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Leaf chemical and spectral diversity in Australian tropical forests

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Abstract. Leaf chemical and spectral properties of 162 canopy species were measured at 11 tropical forest sites along a 6024 mm precipitation/yr and 8.7°C climate gradient in Queensland, Australia. We found that variations in foliar nitrogen, phosphorus, chlorophyll *a* and *b*, and carotenoid concentrations, as well as specific leaf area (SLA), were expressed more strongly among species within a site than along the entire climate gradient. Integrated chemical signatures consisting of all leaf properties did not aggregate well at the genus or family levels. Leaf chemical diversity was maximal in the lowland tropical forest sites with the highest temperatures and moderate precipitation levels. Cooler and wetter montane tropical forests contained species with measurably lower variation in their chemical signatures. Foliar optical properties measured from 400 to 2500 nm were also highly diverse at the species level, and were well correlated with an ensemble of leaf chemical properties and SLA ($r^2 = 0.54$–0.83).

A probabilistic diversity model amplified the leaf chemical differences among species, revealing that lowland tropical forests maintain a chemical diversity per unit richness far greater than that of higher elevation forests in Australia. Modeled patterns in spectral diversity and species richness paralleled those of chemical diversity, demonstrating a linkage between the taxonomic and remotely sensed properties of tropical forest canopies. We conclude that species are the taxonomic unit causing chemical variance in Australian tropical forest canopies, and thus ecological and remote sensing studies should consider the role that species play in defining the functional properties of these forests.

Key words: biological diversity; chemical diversity; imaging spectroscopy; leaf chemistry; leaf optical properties; lowland tropics; Queensland, Australia; rain forest.

Introduction

Leaf chemical properties are key determinants of plant physiology and biogeochemical cycling in ecosystems (Vitousek and Sanford 1986, Reich et al. 1997), yet these properties remain poorly understood in the tropics. Canopy chemistry is of particular interest in tropical forests because (1) canopy species often dominate carbon storage as well as energy balance and water use (Phillips et al. 1998, Clark et al. 2004); (2) the diversity of canopy species is often proportional to total plant diversity (Orians et al. 1996, Lawton et al. 1998); and (3) the diversity of insect and animal life is causally linked to tree diversity (Janzen 1970, Wilson 1992). Given that canopy chemistry is intimately tied to all of these processes, it is surprising that so little is known about the chemical diversity of tropical forests. Our deficient knowledge probably results from a combination of factors including high species diversity, tall, inaccessible trees, and difficult study conditions.

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A recent compilation by Townsend et al. (2007) showed that foliar nitrogen (N) and phosphorus (P) variability may be as high locally in tropical forests as it is globally across biomes. Leaf N and P are critical unknowns in predicting ecological processes, but so are many other properties of foliage. Although N and P are good predictors of basic biogeochemical cycling in forests, multiple pigments are important determinants of light capture, protection from high radiation, and regulation of photosynthetic functions (Björkman and Demmig-Adams 1995, Evans et al. 2004). Leaf water is an important indicator of canopy thermal regulation and moisture stress, which can be particularly acute in tropical systems (Williamson et al. 2000, Nepstad et al. 2002). Specific leaf area (SLA; cm^2/g) is a leaf structural property linked to the entire constellation of leaf chemicals, and leaf photosynthetic processes scale with SLA (Evans and Poorter 2001, Wright et al. 2004, Niinemets and Sack 2006). The combination of foliar N, P, pigments, water, and SLA makes an important contribution to plant canopy and whole-system function (Field and Mooney 1986, Evans 1989, Reich et al. 1997), and yet we know little about these combinations among the myriad tropical forest species throughout the world.

A portfolio of leaf chemicals will not only allow the prediction of the functional attributes of a species within the forest, but also is likely to determine the role that each species plays in ecosystem-level responses to land use and climate change. It seems probable that species maintain unique chemical "signatures" that would leave a canopy imprint on the soil and other biogeochemical compartments, or vice versa (John et al. 2007, Townsend et al. 2007). However, we currently do not know enough about the taxonomic variability in chemical signatures to forecast ecosystem-level responses to environmental change, or to incorporate the detailed chemistry of plants into biogeochemical models. Our knowledge, and thus our models, require additional observations of leaf and canopy chemistry at landscape to regional scales, but few are available for tropical forests.

Past work suggests that the chemical properties of plant foliage can be assessed using airborne imaging spectroscopy, also known as hyperspectral imaging (Wessman et al. 1988, Martin and Aber 1997, Smith et al. 2003). While linkages between spectroscopic measurements and foliar chemicals continue to expand and improve (Horler et al. 1983, Lee et al. 1990, Curran et al. 1992, Ceccato et al. 2001, Kokaly 2001), only very recent work suggests that the chemical variance across a landscape, derived from airborne imaging spectroscopy, may allow for mapping of taxonomic diversity among canopy species. Early steps in this effort show that just a handful of key chemicals can express the presence of species and/or plant functional types (Asner and Vitousek 2005, Asner et al. 2008), and even the number of species per area (richness) in tropical forests (Carlson et al. 2007). Despite these early steps, we do not know how chemical signatures are expressed taxonomically: whether species, genera, or families are unique. Past studies of leaf chemical and spectral variations have included a number of tropical species (Lee et al. 1990, Roberts et al. 1998, Castro-Esau et al. 2004, Zhang et al. 2006), but systematic analyses across plant genera and families are very rare (Castro-Esau et al. 2006). No studies have considered taxonomic variation in leaf optical properties in the context of multi-chemical signatures among species.

Here we report on a study to quantify taxonomic variation of leaf chemical signatures among canopy species found across an elevation and substrate gradient in humid tropical forests of Queensland, Australia. In step with the chemical assays, we also quantified leaf spectral properties of the tropical forest taxa, and explored the causal linkages between the biochemical and spectroscopic properties of species. We ask: (1) Do tropical forest canopy species have unique foliar chemical signatures, and if so, at what taxonomic level of aggregation do plants express these signatures? (2) Do foliar spectral properties of species track their multi-chemical signatures? (3) How variable are chemical and spectral signatures at the site level, and do these signatures track species richness?

Materials and Methods

Study sites and sampling design

Our study was conducted at 11 tropical forest sites located across Queensland, Australia (Table 1). The sites range in elevation from 18 m to 1556 m above sea level, with a concomitant range in mean annual temperature of 15.8–24.5°C. Annual precipitation ranges from 1313 to 7337 mm/yr across the sites, and substrates vary from basalts to granites and meta-sediments. For our purposes, we partitioned the sites into lowland (<100 m), submontane (700–1000 m), and montane (>1000 m) groups. This partitioning is somewhat arbitrary, but it conforms to typical classifications of Australian tropical forests throughout the region (Webb and Tracey 1981, Nightingale et al. 2008).

This study focused on quantifying interspecific and site-level variation in the foliar chemical and spectral properties of forest canopy species. We therefore not only designed our sampling strategy to span a large range of site conditions shown in Table 1, but also compiled a taxonomically diverse data set spanning the sites that included 51 families, 121 genera, and 162 species (Appendix B). A broad sampling across families and genera was an important axis of taxonomic variability, but we also collected multiple species from nine families: Cunoniaceae ($n = 8$), Elaeocarpaceae ($n = 8$), Lauraceae ($n = 9$), Meliaceae ($n = 8$), Myrtaceae ($n = 17$), Proteaceae ($n = 12$), Rutaceae ($n = 7$), Sapindaceae ($n = 7$), and Sapotaceae ($n = 4$). This provided a means to quantify chemical and spectral variation at different levels of taxonomic aggregation.

At each of the 11 field sites, we identified all common canopy tree species, verifying identities when necessary at the herbarium at the CSIRO Tropical Research Centre, Atherton, Queensland, Australia (herbarium code QRS). Although we probably missed very rare species, each collection was designed to obtain all common canopy species, genera, and families at a site representing ~1 ha of tropical forest. The species selected for collection were found in closed-canopy conditions, and only fully sunlit portions of the uppermost tree crowns were selected for foliage collection. A shotgun or slingshot/line was used to remove 3–5 branches and twigs containing sunlit foliage.

Biochemistry.—Our leaf assays were designed to encompass the major chemical properties known to control or contribute to the physiology of species and biogeochemical cycles at the ecosystem level. We selected chlorophyll *a* and *b* (Chl *a*, Chl *b*), total carotenoids (Car) anthocyanins (Anth), leaf water concentration, total N and P, and SLA as the ensemble of parameters to measure. Each of these leaf parameters also has a demonstrated contribution to the spectroscopy and thus optical remote sensing of canopies (Curran 1989, Ustin et al. 2004), the second focus of analysis in our study. Adding other chemicals would further diversify the chemical portfolio of each species, but

TABLE 1. Description of the 11 rain forest research sites visited for foliar spectroscopy and chemistry.

Site	Latitude (S)	Longitude (E)	Elevation (m m.s.l.)	Substrate category	MAP (mm)	PDQ (mm)	MAT (°C)	*n*
Lowland								
Oliver Creek	16°8′16″	145°26′27″	18	meta-sediments	3551	226	24.5	22
Daintree	16°6′12″	145°27′48″	51	meta-sediments	3961	273	24.3	16
Mt. Bellenden Ker, base	17°16′15″	145°51′1″	80	granite	4032	313	23.5	6
Submontane								
Topaz	17°25′43″	145°42′8″	722	basalt	3035	262	20.1	18
CSIRO	17°16′32″	145°29′5″	782	basalt	1313	69	19.8	19
Windsor Tablelands	16°16′19″	145°4′49″	870	granite	1892	120	19.3	12
Mt. Lewis	16°33′55″	145°17′39″	999	granite	2323	168	18.4	12
Montane								
Windsor Tablelands	16°15′9″	145°2′22″	1092	granite	2172	153	18.4	13
Tully Falls National Park	17°41′52″	145°32′8″	1104	basalt	2230	175	18.3	12
Mt. Lewis	16°31′39″	145°16′10″	1248	granite	2323	168	18.4	15
Mt. Bell. Ker, summit	17°16′50″	145°51′15″	1556	granite	7337	729	15.8	10

Note: Unit abbreviations are m.s.l., height above mean sea level; MAP, mean annual precipitation; PDQ, precipitation of the driest quarter; MAT, mean annual temperature; *n*, number of tree species sampled per site.

doing so might create a chemical combination that does not aid in determining the spectroscopic signatures of the plants, and thus would not be remotely sensible.

