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Temporal dynamics and leaf trait variability in Neotropical dry forests

by

Michael Sean Hesketh

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ABSTRACT

This thesis explores the variability of leaf traits resulting from changes in season, ecosystem successional stage, and site characteristics. In chapter two, I present a review of the use of remote sensing analysis for the evaluation of Neotropical dry forests. Here, I stress the conclusion, drawn from studies on land cover characterization, biodiversity assessment, and evaluation of forest structural characteristics, that addressing temporal variability in spectral properties is an essential element in the monitoring of these ecosystems. Chapter three describes the effect of wet-dry seasonality on spectral classification of tree and liana species. Highly accurate classification (> 80%) was possible using data from either the wet or dry season. However, this accuracy decreased by a factor of ten when data from the wet season was classified using an algorithm trained on the dry, or vice versa. I also address the potential creation of a spectral taxonomy of species, but found that any clustering based on spectral properties resulted in markedly different arrangements in the wet and dry seasons. In chapter 4, I address the variation present in both physical and spectral leaf traits according to changes in forest successional stage at dry forest sites in Mexico and Costa Rica. I found significant differences in leaf traits between successional stages, but more strongly so in Costa Rica. This variability deceased the accuracy of spectral classification of tree species by a factor of four when classifying data using an algorithm trained on a different

successional stage. Chapter 5 shows the influence of seasonality and succession on trait variability in Mexico. Differences in leaf traits between successional stages were found to be greater during the dry season, but were sufficient in both seasons to negatively influence spectral classification of tree species. Throughout this thesis, I show clear and unambiguous evidence of the variability of key physical and spectral leaf properties over various temporal scales, with the conclusion that an understanding of this variability must play a central role in the establishment of monitoring techniques for dry forests.

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Chapter 1: Introduction

1.1 Introduction

In recognition of the paucity of research being carried out in the dry forests versus more humid tropical biomes, Sánchez-Azofeifa *et al.* (2003) suggested three areas where concentrated remote sensing research would best contribute to an overall better understanding of these environments. First they advocate for an application of satellite remote sensing techniques to identify and map the extent of dry forests, particularly secondary forests in various stages of regeneration. Second, they suggest that methods of estimating forest biophysical properties such as leaf area index be developed using spectral analysis. These properties are key inputs to models of forest productivity and their estimation using remote sensing allows for inexpensive and efficient evaluation of this variable over large areas (Roughgarden *et al.* 1991). Finally, they point to the increasing availability of hyperspectral data sources as an opportunity to develop techniques for the identification of individual tree species based on their reflectance characteristics (Foody 2003; Kerr and Ostrovsky 2003).

Of these three research areas, the first two have been investigated with good success in the intervening decade. Miles *et al.* (2006) estimated global dry forest cover using MODIS data and Portillo-Quintero and Sánchez-

Azofeifa (2010) focused on the extent and distribution of the tropical dry forests in the Americas, also addressing the issues of forest fragmentation and conservation status. While not incorporated into the above estimations, differentiation among early, intermediate, and late successional stages has been carried out with success by Arroyo-Morra *et al.* (2005) and Hartter *et al.* (2008) using Landsat ETM+ and TM data, respectively. More recently, Castillo-Nunez *et al.* (2012) used high resolution LiDAR Vegetation Imaging System (LVIS) data to differentiate among successional stages, also providing additional detail in the intermediate stage. Leaf area index and biomass have been modeled in the dry forest through the application of empirically derived relationships with spectral indices (Kalácska *et al.* 2005b) as well as Bayesian networks (Kalácska *et al.* 2005a), applied to ETM+ data.

The third research priority highlighted by Sánchez-Azofeifa *et al.* (2003) has proven the most complex. Though the high levels of species diversity in the tropics pose an obvious challenge in spectrally based species detection and biodiversity assessment relative to more species-poor temperate environments, Clark *et al.* (2005) and Asner and Martin (2008) have shown the potential for the distinction between species at both the crown and leaf scales, respectively, in the humid tropics. Ultimately, the process of species classification based on spectral reflectance rests on the assumption that reflectance is more variable between species than among individuals of the same species (Cochrane 2000). Casting this into the context of the highly seasonal tropical dry forests, it is precisely this variability that must be assessed in evaluating the potential for species discrimination.

One of the advantages of a remote sensing approach to the analysis of biophysical properties is the ability to reasonably survey not only across space but time (Cohen and Goward 2004; Nagendra 2001). While the impact of changes in geography and altitude on forest biophysical and spectral properties have been acknowledged and investigated, (Asner and Martin 2008; Asner *et al.* 2009; Castro-Esau *et al.* 2006; Martin *et al.* 2007) and the effects of seasonality on spectral properties have been shown to influence the potential for discrimination between trees and lianas (Castro-Esau *et al.* 2004; Kalácska *et al.* 2007), the temporal domain has been less fully explored. Particularly in highly seasonal environments such as tropical dry forests, this presents an important knowledge gap, which must be filled in order to take full advantage of the potential for remote sensed analysis to contribute to our understanding of forest properties.

The question under investigation in this thesis is whether leaf physical, chemical, and spectral properties show measurable variability in response to changes in season and forest successional stage in tropical dry forest environments. An improved understanding of diversity in leaf chemical and physical characteristics is a necessary component of better understanding ecosystem function (Townsend *et al*. 2007). Similarly, the effect that this variability has on leaf spectral response will influence the potentential for remote sensing of biodiversity and ecosystem function using leaf spectral properties (Asner and Martin 2008).

This thesis is comprised of four separate stand-alone papers, related by the common theme of furthering our understanding of tropical dry forests, and in particular their temporal variability, through exploration of the relationships between biophysical properties and ecosystem characteristics and their impact on leaf spectral properties. Particular emphasis is placed on exploration of temporal variation in leaf-level spectral and biophysical traits between seasons and successional stages at two tropical dry forests located in Mexico and Costa Rica.

Chapter 2, *"Review of remote sensing of tropical dry forests"* (Hesketh and Sánchez-Azofeifa, 2013), contextualizes the discussion with a detailed review of the state of the art in remote sensing in dry tropical environments. As in other disciplines, research in dry tropical remote sensing has lagged behind that conducted in temperate and rainforest ecosystems, and researchers face unique challenges stemming from the extreme variability of these forests. This chapter focuses on 1. The use of satellite remote sensing in detecting and mapping dry forests, 2. The estimation of dry forest biomass and productivity using remotely sensed data, 3. The potential for the assessment of biodiversity through leaf-level spectral discrimination between dry forest species and plant structural groups, and 4. The application of optical remote sensing data in conjunction with LiDAR in the assessment of forest structure and successional stage. The review concludes with a discussion of the obstacles and opportunities related to furthering our understanding of tropical dry forests through remote sensing, including infrastructure and organization, emerging data sources and analysis techniques, and the requirement for better understanding of the temporal domain.

Directly addressing the effect of the extreme seasonality of tropical dry forests, the main objective of Chapter 3, *"The effect of seasonal spectral variation on species classification in the Panamanian tropical forest"* (Hesketh and Sánchez-Azofeifa, 2012)**,** is to quantify the impact of seasonality on the potential for species level discrimination based on leaf-level spectral reflectance. Dendrograms derived from the spectral reflectance of 47 species of trees and lianas area analyzed based on a novel application of Horton's laws as a means to investigate the variation in leaf optical properties of species collected in both wet and dry forest. Additionally, the impact of this spectral variation on species classification using a non-parametric decision tree classifier is explored. In the context of research into the relationship between taxonomy and spectral reflectance (Asner and Martin 2008; Asner 2008; Clark *et al.* 2005; Doughty *et al.* 2011) understanding the potential for temporal variation in leaf spectral properties is an important step towards better understanding the consistency of the relationship and its utility in spectral classification.

Chapter 4, *"Successional variation in spectral and biophysical leaf traits in two American tropical dry forests",* focuses again on temporal variation in leaf spectral properties, looking at the effect of forest successional stage on variation in leaf spectral reflectance as well as a suite of biophysical and chemical variables. This work was carried out at the Chamela Cuixmala Biosphere Reserve (CCBR) in Mexico and Santa Rosa National Park (SRNP) in Costa Rica which, while both dry forests, have different climatic and edaphic characteristics. Most notably, SRNP receives almost twice the growing season precipitation as does CCBR. Through statistical analysis, variability in leaf water content, specific leaf area, pigment concentration, foliar chemistry, and spectral properties are investigated. Classification analysis is used to quantify the extent to which this variability impacts the potential for accurate biodiversity assessment based on spectral reflectance. In the context of a body of influential research that stresses the global consistency of leaf trait relationships across biomes (Reich *et al.* 1997; Wright *et al.* 2004), addressing the variability between successional stages within a single site highlights the importance of microclimate and plot-level differences in growing conditions in controlling leaf traits.

The objective of Chapter 5, *"Effect of season and successional stage on leaf properties in a tropical dry forest",* is to combine the temporal gradients considered in chapters 3 and 4, and investigate seasonal variability in leaf biophysical, chemical, and spectral properties at changing successional stages. This work was conducted over two periods at CCBR encompassing the height of the wet season and the last weeks of the growing season in the period of fall senescence. The methodologies from chapter 4 are adapted to show the changes in the effect of seasonality between early, intermediate, and late stages of dry forest succession at a single site. The importance of secondary forests in the neotropics (Joseph Wright and Muller-Landau 2006; Wright 2005; Wright and Muller-Landau 2006) implies that an understanding of the dynamics of forest succession is an integral part of tropical forest monitoring. As such, an appreciation of the impact of forest succession on leaf traits is a necessary prerequisite for the monitoring of the forest regeneration. This underscores the importance of a thorough understanding of the temporal sources of biophysical (and the resulting spectral) variability before undertaking any kind of biodiversity analysis, particularly in the seasonal tropics.

The four chapters that make up the body of this thesis emphasize the importance of community-scale variability at a time when a focus of influential research (e.g. Asner and Martin 2008; Asner *et al.* 2011; Wright *et al.* 2004) is on the establishment of unifying trends. This thesis closes with a summary outlining the conclusions reached in the preceding chapters in the context of the importance of the temporal domain in understanding biophysical and spectral variability at the leaf level, and the implications and challenges for future research.

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Chapter 2: Review of remote sensing of tropical dry forests*

2.1 Introduction

For more than twenty years, tropical dry forests (TDFs) have been recognized among the world's most threatened ecosystems (Janzen 1988; Murphy and Lugo 1986b; Olson 2000). These forests account for 49 percent of the vegetated land cover in Mesoamerica and the Caribbean and 42 percent of all tropical forest vegetation worldwide (Murphy *et al.* 1995; Van Bloem *et al.* 2004) with a current estimated total global cover of 1,048,700 km^2 (Miles *et al.* 2006), and are often areas of intense human occupation and exploitation (Murphy and Lugo 1986a; Quesada and Stoner 2004; Sánchez-Azofeifa *et al.* 2005a). In spite of this, these forests have been the subject of only a fraction of the research devoted to tropical forests globally, with the majority of study over the past 60 years focused on tropical humid forests or rainforests (Sánchez-Azofeifa *et al.* 2005b). With increasing concern over the health and conservation status of TDFs (Stoner and Sánchez-Azofeifa 2009), there is a need for tools to better map and understand these important resources.

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The benefit of satellite remote sensing to forest ecology is the potential for a systematic, synoptic view of the earth at large spatial scales and at regular intervals (Cohen and Goward 2004; Nagendra 2001; Roughgarden *et al.* 1991). Satellite data have been used for the mapping and monitoring of the distribution and change of plant species and land cover types, deforestation, fire and insect damage, and human impact on the environment. Additionally, these data have been used in the extraction of biophysical characteristics (e.g. total above-ground biomass, leaf and woody area indices (LAI and WAI, respectively), and canopy cover) which are key components in a variety of ecological models, as well as calculations of carbon balance and primary production (Castro-Esau *et al.* 2004; Foody 2003; Kerr and Ostrovsky 2003; Lambin 1999; Nagendra 2001). At smaller scales, the use of high spectral resolution data at the leaf and crown level has allowed for the evaluation the spectral elements of leaf properties and their variation between and among species, structural groups, locations, and seasons (Hesketh and Sánchez-Azofeifa 2012), in a manner that may be adapted to larger spatial scales and used to better understand ecosystems at the plot level and beyond (Asner and Martin 2008b).

The objective of this review is to explore the applications of remote sensing to forest studies and the use of these tools and techniques in better understanding TDFs. Following a discussion of the relationship between spectral reflectance and biophysical and structural properties of vegetation, the chapter will review remote sensing challenges and findings in TDF research, grouped broadly under four main areas: 1. The use of satellite remote sensing data as an input to the classification of TDFs regionally and globally, with the aim of mapping their extent and distribution, 2.The estimation of TDF forest biomass and productivity, 3.The assessment of biodiversity through spectral evaluation of species and plant structural groups and 4. The application of optical and LiDAR remote sensing in the assessment of forest structure and successional stage.

