closely related. Among the samples misidentified, most were mistaken for congenerics (Fig. 5), particularly for pressed and ground specimens, which implies that related species are more spectrally similar (Schweiger et al. 2018; Meireles et al 2020a; Schweiger et al. 2021). Hence, discriminating more closely related species might pose a greater challenge and lead to a higher error rate. Nevertheless, our analysis reinforces that spectral models can often distinguish among species or higher-level taxonomic groups (Meireles et al. 2020b).

The fact that pressed-leaf models showed the highest classification accuracy (>97%) is noteworthy, since pressed specimens are the standard way that field botanists preserve plant samples for later identification. This outcome is surprising since pressed leaves were never best for predicting any single trait. Although all three models performed well, we conjecture that pressed-leaf models have an advantage because they retain aspects of leaf structure that vary among species while, more importantly, also revealing the absorption features of potentially distinctive compounds in the SWIR range. While water content can only vary in one dimension, there are many macromolecules and metabolites whose absorption features in this range might allow finer discrimination of species. The partial preservation of leaf structure, at least relative to ground leaves, might allow for another dimension along which species can be distinguished.

The future of spectroscopic trait estimation

Although trait predictions from spectral models are not perfect, they have a few advantages over conventional trait measurements: they (1) are potentially non-destructive; (2) are fast and require little training, especially compared to measuring multiple traits; and (3) have very low marginal cost, despite the high capital cost of buying a spectrometer. Because of these advantages, the widespread adoption of

leaf spectroscopy could make it easier to address questions that require large datasets of functional traits. But researchers who are attracted to reflectance spectroscopy by the promise of quick, cheap trait estimates may be deterred if researchers must each make their own trait prediction model, which would imply that they must still measure traits conventionally on a subset of their samples. For that reason, we should aim for spectral models to be general.

This aim is not easy to achieve: for multiple reasons, a model trained on one spectral dataset may not always make good trait predictions from a second dataset. The reasons may include, but are not limited to, differences between the training and target data sets in: (1) their representation of plant traits and optical properties; (2) plant sample preparation prior to spectral measurements; and (3) the instruments and foreoptics used. Here we discuss these issues one by one; in the following section, we describe some concerns particular to herbarium specimens.

First, the ranges and the covariance structure of the traits may differ among the datasets. For example, if a researcher wants to apply a model trained on boreal conifers alone to tropical broadleaf species, the conifer model may have to extrapolate well beyond its range. A general trait model must include a broad range of functional variation, which poses a logistical challenge because any one site only encompasses a fraction of global trait variation. Moreover, even portions of trait space that are represented within the training dataset may be poorly predicted—for example, Streher et al. (2020) found that models for LMA among plants in the seasonally dry Neotropics performed poorly above 300 g m^{-2}. A fully general model, if such a thing is possible, would need to represent the full range of leaf structure and chemistry.

Another class of issues with model generality concerns sample preparation before spectral measurements. For example, ground-leaf spectra vary based on the size of the particles in the ground tissue (Foley et al. 1998). Other decisions, such as the choice to include or exclude petioles in the ground tissue sample,

could alter the spectrum and compromise trait estimation from models trained on samples prepared another way.

Yet another class of issues concerns spectrometers and their foreoptics. Spectra of fresh leaves can be measured with different foreoptics, including integrating spheres, contact probes, or leaf clips. Integrating spheres are designed for making measurements that are nearly independent of the angle of the light source or the sensor relative to the sample. They also allow the use of light traps to absorb nearly all transmitted light; in contrast, most leaf clips or contact probes use black backgrounds that invariably reflect a small fraction of light. These features make integrating spheres likely to produce more consistent measurements between instruments or replicate samples (Petibon et al. 2021). Finally, integrating spheres also allow researchers to measure both reflectance and transmittance spectra. On the other hand, leaf clips and contact probes are much faster to use, often produce higher signal-to-noise ratios, and allow measurement of angle-dependent (anisotropic) reflectance when it is desired. The data from these foreoptics are not often directly comparable: spectra measured with leaf clips or contact probes tend to show higher reflectance, probably due to the effects of the viewing and illumination angles (Potůčková et al. 2016; Hovi et al. 2018). Spectrometers and their software may also differ in their sensors and techniques for processing spectra.

In this study, we use integrating spheres with fresh leaves, but leaf clips with pressed specimens; using an integrating sphere requires placing specimens either inside the sphere or in one of the ports, each of which could damage the delicate pressed specimens. Such logistical constraints could limit the choices that researchers can make in data collection. In theory, our use of different foreoptics for measuring fresh-, pressed-, and ground-leaf spectra could confound our comparisons of model accuracy. But given that we calibrated the models separately, we argue that the physical changes caused by pressing or grinding are more important than the foreoptic type for explaining which kind of spectra work best for each trait in this study. Some studies (e.g. Serbin et al. 2019; Meireles et al. 2020) have successfully built models that

combine data collected with multiple kinds of instruments—and indeed, here we use two different models of spectrometers for fresh-leaf estimates. Assuming that these kinds of technical challenges can be overcome—for example, by developing tools to reconcile measurements with different setups, as in Meireles et al. (2020)—we show that there are good reasons to be confident about building general models to estimate traits from a wide variety of plants.

The challenges of reducing the error and improving the generality of trait estimates are not unique to reflectance spectroscopy (Clark & Kellner 2012). Conventional wet lab trait measurements, too, are prone to varying amounts of error, including those used to train the spectral models. For example, different methods of measuring lignin yield different results, and some have high variance between replicate samples (Brinkmann et al. 2002). As with any other technique, the goal for spectroscopic trait estimation is to improve model accuracy and generality as much as they can be jointly improved without making the technique harder to use. The creation of open libraries for spectral data (like EcoSIS; https://ecosis.org/) and spectral models (like EcoSML; https://ecosml.org/) will contribute to this goal.

Some of the same concerns apply to species classification models, even though they are likely to remain unavoidably specific to particular applications. For example, a species classification model must include strong representation in the training dataset for all the species that one might encounter. Researchers may also want to use existing data, as from open libraries, to train models, which would require resolving concerns about sampling—including phenotypic plasticity—as well was the potential influence of instrumentation and measurement protocols (Meireles et al. 2020).

What should herbarium managers do?

If spectroscopic models can reliably estimate traits from pressed-leaf spectra, researchers may be able to use herbarium specimens non-destructively to address core questions in functional trait ecology and evolution, including studies of historical changes in trait composition and diversity (Lang et al. 2018).

This technique may allow researchers to reduce their reliance on measuring traits or reflectance spectra through intensive, logistically challenging field campaigns in order to fill in trait datasets, speeding up progress in plant functional ecology. It could also keep researchers from having to do destructive and time-consuming conventional trait measurements on herbarium specimens. Likewise, spectroscopy may be able to assign species identifications to specimens that are otherwise hard to identify.

However, measuring and interpreting spectra from herbarium specimens presents additional challenges. Perhaps the most concerning is the potential for specimens' optical and chemical properties to degrade during preparation or storage, especially light-sensitive photosynthetic pigments. Such degradation could make it hard to distinguish secular trends in the traits of living plants over time from changes in storage. Even in this study, where no specimens were collected before 2017, many underwent noticeable changes in color, including browning or blackening; ~12% were scored at 2 or higher. Certain preparation and storage conditions could accelerate changes in optical properties. For example, when collecting herbarium specimens in the wet tropics, researchers often treat leaves with an alcohol solution as a preservative (Blanco et al. 2006). Such treatments could discolor leaves—as could the spoilage that would occur in their absence. Exposure to high light, temperature, or humidity could also contribute to discoloration, which underscores the importance of developing and using best practices for preparation and storage. Some species may also be more susceptible than others; for example, in our dataset, ~42% of *Populus grandidentata* specimens were scored at 2 or higher, but no *Betula papyrifera* specimens were.