For each species identified in the field, leaf discs (six per leaf) were immediately taken from 10 randomly selected leaves and frozen on dry ice in the field. They were later transferred to a −80°C freezer until pigment analyses were performed in a laboratory. Frozen leaf discs (1.1 cm^2 area) were ground in a chilled mortar with 100% acetone, a small amount of quartz sand, and $MgCO_3$ to prevent acidification. Following centrifugation for three min at 3000 rpm, the absorbance of the supernatant was measured using a dual-beam scanning UV-VIS spectrophotometer (Lambda 25, Perkin Elmer, Beaconsfield, UK). Chl *a*, Chl *b*, and Car were determined using multiwavelength analysis at 470, 645, 662, and 710 nm (Lichtenthaler and Buschmann 2001). Anthocyanins were measured in a similar manner, but an acidified methanol solution (MeOH:HCl:H_2O 90:1:1 by volume) was substituted for acetone, and anthocyanin concentration was determined from the absorbance at 529 nm (Sims and Gamon 2002).

At each field site, an additional subsample of 25–50 leaves was placed in a polyethylene bag and kept cool for up to 6 h until the leaves were processed for fresh mass and scanned for leaf area based on Martin et al. (2007). Leaf subsamples were selected to represent the range of colors and conditions found among all leaves collected. Occasionally, epiphylls were encountered, but never in high abundance, and those found were removed prior to analyses. Leaf samples were then dried at 70°C for at least 72 h, and weighed to determine leaf H_2O concentration [(fresh mass – dry mass)/dry mass] and SLA. Dried leaves were ground in a 20-mesh Wiley mill, and subsets were analyzed for N and P concentration using a Kjeldahl sulfuric acid/cupric sulfate digest (Jones 1987). Digests were analyzed using an Alpkem colorimetric autoanalyzer (O-I Analytical, College Station, Texas, USA).

Spectroscopy.—Hemispherical reflectance and transmittance from 400–2500 nm was measured on each leaf taken for pigment analysis. The measurements were made immediately after detaching the leaf from each branch at the field site. The measurements were collected with a field spectrometer using 1.4-nm sampling (FR-Pro with Select Test custom detectors; Analytical Spectra Devices, Boulder, Colorado, USA), an integrating sphere modified for high-resolution spectroscopic assays (Labsphere, Durham, New Hampshire, USA), and a custom illumination collimator that we built. The spectra were then calibrated for stray light, and referenced to a calibration block in the integrating sphere (Asner 1998).

Statistical analyses.—We carried out five types of analyses on the chemical and spectral data to (1) quantify site- and species-specific differences in foliar chemistry; (2) quantify the covariances between foliar properties; (3) determine whether tropical forest canopy species have unique foliar chemical signatures, and if so, whether the chemical signatures are aggregated by genus, family, or site; (4) quantify the absolute and relative contributions of each chemical constituent to the spectral properties of the foliage; (5) explore the potential use of hyperspectral data to classify species by spectral properties; and (6) test our ability to simulate taxonomic diversity using chemical and spectral data.

First, we explored the basic statistical differences among chemicals and spectral properties by site and by plant family. All groups were compared using ANOVA with Tukey multiple comparisons on the log-transformed values, to satisfy assumptions of data normality. Second, we defined and then compared the combined chemical signature (α) among species using a standardization approach by which each chemical (x_{ij}) was normalized using the minimum (MIN) chemical value of

the sample population divided by the minimum value of the population:

$$\alpha = \sum X_{ij} = \sum \frac{x_{ij} - \mathrm{MIN}(x_{iJ})}{\mathrm{MIN}(x_{iJ})} \quad (1)$$

where X_{ij} is the standardized leaf property for each leaf constituent i and species j, and J is the total population of species. The standardization step ensures that each chemical will have a comparable statistical distribution.

Hierarchical cluster analysis was next used to examine the chemical as well as the spectral relationships among the 162 canopy species taken from the lowland, submontane, and montane forest sites. Cluster analysis provides a means to simultaneously use multiple plant chemical or spectral characteristics to quantitatively sort species into groups based on their degree of association (Hartigan 1975). Using this method, species cluster more closely if their chemical or spectral signatures are similar. Here, we used Ward's method of clustering (Ward 1963), where the distance between two clusters is the analysis of variance sum of squares summed over all of the variables:

$$D_{KL} = \frac{||\ \bar{\mathbf{x}}_K - \ \bar{\mathbf{x}}_L||^2}{\frac{1}{N_K} + \frac{1}{N_L}} \quad (2)$$

where D is the statistical distance between each cluster pair K and L, $\mathbf{x}_K$ and $\mathbf{x}_L$ are the mean vectors for the clusters, and N_K and N_L are the number of observations per cluster. All data were standardized by the parameter mean and standard deviation prior to analysis. This analysis produced a statistical clustering or dendrogram depicting the organization of species based on either their chemical or spectral signatures.

Following the chemical and spectral cluster analyses, we used partial least squares (PLS) regression to determine the relative contribution of each chemical constituent to the spectral signatures of the species (Smith et al. 2003). Candidate chemicals included Chl *a*, Chl *b*, Car, Anth, N, P, and H_2O, as well as SLA. The PLS approach utilizes the continuous, full-range spectrum rather than a band-by-band analysis. Spectral weightings generated by the PLS calculation directly relate the features in the spectra to the chemical constituents analyzed (Haaland and Thomas 1988). To avoid overfitting, the number of factors used in the PLS analysis was determined by minimizing the Prediction Residual Error Sum of Squares (PRESS) statistic (Chen et al. 2004). The PRESS statistic was calculated through an iterative cross-validation prediction for each model. This cross-validation procedure iteratively generates regression models (n = up to 15 iterations) while reserving one sample from the input data set until the root mean square error (RMSE) of the PRESS statistic is minimized. The PLS-PRESS models were then used to estimate each leaf chemical and SLA from the original spectral data. This provided a means to determine the relative importance of each foliar property in predicting the spectra. Both the cluster and PLS-PRESS analyses were carried out using SAS JMP 7.0 statistical software package (SAS Institute 2003).

Finally, we used a probabilistic diversity model to explore the role that leaf properties play in expressing the plant diversity in the lowland, submontane, and montane tropical forest sites. The model populates a virtual forest with species and their measured chemical and spectral signatures (Asner 2008). First, a single species is randomly selected from the total community of n species collected in the field. The mean leaf chemical and spectral information for this species is taken from the database, along with the field-measured variance to accommodate natural intraspecific variability. Other species are then randomly selected and added to the virtual landscape in the same way. As the species richness of the virtual forest increases, we track the change in the total range (maximum – minimum) of chemical or spectral values until the entire community is populated, or until a prescribed richness level is obtained. The model uses a Monte Carlo simulation to calculate an average change in the chemical and spectral variability of 1000 virtual forests as taxonomic diversity is randomly increased (mean and SD of 1000 forests with one species and its chemical variability; then 1000 forests with two species, each with its chemical variability; and so on, up to 1000 forests with all species, each with its chemical variability). The model can be run for a single chemical (e.g., N), or for a chemical signature index (α; Eq. 1) that combines any number of leaf properties per species. For this study, we simulated the forest canopies using an eight-parameter chemical signature incorporating leaf pigments, nutrients, water, and SLA from Fig. 4 applied to Eq. 1. We then repeated the analysis using the standardized spectral reflectance data to provide an optical equivalent (Λ) for analyzing taxonomic variation. We use the standardized reflectance here because it is equivalent to the standardization of the chemical index used in Eq. 1. It is important to note that the point of this modeling step was not to simulate whole canopies, as might be viewed by an airborne or satellite remote sensing instrument, but rather to compare chemical and spectral diversity relationships among canopy trees as would be encountered throughout a forest. True canopy-level simulations require radiative transfer, ray tracing, or radiosity modeling approaches (Gerstl and Borel 1992, Govaerts et al. 1995, Jacquemoud et al. 2000) that are beyond the scope of this study.

