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Remote Sensing of Environment

journal homepage: www.elsevier.com/locate/rse

The effect of seasonal spectral variation on species classification in the Panamanian tropical forest

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ARTICLE INFO

Article history:

Received 10 August 2010
Received in revised form 15 November 2011
Accepted 17 November 2011
Available online xxxx

Keywords:

Leaf optical properties
Seasonality
Tropical dry forest
Panama
Spectro-temporal variability
Lianas
Leaf spectroscopy

ABSTRACT

Variation in the leaf optical properties imposed by variation in genetics and location has been addressed in recent literature, but those stemming from forest seasonality and phenology have been less well explored. Here, we explore the effect of inter-seasonal spectral variation on the potential for automated classification methods to accurately discern species of trees and lianas from high-resolution spectral data collected at the leaf level at two tropical forest sites. Through the application of a set of data reduction techniques and classification methods to leaf-level spectral data collected at both rainforest and seasonally dry sites in Panama, we found that in all cases the structure and organization of spectrally-derived taxonomies varied substantially between seasons. Using principle component analysis and a non-parametric classifier, we found at both sites that species-level classification was possible with a high level of accuracy within a given season. Classification across season was not, however, with accuracy dropping on average by a factor of 10. This study represents one of the first systematic investigations of leaf-level spectro-temporal variability, an appreciation for which is crucial to the advancement of species classification methods, with broad applications within the environmental sciences.

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1. Introduction

Remote sensed analysis of tropical forest environments has until recently been conducted along two separate lines: The first encompasses moderate-to-coarse resolution, multi-spectral analysis of forest cover, addressing natural and anthropogenic change or disturbance (Coppin and Bauer, 1996), and its properties (ex. forest phenology (Huemmrich et al., 1999; Xiao et al., 2005; Zhang et al., 2003), composition (Castro-Esau et al., 2003), and landcover classification (Adams et al., 1995)). The second relies on leaf- and canopy-level, high spectral resolution data to investigate the relationships between optical characteristics and physiological (Sims and Gamon, 2002), biochemical (Asner, 1998), and structural variables (Sanchez-Azofeifa et al., 2009). One of the promises of the increasing quality and availability of hyperspectral data and accompanying advances in analytical techniques is the potential for investigation not just of species richness and variation, but also of the interrelated biochemical and physiological processes which impact canopy function and productivity at spatial scales which bridge these two lines of inquiry (Asner, 2008).

Classification of plant species at the leaf level has been most commonly attempted using direct spectral–taxonomic relationships (Cochrane, 2000). Variations of this concept have been applied in

boreal (Fuentes et al., 2001) and chaparral (Ustin et al., 2004) environments, though the increased complexity and variability of tropical systems suggest some need for caution in their application. An approach has been put forth to aid in species-level mapping of tropical forest environments coupling hyperspectral reflectance measurements with chemical signatures developed using the relationship between leaf traits and species (Asner and Martin, 2008).

High-resolution leaf spectral reflectance has the potential to allow for estimation of leaf traits (Gamon and Surfus, 1999; Gamon et al., 2005; Sims and Gamon, 2002) as well as for discrimination of structural groups (Castro-Esau et al., 2004; Kalacska et al., 2007) and species type (Clark et al., 2005; Zhang et al., 2003). The fundamental prerequisite to identification of tree and liana species from leaf reflectance data is that spectral variation within species is lower than the variation between species. That this condition can be met has been demonstrated by the above studies, but typically with classification restricted to a single site, and using a dataset collected within a narrow temporal window (Asner and Martin, 2008; Cochrane, 2000).

While exploration of temporal variation in leaf traits across plant genera and families in the tropics has been minimal (Asner et al., 2009), Roberts et al. (1997) have addressed leaf reflectance as a function of leaf age, and Schwartz and Reed (1999), and Zhang et al. (2003) have all used leaf optics to track forest phenology. Investigating spectral variation within species, Castro-Esau et al. (2006) found sufficient difference in the optical properties of species sampled across multiple sites that accurate automated classification was impossible.

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Conversely, Asner et al. (2009) found in a study of 162 canopy species across a wide climatic gradient in Australia that, although biophysical variables were strongly related to leaf reflectance, variation in leaf chemical signatures varied far more in response to taxonomy and species richness than to changes in climate. They did, however, find the greatest chemical variation in lowland sites with warm temperatures and moderate precipitation levels, which echo Townsend et al. (2007), who found maximal N:P variation according to rainfall in highly seasonal sites in Costa Rica. Martin et al. (2007) found strong genetically-attributable variation in pigment and optical characteristics among samples of *M. polymorpha* grown from seed sources collected from a wide environmental (soil type and altitude) gradient. Most recently, Sanchez-Azofeifa et al. (2009) evaluated variation in optical and biophysical leaf traits between structural groups (trees and lianas) and forest types (wet and dry tropical forests) at two sites in Panama. Their results indicate significant differences in pigment content, leaf thickness, and specific leaf area, dry-to-fresh mass ratio, and leaf water content between trees and lianas collected at a dry forest site, but not at a rainforest site. This validates earlier work by Castro-Esau et al. (2004) and Kalacska et al. (2007).

While research up to the present has addressed leaf properties (Asner, 1998) and variability between environmental (Asner et al., 2009; Sanchez-Azofeifa et al., 2009) and structural groups (Castro-Esau et al., 2004; Kalacska et al., 2007), the question of temporal variation in leaf traits has been largely unexplored at the leaf scale. It is clear from previous research (Castro-Esau et al., 2004; Kalacska et al., 2007) that inter-seasonal spectral variation is a major limiter to our ability to accurately classify forest species in an unsupervised or automated setting, and that a better understanding of the nature and extent of this variation will be critical in the refinement of existing classification techniques and the development of new ones. Our objective, therefore, is to evaluate the nature and extent of seasonal spectral variation at both wet and dry tropical forest sites. Specifically, we test whether the clustering of data from the same site yields similar patterns during the wet and dry seasons, then address the effect that seasonal spectral variation has on the accuracy of unsupervised classification of these data.

2. Methods and data collection

2.1. Site description

Data for this study were collected at two sites in Panama, taking advantage of canopy cranes operated by the Smithsonian Tropical Research Institute (STRI) to obtain access to the top of the forest canopy. The first site, Parque Natural Metropolitano (PNM) is located just outside of Panama City. The crane has a height of 42 m with a boom radius of 51 m. Annual rainfall averages approximately 1800 mm, more 90% of which falls between May and December (Gamon et al., 2005). Liana species represent a sizable proportion of canopy species at the park. Avalos and Mulkey (1999) estimated that contributions made by lianas to the canopy area surveyed by the crane were variable between 14.0% during the dry season and 30.9% during the rainy season. The full complement of canopy species considered in this study is detailed in Table 1.