2.2 Remote Sensing of Vegetation

2.2.1 Spectral Characteristics of Green Vegetation

Investigation into leaf optics dates back to the first half of the twentieth century (Billings and Morris 1951; Gates *et al.* 1965; Loomis 1965; Shull 1929), and a prime objective of leaf spectroscopy has been to relate leaf optical properties to chemical and biophysical characteristics. Spectral reflectance at the leaf level is influenced by three characteristics: 1) the internal cellular structure of the leaf, 2) leaf pigment content, and 3) orientation relative to solar radiation (Turner *et al.* 1999). While leaf morphology is highly variable by species and phenological stage, it tends to be characterized by a relatively open structure, with palisade and spongy mesophyll cells sandwiched between an upper and lower epidermis. The upper layer of the epidermis, called the cuticle, is a thin waxy coating regulating transmittance of radiation into the leaf's internal structure. Chloroplasts are found throughout the palisade and mesophyll cells, but tend to be concentrated towards the upper side of the leaf, provided that the leaf has a horizontal orientation relative to the sun (vertically-oriented or erectophile leaves tend to have chloroplasts distributed along both edges (Jensen 2000)).

Radiation interacts with the leaf though absorption and scattering. The cell structures within the leaf are large with respect to the wavelengths of light, though the hair-like strands called grana within the chloroplast are small enough (approximately 0.5x0.05 µm) to induce some scatter. Plants absorb the ultraviolet and visible wavelengths efficiently, though absorption decreases dramatically through the near and mid infrared parts of the spectrum (0.70 - 1.8 µm). The structural components influencing these decreases in the longer wavelengths are the large intercellular spaces in the spongy mesophyll (the site of 0_2 and CO_2 exchange), which result in high internal scattering of radiation in the near infrared region, reducing absorption and increasing reflectance of these wavelengths. This lowered absorption in the higher-energy part of the spectrum is a mechanism to control the thermal properties of the leaf, preventing overheating (Gamon *et al.* 2005). Water vapor saturating these intercellular spaces interacts with mid infrared radiation, resulting in absorption peaks at 0.97, 1.19, 1.45, 1.94, and 2.7 µm, and increased reflectance between them (Gates *et al.* 1965).

Leaf pigment content most directly affects the spectral response in the visible range of the spectrum (0.4-0.7 μm). Chlorophylls *a* and *b* absorb strongly in the blue and red wavelengths, and much less so in the green. The presence of other pigments within the leaf (carotenes and xanthophyll cycle pigments) with similar absorption characteristics broadens these absorption peaks. The ratio between this high absorption in the red region and high reflectance in the near infrared region is exploited in a significant portion of the index-based approaches to vegetation monitoring (le Maire *et al.* 2004). As the leaf matures from initial flush to senescence pigment levels shift, causing an alteration to the measured reflectance and the apparent color of the leaf as chlorophyll levels increase and decrease throughout the growing season, replaced by carotenes and anthocyanin (Gates *et al.* 1965).

2.2.2 Satellite Analysis of Vegetation

Aside from the issues of appropriate spatial, spectral and temporal resolutions associated with all satellite remote sensed analyses (Nagendra 2001), there are challenges associated with mapping and monitoring vegetation using airborne and satellite-derived spectral reflectance data. In particular, the potentially distorting effects of the atmosphere, topography, canopy architecture, and the influence of soil on spectral reflectance must be understood and accounted for as a preliminary to any analysis.

The effect of the atmosphere on reflectance is described as the difference between the actual top of copy (TOC) reflectance and measured top of atmosphere (TOA) reflectance. For remote sensed projects where data are compared over either time or space, the effects of light scatter in the atmosphere due to dust and aerosols must be accounted for (Myneni *et al.* 1995b). Song *et al.* (2001) suggest several means of correction, which are summarized as follows. The first is dark object subtraction (DOS), by which the effects of atmospheric scatter are registered as the brightening of the darkest objects in a scene. The DOS method uses this difference to reduce the brightness of the overall image relative to the difference between the measured and assumed actual reflectance of these dark objects. The path radiance (PARA) approach uses a similar set of relationships between the blue, red, and middle infrared bands to approximate and correct for the effects of atmospheric aerosols. Finally, relative atmospheric correction takes advantage of the presence of pseudo invariant features over a time series of images to account for atmospheric effects. These features, such as rock outcrops or built structures, may be used to adjust each image so that the reflectance of these features is standardized throughout the time series. This method does not require estimation of atmospheric optical properties and can correct for systematic variance as well as atmospheric, but is unsuitable in cases where extreme phenological or environmental changes have take place between the images, or where images are spatially distributed. Similarly, topographic and forest structural characteristics can influence the measured reflectance. Distortions due to bi-directional reflectance from the canopy may cause "hot spots" in imagery (resulting from sun/sensor geometry which places the sensor between the sun and the canopy, resulting in an artificially brightened image), and changes in canopy closure, gap spacing, and leaf clumping with the canopy can also induce variation in reflectance (Myneni *et al.* 1995b).

Because the radiance measured above a vegetated surface is a composite of both the vegetation itself and the background surface (typically soil), it is important to appreciate the contribution of both. Soil effects are most evident in areas of low canopy closure and low leaf area index (LAI), and spectral indexing methods may attempt to reduce sensitivity to its effects for studies where soil registers as background noise, obscuring the object of interest (McDonald *et al.* 1998). Conversely, when surface parameters such as the albedo are under study, it is important to accurately include the soil's contribution to total reflectance.

Table 2-1 details the air- and space-born optical sensors most commonly used in forest analysis. While multispectral sensors still provide the majority of large-scale satellite data, hyperspectral data, characterized by many narrow contiguous spectral bands, have become increasingly common in classification studies and the estimation of forest biophysical characteristics. While the hyperspectral sensors listed are carried aboard aircraft or satellites, important primary research is also carried out at the leaf scale using small, portable field spectrometers.

Sensor	Spatial Resolution (m)	Spectral Coverage (nm)	# Spectral Bands
NOAA AVHRR	1000	580 - 11500	5
MODIS	250 - 1000	620 - 14385	36
Landsat TM and ETM+	28.5	450 - 12500	7
IKONOS	4	445 - 853	4
EO-1 HYPERION	30	400 - 2400	220
CASI	0.25 - 1.5	380 - 1050	288
HyMap	3 – 10	450 - 2500	126
AVIRIS	20	400 - 2500	224

Table 2-1. Overview of the spatial and spectral characteristics of the commonly used platforms for earth remote sensing.

2.3 Mapping the Extent and Distribution of TDFs

The loss lost of TDF cover noted by Janzen (1988) prompted his inclusion of these forests among the world's most threatened biomes. Taking into account the high degree of biodiversity and endemism found in TDFs (Gentry 1982; Gillespie *et al.* 2000; Lott *et al.* 1987) and the concern over forest degradation and fragmentation (Sánchez-Azofeifa *et al.* 2009), the importance of the development of a clear inventory of TDF cover is clear. Previous assessments of ecosystems at risk have largely failed in addressing TDFs status as distinct from other tropical forest biomes (Miles *et al.* 2006), though areas containing high proportions of TDFs have been identified as biodiversity hotspots (Myers *et al.* 2000). Estimates of TDF cover and distribution are important tools not only for planning and conservation, but form part of an overall census of land cover. Inaccuracies in these forest maps, typically manifest as an overestimation of tropical rain forest and the corresponding underestimation of TDF (Portillo-Quintero and Sánchez-Azofeifa 2010), impact the accuracies of estimations of carbon stocks which, in addition to their importance ecologically, are essential for the implementation of conservation strategies based on payments for environmental services (Kalácska 2005).

In addition to methodological challenges which may vary based on the remote sensing platform and the analysis techniques employed in classifying TDF cover, two issues are consistent in the literature: a lack of consensus on just what constitutes TDF, and the timing of image acquisition, taking into account the inherent seasonality of these forests.

There is general agreement that the basic characteristics of TDFs are high temperatures, moderate but seasonal precipitation, and a forest canopy dominated by deciduous trees (Murphy *et al.* 1995; Murphy and Lugo 1986a). Holdridge (1967) defines tropical and sub-tropical TDFs as those in frost free zones with a mean annual temperature > 17°C, between 250 and 2000 mm of annual precipitation and a ratio of potential evapotranspiration to precipitation in the range of 1 – 2. Sánchez-Azofeifa *et al.* (2005b) amend this, specifying a mean temperature of ≥ 25°C, precipitation between 700 and 2000 mm and a minimum of three dry months per year of drought where precipitation does not exceed 100 mm per month. Inconsistency among vegetation classification is of particular importance when comparing across large geographic areas or among studies with varying methodologies (Blasco *et al.* 2000).

The second issue is the variability of vegetation spectral reflectance in response to phenological changes throughout the growing season. Since TDFs are intensely seasonal by definition, it follows that spectral response follows similar annual patterns. A disregard for this variability may be responsible for the traditional misrepresentation of TDFs in satellite-based land cover assessments (Sánchez-Azofeifa *et al.* 2001). Both Kalácska *et al.* (2007b) and Portillo-Quintero and Sánchez-Azofeifa (2010) suggest the superiority of satellite data collected during the dry season for accurate classification of TDFs.

The only remote sensed analysis of TDF cover at a global scale was carried out by Miles *et al.* (2006). Using a biogeographic classification scheme developed by Olsen (2001) and including tropical and subtropical grasslands, savannas and shrublands as well as tropical and subtropical dry broad-leaved forests as locations of *potential* TDF cover, they produced a potential cover map based on Vegetation Continuous Fields data at a 500 m resolution from MODIS. Their findings indicate 104,700 km^2 total potential TDF cover globally with the majority (66.7%) located in the Americas. The authors further analyze the degree of forest change during the period of

1980-2000 using 8 km resolution data from the Advanced Very High Resolution Radiometer (AVHRR). Deforestation during this period was greatest in Latin America, with an estimated 12% total decrease as compared to an average estimated decrease of 2% throughout Asia and similarly low values across most of Africa. Also addressed was the risk to TDF areas from several potential threats: climate change, forest fragmentation, fire, conversion to agriculture, and increasing human population density. The study suggests that only 3.3% of forest cover global is not subject to threat from one of these sources, with 31.7 – 59.2% subject to three or more and >95% subject to at least two.

More recently, Portillo-Quintero and Sánchez-Azofeifa (2010) applied an approach based around spectral classification rather than the use of previously processed land cover products to generate a map of the extent and distribution of TDFs throughout the Neotropics. MODIS surface reflectance data were acquired during the dry season to improve the separability of TDF from surrounding semi-deciduous and evergreen forests (Kalácska *et al.* 2007b) and mosaicked into a continuous image covering the American tropics. Using training sites collected from Landsat TM and ETM+ imagery over known vegetation types and validated using high-resolution imagery from Google Earth (Google Inc. 2012), the MODIS data were processed with the non-parametric decision tree classifier See5 (Rulequest Reseach 2008). The resulting map shows a total of 519,597 km^2 of TDF across the Americas, with the greatest cover found in Mexico (38%), Bolivia (25%) and Brazil (17%). Comparing this extent to the potential extent of the "tropical and subtropical broad-leaf forest" defined by Olson (2001), an average of 66% loss to anthropogenically attributed deforestation or conversion was noted across the study area.

Equally important as an accurate assessment of the extent and loss of TDFs derived from distribution maps is an understanding of the degree of continuity of the biome. Forest fragmentation poses a major risk to the health of both flora and fauna, with smaller fragments (<10 km^2) found to have higher rates of species extinction and a greater risk of conversion to other land covers (Laurance *et al.* 2002; Rodriguez *et al.* 2007a; Rodriguez *et al.* 2007b). Sánchez *et al.* (2009) used a classification of TDFs based on data from NASA's 15 m Advanced Spaceborne Thermal Emission and Reflection Radiometer satellite (ASTER) to evaluate the degree of fragmentation in protected versus unprotected areas around Mexico's Chamela-Cuixmala Biosphere Reserve. They found that while both the size and number of forest fragments remained relatively constant within a 15 km radius of the reserve, beyond that boundary the number of patches increased while the average patch size decreased considerably. Portillo-Quintero and Sánchez-Azofeifa (2010) examined the proportions of forest fragments falling into ≤ 2.5 km^2, 2.5 - 10 km^2, and ≥ 10 km^2 classes across the Neotropics, finding generally high proportions of forest in the > 10 km^2 class, with the lowest found in Peru and Costa Rica. Both the above studies consider the impact of protected areas on TDF conservation and health. Sánchez-Azofeifa *et al.* (2009) note the trend toward increasing fragmentation and decreasing patch size accompanying increasing distance from protected areas, noting the associated loss of connectivity between the residual fragments as a serious threat to forest biodiversity. In spite of this, Portillo-Quintero and Sánchez-Azofeifa (2010) report that only 4.5% of the TDF cover in the Neotropics is subject to protection, compared with a global estimate of 16-18% of humid forests, savannas and grasslands (Hoekstra *et al.* 2005).