Results and Discussion

Leaf chemistry

Our foliar chemical data spanned a globally significant range of values, which is important to the overall focus of the study on chemical signature variance and chemical–spectral linkages in tropical forests. For example, our leaf N and P data ranged from 0.75% to 3.5% and 0.05% to 0.34%, respectively, which nearly

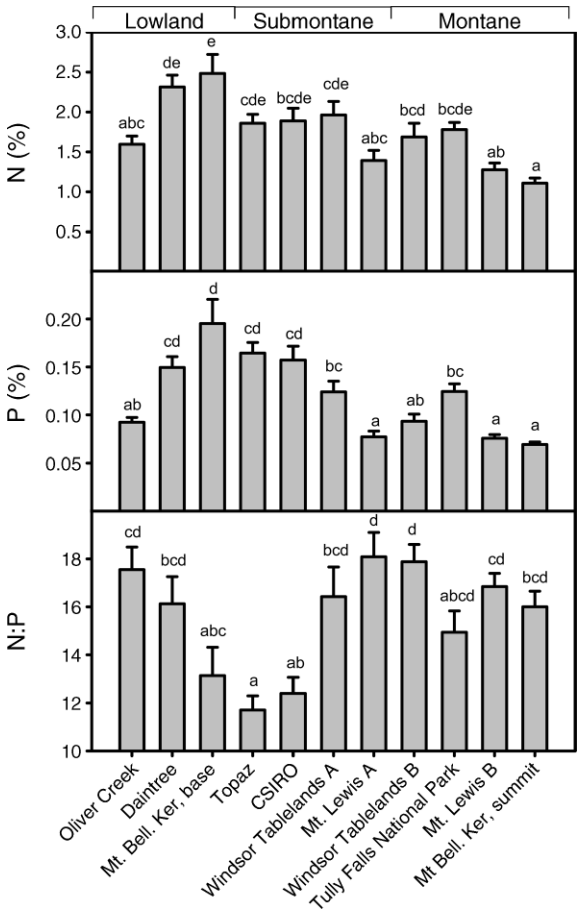

FIG. 1. Mean (+SE) leaf nitrogen (N) and phosphorus (P) concentrations, and N:P ratio, for canopy species collected across an elevation gradient from lowland to montane tropical forests throughout Queensland, Australia. Different lowercase letters denote statistical differences among groups using ANOVA with Tukey multiple-comparison tests ($P < 0.05$).

matched the global tropical forest compilation of Townsend et al. (2007). Similarly, our N:P ratios ranged from 5.6 to 31.6, which nearly encompasses most reports for tropical forests. The SLA data spanned a range of 37–274 cm^2/g, and total chlorophyll concentrations covered an unusually broad 16-fold range of values. As an ensemble of chemical properties, this Australian tropical forest data set is enormously variable from nutrient chemistry, physiological ecology, and remote-sensing perspectives (Asner 1998, McGroddy et al. 2004, Wright et al. 2004).

On a site basis, foliar N and P concentrations each varied more than twofold, and N:P ratios ranged from 11.7 ± 2.4 to 17.6 ± 4.4, mean ± SE (Fig. 1). N:P values <14 may suggest sites where N limitation is more pronounced, whereas N:P > 16 indicate possible P limitation (Hedin 2004, Reich and Oleskyn 2004). Substrate-related differences among sites were inconclusive due to high variability in leaf N and P among species within each site (Fig. 1), but a few basic differences were observed. Among lowland forests, trees in the Oliver Creek site on meta-sediment substrates had the lowest foliar N and P, whereas the granite substrate site (Mt. Bellenden Ker-base) supported the highest values. The submontane Topaz and CSIRO sites had lower N:P ratios than did the other sites in that elevation category. Foliar N and P concentrations decreased slightly, but inconsistently, with increasing elevation from about 722 to 1556 m. Overall, the imprint of different substrates and elevations was only weakly and variably expressed in the leaf N and P data.

The effects of substrate and elevation (climate) on leaf water, pigments, and SLA were also weak and inconsistent at the site level. There was a slight decrease in SLA at higher elevations, and total chlorophyll and carotenoids roughly followed this pattern (Fig. 2). SLA, water, total chlorophyll, and carotenoids peaked in the lowland forest sites at Daintree and Mt. Bellenden Ker, base. However, most patterns were not significant, and leaf water and anthocyanin pigments were even more decoupled from the SLA patterns.

Regrouping the chemical and SLA data among nine plant families found at multiple (or all) forest sites, we found that N varied more between families than it did across all sites combined (Appendix A: Fig. A1, compared to Fig. 1). Similarly, P variability among families nearly matched that of the variation among the 11 forest sites; however, variations in N:P ratios were more conservative at the family level. Species in the Meliaceae had particularly high N and P concentrations in their leaves, whereas the Rutaceae displayed a high N:P ratio (Appendix A: Fig. A1). Plants in both the Meliaceae and Rutaceae had high values for nearly all pigment concentrations and SLA (Appendix A: Fig. A2). In contrast, species in the Proteaceae had the highest concentrations of anthocyanins. Together, these data demonstrate that basic chemical variability at the family level can be as high as or higher than it is across extremely broad environmental gradients of more than 6000 mm rainfall and nearly 9°C in mean annual temperature. This conclusion supports the study by Townsend et al. (2007), showing that variation in N:P ratio is as high taxonomically as it is geographically in Brazil and Costa Rica. Our measurements extend such findings to include a portfolio of pigments, water, and SLA, all of which are key to understanding controls over functional and spectral properties of tropical forest species.

Linear regression analyses showed relatively poor to moderate correlations among most of the leaf chemicals and SLA (Fig. 3). The obvious exception was the tight relationship between Chl *a* and Chl *b* ($r^2 = 0.96$), which was expected given the functional link between these two pigments (Anderson et al. 1995). In addition, carotenoid concentrations were correlated with Chl *a* and Chl *b* ($r^2 = 0.77$, 0.82) and N ($r^2 = 0.61$) concentrations, although it is notable that a number of outliers could readily be identified, even in these highly correlated relationships.

Although N and P are often considered to be linked in temperate ecosystems (Reich and Oleskyn 2004), they were only moderately correlated in the tropical forest trees in our study ($r^2 = 0.54$; Fig. 3).

The standardized leaf chemical signatures revealed enormous variation among species at site and regional scales (Fig. 4). The color codes show the relative levels of leaf constituent concentrations and SLA for each species, here again indicating no obvious covariances among leaf parameters other than for Chl *a* and *b*. The lowland forests showed the greatest degree of variability, with, for example, SLA spanning a nearly fivefold range of relative values among canopy species. Moreover, leaf pigment concentrations were up to three times more variable than either N or P, indicating their contribution to the uniqueness of the chemical signatures among canopy species.

The chemical index (α) provided a convenient marker to further compare species within and across sites (Fig. 4). Variance in α values increased from 6.0 to 8.6 and to 17.9 for montane, submontane, and lowland forests, respectively. In fact, the largest chemical variation was evident just within the lowland forest site at Daintree: from the lowest, $\alpha = 5.0$ in *Argyrodendron peralatum*, to the highest, $\alpha = 27.6$ in *Dysoxylum papuanum*. These results suggest that chemical diversity increases in warmer environments, probably reflecting a wider variety of chemical and physiological strategies among species.

Within families collected from multiple sites, the Sapindaceae and Sapotaceae showed relatively low chemical variance among 10 species (Appendix A: Fig. A3). However, two plant families spanning all 11 forest sites (Elaeocarpaceae and Myrtaceae) displayed high variability among chemical signatures of 25 species. At times, chemical diversity was extremely high among congeners, such as *Dysoxylum* in the Meliaceae, which expressed a range in α of nearly 300% among just six species. In sum, the chemical diversity of lowland forests far exceeds that of cooler montane systems, but the chemical diversity at any given site can be dominated by a single family or even a single genus.

Leaf spectroscopy.—Summary spectral reflectance and transmittance properties of the species are shown by site in Fig. 5 and are regrouped in the nine common plant families found among sites (Appendix A: Fig. A4). At species, family, and site levels, reflectance variation was highest in the near-infrared (NIR; 700–1300 nm), whereas transmittance was most variable in the shortwave-IR (SWIR; 1500–2500 nm). The NIR spectral range is dominated by variation in leaf water content and leaf thickness, related to SLA (Jacquemoud and Baret 1990, Ceccato et al. 2001). Transmittance variation in the SWIR is caused by leaf water concentration, with important contributions from protein N, cellulose, and lignin concentrations (Curran 1989). First derivatives of the spectra in the visible region (400–700 nm) associated with chlorophyll, carotenoid, and anthocyanin pigments were also highly variable (data not shown). Similar to the individual chemical results, there were no obvious trends in the spectra taken from lowland, submontane, or montane forest sites, although the

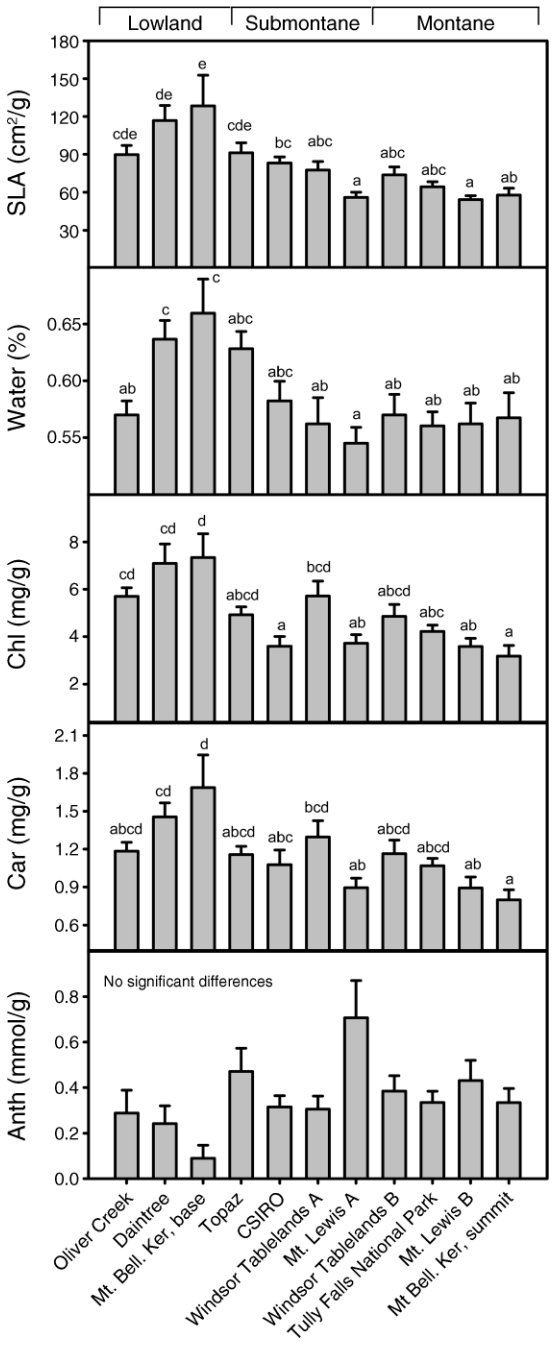

FIG. 2. Mean (+SE) specific leaf area (SLA) and water content, and total chlorophyll (Chl $a+b$), carotenoid (Car), and anthocynanin (Anth) concentrations for canopy species collected across an elevation gradient from lowland to montane tropical forests throughout Queensland, Australia. Different lowercase letters denote statistical differences among groups using ANOVA with Tukey multiple-comparison tests ($P < 0.05$).