A second STRI crane is located in the rain forest at Fort Sherman (FS), along the Caribbean coast near Colón. Annual rainfall at this site is approximately 3300 mm. The crane is 56 m high with a boom radius of 54 m (Castro-Esau et al., 2004). While liana species are important contributors to biodiversity at this site as well, it is to a lesser extent than at PNM (Table 1).

2.2. Data collection and analysis

Leaf spectral data were collected twice at each site: once during the peak of the rainy season (May 2005), and once at the beginning of the dry season (March 2007), but before complete leaf loss. A total of 17

Table 1

Species included in study. Codes beginning with L indicate liana species while those beginning with T indicate tree species.

Fort Sherman (rainforest)		Parque natural metropolitano (dry)	
Species	Code	Species	Code
<i>Dolioscarpus multiflorus</i>	L27	<i>Aristolochia maxima</i>	L1
<i>Arrabidaea verrucosa</i>	L28	<i>Stizophyllum riparium</i>	L2
<i>Pleonotoma variabilis</i>	L29	<i>Serjania atrolineata</i>	L3
<i>Odontadenia punctulosa</i>	L30	<i>Stigmaphyllon hypergyreum</i>	L4
<i>Dioclea wilsonii</i>	L31	<i>Gouania lupuloides</i>	L5
<i>Forsteronia myriantha</i>	L32	<i>Mikania leiostachya</i>	L6
<i>Tontelea ovalifolia</i>	L33	<i>Bonamia trichantha</i>	L7
<i>Maripa panamensis</i>	L34	<i>Jacquemontia sp.</i>	L8
<i>Pouteria reticulata</i>	L41	<i>Passiflora vitifolia</i>	L9
<i>Lonchocarpus longifolium</i>	T11	<i>Dolioscarpus major</i>	L11
<i>Carapa guianensis</i>	T12	<i>Prionostema aspera</i>	L12
<i>Matayba apetala</i>	T14	<i>Dolioscarpus dentatus</i>	L13
<i>Cordia bicolor</i>	T17	<i>Amphilophium paniculatum</i>	L14
<i>Manilkara bidentata</i>	T19	<i>Pithecoctenium crucigerum</i>	L15
<i>Aspidosperma cruenta</i>	T21	<i>Trichostigma octandrum</i>	L16
<i>Brosimum utile</i>	T23	<i>Hiraea reclinata</i>	L17
<i>Ficus nymphaeifolia</i>	T25	<i>Forsteronia spicata</i>	L18
		<i>Arrabidaea patellifera</i>	L19
		<i>Hippocratea volubilis</i>	L21
		<i>Serjania mexicana</i>	L22
		<i>Phryganocydia corymbosa</i>	L23
		<i>Tetracera portobellensis</i>	L24
		<i>Anacardium excelsum</i>	T1
		<i>Luehea seemannii</i>	T2
		<i>Astronium graveolens</i>	T3
		<i>Cordia alliodora</i>	T4
		<i>Annona spraguei</i>	T5
		<i>Castilla elastica</i>	T6
		<i>Ficus insipida</i>	T9
		<i>Chrysophyllum cainito</i>	T10

species were analyzed for FS, and 30 for PNM, with collection and sampling protocols according to Kalacska et al. (2007) and Castro-Esau et al. (2004, 2006), Sanchez-Azofeifa et al. (2009). Leaves were collected from the top of the canopy (all sun-leaves) and selected such that galls and visible epiphytes were avoided. Spectral data were collected using the ASD FieldspecFR spectrometer using the ASD Leaf Clip device (Analytical Spectral Devices, Boulder CO). The spectral range of the instrument is 350–2500 nm with a 3 nm resolution from 350 to 1000 nm and 10 nm from 1000 to 2500 nm. All data was resampled to 1 nm resolution in post-process. Typically, 10 leaves per species were collected with three spectra per leaf measured per sample. A third data set, collected using the same protocols, was gathered during a rainy transitional period (February 2011). These data, as well as those from 2007 (our dry season data), also include leaf area and wet/dry weight measures for calculation of specific leaf area (SLA).

Principal components analysis (PCA) was applied to the hyperspectral signatures to reduce the dimensionality and redundancy inherent in these data (Schowengerdt, 1996). PCA reduces the data to a set of orthogonal eigenvectors, which maximize variation and greatly reduce autocorrelation (Kalacska et al., 2007). The first 4 components in each resulting transformation were retained such that >97% of the expressed variation in the raw data was represented. Because leaf chemical and biophysical characteristics were not available, spectral vegetation indices (SVIs) were calculated from each input spectra to complement the PCA decompositions and stand as proxy for these biophysical variables. Merzylak et al.'s (1999) plant senescence reflectance index (PSRI) increases proportionate to the carotenoid/chlorophyll molar ratio, and serves in comparison of the balance of these key pigments across seasons (Eq. 1). Penuelas et al.'s (1993) water index (WI) stands as proxy for direct leaf water content (Eq. 2).

$$PSRI = \frac{(R_{678} - R_{500})}{R_{750}} \quad (1)$$

$$WI = \frac{R_{900}}{R_{970}} \quad (2)$$

Two resulting spectral datasets were used in subsequent analyses. The first contained leaf spectra, averaged to the species level. The second contained the retained principle components as well as the two SVIs, this time at the level of the individual sample. Each dataset was repeated for each season (dry and rainy) at each site (FS/wet and PNM/dry). The flow diagram in Fig. 1 outlines the paths these two datasets take through the subsequent analyses.

Seasonal variation was tested using a set of two classification procedures to separately evaluate both the structure of data classified during different seasons, and the effect of cross-season classification on the overall accuracy. First, to visualize the effects of seasonal spectral variation, the raw spectral data was classified using an agglomerative hierarchical clustering algorithm implemented in Matlab (V. 7.8, The Mathworks 2009). The resulting dendrograms not only illustrate the impact of seasonality on species-level clustering, but allow evaluation of the difference between clustering of spectral data collected during opposing seasons, directly addressing a fundamental requirement for automated species identification: that each species' spectral "fingerprint" is insensitive to seasonal variation. To address the impact of the mixing of the two principle plant structural groups (trees and lianas), data were clustered first with trees and lianas mixed, and again with trees and lianas considered separately.

