Foliar functional and genetic variation in a keystone Hawaiian tree species estimated through spectroscopy

Seeley, M.[1,2], Stacy, E.A.[3], Martin, R.E.[1,2], & Asner, G.P.[1,2]

1. *Center for Global Discovery and Conservation Science, Arizona State University, Hilo, HI 96720, USA*
2. *School of Geographical Sciences and Urban Planning, Arizona State University, Tempe, AZ 85281, USA*
3. *School of Life Sciences, University of Nevada, Las Vegas, NV 89154, USA*

Highlighted Student Paper Statement: *By quantifying the spectral variability of Metrosideros polymorpha, an endemic, keystone species of the Hawaiian Islands, we take the first step in using remote sensing to spatially map genetic varieties of this species and to more generally study the genetic basis of biodiversity.*

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Abstract

Imaging spectroscopy has the potential to map closely related plant taxa at landscape scales. While spectral investigations at the leaf and canopy levels have revealed relationships between phylogeny and reflectance, understanding how spectra differ across, and are inherited from, genotypes of a single species has received less attention. We used a common-garden population of four varieties of the keystone canopy tree, *Metrosideros polymorpha*, from Hawaii Island and four F1-hybrid genotypes derived from controlled crosses to determine if reflectance spectra discriminate sympatric, conspecific varieties of this species and their hybrids. With a single exception, pairwise comparisons of leaf reflectance patterns successfully distinguished varieties of *M. polymorpha* on Hawaii Island as well as populations of the same variety from different islands. Further, spectral variability within a single variety from Hawaii Island and the older island of Oahu was greater than that observed among the four varieties on Hawaii Island. F1 hybrids most frequently displayed leaf spectral patterns intermediate to those of their parent taxa. Spectral reflectance patterns distinguished each of two of the hybrid genotypes from one of their parent varieties, indicating that classifying hybrids may be possible, particularly if sample sizes are increased. This work quantifies a baseline in spectral variability for an endemic Hawaiian tree species and advances the use of imaging spectroscopy in biodiversity studies at the genetic level.

Key Words

Spectroscopy, plant evolution, leaf spectra, genetic diversity, Hawaii, *Metrosideros polymorpha*

Introduction

Genetic diversity of forests provides a foundation for resilience to climate change, biological invasions, and other anthropogenic threats (Crutsinger et al., 2008; Schaberg et al., 2008). High genetic diversity of overstory forest species has been linked to increased productivity and fitness (Aravanopoulos & Zsuffa, 1998; Arcade et al., 1996; Jelinski, 1993; Knowles & Grant, 1981; Mitton et al., 1981), a higher tolerance to pollutants (Bergmann & Hosius, 1996; Müller-Starck, 1985; Oleksyn et al., 1994), and cascading trophic effects on arthropod (Johnson et al., 2006) and fungal (Tang et al., 2022) biodiversity. As genetic diversity is a basis for adaptation and enhanced resilience, it is vital to preserving forest ecosystems, yet anthropogenic disturbances have resulted in significant declines in forest genetic diversity, reducing the future resistance of affected species (Schaberg et al., 2008). While the 15th Sustainable Development Goal of the United Nation includes aims to stop biodiversity loss, including loss of genetic diversity (Le Blanc, 2015), the resources with which to quantify and map genetic diversity are constrained because genetic analyses of forest species require extensive field and lab work (Walters & Scholes, 2017).

Remote sensing, in particular imaging spectroscopy, has emerged as a powerful tool for quantifying biodiversity at large spatial scales to understand drivers of biodiversity and inform protection priorities (Asner et al., 2017; Féret & Asner, 2011). Imaging spectroscopy generates high-spectral-resolution data spanning the visible to shortwave-infrared (SWIR; 400-2500 nm) electromagnetic spectrum. Applied to vegetation, spectroscopy captures the molecular constituents of leaves, mediated by leaf structure. Leaf traits such as leaf mass per area (LMA), chlorophyll content, and secondary compounds, among others are an expression of adaptation of a species to its environment (Ordoñez et al., 2009; I. J. Wright et al., 2004). While such

quantitative traits often have a substantial allelic basis (Hallgren et al., 2003; Marron & Ceulemans, 2006) that is predominantly polygenic (Bourgaud et al., 2001; Orians et al., 2000), heritability of leaf traits derived from spectroscopy has not been widely tested. Due to the capability of spectroscopy to capture these traits, spectral variation tracks genetic variation among and within forest stands (Blonder et al., 2020; Cavender-Bares et al., 2016; Deacon et al., 2017; Madritch et al., 2014; Martin et al., 2007). According to the spectral variability hypothesis, the variability of canopy reflectance spectra within an area is positively related to plant diversity (Palmer et al., 2000, 2002). Consistent with this hypothesis, leaf-level spectroscopy has revealed heritable spectral differences within and among species of *Quercus* (oak; Cavender-Bares et al., 2016) and *Dryas* (an Arctic shrub; Stasinski et al., 2021) as well as within populations of *Populus tremuloides* (aspen; Deacon et al., 2017) and *Metrosideros polymorpha* (ohia, Martin et al., 2007). Ploidy levels and genetic varieties of *P. tremuloides* have been successfully classified using canopy-level imaging spectroscopy (Blonder et al., 2020; Madritch et al., 2014). Further, some studies have revealed patterns of leaf spectra consistent with phylogeographic variation within species (e.g., *Quercus oleoides*, *Fagus sylvatica*—European beach—and *P, tremuloides*; Cavender-Bares et al., 2016; Blonder et al., 2020; Czyż et al., 2020; Madritch et al., 2014) or among species (e.g., Neotropical trees; McManus et al., 2016). To develop imaging spectroscopy as a tool for characterizing genetic variation at the landscape level, we must first understand how spectra vary within continuous forest stands, including variation among conspecific varieties and their hybrids, especially at fine spatial and taxonomic scales. This gap in our understanding of how spectroscopy captures functional variation challenges conservation agendas that seek to include genetic diversity.