Whether such degradation could affect trait estimation is an open empirical question. We find no evidence that it does, even for badly discolored specimens, but our specimens were only collected as early as 2017, while ecologists may want to use specimens collected decades ago. Long-term studies of the same specimens over time—especially with sets of specimens subjected to varying preparation and storage techniques—would help establish how optical properties tend to change, and may establish predictable patterns of degradation over time that can be corrected to estimate the original spectrum. In any case, it

seems likely that estimating traits in old or degraded specimens will require including equally old or degraded specimens in the dataset used to build the trait model.

There may be other challenges besides sample degradation. Measuring reflectance usually requires a black absorbing background so that any transmitted light is not reflected back into the sensor. However, many herbarium specimens are glued to a paper backing. The availability of loose whole leaf material would make measuring reflectance spectra much easier. However, researchers may be able to develop and validate methods to measure and correct for reflectance from the paper backing, a potential subject for future research.

If taking spectral measurements during field collections is too challenging, the best way to incorporate spectroscopy into the operation of a working herbarium may be to measure the spectra of recently pressed and collected specimens that have not yet been mounted—ideally, immediately after drying. The spectrum could then be linked to the herbarium record for future analyses and made available to download, together with metadata about the instrument, foreoptic, specimen preparation, and date of spectral measurements. While the ideal situation would be for specimen collectors to gather abundant loose tissue for trait (or spectral) measurements (Heberling and Isaac 2017), doing so is not always feasible. Linking spectra to herbarium records may also require new developments in biodiversity informatics, since the hyperdimensionality of the reflectance spectrum could make it hard to accommodate within existing standards such as the Darwin Core (Wieczorek et al. 2012) or the ABCD schema (Holetschek et al. 2012). Coordination between herbarium managers and researchers who use reflectance spectroscopy could advance protocol development and build agreement about best practices for measurement and curation (Heberling and Isaac 2017).

Validating the spectral estimation of traits from older herbarium specimens will require extensive further research, but we take a first step towards this goal by showing that non-destructively measured pressed-

leaf spectra retain information about many leaf functional traits. This finding raises the possibility of using herbaria as tools to understand plant functional trait ecology and evolution in a much more comprehensive way. Our study has far-reaching implications for capturing the wide range of functional and phenotypic information in the world's preserved plant collections and broadly advancing digital taxon identification in collected specimens.

Acknowledgements

We performed this research on the ancestral and contemporary land of Native people: primarily the Kanien'kehá:ka (Mohawk) and Omàmiwinini (Algonquin) First Nations for the Beauchamp-Rioux, Dessain, and Boucherville projects, and the Noongar people for the Warren project. For permissions and assistance, we thank le Jardin botanique de Montréal; la Station de biologie des Laurentides (SBL) de l'Université de Montréal; l'Institut de recherche d'Hydro-Québec (IREQ); the National Capital Commission for sampling at Mer Bleue Bog; la Société des établissements de plein air du Québec (SÉPAQ) for sampling at Parc national d'Oka, Parc national du Mont-Saint-Bruno, and Parc national des Îles-des-Boucherville; and the Parks and Wildlife Service of Western Australia for sampling in D'Entrecasteaux National Park. We would like to thank Antoine Mathieu, Alexandra Massey, Florence Blanchard, Elisabeth Hardy-Lachance, Zachary Bélisle, Sandra Jooty, Myriam Cloutier, Fabien Cichonski, Madeleine Trickey-Massé, Aurélie Dessain, Xavier Guilbeault-Mayers, Jocelyne Ayotte, and especially Sabrina Demers-Thibeault for contributing to trait measurements. Trait and fresh-leaf spectral data from Canadian and Australian sites were collected as part of the Canadian Airborne Biodiversity Observatory (CABO), which is funded by NSERC Discovery Frontiers grant 509190-2017, as well as NSERC Discovery grants (RGPIN-2014-06106, RGPIN-2019-04537) and a Canada Research Chair to EL. Other spectral data were collected with support from the NSF/NASA under DEB #1342778, an International Thesis Research Travel Grant from the University of Minnesota's Graduate School, and a grant distributed through an NSF-funded Research Coordination Network (DEB-1745562). S.K. was supported by an NSF Graduate Research Fellowship (Grant No. 00039202) and a UMN Doctoral

Dissertation Fellowship. R. B.-R. was supported by NSERC through the Canada Graduate Scholarships—Master's program.

Tables

Table 1. Summary statistics of PLSR model calibration and validation from fresh-leaf spectra. %RMSE is calculated as RMSE divided by the range of data within either the training data set (calibration) or the testing data set (validation), each of which may be smaller than the range reported in the complete data set. Global ranges are derived from the TRY database (Kattge et al. 2020; accessed 13 April 2021), omitting data with error risk greater than 3. Carbon fractions (solubles, hemicellulose, cellulose, and lignin) are each represented by <1250 datapoints in TRY, which may curb the estimates of the global range.

	Range	Global range	No. comps	R^2		RMSE		%RMSE	
				Cal.	Val.	Cal.	Val.	Cal.	Val.
Solubles (%)	38.5-88.9	20.0-74.6	13	0.526	0.457	5.63	5.92	11.2	15.2
Hemicellulose (%)	2.77-34.1	8.71-45.0	10	0.517	0.479	3.36	3.43	10.7	14.6
Cellulose (%)	5.19-25.8	7.65-42.6	24	0.644	0.597	1.90	2.14	9.46	11.0
Lignin (%)	0.918-21.5	2.27-32.4	10	0.513	0.498	2.34	2.56	11.4	13.0
C_{mass} (%)	39.5-53.6	25.5-60.3	12	0.708	0.715	1.16	1.21	8.67	10.0
N_{mass} (%)	0.883-5.62	0.14-7.27	11	0.689	0.695	0.328	0.338	7.92	7.32
LMA (kg m^{-2})	0.0233-0.217	0.00866-0.354	17	0.955	0.950	0.00783	0.00818	4.03	4.58
LDMC (mg g^{-1})	159-573	68.2-857	19	0.877	0.800	22.5	28.2	5.50	7.42

Table 2. Summary statistics of PLSR model calibration and validation from pressed-leaf spectra. Data ranges in the complete data set are reported in Table 1.

	No. comps	R^2		RMSE		%RMSE	
		Cal.	Val.	Cal.	Val.	Cal.	Val.
Solubles (%)	12	0.690	0.657	4.57	4.54	9.07	11.7
Hemicellulose (%)	12	0.711	0.666	2.64	2.65	8.42	11.3
Cellulose (%)	18	0.803	0.701	1.42	1.63	7.05	8.78
Lignin (%)	13	0.586	0.621	2.16	2.24	10.5	11.4
C_{mass} (%)	17	0.815	0.844	0.935	0.881	7.00	7.31
N_{mass} (%)	16	0.821	0.744	0.247	0.312	5.97	6.75
LMA (kg m^{-2})	10	0.924	0.916	0.0102	0.0102	5.24	5.72
LDMC (mg g^{-1})	19	0.738	0.701	33.8	34.9	8.16	9.20

Table 3. Summary statistics of PLSR model calibration and validation from ground-leaf spectra. Data ranges in the complete data set are reported in Table 1.