Evaluation of seasonal variation in the structure of the dendrograms was by comparison of the bifurcation ratio (R_b). This ratio was established by Horton (1932, 1935) to describe the branching pattern of drainage networks as they progressed toward a confluence, and is used here to quantify the structure of the dendrograms in a way that can be compared across seasons. R_b , has been used to quantify not only the complexity of river systems, but also variation in the branching of vegetative shoots as a means of evaluating genotypic plasticity (Oohata and Shidei, 1971; Whitney, 1976). To calculate, branches are ordered according to Strahler's streambed organization of the tributaries of a trunk stream channel (Strahler, 1952). The value for R_b at a given order μ is the ratio of the number of branches at that order (N) to the number at the next order higher (Eq. 3) and is proportionate to the complexity of the network. This ratio may also be averaged across all orders for a general measure of dendrogram complexity (Fig. 2)

$$R_b = \frac{N_\mu}{N_{\mu+1}} \quad (3)$$

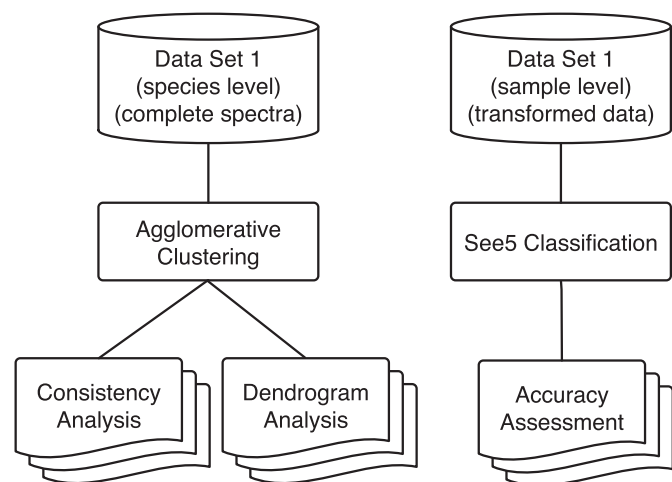


Fig. 1. General workflow of analysis showing the progress of Dataset 1 through hierarchical clustering and dendrogram analysis, and Dataset 2 through unsupervised classification.

As well as variation in the branching structure of the dendrograms generated from the clustering procedure, changes in the species composition of each cluster were evaluated. Each species' nearest neighbors were compared for each season and each site, and the consistency between seasons was calculated as the percentage of species with the same nearest neighbors in each season. This metric of consistency quantifies the tendency for spectrally similar species to cluster together at the lowest level of the dendrograms. If reflectance is similar between seasons, the same species will be found clustered together resulting in a high level of consistency. Greater seasonal spectral variation will result in lower consistency between dendrograms generated for different seasons at a given site.

Second, to quantify the impact of seasonal variation on classification accuracy, we adapted the method of Kalacska et al. (2007). As inputs, we used the four retained principal components and the 2 SVIs, aggregated to the sample level. For the classifier, we chose the non-parametric decision tree classifier See5 (Rulequest Research 2008). See5's cross-validation function, which allows for a quick and direct evaluation of the overall accuracy of within- and between-season classification, was used to evaluate the effect of inter-seasonal spectral variation on overall classification accuracy. The classifier was applied twice to the data from each site: First, the dataset from each season was split in half, with one half used to train the classifier and the other half used to test its accuracy. Second, to test the impact of seasonal variation on the accuracy of the classifier, the entire dataset from the wet season was used as the training set, then tested on the entire dry season dataset, and vice versa. Classification accuracy was calculated as the percentage of data in the testing set classified correctly.

3. Results

3.1. Inter-seasonal differences in spectral reflectance

The average spectral signatures of all species included in the investigation are shown in Fig. 3a and b for the wet and dry seasons, respectively. Spectral features in the visible region (400–700 nm) are reflective of leaf chemistry and pigment content, specifically chlorophyll content, which results in strong absorption features both above and below the 550 nm green peak (Boyer et al., 1988). Features in the near-infrared (700–1100 nm) range most related to leaf structure (Woolley, 1971), with weak water absorption features at ~1000 and 1200 nm (Gao and Goetz, 1995). Reflectance in the shortwave region (1500–2400 nm) is controlled largely by water absorption (Gates et al., 1965). Where Fig. 3a and b demonstrates spectral variability among the species examined, Fig. 3c details the seasonal difference in reflectance at each wavelength for each of the species tested. There is a difference here in the regions of greatest seasonal spectral variation between the wet and dry forest sites. At FS, the greatest spectral variation is found occur along the red edge (~720 nm). This spectral range is important in this study as we employ SVIs as proxy for measured leaf biophysical properties, and this region is sensitive to leaf chlorophyll content (Curran et al., 1990). At PNM, the difference in this spectral region was muted in comparison to the short wave infrared region (1350–2300 nm), which is governed largely by water absorption. This general pattern in the seasonal variation is found in both tree and liana species at both sites.

3.2. Variation in classification structure

Dendrograms resulting from hierarchical clustering of the full-range spectra from both sites show marked variation between wet and dry seasons (Fig. 4a–d). The values for Horton's bifurcation ratio (R_b) at both sites and seasons are presented in Table 2. While the mean R_b (calculated across all orders of μ) shows some variation between wet and dry seasons at each site (0.12 and 0.19 difference at FS and PNM respectively), these differences are muted in comparison to the differences