Metrosideros polymorpha Gaudich. (Myrtaceae) is an ideal model species for testing the capacity of spectroscopy to characterize functional genetic variation of forest canopies at fine spatial and taxonomic scales. This dominant tree species comprises a large number of vegetatively distinct varieties and races distributed nonrandomly within continuous forests that span environmental gradients and ecotones within the climatically variable Hawaiian Islands (Dawson & Stemmermann, 1990; Stacy et al., 2020; Stacy & Sakishima, 2019; Treseder & Vitousek, 2001). The many forms of *M. polymorpha*, along with the four other species of Hawaiian *Metrosideros*, appear to derive from a single colonization of Hawaii by the genus ~2.6-3.9 million years ago (Choi et al., 2021; Dupuis et al., 2019; Percy et al., 2008; S. D. Wright et al., 2000). Diversification within this group is largely the result of adaptive radiation associated with Hawaii's diverse abiotic conditions (Ekar et al., 2019; Izuno et al., 2022; Morrison & Stacy, 2014; Stacy et al., 2014, 2020; Stacy & Sakishima, 2019). On Hawaii Island, the youngest and largest island in the chain, *Metrosideros* occurs continuously (barring deforestation) from sea level to 2470 m above sea level wherever mean precipitation exceeds 50 cm annually (Stemmermann & Ihsle, 1993). The *Metrosideros* community on Hawaii Island comprises just four varieties of *M. polymorpha* associated with different environments: *M. polymorpha* var. *incana* (new lava flows at low-to-middle elevations and dry areas), *M. polymorpha* var. *glaberrima* (mature substrates at all but lowest and highest elevations), *M. polymorpha* var. *polymorpha* (all substrates at high elevations), and *M. polymorpha* var. *newellii* (riparian zones; Dawson & Stemmermann, 1990). All taxon pairs can be crossed to make F1 hybrids (Corn, 1979; Rhoades, 2012; Stacy et al., 2017), and hybridization between varieties occurs to varying degrees where ranges overlap (Corn & Hiesey, 1973; Stacy et al., 2016). Thus, *M. polymorpha* on Hawaii Island presents the opportunity to examine the utility of spectroscopy to discern very

closely related, co-occurring tree taxa and their hybrids and to examine the expression and differentiability of leaf traits in the hybrids.

Here, we use a common-garden population of the four varieties of *M. polymorpha* on Hawaii Island and their F1 hybrids derived from controlled crosses to address the following questions: Do leaf-level reflectance spectra differentiate the four varieties of *M. polymorpha* on Hawaii Island? Are patterns of spectral inheritance in F1 hybrids distinct and intermediate to those of their parental varieties, as expected for highly polygenic traits? Finally, for a single variety occurring on multiple islands, we ask: do the reflectance spectra differ between common-garden trees from Hawaii Island and Oahu? We include a discussion of the spectral data in light of evidence of differential adaptation of the four varieties to contrasting environments and to islands of different ages.

Methods

Common Garden Population

The 54 reproductively mature trees used in this study were raised from seed at Panaewa Farm, College of Agriculture, Forestry, and Natural Resources Management, University of Hawaii Hilo, located 75 m above sea level on east Hawaii Island. Seeds were derived from controlled crosses in natural populations on Hawaii Island and Oahu (Rhoades, 2012; Stacy et al., 2017, unpub. data*)*, supplemented by open-pollinated seeds, and all trees were maintained at the farm for use in studies of life history traits and hybrid fertility (Stacy et al., unpub. data). The 8-to-14-year-old trees represented the four varieties of *M. polymorpha* on Hawaii Island (hereafter designated glaberrima, incana, newellii, and polymorpha; Fig. SI 1), four inter-varietal F1 hybrid genotypes from Hawaii Island, and a single variety (incana) from Oahu (Table 1).

With two exceptions, all genotypes comprised trees derived from >1 site, or trees for which parents were derived from >1 site in the case of F1 hybrids; the exceptions were individuals of incana from Hawaii Island and Oahu that were derived from controlled field crosses at a single site on each island. All trees were maintained within a 72'x35' coldframe until 2020 when some Hawaii Island-derived trees were outplanted in a common garden adjacent to the coldframe. We assessed the effect of this outplanting on leaf spectra by comparing greenhouse and common-garden trees of incana-polymorpha F1 hybrids following the methods below and found no significant differences. Thus, we determined that outplanting had negligible effects on the spectra, and all samples were combined for analysis of spectra among genotypes.

Leaf Measurements

We measured leaf reflectance spectra on six trees from each of the nine genotypes (treating incana from Hawaii Island and Oahu as separate genotypes). A minimum of 11 leaves were collected from each plant, placed in zip lock bags, and stored on ice for transport to the laboratory for analysis within four hours. We selected leaves from sunlit portions of the plant with minimal discoloration (e.g. chlorosis) and sooty mold. Five representative leaves per tree were selected and wiped clean with water and patted dry prior to spectral measurements. Spectral measurements were collected using a leaf clip and field spectrometer at 1-nm intervals from 350 to 2500 nm (Analytical Spectra Devices Inc., Boulder, CO, USA). Spectra were calibrated using a white reference and corrected using parabolic correction to optimize spectrometer measurements (Hueni & Bialek, 2017). Parabolic correction was performed to correct for differences in temperature sensitivity of sensors within the field spectrometer. A jump in the spectra often occurs around 1000 nm due to the silicon-based sensors for the visible to near

infrared and can be corrected post hoc according to Hueni & Bialek (2017). Finally, brightness normalization was applied to all spectral measurements, as it minimizes noise (Kruse et al., 1993; Myneni et al., 1989). Reflectance values below 400 nm were removed, as wavelengths between 350 and 400 nm have a low signal-to-noise ratio. Leaf spectra were averaged by plant. Following spectral measurements of all leaves, leaf area was calculated using ImageJ from a leaf scan collected with an EPSON scanner at 600 dots per square inch. Once dried for 72 hours at 65 degrees Celsius, leaves were weighed, and leaf mass per area (LMA) was quantified for each plant.

Analysis

To assess whether leaf spectra can differentiate the varieties of *M. polymorpha* and their hybrids, we used principal component analysis (PCA) and analysis of variance (ANOVA). Using the *pca* function from the *scikit learn* python package (version 0.24.1; Virtanen et al., 2020), which uses a covariance matrix for the eigen decomposition, we reduced the 2100 dimensions of the reflectance data to the first 10 principal components (PC). PCA was applied spearately to different genotype groupings detailed below. For each of the first 10 PCs, we evaluated its ability to separate the genotypes using an ANOVA according to the methods in Cavender-Bares et al. (2016), followed by Tukey's pairwise HSD tests. These methods were performed in python using the *statsmodels* package (version 0.12.2; Seabold & Perktold, 2010) to compare genotypes separately for each of the following six groups: 1) Hawaii Island incana, glaberrima, newellii, and polymorpha; 2) glaberrima-incana, glaberrima, and Hawaii Island incana; 3) incana-polymorpha, Hawaii Island incana, and polymorpha; 4) newellii-polymorpha, newellii, and