	No. comps	R^2		RMSE		%RMSE	
		Cal.	Val.	Cal.	Val.	Cal.	Val.
Solubles (%)	24	0.806	0.809	3.66	3.54	7.26	9.12
Hemicellulose (%)	20	0.736	0.716	2.54	2.54	8.12	10.8
Cellulose (%)	27	0.912	0.914	0.916	0.941	4.56	4.83
Lignin (%)	23	0.764	0.812	1.64	1.57	7.98	8.01
C_{mass}	22	0.914	0.932	0.641	0.589	4.80	4.89
N_{mass}	23	0.954	0.966	0.123	0.114	2.98	2.47
LMA (kg m^{-2})	19	0.866	0.804	0.0136	0.0154	6.97	8.67
LDMC (mg g^{-1})	20	0.797	0.778	29.9	30.3	7.21	8.44

Figure captions

Fig. 1: Distributions of spectral reflectance and its coefficient of variation (CV) among fresh (left), pressed (middle), and ground (right) leaf samples. A solid black line connects the median reflectance at each wavelength, around which dark blue and light blue ribbons denote the middle 50% and 95%. The solid red line shows the coefficient of variation among spectra at each wavelength.

Fig. 2: Independent validation results for predictions of soluble cell contents, hemicellulose, cellulose, and lignin from fresh- (left), pressed- (middle), and ground-leaf (right) spectra. In each panel, a separate regression line is shown for each functional group, overlaid on top of the thick dashed 1:1 line. The error bars for each data point are 95% confidence intervals calculated from the distribution of predictions based on the model coefficients from the 100 jackknife iterations.

Fig. 3: Independent validation results for predictions of C_{mass}, N_{mass}, LMA, and LDMC from fresh- (left), pressed- (middle), and ground-leaf (right) spectra, displayed as in Fig. 3.

Fig. 4: The variable importance of projection (VIP) metric calculated based on fresh- (A), pressed- (B), and ground-leaf (C) models. Each panel shows carbon fractions (top) and traits related to structure or elemental composition (bottom). Abbreviations: sol = solubles, hemi = hemicellulose, cell = cellulose, lign = lignin.

Fig. 5: Confusion matrices for partial least-squares discriminant analysis from fresh- (left), pressed- (middle), and ground-leaf (right) spectra. Numbers on the diagonal represent correctly classified species, while off-diagonal numbers represent misclassified species; the row denotes the true species identity, while the column denotes the model's prediction.