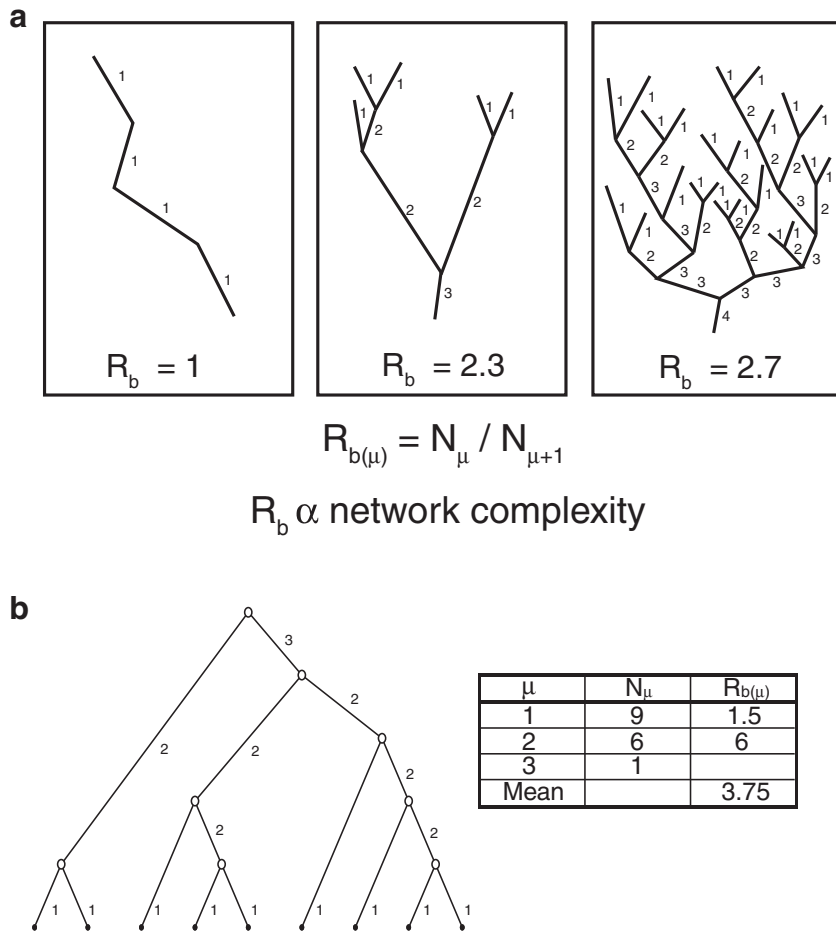


Fig. 2. Diagrammatic explanation of Horton's bifurcation ratio (R_b). a. Demonstration of the scaling of R_b relative to drainage network complexity. b. Representation of R_b in the context of the dendrogram analysis used in this study.

found at each level of μ . Difference in R_b between wet and dry seasons at the level of each order (μ) ranges between R_b 0.2 and 1.0, with the exception of the value for $\mu = 2$ at FS site of R_b 2.2 (Table 2).

Seasonal variation in the organization of species within the dendrogram, as demonstrated in Fig. 4, was also noted at both study sites. Similar groupings are uncommon at both sites, with consistency found to be lower at PNM, with a value of 6.7%, than at FS, where just under one quarter of species shared at least one neighbor between seasons.

While differences in R_b are found at all levels of μ (including the overall mean) at both sites when the entire datasets (all tree and liana species) are included, these differences are greatly muted when only the liana species were considered. Tree species were not considered independently as the number of species in this group did not provide an adequate sample. The resulting dendrograms are presented in Fig. 4e–h. Inter-seasonal variation in R_b was found only in the data from PNM, while the smaller FS sample showed no measurable difference between dendrograms calculated for the wet and dry seasons (Table 3). The absolute difference in R_b between seasons also shows a relationship to the sample size taxonomic complexity of the data being processed. The liana group from FS showed no difference in R_b between seasons (Table 3), indicating that there may be a sample size threshold below which R_b comparisons are not a realistic method of analysis. The consistency of species composition at the lowest dendrogram level was also lower at PNM than FS, though these results are not substantially altered from those found when all species were considered together, with lianas at PNM having a consistency of 4.6% and trees a consistency of 22.2%.

3.3. Effect of seasonality on classification accuracy

Figs. 5 and 6 explore the spectral vegetation indices used in the See5 classification, separating by functional group (lianas and trees) as well as by site and season.

Higher plant senescence reflectance index (PSRI) values were observed at the dry forest site and lower values at the rainforest site (Fig. 5). The effect of seasonality on this index seems to be inverted at the two sites, however, with the dry season having generally higher PSRI values than the wet season at PNM and the reverse at FS, with the exception of tree species at FS. This seems to echo the influence of moisture-induced senescence on spectral response reflected in Fig. 3. Differences among PSRI values (Evaluated using Student's *t*-test) tended to be significant between sites (Table 4) but not between seasons at a single site. Water index (WI) values are unsurprising at the structural group level, though the variation in water content between trees and lianas masks the effect of seasonality at each when all species are taken together (Fig. 6). Values here showed little trend toward significant difference, either between sites or seasons (Table 4).

Fig. 7 shows the impact of seasonal variation in spectral properties on species-level classification accuracy. Using the PCA transformed data PSRI, and WI, accuracy was evaluated as the percentage of correct classification, splitting the data 50/50 for training and testing. In all cases, accuracy was dramatically higher where the classification was tested using a classification trained on data collected during the same season as the test data. The rainforest site (FS) showed accuracy of 80.4–83.5% when training and testing data were from the same season, but dropped to 8.6–10.9% when opposing seasons were