168 polymorpha; 5) glaberrima-polymorpha, glaberrima, and polymorpha ; and 6) incana from Oahu
169 and Hawaii Island (Table 1).
170 While the PCA allowed us to determine if the spectra were differentiable, we used the
171 spectral similarity index (SSI; Eq. 1; Somers et al., 2009, 2012, 2015) to quantify spectral
172 overlap between varieties. The SSI calculates the spectral distance between populations *i* and *j*
173 for each wavelength:
174
175 Eq. 1.
176 $$SSI = \frac{\left|\bar{R}_{b,i} - \bar{R}_{b,j}\right|}{sd\left(R_{b,i}\right) + sd\left(R_{b,j}\right)}$$
177
178 where *R* is the brightness-normalized reflectance for each group over *n* spectral bands. Rather
179 than performing pairwise comparisons, population *j* was represented by pooled reflectance data
180 from all varieties (including Oahu and Hawaii Island incana). In doing so, we estimated the
181 degree to which each variety diverged spectrally from all varieties. SSI has been used to estimate
182 species turnover (Somers et al., 2015) and as a means of determining which wavelengths
183 distinguish classes (Asner et al., 2018). Here we plotted SSI across the entire spectrum to
184 quantify the degree of separation, with higher SSI values indicating a higher degree of spectral
185 overlap, between spectra of the *M. polymorpha* varieties. Further, we calculated the mean SSI by
186 taking an average of 1/SSI across all bands (Eq. 2).
187
188 Eq. 2
189 $$mean\ SSI = \frac{1}{n}\sum_{b=0}^{n}\frac{sd\left(R_{b,i}\right) + sd\left(R_{b,j}\right)}{\left|\bar{R}_{b,i} - \bar{R}_{b,j}\right|}$$

To understand within-variety variation, we calculated the mean spectra and coefficient of variation (CV) for each variety across the spectra. The CV is a standardized measure of variation that allows for visual comparison among samples across the full spectrum. Here, we use the CV to visually assess regions of the spectrum that show the greatest variation within each genotype of *M. polymorpha*. While this investigation is useful for visualizing diversity in terms of reflectance between varieties, band-by-band assessments of CV are limited because spectra are derived from broader features related to chemical interactions with light.

Lastly, we examined variation among genotypes in leaf traits derived from the reflectance data. Leaf chemical traits were estimated from reflectance spectra using chemometric equations specific to *M. polymorpha* developed by Asner et al. (2018). These spectral-chemical relationships were determined using the partial least squares regression (PLSR) – prediction residual error sum of squares (PRESS) method that has been used to develop universal chemometric equations for broadleaf species (Asner et al., 2009, 2015; Asner & Martin, 2008). As these methods approximate leaf traits, we use them as a means of comparing leaf traits between groups rather than interpreting their absolute value. We estimated eight chemical traits (Table SI 1) using the equations specific to *M. polymorpha*, including the photosynthetic pigments chlorophylls a and b, the structural molecules lignin and cellulose, and the secondary traits phenols and tannins. Chlorophylls a and b were summed and represented as chlorophyll a+b. Further, nonstructural carbohydrates (NSC) like sugars and starch were estimated along with total nitrogen (N) and total carbon (C). Leaf mass per unit area (LMA) was calculated using leaf area and dry weights quantified from the collected leaves, described above. When discussing leaf trait data, we refer to the chemical leaf traits estimated from the reflectance data as well as

the LMA calculated from leaves. Significance of differences in leaf traits between genotypes in the groupings described above was quantified using ANOVA and Tukey HSD tests. All analyses were done using python version 3.6.9.

In summary, we first used principal component analysis (PCA) to reduce this highly dimensional dataset into fewer components that captured a larger proportion of the variance. We then determined whether any of the components could separate the varieties as well as F1 hybrids from their parent varieties using ANOVA and Tukey HSD. To understand differences in reflectance between and within the varieties, we used the spectral similarity index (SSI) and compared their coefficient of variation (CV) and leaf traits.

Results

Spectral Divergence Among Varieties

PCAs of leaf spectra (Table 2) separated all varieties in pairwise comparison except glaberrima and incana. Two PCs (PC1 and PC5) derived from the reflectance spectra significantly differentiated the varieties ($p = 0.003$ for each; Table 2). In the pairwise comparison, incana and newellii were separable in both PC1 and PC5. PC1 additionally separated glaberrima and polymorpha as well as newellii and polymorpha. Glaberrima and newellii as well as incana and polymorpha were differentiable in PC5. The only taxon pair that was not separable in the first 10 PCs of the reflectance data was glaberrima and incana.

When visually comparing spectra of the four Hawaii Island varieties, the mean brightness-normalized spectra vary most in the visible (400-700 nm) and shortwave infrared (SWIR; 1500- 2500 nm) wavelength regions (Fig. 1a). According to the spectral separability index (SSI), separation between the varieties occurred across the spectra (Fig. 1b), with

polymorpha having the greatest mean SSI (29; Table 3). Both polymorpha and Hawaii Island incana were most distinct in the visible and parts of the infrared while glaberrima had the greatest separability in the SWIR and infrared (Fig. 1b). Glaberrima and incana were similar in their degree of spectral overlap with SSI values of 9 and 11, respectively (Table 3). Newellii, which had the highest separability after ~1800 nm, had the lowest mean SSI (7; Fig. 1b; Table 3).

Among Hawaii Island varieties, incana had the least within-variety variation among the spectra, while newellii and polymorpha displayed the most variation according to the CV (Fig. 1c).Within-variety variation was greatest in the visible and SWIR regions of the spectrum (Fig. 1c). The CV of newellii peaked in the visible region, where newellii had not only the greatest within-variety variation but also the highest reflectance values. This result is also expressed in the estimated chemical data (Fig. 2), where newellii had a greater variability relative to the other varieties and lower values of chlorophyll a+b than polymorpha. While polymorpha likewise had a high CV in the visible, this variety had the lowest reflectance in this region compared to the other varieties, and this corresponded to high chlorophyll a+b. In the SWIR region, which is influenced by many leaf traits, within-variety variation was greatest for polymorpha and newellii, followed by glaberrima. Newellii had higher total N than all other varieties but lower LMA, total phenols, and lignin than some other varieties. Polymorpha had lower cellulose than newellii and higher LMA than glaberrima and newellii. Both polymorpha and glaberrima had a wide variation in NSC, and polymorpha had high variation in LMA. Incana had low variation in all the leaf traits except for tannins. Leaf traits were less useful than PCA for discriminating the varieties (Fig. 2). Cellulose, chlorophyll a+b, lignin, phenols, total N, and LMA separated newellii from

all other varieties (Fig. 2). Beyond this, only polymorpha and glaberrima differed significantly in leaf traits (chlorophyll a+b and LMA; Fig. 2).

Spectral Patterns in Hybrids

The four F1 hybrid genotypes demonstrated different patterns of leaf reflectance relative to their parental taxa. Spectral PC1 scores separated glaberrima and incana as well as glaberrima and glaberrima-incana hybrids (Table 4). Mean spectra of glaberrima-incana F1s fell between the mean spectra of their parent varieties but were closer to glaberrima in the visible and closer to incana between approximately 2000 and 2500 nm (Fig. 3a). Overall, the shape of the CV across the spectrum within glaberrima-incana F1s mirrored that of incana (Fig. SI 4a). None of the leaf traits differed between the glaberrima-incana F1s and either of their parent varieties (Fig. 4a). Variation in F1 leaf traits was often intermediate to or less than that of the parent varieties, except for total C and LMA (Fig. 4a).

