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69	Abstra	ct
70	1.	Spectral reflectance measured from herbarium specimens represents a vast source of plant
71		phenotypic and functional trait data.
72	2.	The potential to capture data from specimens to enhance knowledge of plant function and taxon
73		identification has inspired many laboratories worldwide to initiate next-generation spectral
74		digitization from specimens.
75	3.	Combining these datasets into a coordinated global database would enable prediction of traits
76		from the world's plants and allow novel, impactful scientific questions to be addressed at global
77		scale. These novel data streams will generate new capacity to model plant traits globally, enabling
78		connection with remote sensing and ecological and biosphere models and to reconstruct their
79		evolutionary history.
80	4.	Coordination is needed to avoid downstream problems in data aggregation due to variation in data
81		standards and technical specifications of the instruments, optical setups, or measurement
82		protocols. The International Herbarium Spectral Digitization (IHerbSpec) working group has
83		initiated a globally collaborative program, outlining the central issues to address in establishing
84		protocols, standards, and best practices, and next steps. This collaborative effort will allow
85		generation of replicable spectral reflectance data from plant specimens housed in herbaria around
86		the world within ongoing digitization programs following community-defined standards and
87		Findable, Accessible, Interoperable and Reusable (FAIR) principles.
88		
89	Keywo	rds: methodological standards, collection management, plant functional traits, spectral reflectance,
90	next-ge	eneration digitization, global herbarium
91		

93 Introduction

94 In an era of rapid global change and biodiversity loss, safeguarding our knowledge of plant diversity is

- 95 essential. Herbaria serve as foundational repositories for this knowledge, both through their traditional
- 96 applications to document species morphology, distribution, use, and phenology (National Academies of
- 97 Sciences and Medicine 2020, Heberling et al. 2021, Davis 2023, Mandrioli 2023). Advancing
- 98 technologies have now enabled investigations that apply whole-genome sequencing and metabolomic
- 99 methods of herbarium specimens that are up to hundreds of years old (Burbano and Gutaker 2023, Davis
- 100 2023, Medeiros et al. 2024, Davis and Knapp 2025). These advances are now routinely used in
- 101 macroecological and biogeographical studies providing a vast botanical record of species distributions in
- space and time relevant to monitoring biodiversity change in the Anthropocene (Willis et al. 2008,
- 103 Meineke et al. 2018a, Meineke et al. 2018b). We broaden the extended specimen concept, which
- 104 reenvisions the role of specimen data as a vast, connected repository of information about individual
- 105 organisms (Webster 2017, National Academies of Sciences and Medicine 2020), to include spectral
- 106 reflectance from dried plant specimens. A wealth of information about plant chemistry, function, structure
- 107 can be inferred from plant reflectance spectra—the pattern of light's reflectance from plant tissues across
- 108 wavelengths (Elvidge 1990, Gitelson and Merzlyak 1994, Sims and Gamon 2002, Ustin et al. 2004, Asner
- and Martin 2011, Serbin et al. 2014b, Cavender-Bares et al. 2017, Chlus and Townsend 2022, Wang et al.
- 110 2023). Spectra can provide information about taxonomic identity (Durgante et al. 2013), phylogenetic
- 111 placement (Meireles et al. 2020a), defense chemistry in diverse genera (Fine et al. 2021), and phenotypic
- 112 variation linked to genetic or phylogeographic variation (Cavender-Bares et al. 2016b, Deacon et al.
- 113 2017a, Stasinski et al. 2021, Hernandez-Leal et al. 2025). The contribution of spectral information to
- 114 obtaining both functional and phylogenetic information in plants centers reflectance spectra as a critical
- 115 data type in the plant sciences with high potential to integrate information about plant diversity across
- scales, from leaves to ecosystems and the biosphere (Cavender-Bares et al. 2017, Cavender-Bares et al.
- 117 2025, Wang et al. 2022, National Academies of Science and Medicine 2025).

118 Advances in spectroscopic technology and analytical approaches across scales that have enabled 119 spectral data capture across scales (Jetz et al. 2016b, Jacquemoud and Ustin 2019, Serbin and Townsend 120 2020, Wang et al. 2023) have led to the development of the growing field of spectral biology. This field 121 enables trait models and methods of taxonomic identification from spectral measurements of fresh or 122 dried, pressed leaves (Durgante et al. 2013, Meireles et al. 2020a, Kothari et al. 2023), including actual 123 herbarium collections (Kühn et al. 2025, Neto-Bradley et al. 2025, White et al. 2025), to address myriad 124 questions in ecology, evolution, taxonomy, phylogeography, historical biogeography, biochemistry, and 125 related realms of inquiry. When combined with even a fraction of the world's 400 million herbarium 126 specimens (Thiers 2024), reflectance spectroscopy provides a new means to extend our inferences of 127 plant phenotypic and functional variation across space and time – and across the entirety of plant 128 taxonomic and phylogenetic diversity.

129 As with digitization standards for herbarium imaging over the past 25 years (Nelson et al. 2015, 130 Hedrick et al. 2019, Davis et al. 2021), similar consensus on protocols and standards is now needed to 131 incorporate spectral data in the 'global metaherbarium' (Davis 2023). Standardized workflows for 132 spectral data and metadata collection will enable integration across herbarium collections, linking trait 133 estimates to individual specimens and the time, place and environment in which they were collected 134 (Davis et al. 2015, Willis et al. 2017, Meineke et al. 2018a, Meineke et al. 2018b, Pearson et al. 2020). 135 This article documents the purpose and vision of the newly established international herbarium 136 spectral digitization (IHerbSpec) working group, our progress to date in advancing spectral digitization 137 within herbaria across all green plant groups as a scalable tool for biodiversity science. We describe 138 the nature of spectra, the prospect of measuring them in the world's herbaria, highlight the benefits of 139 this massive effort, and address key challenges and next steps.

140

141 The expanding use of plant traits in ecology and evolution

142 Extensive work has been carried out by plant systematists to describe new species based on plant traits,

143 often in conjunction with genetic and genomic data. Plant traits have become fundamental to

144 understanding ecological processes such as interactions with the abiotic environment, interactions with

145 herbivores and pollinators, growth responses to resource availability, community assembly processes, and

- 146 the contributions to ecosystem and biosphere functions (Lavorel and Garnier 2002, Violle et al. 2007,
- 147 Cavender-Bares et al. 2016a, Funk et al. 2017, Shipley et al. 2017, Dechant et al. 2024). Traits provide
- 148 key insights into consistent patterns of resource acquisition and ecological functions across the green
- plant tree of life, termed the Leaf Economics Spectrum (Wright et al. 2004, Díaz et al. 2016). These
- 150 patterns influence species distributions across environmental gradients and inform models of community
- 151 assembly, vegetation dynamics, and biosphere function (Cavender-Bares et al. 2016a). Consequently,

152 significant effort has been invested in developing global plant trait databases (e.g., TRY (Kattge et al. 153 2020), BIEN (Enquist et al. 2016), AusTraits (Falster et al. 2021, Wenk et al. 2024)), and scaling plant 154 traits to global maps to model biosphere dynamics (Dechant et al. 2024). Frequently collected functional 155 traits for leaves include nitrogen and other nutrients, leaf mass per area, cellulose, lignin, pigments, stable 156 carbon isotopies, and other bioechemical compounds, such as carbohydrates, proteins, lipids, and 157 secondary metabolites (Fig. 1). Plant functional traits are well-studied and measured in temperate and 158 arctic regions in well-resourced, easily accessible regions of North America, Europe, China, and 159 Australia, but are undersampled in tropical regions (Bjorkman et al. 2018, Jetz et al. 2016). Common and 160 widespread species are much better represented in plant functional trait databases than rare species, 161 seasonally ephemeral species and species from understudied biogeographic regions. Given that herbaria 162 provide access to plant specimens collected by generations of botanists, they offer a means to 163 systematically obtain trait data from plants in taxa that are rare and/or occur in ecosystems or regions of 164 the world that are difficult to access, filling in global gaps in plant functional traits of the known plant 165 species and lineages (Heberling 2021). Although sampling biases also exist within herbaria (Daru et al. 166 2018), their coverage is better than that of trait databases.

167

168 What are reflectance spectra?

169 Reflectance spectra are observations of the interaction of electromagnetic radiation (light) with get 170 reflected from surfaces resolved to narrow wavelength bands representing a few nanometers each (Fig. 1). 171 Plant tissues from across the tree of life have various structural, anatomical, organellar and biomolecular 172 compositions that influence their spectral properties. As a result, spectral data can be used to estimate a 173 wide array of plant traits, including structural, chemical, and physiological properties (Ustin et al. 2009, 174 Serbin et al. 2014a, Féret et al. 2017), and provide a holistic view of plant phenotypes (Kothari and 175 Schweiger 2022). Reflectance spectra spanning visible, near-infrared and short-wave infrared light (400 -176 2500 nm) from freshly-harvested leaves differ from those obtained from pressed and dried leaves because 177 of changes in water content, pigments and structure during drying (Fig. 1). Spectra from fresh leaves can 178 provide accurate estimates of plant pigments, traits related to water content, and mobile, degradable or 179 small molecular weight carbon compounds (Wang et al. 2023). Dry leaves often provide more accurate 180 prediction of macronutrients, micronutrients and carbon or nitrogren stable isotopes (Chlus and Townsend 181 2022, Kothari et al. 2023) but are unsuitable for predicting water-related traits and some pigments that 182 degrade during drying (Ustin et al. 2009). Spectra also vary with changes in environmental conditions, 183 including light, water availability, carbon dioxide, temperature and soil conditions (Cavender-Bares et al. 184 2016b, Jacquemoud and Ustin 2019, Kühn et al. 2025, Stefanski et al. 2025).

- 185 Given their utility in detecting variation in plants, reflectance spectra are widely used in applied
- 186 contexts. High throughput methods of spectral detection are increasingly used in plant breeding programs
- 187 to detect phenotype variation (e.g., Ge et al. 2016, Meacham-Hensold et al. 2019). Spectral signatures of
- 188 plants are also used to detect heavy metals and other environmental contaminants in plant tissues (Zhang
- 189 et al. 2022), to scale up detection of pathogen infections via remote sensing (e.g., Fallon et al. 2020, Sapes
- 190 et al. 2024) and for detection of invasive species (e.g., Dao et al. 2021, Mallmann et al. 2023). Currently,
- 191 major efforts are underway to scale up spectral data from plants for biodiversity monitoring (Jetz et al.
- 192 2016a, Cavender-Bares et al. 2020, Wang et al. 2020, Williams et al. 2021, Cavender-Bares et al. 2022,
- 193 Blanchard et al. 2024, Dechant et al. 2024, Hadlich et al. 2025).
- 194

195 Estimating plant traits from spectra of herbarium specimens

196 Advancing technologies and a suite of studies over the past decade have set the stage for applying spectral 197 biology to herborized specimens to predict plant function (Fig. 1). Some of the first spectral studies of 198 dried leaves were conducted in the early 1990s and contributed to early vegetation index-based or 199 radiative transfer model-based approaches to linking spectra with traits (Elvidge 1990; Fourty et al. 1996). 200 More recently, Costa et al. (2018) showed the potential of using machine learning models trained on 201 spectra on silica-dried leaves to predict traits of tropical tree species. Building on this work, Kothari et al. 202 (2023) showed similar results for a wide range of traits in a large data set of unmounted pressed leaves 203 from temperate forest species; indeed, for most chemical traits, spectra of pressed leaves performed better 204 than the more conventional approach of using spectra of fresh leaves. Sampling the same species as 205 Kothari et al. (2023), White et al. (2025) subsequently showed that spectral models trained using detached 206 leaves from decades-old herbarium specimens accurately predicted key traits such as leaf mass per area. 207 Kühn et al. (2025) applied a spectral model to 20th-century herbarium specimens to show temporal trends 208 in leaf carbon, nitrogen, and phosphorus content associated with intensifying agricultural practices in 209 Germany.

210

211 Figure 1. Plant traits derived from full-range spectral reflectance (400 - 2500 nm) from fresh leaf tissue 212 (A) and dried, pressed leaves (B). Spectral regions can be characterized into the visible range (VIS, 400-213 700 nm, shown in rainbow colors in both A and B), the near infrared (NIR, ~700-1100), and the short-214 wave infrared (SWIR1 ~1100-2000, and SWIR2, ~2000-2500, shown by the gray shaded areas in (A). 215 Plant pigments (chlorophyll a and b, carotenoids, anthocyanins), traits related to water content and water 216 potential, including leaf dry mass concentration (LDMC), equivalent water thickness (EWT), and mobile, 217 degradable or small molecular weight carbon compounds (sugars, nonstructural carbohydrates) can be 218 derived from spectra in fresh leaves. In dry leaves, water-related and pigmentation traits may not be 219 reliably derived due to degradation. In both fresh and dry leaves, it may be possible to estimate 220 macronutrients (nitrogen (N), carbon (C), phosphorus (P), potassium (K)) and micronutrients (calcium 221 (Ca), zinc (Zn), iron (Fe), copper (Cu), sulfur (S), boron (B), magnesium (Mg), manganese (Mn), 222 aluminum (Al), sodium (Na)), as well as large stable carbon-based molecules (phenolics, lignin, cellulose 223 or hemicellulose), defense compounds (phenolics, flavonoids) and stable isotopes (δ^{13} C, δ^{15} N). Indeed,

they may be more accurately detected from dried tissue due to the absence of water. Water absorbs energy throughout the spectral range shown, but particularly in the bands indicated in A. Some of the spectral

features that are important for predicting traits are indicated in B, adapted from Cavender-Bares et al.
 2025.