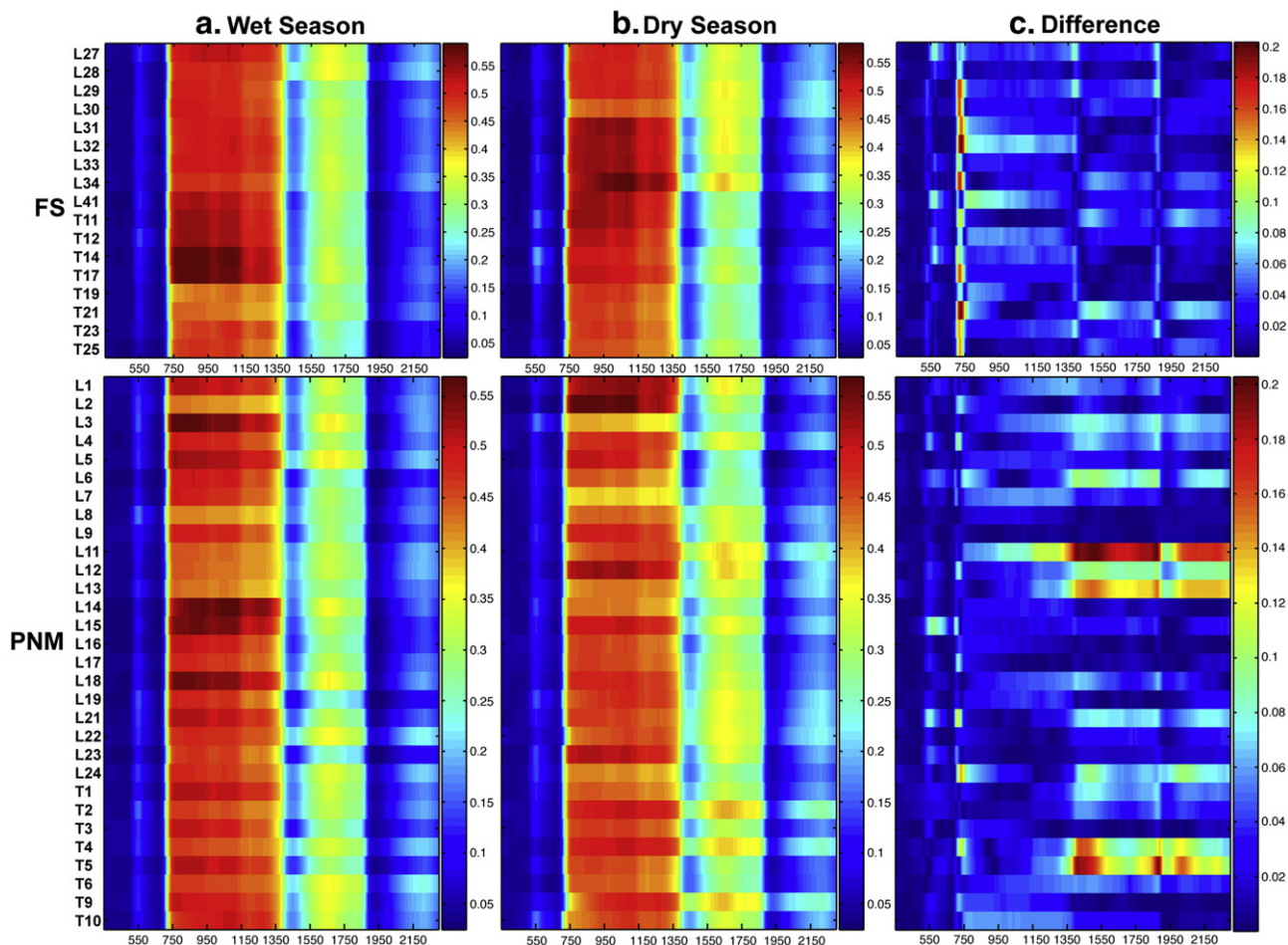


Fig. 3. Reflectance by wavelength of liana and tree species at both forest sites. a. Wet season; b. Dry season; c. Absolute difference between wet and dry reflectance. Species labels correspond to the codes in Table 1.

evaluated. This decrease was more pronounced at the dry forest (PNM) site, where within-season accuracy was between 80.7 and 83.0%, but between-season accuracy fell by more than a factor of 10, to 4.6–7.7%.

Classifying each structural group separately yielded similar results, though the smaller input datasets returned slightly higher accuracies in almost all cases. Considering only lianas, the classifier returned average accuracies of 84.2% for within-season classification at FS, and 19.3% for between-season classification, with results of 85.5% and 11.6% for within- and between-season classification at PNM. Accuracy for trees only was higher still, with average accuracies of 89.5% and 16.3% for within- and between-season classification at FS and 91.2% and 16.6% at PNM.

3.4. Specific leaf area

While no biophysical data were collected along with our wet season (2005) spectral data, we were able to compare specific leaf area (SLA) data from the dry season with unpublished data from the same sites collected during a rainy period during the wet–dry transition in February 2011. SLA is relevant, as it has been shown to be an important predictor of other traits in the leaf economics spectrum (Wright et al., 2004) as well as a linkage between chemical and spectral signatures (Asner et al., 2009). A paired *t*-test comparing SLA between these growing periods showed a strongly significant difference ($P < 0.001$).

4. Discussion

Throughout this investigation, we found consistent evidence that leaf spectral properties vary between seasons to a sufficient extent that the results of spectral clustering for wet and dry seasons were measurably dissimilar and classification accuracy was dramatically affected. Methodologies using only spectral data for the classification of species and assessment of biodiversity are still a subject of exploration and development and this evidence of the impact of seasonality on leaf spectral response suggests that an understanding of the spectro-temporal domain is an essential step in their refinement.

4.1. Variation of leaf spectral reflectance with season

Variation in leaf spectra between seasons indicates a fundamental difference in plant function between wet and dry forest environments, also noted in Sanchez-Azofeifa et al. (2009). Rather than the actual reflectance or spectral signature of a given species, we are concerned with the extent and location of the greatest variation in the signature between seasons. At Fort Sherman, our wet forest site, this variation was most pronounced along the red edge, between 550 and 750 nm. This spectral region encompasses the wavelengths typically used in the estimation of chlorophyll (Boyer et al., 1988, Gates et al., 1965), indicating that the primary driver of spectral variation in this ecosystem is likely variation in the relative abundances of leaf pigments. Conversely, the dry site, Parque Natural Metropolitano, showed substantially less variation in this spectral region, with the greatest sources of variation