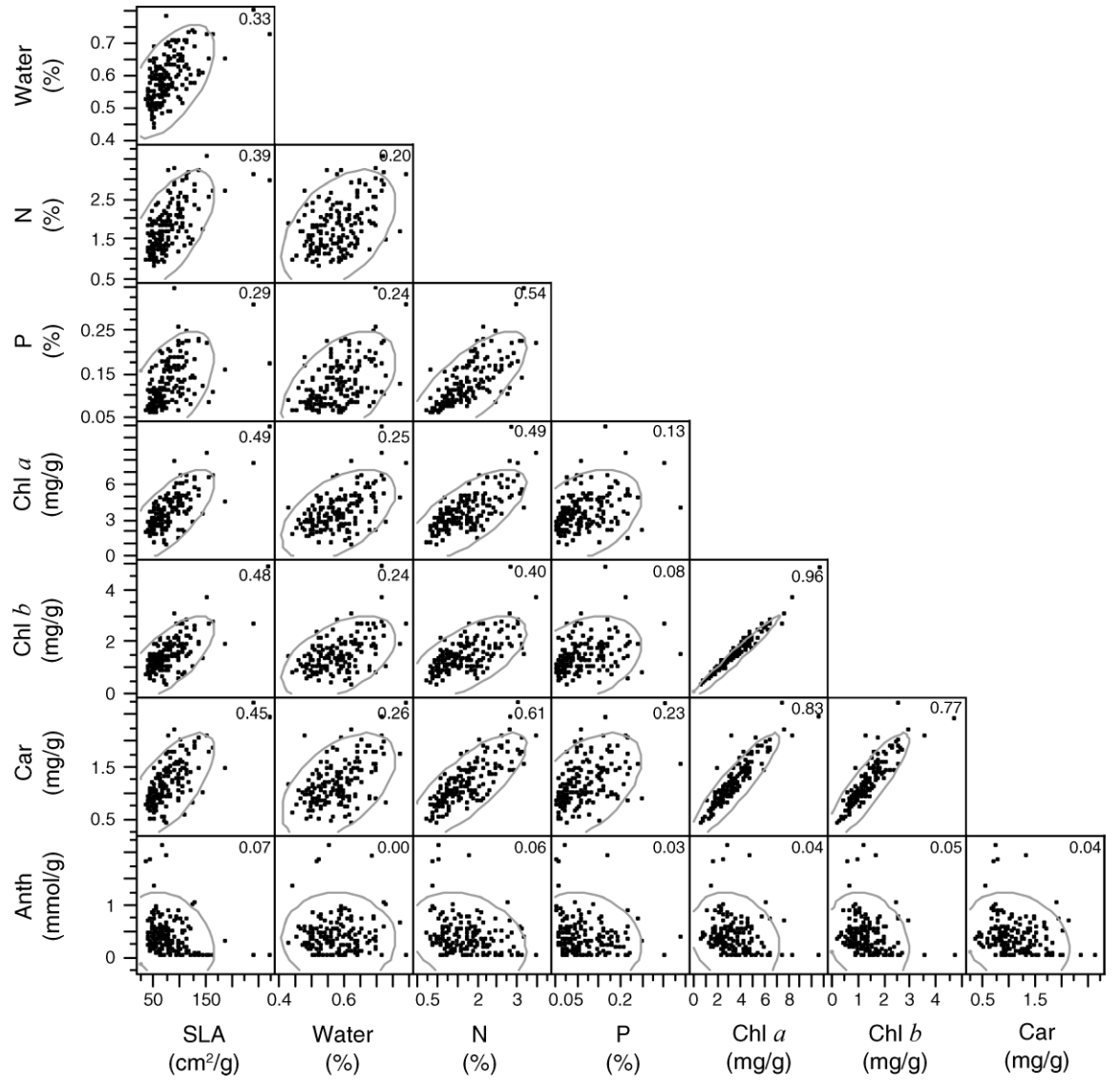

FIG. 3. Scatterplots (with regression coefficients, r^2, in upper right corner) showing relationships between leaf properties measured in Figs. 1 and 2. Gray lines represent bivariate density ellipses that enclose ~95% of the data. More closely correlated variables are contained in narrower ellipses with a stronger orientation along the diagonal.

Daintree (lowland) site showed particularly high variability in both reflectance and transmittance compared to all other sites.

PLS regression analyses revealed wavelength-specific spectral regions predicting the foliar properties among tree species (Fig. 6). Spectral reflectance weightings greater than or less than about 25 in the visible range indicate strong, but variable, contributions by Chl *a*, Chl *b*, Car, N, and P. Anthocyanins were poorly represented in the visible range, probably due to the overall low values for this pigment in the leaf material (Fig. 2). SLA was heavily weighted at very short wavelengths (<500 nm), and again in the NIR, but the most important spectral region for predicting SLA was the SWIR (>1500 nm). In this range, small changes in leaf thickness, and thus path length of light travel through the leaf material, cause important variations in leaf reflectance (Jacquemoud and Baret 1990). N, P, and most pigments followed SLA in predicting the SWIR reflectance among species. Similarity in the SWIR spectral weightings for these chemicals results from a combination of direct chemical expression in the spectrum (Curran 1989) and broad stoichiometric covariance with SLA (Asner 2008). Nonetheless, the magnitude of the PLS reflectance weightings in the SWIR indicated that N and pigments were the most important contributors, with P, water, and anthocyanin less so.

Equations developed from PLS and cross-validation procedures demonstrated that the reflectance spectra

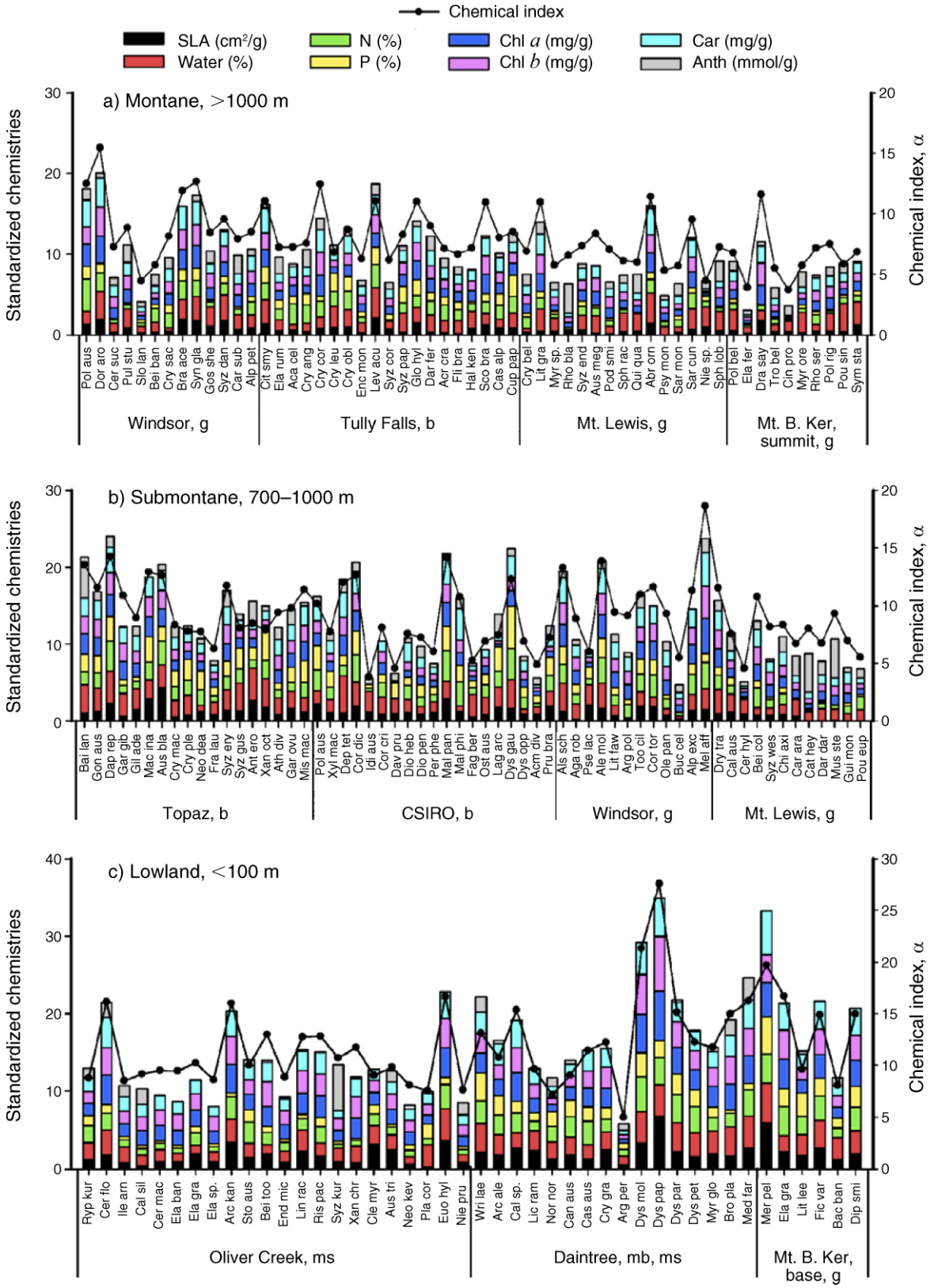

FIG. 4. Standardized chemical signatures (Eq. 1) for all canopy tree species. Data are organized by site, and then by family-genus-species with each site. See Appendix B for full species names. Sites are grouped by lowland, submontane, and montane classes, and substrate origin is denoted in letters following the site name (b, basalt; g, granite; ms, meta-sediments; mb, meta-basalts). Color bars quantitatively show differences among leaf properties. The black line and dots show a chemical index α, as shown in Eq. 1.