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230 Box 1 Brief glossary of terminology applied to plant reflectance

- **Band** a range of wavelengths within a spectrum for which the radiation is detected.
- **Bandwidth** the range or span of the spectral band.
- Dark current the small electric current that flows through a spectroradiometer even in the absence of incident
 light.
- Electromagnetic spectrum Wavelengths of electromagnetic radiation that span short wavelength (high frequency)
 to long-wavelength (low frequency) wavebands.
- Fiber optic strands of glass or plastic (optical fibers) used to transmit light, for example as reflectance from a leaf
 to an instrument detector.
- Full range spectrum Spectroradiometers used for foliar spectroscopy commonly span the range of 400 2500 nm
 covering the visible (VIS), near-infrared (NIR) and shortwave infrared (SWIR), which is considered full
 range (VIS-NIR-SWIR) wavelengths. Alternately, some instruments only span 400-1000 nm, which covers
- only the visible and near-infrared.
- Herbarium specimen a specimen with associated taxonomic, geographic, temporal and other collection metadata
 stored in an herbarium, for which we might not know how it was dried or conserved

- Hyperspectral Spanning the visible to near-infrared or shortwave infrared measuring narrow wavelength bands
 every 3 to 5 nm.
- 247 InGaS sensor photodiodes for near-infrared (NIR) and short wavelength infrared (SWIR) regions.
- 248 Near-infrared (NIR) the range of electromagnetic spectrum from 700 1100 nm.
- Partial Least Square Discriminant Analysis (PLS-DA) a machine learning approach used for the classification
 of high-dimensional datasets such as spectral data. PLS-DA utilizes spectral phenotypic data to create a
 simplified classification method to distinguish among taxonomic or functional groups.
- 252 Pressed plant a controlled dried specimen that was pressed flat to dry
- Partial Least Square Regression (PLSR) is a statistical method (multivariate regression) used to model the
 relationship between X (predictor) and Y (response) variables. In spectral biology, PLSR is used to
 estimate foliar traits due to its ability to handle multicollinearity and reduce the dimensionality of spectral
 data.
- 257 Radiative transfer models (RTM) - physical models using computer programs to simulate the reflectance, 258 transmittance, and absorption of solar radiation in various media, including leaves. Governed by physical 259 laws, RTMs can operate in forward mode to predict spectral responses of leaves based on material 260 characteristics or in backward mode to infer material properties from leaf spectra. These models vary in 261 complexity, balancing computational demand, accuracy, and scalability. The number of traits that can be 262 simultaneously identified is limited. For remotely sensed imagery, inputs such as solar radiation and 263 elevation angle, and parameters like leaf angle distribution and leaf area index help to solve the radiative 264 transfer equation in optical or thermal domains.
- Reflectance standards A reflectance standard is a physical reference sample that includes ratio values between the
 total amount of radiation, as of light, reflected by a surface, and the total amount of radiation incident on
 the surface across the measured spectrum. Reflectance standards are used for the calibration and
 verification of spectrometers.
- Spectral reflectance Reflectance expressed as a function of wavelength (i.e., as a spectrum). Spectral reflectance
 is the fraction of the incident radiant flux that is reflected as a function of wavelength

271 Short wave infrared (SWIR) - The range of the electromagnetic spectrum between 1100 and 3000 nm.

- Spectral biology -The study of biological processes and biodiversity using spectral resolved observations of light's
 interaction with biological systems to reveal plant chemistry, structure, and function across scales, from
 genomes to ecosystems.
- 275 Spectroradiometer -A device designed to measure electromagnetic radiation across a range of wavelengths; a
 276 radiometrically calibrated spectrometer (c.f. spectrometer).
- Spectroscopy The study of interactions between electromagnetic radiation and matter, used in imaging to measure
 reflected radiation from image pixels that is used to analyze the properties of leaves, canopies, ecosystems,
 and landscapes over time and space.
- 280 Spectral resolution the measure of a sensor or spectrometer's ability to distinguish between closely spaced
 281 wavelengths within the electromagnetic spectrum. It is quantified by the width of the spectral bands, the

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minimum resolvable wavelength interval, or, specifically for spectrometers, as the ratio of the measured wavelength to the full width at half maximum (FWHM) of a spectral peak ($R = \lambda/\Delta\lambda$). Higher spectral resolution, characterized by narrower bands or smaller FWHM values, allows for finer discrimination of spectral features and thus, more detailed identification of materials.

286 Spectral range - Span between the smallest and largest wavelengths that a sensor or system can detect.

Taxon discrimination methods - analytic methods aimed at classifying unidentified samples into taxonomic
 categories, or clustering samples according to taxonomic affinities.

Trait models - physical, statistical or machine learning models used to predict foliar traits from leaf spectra
 Visible range (VIS) - the range of electromagnetic spectrum from 400 - 700 nm.

291 292

293 Applying spectral signatures to phenotype-genotype associations and evolutionary models

294 Spectroscopy provides a source of phenotypic data that can be measured on samples across the plant tree 295 of life and coupled with genetic and genomic information (Fig. 2) or to scale up the study of phenotypic 296 variation across large spatial, temporal, and phylogenetic scales from variation within species to the plant 297 tree of life (Meireles et al. 2020, Cavender-Bares et al. 2025). Integrating spectra with genomic data 298 allows researchers to determine factors and evolutionary processes involved in shaping underlying traits 299 (Matsuda et al. 2012; Čepl et al. 2018). For example, spectral data can reveal variations in traits relevant 300 to ecological niches, while genomic data provides insight into the genetic architecture underlying these 301 traits (Blonder et al. 2020; Madritch et al. 2014). Together, these kinds of datasets can reveal how 302 populations adapt to environmental pressures (Ge et al. 2019, Galan et al. 2020). Such integration 303 revolutionizes our understanding of how adaptation unfolds at both microevolutionary and 304 macroevolutionary scales. By comparing spectral data with genomic markers, it is possible to identify 305 genetic loci associated with key traits, such as cold tolerance or drought resistance, and assess how these 306 loci vary across populations (Madritch et al. 2014, Cavender-Bares et al. 2016b, Czyż et al. 2020). This 307 integration illuminates the demographic history and selective pressures driving adaptation. For instance, 308 closely related species of *Quercus* subsection *Virentes* exhibit various forms of evolutionary divergence, 309 including sympatry, allopatry, and parapatry (Cavender-Bares et al. 2015). These species show spectral 310 phenotypic divergence associated with specific microhabitats, shaped by local adaptation to ecological 311 variation (Hernandez-Leal et al., 2025). This example highlights how demographic processes and 312 selective pressures differ across spatial scales. Phenotype-genotype associations offer a robust framework 313 for uncovering the interplay between natural selection, demographic processes, and adaptation (Kokaly et 314 al. 2009, Cavender-Bares et al. 2016b, Deacon et al. 2017b, Blonder et al. 2020, Stasinski et al. 2021, 315 Hernandez-Leal et al. 2025). Coupling spectral data with genomic and genetic information expands 316 opportunities for understanding evolutionary processes across the plant tree of life (Ge et al. 2016).



318 Figure 2. An example of combining genotypic and phenotypic data derived from pressed leaf spectra. 319 The spatial distribution of genetic (left) and phenotypic (right) variation in *Quercus oleoides*. Four genetic 320 groups were identified using STRUCTURE (Pritchard 2000) from 123 individuals and five phenotypic 321 groups identified by GENELAND (Guillot et al., 2009) using six spectrally derived leaf traits (leaf mass 322 area, thickness, solubles, hemicellulose, cellulose, lignin) derived from dried leaf spectra from all 323 individuals. The percentage assignment to genetic or phenotypic groups is represented at both the 324 individual tree level (upper bar plots) and subpopulation level (pie charts). Genetic data are from 325 Cavender-Bares et al. 2015. Figure is adapted from Hernandez-Leal et al. 2025.

326 Spectral data can also be used to model species traits on phylogenetic trees using comparative 327 methods (Harvey and Purvis 1991, Cavender-Bares 2019, Meireles et al. 2020a). Traits derived from 328 spectra, or spectral indices and variables themselves, can be used to model evolutionary rates, constraints 329 and selection when spectra are consistently measured across species. In a simulation exercise, we show 330 how dried leaf spectra are expected to evolve according to four models of evolution, Brownian motion 331 random walk model with fast and slow rates of evolution, a single-optimum Ornstein-Uhlenbeck model of 332 adaptive evolution, and an Early Burst model with declining rates of evolution through time (Fig. 3). A 333 major challenge, however, is the high dimensionality of full-range spectral data, which are comprised of 334 hundreds of interdependent variables per measurement. Incorporating this high-dimensionality within a 335 phylogenetic comparative framework is challenging both statistically and computationally because of the 336 high number of coevolving traits in a lower number of taxa (Clavel et al. 2019). Novel comparative 337 methods such as penalized likelihood approaches can now be applied to spectral data in phylogenetic 338 frameworks (Clavel et al. 2015, Clavel et al. 2019). 339



342 Figure 3. Simulated evolution of leaf structure (i.e., numbers of cell layers, N) under three models of 343 evolution and the corresponding dried leaf spectra predicted from leaf traits using the PROSPECT D 344 radiative transfer models (Féret et al. 2017) following Meireles et al. (2020a). Each row represents one 345 model: Brownian motion with fast (A, B) or slow (C,D) evolutionary rates, a single-optimum Ornstein-Uhlenbeck adaptive evolution process (E,F), and an Early Burst process with declining rates over time 346 347 (G,H). The graphs on the left show phylogenetic trees through relative time (x-axis) with y-values and 348 branch colors indicating simulated leaf structure values. The graphs on the right show simulated 349 reflectance spectra (400–2500 nm) for the final trait values of each lineage. (Starting values for the traits 350 in the models are as follows: N=0.5, chlorophyll, Cab=10; carotenoids, Car=12.0; LMA/leaf dry matter 351 content, Cm = 0.0005; water, Cw=0; leaf structure; sigma value for N=0.1.) 352

353

354 Taxon discovery and discrimination

Spectral data have become a valuable tool for evaluating and refining taxonomic hypotheses, enabling
rapid, non-destructive assessment of phenotypic cohesion and differentiation among taxa (2024). Across
taxonomic ranks, spectra from dry leaves have been used to supplement DNA-based methods and support

- 358 systematic studies, particularly in morphologically complex clades. In the Amazon, researchers have
- analyzed spectral absorption profiles with a Fourier-transform near-infrared (FT-NIR) spectrometer on
- 360 unmounted leaf samples to distinguish closely related species within the genus *Eschweilera*
- 361 (Lecythidaceae) (Durgante et al. 2013) as well as among different developmental stages of species in the
- 362 Burseraceae (Lang et al. 2015). Paiva et al. (2021) applied spectroscopy to pressed fern fronds to classify
- 363 species in the genus *Microgramma* with over 90% accuracy Both Prata et al. (2018) and Damasco et al.
- 364 (2019) integrated DNA and spectral data to suggest taxonomic solutions in species complexes, including
- the reestablishment of *Protium cordatum* (Burseraceae) to species rank (Damasco et al. 2019).
- 366 Spectroscopy is now being used to support new species hypotheses based on morphological and
- 367 morphometric characteristics (Vasconcelos et al. 2020, da Cruz Vasconcelos et al. 2021, Gaem et al.
- 368 2022, Costa et al. 2025) or to differentiate hybrids from parental species (Deacon et al. 2017a).
- 369 Applications of these approaches to herbarium specimens are more recent. White et al. (2025)
- 370 showed that spectral models from herbarium specimen leaves of diverse ages and sources (with a median
- age of 91 years) could classify specimens to species with up to 74% accuracy and genera up to 84%
- accuracy. In a broad study of stone oak (*Lithocarpus*) specimens from across Asia, Neto-Bradley et al.
- 373 (2025) found that machine learning models trained on spectra work nearly as well as those from digitized
- 374 images for identifying taxa. They concluded that spectra may be particularly important for identifying
- 375 incomplete specimens of historical significance that may otherwise only be identified to family. In the
- 376 future, it should be possible to combine digital images with spectral profiles to increase our power of
- 377 discrimination at different taxonomic levels.

These studies highlight the feasibility of using spectra from herbarium specimens for species delimitation, taxonomic revision, and the detection of cryptic diversity. Discriminatory models offer an additional dimension of variation—rooted in chemistry and structure—that can complement morphology and genetics. Moreover, spectral classification can serve as a test of taxonomic validity, revealing whether phenotypic variation aligns with expected groupings. High within-taxon variation or convergent phenotypes can reduce accuracy, while informative spectral regions may shed light on the evolutionary processes shaping trait divergence.

385 For plant systematists, these advances represent a major opportunity to re-explore herbarium 386 collections with new eyes. Spectral profiles can uncover consistent differences in chemical and structural 387 traits in specimens that may not be evident through morphology. This offers an additional dimension of 388 variation for species delimitation, taxonomic revisions, and the identification of cryptic diversity, 389 especially in groups with limited diagnostic characters or incomplete material. As the number of spectral 390 studies increases, the development of shared protocols and metadata standards will be essential to ensure 391 reproducibility and cross-study comparability, enabling broader synthesis and integration into taxonomic 392 workflows.

393 Operational workflows in botanical taxonomy are advancing rapidly, driven by the robustness 394 and consistency of the results achieved across diverse taxonomic groups. This momentum will be further 395 amplified by the development of compact handheld and benchtop spectrometers that have the potential to 396 greatly expand the use of spectroscopy in herbarium collections worldwide. These devices enable 397 individual herbaria to conduct spectral analysis of type specimens and other restricted materials, 398 overcoming common barriers such as loan limitations and fragility of historical collections. Yet there are 399 challenges to every aspect of this work, which if not addressed will make using the data collected 400 difficult. Through an international collaborative working group, Meireles et al. (2020a) combined spectral 401 data from fresh leaves collected from three different instruments models in different parts of the world by 402 different investigators. By reconciling the instrument-specific variation and converting the data to a 403 common format, they showed that reflectance signatures show phylogenetic signal and are tightly coupled 404 to the tree of life with the capacity to reveal evolutionary history. This effort demonstrated both the 405 difficulty and the benefits of aggregating data.

Generating spectral measurements from specimens of known identity will enable the
development of reference datasets for identification purposes and may help to provide a preliminary
taxonomic classification of the multitudes specimens in herbaria that have not been identified because
they lack diagnostic structures, or have not yet been studied by sufficiently knowledgeable experts (NetoBradley et al. 2025). Fresh leaf spectra show promise for classifying species to all taxonomic ranks
(Meireles et al. 2020b, Mallmann et al. 2023, Blanchard et al. 2024, Hadlich et al. 2025), and dried leaf

- 412 spectra may show even greater promise because the removal of the water absorption bands in the SWIR
- 413 may reveal more unique absorption features (Kothari et al. 2023). Stepwise hierarchical approaches for
- 414 classifying taxa first to broader and then narrower clades is a promising approach to accelerate the
- 415 identification of undetermined (dried) herbarium material (Fig. 4). Such an approach would get around
- 416 the problem of computational limits and reduced accuracy when large numbers of classes are
- 417 discriminated among. To achieve high accuracies, highly populated spectral libraries at each phylogenetic
- 418 scale would be necessary.
- The success of taxonomic discrimination depends on the similarity of leaf and other plant part
- 420 morphologies within each of the assigned taxa and can be interpreted as a test of cohesion within groups
- 421 and distinction between them. As such, an application of taxonomic discrimination models can be used to
- 422 test the taxonomy itself. If there is nearly as much phenotypic variation within as between taxa, or if
- 423 phenotypes evolve convergently, the success of taxonomic discrimination may be limited. Discriminatory
- 424 models can potentially reveal the spectral regions that can best distinguish taxa, which may contribute to
- 425 deciphering how taxa have diverged or converged and the evolutionary forces that have shaped their
- 426 phenotypes.
- 427

428 Figure 4. Stepwise hierarchical approach to taxon classification to place specimens within the plant tree 429 of life. Classification algorithms, such as partial least squares discriminant analysis (PLSDA), are limited 430 by statistical power in the number of entities they can accurately discriminate between. One possible 431 solution is a nested approach where spectral signatures are used to differentiate broad clades within the 432 vascular plants, and stepwise within increasingly smaller clades, such as orders, families, genera-or 433 increasingly narrow phylogenetic lineages. Shown are confusion matrices from broad to increasingly 434 narrow taxonomic groups, starting with A) broad clades in the vascular plants, including the Rosid clade, 435 B) orders within the Rosids, including the Fagales, C) sections of the genus *Quercus* within the Fagales, 436 and D) species within *Quercus* section *Virentes*. Correct assignments are on the diagonal, incorrect 437 assignments are in the off-diagonal cells. Data used in A and B and the vascular plant phylogeny are from 438 Meireles et al. 2020; spectral data used C are from Cavender-Bares et al. 2016, spectral data in D are from 439 Hernandez-Leal et al. 2025. The *Quercus* phylogeny is from Hipp et al. 2020.