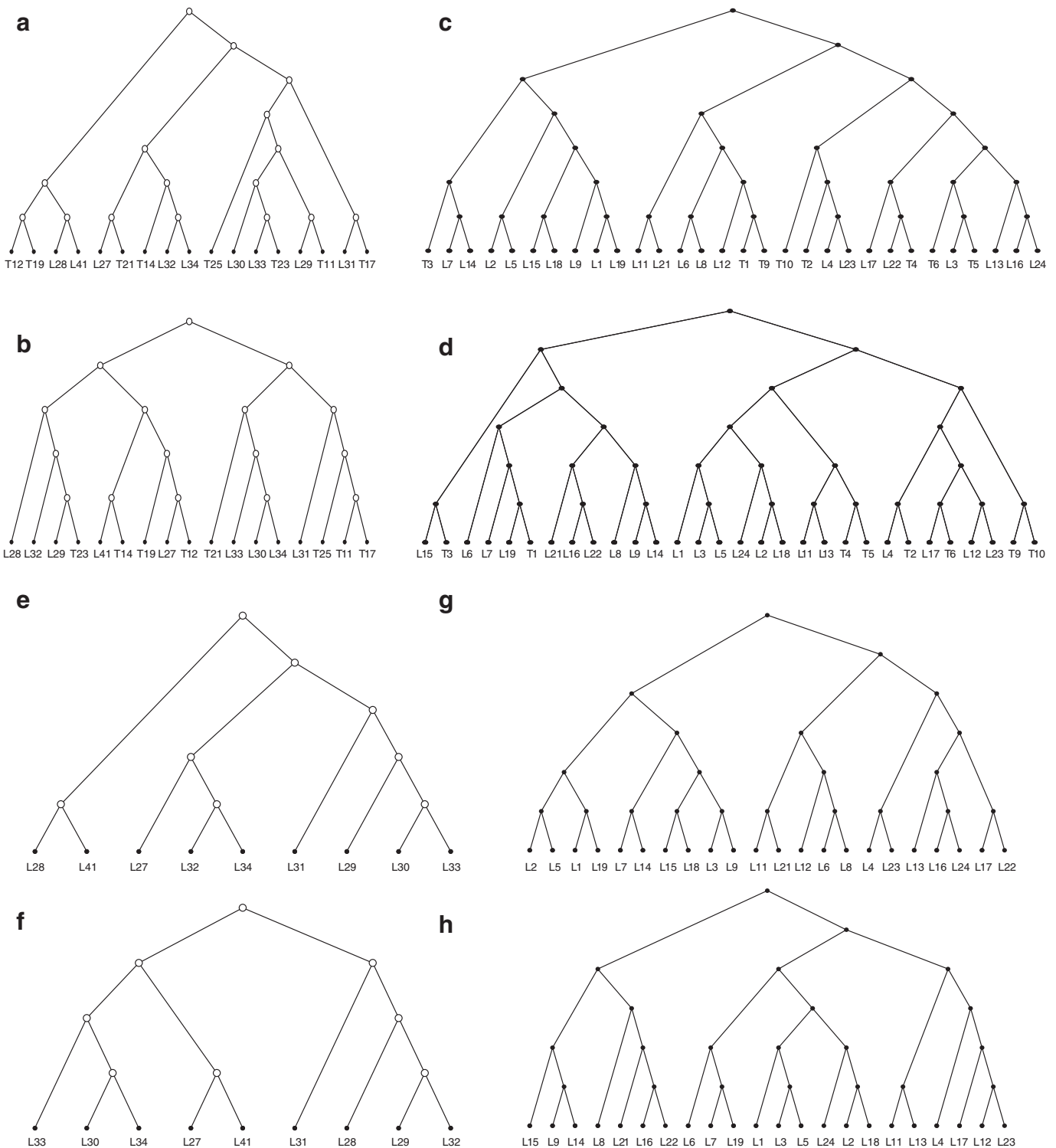


Fig. 4. Dendrograms resulting from hierarchical clustering of Dataset 1: All species (a. FS wet, b. FS dry, c. PNM wet, d. PNM dry); Lianas only (d. FS wet, e. FS dry, f. PNM wet, g. PNM dry).

found at longer wavelengths in the near- and shortwave-infrared regions, where reflectance is governed largely by absorbance by water. This is perhaps unsurprising in a drier forest environment where species are particularly sensitive to moisture variation (Murphy and Lugo, 1986), but it indicates that spectral variation cannot be simply tied to a common source, and should be considered in the context of local-scale ecology.

While our results are based strictly on spectral data, the inclusion of two spectral indices is intended to impart a component to the

classification procedure that has a more direct ecological interpretation. Of the two indices used, PSRI values more clearly indicate a relationship between seasonality and leaf spectral response. The inversion in response to seasonality at the two sites indicates a fundamental difference in the composition and functional ecologies of wet vs. dry forest environments. Notably, the seasonally dry site at PNM carries a much higher liana load than does the wet site at FS (73% vs. 53% of species accessible by the crane in our sample). Schnitzer (2005) suggests that the evolution of a more efficient vascular system

Table 2
Bifurcation ratios—complete species set considered.

	μ	R_b		
		Wet season	Dry season	Difference
PNM	1	1.43	1.58	0.15
	2	3.5	2.72	0.78
	3	6	7	1
	Mean	3.64	3.76	0.12
FS	1	1.89	1.42	0.47
	2	1.8	4	2.2
	3	5		
	Mean	2.9	2.71	0.19

Table 3
Bifurcation ratios—only liana species considered.

	μ	R_b		
		Wet season	Dry season	Difference
PNM	1	1.83	1.47	0.36
	2	2	3.75	1.75
	3	3	4	1
	Mean	2.61	3.07	0.46
FS	1	1.5	1.5	0
	2	6	6	0
	3			
	Mean	3.75	3.75	0

and deeper root network impart a competitive advantage to liana species in dry environments that they don't enjoy in wetter ecosystems where water stress is less prevalent. The implication for classification is that their drought adaptation allows lianas to respond differently to seasonality in precipitation than trees, with later leaf loss following the onset of the dry season (Kalacska et al., 2005), and generally higher leaf water content (Andrade et al., 2005; Schnitzer, 2005). Sanchez-Azofeifa et al. (2009) propose a *liana syndrome*, referring to a distinct set of plant traits exhibited by liana species in dry forest environments. They found that the drought adaptations noted by Schnitzer (2005) and Andrade et al. (2005) manifest in lianas as higher spectral reflectance, higher transmittance, and lower absorbance, producing reduced heat load, leaf-to-air vapor

pressure difference and potential for water stress. These traits are revealed in dry forest environments, where they confer an advantage to lianas relative to the surrounding tree species (Sanchez-Azofeifa et al., 2009). More efficient resource allocation in these drier environments results in lower susceptibility to drought and a longer growing season than the surrounding trees. This has led to a general increase in liana load in tropical dry forests (Schnitzer et al., 2011). As well as registering at the leaf level, this syndrome potentially affects remote monitoring of forest phenology where a positive shift in the liana/tree ratio causes an apparent increase in greenness during the onset of the dry season. This variation is reflected in the estimated leaf water content (from spectral water index values) when the two structural groups are considered independently, though it is masked when the groups are merged.

4.2. Classification structure

Our results indicate that the variation imposed by seasonality on leaf optical properties is more than sufficient to affect the results of clustering and classification processes applied to the spectral data. Dendrograms resulting from agglomerative clustering of the full spectral dataset (Fig. 4) were analyzed for variation between seasons in both the composition of the clusters produced and the branching structure of the dendrograms themselves.

If seasonality were unimportant, the same type of spectral data, clustered in the same manner, would yield clusters where the same species were found in close association to each other in both seasons. Our results indicate that this is seldom the case, with consistency between the seasons not exceeding 25%, and as results as low as 6.6% at the dry forest site. That consistency should be lower at the drier of our study sites is generally consistent with Asner et al. (2009), who found stronger associations between biological and spectral properties in wetter, cooler forest environments. There is, however, some inconsistency in the literature on this count. Both Castro-Esau et al. (2004) and Kalacska et al. (2007) found that classification at a structural group level (separating tree and liana species) was more accurate in dry forest environments. It's possible that this seeming contradiction is a function of the level of analysis (structural group vs. species) related to differing adaptive strategies of lianas and trees. In Castro-Esau et al. (2004) lianas were found to have lower overall chlorophyll