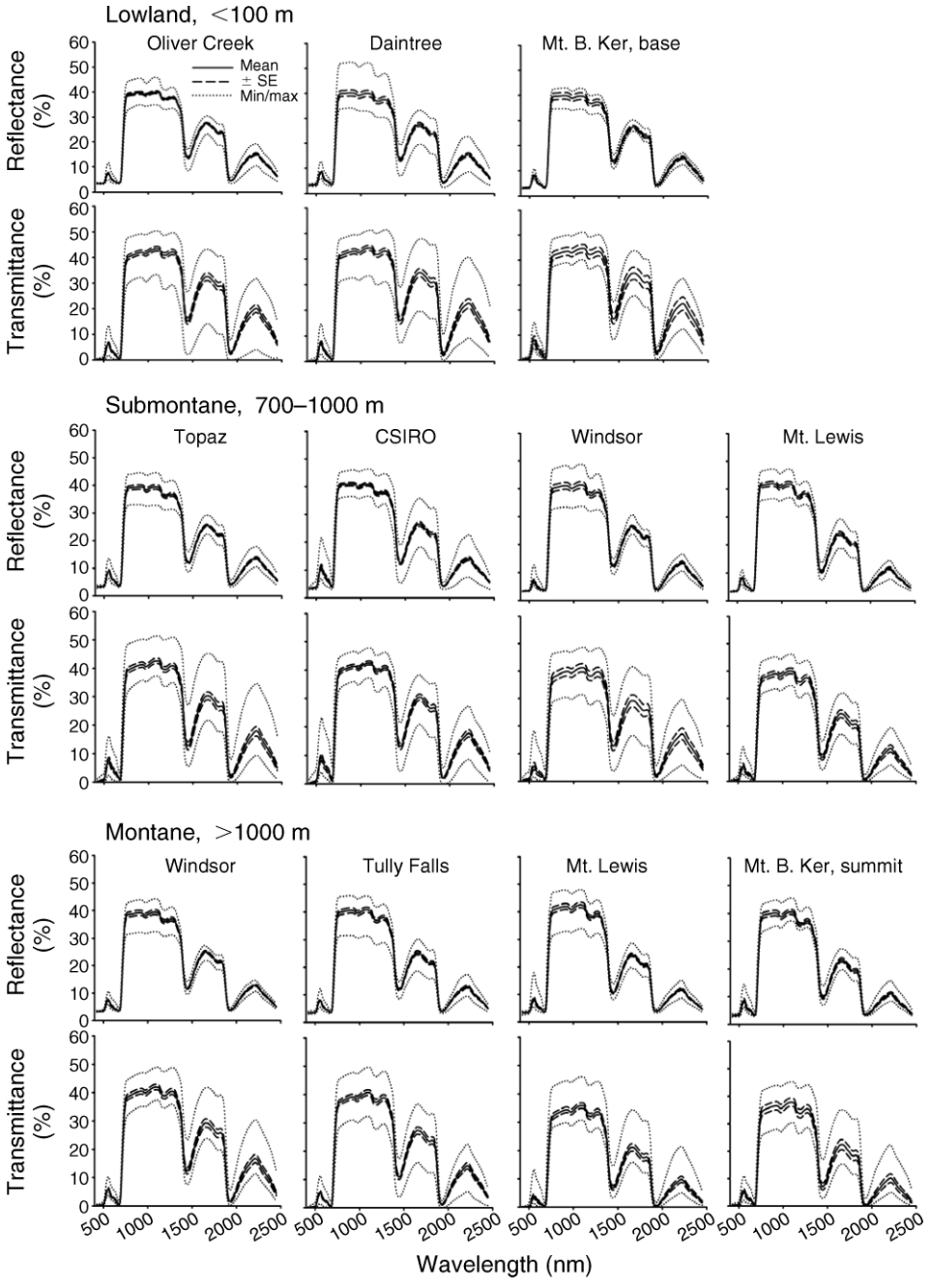

FIG. 5. Mean (±SE), minimum, and maximum values of leaf hemispherical reflectance and transmittance of canopy species. Data are arranged by sites in lowland, submontane, and montane tropical forests.

could be used to provide parameters that were strong predictors of an ensemble of leaf properties (Fig. 7). Comparison of regression results for each leaf constituent indicated their relative contribution to the spectral data: (1) SLA, Chl *a*, Chl *b*, and Car were very strongly expressed in the spectral data ($r^2 = 0.81$–0.83); (2) N and water concentrations were also well predicted ($r^2 = 0.71$–0.72); and (3) P concentration was relatively well represented in the reflectance data ($r^2 = 0.54$). Anthocyanins were poorly expressed in the spectra.

Analogous tests with leaf transmittance and absorbance spectra showed similar results, although the regressions against P concentration were weaker than with the reflectance data (Appendix A: Figs. A5 and A6). In addition, transmittance- and absorbance-based spectral weightings in the PLS results showed that the

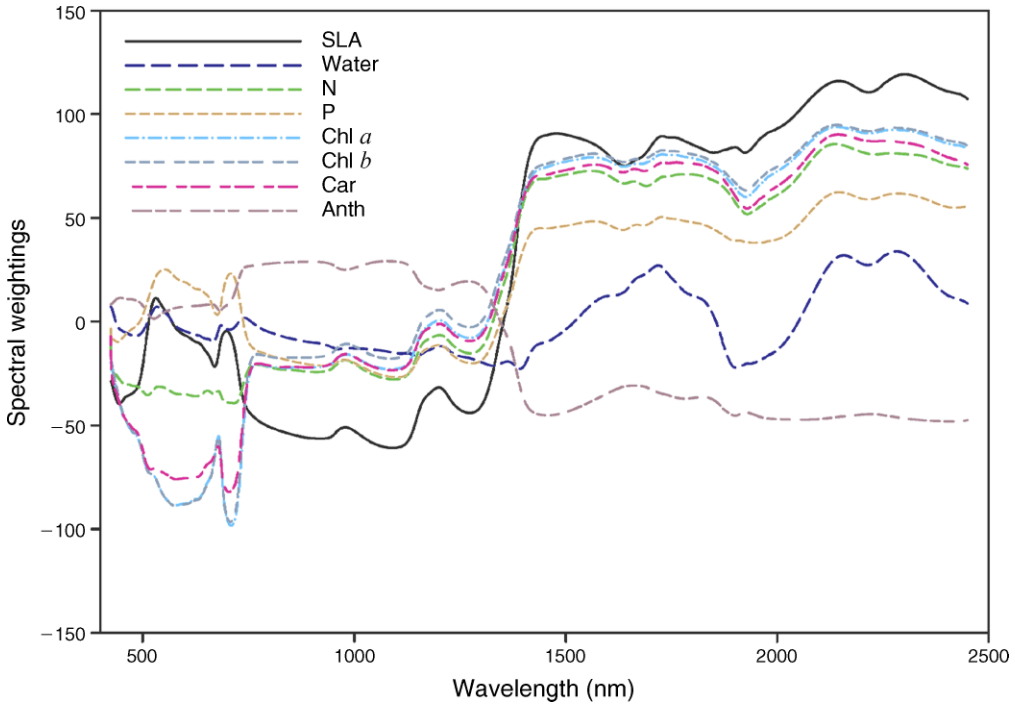

FIG. 6. Partial least squares (PLS) reflectance weighting factors for each leaf chemical property and specific leaf area (SLA). Wavelengths of maximum importance in determining leaf properties are those with spectral weightings that diverge from zero.

visible and SWIR spectral regions were differentially sensitive to leaf properties in a way consistent with our understanding of leaf optics and chemical spectroscopy (Appendix A: Fig. A7).

Interpreted together, the results indicate that a constellation of leaf properties is quantitatively represented by the reflectance and transmittance spectra. Previous studies have often focused on the estimation of one leaf chemical from reflectance spectra or from a few bands taken from the spectra (Gitelson et al. 2001, 2002, Kokaly 2001, Sims and Gamon 2002, Smith et al. 2003). Here we show that PLS statistics can provide estimates of multiple chemicals at the leaf level, without statistical overfitting (Haaland and Thomas 1988). Our findings also suggest that, to quantify the contribution of a specific chemical to the spectral properties of a species, it may be useful to apportion the contributions of several chemicals to the spectrum, thereby indicating the relative importance of each chemical in driving the spectral variation among species.