440



442 The beauty of the 'global metaherbarium' lies in its ability to connect a wide array of data types that

together comprise the extended specimen (Webster 2017, Lendemer et al. 2020, National Academies of

444 Sciences and Medicine 2020, Davis 2023, Davis and Knapp 2025). These include not only the physical

specimen itself, but also associated data such as digital images, species distribution models (SDMs), DNA

446 sequences, and ecological trait measurements (Webster 2017, Lendemer et al. 2020) promising layers of

447 information that can significantly enhance the value of the specimen and the power of global-scale 448 analyses if consistent or interoperable approaches can be established. While there is considerable 449 excitement for the application of spectrometry in herbaria, a series of methodological, material, and data 450 processing challenges must be addressed. Differences in instrumentation, specimen preservation, and data 451 processing pipelines introduce variability that complicates large-scale data aggregation and cross-452 institutional compatibility (Fig. 5, (White et al. 2025). These issues were encountered and overcome by 453 the NIMBioS (National Institute for Mathematical and Biological Synthesis) working group (Meireles et 454 al. 2020c), which aggregated and harmonized spectral data collected from fresh leaves using different 455 instruments and protocols around the world. Their work demonstrated both the promise and difficulty of 456 harmonizing spectral data, given that spectra can vary significantly based on optical system design and 457 measurement conditions. A further set of challenges arises from the varied nature of how herbarium 458 specimens are treated upon intake, their age, their storage conditions, the impact of chemical additives 459 such as glues, pest prevention treatments, and other specimen conservation factors (Kühn et al. 2025, 460 White et al. 2025). To maximize the utility of spectral data in functional trait estimation, taxonomic 461 classification, and evolutionary modeling, standardized measurement protocols across institutions, data 462 quality controls, and robust correction methods are critical.



Figure 5. The herbarium spectral digitization workflow from specimen collection to global integration, and challenges. A) Variation in specimen condition and mounting methods, including changes in leaf preservation quality, use of adhesives or sewing, and presence of detached leaves in packets. B) Spectral digitization process using different spectroradiometers, with emphasis on black background placement and data upload; differences in instruments, optics, and data processing. C) Global integration of spectral data across herbaria, linked to specimen records and trait data such as carbon (C), nitrogen (N), and leaf mass per area (LMA).

471

472 *Spectral regions to measure*

473 Spectroscopy has been widely used to analyze fresh leaf tissue in the range of 400-2500 nmto obtain 474 information about plant function and phenotype (Table S1). The visible range (400 - 700 nm) is strongly 475 associated with chlorophyll, carotenoids, anthocyanins and other pigments (Gitelson et al. 1998, Gamon 476 and Surfus 1999). Chlorophyll is partially degraded when exposed to light and may be reduced in 477 herborized leaves, but it can still be detected. Spectral information related to carotenoids, flavonoids, 478 lignin, and other chemical compounds remain readily detected and may be less susceptible to degradation. 479 In the near-infrared-region (NIR, 1000-2500 nm), vibration frequencies associated with molecular 480 functional groups (-CH, -NH, -OH) show features of primary metabolites (carbohydrates, lipids, and 481 proteins) as well as of secondary metabolites (phenolic compounds, terpenoids, and alkaloids) (Türker-482 Kaya and Huck 2017, Jacquemoud and Ustin 2019, Ustin and Jacquemoud 2020). Spectral regions 483 beyond 2500 nm reveal additional information. In the mid- and long-wave infrared regions (2500-25000 484 nm), for example, detailed chemical identification is possible, particularly for pure chemical compounds, 485 (Türker-Kaya and Huck 2017), providing opportunities to detect ecologically and evolutionarily 486 important differences among organisms, even when we do not have their taxonomic names. 487 488 Variation in foliar phenotypes. Spectral data capture variation in leaves due to a multitude of factors,

variation in joint prenotypes. Speetra data captare variation in feaves due to a maintaide of factors,

including leaf developmental and ontogenetic stage, light exposure and environmental conditions during

growth, and a range of other factors (Fajardo and Siefert 2016). Exposure of the foliar tissue to a range of

491 environmental conditions within a single tree canopy, across environmental gradients or in response to

492 temperature, water availability or CO₂ concentrations can modify tissue properties (Stefanski et al. 2025)

493 and thus influence the reflection of light. Spectral properties and traits change over the lifecycle of the

leaf (e.g., Fajardo and Siefert 2016, Chlus and Townsend 2022). Within a single leaf, tissue properties

- will change from the edges to the vascular tissue. With age, leaves add secondary cell wall material,
- 496 cuticles become more structurally complex and thicker, and concentrations of biochemical compounds
- 497 change, including compounds that are upregulated or produced in response to stress. Herbarium
- 498 specimens representing species with asynchronous flowering or fruiting may disproportionately contain
- 499 young, developing leaves or reproductive structures, leading to spectra that do not adequately represent

mature leaves (Fig. 6). Predictive models show promise in accommodating such biological variation as
long as models are trained to "see" the full range of variation (Lang et al. 2015, Wang et al. 2023).

502

503 Adaxial and abaxial leaf surfaces. Adaxial and abaxial surfaces on vascular plants are often distinct. 504 Given that leaf spectroscopy is often used to ground-truth remote sensing measurements, many protocols 505 only involve measuring the adaxial surface of the leaf, which is the side most likely to be observed from 506 above. Measurements of herbarium specimens have different goals, however, and the option is available 507 to measure both leaf surfaces. The abaxial surface may contain important features for taxonomic 508 identification, including pubescence or differing pigmentation. Whether both leaf surfaces should be 509 measured as standard is an area for further investigation and discussion. In any case, it is important to 510 document the leaf surface measured, given their spectral signatures are different (Ustin et al. 2001). 511

Leaf size. Small leaves present challenges to spectral measurement because they require adaptations to
optical detection arrangements, such as fine leaf probes, small radius fiber optic cables, and/or small
detection windows, which can reduce the signal-to-noise ratio and quality of the reflectance signal.
Conifer needles, vascular plants with tiny leaves, and non-vascular plants such as mosses and lycopodia,
can be difficult to measure due to their small size and the fact that many of them do not lie flat (Fig. 6).
When leaves are not flat, the 'geometry' or angle of the light source relative to the surface of the leaf often
varies from one measurement to the next, influencing the specular component of reflectance. When

519 possible, it may help to measure the flattest parts of the leaves. Alongside these challenges, lichens also

520 pose challenges due to their changing form and physiology when they dehydrate.

521

522 *Specimen age.* Over time specimens may undergo chemical or even structural degradation due to

523 exposure to environmental factors. Although some aspects of this process have been documented,

524 including the breakdown of chlorophyll and accumulation of brown pigments (Fourty et al. 1996), in

525 general little is known about the suite of changes through time and the factors that slow or accelerate

them. White et al. (2025) found that older specimens exhibit a slight decline in correct classification

527 probability from spectra. The potential influences of degradation on spectra reinforces the need for careful

528 specimen selection and possibly data filtering strategies that account for specimen age and preservation

529 history when integrating spectral datasets (Durgante et al. 2013, Lang et al. 2015).

530

Figure 6. Variation in the challenges of measuring reflectance spectra on mounted specimens. A) An
example of a relatively straightforward measurement of a specimen leaf against a black background using
a one-sided leaf clip with a fiber optic probe that is smaller diameter than the leaf laminar surface area.

534 Isotypes of (B) *Restio arcuatus* and (C) *Vulpia microstachys* are taxa with small leaves. D) *Abies*

- *balsamea*, a conifer with typical narrow needles that is taped to the herbarium sheet but has also been
- 536 glued, evidenced by the discolored glue indicated with the arrow. A fully taped *Salix arctica* (E) specimen
- 537 from the Botanical Museum of Copenhagen with conspicuously gray discoloration representing
- variability in preservation techniques and quality. An herb (F) with delicate, thin leaves that are fully
- 539 glued to the sheet with no packet containing extra material to measure. A *Sticta* lichen (G) with a non-flat 540 thallus that will distort optical geometry. Circles on the top left demonstrate various aperture sizes (mm)
- 540 that will distort optical geometry. Circles on the top left demonstrate various aperture sizes (min) 541 typical of different optical instruments. For comparison, scale bars are shown in B, C and F, with a 2 cm
- 542 bar in B.
 - Representative 22 D В optical aperture 6 sizes (mm) 2 2 cm G BOSTON SOCIETY Е F OF NATURAL HIST anthralis reschata nemel" Filamesk Dolley, Oregou Coll. (uns Summers (boy) +98

545 *Variation in preservation among specimens in herbarium collections.*

546 Herbarium collections are generally curated to maximize the taxonomic and/or geographical diversity of

- 547 specimens for given regions, and may represent centuries of collection efforts across biomes. The
- 548 historical and biogeographic breadth has led to heterogeneity in specimen preservation techniques.
- 549 Variability in storage conditions among herbarium facilities compounds the variation due to preservation
- 550 methods. The most significant factors affecting specimen degradation and quality are the protocols
- implemented to initially press and dry the plant collection in the field. The best case scenario would be
- 552 that a collected specimen is immediately pressed inside acid-free paper and dried with forced-air at
- ambient temperatures. However, this practice is rarely followed, and specimens, especially in the humid

tropics, are usually sealed in a bag containing 60-95% ethanol to kill fungus and prevent rotting. In all
latitudes, pressed specimens are routinely dried using industrial ovens.

556 Glue is a particularly significant contaminating source for herbarium spectra due to its potential 557 for direct contamination. For example, White et al. (2025) found that glue reduced the probability of 558 correct species identification from spectra. Sewing specimens to the herbarium sheets or using archival 559 grade mounting tape, both durable and secure mounting practices commonly used in European and South 560 American herbaria, can avoid this problem. However, it is highly labor-intensive and has not been a 561 standard practice in many herbaria. Consequently, glues are the most widely used adhesive in North 562 America. The leaves of some specimens may contain multiple layers of different adhesives. Other 563 common historical practices that may have resulted in the contamination of spectra include methyl 564 bromide fumigation (now banned) and the sprinkling of diatom powder and various poisons such as 565 arsenic or naphthalene. Efforts to 'unmix' or 'subtract' the glue or paper spectra from the leaf using 566 spectral libraries of these contaminants have not yet yielded solutions to isolating the leaf signal from a 567 spectral profile that contains these extra materials (A. Guzmán, B. Neto-Bradley, JE Meireles, 568 unpublished data). Although many large herbaria in well-funded institutions can maintain temperature 569 and humidity controls, humidity fluctuations remain a significant challenge worldwide, potentially 570 accelerating specimen degradation.

571

572 Instruments and optics. Variation in detectors and optical setups among instruments create differences in 573 spectral signatures, adding complexity to the use of herbaria for spectral data collection (Fig. 5, Table 1). 574 The signal-to-noise ratio, field-of-view, and spectral resolution and range are inherent instrument 575 variables that can influence optimal measurements on specimens. Standardization of spectral digitization 576 protocols and proper documentation is thus crucial for ensuring interoperability among spectral 577 digitization efforts and integration into broader analytical frameworks. The signal-to-noise ratio (SNR), 578 for instance, determines the quality and reliability of the measurements, where higher values mean clearer 579 and more precise spectral data. SNR has a major effect on the comparison of measurements between 580 instruments and, thus, the transferability of potential models derived from them. SNR is mostly 581 influenced by light detector sensitivity (particularly affecting the wavelengths near the edges of the 582 detector's spectral range) and light source intensity. Inappropriate selection of integration times, incorrect 583 calibration, or variation in viewing and illumination angles can impact the SNR. The removal of bands 584 with low SNR at the edge of the spectral range can help integrate data from different instruments into 585 models (White et al. 2025). However, removing bands should not be a default approach and should be 586 carefully considered in relation to project goals.

587 The field-of-view (FOV) – the angular extent of the observable area that can be seen through 588 foreoptics or lenses – is another variable that often differs between instruments and influences how well 589 specimens are captured in a single measurement. A wider FOV allows for the capture of a larger portion 590 of the specimen, thereby increasing the representation of intraspecific variability. However, wider FOVs 591 are usually associated with lower SNR measurements and are not ideal for small leaves and make it 592 difficult to target specific leaf regions such as the blade rather than the central vein. Additionally, some 593 instruments present a non-uniform FOV due to the optical fiber bundle and its integration with the light 594 detector, which does not uniformly cover the viewing area (Lévesque et al. 2014). Using instruments with 595 a narrow or non-uniform FOV might require several measurements of a leaf specimen to adequately 596 capture the variability of optical information.