2.4 Assessment of Forest Health (Biomass, Lear Area index, Productivity)

Remote sensed analysis of forests has the potential to provide strong linkages between spectral reflectance and forest biophysical characteristics which may then be used as proxy for physical inputs to models of ecosystem process, biosphere-atmosphere transfer, and carbon exchange (Hall *et al.* 1995; Treitz and Howarth 1999). The goal of studies in this area has been to develop methodology by which to accurately extract characteristics that are not commonly included in forest inventories, such as leaf area index (LAI), total above ground biomass (TAGB), and the fraction of absorbed photosynthetically active radiation (fAPAR) from remotely sensed data over large areas without further fieldwork. Though techniques involving neural networks have shown promise (Carpenter 1997; Kalácska *et al.* 2005; Running *et al.* 1986), research here has focused primarily on two methods. Index-based approaches, based on empirical relationships between the ground-sampled measurements and their spectral properties, exploit differences in characteristic spectral regions of the reflected electromagnetic radiation (Kalácska *et al.* 2004; Running *et al.* 1986; Turner *et al.* 1999). Due to the relative simplicity of application, these methods have been by far the most common (McDonald *et al.* 1998).

Conversely, methods employing the inversion of physically based radiative transfer models have been investigated with some success (Jacquemoud *et al.* 1996; Kuusk and Nilson 2000; le Maire *et al.* 2004). These models, such as those by Li and Strahler (Li and Strahler 1986, 1992), use reflectance as an input to derive biophysical variables such as LAI. Such models calculate canopy reflectance by incorporating nested models of the spectral properties of the individual contributing factors. An example is the model by Kuusk and Nilson (2000), which incorporates the PROSPECT2 leaf optical model (Jacquemoud *et al.* 1996), the 6S atmospheric transfer model (Vermote *et al.* 1997), and the MCRM canopy reflectance model (Kuusk 1995), accounting also forest inventory and structural characteristics, as well as the effects of soil and ground BDRF. Rautainen (2005) found that, while this process only slightly over-predicted LAI values, such an approach is subject to error from a multitude of sources, most importantly the input parameter relating to stand characteristics, which must be taken from forest inventories (if available) or generalized. The ground truth LAI data is also subject to error, as they are generally modeled as well, taken form allometric equations. Even optical methods used for measuring LAI *in situ* operate on the often-incorrect assumption of randomly dispersed clumping in the canopy. Privette *et al.* (1996) similarly note that while inverted physical models have the virtue of accounting for bidirectional effects, and requiring potentially less prior calibration than vegetation indices, they are computationally more demanding, and require *a priori* knowledge of the vegetation characteristic of the study site, which may not be readily available.

Reliable estimates of forest biomass are essential for understanding the importance of forest environmental processes and in regional and global carbon budgeting (Foody 2003; Houghton *et al.* 2001). The most direct method typically employed for biomass estimation is direct correlation with spectral reflectance or, more commonly, with a spectral vegetation index (SVI) such as the normalized difference vegetation index (NDVI), then validation through either destructive forest sampling or comparison with allometric equations derived from previous sampling (Castro-Esau *et al.* 2003). While this approach has been employed with some success in temperate forests (Curran *et al.* 1992; Danson and Curran 1993; Peterson *et al.* 1987), the relationship between SVIs and biomass has been found to be generally poor in the tropics, with their overall denser forest cover, as most indices tend to saturate at higher LAIs (Kalácska *et al.* 2005; Myneni *et al.* 1995a). Foody *et al.* (2001) note only insignificant correlations ($p<0.95$) between forest biomass and 230 permutations of six common ratio-based SVIs calculated from Landsat TM data collected over Borneo, confirming earlier results by Sader *et al. (1989)*.

An alternative to direct correlation with TDF spectral properties has been to exploit the relationship between forest age (or successional stage) and biomass. Issues here, however stem from a poor understanding on the age of succession and the site specificity of the equations used to derive biomass from stand age (Castro-Esau *et al.* 2003)

Leaf area index, defined as the total one sided surface area of all leaves in the canopy within a defined region (typically expressed m^2m^{-2}) (Gong *et al.* 2003), is a key indicator of potential evapotranspiration and thus photosynthesis and stand productivity (Chason *et al.* 1991). As with forest biomass, LAI has often been estimated by the application of regressions between sampled LAI and SVIs, which show high sensitivity to changes in leaf area at low to moderate values, reaching an asymptote at LAI values of 3-5 (Chen and Cihlar 1996; Turner *et al.* 1999; White *et al.* 1997). Numerous ratio-based vegetation indices have been statistically related to LAI, typically exploiting the variation in the red and near infrared reflectance of green plants. Turner *et al.* (1999) suggest that the relationships between vegetation indices from satellite multispectral data may be useful for retrieving LAI, and note some issues that must me managed if these estimations are to be accurate. They remark on the impact of image processing procedures and the importance of corrections for atmospheric effects, particularly when carrying out analyses between multiple sites or dates. They found that, while topographic corrections had a marked effect on the raw vegetation index values, they had little to no effect on the strength of the index-LAI relationships. The prime issue with the SVI approach is the tendency for the SVIs to saturate at the higher LAI levels found in tropical systems (Birky 2001), attributed to the saturation of the individual spectral bands when the forest reaches a certain level of green biomass (Kalácska *et al.* 2004). Turner *et al.* (1999) recognize the tendency for vegetation index values to reach an asymptote at LAIs greater than 5 and note the importance of selecting vegetation indices that are appropriate for the cover type under evaluation. The goal in the development of these indices is to maximize the sensitivity to changes in the characteristic under study (like chlorophyll content) while minimizing sensitivity to background effects (such as the influence of soil or the atmospheric) (Sims and Gamon 2002).

Kalácska *et al.* (2005b) test the relationship of SVIs to LAI in the TDFs at two sites in Costa Rica and a third in Pacific Mexico. They found highly significant correlations between LAI and SVIs (calculated from 28.5 m Landsat ETM+ data) using nonlinear regression with a Lorentzian

Cumulative Function. The best-fit spectral index varied slightly, with the modified simple ratio (MSR) (Chen 1996) providing the strongest result at two sites and the soil adjusted vegetation index 2 (SAVI2) (Qi *et al.* 1994) providing the best fit at the third. Kalácska *et al.* (2005) also explore the use of Bayesian and neural network classifiers applied to Landsat ETM+ data as an alternative approach to estimating dry tropical LAI. Both Bayesian and neural network approaches were found to have a lower testing error than the SVI approach (48.7% and 56.9%, respectively, vs. 64.9%) when tested during the wet season at a TDF site in Costa Rica.

While estimations of both forest biomass and LAI stand as either proxies for forest productivity or inputs into further calculations, there has been some research into more directly measuring productivity via investigation of the relationship between spectral reflectance and photosynthetic rates, both in terms of absorbed photosynthetically active radiation (APAR) and light use efficiency (LUE) at the leaf level. Gamon *et al.* (2005) break down the gross photosynthetic rate into the product of LUE and APAR, adapted from earlier work by Monteith (1977) on the components of net primary productivity (NPP). APAR can be evaluated with commonly used SVIs such as the simple ratio (SR) and NDVI (Gamon *et al.* 1995), and the authors found consistently strong relationships between SR and the measured fraction of absorbed photosynthetically active radiation (fAPAR) at the crown scale at a TDF site in Panama (NDVI was found to saturate over dense canopies). Estimation of the LUE term was through the hyperspectral photochemical reflectance index (PRI), which has been found to be sensitive to xanthophyll cycle activity (Gamon *et al.* 1997; Gamon *et al.* 1992) and LUE at leaf (Gamon *et al.* 1992; Penuelas *et al.* 1995), canopy (Stylinski *et al.* 2002) and stand (Nichol *et al.* 2000; Rahman *et al.* 2001) scales. In the TDF context, leaf scale PRI showed close correlation with measures of leaf fluorescence (a measure of radiation use efficiency (Genty *et al.* 1989)). The authors also noted a depression in PRI coincident with increased incident photosynthetic photon flux densities (PPFD) at the crown scale, supporting the relationship between PRI and LUE noted at the leaf scale and furthering the prospect of the evaluation of net photosynthesis via optical remote sensing.

2.5 Distinguishing Between Species and Structural Groups

Though sensors with moderate spatial and spectral resolution have been effective in identifying and classifying broad forest classes and estimating some forest properties, the high species densities found in both the humid and dry tropics (Myers *et al.* 2000; Zhang *et al.* 2006), as well as the general similarity in leaf reflectance among green vegetation (Portigal *et al.* 1997) make the identification of individual plant species difficult or impossible using these coarser-resolution data. The issue of pixel resolution may be solved by the application of higher resolution data, such as from

IKONOS or Digital Globe's Quickbird satellites, which are able to resolve individual tree crowns for analysis (Clark *et al.* 2005). While these sensors have been used with moderate success in temperate forests (Fuentes *et al.* 2001; Nagendra 2001; Nagendra and Gadgil 1999; Ustin *et al.* 2004), the spectral resolution is insufficient in the context of the high species diversity found in TDFs. As such, the majority of studies have been conducted using leaf-level data collected using laboratory spectro-radiometers.

While the results of studies evaluating species discrimination using optical data in wet tropical environments are encouraging (Clark *et al.* 2005; Cochrane 2000; Gamon *et al.* 2006; Rivard *et al.* 2008) similar studies in TDF environments have been slower in coming. Rather than work at the level of discriminating among individual species, some studies have concentrated on distinguishing between leaves of the two principle structural groups in tropical canopies: trees and lianas (woody, self-supporting vines). This distinction is important as the impact of lianas at the leaf level bears heavily on the potential for automated species detection at the crown level and coarser. Both Castro-Esau *et al.* (2004) and Kalácska *et al.* (2007a) evaluated the spectral separability of these groups at sites in Panama. Both found that trees and lianas could be distinguished accurately using data collected at a dry forest site using a selection of supervised classifiers to process principle component- and wavelet-transformed data. Using the same procedures at a rainforest site, Castro-Esau *et al.* found they were less able to separate the two structural groups. To explain this difference in separability between trees and lianas in TDFs versus tropical rainforests, Sánchez-Azofeifa *et al.* (2009) proposed a liana syndrome, referring to a distinct set of plant traits exhibited by liana species in dry forest environments. They suggest that evolutionary adaptations made by liana species to contend with increased water stress due to seasonal drought, such as delayed leaf loss at the end of the rainy season (Kalácska *et al.* 2005a) and increased leaf water content (Andrade *et al.* 2005; Schnitzer 2005), manifest spectrally as higher spectral transmittance, lower absorbance, and overall increased reflectance. The competitive advantage conferred by these adaptations in dry forests implies the potential that increasing liana cover will be an ongoing consideration for the automated evaluation of TDF biodiversity.

The basis for the differentiation between species or functional groups is that leaf (or canopy) biochemistry is unique for a given species, resulting in a chemical signature that may be used to identify that species, and that this signature is expressed in that species' spectral reflectance (Asner and Martin 2008a; Peterson *et al.* 1988). Though the studies mentioned here have demonstrated strong correlations between taxonomy and leaf optical properties, it is clear that understanding the scope of variation both in leaf spectral properties and the biophysical traits that control them must be a priority. Castro-Esau *et al.* (2006) found sufficient difference between spectra of given species sampled at multiple sites in Costa Rica that accurate classification across sites was impossible. Martin *et al.* (2007) similarly found strong environmentally-attributable variation in pigment and optical characteristics among samples of *M. polymorpha* grown from seed sources spanning a wide environmental (soil-altitude) gradient. Asner *et al.* (2009) found in a study of 162 canopy species across a wide climatic gradient in Australia that, though 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 moderate precipitation levels, which echoes Townsend *et al.* (2007), who found maximal N:P variation according to rainfall in highly seasonal sites in Costa-Rica.

The impact of this chemical and spectral variability is particularly important in the TDF due to its strong seasonality and the accompanying variation in leaf properties. Though the impact of leaf phenology has been well noted (Kalácska *et al.* 2007a; Portillo-Quintero and Sánchez-Azofeifa 2010), spectral analysis has largely been limited to the tracking of NDVI (or similar spectral indices) throughout the growing season using space-borne sensors (Schwartz and Reed 1999; Zhang *et al.* 2003). The importance of season on spectral response in the dry tropics have been demonstrated, however, by Roberts *et al.* (1998) who documented spectral changes associated with leaf senescence in the Brazilian caatinga, and Hesketh and

Sánchez-Azofeifa (2012), who found a tenfold decrease in classification accuracy when applying a single nonparametric classifier across both rainy and dry seasons. Underscoring the impact of seasonal and phenological cycles on forest monitoring. This leaf level spectral variation has been found to be exaggerated when scaled up to the level of the forest canopy (Zhang *et al.* 2006).