Figures

Fig. 1

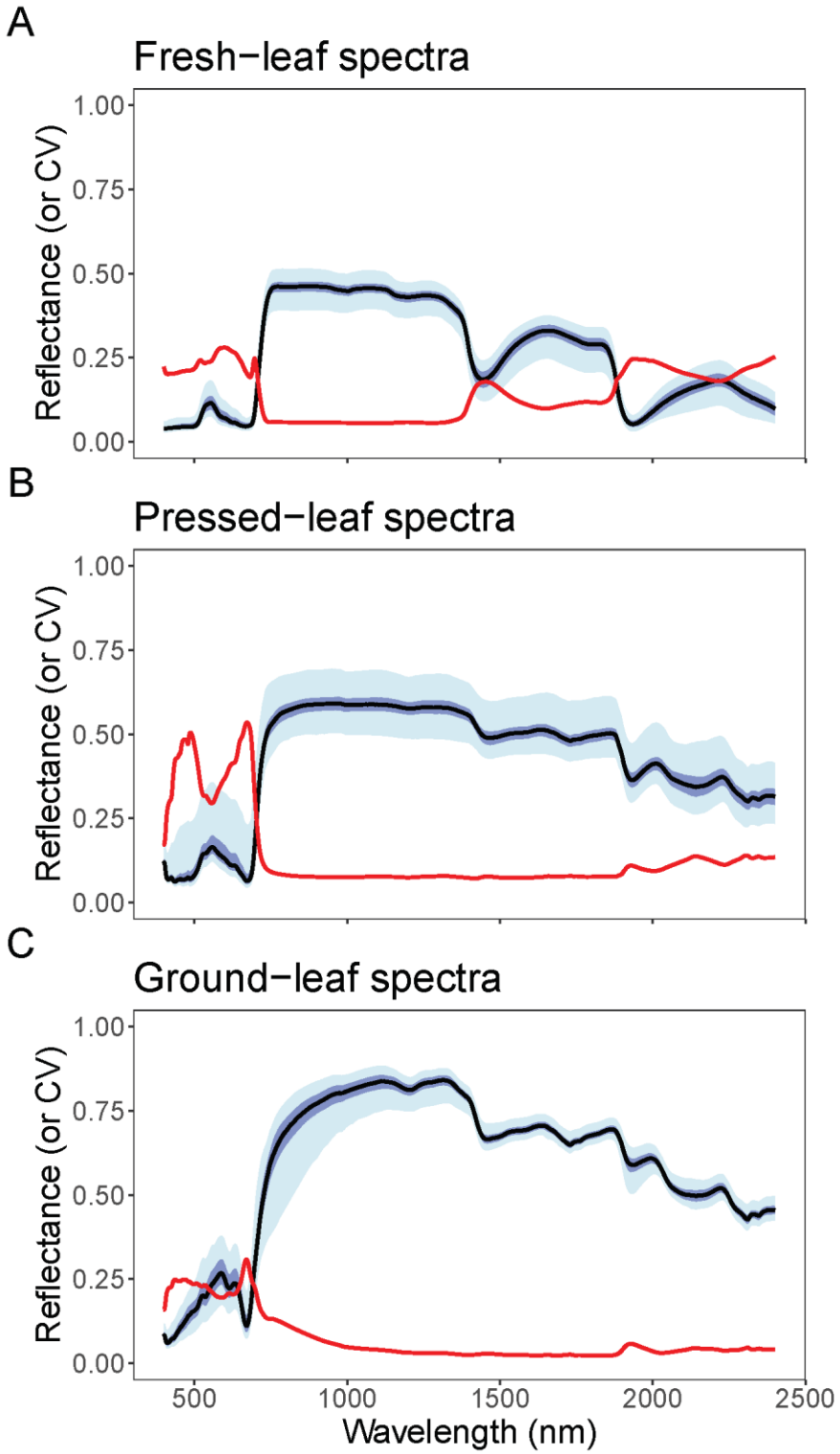


Fig. 2

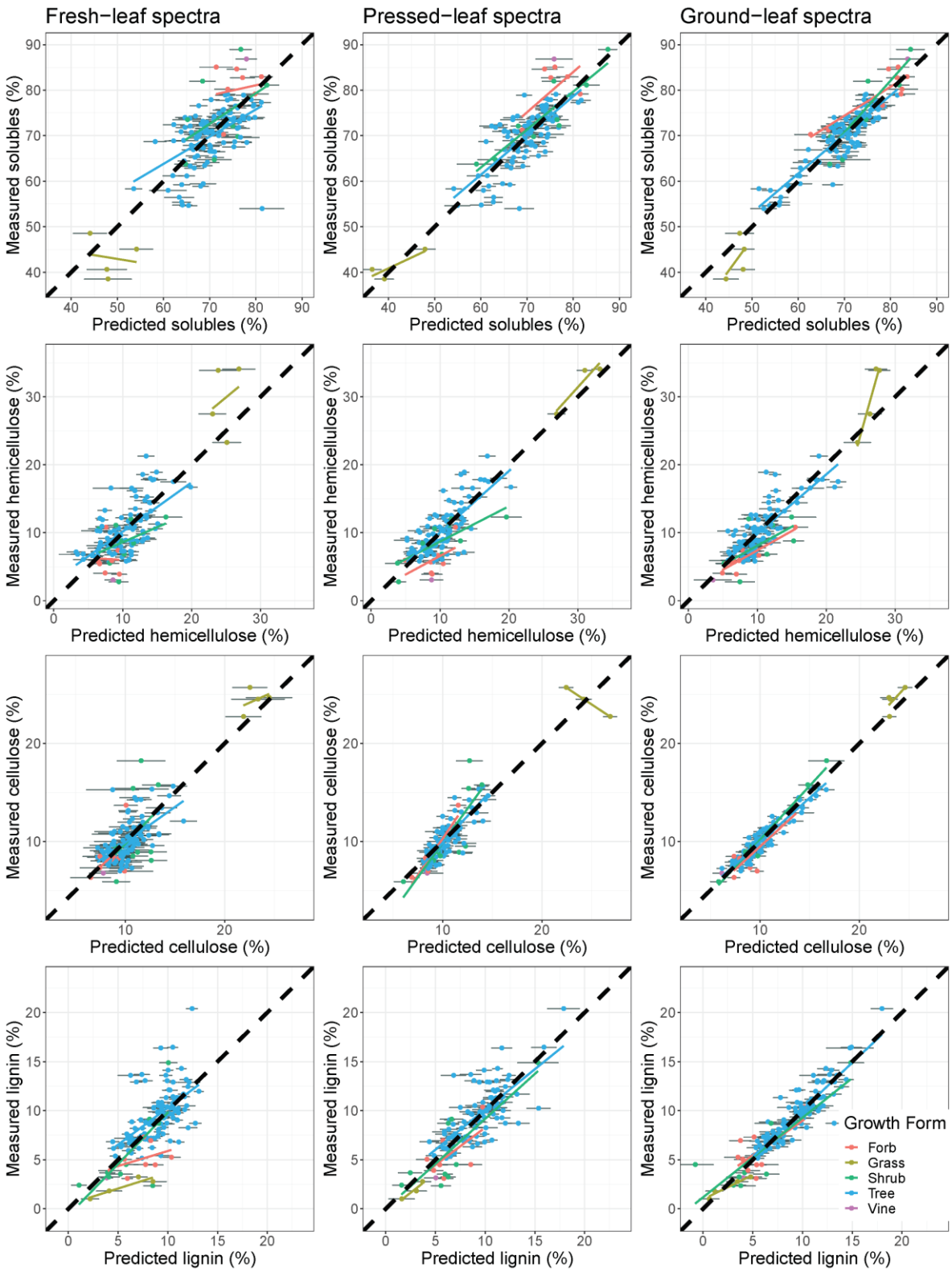

Fresh−leaf spectra
Pressed−leaf spectra
Ground−leaf spectra
Measured solubles (%)
Predicted solubles (%)
Measured hemicellulose (%)
Predicted hemicellulose (%)
Measured cellulose (%)
Predicted cellulose (%)
Measured lignin (%)
Predicted lignin (%)
Growth Form
Forb
Grass
Shrub
Tree
Vine

Fig. 3

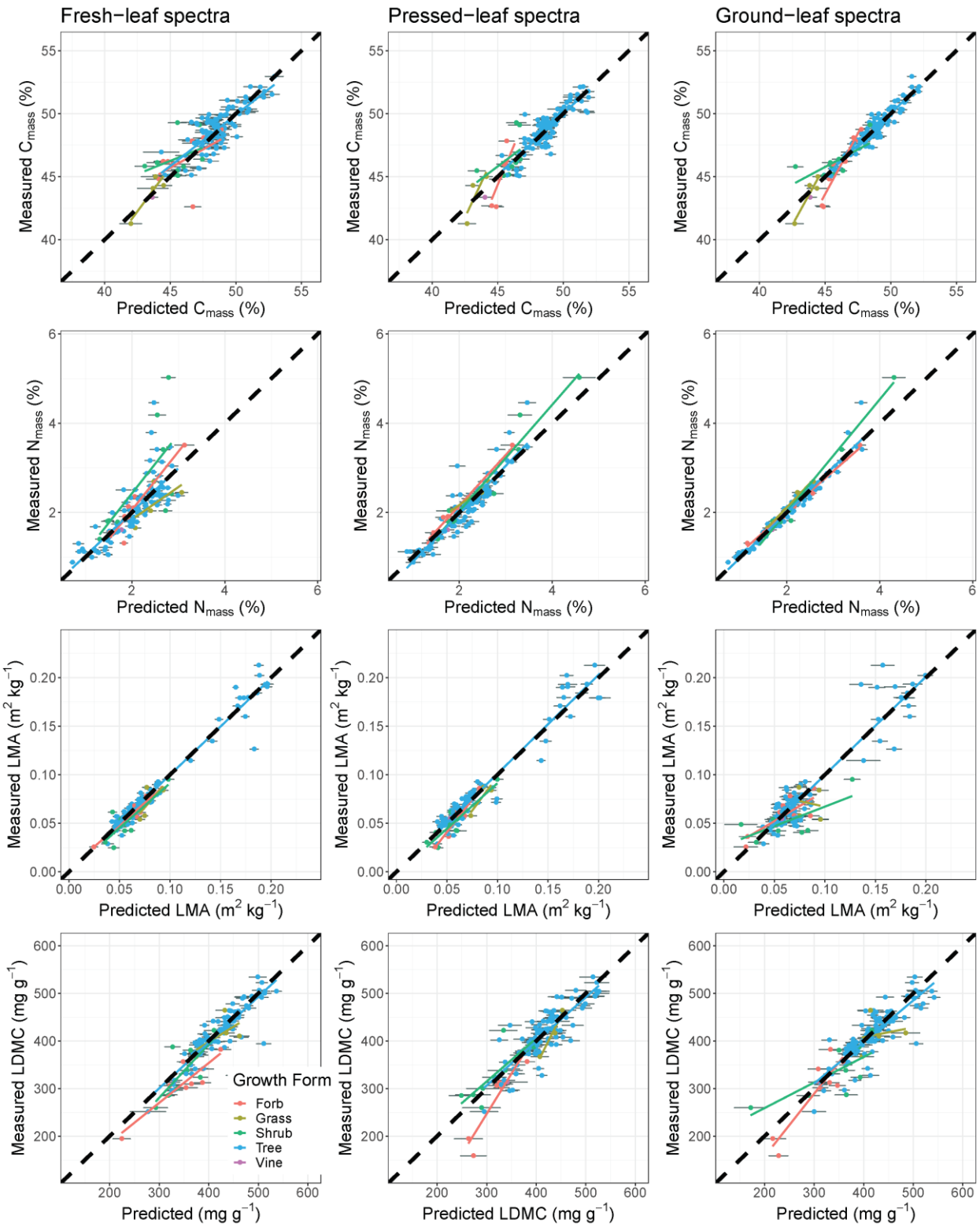

Fresh−leaf spectra
Pressed−leaf spectra
Ground−leaf spectra
Measured Cmass (%)
Predicted Cmass (%)
Measured Nmass (%)
Predicted Nmass (%)
Measured LMA (m2 kg−1)
Predicted LMA (m2 kg−1)
Measured LDMC (mg g−1)
Predicted LDMC (mg g−1)
Predicted (mg g−1)
Growth Form
Forb
Grass
Shrub
Tree
Vine