597 The spectral range and resolution are additional instrument variables inherent in instrument 598 design. The spectral range of instruments is distinct from spectral resolution and is commonly used to 599 differentiate between VIS-NIR spectrometers (e.g., 350 – 1000 nm), VIS-SWIR spectrometers (e.g., 350 600 -2500 nm; also known as 'full range'), MIR (2.5-6.0 μ m and and TIR spectrometers (e.g., 6.0 - 16 μ m) 601 because these detect photons in different regions of the electromagnetic spectrum. Although VIS-NIR 602 spectrometers are less expensive than full-range spectrometers, some studies on dried leaves have begun 603 to suggest that full-range spectra perform better for trait prediction (Kothari et al. 2024; White et al. 604 2025). Moreover, the spectral resolution of many full-range instruments varies within instruments because 605 there are usually two or more light detectors, a silicon sensor (VIS and NIR) and an InGaAs (indium 606 gallium arsenide) detector for the 1000–1700 nm wavelength region (e.g. Spectral Evolution) or an 607 extended InGaAs covering the spectrum to 2500 nm (NIR and SWIR), each with distinct sensitivity and 608 band sampling. Due to a trade-off between sensitivity and signal availability (i.e., light), silicon detectors 609 designed for VIS-NIR wavelengths (e.g., 400 – 1000 nm)-where halogen bulbs generate peak irradiance-610 have high wavelength resolution. Consequently, they often achieve higher spectral resolution than 611 detectors that span NIR-SWIR wavelengths (e.g., 1000 - 2500 nm). Instruments commonly report data 612 output in 1-nm increments for spectral sampling of bandwidths 3 to 6 nm wide. Given the variation in 613 design among instruments, the scanning interval (band center and band width) at different spectral ranges 614 should be recorded in the metadata (see below). 615 Additional factors influencing the quality of spectral measurement data include the sensitivity of

616 fiber optics, quality of standards, viewing and illumination angles, and the intensity of light sources 617 (Grant 1987). Variations in fiber optic alignment can impact the signal-to-noise ratio, requiring careful 618 handling and regular replacement (manufacturer maintenance should confirm whether all fibers are 619 functional or, if not, determine the percentage of remaining fibers and their locations in the cluster). Use 620 of a standardized leaf probe with integrated illumination avoids problems with the positioning of the

- 621 lights and the spectrometer. Regular replacement of calibration standards and routine instrument
- 622 maintenance, such as cleaning and recalibrating sensors, are essential for maintaining instrument
- 623 performance. The proper selection of light source intensity along with optimized integration times is
- 624 critical to avoiding heat effects on specimens, which can alter both their optical properties and integrity.
- 625 Lamps should be operated on direct current, not alternate current power supplies.
- 626

627 Table 1. Challenges to aggregating spectral data across specimens and institutions and potential

628 solutions.

Challenge	Description	Mitigation Strategy
Variation in leaf phenotypes due to environment	Environmental variation (light, temperature, CO ₂ , nutrients) can influence spectra. Variation within a leaf	Collect spectra on specimens across a range of conditions to capture the full variation; avoid midrib and leaf edges, avoid leaves with herbivory and pathogens.
Variation in leaf phenotypes due to leaf age/development stage	Spectral properties and traits change over the lifecycle of the leaf	Capture information about leaf age in the metadata
Specimen age	Chemical degradation, pigmentation loss, and exposure to environmental factors over time can influence spectral signatures	Collect spectra on specimens across a range of ages to capture the full variation. When possible,conduct analyses on specimen age effects. Effects may not be large. Metadata should include date that specimen was acquired and location.
Preservation and storage variation	Drying, decontamination methods, adhesives, and storage environments can influence spectra	Standardize methods for incoming specimens. Conduct analyses on preservation and storage effects to determine if effects are large or small. Increase sample size to capture range of preservation methods.
Adhesives	Glues contaminate the reflectance spectrum	Prioritize leaves (or other tissues) without adhesives.
Leaf size	Small leaves are difficult to measure with standard set-ups	Fine leaf probes, narrow fiber optic cables, small detection windows
Leaf surface	Adaxial and abaxial surfaces differ	Measure both sides of the leaf. Capture metadata on which side(s) was/were measured
Measuring plant surfaces beyond leaves	Flowers, stems and other organs are also important	Measure on a project-by-project basis or using criteria at individual herbaria
Specimen geometry	Non-flat foliar surfaces generate specular influences on reflectance spectra ("noisy spectra")	Prioritize flat tissues. Increase sample size. Use probe with a small optical aperture.
Instruments and optics	Different instruments give different spectral reflectance signatures. Optical setups also influence spectra.	Adhere to agreed upon protocols, instrument setup and metadata standards. Establish interconversion

		methods. Use the same reflectance standards across all labs
Reflectance background	White mounting paper introduces artefacts into the reflectance spectrum	Use a black non-reflective background on detached leaves or insert under a mounted leaf that is loose
Measurement settings and protocols	Sample count, leaf surface choice, integration times and other user- defined parameters influence SNR, data comparability and the time it takes to measure a specimen	Develop minimum sample counts and data standards across herbaria that can be surpassed for specific purposes and/or within individual herbaria
Destructive sampling for traits	Validation of models for many functional traits (N, P, cellulose, etc.) requires destroying leaf tissue to make direct observations.	Models may be generated from pressed leaves or tissues will need to be sampled ethically and in a coordinated manner.
Metadata collection and access	Data incompatibility due to the range of variables affecting herbarium spectral measurements	Establish metadata standards, similar to Darwin Core format, agreed upon, recorded and shared by all institutions.

630 Considerations for standardization of protocols and metadata

While detailed protocols and metadata standards remain to be established and agreed upon across institutions, it is important to provide some guidance at this stage, given the rapid increase in the use of spectroscopy for plant studies (Cavender-Bares et al. 2025). Here we make a series of recommendations and pose questions for consideration. Standardizing spectral digitization setups—including light source positioning, spectral calibration, and reference materials—will be essential to ensure compatibility between datasets collected using different instruments and workflows. Standardization of measurement protocols and metadata will be critical to maintaining consistency across instruments, collections, and

- 638 institutions.
- 639

640 *Sampling.* We recommend sampling the variation among leaves on a specimen as well as variation within

641 a single leaf, avoiding leaves with blemishes, contaminants or pathogens (unless these are relevant to the

642 objectives of the study). When selecting among specimens for sampling, prioritizing those that have

643 leaves where a black, non-reflective background can be readily inserted under the leaf will enable

aggregation of data. Measuring sufficient leaf area to ensure that the spectra are representative of the

645 variation within the specimen should be balanced against the care required to ensure non-destructive

sampling. Taking 3-5 measurements across the leaf and 3-5 leaves per specimen can help capture relevant

647 variation, but one fully expanded leaf per specimen may be a sufficient compromise to save time and

648 avoid damage.

650 *Reflectance standards.* Given the variation in instrument and optical arrangements across institutions,

651 regular measurement of standard cards of known reflectance using a set of certified reflectance standards

652 ranging from black to gray to white provides a means to standardize data, making them comparable. A

653 rare earth panel can be used to confirm wavelength calibration, especially if the instrument is returned for

654 manufacturer recalibration infrequently. The black and gray standards would be measured regularly, such

as once daily during measurement sessions. The white standard should be measured with each specimen,

or at least every two to three specimens if spectral measurements are taken very rapidly.

657

658 Reflectance background. Background effects also play a critical role in spectral consistency across 659 institutions. Light transmitted through leaves may reflect from the background (glue, paper, and even lab 660 benches), which 'contaminates' the spectrum. The degree of contamination depends on the optical 661 thickness of the leaves, which governs how much light is transmitted. Measuring leaves against a non-662 reflective black background to avoid contamination from other reflective surfaces is critical. This may 663 create challenges for some specimens, which are often affixed to paper. If the specimen has been sewn or 664 taped, it should be possible to slide a thin black sheet between the attached leaf and the paper. Herbarium 665 specimens with loose leaves available in packets may be selected, and those leaves checked for glue 666 before measuring them against a black background. A contact probe or modified leaf clip with the bottom 667 portion removed will facilitate measurements. The importance of using a non-reflective black background 668 will likely prevent automation through conveyor belts, as has been used for digitization of specimen 669 images.

670 The selection of non-reflective black backgrounds is a critical component of standardization. A 671 rule of thumb is that the background should have less than 4% reflection. EVA foam, black plastic, black 672 card stock painted with Krylon® Camouflage Matte Black spray paint, and SpectralBlack® foil, or black 673 backgrounds of manufacturer leaf clips for portable spectroradiometers (Malvern Panalytical [ASD], 674 Spectra Vista Corporation, Spectral Evolution) are among the materials that have been used. It is 675 worthwhile collecting a spectrum (or spectra) of the non-reflective black material to have as a reference if 676 there are questions about the leaf measurements later. The identification and adoption of a universal 677 background standard is an important objective of protocol development. 678 However, as noted above, vast portions of specimens already present in collections have been

However, as noted above, vast portions of specimens already present in collections have been
mounted with glue, such that using a non-reflective black background is not a feasible solution in all cases
(Neto-Bradley et al., 2025). The extent to which measurements on mounted leaves should still be
collected is an open area of discussion.

682

683 *Metadata standards*. Metadata standardization is critical for harmonizing datasets, as it facilitates the 684 integration of phenomic data with associated specimen metadata, such as taxonomy, collection locality, 685 and ecological context. Metadata will also provide users critical data on specimen preservation method, 686 and potential artefacts due to glues or reflective backgrounds, that can inform aggregation and integration 687 efforts. By adopting common metadata schemas and persistent identifiers (DOIs), researchers can link 688 spectral data directly to digital databases, fostering seamless collaboration and data reuse. Experience 689 gained from successful protocol standardization and data aggregation initiatives (e.g. Darwin Core and 690 iDigBio; (Wieczorek et al. 2012, Soltis 2017) can be leveraged to implement a strategy for herbarium 691 spectroscopic data. Recording metadata for instrument type, including the spectrometer brand and model, 692 date of last maintenance and calibration by the manufacturer, whether the instrument is internally 693 calibrated, as well as the spectral resolution and wavelength interval the instrument actually measures. 694 The setup and standard measurements across a set of standard reference cards (white, gray, black) will be 695 essential for aggregation. Also important is recording developmental, phenological, and ecological factors 696 that influence leaf structure and physiology, as these are critical to untangling the effects of specimen 697 processing and storage. Effective metadata curation and cyberinfrastructure development will be critical 698 for integrating herbarium spectral data into global biodiversity platforms. Standardizing metadata fields 699 for specimen age, preservation method, mounting medium, and measurement conditions will facilitate 700 dataset comparison and allow researchers to apply appropriate quality control measures. Advances in 701 spectral data repositories, such as linking reflectance data to GBIF, speciesLink and iDigBio records, will 702 enhance accessibility and ensure that spectral datasets are fully interoperable with existing biodiversity 703 databases (Heberling 2021, Davis 2023) and further enhance the extended specimen concept (Lendemer 704 et al. 2020).

705

706 Data processing. Raw data should be stored as collected directly from the instrument to avoid artifacts in 707 the data that cannot be undone, e.g., resampling or binning bands to achieve higher SNR. Processed data 708 may also be included. There are various approaches to processing raw spectra through resampling and 709 normalization or transformation using derivatives or continuous wavelet transforms (CWT) to standardize 710 datasets from different instruments. Band resampling at a higher resolution than the true resolution could 711 introduce artificial data to the spectrum but resampling to reduce the number of bands is common. A 712 resampling interval of 5 nm is reasonable for accommodating the differences in spectral resolution 713 between instruments (e.g., Spectral Evolution NaturaSpec, PSR+, or SVC HR-1024i). Resampling 714 reduces the number of correlated bands for predictive models. Although small absorption features may be 715 perceived as insignificant or as noise, they may be important for taxonomic classification, and binning or 716 resampling to lower resolution could result in loss of information. If the raw spectral data is preserved,

- any loss of information can be assessed by comparison to the raw data. In full-range instruments, the
- 718 350–400 nm and 2,400–2,500 nm regions are often trimmed due to the low signal to noise ratio in those
- regions. Reflectance values at the wavelengths where the silicon and InGaAs sensors meet need to be
- 720 joined consistently. R packages like spectrolab (Meireles et al. 2017) have options for resampling,
- trimming, and joining data between the silicon and InGaAs sensors.
- 722 The success of aggregating herbarium reflectance spectra will depend on practices that ensure 723 consistency, interoperability, and accessibility across institutions. Data quality controls must be ensured 724 to include careful adherence to measurement protocols. It should also involve the preprocessing of 725 spectral datasets to remove spectra of low quality. Routine validation processes will ensure that data 726 meets the necessary standards for reproducibility and analysis. Finally, implementing analysis engines 727 capable of handling high-dimensional datasets will be transformative. These engines should integrate 728 spectral data with complementary datasets, such as genomic or spatial data, and provide tools for 729 advanced modeling and visualization. Open-source analysis platforms with user-friendly interfaces will 730 democratize access to these tools and foster collaboration across disciplines.
- 731

732 Fitting appropriate functional trait models to specimens. A key consideration is the transferability of 733 spectral models between fresh, pressed, and herbarium specimens. While recent studies have confirmed 734 that these specimen types are all informative for functional trait prediction and taxonomic classification 735 (Wagner et al. 2019, Kothari et al. 2023), accounting for minor spectral shifts due to aging and 736 preservation during model building will be important. Spectral models trained on recently pressed 737 specimens may perform well on herbarium specimens, but in some cases where degradation will likely 738 bias trait modeling (e.g., chlorophyll). Developing correction factors and harmonization techniques for 739 cross-specimen spectral applications will be necessary to expand the utility of herbarium spectral 740 databases. When seeking to apply a trait model, it is critical to collect some ground-truth data to ensure 741 that it can yield accurate predictions for a particular class of specimens, based on taxa sampled, the 742 herborization process, and instrumental set up. Without validation, there is no basis for ensuring that 743 models developed for one set of specimens will accurately predict traits in another set of specimens. 744 745 **Database sufficiency**

The development of cyberinfrastructure has been pivotal in enabling large-scale aggregation of
spectral data. Platforms like iDigBio, *species*Link and GBIF provide centralized repositories for
biodiversity data, but dedicated cyberinfrastructure for spectral datasets, integrated with existing
platforms, will be essential for advancing collections-based research. These systems should support realtime synchronization of available data from herbarium institutions, cross-referencing, and retrieval for

- 751 global accessibility. Any dedicated spectral cyberinfrastructure platforms will require Application
- 752 Programming Interfaces (APIs) to enable researchers to query, retrieve, and contribute spectral datasets
- 753 programmatically; facilitating the large-scale synthesis of data. While several cyberinfrastructure
- platforms have been developed specifically for spectral data and models, such as EcoSIS and EcoSML
- 755 (Wagner et al. 2019), they are not appropriate or sufficient for capturing taxonomic information required
- in biodiversity collections. A more efficient solution will be to ensure that the databases from natural
- 757 history museums can incorporate spectral data and the critical associated metadata.
- 758
- Darwin Core. The Darwin Core (DC) standard is a compendium of terms and definitions that describe the observation of an organism at a particular time and space (Wieczorek et al. 2012). Broadening the Darwin Core to incorporate herbarium reflectance spectroscopy will enable data sharing across existing biodiversity informatics platforms (e.g., GBIF, *species*Link) and the use of spectral information in biodiversity research and conservation. In addition, extending DC for spectroscopy will supply new means for sharing protocols and practices for data aggregation that are critical for improving the quality of the herbarium reflectance data collection, storage, and distribution.
- 766

767 Effective, Equitable and Ethical Sampling

768 Herbarium users and stewards must balance innovation with preservation. Guidelines for the effectiveness 769 and ethics of these global biodiversity heritage have been recently formalized to provide a path to better 770 utilize and steward these collections to safeguard their continued use (Davis et al. 2025). Many of these 771 guidelines have already been in place for decades and are often included in institutional policies that have 772 been deliberated by stewards and shared with users (Richard et al. 2019, Shah 2023). The herbarium and 773 Natural History Museum community has become increasingly aware of what we call the 'destructive 774 sampling conundrum': how to foster innovative research that includes destructive sampling of specimens 775 that are meant to be protected permanently (Davis et al. 2025). We additionally recognize that herbaria 776 are differentially concentrated around the world, especially the global north (Heberling et al. 2019, Park et 777 al. 2023), and are of different sizes and face a range of operating circumstances and challenges.