2.6 Forest Structure and Successional Stage

TDFs have long been areas of intense human activity (Miles *et al.* 2006), resulting in the high levels of deforestation and fragmentation driven by fire, conversion for agriculture and habitation, and commercial logging (Calvo-Alvarado *et al.* 2009; Colon and Lugo 2006). These forests in the stages of recovery from human-induced disturbance are termed secondary forests (Brown and Lugo 1990). Changes in the economic climate that drove forest degradation in many areas of the dry tropics have resulted in a increased rate of return of these forest on what was once cleared land (Calvo-Alvarado *et al.* 2009). These secondary forests represent an important element of the global capacity for carbon sequestration, but also a source of potential error in the estimation of carbon budgets, as the capacity for carbon uptake is dependent on forests' species composition and the age of the secondary growth (Brown and Lugo 1990; Foody *et al.* 1996; Uhl *et al.* 1988). In response to increasing interest in these young forests and the role they play in ecological and economic models (Feldpausch *et al.* 2004; Koning *et al.* 2005), a need for remote sensing tools to asses the structure and characteristics has been identified (Chambers *et al.* 2007).

The discrimination and mapping of forest structure in the tropics shares some of the challenges associated with the mapping forest extents and Total Above Ground Biomass (TAGB) addressed above, namely the availability of quality, cloud free satellite imagery over the often-cloudy tropics, and the site-specificity of the empirical relationships between stand age and reflectance. Nonetheless, mapping of secondary forests and the contained age classes or successional stages has been carried out with some success in both the wet and dry tropics. Where remote sensing analysis in the Brazilian Amazon using various data types and classification methods has shown the potential for differentiating among primary and secondary forest classes (Alves and Skole 1996) as well as successional stages (e.g. Kimes *et al.* 1999; Lucas *et al.* 2000), the increased variability in TDF sites has complicated similar studies in the seasonally dry tropics (Kalácska *et al.* 2005b).

The pronounced seasonality characteristic of TDFs presents an additional challenge to discriminating between age classes in these forests using optical data. As with studies mapping dry forest extent (Portillo-Quintero and Sánchez-Azofeifa 2010), and discriminating between plant functional groups (Castro-Esau *et al.* 2004; Kalácska *et al.* 2007a), Arroyo-Mora *et al.*(2005) used data from the dry or rainy-to-dry transitional seasons to map dry forest succession in Costa Rica using Landsat ETM+. Their work also addresses the issue of poor spectral correspondence with age-based definitions of successional classes. Rather, they define forest successional stages with respect to their structural elements (*eg*. stem density, basal area, and the number of canopy layers). Comparing the separability of these structural classes to that of stages based on time since abandonment (*ca*. 5-10 yr, 19-22 yr, 22-30 yr, and primary growth) using cluster analysis and pattern recognition techniques, they found that the structural classes were consistently discernable while the age-based classes tended to overlap considerably, precluding accurate classification.

Hartter *et al.* (2008) also used dry season data acquired from Landsat for successful discrimination of TDF successional stages. Rather than age-based definitions of secondary forest classes, they define early successional classes as those with a woody basal area (BA) of < 15 m^2ha^{-1} and mid-late classes as those with a BA of > 30 m^2ha^{-1}. Landsat TM data was acquired over two TDF sites in Mexico, and validated with BA surveys at 28 field plots. The authors report an overall classification accuracy of 81% using a multi-stage classification approach. First, the land cover was segmented into forest, crops, and other categories using an SVI. Second, the forest class was further divided into early and mid-late successional classes using the SVI data plus the first three principle components calculated from the Landsat TM data and a texture layer (derived from the variance in spectral properties) as inputs. Despite the high accuracy of the classification, the authors echo the sentiment expressed in the previously-cited studies that consideration of the variability imparted by changing phenology is an essential element of remote sending analysis of TDFs.

Remote sensed analysis can also contribute to an improved understanding of the ecological characteristics related to changing forest successional stage. Using a 56 year chronosequence derived from orthorectified aerial photography and Landsat ETM+ data acquired between 1944 and 2000 over Providencia Island, Columbia, Ruiz *et al.* (2005) evaluated variability in species richness and diversity according to six age classes (<6, 6 – 10, 11 – 16, 17 – 31, 32 – 56, and >56 years since abandonment) derived from the remote-sensed investigation. Through comparison of diversity metrics (Shannon's H, Simpson's D) calculated from field surveys carried out at plots located within the age classes identified using the chronosequence, they found that while species density reached a peak in the intermediate successional stages (32 – 56 years since abandonment), the overall species richness increased linearly with stand age, reaching a maximum in stands over 56 years old. While they acknowledge the limitation of this method relative to traditional chronosequence methodologies (interviews of residents, sometimes coupled with visual interpretation of air photos) in identifying exact stand ages, the authors note the efficiency and effectiveness of the combination of Landsat data and orthophotos in surveying and classifying a large geographic area, while maintaining the benefit of the long (more than 50 years in this example) time series available using archived aerial photographs.

Data outside the optical wavelengths has also been incorporated into the analysis of forest structure and succession. In a TDF site in Yuccatan, Mexico, Southworth (2004) explored the incorporation of the thermal infrared data in Landsat TM band 6 to improve land cover classification, including discrimination between early-mid and mid-late successional stages. Using the relationship between surface temperature and successional stage, they developed a series of hybrid optical/thermal indices that they found allowed for a visually superior classification of primary and secondary forests, though the addition of the thermal band did not statistically improve the accuracy of the analysis.

An alternative with the potential to overcome some of the shortcomings of optical data, namely the poor availability of cloud-free data, saturation of vegetation indices, and confusion between forest classes (Castro-Esau *et al.* 2003) is actively remote sensed data such as LiDAR (Light Detection and Ranging). LiDAR measures the strength of the return of an emitted signal to estimate the distance between the target and sensor (Lefsky *et al.* 2002). Waveform LiDAR, such as the LVIS (LiDAR Vegetation Imaging System) measures multiple returns, providing not only canopy height but also an estimation of the internal structure of the forest, providing a three-dimensional picture of the canopy (Castillo-Nunez *et al.* 2011). Castillo *et al.* (2012) used LVIS data to discriminate and map successional stages in Costa Rica. Using an unsupervised classification of three return levels, they generated a map of successional stages which corresponded well to a "literature based" map extrapolated from the relationship between measured canopy height in 20 field plots and the estimated canopy height from the 100% return level. Furthering this, they focused on the intermediate successional stage identified by the classification and identified three classes within this, better characterizing the areas of transition between the early/intermediate and intermediate/late successional stages.

2.7 Conclusion

In 2003, noting a paucity of research in TDFs relative to temperate wet tropical environments, Sánchez-Azofeifa *et al.* (2003) identified three principle research priorities for remote sensing in TDF environments. First, the application of remote sensing tools and spectral analysis to the discrimination and characterization of secondary forest, citing the rapid regrowth and biomass accumulation of TDFs following abandonment and their then-unquantified potential as carbon sinks. Second, the characterization of forest biophysical parameters, particularly LAI, using remotely sensed spectral proxies. Third, the development of hyperspectral analysis techniques to characterize individual tree species based on their spectral reflectance. Subsequent work has demonstrated progress in all three of these research areas, but also highlighted new areas of importance in the remote sensing of TDFs. Of particular importance is investigation into the temporal and especially phenological characteristics of these forests. TDFs are intensely seasonable by definition, and the studies cited here have cited this source of variation as an obstacle to accurate spectral characterization of land cover, biophysical characteristics, and forest structure and composition. A second area of exploration is the integration of multi-scale and multi-sensor data sources, taking advantages of overlapping spatial, spectral, and temporal characteristics to better address the challenges of remote analysis of TDFs.

The question of spectral variation in response to TDF phenology has been shown to impact the characterization of all aspects of these forests by optical remote sensing. Figure 2-1 summarizes the annual trajectory of NDVI (as a proxy for LAI) and the related utility of spectral data for TDF research. During the dry season, LAI (and accordingly NDVI) are low, and the overall forest vertical structure less obscured by the leafy canopy. As such, discrimination of TDF boundaries (e.g. Portillo-Quintero and Sánchez-Azofeifa 2010) as well as structure and successional stage (e.g. Arroyo Mora *et al.* 2005; Hartter *et al.* 2008; Kalácska *et al.* 2005b) have been most accurate during this phenophase. Conversely, LAI studies for forest biomass estimation may best be conducted during the rainy season when consistent NDVI values may be translated into more reliable biomass estimates. A complication to this is the potential for spectral indices to reach an asymptote or saturate in response to high LAI (Turner *et al.* 1999). Figure 2-2 generalizes the potential for NDVI saturation as a function of stand age in both tropical dry and wet forest environments. Due to more rapid growth rates, tropical wet forest canopies reach sufficient LAI values to saturate the index relatively early, where TDFs follow a slower successional trajectory, resulting a larger temporal window within which NDVI remains sensitive to variation in LAI.

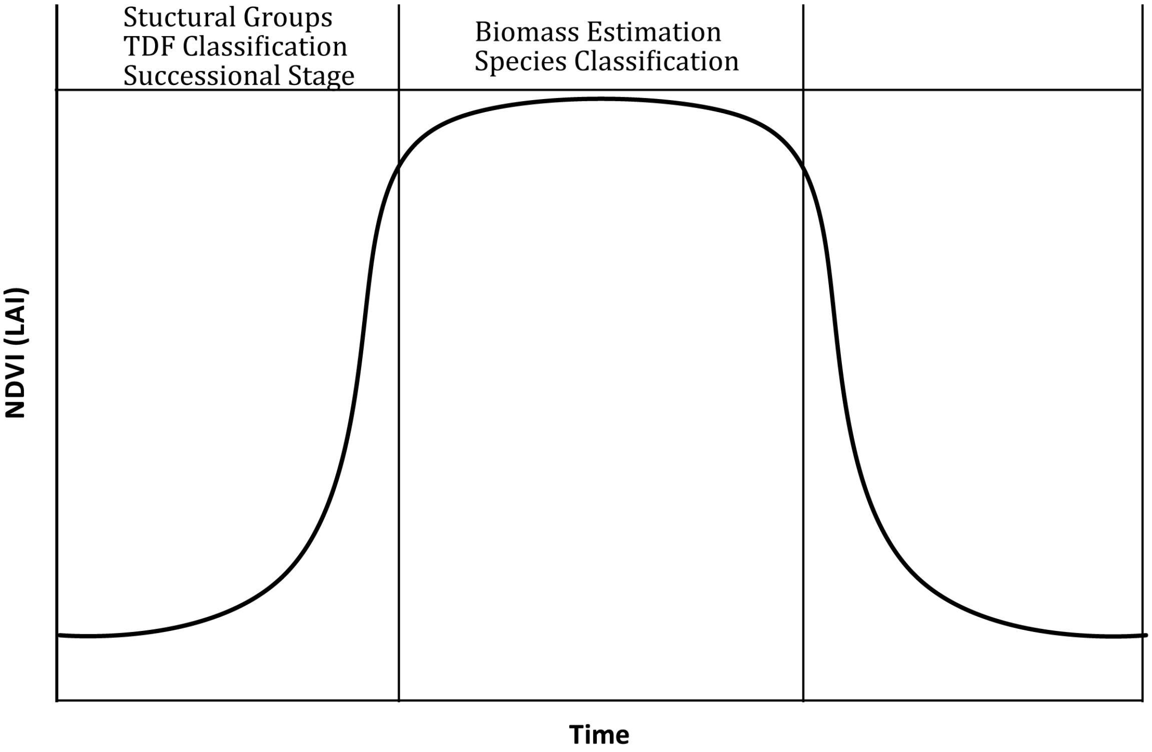

Figure 2-1. Summary of ideal times for remotely sensed analysis of TDF characteristics, in terms of annual phenology as measured by NDVI.

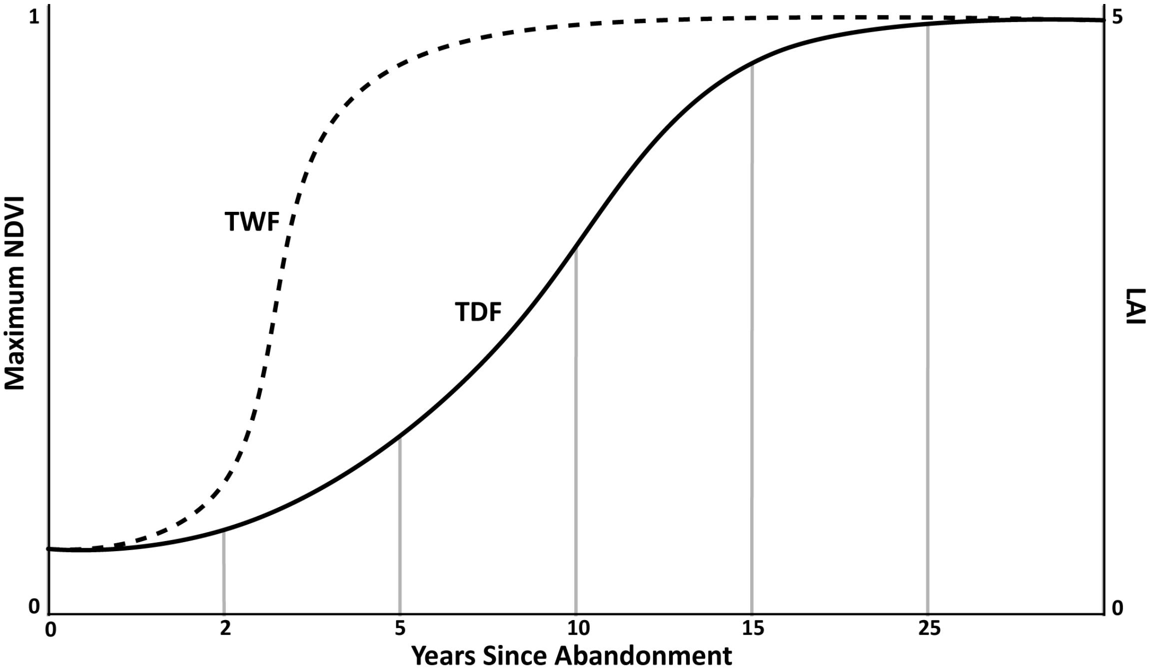

Figure 2-2. Representation of the relative regeneration times of tropical wet forests (TWF) versus tropical dry forests (TDF) in terms of years since abandonment.