Fig. 4A

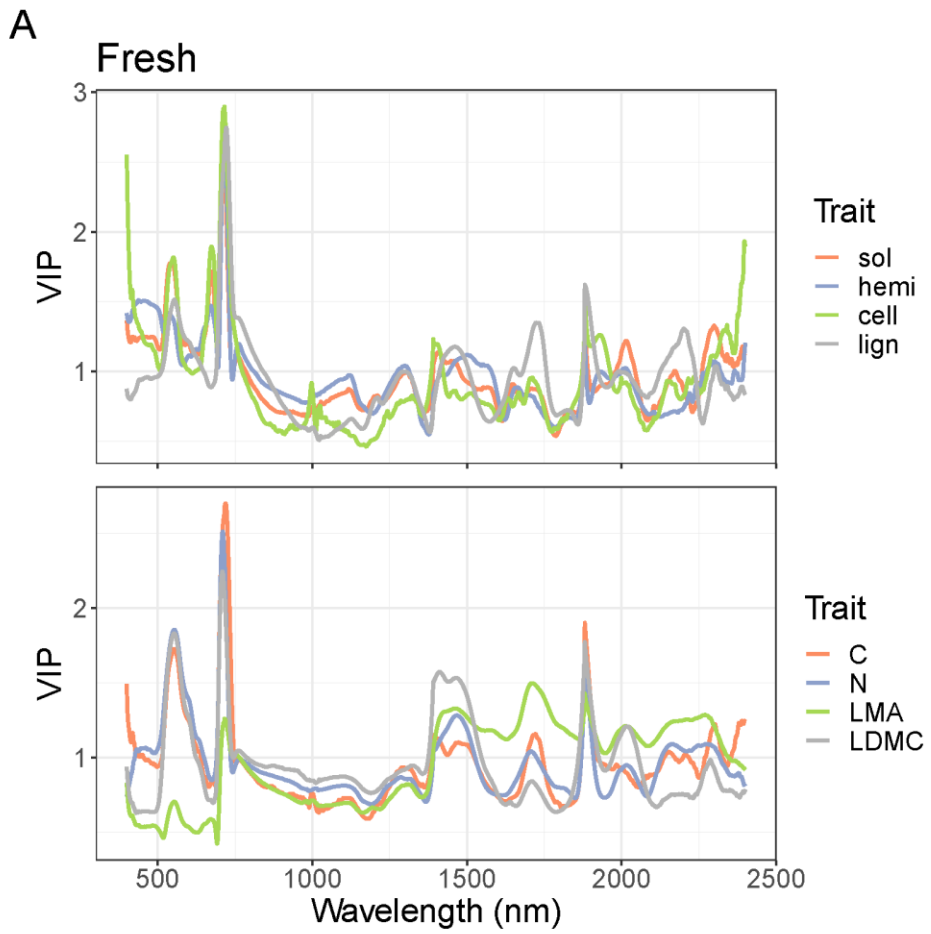

A
Fresh
VIP
3
2
1
Trait
sol
hemi
cell
lign
VIP
2
1
Trait
C
N
LMA
LDMC
500
1000
1500
2000
2500
Wavelength (nm)

Fig. 4B

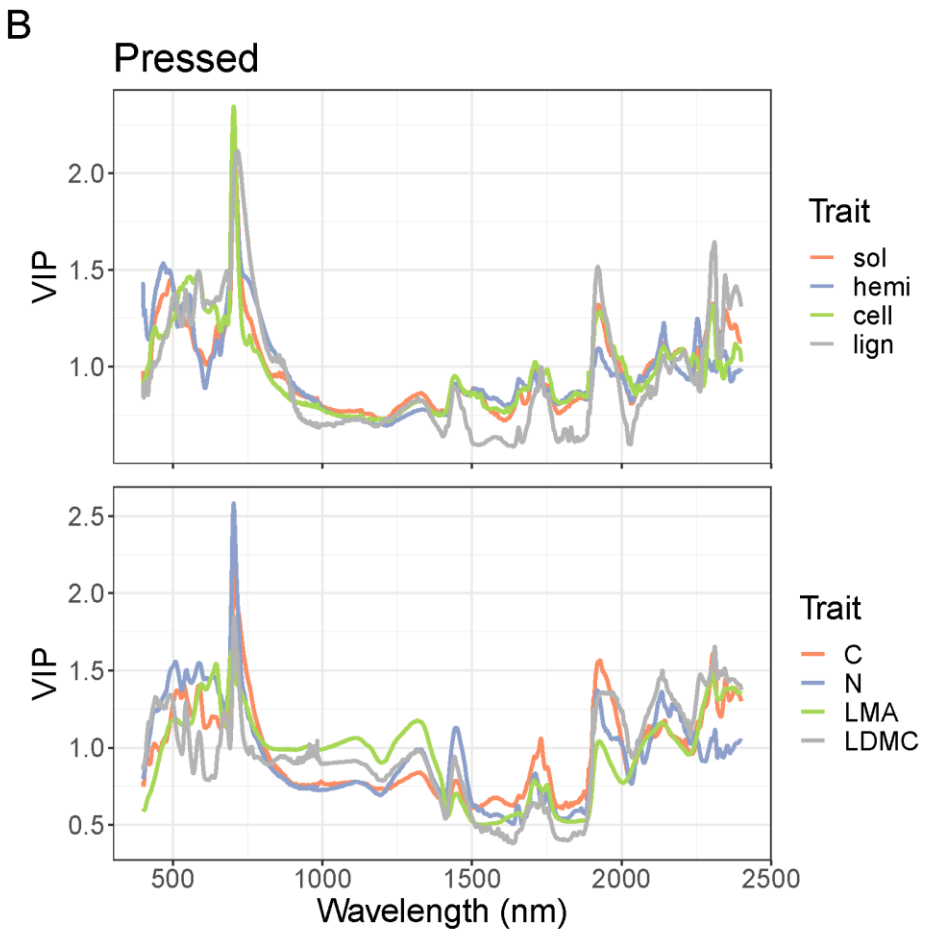

B
Pressed
VIP
2.0
1.5
1.0
Trait
sol
hemi
cell
lign
VIP
2.5
2.0
1.5
1.0
0.5
Trait
C
N
LMA
LDMC
500
1000
1500
2000
2500
Wavelength (nm)