The effective implementation of spectroscopic sampling and measurement strategies requires meticulous consideration of both the specimen's characteristics and the preservation and repair methods that the world's herbaria employ. Under ideal conditions, spectral digitization provides a non-destructive alternative to traditional trait measurements, allowing researchers to infer key plant traits without physically altering specimens (White et al. 2025). By non-destructive, we mean the specimen is left the way it was previously with no trace of tissue removal (Davis et al. 2025). Spectral data can be collected non-destructively when either detached leaves are available or when some leaves on a specimen are not 785 fully glued. Damage or removal of specimen tissue for spectroscopy efforts are likely to occur if non-786 destructive protocols are not enforced. While spectral measurements can be made non-destructively, if 787 trait prediction is the ultimate goal, some amount of trait validation is necessary to improve the accuracy 788 of trait prediction. Trait validation will require the destructive use of tissue from similar taxa ideally with 789 similar herborization processes. The high performance of trait models from pressed leaves (Kothari et al. 790 2023) applied to herborized specimens (White et al. 2025) indicates that pressed leaves of the same taxa 791 can probably be used for validation rather than herbarium specimens. If tissue removal from specimens is 792 required, it should be absolutely minimal, avoiding key features for identification or possible future use, 793 and documenting what was removed. It is crucial to seek permission for these efforts. To minimize the 794 need for destructive sampling, unwanted duplicates could be retained for spectroscopic and destructive 795 trait measurements for calibration of trait models. Alternatively, excess duplicate specimens could be set 796 aside and offered to the network of herbarium spectroscopy researchers, advancing the development of 797 spectroscopic libraries for trait estimation. Adhering to non-destructive measurement approaches should 798 be strongly encouraged to maintain the structural and historical integrity of the specimens. Researchers 799 should obtain explicit permission before conducting studies and ensure that their methodologies align 800 with the conservation objectives of the hosting institutions.

801 Finally, it is important that any databased content or spectral signatures harvested be offered as 802 shared data to the host institution as part of the global metaherbarium, making the data available 803 immediately. Doing so will help to avoid duplication of efforts with the same collection at other 804 institutions. Finally, it is essential to promote collaboration, including by recognizing herbaria scholars, 805 staff, and scientists formally as contributors, collaborators, funded partners, and coauthors (Edwards et al. 806 2024, Davis et al. 2025) following practices that require extraction of tissue or other alterations to 807 herbarium specimens (e.g., Burbano and Gutaker 2023, Davis 2023, Davis and Knapp 2025). Establishing 808 clear guidelines for sampling ensures that scientific investigations do not compromise the preservation of 809 these collections. For example, the American Society of Plant Taxonomists has outlined best practices 810 that emphasize the importance of minimizing physical alterations to specimens. By adhering to such 811 protocols, researchers can foster trust and facilitate ongoing access to these vital resources. All of these 812 considerations are paramount in the application of leaf spectroscopy to herbarium specimens and for 813 effective, equitable, and ethical sampling strategies (Davis et al. 2025). Sharing findings and derivative 814 data with the broader scientific community will promote transparency and collective advancement of the 815 plant sciences.

816

817 Next Steps

- 818 In closing, we declare that the international herbarium spectral digitization (IHerbSpec) working group is
- ready to go spectral! We acknowledge that we have considerable work to do as a community to ensure
- 820 that data can be aggregated and to ensure fair and equitable practices. Our next step is to develop agreed
- 821 upon protocols and metadata standards for the community. We pledge to be collaborative, inclusive and
- 822 equitable in our work and to generate data from the world's herbaria to advance understanding and
- 823 protection of the world's biodiversity.

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834 Author contributions

- All authors contributed intellectually to the manuscript, including the writing, editing and tables. JCB,
- **B36** JPL, DW, MHL, JEM and BNB prepared or contributed to the figures. JCB organized and led the writing
- 837 process with help from DW.
- 838
- 839

840 References Cited

- Asner, G., and R. Martin. 2011. Canopy phylogenetic, chemical and spectral assembly in a lowland
 Amazonian forest. . New Phytologist 189:999-1012
- Blanchard, F., A. Bruneau, and E. Laliberté. 2024. Foliar spectra accurately distinguish most temperate
 tree species and show strong phylogenetic signal. American Journal of Botany 111:e16314.
- Blonder, B., B. J. Graae, B. Greer, M. Haagsma, K. Helsen, R. E. Kapás, H. Pai, J. Rieksta, D. Sapena, C.
 J. Still, and R. Strimbeck. 2020. Remote sensing of ploidy level in quaking aspen (Populus tremuloides Michx.). Journal of Ecology 108:175-188.
- Burbano, H. A., and R. M. Gutaker. 2023. Ancient DNA genomics and the renaissance of herbaria.
 Science 382:59-63.
- Cavender-Bares, J. 2019. Diversification, adaptation, and community assembly of the American oaks
 (*Quercus*), a model clade for integrating ecology and evolution. New Phytologist 221:669-692.
- 852 Cavender-Bares J, Gonzalez-Rodriguez A, Eaton DAR, Hipp AAL, Beulke A, Manos PS. 2015.
 853 Phylogeny and biogeography of the American live oaks (Quercus subsection Virentes): a
 854 genomic and population genetics approach. Molecular Ecology 24(14): 3668-3687.
- Cavender-Bares, J., D. Ackerly, S. Hobbie, and P. Townsend. 2016a. Evolutionary legacy effects on
 ecosystems: Biogeographic origins, plant traits, and implications for management in the era of
 global change. Annual Review of Ecology, Evolution, and Systematics 47:433-462.
- 858 Cavender-Bares, J., J. A. Gamon, S. E. Hobbie, M. D. Madritch, J. E. Meireles, A. K. Schweiger, and P.
 859 A. Townsend. 2017. Harnessing plant spectra to integrate the biodiversity sciences across
 860 biological and spatial scales. American Journal of Botany 104:1-4 doi: 10.3732/ajb.1700061.
- 861 Cavender-Bares, J., A. Gonzalez-Rodriguez, D. A. R. Eaton, A. A. L. Hipp, A. Beulke, and P. S. Manos.
 862 2015. Phylogeny and biogeography of the American live oaks (*Quercus* subsection *Virentes*): a
 863 genomic and population genetics approach. Molecular Ecology 24:3668-3687.
- Cavender-Bares, J., J. E. Meireles, J. J. Couture, M. A. Kaproth, C. C. Kingdon, A. Singh, S. P. Serbin,
 A. Center, E. Zuniga, G. Pilz, and P. A. Townsend. 2016b. Associations of leaf spectra with
 genetic and phylogenetic variation in oaks: prospects for remote detection of biodiversity.
 Remote Sensing 8:221-238.
- Cavender-Bares, J., J. E. Meireles, J. Pinto-Ledezma, P. B. Reich, M. C. Schuman, P. A. Townsend, and
 A. Trowbridge. 2025. Spectral biology across scales in changing environments. Ecology in press.
- 870 Cavender-Bares, J., F. Schneider, M. Santos, A. Armstrong, A. Carnaval, K. Dahlin, L. Fatoyinbo, G.
 871 Hurtt, D. Schimel, and P. Townsend. 2022. Integrating remote sensing with ecology and
 872 evolution to advance biodiversity conservation. Nature Ecology & Evolution1-14.
- 873 Cavender-Bares, J., P. A. Townsend, and J. A. Gamon. 2020. Remote Sensing of Plant Biodiversity.
 874 Springer International Publishing.
- 875 Chlus, A., and P. A. Townsend. 2022. Characterizing seasonal variation in foliar biochemistry with
 876 airborne imaging spectroscopy. Remote Sensing of Environment 275:113023.
- Clavel, J., L. Aristide, and H. Morlon. 2019. A Penalized Likelihood Framework for High-Dimensional
 Phylogenetic Comparative Methods and an Application to New-World Monkeys Brain Evolution.
 Systematic biology 68:93-116.
- Clavel, J., G. Escarguel, and G. Merceron. 2015. mvmorph: an r package for fitting multivariate
 evolutionary models to morphometric data. Methods in Ecology and Evolution 6:1311-1319.
- Costa, D. d. S., E. F. S. Rossetto, and L. L. Giacomin. 2025. Neea contracta (Nyctaginaceae), a New
 Species from Amazonia. Systematic Botany 49:743-748.
- Czyż, E. A., C. Guillén Escribà, H. Wulf, A. Tedder, M. C. Schuman, F. D. Schneider, and M. E.
 Schaepman. 2020. Intraspecific genetic variation of a Fagus sylvatica population in a temperate
 forest derived from airborne imaging spectroscopy time series. Ecology and Evolution 10:7419 7430.

- da Cruz Vasconcelos, C., I. D. K. Ferraz, M. U. Adrianzén, J. L. C. Camargo, and M. H. Terra-Araujo.
 2021. Chromolucuma brevipedicellata (Sapotaceae, Chrysophylloideae), a new tree species from
 central Amazonia, Brazil. Brittonia 73:211-219.
- Bamasco, G., D. C. Daly, A. Vicentini, and P. V. A. Fine. 2019. Reestablishment of Protium cordatum
 (Burseraceae) based on integrative taxonomy. Taxon 68:34-46.
- Bao, P. D., Y. He, and C. Proctor. 2021. Plant drought impact detection using ultra-high spatial resolution
 hyperspectral images and machine learning. International Journal of Applied Earth Observation
 and Geoinformation 102:102364.
- Baru, B. H., D. S. Park, R. B. Primack, C. G. Willis, D. S. Barrington, T. J. S. Whitfeld, T. G. Seidler, P.
 W. Sweeney, D. R. Foster, A. M. Ellison, and C. C. Davis. 2018. Widespread sampling biases in herbaria revealed from large-scale digitization. New Phytologist 217:939-955.
- B99 Davis, C. C. 2023. The herbarium of the future. Trends in Ecology & Evolution 38:412-423.
- Davis, C. C., J. A. Kennedy, and C. J. Grassa. 2021. Back to the future: A refined single-user photostation
 for massively scaling herbarium digitization. Taxon 70:635-643.
- Davis, C. C., and S. Knapp. 2025. Exploring biodiversity through museomics. Nature Reviews Genetics
 26:149-150.
- Davis, C. C., E. Sessa, A. Paton, A. Antonelli, and J. K. Teisher. 2025. Guidelines for the effective and
 ethical sampling of herbaria. Nature Ecology & Evolution 9:196-203.
- Davis, C. C., C. G. Willis, B. Connolly, C. Kelly, and A. M. Ellison. 2015. Herbarium records are reliable
 sources of phenological change driven by climate and provide novel insights into species'
 phenological cueing mechanisms. Am J Bot 102:1599-1609.
- Deacon, N. J., J. J. Grossman, A. K. Schweiger, I. Armour, and J. Cavender-Bares. 2017a. Genetic,
 morphological, and spectral characterization of relictual Niobrara River hybrid aspens (Populus × smithi). American Journal of Botany 104:1878-1890.
- Deacon, N. J., J. J. Grossman, A. K. Schweiger, I. Armour, and J. Cavender-Bares. 2017b. Genetic,
 morphological, and spectral characterization of relictual Niobrara River hybrid aspens (Populus x smithii). American Journal of Botany 104:1878-1890.
- 915 Dechant, B., J. Kattge, R. Pavlick, F. D. Schneider, F. M. Sabatini, Á. Moreno-Martínez, E. E. Butler, P.
 916 M. van Bodegom, H. Vallicrosa, T. Kattenborn, C. C. F. Boonman, N. Madani, I. J. Wright, N.
 917 Dong, H. Feilhauer, J. Peñuelas, J. Sardans, J. Aguirre-Gutiérrez, P. B. Reich, P. J. Leitão, J.
- 918 Cavender-Bares, I. H. Myers-Smith, S. M. Durán, H. Croft, I. C. Prentice, A. Huth, K. Rebel, S.
- Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I.
 Colin Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J.
 Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. Joseph Wright, S. N. Sheremet'ev, H. Jactel, C.
 Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther,
 V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form
 and function. Nature 529:167-171.
- Durgante, F. M., N. Higuchi, A. Almeida, and A. Vicentini. 2013. Species Spectral Signature:
 Discriminating closely related plant species in the Amazon with Near-Infrared Leaf Spectroscopy. Forest Ecology and Management 291:240-248.
- Edwards, E. J., B. D. Mishler, and C. D. Davis. 2024. University herbaria are uniquely important. Trends
 in Plant Science 29:825-826.
- Bividge, C. D. 1990. Visible and near infrared reflectance characteristics of dry plant materials.
 International Journal of Remote Sensing 11:1775-1795.
- Enquist, B. J., R. Condit, R. K. Peet, M. Schildhauer, and B. M. Thiers. 2016. Cyberinfrastructure for an
 integrated botanical information network to investigate the ecological impacts of global climate
 change on plant biodiversity. PeerJ Preprints 4:e2615v2612.