Classification at the species and structural group level seem to be optimally performed during different periods of the growing season. The potential for discrimination between tree and liana species, as explored by Castro-Esau *et al.* (2004) and Kalácska *et al.* (2007a) was highest during the dry season, likely due to different adaptations to water stress among the two structural groups, as proposed by Sánchez *et al.* (2009). While aircraft and satellite sensors do not currently have the combination of high spatial and spectral resolution required for species-level classification in the species-rich tropics (Asner and Martin 2008a), studies conducted at the leaf level with hyperspecral instruments have demonstrated high accuracies in distinguishing between species at single sites within narrow temporal windows. Issues of temporal variability become an obstacle when these classifications are extended beyond the parameters of the original study, to the extent that at this stage, the likelihood of automated classification of species using a library of consistent spectral signatures seems low (Castro-Esau *et al.* 2006; Hesketh and Sánchez-Azofeifa 2012). It is clear that virtually any remote sensed analysis of TDFs is subject to phenologically-induced spectral variability, and that a clearer understanding of the nature and scope of this variation must be one of the priorities of future work.

A second area with the potential to advance our understanding of TDFs is the fusion of various data sources to provide a more detailed and accurate estimation of TDF characteristics. While the use of optical data at multiple spatial resolutions is discussed here (e.g. Arroyo Mora *et al.* 2005), the use of complementary data types has been under-explored in TDF studies. Synthetic aperture radar imagery has the benefit of cloud penetration, with the potential to fill imagery gaps in low temporal resolution that result from high cloud cover over tropical areas, particularly during the wet season (Sánchez-Azofeifa *et al.* 2003). Radar data also provide complementary information content to optical data. While data in the visible and infrared spectral regions provide information on the chemical and structural characteristics of vegetation, radar data can supplement this with additional information on surface texture and dielectric properties, aiding in particular the separation of vegetation from bare soil and the estimation of moisture content (Held *et al.* 2003). Similarly, LiDAR data, which can provide detailed information on forest canopy structure (Skowronski *et al.* 2007) have shown to increase the accuracy of species classification when combined with airborne hyperspectral data set over a temperate forest (Dalponte *et al.* 2008), though this fusion is as-yet untried in the TDF.

A challenge to the application of these data fusions lies in the lack of infrastructure for the organization and integration of the various spectral and ecological data and metadata used in the analysis of forest characteristics and processes (Quesada *et al.* 2009). The establishment of this infrastructure has the potential to provide researchers with the opportunity to work at nested spatial, spectral and temporal scales. For example, fPAR data can be collected in near real-time by ground based sensor networks, linked to time-stamped daily phenological data from nearby sensor stations, which can be further linked via GPS coordinates to air- and satellite-borne remote sensed products. This coupling of field data and remote sensed imagery both validates remote sensed products but addresses directly the temporal dynamics of TDF ecosystems (Gamon *et al.* 2006).

Remote sensing analyses have become a critical component of ecological research (Kerr and Ostrovsky 2003), particularly in the assessment of remote regions and the exploration of patterns at various spatial and temporal scales. Continued research and development, particularly in the area of temporal and phenological variability, will be crucial in better understanding TDFs and providing linkages between forest biophysical and structural characteristics and the environmental factors that govern them.

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Chapter 3: The effect of seasonal spectral variation on species classification in the Panamanian tropical forest†

3.1 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 (Sánchez-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).

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† A version of this chapter has been published. Hesketh, M., & Sánchez-Azofeifa, G.A. (2012). The effect of seasonal spectral variation on species classification in the Panamanian tropical forest. *Remote Sensing of Environment, 118*, 73-82.

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 suggests 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 *et al.* 2005; Gamon and Surfus 1999; Sims and Gamon 2002) as well as for discrimination of structural groups (Castro-Esau *et al.* 2004; Kalácska *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. 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 echoes 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, Sánchez-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 Kalácska *et al.* (2007).

While research up to the present has addressed leaf properties (Asner 1998) and variability between environmental (Asner *et al.* 2009; Sánchez-Azofeifa *et al.* 2009) and structural groups (Castro-Esau *et al.* 2004; Kalácska *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; Kalácska *et al.* 2007) that interseasonal 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.

3.2 Methods

3.2.1 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 than 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 Mulky (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 3-1.

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 FS as well, it is to a lesser extent than at PNM (Table 3-1).

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

Table 3-1. Species included in study. Codes beginning with L indicate liana species while those beginning with T indicate Tree Species

3.2.2 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 species were analyzed at FS, and 30 at PNM, with collection and sampling protocols according to Kalacsca *et al.* (2007) and Castro-Esau *et al.* (2006; 2004; Sánchez-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 350-1000 nm and 10 nm from 1000-2500 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 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 (Kalácska *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 caratenoid/chlorophyll molar ratio, and serves in comparison of the balance of these key pigments across seasons (Equation 1). Penuelas *et al.*'s (1993) water index (WI) stands as proxy for direct leaf water content (Equation 2).

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

[2] $$WI = \frac{R_{900}}{R_{970}}$$

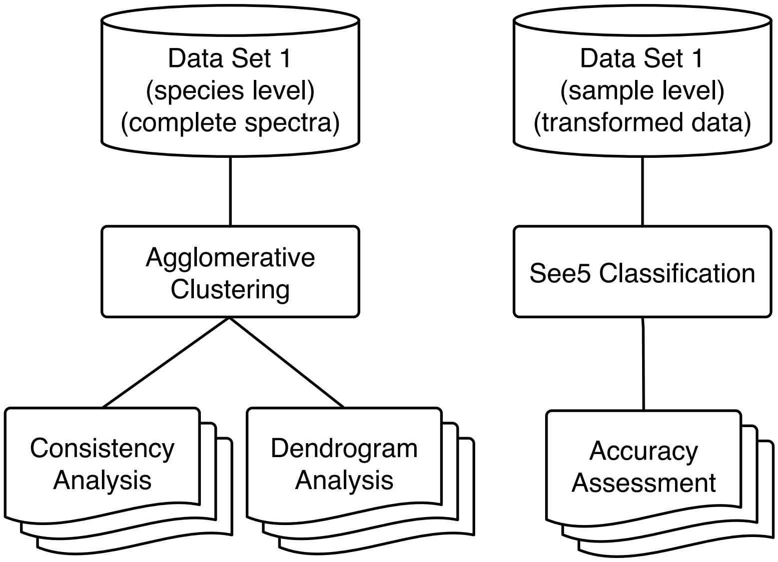


Figure 3-1. General workflow of analysis showing the progress of Dataset 1 through hierarchical clustering and dendrogram analysis, and Dataset 2 through unsupervised classification.

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 Figure 3-1 outlines the paths these two datasets take through the subsequent analyses.

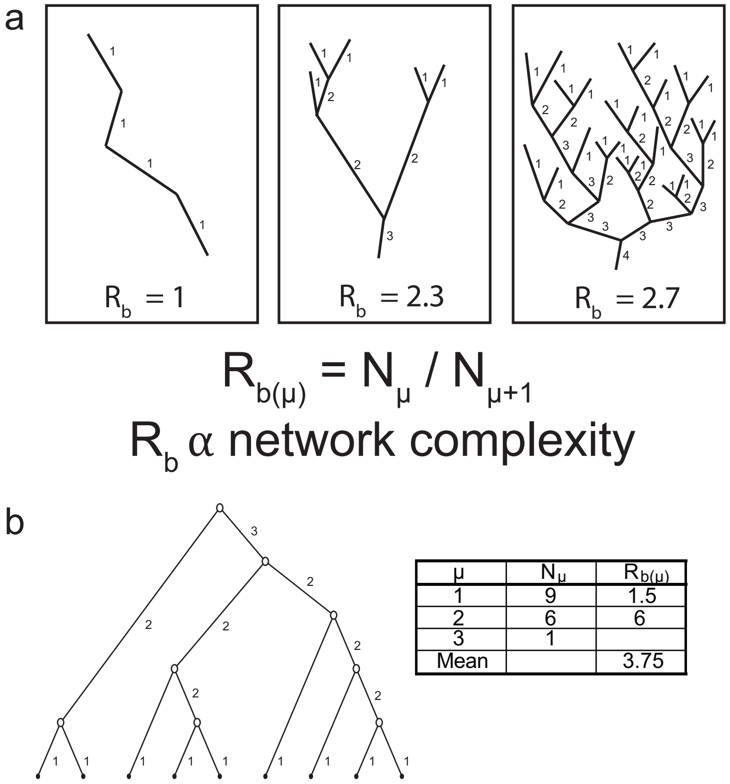
Seasonal variation was tested using a set of two classification procedures to separately evaluate both the structure of data classified during different seasons, and the effect of cross-season classification on the overall accuracy. First, to visualize the effects of seasonal spectral variation, the 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 (Equation 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 (Figure 3-2)

$$[3] \quad R_b = \frac{N_\mu}{N_{\mu+1}}$$

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.



μ	N_μ	$R_{b(\mu)}$
1	9	1.5
2	6	6
3	1	
Mean		3.75

Figure 3-2. 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.

Second, to quantify the impact of seasonal variation on classification accuracy, we adapted the method of Kalácska *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 Reseach 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 interseasonal 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 will be 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.

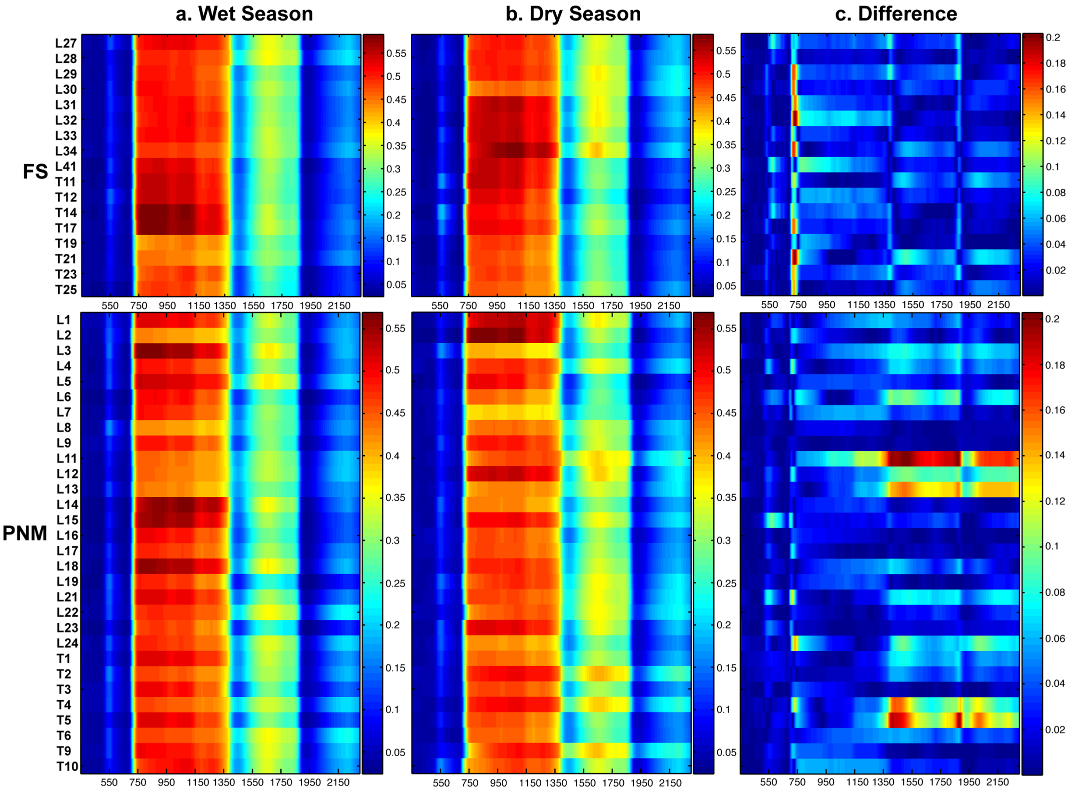
3.3 Results

3.3.1 Interseasonal differences in spectral reflectance

The average spectral signatures of all species included in the investigation are shown in Figure 3-3a and 3-3b 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) region is controlled largely by water absorption (Gates *et al.* 1965). Where Figures 3-3a and b demonstrate spectral variability among the species examined, Figure 3-3c 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 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, 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.