Fig. 4C

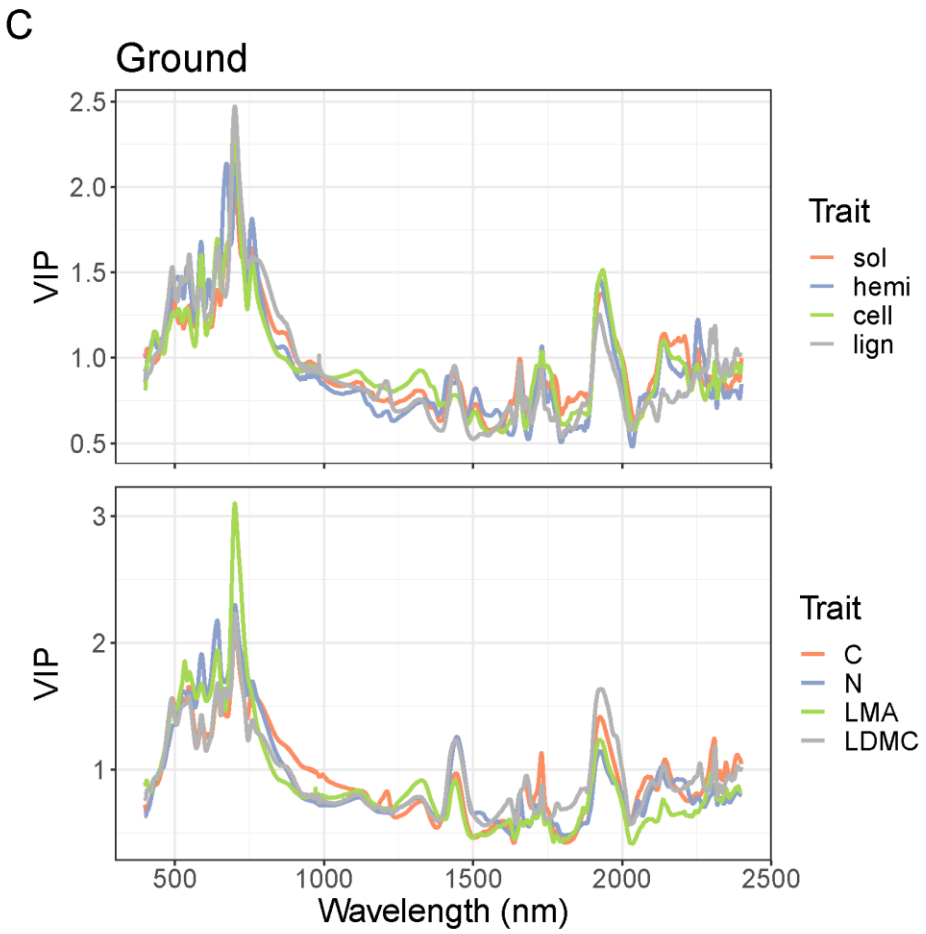

C
Ground
VIP
2.5
2.0
1.5
1.0
0.5
Trait
sol
hemi
cell
lign
VIP
3
2
1
Trait
C
N
LMA
LDMC
500
1000
1500
2000
2500
Wavelength (nm)

Fig. 5A

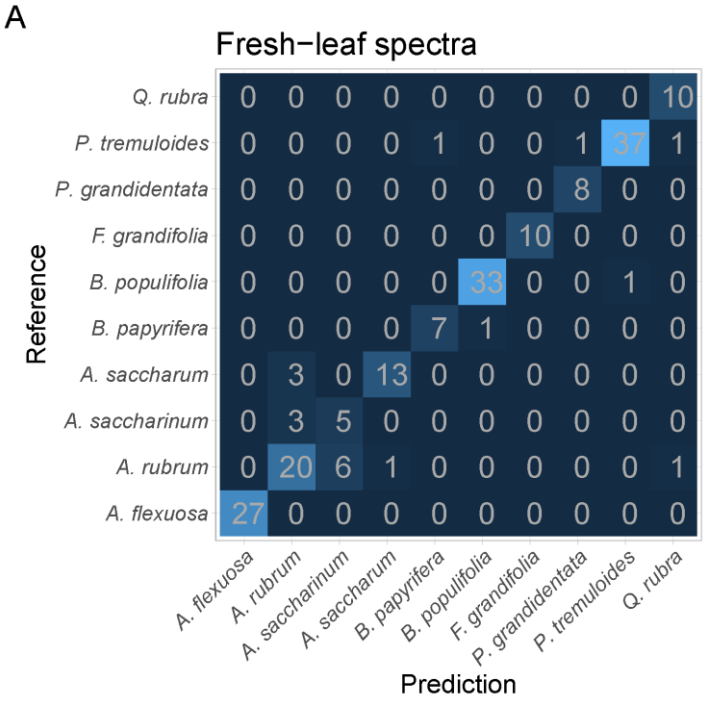

A
Fresh−leaf spectra
Reference
Prediction
Q. rubra 0 0 0 0 0 0 0 0 0 10
P. tremuloides 0 0 0 0 1 0 0 1 37 1
P. grandidentata 0 0 0 0 0 0 0 8 0 0
F. grandifolia 0 0 0 0 0 0 10 0 0 0
B. populifolia 0 0 0 0 0 33 0 0 1 0
B. papyrifera 0 0 0 0 7 1 0 0 0 0
A. saccharum 0 3 0 13 0 0 0 0 0 0
A. saccharinum 0 3 5 0 0 0 0 0 0 0
A. rubrum 0 20 6 1 0 0 0 0 0 1
A. flexuosa 27 0 0 0 0 0 0 0 0 0
A. flexuosa
A. rubrum
A. saccharinum
A. saccharum
B. papyrifera
B. populifolia
F. grandifolia
P. grandidentata
P. tremuloides
Q. rubra

Fig. 5B

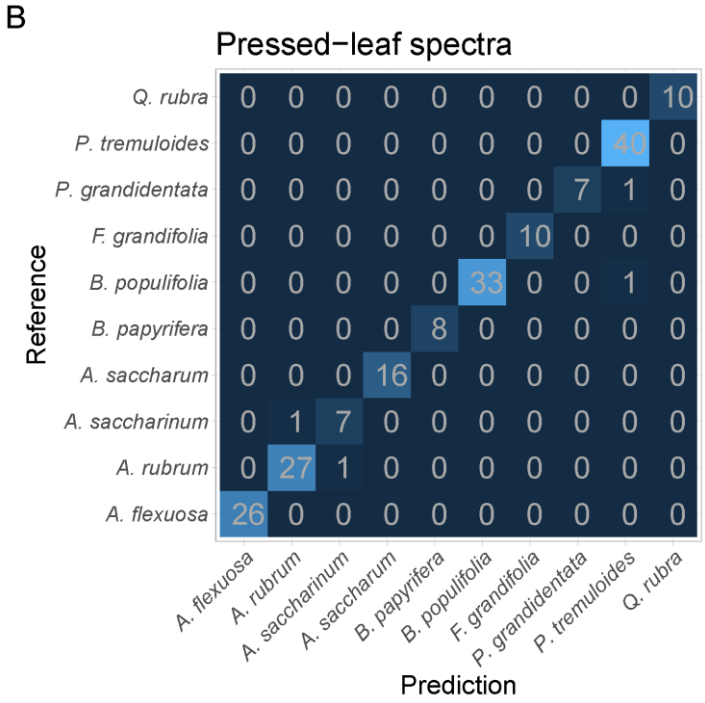
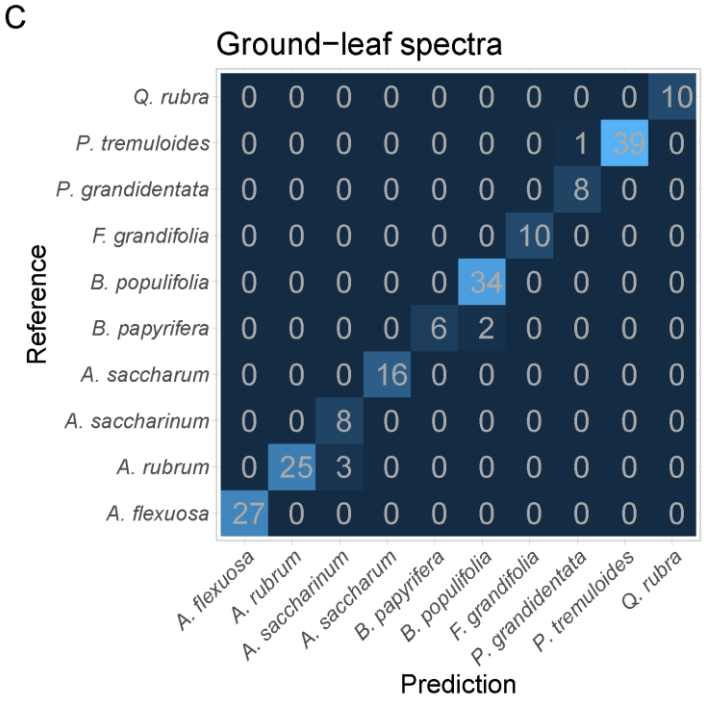