- Fajardo, A., and A. Siefert. 2016. Phenological variation of leaf functional traits within species.
 Oecologia 180:951-959.
- Fallon, B., A. Yang, C. Lapadat, I. Armour, J. Juzwik, R. A. Montgomery, and J. Cavender-Bares. 2020.
 Spectral differentiation of oak wilt from foliar fungal disease and drought is correlated with
 physiological changes. Tree Physiology 40:377-390.
- 944 Falster, D., R. Gallagher, E. H. Wenk, I. J. Wright, D. Indiarto, S. C. Andrew, C. Baxter, J. Lawson, S. 945 Allen, A. Fuchs, A. Monro, F. Kar, M. A. Adams, C. W. Ahrens, M. Alfonzetti, T. Angevin, D. 946 M. G. Apgaua, S. Arndt, O. K. Atkin, J. Atkinson, T. Auld, A. Baker, M. von Balthazar, A. Bean, 947 C. J. Blackman, K. Bloomfield, D. M. J. S. Bowman, J. Bragg, T. J. Brodribb, G. Buckton, G. 948 Burrows, E. Caldwell, J. Camac, R. Carpenter, J. A. Catford, G. R. Cawthray, L. A. Cernusak, G. 949 Chandler, A. R. Chapman, D. Cheal, A. W. Cheesman, S.-C. Chen, B. Choat, B. Clinton, P. L. 950 Clode, H. Coleman, W. K. Cornwell, M. Cosgrove, M. Crisp, E. Cross, K. Y. Crous, S. 951 Cunningham, T. Curran, E. Curtis, M. I. Daws, J. L. DeGabriel, M. D. Denton, N. Dong, P. Du, 952 H. Duan, D. H. Duncan, R. P. Duncan, M. Duretto, J. M. Dwyer, C. Edwards, M. Esperon-953 Rodriguez, J. R. Evans, S. E. Everingham, C. Farrell, J. Firn, C. R. Fonseca, B. J. French, D. 954 Frood, J. L. Funk, S. R. Geange, O. Ghannoum, S. M. Gleason, C. R. Gosper, E. Gray, P. K. 955 Groom, S. Grootemaat, C. Gross, G. Guerin, L. Guja, A. K. Hahs, M. T. Harrison, P. E. Hayes, 956 M. Henery, D. Hochuli, J. Howell, G. Huang, L. Hughes, J. Huisman, J. Ilic, A. Jagdish, D. Jin, 957 G. Jordan, E. Jurado, J. Kanowski, S. Kasel, J. Kellermann, B. Kenny, M. Kohout, R. M. 958 Kooyman, M. M. Kotowska, H. R. Lai, E. Laliberté, H. Lambers, B. B. Lamont, R. Lanfear, F. 959 van Langevelde, D. C. Laughlin, B.-A. Laugier-Kitchener, S. Laurance, C. E. R. Lehmann, A. 960 Leigh, M. R. Leishman, T. Lenz, B. Lepschi, J. D. Lewis, F. Lim, U. Liu, J. Lord, C. H. Lusk, C. 961 Macinnis-Ng, H. McPherson, S. Magallón, A. Manea, A. López-Martinez, M. Mayfield, J. K. 962 McCarthy, T. Meers, M. van der Merwe, D. J. Metcalfe, P. Milberg, K. Mokany, A. T. Moles, B. 963 D. Moore, N. Moore, J. W. Morgan, W. Morris, A. Muir, S. Munroe, A. Nicholson, D. Nicolle, 964 A. B. Nicotra, Ü. Niinemets, T. North, A. O'Reilly-Nugent, O. S. O'Sullivan, B. Oberle, Y. 965 Onoda, M. K. J. Ooi, C. P. Osborne, G. Paczkowska, B. Pekin, C. Guilherme Pereira, C. 966 Pickering, M. Pickup, L. J. Pollock, P. Poot, J. R. Powell, S. A. Power, I. C. Prentice, L. Prior, S. 967 M. Prober, J. Read, V. Reynolds, A. E. Richards, B. Richardson, M. L. Roderick, J. A. Rosell, M. 968 Rossetto, B. Rye, P. D. Rymer, M. A. Sams, G. Sanson, H. Sauquet, S. Schmidt, J. 969 Schönenberger, E.-D. Schulze, K. Sendall, S. Sinclair, B. Smith, R. Smith, F. Soper, B. Sparrow, 970 R. J. Standish, T. L. Staples, R. Stephens, C. Szota, G. Taseski, E. Tasker, F. Thomas, D. T. 971 Tissue, M. G. Tjoelker, D. Y. P. Tng, F. de Tombeur, K. Tomlinson, N. C. Turner, E. J. 972 Veneklaas, S. Venn, P. Vesk, C. Vlasveld, M. S. Vorontsova, C. A. Warren, N. Warwick, L. K. 973 Weerasinghe, J. Wells, M. Westoby, M. White, N. S. G. Williams, J. Wills, P. G. Wilson, C. 974 Yates, A. E. Zanne, G. Zemunik, and K. Ziemińska. 2021. AusTraits, a curated plant trait 975 database for the Australian flora. Scientific Data 8:254. 976 Féret, J. B., A. A. Gitelson, S. D. Noble, and S. Jacquemoud. 2017. PROSPECT-D: Towards modeling 977 leaf optical properties through a complete lifecycle. Remote Sens. Environ. 193:204-215. 978 Fine, P. V. A., D. Salazar, R. E. Martin, M. R. Metz, T. M. Misiewicz, and G. P. Asner. 2021. Exploring 979 the links between secondary metabolites and leaf spectral reflectance in a diverse genus of 980 Amazonian trees. Ecosphere 12:e03362.
- Funk, J. L., J. E. Larson, G. M. Ames, B. J. Butterfield, J. Cavender-Bares, J. Firn, D. C. Laughlin, A. E.
 Sutton-Grier, L. Williams, and J. Wright. 2017. Revisiting the Holy Grail: using plant functional traits to understand ecological processes: Plant functional traits. Biological Reviews 92:1156-1173.
- Gaem, P. H., A. Andrade, F. F. Mazine, and A. Vicentini. 2022. Tree species delimitation in tropical
 forest inventories: Perspectives from a taxonomically challenging case study. Forest Ecology and
 Management 505:119900.
- Gamon, J., and J. Surfus. 1999. Assessing leaf pigment content and activity with a reflectometer. New
 Phytologist 143:105-117.

- Ge, Y., G. Bai, V. Stoerger, and J. c. Schnable. 2016. Temporal dynamics of maize plant growth, water
 use, and leaf water content using automated high throughput RGB and hyperspectral imaging.
 Comput. Electron. Agric. 127:625-632.
- Gitelson, A. A., C. Buschmann, and H. K. Lichtenthaler. 1998. Leaf chlorophyll fluorescence corrected
 for re-absorption by means of absorption and reflectance measurements. J. Plant Physiol.
 152:283-296.
- Gitelson, A. A., and M. N. Merzlyak. 1994. Spectral reflectance changes associate with autumn
 senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and
 relation to chlorophyll estimation. Journal of Plant Physiology 143:286–292.
- Grant, L. 1987. Diffuse and specular characteristics of leaf reflectance. Remote Sensing of Environment
 22:309-322.
- Guillot G, Santos F. 2009. A computer program to simulate multilocus genotype data with spatially
 autocorrelated allele frequencies. Molecular Ecology Resources 9: 1112-1120.
- Hadlich, H. L., J. Schöngart, F. Wittmann, C. C. Vasconcelos, C. L. Mallmann, M. L. G. Conde, P.
 Amaral de Sá, L. O. Demarchi, G. B. Mori, M. T. F. Piedade, and F. M. Durgante. 2025. Scaling up tree diversity inventories across Amazonian ecosystems using field spectroscopy.
 bioRxiv:2025.2003.2026.645444.
- Harvey, P. H., and A. Purvis. 1991. Comparative methods for explaining adaptations. Nature 351:619 624.
- Heberling, J. M. 2021. Herbaria as Big Data Sources of Plant Traits. International Journal of Plant
 Sciences 183:87-118.
- Heberling, J. M., J. T. Miller, D. Noesgaard, S. B. Weingart, and D. Schigel. 2021. Data integration
 enables global biodiversity synthesis. Proceedings of the National Academy of Sciences
 118:e2018093118.
- Heberling, J. M., L. A. Prather, and S. J. Tonsor. 2019. The Changing Uses of Herbarium Data in an Era of Global Change: An Overview Using Automated Content Analysis. BioScience 69:812-822.
- Hedrick, B., M. Heberling, E. Meineke, K. Turner, C. Grassa, D. Park, J. Kennedy, J. Clarke, J. Cook, D.
 Blackburn, S. Edwards, and C. Davis. 2019. Digitization and the future of natural history
 collections.
- Hernández-Leal MS, Guzmán Q. JA, González-Rodríguez A, Cavender-Bares J. 2025. Foliar spectral
 signatures reveal adaptive divergence in live oaks (Quercus1section Virentes)across species and
 environmental niches. EcoEvoRxiv https://ecoevorxiv.org/repository/view/9054/:
 https://doi.org/10.32942/X32942BK32990.
- Hipp AL, Manos PS, Hahn M, Avishai M, Bodénès C, Cavender-Bares J, Crowl AA, Deng M, Denk T,
 Fitz-Gibbon S, et al. 2020. Genomic landscape of the global oak phylogeny. New Phytologist
 226(4): 1198-1212.
- 1026 Jacquemoud, S., and S. Ustin. 2019. Leaf Optical Properties. Cambridge University Press, New York.
- Jetz, W., J. Cavender-Bares, R. Pavlick, D. Schimel, F. W. Davis, G. P. Asner, R. Guralnick, J. Kattge, A.
 Latimer, P. Moorcroft, M. E. Schaepman, and S. L. Ustin. 2016a. Monitoring plant functional
 diversity from space. Nature Plants 2.
- Jetz, W., J. Cavender-Bares, R. Pavlick, D. Schimel, F. W. Davis, G. P. Asner, R. Guralnick, J. Kattge, A.
 M. Latimer, P. Moorcroft, M. E. Schaepman, M. P. Schildhauer, F. D. Schneider, F. Schrodt, U.
 Stahl, and S. L. Ustin. 2016b. Monitoring plant functional diversity from space. Nature Plants
 2:16024.
- Kattge, J., G. Bonisch, S. Diaz, S. Lavorel, I. C. Prentice, P. Leadley, S. Tautenhahn, G. D. A. Werner, T.
 Aakala, M. Abedi, A. T. R. Acosta, G. C. Adamidis, K. Adamson, M. Aiba, C. H. Albert, J. M.
 Alcantara, C. C. Alcazar, I. Aleixo, H. Ali, B. Amiaud, C. Ammer, M. M. Amoroso, M. Anand,
 C. Anderson, N. Anten, J. Antos, D. M. G. Apgaua, T. L. Ashman, D. H. Asmara, G. P. Asner,
 M. Aspinwall, O. Atkin, I. Aubin, L. Baastrup-Spohr, K. Bahalkeh, M. Bahn, T. Baker, W. J.
 Baker, J. P. Bakker, D. Baldocchi, J. Baltzer, A. Banerjee, A. Baranger, J. Barlow, D. R.
 Barneche, Z. Baruch, D. Bastianelli, J. Battles, W. Bauerle, M. Bauters, E. Bazzato, M.

1041 Beckmann, H. Beeckman, C. Beierkuhnlein, R. Bekker, G. Belfry, M. Belluau, M. Beloiu, R. 1042 Benavides, L. Benomar, M. L. Berdugo-Lattke, E. Berenguer, R. Bergamin, J. Bergmann, M. B. Carlucci, L. Berner, M. Bernhardt-Romermann, C. Bigler, A. D. Bjorkman, C. Blackman, C. 1043 1044 Blanco, B. Blonder, D. Blumenthal, K. T. Bocanegra-Gonzalez, P. Boeckx, S. Bohlman, K. 1045 Bohning-Gaese, L. Boisvert-Marsh, W. Bond, B. Bond-Lamberty, A. Boom, C. C. F. Boonman, 1046 K. Bordin, E. H. Boughton, V. Boukili, D. Bowman, S. Bravo, M. R. Brendel, M. R. Broadley, K. 1047 A. Brown, H. Bruelheide, F. Brumnich, H. H. Bruun, D. Bruy, S. W. Buchanan, S. F. Bucher, N. 1048 Buchmann, R. Buitenwerf, D. E. Bunker, J. Burger, S. Burrascano, D. Burslem, B. J. Butterfield, C. Byun, M. Marques, M. C. Scalon, M. Caccianiga, M. Cadotte, M. Cailleret, J. Camac, J. J. 1049 1050 Camarero, C. Campany, G. Campetella, J. A. Campos, L. Cano-Arboleda, R. Canullo, M. 1051 Carbognani, F. Carvalho, F. Casanoves, B. Castagnevrol, J. A. Catford, J. Cavender-Bares, B. E. 1052 L. Cerabolini, M. Cervellini, E. Chacon-Madrigal, K. Chapin, F. S. Chapin, S. Chelli, S. C. Chen, 1053 A. P. Chen, P. Cherubini, F. Chianucci, B. Choat, K. S. Chung, M. Chytry, D. Ciccarelli, L. Coll, 1054 C. G. Collins, L. Conti, D. Coomes, J. H. C. Cornelissen, W. K. Cornwell, P. Corona, M. Coyea, 1055 J. Craine, D. Craven, J. Cromsigt, A. Csecserits, K. Cufar, M. Cuntz, A. C. da Silva, K. M. 1056 Dahlin, M. Dainese, I. Dalke, M. Dalle Fratte, T. D. L. Anh, J. Danihelka, M. Dannoura, S. 1057 Dawson, A. J. de Beer, A. De Frutos, J. R. De Long, B. Dechant, S. Delagrange, N. Delpierre, G. 1058 Derroire, A. S. Dias, M. H. Diaz-Toribio, P. G. Dimitrakopoulos, M. Dobrowolski, D. Doktor, P. 1059 Drevojan, N. Dong, J. Dransfield, S. Dressler, L. Duarte, E. Ducouret, S. Dullinger, W. Durka, R. 1060 Duursma, O. Dymova, A. E-Vojtko, R. L. Eckstein, H. Ejtehadi, J. Elser, T. Emilio, K. 1061 Engemann, M. B. Erfanian, A. Erfmeier, A. Esquivel-Muelbert, G. Esser, M. Estiarte, T. F. 1062 Domingues, W. F. Fagan, J. Fagundez, D. S. Falster, Y. Fan, J. Y. Fang, E. Farris, F. Fazlioglu, 1063 Y. H. Feng, F. Fernandez-Mendez, C. Ferrara, J. Ferreira, A. Fidelis, B. Finegan, J. Firn, T. J. Flowers, D. F. B. Flynn, V. Fontana, E. Forey, C. Forgiarini, L. Francois, M. Frangipani, D. 1064 1065 Frank, C. Frenette-Dussault, G. T. Freschet, E. L. Fry, N. M. Fyllas, G. G. Mazzochini, S. 1066 Gachet, R. Gallagher, G. Ganade, F. Ganga, P. Garcia-Palacios, V. Gargaglione, E. Garnier, J. L. 1067 Garrido, A. L. de Gasper, G. Gea-Izquierdo, D. Gibson, A. N. Gillison, A. Giroldo, M. C. 1068 Glasenhardt, S. Gleason, M. Gliesch, E. Goldberg, B. Goldel, E. Gonzalez-Akre, J. L. Gonzalez-Andujar, A. Gonzalez-Melo, A. Gonzalez-Robles, B. J. Graae, E. Granda, S. Graves, W. A. 1069 1070 Green, T. Gregor, N. Gross, G. R. Guerin, A. Gunther, A. G. Gutierrez, L. Haddock, A. Haines, J. 1071 Hall, A. Hambuckers, W. X. Han, S. P. Harrison, W. Hattingh, J. E. Hawes, T. H. He, P. C. He, J. 1072 M. Heberling, A. Helm, S. Hempel, J. Hentschel, B. Herault, A. M. Heres, K. Herz, M. Heuertz, 1073 T. Hickler, P. Hietz, P. Higuchi, A. L. Hipp, A. Hirons, M. Hock, J. A. Hogan, K. Holl, O. 1074 Honnay, D. Hornstein, E. Q. Hou, N. Hough-Snee, K. A. Hovstad, T. Ichie, B. Igic, E. Illa, M. 1075 Isaac, M. Ishihara, L. Ivanov, L. Ivanova, C. M. Iversen, J. Izquierdo, R. B. Jackson, B. Jackson, 1076 H. Jactel, A. M. Jagodzinski, U. Jandt, S. Jansen, T. Jenkins, A. Jentsch, J. R. P. Jespersen, G. F. 1077 Jiang, J. L. Johansen, D. Johnson, E. J. Jokela, C. A. Joly, G. J. Jordan, G. S. Joseph, D. Junaedi, 1078 R. R. Junker, E. Justes, R. Kabzems, J. Kane, Z. Kaplan, T. Kattenborn, L. Kavelenova, E. 1079 Kearsley, A. Kempel, T. Kenzo, A. Kerkhoff, M. I. Khalil, N. L. Kinlock, W. D. Kissling, K. 1080 Kitajima, T. Kitzberger, R. Kjoller, T. Klein, M. Kleyer, J. Klimesova, J. Klipel, B. Kloeppel, S. 1081 Klotz, J. M. H. Knops, T. Kohyama, F. Koike, J. Kollmann, B. Komac, K. Komatsu, C. Konig, N. 1082 J. B. Kraft, K. Kramer, H. Kreft, I. Kuhn, D. Kumarathunge, J. Kuppler, H. Kurokawa, Y. 1083 Kurosawa, S. Kuyah, J. P. Laclau, B. Lafleur, E. Lallai, E. Lamb, A. Lamprecht, D. J. Larkin, D. 1084 Laughlin, Y. Le Bagousse-Pinguet, G. le Maire, P. C. le Roux, E. le Roux, T. Lee, F. Lens, S. L. 1085 Lewis, B. Lhotsky, Y. Z. Li, X. E. Li, J. W. Lichstein, M. Liebergesell, J. Y. Lim, Y. S. Lin, J. C. 1086 Linares, C. J. Liu, D. J. Liu, U. Liu, S. Livingstone, J. Llusia, M. Lohbeck, A. Lopez-Garcia, G. 1087 Lopez-Gonzalez, Z. Lososova, F. Louault, B. A. Lukacs, P. Lukes, Y. J. Luo, M. Lussu, S. Y. 1088 Ma, C. M. R. Pereira, M. Mack, V. Maire, A. Makela, H. Makinen, A. C. M. Malhado, A. Mallik, 1089 P. Manning, S. Manzoni, Z. Marchetti, L. Marchino, V. Marcilio-Silva, E. Marcon, M. 1090 Marignani, L. Markesteijn, A. Martin, C. Martinez-Garza, J. Martinez-Vilalta, T. Maskova, K. 1091 Mason, N. Mason, T. J. Massad, J. Masse, I. Mayrose, J. McCarthy, M. L. McCormack, K.