Spectral signatures of species.—Given the chemical diversity of tropical forest canopy species (Fig. 4), along with the demonstrable linkages between multiple leaf properties and their spectroscopy (Figs. 6 and 7), it should be possible to cluster the species based on their spectral signatures. We attempted to cluster them by site and elevation as shown in Figs. 8–10 for lowland, submontane, and montane forests. The graphic colors show absolute differences in reflectance by wavelength, and the dendrogram to the far right is color-coded to match the site from which each species was collected.

The most prominent feature among these cluster diagrams is the unique nature of most spectral signatures. Here, even small variations in color indicate quantitative differences among spectral features, so there are very few species that have the same spectral signature. The uniqueness of each signature, in turn, results in a generally weak overall clustering solution in the dendrograms. Nonetheless, some notable trends are apparent in the data; for instance, there are two groups of species with similar spectral properties at the Oliver Creek site (names in red; Fig. 8). There are also a few groups with spectral similarity at the montane Tully (names in green) and Mt Lewis (blue) sites (Fig. 10).

More interesting is the number of unique clusters generated by the hierarchical analysis: the lowland, submontane, and montane groups required 19, 19, and 13 clustering levels, respectively. The lowland group required the same number of clustering levels as that of the submontane forests, despite the fact that our lowland data set contained 30% fewer species. In fact, the ratio of the sample size to the number of spectral clustering levels was 2.2, 3.1, and 4.2 for lowland, submontane, and montane forests, respectively. Because this ratio goes up with decreasing cluster complexity in the dendrogram, these results suggest that the montane sites contain the fewest number of unique spectral clusters per unit of species richness, which may be due to tighter environmental constraints over leaf and canopy

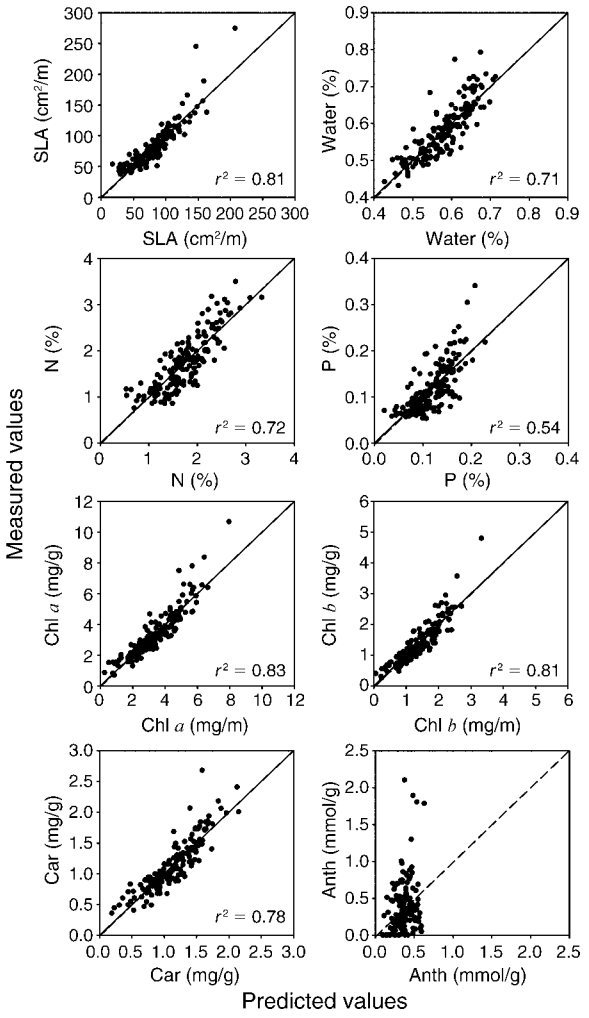

FIG. 7. PLS scatterplots showing absolute prediction strength of the spectral reflectance data for multiple leaf chemicals and specific leaf area (SLA). Measured values are on the *y*-axes; predicted values on the *x*-axes. A comparison of r^2 values among plots provides a relative measure of the importance of each leaf constituent in determining the spectral reflectance of all species (the relationship was not significant for anthocyanin).

optical properties among species at higher elevation, just as we found in the chemical properties of the montane forest foliage (Figs. 1 and 2). In contrast, the lowland sites contained the largest number of clusters per sample size, indicating fewer spectral similarities among species that matched the increased diversity of foliar chemistry in these warmer environments (Fig. 4).

In sum, these clustering results demonstrate the uniqueness of leaf spectral signatures among tropical forest canopy species in Australia, a result that echoes work presented from tropical forests in Central and South America (Roberts et al. 1998, Castro-Esau et al. 2004). Our results are the first to show that the diversity of leaf spectral properties parallels the chemical variability among species, which was especially apparent when comparing lowland and montane tropical forests (Fig. 4). We therefore contend that spectral diversity can serve as a fundamental surrogate for chemical diversity, opening new doors for spectral remote sensing of canopy chemical variation in rain forest ecosystems. Does chemical and/or spectral variation track taxonomic diversity? We used the diversity modeling approach to explore this possibility.

Canopy diversity modeling.—Our model demonstrated a nonlinear increase in both the chemical and spectral variability of tropical forest communities with increasing taxonomic diversity (Fig. 11). This is not surprising, but for our purposes, we focus on the rate of change, saturation point, and differences among tropical forest types. At high levels of species richness, the chemical diversity of the lowland systems was up to twofold greater than that of the other sites sampled (Fig. 11A). Although the initial rate of change in chemical variation in a forest type was only slightly different at low species counts, it was nearly an order of magnitude higher in the lowland forests when 25 or more species were considered (Fig. 11B). Saturation of the chemical variance among species was never achieved in any of the forests (Fig. 11B). The uncertainty around these estimates was greatest in the lowland forests, and very small in submontane and montane systems (Fig. 11A).

Spectral variation also increased as species richness increased (Fig. 11C). As observed with the chemical results, the spectral variability among the species was up to twofold higher in lowland than in montane forests. Absolute uncertainties in the spectral variance simulations were highest in the lowland forest sites and smallest in the submontane (Fig. 11C), results similar to those derived from the chemical diversity simulations (Fig. 11A).

Initial changes in spectral variance (at low richness levels) were also a magnitude greater in the lowland forest simulations than in the montane systems (Fig. 11D). However, once canopy richness exceeded about five species in the montane sites, further increases in plant diversity were almost linearly tracked by spectral diversity. Although the rate of change in spectral variance was nonlinear in the lowland sites, it remained positive up to 40 species. Similar analyses with up to 150 species from the central Brazilian Amazon also showed non-saturating patterns in chemical and spectral diversity (Asner 2008). We also observed a crossover in the rate of change in both chemical and spectral diversity at richness values of 12–14 species in the submontane and montane systems (Fig. 11B vs. D). This result suggests that localized spatial variation in canopy richness is expressed more strongly in submontane than in montane forests. In contrast, the montane systems undergo a more consistent change in spectral variance as richness increases (up to 40 species modeled in this case).

In combination, these simulations serve as predictions on how chemical and spectral diversity might change spatially with increasing species richness in Australian