B
Pressed−leaf spectra
Reference
Prediction
Q. rubra 0 0 0 0 0 0 0 0 0 10
P. tremuloides 0 0 0 0 0 0 0 0 40 0
P. grandidentata 0 0 0 0 0 0 0 7 1 0
F. grandifolia 0 0 0 0 0 0 10 0 0 0
B. populifolia 0 0 0 0 0 33 0 0 1 0
B. papyrifera 0 0 0 0 8 0 0 0 0 0
A. saccharum 0 0 0 16 0 0 0 0 0 0
A. saccharinum 0 1 7 0 0 0 0 0 0 0
A. rubrum 0 27 1 0 0 0 0 0 0 0
A. flexuosa 26 0 0 0 0 0 0 0 0 0
A. flexuosa
A. rubrum
A. saccharinum
A. saccharum
B. papyrifera
B. populifolia
F. grandifolia
P. grandidentata
P. tremuloides
Q. rubra

Fig. 5C


C
Ground−leaf spectra
Reference
Prediction
Q. rubra 0 0 0 0 0 0 0 0 0 10
P. tremuloides 0 0 0 0 0 0 0 1 39 0
P. grandidentata 0 0 0 0 0 0 0 8 0 0
F. grandifolia 0 0 0 0 0 0 10 0 0 0
B. populifolia 0 0 0 0 0 34 0 0 0 0
B. papyrifera 0 0 0 0 6 2 0 0 0 0
A. saccharum 0 0 0 16 0 0 0 0 0 0
A. saccharinum 0 0 8 0 0 0 0 0 0 0
A. rubrum 0 25 3 0 0 0 0 0 0 0
A. flexuosa 27 0 0 0 0 0 0 0 0 0
A. flexuosa
A. rubrum
A. saccharinum
A. saccharum
B. papyrifera
B. populifolia
F. grandifolia
P. grandidentata
P. tremuloides
Q. rubra

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Supplementary figure captions

Fig. S1: Fresh-leaf spectral model performance statistics for each trait based on 100 jackknife resamples from the calibration data set. Abbreviations: sol = solubles, hemi = hemicellulose, cell = cellulose, lign = lignin.

Fig. S2: Pressed-leaf spectral model performance statistics for each trait based on 100 jackknife resamples from the calibration data set. Abbreviations: sol = solubles, hemi = hemicellulose, cell = cellulose, lign = lignin.

Fig. S3: Ground-leaf spectral model performance statistics for each trait based on 100 jackknife resamples from the calibration data set. Abbreviations: sol = solubles, hemi = hemicellulose, cell = cellulose, lign = lignin.

Supplementary figures

Fig. S1

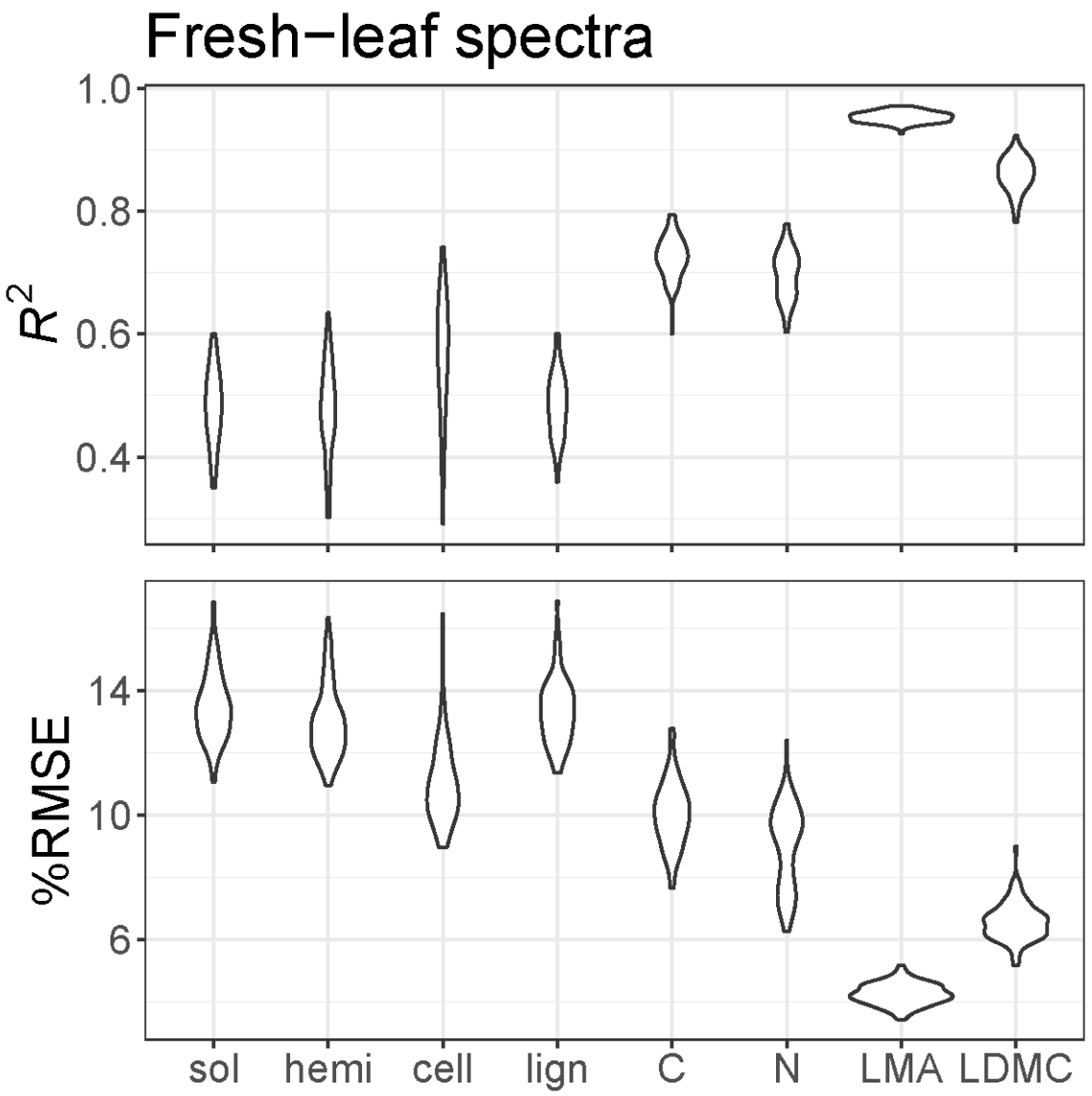
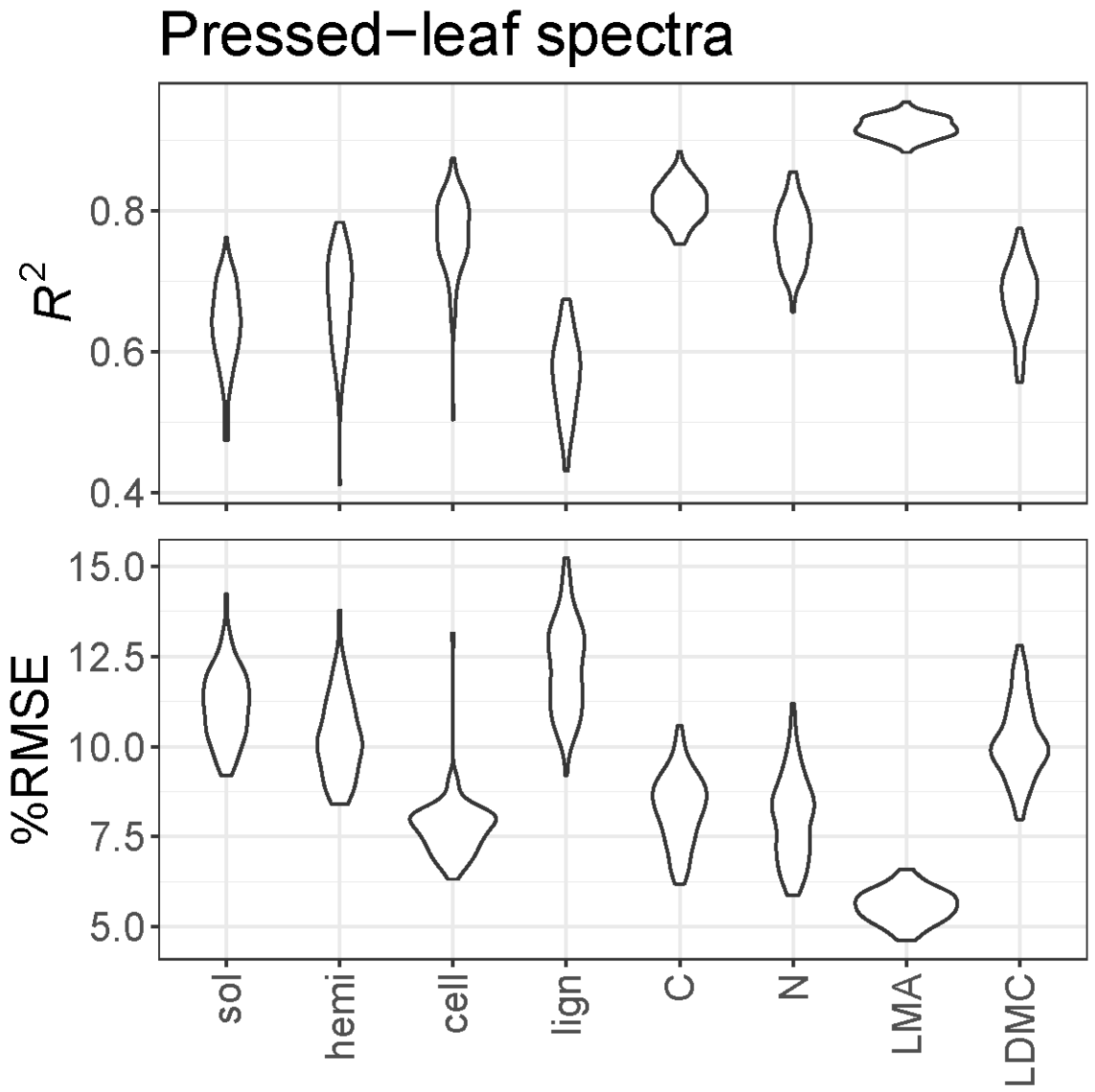