3.3.2 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 (Figure 3-4a-d). The values for Horton's bifurcation ratio (R_b) at both sites and seasons are presented in Table 3-2. 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 found at each level of μ. Difference in R_b between wet and dry seasons at the level of each order (μ) range between R_b 0.2 and 1.0, with the exception of the value for μ=2 at FS site of R_b 2.2 (3-2).


a. Wet Season

b. Dry Season

c. Difference

Figure 3-3. 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.

Seasonal variation in the organization of species within the dendrogram, as demonstrated in Figure 3-4, 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.

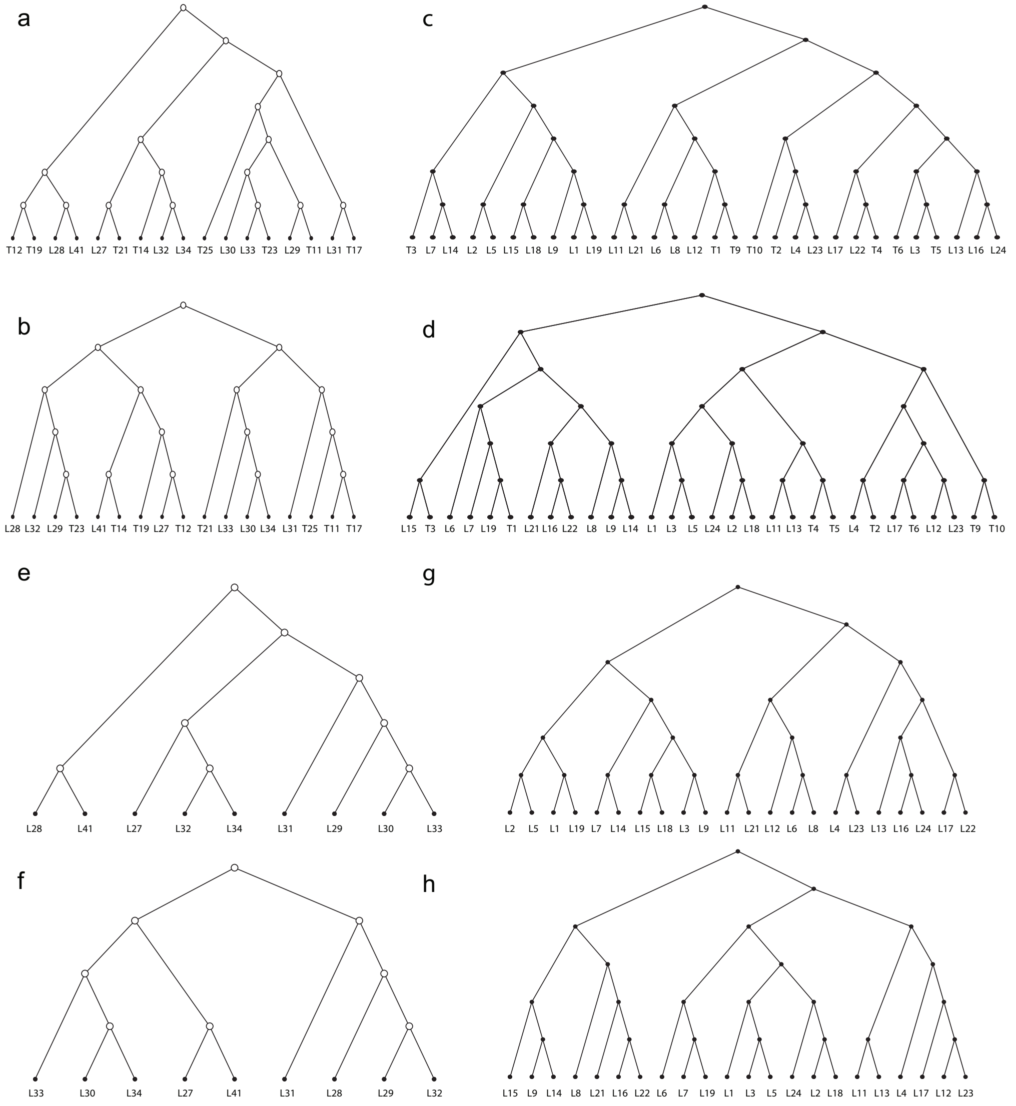


Figure 3-4. Dendrograms resulting from hierarchical clustering of Dataset 1: All species (a. FS Wet, b. FS Dry, c. PNM Wet, d. PNM Dry); Lianas only (d. FS Wet, e. FS Dry, f. PNM Wet, g. PNM Dry).

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 Figure 3-4e-h. Interseasonal 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 3-3). 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 3-3), 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%.

	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 3-2. Bifurcation ratios – complete species set 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

Table 3-3. Bifurcation ratios – only liana species considered

3.3.3 Effect of seasonality on classification accuracy

Figures 3-5 and 3-6 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 (Figure 3-5). 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 shown in Figure 3-3. Differences among PSRI values (Evaluated using Student's t-test) tended to be significant between sites

(Table 3-4) 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 (Figure 3-6). Values here showed little trend toward significant difference between sites or seasons (Table 3-4).

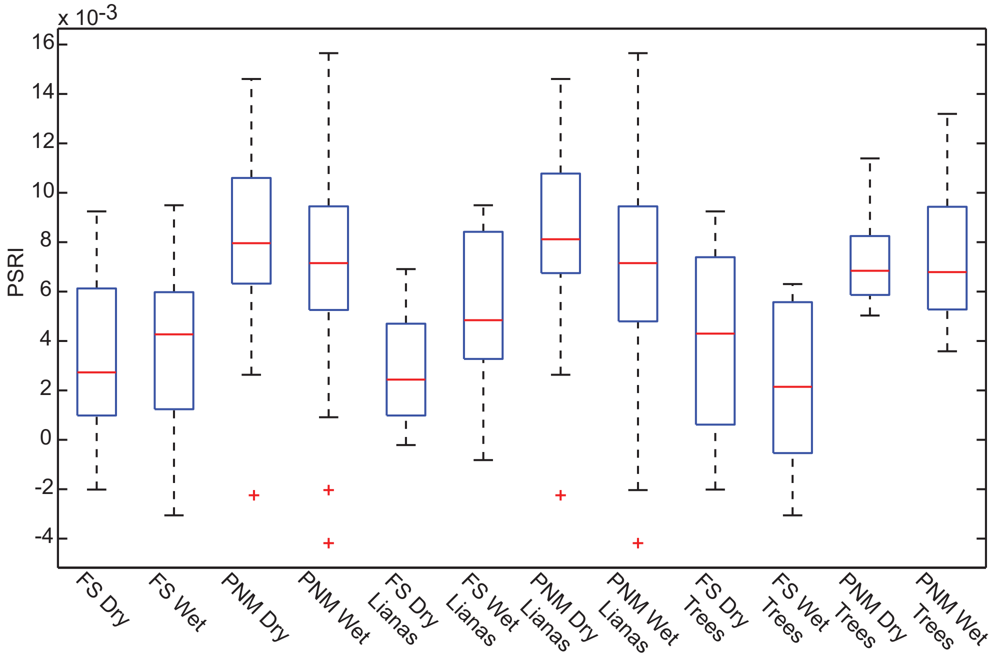


Figure 3-5. PSRI compared between seasons and structural groups. Outliers are represented by crosses.

	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
FS WET		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		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**		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		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**		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		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*		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		**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		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		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**		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	

WI

PSRI

Table 3-4. Significance values for differences between sites and seasons for the two SVIs used as inputs to classification. Stronly significand ($p<0.05$) 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 †.

Figure 3-7 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 classifier 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 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%.

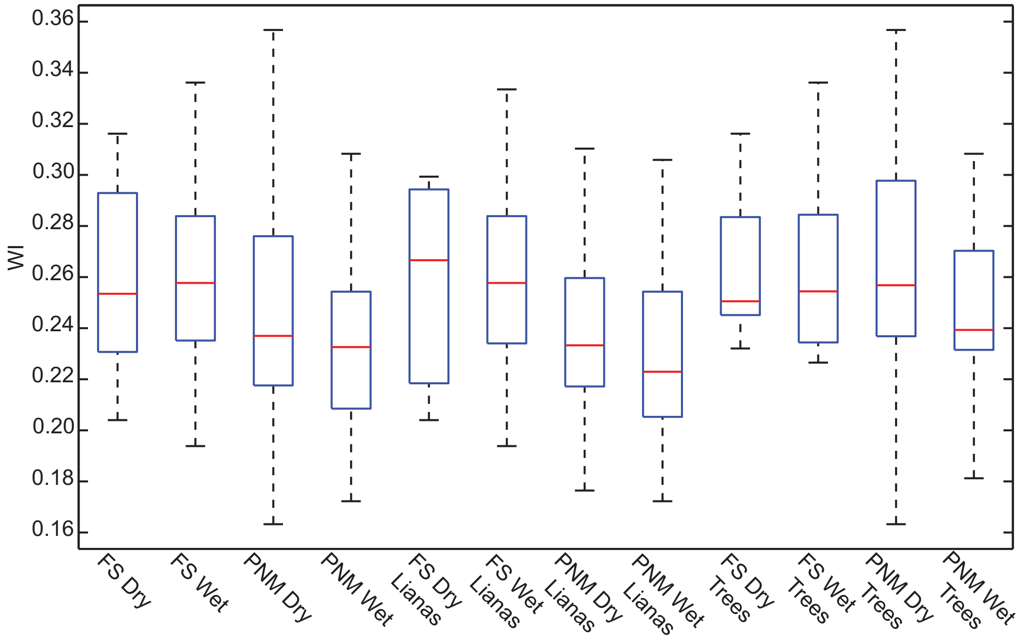


Figure 3-6. WI compared between seasons and structural groups

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.

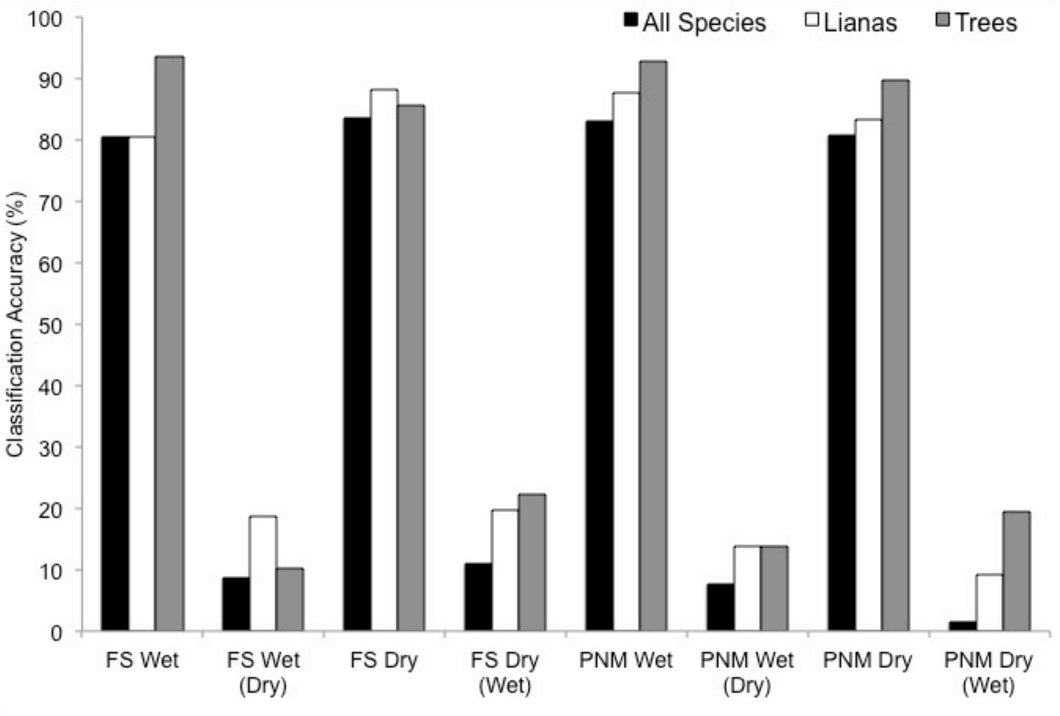


Figure 3-7. See5 classification accuracy for both wet (FS) and dry (PNM) sites, expressed as percentage. Where training and testing data are taken from the same season, only that season is labeled. Where training and testing data are from opposing seasons, the label reflects training and testing seasons as: train (test).

3.3.4 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 ($P < 0.001$).

3.4 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.

3.4.1 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 Sánchez-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 FS, 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 (Gates *et al.*, 1965, Boyer *et al.*, 1988), indicating that the primary driver to spectral variation in this ecosystem is likely variation in the relative abundances of leaf pigments. Conversely, the dry site PNM showed substantially less variation in this spectral region, with the greatest sources of variation 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 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 (Kalácska *et al.* 2005), and generally higher leaf water content (Andrade *et al.* 2005; Schnitzer 2005). Sánchez-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 (Sánchez-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.

3.4.2 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 (Figure 3-4) 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 Kalácska *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 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 Sánchez *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 interseasonal 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.