The incana-polymorpha F1 trees showed intermediate values for many of the leaf traits and within-genotype spectral variation, though their mean spectra most often mirrored those of polymorpha (Fig. 3b). Consistent with this trend, PC3 scores (but not PC1 or PC2 scores) separated incana-polymorpha F1s from incana but not polymorpha (Fig. 3b; Table 4). Similar to the glaberrima-incana F1s, spectral variation (CV) of incana-polymorpha most resembled that of incana in shape but was often intermediate or closer to the other parent (here, polymorpha) in magnitude (Fig. SI 4b). Both incana-polymorpha F1s and polymorpha had higher chlorophyll a+b than incana (Fig. 4a). Many of the other leaf traits of the F1s displayed values intermediate to those of the parent values, though the variation of the hybrid data was often greater than that of either parent.

281 Mean spectral values of newellii-polymorpha F1s were intermediate in the visible,
282 closely followed polymorpha in the infrared and beyond ~1700 nm, and were lower than either
283 parent between 1500 and 1700 nm (Fig. 3c). Newellii and polymorpha was the only pair of
284 genotypes in the newellii-polymorpha group that was separable by any PC scores (Table 4). Leaf
285 trait data indicated that many of the F1 traits were intermediate to those of the parent varieties,
286 but within-F1 variation was lower than variation within either parent for lignin, NSC, and total C
287 (Fig. 4b). In contrast, tannin levels varied more among F1 trees than among trees of either parent.
288 Four of the leaf traits separated newellii and polymorpha, while total N, lignin, and LMA
289 separated newellii and newellii-polymorpha (Fig. 4b). Polymorpha and newellii-polymorpha F1s
290 did not differ in any of the leaf traits.
291 Mean spectra of glaberrima-polymorpha F1s largely fell between those of the parent
292 varieties, but more closely followed glaberrima in the visible and polymorpha in the SWIR (Fig.
293 3d). Only the parent varieties were differentiable using reflectance spectra (Table 4). Glaberrima-
294 polymorpha F1s had lower total C relative to polymorpha, though within-group variation of total
295 C was greater in the hybrid than either parent (Fig. 4b). LMA was the only leaf trait for which
296 glaberrima-polymorpha F1s were intermediate to the parents in both median value and within-
297 group variation. The glaberrima-polymorpha outlier values for tannins, lignin, and cellulose were
298 taken from the same plant (Fig. 4b).
299
300 *Comparing Populations across Islands*
301 Trees of incana from Oahu and Hawaii Island were compared to assess inter-island
302 divergence of leaf spectra (Fig. 1). Across the full spectrum, except in the infrared (~750 - 1700
303 nm), mean spectral reflectance of incana was greater for trees from Oahu than those from Hawaii

304 Island (Fig. 1a). The CV was similarly greater for Oahu trees across the spectra, and the shapes
305 of the CV were similar only in the visible (Fig. 1c). PC1 scores significantly differentiated leaf
306 spectra of incana from the different islands (p-value < 0.05), and Oahu incana, with an SSI of 4,
307 had the lowest SSI of all the varieties by nearly a factor of four (Fig. 1b; Table 3). Six of the leaf
308 chemical traits differed between islands (Fig. 2). Oahu incana had higher cellulose and total N
309 concentrations, but lower lignin, phenols, LMA, and tannins. Qualitative comparisons suggested
310 that within-group trait variation was greater for Oahu incana in cellulose, lignin, NSC, and
311 tannins.
312
313 **Discussion**
314 We measured the leaf spectra of several genotypes of a landscape-dominant tree species
315 and demonstrated separation of ecologically diverged varieties across the geographic scale of
316 east Hawaii Island. Leaf reflectance data successfully distinguished all but one pair of varieties
317 of *M. polymorpha* on Hawaii Island as well as populations of the same variety from different
318 islands. Spectral reflectance measures from four classes of F1 hybrids led to less successful
319 discrimination of intraspecific hybrids from their parental varieties, as expected. However, the
320 results suggest that reflectance spectra should be useful for the detection of *M. polymorpha*
321 hybrid zones using airborne imaging spectroscopy and that with increased sample size,
322 discrimination of individual F1 hybrids from parental taxa may be possible.
323
324 *Spectral Divergence Among Varieties*
325 Biodiversity estimates based on imaging spectroscopy, in accordance with the spectral
326 variability hypothesis, have been made across many landscapes (Féret & Asner, 2011; Schäfer et

al., 2016), but few studies have investigated how spectral variability captures intraspecific variation at finer scales (Cavender-Bares et al., 2016; Czyż et al., 2023; McManus et al., 2016). The current study demonstrates the potential of reflectance spectra to capture the genetic variation within a single hyperdominant tree species. Cavender-Bares (2016) similarly demonstrated separability of common-garden *Quercus oleoides* from populations across Central America where gene flow was limited due to the geographic separation of populations. Here we demonstrate spectral differentiation at scales much smaller than several hundred kilometers as monodominant stands of different *M. polymorpha* variants can exist directly adjacent to one another – a promising first step toward landscape-scale mapping of this species. Further, the leaf reflectance and derived trait data may reflect the differential adaptation of the four varieties of *M. polymorpha* to contrasting environmental niches in accordance with the spectral variability hypothesis (Palmer et al., 2000, 2002), as is discussed below.

The spectral signatures of the four varieties of *M. polymorpha* on Hawaii Island were separable in pairwise comparisons, except for those of the two successional varieties, incana and glaberrima. Despite their distinct leaf phenotypes, pubescent incana and glabrous glaberrima are the most weakly genetically differentiated pair of varieties on Hawaii Island (DeBoer & Stacy, 2013; Stacy et al., 2014) and Oahu (Stacy et al., 2020). Weak differentiation is consistent with their likely multi-million-year history of alternating periods of isolation by selection on new (incana) and old (glaberrima) lava flows and periods of hybridization on intermediate-aged flows (Corn & Hiesey, 1973; Drake & Mueller-Dombois, 1993; Kitayama et al., 1997; Stacy et al., 2017; Stacy & Sakishima, 2019). Glaberrima and incana were not differentiable when all four varieties were included in the analysis; however, they were separable in the analysis comprising just these varieties and their hybrids. This result suggests that classifying glaberrima and incana

using airborne imaging spectroscopy will be possible, but it may require the training of a secondary classification model on these varieties alone. In addition to their lack of separability via reflectance data, these varieties had a similar degree of spectral overlap (SSI) with the other varieties. Notably, incana had the lowest CV of reflectance of any variety. This low variation may be due to lower genetic variation among sampled incana due to purifying selection (Cvijović et al., 2018) in the harsh abiotic environments of new lava flows or due to the narrow sampling of incana for this study (i.e., from a single population) relative to the other varieties.