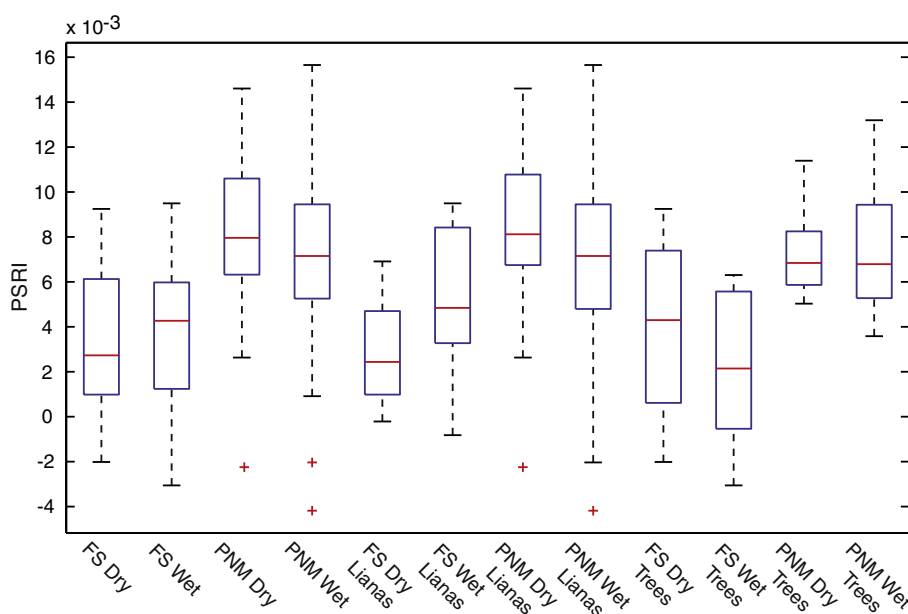


Fig. 5. PSRI compared between seasons and structural groups.

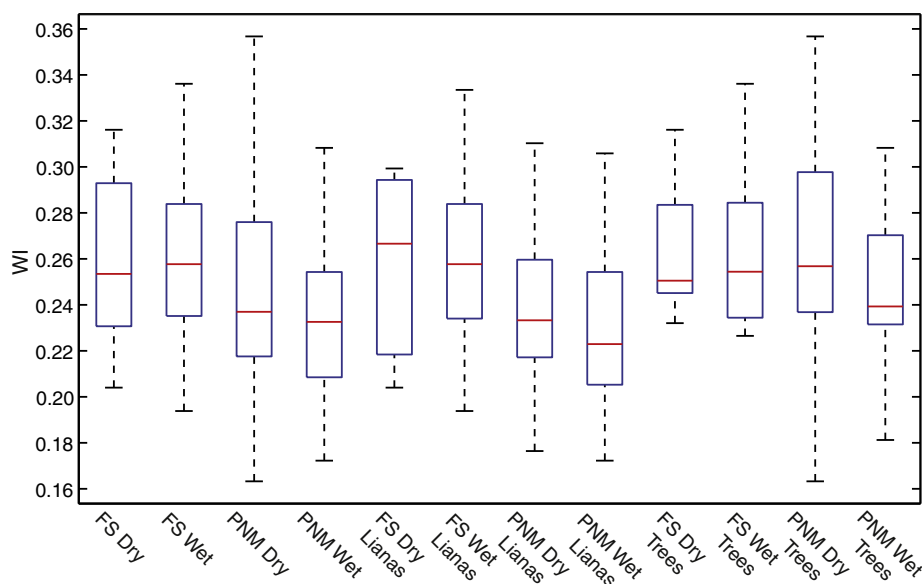


Fig. 6. WI compared between seasons and structural groups. Outliers are represented by crosses.

concentration which, when coupled with and offset phenological cycle and a greater tendency towards deciduousness (Avalos and Mulkey, 1999), may help explain why lianas were more easily distinguished from trees during the dry season. This ecophysiological distinction between liana and tree species in tropical dry forest has since been expanded upon by Sanchez-Azofeifa et al. (2009).

The changes imposed on clustering results imposed by seasonal spectral variation were addressed in this paper using Horton's bifurcation ratio (Strahler, 1957). This approach allows for comparison of both the internal and overall structural variability of the dendrograms generated by the clustering process with time as the independent variable. In comparing R_b between seasons at our two sites, we find that not only are the final clusters affected by inter-seasonal variation (wet vs. dry), but also the internal structure of the dendrograms. The absolute difference in R_b between seasons also shows a relationship to the sample size and taxonomic complexity of the data being processed. While differences in R_b are found at all levels of μ (including the overall mean) at both sites when the entire datasets (all tree and liana species) are included, these differences are greatly muted when only the liana species were considered.

4.3. Effects of seasonality on classification accuracy

Classification was successful at each site, with accuracies ranging from 80.43% to 93.48%, provided that both training and testing data were drawn from the same season. This is consistent with the accuracies reported by Clark et al. (2005), with an accuracy of 92.0% in classifying a set of 7 tree species in Costa Rica, and Castro-Esau et al. (2006), who reported accuracy of better than 80% at each of six sites in Costa Rica, Panama, and Mexico. Certainly sample size has an influence on overall accuracy. Castro-Esau et al. (2006) report a decreasing trend in classification test accuracy from approximately 85% for 20 species to approximately 80%, projecting a linear decrease to 69% with 100 species, with accuracy eroding beyond that. Our results show a similar relationship between sample size and accuracy, though our sample size is smaller overall and the decrease in sample size when structural groups are considered separately is accompanied by a decreased taxonomic complexity, which may also influence the accuracy of the classification. Our highest classification accuracies, both within-season and between-season, are found where the sample size is smallest. Where only trees are considered, dropping the overall sample to 8 species at both sites, we achieve an average within-season accuracy of approximately 90%,

in line with Clark et al. (2005). Where the sample is largest, accuracy drops to values similar to Castro-Esau et al. (2006), with an average of 82% found for a sample of 30 species of mixed trees and lianas.

Recent work has made it clear both that liana species differ at the leaf trait level from tree species (Castro-Esau et al., 2004, 2006; Kalacska et al., 2007) and lianas exhibit a different set of traits and adaptations that seem to be tied to the local environment and manifest both physiologically and spectrally (Sanchez-Azofeifa et al., 2009). In the context of this study, this implies that not only an understanding of within-species inter-seasonal variation, but the effects of environment (particularly the contrast between rainforest and dry tropical forests) are essential for the implementation of accurate automated classification on a broad scale. This issue is exacerbated by what appears to be increases in overall liana abundance in American tropical forests (Schnitzer et al., 2011).

While the contributions of liana species to the mix of spectral reflectance were found at the leaf level, the unique characteristics of lianas suggest a greater need for caution in the process of scaling these leaf-level results to the canopy and landscape scales. Sanchez-Azofeifa and Castro-Esau (2006) noted two impacts of increased liana abundance at the canopy scale: First, that overall reflectance near the green peak (550 nm) was higher in canopies with higher levels of liana infestation and; Second, that liana infestation reduced the difference in spectral between tree species. They note in particular the difficulty that this implies for the potential use of SVIs in differentiating tree species. This complication may be added to the issue of seasonal spectral variation addressed in this paper. Not only are the leaf-level spectra of trees and lianas highly variable between wet and dry seasons, but these two structural groups respond to seasonality differently in changing environments and further variability in liana infestation can obscure spectral characteristics at the canopy (and coarser) scales.