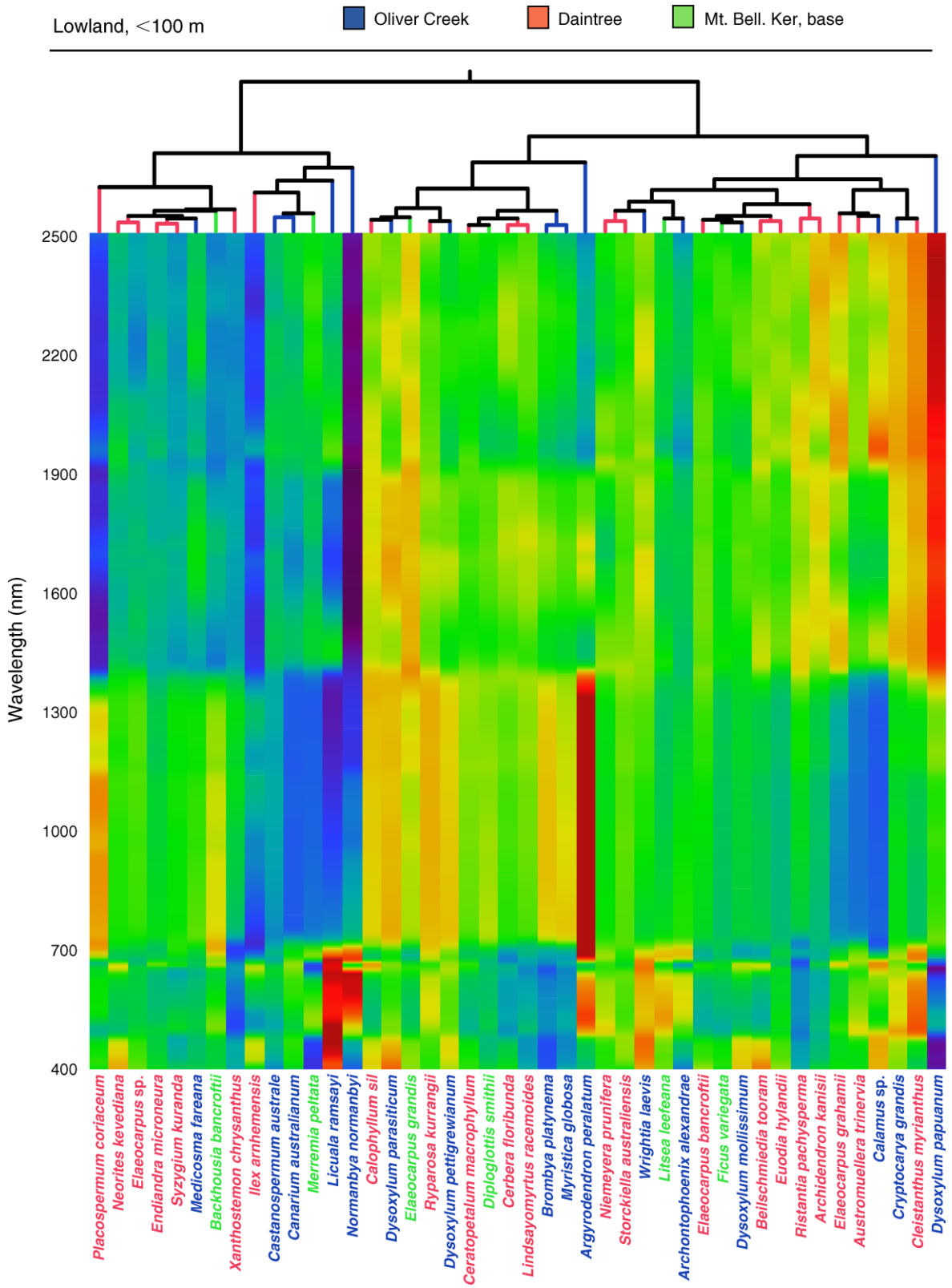

FIG. 8. Hierarchical clustering of canopy species in lowland tropical forests based on their spectral reflectance signatures in the 400–2500 nm range. The colors within the cluster diagram quantitatively depict properties within the reflectance signature that are similar (same colors, hues) or different among species. The dendrogram at the top shows the statistical similarity among species. Color codes in the species names relate them to their collection site (in the key); see Appendix B for full species names.

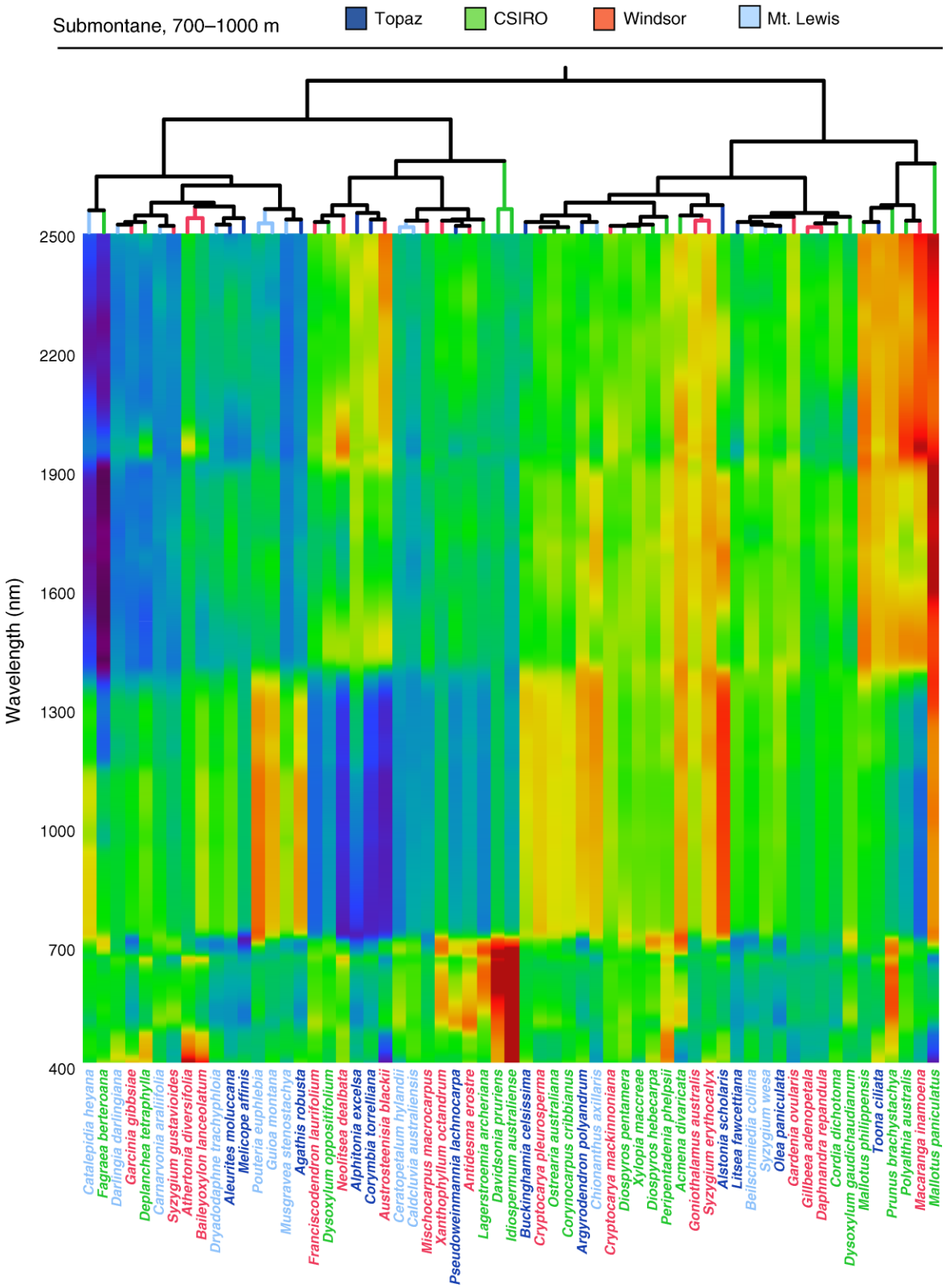

FIG. 9. Hierarchical clustering of canopy species in submontane tropical forests based on their spectral reflectance signatures in the 400–2500 nm range. Descriptions are as in Fig. 8.

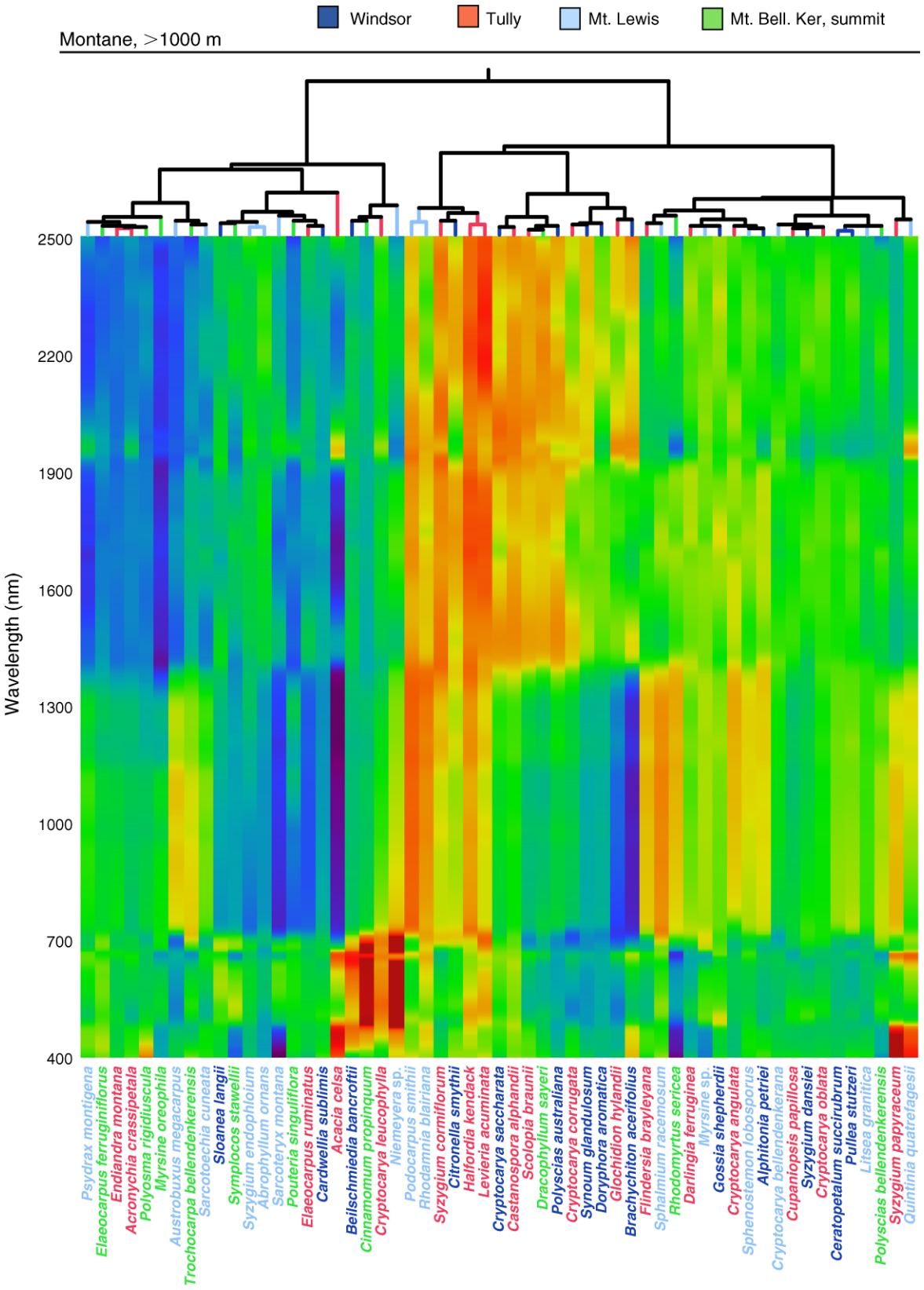

FIG. 10. Hierarchical clustering of canopy species in montane tropical forests based on their spectral reflectance signatures in the 400–2500 nm range. Descriptions are as in Fig. 8.