The spectral signatures and leaf traits recorded for polymorpha were consistent with values expected for high-elevation plants. Polymorpha dominates forests above ~1400 m and exhibits many traits associated with high-elevation plants, such as slow growth, compact form, and highly pubescent leaves (Dawson & Stemmermann, 1990; Homeier et al., 2010; King et al., 2013; Yang et al., 2008). The high LMA observed in polymorpha compared to the other varieties is consistent with expectations, as thicker leaves are often associated with high-elevation plants (Read et al., 2014). Lignin is associated with tensile strength (Trupiano et al., 2012; Zhang et al., 2014), and high lignin in polymorpha may be an adaptation to the mechanical stress of wind at high elevations (Zaborowska et al., 2023). High LMA and chlorophyll a+b in polymorpha are consistent with the relatively higher total chlorophyll and lower leaf surface area observed in common-garden trees of *M. polymorpha* derived from high-elevation, open-pollinated seeds (Martin et al., 2007). High chlorophyll a+b in polymorpha may be related to leaf pubescence, as pubescent polymorpha leaves self-shade to reduce damage to photosystems (Martin et al., 2007). Low peak reflectance in the visible spectrum is supported by the high chlorophyll content and suggests that polymorpha captures more light than the other varieties (Martin et al., 2007).

372 The CV of the reflectance spectra was high for polymorpha, which was unexpected given
373 the lower genetic variation of polymorpha relative to other varieties of *M. polymorpha* on Hawaii
374 Island (Stacy et al., 2014). Moreover, despite its high genetic differentiation relative to incana
375 and glaberrima (DeBoer & Stacy, 2013), polymorpha had the greatest spectral overlap with these
376 taxa. While lower trait variability at high elevations has been observed using imaging
377 spectroscopy data in Peru (Asner et al., 2016) and Hawaii (Seeley et al., *unpublished*),
378 polymorpha had high variability in its reflectance spectra. This appears to be due primarily to
379 high variation in NSC and LMA, which may be a result of growing plants adapted to high
380 elevations in low elevations or to sampling from young potted plants as opposed to full-grown
381 trees. Comparisons of high- versus low-elevation *M. polymorpha in situ* revealed lower CV of
382 canopy reflectance and trait variability at high elevations (Martin & Asner, 2009; Seeley et al.,
383 *unpublished*), supporting the conclusion that the greenhouse growing conditions affected
384 polymorpha.
385 Reflectance spectra for newellii were generally consistent with isolation of small
386 populations in separate riparian environments. Newellii is restricted to small, linear populations
387 along riparian corridors on east Hawaii Island (Dawson & Stemmermann 1990, Ekar et al. 2019).
388 The relatively strong genetic isolation of newellii from the other varieties (mean pairwise F_{ST}
389 between newellii populations and populations of all other varieties = 0.13; max = 0.25; pairwise
390 F_{ST} between glaberrima and polymorpha = [0.040, 0.137], incana and glaberrima = [0.029,
391 0.117], and incana and polymorpha = [0.051, 0.079] on young and old substrates, respectively;
392 (Stacy et al., 2014) likely explains the low degree of spectral overlap observed in the SSI.
393 Newellii populations are significantly diverged from each other due to genetic drift (Stacy et al.
394 2014), and individuals included in this study originated from different populations. Structural

flexibility reduces drag in water and is a common adaptation in plants contending with flowing water (Dittrich et al., 2012). As lignin adds rigidity to foliage (dos Santos Abreu et al., 1999), the low lignin observed in leaves of newellii is consistent with adaptation of this variety to high river discharge events (Ekar et al. 2019) as well as the lignification suppression observed in the roots of flood-stressed soybeans (Komatsu et al., 2010). The relatively high total N in newellii leaves may indicate that newellii has higher protein concentration than the other varieties. While some riparian plants use specialized proteins to withstand flooding events (Xue et al., 2020), this has yet to be investigated in newellii. Further, reflectance of visible light was greatest for newellii, which may be a means of photoprotection. Newelli leaves, like those of glaberrima which had the second highest reflectance in the visible, are typically glabrous and therefore do not self-shade via pubescence.

Spectral Patterns in Hybrids

Patterns of spectra and leaf traits varied across the four F1 genotypes. The glaberrima-incana, incana-polymorpha, and newellii-polymorpha F1s largely showed levels intermediate to those of the parent varieties, whereas the glaberrima-polymorpha F1s did not. Leaf traits of glaberrima-polymorpha often ranged higher or lower than those for either parent, although few of the differences were significant. Interestingly, these same patterns match those observed in the phenotypes of 2-year-old seedlings of these same four F1 genotypes, which were intermediate for all F1s except glaberrima-polymorpha (Stacy et al., 2016, unpub. data).

Phylogenetic signal in reflectance spectra has been demonstrated in multiple genera (Blonder et al., 2020; Cavender-Bares et al., 2016; Czyż et al., 2020; Madritch et al., 2014; McManus et al., 2016; Meireles et al., 2020). Here, we show inheritance patterns of reflectance

spectra in intraspecific F1 hybrids of *M. polymorpha*. Through this study, we hope to understand the applicability of imaging spectroscopy in classifying hybrids in landscape-wide mapping efforts. Of the four F1 genotypes included in this study, only glaberrima-incana and incana-polymorpha were separable from one of the parent varieties using leaf spectra. In the case of glaberrima-incana, spectra could distinguish the hybrid from glaberrima, whereas individual leaf traits could not. For this hybrid and its parents, leaf traits were not distinct enough to discriminate the genotypes. For the other F1 genotypes, at least one of the leaf traits differed significantly between the hybrid and one parent variety. These results indicate that classifying hybrids using airborne imaging spectroscopy may be possible with an increased sample size but will likely require both PCA and leaf trait estimations from spectral data to capitalize on all the information present in the data.

Comparing Populations across Islands

We assessed whether spectra of *M. polymorpha* var. *incana* from islands of differing ages are differentiable and consistent with their contrasting environments. As found in other studies of conspecific populations sampled across a broad spatial scale (Cavender-Bares et al., 2016; Madritch et al., 2014), we found that the populations of incana from Hawaii Island and Oahu had distinct spectral signatures. Further, the SSI indicated that Oahu incana were more distinct spectrally than any of the Hawaii varieties. These results are consistent with a higher genetic similarity of populations within islands than among islands (Choi et al., 2021; Percy et al., 2008; Stacy & Sakishima, 2019). While the spectra of Oahu and Hawaii Island incana are separable, they share a characteristic shape in the CV between 500 and 750 nm. This shape was also present in all incana hybrids and includes a rounded peak around the red wavelengths as well as a sharp

peak at the red edge. As the red edge defines the inflection point between red and infrared and has been linked to chlorophyll content, mesophyll structure, and leaf water content (Collins, 1978; Horler et al., 1983), it is likely that variability patterns for one or all of these traits are present in incana and inherited by incana hybrids. Within-variety variation of leaf spectra was greater for Oahu incana, which may be due to the weaker purifying selection there relative to that on new lava flows on volcanically active Hawaii Island, the relatively narrow sampling of Hawaii Island incana in this study, or simply the older age of Oahu. Oahu, being approximately 3 million years older than Hawaii Island, has more available nitrogen in its soils (Vitousek et al., 1997), which results in greater trait variability (Asner et al., 2016; Ordoñez et al., 2009) and therefore spectral variability (Seeley et al., *unpublished*).