3.4.3 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 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 *et al.* (2006) report a decreasing trend in classification test accuracy from approximately 85% for 20 species, 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 *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 *et al.* 04, 06, Kalakska *et al.* 07)) and that lianas exhibit a different set of traits and adaptations that seem to be tied to the local environment and are manifest both physiologically and spectrally (Sánchez-Azofeifa *et al.* 2009). In the context of this study, this implies that not only an understanding of within-species interseasonal variation, but the effect 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 and Bongers 2011).

The unique characteristics of lianas suggest a need for caution in the process of scaling the results of leaf level spectral analyses to the canopy and landscape scales. Sánchez-Azofeifa and Castro (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 reflectance 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.

3.5 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 show 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 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; Kalácska *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 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.

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Chapter 4: Successional variation in spectral and biophysical leaf traits in two Latin American tropical dry forests.

4.1 Introduction

Leaves play an important role in moderating the thermal, light, and moisture environment within the forest canopy (Fernandes *et al.* 2003; Fournier *et al.* 2003; Gamon *et al.* 2005b). Structural and biochemical traits, which are indicators of leaf function, tend to vary in coordinated ways, reflecting differences among and between species, as well as the influence of environmental conditions (Martin *et al.* 2007). Further, this covariance has been shown to describe general patterns in leaf resources allocation and longevity (Osnas *et al.* 2013; Wright *et al.* 2004a)

Relationships between these biophysical traits and leaf spectral properties (Asner 1998; Gates *et al.* 1965; Woolley 1971) allow for the use of remotely sensed data to aid in forest characterization in situations where field-based assays may be unfeasible for reasons of economics or logistics (Kalácska *et al.* 2005). High-resolution spectroscopy has been used to explore the relationship between leaf reflectance and photosynthetic capacity (Gamon *et al.* 2005a), plant functional traits (Alvarez-Añorve *et al.* 2012), and leaf chemistry (Asner *et al.* 2011; Doughty *et al.* 2011). The increasing availability of higher spatial and spectral resolution data have provided opportunities to apply these relationships between leaf physical and spectral characteristics to the assessment and modeling of species diversity and composition at both leaf and canopy scales (Asner and Martin 2008; Clark *et al.* 2005).

Because foliage adapt to changing environmental conditions such as variability in illumination, moisture availability, and soil properties (Welles 1990); leaf traits and spectral properties can be expected to vary across geographic and temporal gradients. In tropical forests, characterized by high species diversity and endemism, the extent and nature of variation in leaf physical and chemical traits are still poorly understood (Asner *et al.* 2009). Townsend *et al.* (2007) found a pattern of N:P variation associated with precipitation rates at highly seasonal sites in Costa Rica. Martin et al (Martin *et al.* 2007) explored patterns in pigment and optical properties of greenhouse-grown *M. Polymorpha* grown from seed sources spanning a wide variety of soil types and elevations. Variation in chemical and spectral characteristics of 162 canopy species in Australia was investigated by Asner *et al.* (2009), who point to strong taxonomic controls on leaf chemical signatures. While they suggest that taxonomy may be the prime driver to variation, they also found patterns associated with climate, with the maximum chemical and spectral diversity found in warm lowland sites with moderate annual precipitation.

Castro-Esau *et al.* (2004), Kalácska *et al.* (2007a), and Sánchez-Azofeifa et al (2009) have all addressed variation in leaf traits and spectral properties in tree and liana species between dry- and rainforest environments in Panama. The earlier studies both report differences in the ability to discriminate between leaves of tree and liana species based on their spectral reflectance at dry and wet forest sites. Sánchez-Azofeifa *et al.* (2009) explain this trend through exploration of leaf traits and internal structure, noting specific physiological adaptations in liana species collected in dry environments that allow for more accurate spectral discrimination between the structural groups when compared with the same type of structural element in tropical rainforests. Attempting to explain Schnitzer's (2011) comments on increasing liana abundance in tropical forests, Asner and Martin (2012) later also found physiological differences between liana and tree leaves in the humid tropics, especially among traits related to light capture and growth. Their survey of 22 chemical and physical leaf traits from more than 7200 species of trees and lianas reinforces the conclusions of Sánchez-Azofeifa *et al.* (2009), and suggest a physiological advantage of lianas in forests undergoing disturbance.

Trait and spectral variation in response to temporal gradients has been particularly under-explored, though Roberts *et al.* (1998) show patterns in leaf reflectance related to leaf age in *Caatinga* forests, and numerous studies have used variation in pigment-sensitive vegetation indices to monitor phenology at stand scales (e.g. Eklundh and Olsson 2003; Zhang *et al.* 2003). This lack of consideration for the temporal dimension may be related to a bias in the literature toward studies on Amazonian and other rainforest environments, where phenological patterns are muted compared with more seasonal environments (Morellato *et al.* 2000). The less pronounced phenological curve of these largely evergreen forests is less likely to result in significant variation in leaf physical and spectral properties.

Though they receive less attention than rainforests (Sánchez-Azofeifa *et al.* 2005b), tropical dry forests are among the world's most threatened ecosystems (Janzen 1988; Murphy and Lugo 1986b; Olson 2000), accounting for 48% of vegetation cover in Mesoamerica and the Caribbean and 42% of all tropical forests worldwide (Murphy *et al.* 1995; Van Bloem *et al.* 2004). In the Americas, dry forests occupy 519,597 km^2 of the total landcover (Portillo-Quintero and Sánchez-Azofeifa 2010). These dry environments can exhibit a distinct set of leaf traits (Sánchez-Azofeifa *et al.* 2009) and an intense wet/dry seasonality that can directly influence spectral analysis and classification (Hesketh and Sánchez-Azofeifa 2012).

Because tropical dry forests are often areas of intense human occupation and exploitation (Murphy and Lugo 1986a; Quesada and Stoner 2004; Sánchez-Azofeifa *et al.* 2005a), secondary forests are common, creating a temporal gradient as mature forests are converted to a mosaic of successional stages related to the time since abandonment at the site scale. This successional variability is expressed as changes in species composition and stand structure (Feeley *et al.* 2005; Kalácska *et al.* 2007b; Ruiz *et al.* 2005), as well as spectral reflectance from a stand scale (Arroyo Mora *et al.* 2005). As such, the primary objective in this study is to investigate the extent to which this successional gradient is expressed in leaf traits and spectral properties of a set of species common to all successional stages at two tropical dry forest sites. Specifically, we ask first whether, when controlling for species composition, changing forest successional stage results in variability in (1) leaf structural and chemical traits, (2) leaf pigment levels and the associated spectral indices, and (3) the strength of correlations between the above leaf traits. Secondly, we ask if the same successional changes result in a corresponding variability in leaf spectral properties, and whether this variation is sufficient to affect the accuracy of species classification based on spectral reflectance.

4.2 Methods

4.2.1 Site Description

Leaf samples were collected during the wet seasons of 2009 and 2010 at the Chamela-Cuixmala Biosphere Reserve in Mexico (CCBR) and Santa Rosa National Park in Costa Rica (SRNP), respectively (Figure 4-1). The

Chamela-Cuixmala Biosphere Reserve (CCBR) is on the west coast of Mexico in the province of Jalisco (19°22' – 19°35'N, 104°56" – 105°03'W) with a mean annual temperature (1978-2007) of 25.2° C (±1.3° C) (http://www.ibiologia.unam.mx/ebchamela/). The area experiences a highly seasonal pattern of rainfall (García-Oliva *et al.* 2002), with a mean annual precipitation (1977-2006) of 741 mm (±256 mm) occurring mainly during the wet season months of June through October (http://www.ibiologia.unam.mx/ebchamela/).

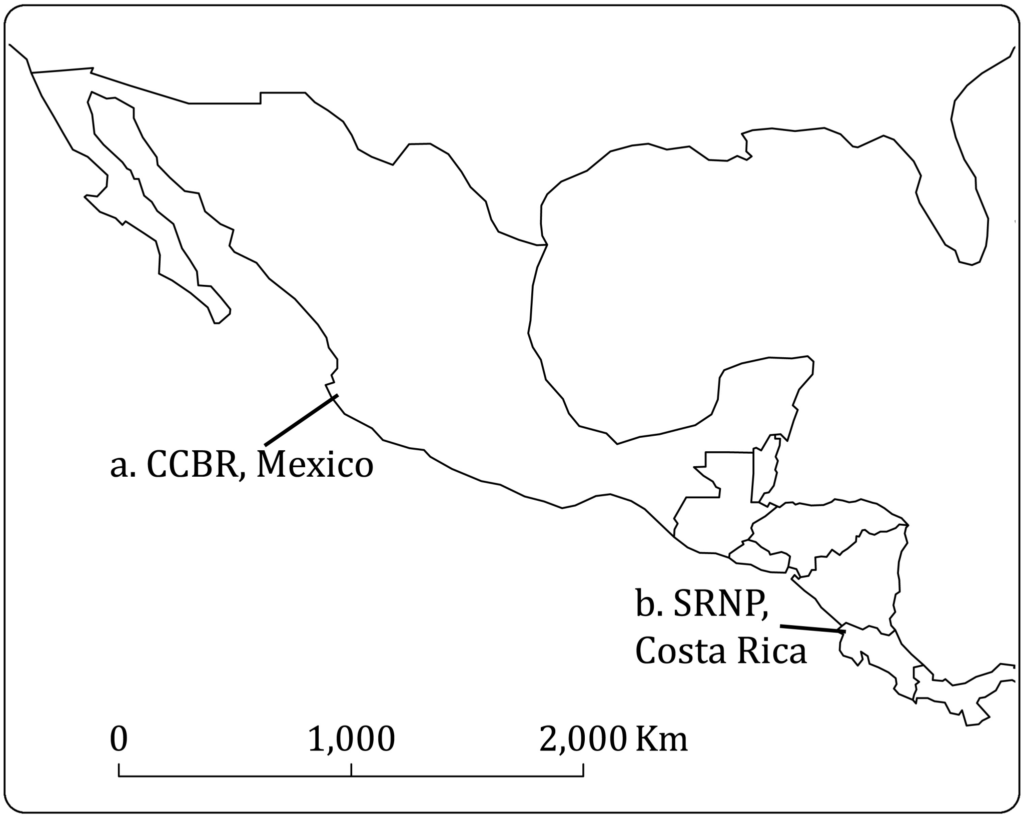

Figure 4-1. Map showing study areas. a. Chamela Cuixmala Biosphere Reserve (CCBR), Jalisco, Mexico. b. Santa Rosa National Park (SRNP), Guanacaste, Costa Rica.

The Chamela-Cuixmala Biological Station was established by the National Autonomous University of Mexico in 1971, with the objective of researching and protecting dry forest environments (Sarukhán *et al.* 1979). The present day CCBR was established in 1993 with the addition of surrounding areas to the 3,319 hectares of land attached to the Biological Station in acknowledgement of their ecological importance (Gentry *et al.* 1995), resulting in a total protected area of 13,142 hectares (Ceballos *et al.* 1999). Conversion from primary to secondary forest in the area surrounding the CCBR is the result of a push by the Mexican government beginning in 1943 encouraging agriculture, cattle raising, and tourism (Castillo *et al.* 2005; Sánchez-Azofeifa *et al.* 2009).

The dry forest successional gradient at the CCBR is the result of the abandonment of cultivated areas following a pattern of slash and burn clearing, cultivation, then use as pasture for cattle (Avila-Cabadilla *et al.* 2009). Within the mosaic created by varying the time-since abandonment, permanent plots have been established at early, intermediate, and late (mature) successional stage. A description of the physiognomy and forest structure of these plots can be found in Avila-Cabadilla *et al.* (2009) In order to minimize variation resulting from changes in altitude and aspect, sites were all located on slopes of 15° to 25° at an average height of 23 meters above sea level (masl). North facing slopes were avoided as they have shown increased altitude-related heterogeneity in plant community composition

(Balvanera *et al.* 2002). The successional stages at CCBR were based on the time since final abandonment, with early stage plots set at 3 – 5 years, intermediate at 8 – 12 years, and late stage plots at > 50 years without disturbance. Shrubs dominated the early stage plots, with an emergent canopy averaging 5 m. The intermediate and late stages share an average canopy height of 10 m. Both stages are dominated by deciduous species, though overall species richness is 15% greater in the late stage (Chazdon *et al.* 2007). Early and intermediate stage plots were located a minimum of 1000 m away from the surrounding mature forest to avoid edge effects.

Santa Rosa National Park (SRNP) occupies 49,500 hectares in the northeast of Costa Rica in the Guanacaste Province (10°48'53"N, 85°'36"54W), within the Guanacaste Conservation Area, which contains all of the dry forest in Costa Rica (Allen 2001; Janzen 2000; Portillo-Quintero and Sánchez-Azofeifa 2010). Typical of dry forests, precipitation is concentrated during the wet season, experienced here from December through May. The average annual rainfall is 1500 mm (Arroyo Mora *et al.* 2005), though this values is highly variable ranging from 915 – 2558 mm per year (Janzen 1993).