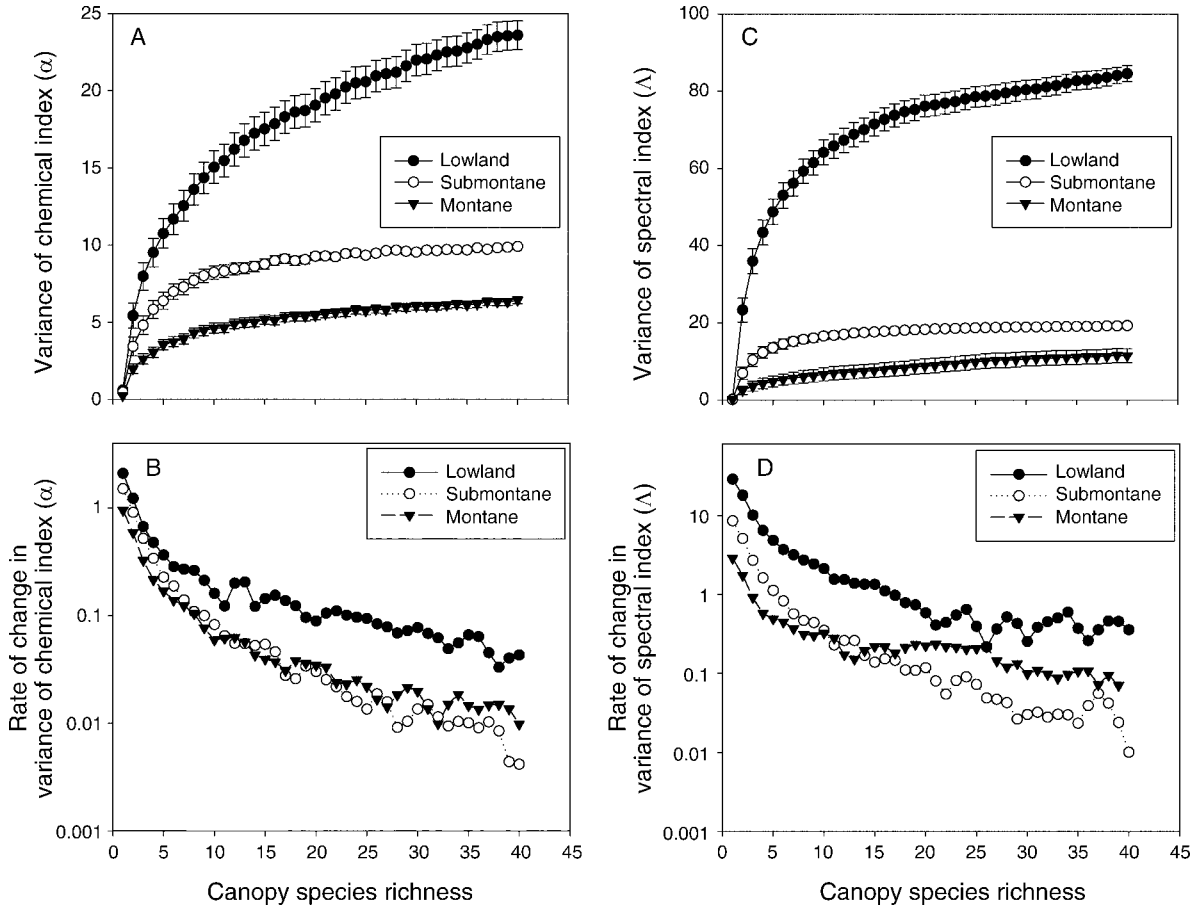

FIG. 11. Modeling results showing the sensitivity of species richness to chemical and spectral diversity. Panels A and B quantify the rate of increase and total dynamic range of the chemical index α (Eq. 1) as species richness is increased for lowland, submontane, and montane tropical forests. Panels C and D are similar to A and B but use the standardized spectral reflectance data (Λ) for each species. Note the *y*-axis log scale in panels B and D.

tropical forests. Chemical and spectral variability are highest at all richness levels in lowland forests, and they increase rapidly at a local scale (10–15 species), then taper off but continue to increase even at a high richness levels. Submontane and montane forests follow similar, but more subtle, patterns of increasing chemical and spectral diversity. Similar predictions were recently tested and applied in lowland rain forests on Hawaii Island (Carlson et al. 2007), showing that the spectral diversity of canopies, derived from actual airborne imaging spectroscopy, tracked taxonomic diversity. Our current study provides detail on the chemical sources of this spectral diversity as well as the interconnections among a large number of species.

It is important to note that our simulations were not designed to represent whole canopies. Nonetheless, leaf-level chemical and spectral variation among species does represent basic differences at the canopy scale. For example, the composition and architecture of highly foliated canopies (e.g., tropical forest trees) amplify the expression of leaf optical properties when measured from overhead by an airborne or satellite sensor (Baret et al. 1994, Asner 1998). We included some degree of intraspecific variation in leaf optical and chemical properties, but additional variation occurs along vertical gradients from fully sunlit to full shade conditions (Lee et al. 1990). Our simulations do not capture this source of variation among leaves within a canopy. However, our study used top-of-canopy, full-sunlight leaves: this is useful because optical remote sensing systems are far more sensitive to upper-canopy foliage in the spectral regions dominated by contributions from pigments, nutrients, and SLA (reviewed by Ustin et al. 2004). We also recognize that new foliar growth (flush), coordinated senescence, and epiphyll growth would confer different spectral and chemical signatures on the foliage of tropical canopy species. These effects remain unmeasured here, and thus should be quantified and included in future simulations. Despite these recognized limitations, we suggest that leaf-level chemical and spectral properties (and the relationships among them) are basic proxies for the taxonomic variability encountered in a tropical forest setting.

Conclusions

The chemical and taxonomic diversity of tropical forest canopies remains difficult to assess at any ecological or geographic scale. We need to increase our understanding of how chemical signatures vary taxonomically and spatially among species, because their variability affects physiological function and biogeochemical processes, and the response of forests to land-use and climate change. Our results indicate that, although climate exerts a measurable impact on foliar chlorophyll, N and P concentrations, and SLA, these effects are modest in comparison to taxonomic sources of variation in leaf properties. In most cases, the integrated chemical signatures, or any single leaf constituent contributing to them, did not aggregate well even at the genus or family levels. We also found that the chemical diversity of species within certain plant families can nearly match that of a forest site or a group of sites. We conclude that species are the taxonomic unit causing chemical variance in Australian tropical forests.

We also considered leaf chemical variability across a pronounced gradient of climate conditions in Australian tropical forests. The more than 6024 mm annual precipitation and 8.7°C temperature range approaches the full range of tropical forest conditions worldwide (Holdridge 1947). Using this gradient, we showed that leaf chemical diversity is maximal in the lowland tropical forest sites with the highest temperatures and moderate precipitation levels. Cooler and wetter montane tropical forests contained species with measurably lower variation in their chemical signatures. The diversity model highlighted quasi-spatial patterns in leaf chemical signatures between species, revealing that lowland forests maintain a far more diverse chemical canopy per unit richness than other forest sites in Australia.

We showed that the leaf optical properties of Australian tropical forest species were also highly diverse, and were well correlated with the ensemble of leaf properties contributing to their chemical signatures. With the exception of anthocyanins, the spectral reflectance and transmittance of the species quantitatively determined all chemicals and SLA at high precision ($r^2 = 0.54$–0.83). These results indicate that the portfolio of leaf properties comprising the chemical signatures is retrievable from the leaf spectral data, and that the spectral variability of tropical forest canopies is driven at the species level more so than at genus or family levels.

Our foliar chemical findings suggest that tropical forest canopies are composed of spatially explicit, crown-scale mosaics of nutrient demand and turnover, as well as photosynthesis and primary production. Such extraordinary chemical variation is difficult to explain ecologically, as it is for taxonomic variation in most humid tropical forests (Wiegand et al. 2007). Independent of the cause for high taxonomic diversity, we show that chemical and spectral diversity follows a similar pattern, at least in humid tropical forests of Australia. Field studies should thus consider the importance of local-scale chemical variation in relation to taxonomic composition. In addition, landscape-scale modeling studies might be improved by incorporating knowledge of chemical variability into statistical analyses and model simulations. Moreover, landscape extrapolations and predictions of ecophysiological and biogeochemical processes might improve by treating the functional properties of canopies as distributions based on chemical and optical properties resulting from taxonomic diversity.

From the remote sensing perspective, high chemical and spectral diversity presents both challenges and opportunities in the context of a new generation of airborne and space-based technologies. Our leaf chemical and spectral data make a robust linkage to the taxonomic variability of humid tropical forests. The interrelationships between the foliar chemical and spectral properties help to explain the successful results reported in developing species-level classifications from leaf and canopy spectral data (Cochrane 2000, Castro-Esau et al. 2004, Clark et al. 2005, Zhang et al. 2006). Foliar data also help to interpret recent canopy richness mapping results from airborne systems that combine imaging spectroscopy with other technologies, such as lidar, for crown-by-crown spectral analysis of tropical forest canopies (Carlson et al. 2007, Asner and Martin 2009). These early developments are promising, but additional studies are needed to scale from leaf and canopy to larger (e.g., satellite) pixel levels in order to understand the relationships among the chemical, spectroscopic, and taxonomic properties of tropical forests.

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APPENDIX A

Eight figures, providing complementary data rearranged by species or measurement technique, referenced in the main text as key additional illustrations pertinent to the interpretation of the role that species play in determining the chemical and spectral diversity of Australian tropical forests (*Ecological Archives* A019-010-A1).

APPENDIX B

A list of species measured in the study (*Ecological Archives* A019-010-A2).