Conclusion

Using the highly variable, landscape-dominant tree species, *M. polymorpha*, grown in a common garden on Hawaii Island, we used leaf reflectance spectra and derived leaf traits to distinguish four ecologically diverged varieties and their hybrids with varying degrees of success. Further, we discussed the possible associations between the reflectance spectra and leaf trait data with local adaptation of the four varieties to their respective environments. The intersection of genetic analyses and geographical information systems (GIS) has been important in informing biogeographical research and conservation decisions that seek to protect genetic diversity (Koskela et al., 2013; Zonneveld et al., 2012); however, spatial genetic data of forests are limited. This study demonstrates that reflectance spectra can discriminate genotypes of *M. polymorpha,* suggesting that while the varieties and hybrids can be spatially mapped using airborne imaging spectroscopy, further investigations are necessary to determine if the resolution

464 from canopy-level data will be stronger or weaker relative to leaf-level data (Jacquemoud et al.,
465 2009; Jacquemoud & Baret, 1990). As we plan for the use of imaging spectroscopy in
466 biodiversity studies, the *M. polymorpha* model system will help us incorporate genetic variation
467 rather than land-cover or morpho-taxonomic variation and pattern into conservation science and
468 management.

Data Availability

The datasets generated during and/or analysed during the current study will be made available on Figshare upon acceptance of the manuscript.

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765

Tables

Table 1: *M. polymorpha* varieties and F1 hybrids used in this study. Varieties are not highlighted, while hybrids are highlighted. Island of origin is noted, though all individuals were grown in a greenhouse/common garden on Hawaii Island. Groupings used to assess separability with principal component analysis and analysis of variance are noted in columns three through eight with "X" denoting membership in each grouping. Six plants per variety/F_1 hybrid were included in each grouping.

Island	Variety/F_1 hybrid	Hawaii Island Varieties	GI Hybrid	IP Hybrid	NP Hybrid	GP Hybrid	Inter-island
Hawaii	glaberrima	X	X			X	
	incana	X	X	X			X
	newellii	X			X		
	polymorpha	X		X	X	X	
	glaberrima-incana		X				
	incana-polymorpha			X			
	newellii-polymorpha				X		
	glaberrima-polymorpha					X	
Oahu	incana						X

Table 2: Results showing the statistical separability of *M. polymorpha* varieties using spectra. Pairwise Tukey results of significant PC axes according to the ANOVA are displayed. ANOVA p-value is presented in column one. The genotypes being compared in the pairwise Tukey are listed in columns two and three. Following this, their mean difference, adjusted p-value (P-adj), and their lower and upper bounds are presented. The second to last column (Reject H_0) indicates whether the null hypothesis that the two genotypes do not differ along the listed PC is rejected. Variety pairs differentiable according to Tukey's tests are highlighted. See Fig. SI 3 for data plotted in PC space and PC loadings across VSWIR spectra.

ANOVA p-value	Genotype 1	Genotype 2	Mean Difference	P-adj	Lower	Upper	Reject H_0
Principal Component 1							
p-value = 0.003	glaberrima	incana	-3.5	0.109	-7.6	0.6	FALSE
	glaberrima	newellii	0.7	0.9	-3.4	4.8	FALSE
	glaberrima	**polymorpha**	**-4.7**	**0.020**	**-8.8**	**-0.6**	**TRUE**
	incana	**newellii**	**4.2**	**0.044**	**0.1**	**8.3**	**TRUE**
	incana	polymorpha	-1.2	0.825	-5.3	2.9	FALSE
	newellii	**polymorpha**	**-5.4**	**0.007**	**-9.5**	**-1.3**	**TRUE**
Principal Component 5							
p-value = 0.003	glaberrima	incana	-0.1	0.9	-0.6	0.4	FALSE
	glaberrima	**newellii**	**0.6**	**0.018**	**0.1**	**1.1**	**TRUE**
	glaberrima	polymorpha	0.4	0.112	-0.1	0.9	FALSE
	incana	**newellii**	**0.7**	**0.006**	**0.2**	**1.1**	**TRUE**
	incana	**polymorpha**	**0.5**	**0.043**	**0.0**	**1.0**	**TRUE**
	newellii	polymorpha	-0.2	0.789	-0.7	0.3	FALSE

Table 3: Within-variety spectral similarity of samples on Hawaii Island and Oahu. Results of mean spectral similarity index (SSI) calculated according to Equation 2. For each spectral channel, the summed standard deviation was divided by the difference between means. These results were summed across the VSWIR spectra and divided by the total number of channels. SSI denotes spectral similarity of the mean spectra and spectral variance between the indicated variety and all varieties on Hawaii Island (and Oahu). Lower values indicate less spectral overlap.

Island	Variety	SSI
Hawaii	glaberrima	9
	incana	11
	newellii	7
	polymorpha	29
Oahu	incana	4

Table 4: Results showing the statistical separation of *M. polymorpha* F1 hybrids and their parent varieties. Each grouping had only one significant principal component (PC) according to ANOVA. The results of the Tukey's pairwise test for these PCs are shown. For each genotype pairing (columns two and three), their mean difference, adjusted p-value (P-adj), and lower and upper bounds are presented. The final column indicates whether the null hypothesis that the genotype pairings do not differ is rejected. See Fig. SI 5 for samples plotted in PC space.