1092 McCulloh, I. R. McFadden, B. J. McGill, M. Y. McPartland, J. S. Medeiros, B. Medlyn, P. 1093 Meerts, Z. Mehrabi, P. Meir, F. P. L. Melo, M. Mencuccini, C. Meredieu, J. Messier, I. Meszaros, 1094 J. Metsaranta, S. T. Michaletz, C. Michelaki, S. Migalina, R. Milla, J. E. D. Miller, V. Minden, R. 1095 Ming, K. Mokany, A. T. Moles, V. A. Molnar, J. Molofsky, M. Molz, R. A. Montgomery, A. 1096 Monty, L. Moravcova, A. Moreno-Martinez, M. Moretti, A. S. Mori, S. Mori, D. Morris, J. 1097 Morrison, L. Mucina, S. Mueller, C. D. Muir, S. C. Muller, F. Munoz, I. H. Myers-Smith, R. W. 1098 Myster, M. Nagano, S. Naidu, A. Narayanan, B. Natesan, L. Negoita, A. S. Nelson, E. L. 1099 Neuschulz, J. Ni, G. Niedrist, J. Nieto, U. Niinemets, R. Nolan, H. Nottebrock, Y. Nouvellon, A. 1100 Novakovskiy, K. O. Nystuen, A. O'Grady, K. O'Hara, A. O'Reilly-Nugent, S. Oakley, W. Oberhuber, T. Ohtsuka, R. Oliveira, K. Ollerer, M. E. Olson, V. Onipchenko, Y. Onoda, R. E. 1101 1102 Onstein, J. C. Ordonez, N. Osada, I. Ostonen, G. Ottaviani, S. Otto, G. E. Overbeck, W. A. Ozinga, A. T. Pahl, C. E. T. Paine, R. J. Pakeman, A. C. Papageorgiou, E. Parfionova, M. Partel, 1103 1104 M. Patacca, S. Paula, J. Paule, H. Pauli, J. G. Pausas, B. Peco, J. Penuelas, A. Perea, P. L. Peri, A. 1105 C. Petisco-Souza, A. Petraglia, A. M. Petritan, O. L. Phillips, S. Pierce, V. D. Pillar, J. Pisek, A. Pomogavbin, H. Poorter, A. Portsmuth, P. Poschlod, C. Potvin, D. Pounds, A. S. Powell, S. A. 1106 1107 Power, A. Prinzing, G. Puglielli, P. Pysek, V. Raevel, A. Rammig, J. Ransijn, C. A. Ray, P. B. 1108 Reich, M. Reichstein, D. E. B. Reid, M. Rejou-Mechain, V. R. de Dios, S. Ribeiro, S. 1109 Richardson, K. Riibak, M. C. Rillig, F. Riviera, E. M. R. Robert, S. Roberts, B. Robroek, A. 1110 Roddy, A. V. Rodrigues, A. Rogers, E. Rollinson, V. Rolo, C. Romermann, D. Ronzhina, C. 1111 Roscher, J. A. Rosell, M. F. Rosenfield, C. Rossi, D. B. Roy, S. Royer-Tardif, N. Ruger, R. Ruiz-1112 Peinado, S. B. Rumpf, G. M. Rusch, M. Ryo, L. Sack, A. Saldana, B. Salgado-Negret, R. 1113 Salguero-Gomez, I. Santa-Regina, A. C. Santacruz-Garcia, J. Santos, J. Sardans, B. Schamp, M. 1114 Scherer-Lorenzen, M. Schleuning, B. Schmid, M. Schmidt, S. Schmitt, J. V. Schneider, S. D. 1115 Schowanek, J. Schrader, F. Schrodt, B. Schuldt, F. Schurr, G. S. Garvizu, M. Semchenko, C. 1116 Seymour, J. C. Sfair, J. M. Sharpe, C. S. Sheppard, S. Sheremetiev, S. Shiodera, B. Shipley, T. A. 1117 Shovon, A. Siebenkas, C. Sierra, V. Silva, M. Silva, T. Sitzia, H. Sjoman, M. Slot, N. G. Smith, 1118 D. Sodhi, P. Soltis, D. Soltis, B. Somers, G. Sonnier, M. V. Sorensen, E. E. Sosinski, N. A. 1119 Soudzilovskaja, A. F. Souza, M. Spasojevic, M. G. Sperandii, A. B. Stan, J. Stegen, K. 1120 Steinbauer, J. G. Stephan, F. Sterck, D. B. Stojanovic, T. Strydom, M. L. Suarez, J. C. Svenning, I. Svitkova, M. Svitok, M. Svoboda, E. Swaine, N. Swenson, M. Tabarelli, K. Takagi, U. 1121 1122 Tappeiner, R. Tarifa, S. Tauugourdeau, C. Tavsanoglu, M. te Beest, L. Tedersoo, N. Thiffault, D. 1123 Thom, E. Thomas, K. Thompson, P. E. Thornton, W. Thuiller, L. Tichy, D. Tissue, M. G. 1124 Tjoelker, D. Y. P. Tng, J. Tobias, P. Torok, T. Tarin, J. M. Torres-Ruiz, B. Tothmeresz, M. 1125 Treurnicht, V. Trivellone, F. Trolliet, V. Trotsiuk, J. L. Tsakalos, I. Tsiripidis, N. Tysklind, T. 1126 Umehara, V. Usoltsev, M. Vadeboncoeur, J. Vaezi, F. Valladares, J. Vamosi, P. M. van 1127 Bodegom, M. van Breugel, E. Van Cleemput, M. van de Weg, S. van der Merwe, F. van der Plas, 1128 M. T. van der Sande, M. van Kleunen, K. Van Meerbeek, M. Vanderwel, K. A. Vanselow, A. 1129 Varhammar, L. Varone, M. Y. Valderrama, K. Vassilev, M. Vellend, E. J. Veneklaas, H. 1130 Verbeeck, K. Verheyen, A. Vibrans, I. Vieira, J. Villacis, C. Violle, P. Vivek, K. Wagner, M. Waldram, A. Waldron, A. P. Walker, M. Waller, G. Walther, H. Wang, F. Wang, W. Q. Wang, 1131 1132 H. Watkins, J. Watkins, U. Weber, J. T. Weedon, L. P. Wei, P. Weigelt, E. Weiher, A. W. Wells, 1133 C. Wellstein, E. Wenk, M. Westoby, A. Westwood, P. J. White, M. Whitten, M. Williams, D. E. 1134 Winkler, K. Winter, C. Womack, I. J. Wright, S. J. Wright, J. Wright, B. X. Pinho, F. Ximenes, T. Yamada, K. Yamaji, R. Yanai, N. Yankov, B. Yguel, K. J. Zanini, A. E. Zanne, D. Zelenv, Y. 1135 P. Zhao, J. M. Zheng, J. Zheng, K. Zieminska, C. R. Zirbel, G. Zizka, I. C. Zo-Bi, G. Zotz, C. 1136 1137 Wirth, and N. Nutrient. 2020. TRY plant trait database - enhanced coverage and open access. 1138 Global Change Biology 26:119-188. 1139 Kokaly, R., G. Asner, S. Ollinger, M. Martin, and C. Wessman. 2009. Characterizing canopy 1140 biochemistry from imaging spectroscopy and its application to ecosystem studies. Remote

1141 Sensing of Environment 113:S78-S91.

- 1142 Kothari, S., R. Beauchamp-Rioux, E. Laliberté, and J. Cavender-Bares. 2023. Reflectance spectroscopy
 1143 allows rapid, accurate and non-destructive estimates of functional traits from pressed leaves.
 1144 Methods in Ecology and Evolution 14:385-401.
- Kothari, S., and A. K. Schweiger. 2022. Plant spectra as integrative measures of plant phenotypes. Journal
 of Ecology 110:2536-2554.
- Kühn, P., R. Umazekabiri, C. Römermann, H. Bruelheide, and K. Wesche. 2025. Nitrogen content of
 herbarium specimens from arable fields and mesic meadows reflect the intensifying agricultural
 management during the 20th century. Journal of Ecology 113:555-569.
- Lang, C., F. R. C. Costa, J. L. C. Camargo, F. M. Durgante, and A. Vicentini. 2015. Near Infrared
 Spectroscopy Facilitates Rapid Identification of Both Young and Mature Amazonian Tree
 Species. Plos One 10:e0134521.
- Lavorel, S., and E. Garnier. 2002. Predicting changes in community composition and ecosystem
 functioning from plant traits: revisiting the Holy Grail. Functional Ecology 16:545-556.
- Lendemer, J., B. Thiers, A. K. Monfils, J. Zaspel, E. R. Ellwood, A. Bentley, K. LeVan, J. Bates, D.
 Jennings, D. Contreras, L. Lagomarsino, P. Mabee, L. S. Ford, R. Guralnick, R. E. Gropp, M.
 Revelez, N. Cobb, K. Seltmann, and M. C. Aime. 2020. The Extended Specimen Network: A
 Strategy to Enhance US Biodiversity Collections, Promote Research and Education. BioScience
 70:23-30.
- Madritch, M. D., C. C. Kingdon, A. Singh, K. E. Mock, R. L. Lindroth, and P. A. Townsend. 2014.
 Imaging spectroscopy links aspen genotype with below-ground processes at landscape scales.
 Philosophical Transactions of the Royal Society of London B: Biological Sciences 369.
- Mallmann, C. L., W. Pereira Filho, J. B. B. Dreyer, L. A. Tabaldi, and F. M. Durgante. 2023. Leaf-Level
 Field Spectroscopy to Discriminate Invasive Species (Psidium guajava L. and Hovenia dulcis
 Thunb.) from Native Tree Species in the Southern Brazilian Atlantic Forest. Remote Sensing.
- Mandrioli, M. 2023. From Dormant Collections to Repositories for the Study of Habitat Changes: The
 Importance of Herbaria in Modern Life Sciences. Life (Basel) 13.
- Meacham-Hensold, K., C. M. Montes, J. Wu, K. Guan, P. Fu, E. A. Ainsworth, T. Pederson, C. E. Moore,
 K. L. Brown, C. Raines, and C. J. Bernacchi. 2019. High-throughput field phenotyping using
 hyperspectral reflectance and partial least squares regression (PLSR) reveals genetic
 modifications to photosynthetic capacity. Remote Sensing of Environment 231:111176.
- Medeiros, L. S., S. M. Q. Lima, M. d. Pinna, I. C. A. Souto-Santos, S. Pirro, and W. M. Berbel-Filho.
 2024. Whole-Genome Sequencing of Two Listrura and Five Microcambeva Species
 (Trichomycteridae, Siluriformes), Rare and Threatened Catfishes from the Atlantic Forest.
 Biodiversity Genomes December.
- Meineke, E. K., T. J. Davies, B. H. Daru, and C. C. Davis. 2018a. Biological collections for
 understanding biodiversity in the Anthropocene. Philosophical Transactions of the Royal Society
 B: Biological Sciences 374:20170386.
- Meineke, E. K., C. C. Davis, and T. J. Davies. 2018b. The unrealized potential of herbaria for global
 change biology. Ecological Monographs 88:505-525.
- Meireles, J. E., J. Cavender-Bares, P. A. Townsend, S. Ustin, J. A. Gamon, A. K. Schweiger, M. E.
 Schaepman, G. P. Asner, R. E. Martin, A. Singh, F. Schrodt, A. Chlus, and B. O'Meara. 2020a.
 Leaf reflectance spectra capture the evolutionary history of seed plants. New Phytologist
 228:485-493.
- Meireles, J. E., B. O'Meara, and J. Cavender-Bares. 2020b. Linking leaf spectra to the plant tree of life.
 Pages 155-172 *in* J. Cavender-Bares, J. A. Gamon, and P. A. Townsend, editors. Remote Sensing of Plant Biodiversity. Springer.
- Meireles, J. E., B. O'Meara, and J. Cavender-Bares. 2020c. Linking Leaf Spectra to the Plant Tree of Life. Pages 155-172 *in* J. Cavender-Bares, J. A. Gamon, and P. A. Townsend, editors. Remote Sensing of Plant Biodiversity. Springer International Publishing, Cham.
- Meireles, J. E., A. K. Schweiger, and J. M. Cavender-Bares. 2017. spectrolab: Class and Methods for
 Hyperspectral Data. R package version 0.0.2.