5. Conclusions and directions for expansion

Our results confirm that inter-seasonal variation in leaf optical properties is measurable and sufficient to preclude automated classification of species at the leaf level using a "database" approach. Analysis of dendrograms derived from an agglomerative clustering of full-range spectra shows strong differences in the arrangement of species when spectral data is collected in the wet versus the dry season, and that neither clustering seems to follow any pattern consistent with

Table 4

Significance values for differences between sites and seasons for the two SVIs used as inputs to classification. Strongly significant ($P < 0.05$) differences in bold. Weakly significant ($P < 0.10$) differences indicated with *. Cases where t -test assumptions were not met and Mann–Whitney used in place indicated with †.

	FS wet	FS dry	PNM wet	PNM dry	FS L wet	FS L dry	PNM L wet	PNM L dry	FS T wet	FS T dry	PNM T wet	PNM T dry	IM
FS wet	1.000	0.955	0.204	0.023	0.850 †	0.900	0.051*	0.011	0.810	0.806	0.874	0.397	
FS dry	0.779	1.000	0.202	0.026	0.821	0.875	0.059*	0.014	0.865	0.857	0.911	0.415	
PNM wet	<0.001	0.001	1.000	0.311	0.850 †	0.409	0.539	0.183	0.240	0.247	0.317	0.397	
PNM dry	0.007	0.016	0.216	1.000	0.121	0.113	0.709	0.663	0.046	0.974	0.093*	0.416	
FS L wet	0.706	0.055	0.001	0.013	1.000	0.958	0.185	0.073*	0.713	0.724	0.806	0.604	
FS L dry	0.214	0.346	0.045	0.293	0.129	1.000	0.175	0.07*	0.810	0.776	0.845	0.584	
PNM L wet	<0.001	0.001	0.818	0.235	0.001	0.055*	1.000	0.433	0.065*	0.079*	0.150	0.558	
PNM L dry	0.022	0.046	0.227	0.847	0.034	0.415	0.216	1.000	0.023	0.030	0.068*	0.278	
FS T wet	0.729	0.902	<0.001	0.097*	0.542	0.519	0.017	0.170	1.000	0.984	0.984	0.352	
FS T dry	0.394	0.314	0.001	0.007	0.603	0.091*	<0.001	0.021	0.366	1.000	0.978	0.390	
PNM T wet	0.006	0.019	0.618	0.761	0.002	0.148	0.546	0.677	0.105 †	0.003	1.000	0.522	
PNM T dry	0.008	0.021	0.727	0.684	0.004	0.166	0.643	0.615	0.077*	0.006	0.864	1.000	
PSRI													

species taxonomy or structural group. Further, even using the moderate sample size of the current study, we found differences in the internal structure of the dendrograms, quantified by comparison of Horton's bifurcation ratio. The effect that these seasonal differences in spectral properties has on the potential for automated species classification is reflected in the dramatic decrease in accuracy found when comparing within- and between-season classification accuracy using a non-parametric classifier. Our results here indicate a general ten-fold decrease in overall accuracy when a classifier trained using

data from the wet season is applied to data from the dry season, or vice versa. This result reinforces previous work, which found a strong influence of environment on classification accuracy (Castro-Esau et al., 2004; Kalacska et al., 2007).

We recognize, however, that our results here are based on analysis of purely optical data, with spectral indices standing in for measured biochemical data. The promising results of Asner and Martin (2008) and Asner et al. (2009) build on relationships between leaf optical properties and leaf chemistry, but exploration of the strength and consistency of

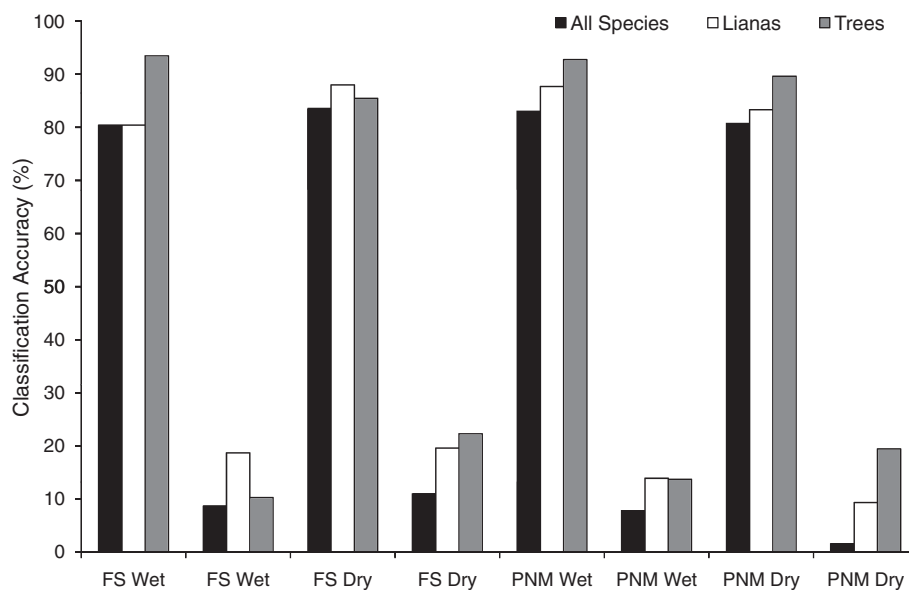


Fig. 7. See5 classification accuracy for both wet (FS) and dry (PNM) sites, expressed as percentage. Where training and testing data are taken from the same season, only that season is labeled. Where training and testing data are from opposing seasons, the label reflects training and testing seasons as: train (test).

these relationships across temporal and geographic gradients must be a priority of research to come. Our findings using purely spectral data point to the value of further study, expanding the seasonal data collection to include a biochemical survey of the leaves collected to complement the spectral analysis. Such an expanded analysis would allow for the exploration of not just the extent of seasonal spectral variation, but also the sources and drivers to this change.

Acknowledgements

We thank Margaret Kalacska and Gregory Asner for their comments and suggestions to early versions of this manuscript. We also thank Ms. Mirna Samarniego for her assistance during the collection of the field data at the Panama canopy cranes. This work was supported by a Discovery Grant from the Canadian Natural Sciences and Engineering Research Council (NSERC), and by a grant from the Inter-American Institute for Global Change Research (IAI) CRN II # 021 which is supported by the US National Science Foundation (Grant GEO-0452325).

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