ANOVA p-value	Genotype 1	Genotype 2	Mean Difference	P-adj	Lower	Upper	Reject H_0
glaberrima-incana Principal Component 1							
p-value = 0.001	**glaberrima**	**glaberrima-incana**	**-2.9**	**0.047**	**-5.9**	**0.0**	**TRUE**
	glaberrima	**incana**	**-5.2**	**0.001**	**-8.1**	**-2.3**	**TRUE**
	incana	glaberrima-incana	-2.2	0.150	-5.1	0.7	FALSE
incana-polymorpha Principal Component 3							
p-value = 0.03	**incana**	**incana-polymorpha**	**1.4**	**0.0363**	**0.1**	**2.7**	**TRUE**
	incana	polymorpha	1.2	0.0791	-0.1	2.5	FALSE
	polymorpha	incana-polymorpha	-0.2	0.9	-1.5	1.1	FALSE
newellii-polymorpha Principal Component 1							
p-value = 0.03	newellii	newellii-polymorpha	-3.1	0.2101	-7.7	1.4	FALSE
	newellii	**polymorpha**	**-5.4**	**0.0202**	**-10.0**	**-0.8**	**TRUE**
	polymorpha	newellii-polymorpha	-2.3	0.4263	-6.9	2.3	FALSE
glaberrima-polymorpha Principal Component 1							
p-value = 0.01	glaberrima	glaberrima-polymorpha	3.1	0.102	-0.5	6.8	FALSE
	glaberrima	**polymorpha**	**4.9**	**0.01**	**1.2**	**8.5**	**TRUE**

	polymorpha	glaberrima-polymorpha	1.7	0.452	-1.9	5.4	FALSE

797

798 **Figures**

799 **Figure 1:** (a) Mean brightness-normalized reflectance (represented as a percentage) and (b)
800 coefficient of variation (CV) of reflectance values for the four *M. polymorpha* Hawaii Island
801 varieties and Oahu incana. See Fig. SI 2 for reflectance prior to brightness-normalization. (c)
802 Spectral separability of all Hawaii Island and Oahu genotypes. Spectral separability was
803 calculated for each wavelength (Eq. 1). Higher values indicate less spectral overlap. See Table 3
804 for average SSI values.

805 **Figure 2:** Boxplots of leaf traits for the Hawaii Island varieties glaberrima (G), polymorpha (P),
806 newellii (N), and incana (I) as well as Oahu incana (OI). Hawaii Island varieties with traits that
807 differed at a significance of $p < 0.05$ as determined by ANOVA and Tukey HSD are noted with
808 an asterisk. Incana from Hawaii Island and Oahu were also compared using ANOVA, and
809 significant differences are likewise noted with an asterisk. Boxplots denote quartile ranges, with
810 the lower and upper bounds of the box indicating the 25^{th} and 75^{th} percentile. Middle lines in the
811 box represent the median of the data, and the whiskers end at the group minimum and maximum.
812 Outliers are shown as points.

813 **Figure 3:** Mean brightness-normalized reflectance (represented as a percentage) of a) the F1
814 hybrids glaberrima-incana (GI) and its parents, glaberrima (G) and incana (I); b) the hybrid
815 incana-polymorpha (IP) and its parents, incana (I) and polymorpha (P); the hybrid newellii-
816 polymorpha (NP) and its parents, newellii (N) and polymorpha (P); d) the hybrid glaberrima-
817 polymorpha (GP) and its parents, glaberrima (G) and polymorpha (P). See supplementary
818 information Figure SI 4 for CV of F1 hybrids.

819 **Figure 4:** Boxplots of nine leaf traits for all F1 hybrids measured and their parents. The left
820 figure (a) represents glaberrima (G), the F1 hybrid glaberrima-incana (GI), incana (I), the hybrid

incana-polymorpha (IP), and polymorpha (P). The right figure (b) displays newellii (N), the hybrid newellii-polymorpha (NP), polymorpha (P), the hybrid glaberrima-polymorpha (GP), and glaberrima (G). Genotypes with traits that differed at a significance of $p < 0.05$ as determined by ANOVA and Tukey HSD are noted with an asterisk. Only groups of the F1 hybrid and their parents (Table 1) were compared using ANOVA and Tukey HSD. None of the traits differed significantly among GI, I, and G according to ANOVA.

Figure 1

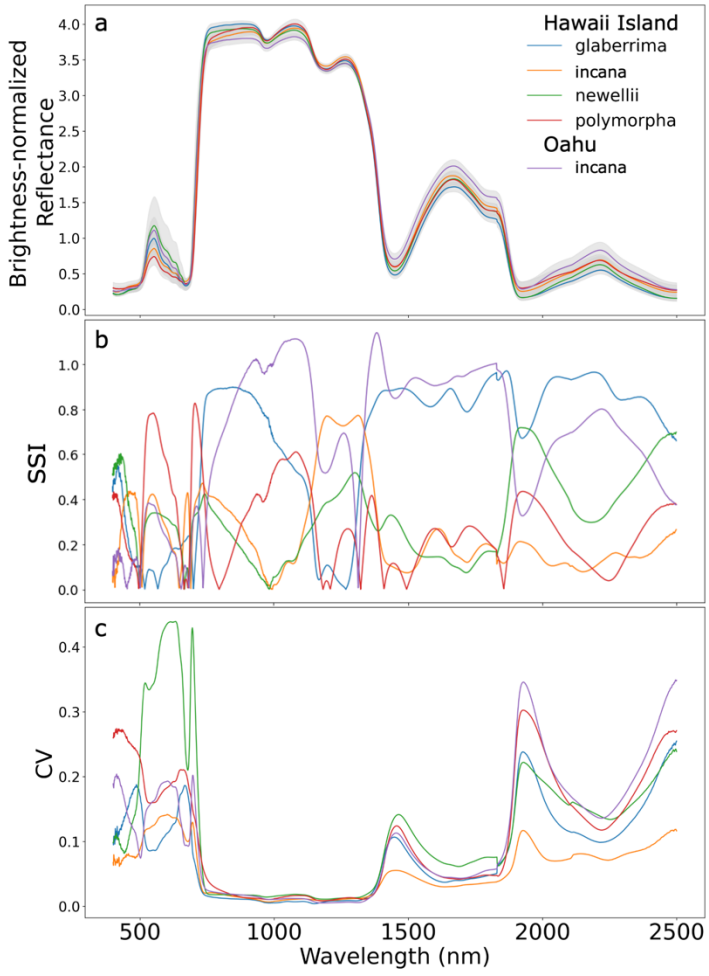
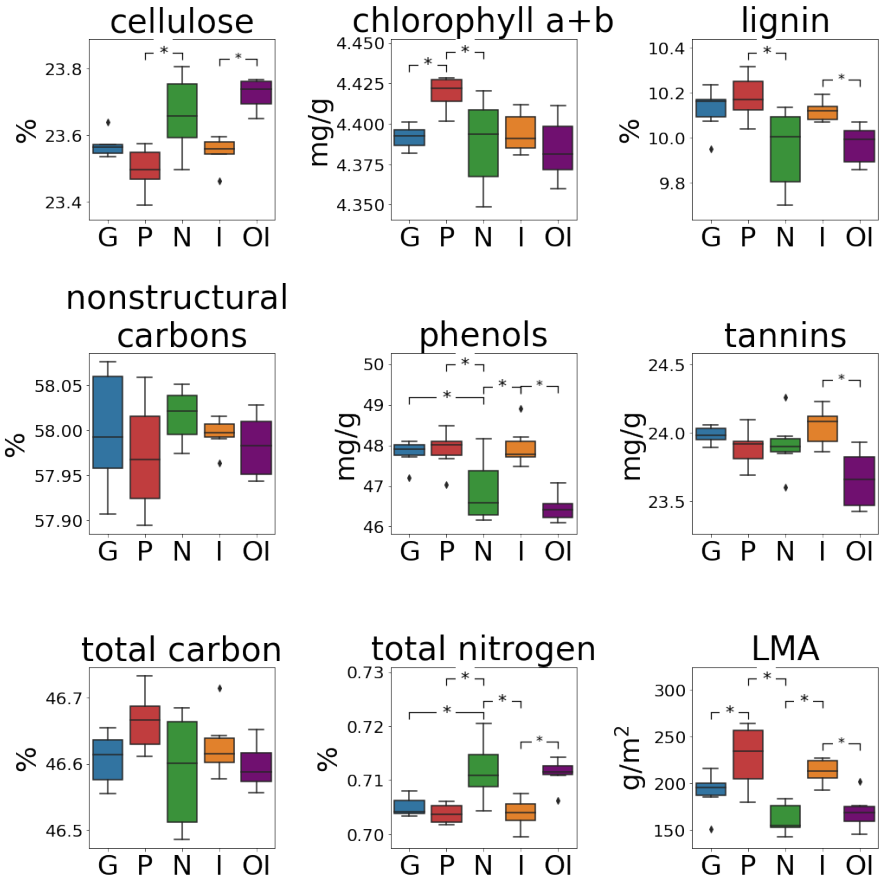