Fig. S2


Pressed−leaf spectra
R^2
0.8
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%RMSE
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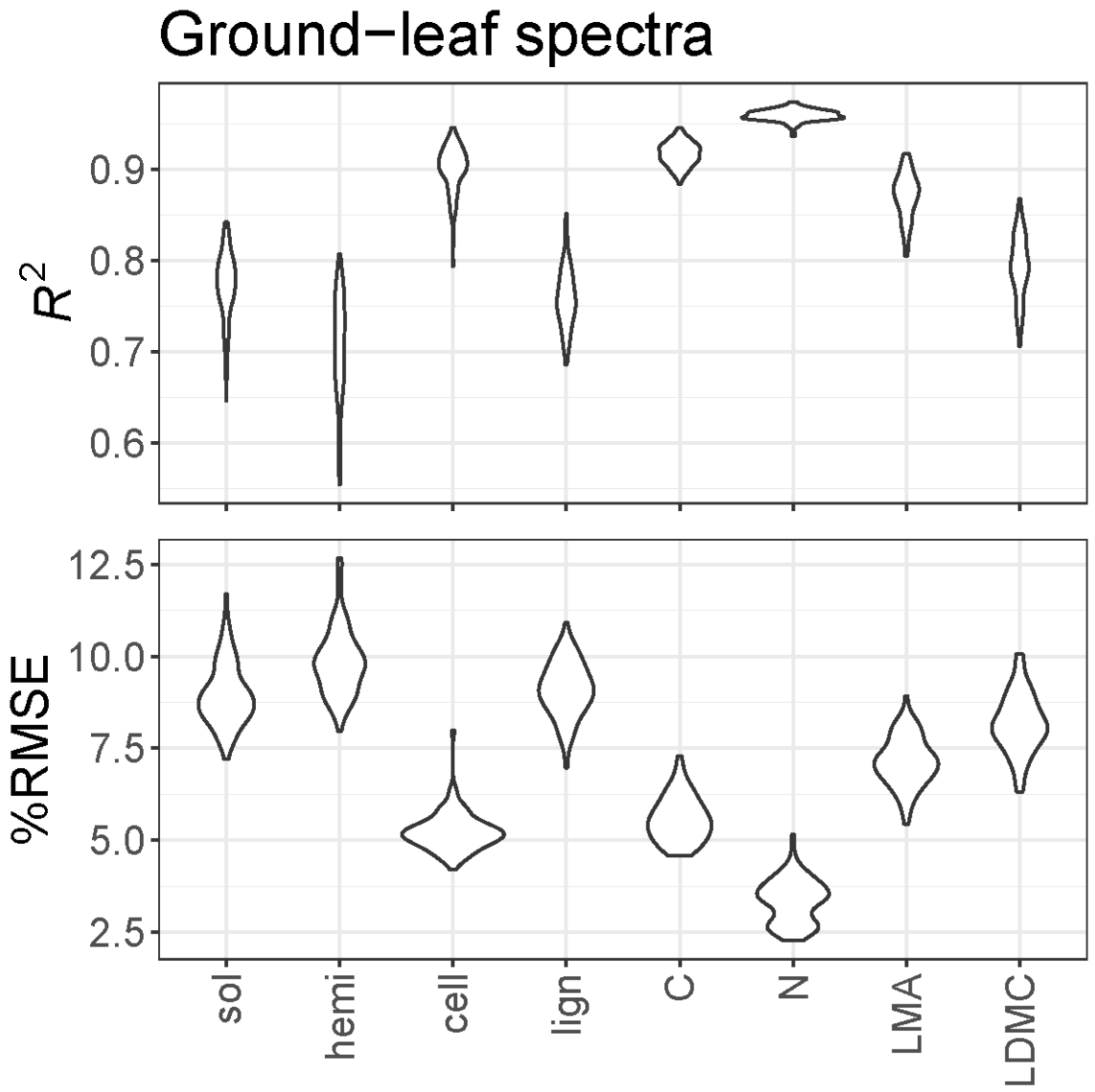
Fig. S3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0.9
0.8
0.7
0.6
%RMSE
12.5
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