- 1193 National Academies of Sciences, E., and Medicine. 2020. Biological Collections: Ensuring Critical
 1194 Research and Education for the 21st Century. The National Academies Press, Washington, DC.
- 1195 Nelson, G., P. Sweeney, L. Wallace, R. Rabeler, D. Allard, H. Brown, R. Carter, M. Denslow, L.
 1196 Ellwood, C. Germain-Aubrey, E. Gilbert, E. Gillespie, L. Goertzen, B. Legler, T. Marsico, A.
 1197 Morris, Z. Murrell, M. Nazaire, and A. Mast. 2015. Digitization Workflows for Flat Sheets and
 1198 Packets of Plants, Algae, and Fungi. Applications in Plant Sciences 3.
- Paiva, D. N. A., R. d. O. Perdiz, and T. E. Almeida. 2021. Using near-infrared spectroscopy to
 discriminate closely related species: a case study of neotropical ferns. Journal of Plant Research
 134:509-520.
- 1202 Park, D. S., X. Feng, S. Akiyama, M. Ardiyani, N. Avendaño, Z. Barina, B. Bärtschi, M. Belgrano, J. 1203 Betancur, R. Bijmoer, A. Bogaerts, A. Cano, J. Danihelka, A. Garg, D. E. Giblin, R. Gogoi, A. 1204 Guggisberg, M. Hyvärinen, S. A. James, R. J. Sebola, T. Katagiri, J. A. Kennedy, T. S. Komil, B. 1205 Lee, S. M. L. Lee, D. Magri, R. Marcucci, S. Masinde, D. Melnikov, P. Mráz, W. Mulenko, P. Musili, G. Mwachala, B. E. Nelson, C. Niezgoda, C. Novoa Sepúlveda, S. Orli, A. Paton, S. 1206 1207 Payette, K. D. Perkins, M. J. Ponce, H. Rainer, L. Rasingam, H. Rustiami, N. M. Shiyan, C. S. 1208 Bjorå, J. Solomon, F. Stauffer, A. Sumadijaya, M. Thiébaut, B. M. Thiers, H. Tsubota, A. 1209 Vaughan, R. Virtanen, T. J. S. Whitfeld, D. Zhang, F. O. Zuloaga, and C. C. Davis. 2023. The 1210 colonial legacy of herbaria. Nature Human Behaviour 7:1059-1068.
- Pearson, K. D., G. Nelson, M. F. J. Aronson, P. Bonnet, L. Brenskelle, C. C. Davis, E. G. Denny, E. R.
 Ellwood, H. Goëau, J. M. Heberling, A. Joly, T. Lorieul, S. J. Mazer, E. K. Meineke, B. J.
 Stucky, P. Sweeney, A. E. White, and P. S. Soltis. 2020. Machine Learning Using Digitized
 Herbarium Specimens to Advance Phenological Research. BioScience 70:610-620.
- Prata, E. M. B., C. Sass, D. P. Rodrigues, F. M. C. B. Domingos, C. D. Specht, G. Damasco, C. C. Ribas,
 P. V. A. Fine, and A. Vicentini. 2018. Towards integrative taxonomy in Neotropical botany:
 disentangling the Pagamea guianensis species complex (Rubiaceae). Botanical Journal of the
 Linnean Society 188:213-231.
- Guillot G, Santos F. 2009. A computer program to simulate multilocus genotype data with spatially
 autocorrelated allele frequencies. Molecular Ecology Resources 9: 1112-1120.
- Richard, K. R., T. S. Harlan, T. Barbara, L. A. Prather, A. M. James, P. L. Laura, C. M. Lucas, and J. F.
 Carolyn. 2019. Herbarium Practices and Ethics, III. Systematic Botany 44:7-13.
- Sapes, G., L. Schroeder, A. Scott, I. Clark, J. Juzwik, R. A. Montgomery, J. A. Guzmán Q, and J.
 Cavender-Bares. 2024. Mechanistic links between physiology and spectral reflectance enable
 previsual detection of oak wilt and drought stress. Proceedings of the National Academy of
 Sciences 121:e2316164121.
- Serbin, S. P., A. Singh, B. E. McNeil, C. C. Kingdon, and P. A. Townsend. 2014a. Spectroscopic
 determination of leaf morphological and biochemical traits for northern temperate and boreal tree
 species. Ecological Applications 24:1651-1669.
- Serbin, S. P., A. Singh, B. E. McNeil, C. C. Kingdon, and P. A. Townsend. 2014b. Spectroscopic
 determination of leaf morphological and biochemical traits for northern temperate and boreal tree
 species. Ecological Applications 7:1651-1669.
- Serbin, S. P., and P. A. Townsend. 2020. Scaling functional traits from leaves to canopies.*in* J. Cavender
 Bares, G. JA, and P. A. Townsend, editors. Remote Sensing of Plant Biodiversity. Springer, New
 York.
- 1236 Shah, T. 2023. The Herbarium Handbook. Kew Publishing.
- Shipley, B., M. Belluau, I. Kuhn, N. A. Soudzilovskaia, M. Bahn, J. Penuelas, J. Kattge, L. Sack, J.
 Cavender-Bares, W. A. Ozinga, B. Blonder, P. M. van Bodegom, P. Manning, T. Hickler, E.
 Sosinski, V. D. Pillar, V. Onipchenko, and P. Poschlod. 2017. Predicting habitat affinities of plant
 species using commonly measured functional traits. Journal of Vegetation Science 28:1082-1095.
- Sims, D. A., and J. A. Gamon. 2002. Relationships between leaf pigment content and spectral reflectance
 across a wide range of species, leaf structures and developmental stages. Remote Sensing of
 Environment 81:337-354.

- 1244 Soltis, P. S. 2017. Digitization of herbaria enables novel research. Am J Bot 104:1281-1284.
- Sousa da Silva, G., and M. J. G. Hopkins. 2024. Utilização da espectroscopia do infravermelho próximo
 (NIRS) como ferramenta na discriminação de espécies do gênero Dimorphandra Schott
 (Leguminosae: Caesalpinioideae). Scientia Plena 20.
- Stasinski, L., D. M. White, P. R. Nelson, R. H. Ree, and J. E. Meireles. 2021. Reading light: leaf spectra capture fine-scale diversity of closely related, hybridizing arctic shrubs. New Phytologist n/a.
- Stefanski, A., E. E. Butler, L. J. Williams, R. Bermudez, J. A. Guzmán Q., A. Larson, P. A. Townsend, R.
 A. Montgomery, J. Cavender-Bares, and P. Reich. 2025. All the light we cannot see: Climate
 manipulations leave short and long-term imprints in spectral reflectance of trees. Ecology in
 press.
- 1254 Thiers, B. M. 2024. Strengthening Partnerships to Safeguard the Future of Herbaria. Diversity.
- Türker-Kaya, S., and C. W. Huck. 2017. A Review of Mid-Infrared and Near-Infrared Imaging:
 Principles, Concepts and Applications in Plant Tissue Analysis. Molecules.
- 1257 Ustin, S. L., A. A. Gitelson, S. Jacquemoud, M. Schaepman, G. P. Asner, J. A. Gamon, and P. Zarco 1258 Tejada. 2009. Retrieval of foliar information about plant pigment systems from high resolution
 1259 spectroscopy. Remote Sensing of Environment 113:S67-S77.
- Ustin, S. L., and S. Jacquemoud. 2020. How the Optical Properties of Leaves Modify the Absorption and
 Scattering of Energy and Enhance Leaf Functionality. Pages 349-384 *in* J. Cavender-Bares, J. A.
 Gamon, and P. A. Townsend, editors. Remote Sensing of Plant Biodiversity. Springer
 International Publishing, Cham.
- Ustin, S. L., S. Jacquemoud, and Y. Govaerts. 2001. Simulation of photon transport in a three dimensional leaf: implications for photosynthesis. Plant, Cell & Environment 24:1095-1103.
- Ustin, S. L., D. A. Roberts, J. A. Gamon, G. P. Asner, and R. O. Green. 2004. Using Imaging
 Spectroscopy to Study Ecosystem Processes and Properties. BioScience 54:523-534.
- 1268 Vasconcelos, C. C., M. U. Adrianzén, J. L. C. Camargo, and M. H. Terra-Araujo. 2020. Pouteria
 1269 kossmanniae (Sapotaceae): a new species from Central Amazonia, Brazil. Phytotaxa 447 265–
 1270 275.
- 1271 Violle, C., M.-L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the
 1272 concept of trait be functional! Oikos 116:882-892.
- Wagner, E. P., J. Merz, and P. A. Townsend. 2019. EcoSIS: A Spectral Library and the Tools to Use It.
 American Geophysical Union, Fall Meeting 2019, abstract #B11F-2396
 2019AGUFM.B11F2396W.
- Wang, R., J. A. Gamon, and J. Cavender-Bares. 2022. Seasonal patterns of spectral diversity at leaf and
 canopy scales in the Cedar Creek prairie biodiversity experiment. Remote Sensing of
 Environment 280:113169.
- Wang, Z., A. Chlus, R. Geygan, Z. Ye, T. Zheng, A. Singh, J. J. Couture, J. Cavender-Bares, E. L.
 Kruger, and P. A. Townsend. 2020. Foliar functional traits from imaging spectroscopy across biomes in eastern North America. New Phytologist 228:494-511.
- Wang, Z., J.-B. Féret, N. Liu, Z. Sun, L. Yang, S. Geng, H. Zhang, A. Chlus, E. L. Kruger, and P. A.
 Townsend. 2023. Generality of leaf spectroscopic models for predicting key foliar functional
 traits across continents: A comparison between physically- and empirically-based approaches.
 Remote Sensing of Environment 293:113614.
- Webster, M. S. 2017. The Extended Specimen: Emerging Frontiers in Collections-Based Ornithological
 Research (1st ed.). CRC Press.
- Wenk, E. H., H. Sauquet, R. V. Gallagher, R. Brownlee, C. Boettiger, D. Coleman, S. Yang, T. Auld, R.
 Barrett, T. Brodribb, B. Choat, L. Dun, D. Ellsworth, C. Gosper, L. Guja, G. J. Jordan, T. Le
 Breton, A. Leigh, P. Lu-Irving, B. Medlyn, R. Nolan, M. Ooi, K. D. Sommerville, P. Vesk, M.
 White, I. J. Wright, and D. S. Falster. 2024. The AusTraits plant dictionary. Scientific Data
 11:537.

- White, D. M., J. Cavender-Bares, C. Davis, J. A. G. Q., S. Kothari, J. Robles, and J. E. Meireles. 2025.
 Seeing herbaria in a new light: leaf reflectance spectroscopy unlocks predictive trait and classification modeling in plant biodiversity collections. EcoEvoRxiv:1-41.
- Wieczorek, J., D. Bloom, R. Guralnick, S. Blum, M. Döring, R. Giovanni, T. Robertson, and D. Vieglais.
 2012. Darwin Core: an evolving community-developed biodiversity data standard. Plos One
 7:e29715.
- Williams, L. J., J. Cavender-Bares, P. A. Townsend, J. J. Couture, Z. Wang, A. Stefanski, C. Messier, and
 P. B. Reich. 2021. Remote spectral detection of biodiversity effects on forest biomass. Nature
 Ecology & Evolution 5:46-54.
- Willis, C. G., E. R. Ellwood, R. B. Primack, C. C. Davis, K. D. Pearson, A. S. Gallinat, J. M. Yost, G.
 Nelson, S. J. Mazer, N. L. Rossington, T. H. Sparks, and P. S. Soltis. 2017. Old Plants, New
 Tricks: Phenological Research Using Herbarium Specimens. Trends in Ecology & Evolution
 32:531-546.
- Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. David. 2008. Phylogenetic
 patterns of species loss in Thoreau's woods are driven by climate change. Proceedings of the
 National Academy of Sciences of the United States of America 105:17029 17033.
- Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T.
 Chapin, J. H. Cornelissen, and M. Diemer. 2004. The worldwide leaf economics spectrum.
 Nature 428:821-827.
- 1312 Zhang, J., M. Wang, K. Yang, Y. Li, Y. Li, B. Wu, and Q. Han. 2022. The New Hyperspectral Analysis
 1313 Method for Distinguishing the Types of Heavy Metal Copper and Lead Pollution Elements. Int J
 1314 Environ Res Public Health 19.
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1319 Supplemental Tables

- 1320 Table S1. Previous studies using dry-leaf spectra.

Application (dry leaves)	Spectral Range	Author
C, N, P, K, micronutrients, leaf pigments, d13C (see Fig. 1)	VIS-NIR: 400-2500 nm	Elvidge 1990, Fourty et al. 1996, Serbin et al. 2014, Prananto et al. 2020, Chlus and Townsend 2022, Chen et al. 2002, Kothari et al. 2023, 2024, Wang et al. 2023, Kühn et al. 2025
Nutrient contents and leaf traits	NIR: 1000-2500 nm	Prananto et al. 2021, Costa et al. 2018, Kothari et al. 2024
Species identification	VIS-NIR: 400-2,500 nm	Meireles et al. 2020; Vasconcelos et ali. 2025
Species identification	NIR: 1.000-2.500nm	Durgante et al. 2013; Lang et al. 2015;Paive et al. 2021; Vasconcelos et al. 2020, 2021
Species identification	IR: 1.250-25.000nm	Kim et al. 2004; Krajsek et al. 2008
Species characterization and chemical properties	Thermal IR: 2.5 - 20 μm	Elvidge 1990; Richardson et. al. 2000