Figure 2


cellulose
%
23.8
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chlorophyll a+b
mg/g
4.450
4.425
4.400
4.375
4.350
lignin
%
10.4
10.2
10.0
9.8
nonstructural carbons
%
58.05
58.00
57.95
57.90
phenols
mg/g
50
49
48
47
46
tannins
mg/g
24.5
24.0
23.5
total carbon
%
46.7
46.6
46.5
total nitrogen
%
0.73
0.72
0.71
0.70
LMA
g/m2
300
250
200
150
G P N I OI

833 **Figure 3**

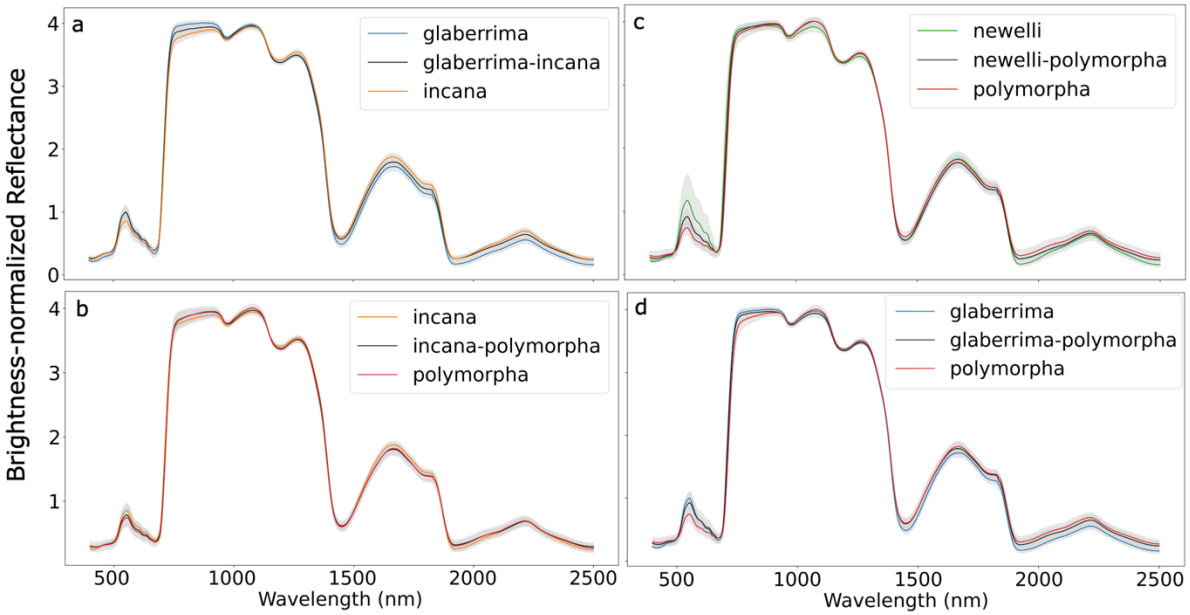

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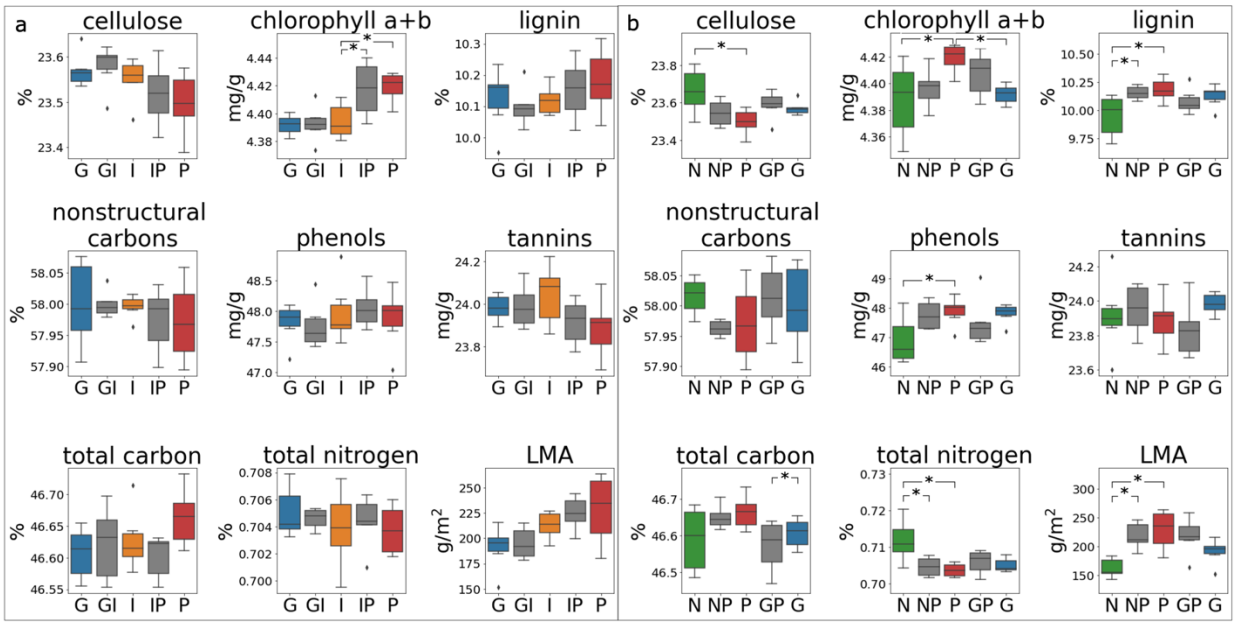
Figure 4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