Historically, SRNP followed a similar pattern of land use as CCBR, with the land serving as a cattle raising hacienda for almost 200 years prior to the establishment of the national park (Castillo-Nunez *et al.* 2011). During the second half of the 20th Century, the area was heavily logged, primarily for *Swietenia macrophylla* (Burnham 1997). The present day mosaic of biotic communities, composed of pastures and secondary dry forests in different stages of succession, has evolved since the park's establishment according to varying histories and intensities of land use in the area (Janzen 1988; Kalácska *et al.* 2004a; Kalácska *et al.* 2004b).

Definition of successional stages at SRNP was via stand structural characteristics rather than time since abandonment. The early stage was defined as areas made up of patches of woody vegetation and shrubs with a single layer canopy having a maximum height of 6 – 8 m. Dominant species are almost entirely deciduous, losing the majority of their leaves during the dry season. Canopy trees in the intermediate stage are between 10 – 15 meters in maximum height and deciduousness drops to approximately 80 %. In the late stage, the canopy is taller (15 – 30 m) and more complex, with evergreen species dominating from 50 to 90 % of the upper crown. A more detailed account of the structure and composition of the successional stages may be found in Kalácska *et al.* (2004a) and Arroyo-Mora *et al.* (2005). The topography of the region of the park where the study sites were located is relatively flat, with an average slope of 7°, so the effects of topography were minimized, and the plots were located with the same considerations as at CCBR.

An overview of the common species found in the established permanent plots may be found for CCBR in Alvarez-Añorve *et al.* (2012) and for SRNP in Arroyo Mora et al (2005). For this study, we concentrated at each site on species that were found in all three successional stages, detailed in Table 4-1. No trends in leaf traits separate the species chosen from those not found in all successional stages, but the species selected tended to be among the dominant species in each stage. While this limits the size of the sample, it allows for the standardization of stand complexity and composition across sites, providing a clearer picture of physiological changes between successional stages.

A focus on species common to all successional stages was an essential element of the study design. A majority of studies addressing variation in leaf traits (e.g. Asner and Martin 2008; Asner and Martin 2012; Wright *et al.* 2004b) have addressed large datasets and include taxonomy as an explanatory, rather than controlled, variable, though Martin *et al.* (2007) is a notable exception. Because our objective is to evaluate trait variability with respect to a temporal gradient (in this case forest successional stage), standardizing the taxonomic composition of our sample was an essential element of the experimental design.

	Family	**Genus species**
a.	Achatocarpaceae*	*Achatocarpus gracilis**
	Boraginaceae	*Cordia alliodora*
	Polygonaceae	*Coccoloba liebmanii*
	Euphorbiaceae*	*Croton suberosus**
	Flacourtiaceae	*Casearia tremula*
	Leguminosae	*Haematoxylum brasiletto*
	Leguminosae	*Lonchocarpus constrictus*
	Leguminosae	*Lonchocarpus eriocarinalis*
	Polygonaceae	*Ruprechtia fusca*

	Family	**Genus species**
b.	Fabaceae*	*Acosmium panamense**
	Rubiaceae	*Alibertia edulis*
	Fabaceae	*Ateleia herbert-smithii*
	Burseraceae	*Bursera simaruba*
	Cochlospermaceae	*Cochlospermum vitifolium*
	Ebenaceae	*Diospyros salicifolia*
	Fabaceae*	*Gliricidia sepium**
	Rubiaceae	*Guettarda macrosperma*
	Malvaceae*	*Luehea candida**
	Malvaceae	*Luehea speciosa*
	Nyctaginaceae	*Pisonia aculeata*
	Verbenaceae*	*Rehdera trinervis**
	Hippocrateaceae	*Semialarium mexicanum*
	Meliaceae	*Swietenia macrophylla*

Table 4-1. Common species surveyed at the CCBR in Mexico (a.) and SRNP in Costa Rica (b.). Species marked with an * were removed from the second phase of the classification analysis

4.2.2 Data Collection.

Collection protocols were the same at each site, with all samples collected during the peak of the rainy season to minimize any variability associated with annual phenological cycles (e.g. leaf stress at the beginning and end of the growing season). Trees were located in plots previously established at each of the two sites, with no replication of species made between plots of the same successional stage. Leaf-bearing branches were harvested from sun-exposed canopy trees and stored for transportation in plastic bags containing moist paper towel to prevent drying. Leaves were left on the branch to aid in preservation. Samples were then transported to an onsite lab for immediate initial processing. The interval between harvest and initial analysis was never to exceed 6 hours as Foley (2006) indicates that spectral features related to leaf pigments, structure, and water content are relatively stable within this time frame, provided steps are taken to maintain moisture content, as described above. The number of samples measured per species, per stage, are summarized in Table 4-2.

	Samples Collected
Spectra	15*
Structural Variables	5
Pigment Concentration	5
Foliar Chemistry	1

Table 4-2. Number of samples collected for each species at each successional stages at both CCBR and SRNP. Structural variables were SLA, water content, and leaf thickness. Pigments sampled were total chlorophyll and carotenoid levels. Foliar chemical analysis was for nitrogen.

The suit of biophysical measurements used in the study is indicated as Table 4-3. All leaf physical and spectral measurements were conducted in lab facilities at CCBR and SRNP, respectively. For these traits, five leaves per species, per stage were selected, and the measurements carried out within 6 hours of collection in the field, as described above. Additionally, five leaves per species, per stage were selected for pigment analysis, to be carried out at the University of Alberta upon return from the field. Leaf cores 3.8 cm^2 in area were extracted from these additional five leaves and immediately frozen at -10 °C for later pigment analysis. These samples were kept frozen in transit between the study sites and the university facilities. Finally, leaf material from each species and stage was dried for chemical analysis, also carried out at the University of Alberta upon return from the field. In all cases, healthy (free of insect damage, noticeable epiphylls, and galls) and mature leaves were selected.

The leaf structural traits recorded were leaf thickness, fresh and dry weight, and leaf area, from which water content and specific leaf area (SLA) were also derived. Leaf thickness, which has been shown to vary with successional stage and impact chlorophyll and nitrogen estimation (Campbell *et al.* 1990; Marenco *et al.* 2009), was measured using a digital micrometer (model 293-344. Mitutoyo, Takatsu-ku, Japan). Measurements were collected at three points on each leaf, between the veins and equidistant between the leaf edge and midrib, and the average of the three measurements recorded to the nearest 0.001 mm (Sánchez-Azofeifa *et al.* 2009).

Leaf area was recorded in the field using digital scans of the fresh leaves at 300 dpi. These scans were later converted to aerial measurements by using a pixel counting approach carried out in Adobe Photoshop, and reported in mm^2. Leaf fresh and dry weights were determined by weighing the fresh leaves immediately upon their removal from the branch, then again after drying for a period of 36 hours in a 60°C drying oven.

Leaf water content and SLA were derived from the above. Water content was expressed as the percent ratio of the difference between the fresh and dry weights and the fresh weight (equation 1). SLA was recorded as the ratio of fresh surface area to dry weight.

[1] (((fresh weight – dry weight) / fresh weight) * 100)

Chemical analysis was carried out on leaf material dried as described above and transported back from the field sites and comprised estimation of the nitrogen and phosphorous content of each species collected at each successional stage. These chemicals are necessary for leaf light capture and growth (Asner 1998) and have been shown to be related to forest primary production (Townsend *et al.* 2007). Nitrogen (as % mass) was estimated using a combustion element analyzer (Richards 1993). Phosphorus (also as % mass) was estimated by digestion followed by colorimetric analysis (Richards 1993). Because the chemical analyses were performed on dried and pulverized leaf material, only one measure per species, per successional stage, was recorded. As a result, only the variation between species or sites/successional stages is determinable for these variables, as the within-species variation for a given site/successional stage is not estimable from a sample containing leaf matter from several individuals. Additionally, the resulting decrease in the size of the dataset for these variables decreases the likelihood of a significant difference between successional stages according to the statistical tests used.

Chlorophyll and carotenoid pigments are likewise related to leaf light capture and growth. Leaf pigment analysis was carried out using a process of dimethyl sulfoxide (DMSO) extraction and analysis using a spectrophotometer (SMART Spectrometer, LaMotte Company, Chestertown, MD, USA) with a spectral range of 300 – 1000 nm and resolution of 1 nm (2 nm accuracy) (Hiscox and Israelstam 1979; Richardson *et al.* 2002). The leaf cores collected and frozen in the field were removed from cold storage, immersed in a 10 ml DMSO solution and warmed in a water bath at 65°C for 20 minutes. This solution was then cooled and transferred to disposable cuvettes (~3 ml) for spectral analysis. Using the spectrophotometer, absorbance was measured at 447, 645, and 663 nm wavelengths selected via calibration using pure chlorophyll a, and b extracts. Estimates of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content were calculated based on Arnon's (1949) relationships, using the equations below, and converted to per-area units.

$$[2] \quad TotalChl(Chl_a + Chl_b) = (12.15 * A_{663} - 2.79 * A_{645}) + (21.15 * A_{645} - 5.1 * A_{663})$$

[3] $$Carotenoid = (1000 * A_{470} - 1.82 - Chl_a - 85.2 - Chl_b)$$

Spectral reflectance of the five leaves measured for thickness and water content in the field was carried out at the field site within the 6 hour window described above as per Foley *et al.* (2006).

Spectra were collected using a portable field spectrometer (Unispec SC, PPSystems, Amesbury, MA, USA). The device uses a bifurcated fiber optic connected to a leaf clip to measure a 3.46 mm^2 area in the range of 400 – 1100 nm with a spectral resolution of 10 nm and an accuracy of < 0.3 nm. Measurements were taken three times for each leaf, at the locations used for thickness measurements described above, and later averaged per leaf.

4.2.3 Statistical Analysis

Summary statistics were calculated for each of the measured variables as described in Table 4-3. Variability in leaf traits was assessed at each site in two ways, first at the site level between all three successional stages, and then between paired successional stages (early-intermediate, early-late, and intermediate-late) to assess the point along the successional pathway at which the variability was greatest. ANOVA analyses were performed to evaluate the difference in leaf traits among the tree successional stages at the site level. Then, paired t-tests (McDonald 2009) were used to investigate the extent of variation between the paired stages. Normality tests were conducted before the t-tests using SigmaPlot 11.0 (Systat Software, San Jose, California 2008). Wherever a variable failed to meet the assumptions for a given parametric test, the non-parametric alternative was used (Kruskal-Wallis H-test and Mann-Whitney U-test for ANOVA and t-tests, respectively (Sokal and Rohlf 1995)). Finally, the interrelatedness of leaf traits at each site was examined using Pearson correlation analysis (Daniel 2005)

a.	Stage	Mean	SD
Water Content (%)	e	62.87	6.18
	i	62.62	5.17
	l	64.99	7.22
Thickness (um)	e	181.75	49.56
	i	164.40	37.64
	l	175.42	53.00
SLA (cm2/g)	e	157.89	35.90
	i	172.05	56.33
	l	181.20	64.54
Chl (m-mol/m^2)	e	419.63	132.43
	i	363.71	113.09
	l	386.03	150.32
Car (m-mol/m^2)	e	173.19	47.17
	i	150.94	49.79
	l	154.02	56.78
mND705	e	0.48	0.06
	i	0.48	0.06
	l	0.45	0.09
DD	e	0.03	0.05
	i	0.04	0.05
	l	0.00	0.06
N %	e	3.86	0.83
	i	3.59	0.75
	l	3.68	0.41
P %	e	0.20	0.05
	i	0.19	0.05
	l	0.23	0.05

b.	Stage	Mean	SD
Water Content (%)	e	63.05	7.20
	i	64.45	4.69
	l	63.80	8.86
Thickness (um)	e	281.35	58.61
	i	252.82	61.71
	l	238.18	49.78
SLA (cm2/g)	e	140.92	80.82
	i	148.88	57.04
	l	158.89	81.53
Chl (m-mol/m^2)	e	304.63	124.99
	i	504.13	148.33
	l	556.40	190.91
Car (m-mol/m^2)	e	138.68	53.91
	i	190.26	61.98
	l	226.12	110.49
mND705	e	0.43	0.09
	i	0.59	0.07
	l	0.59	0.06
DD	e	-0.02	0.07
	i	0.11	0.04
	l	0.11	0.04
N %	e	1.81	0.69
	i	2.68	0.77
	l	2.55	0.79
P %	e	0.11	0.08
	i	0.11	0.03
	l	0.10	0.04

Table 4-3. The assemblage of leaf traits collected at the three successional stages at each of the two study sites. a. CCBR. b. SRNP.

4.2.4 Spectral Analysis

To link the leaf physical and biochemical traits to leaf spectral properties, we used two Spectral Vegetation Indices (SVIs). While empirically-derived hyperspectral indices have shown in general to exhibit greatly varying performance in terms of accurately reflecting measured pigment contents when applied outside the dataset with which they were developed (le Maire *et al.* 2004), we have selected two indices shown to perform consistently across environments. The first is based on the Normalized Difference Vegetation Index (NDVI), generally expressed as the ratio of the difference to the sum of the Near Infrared and Visible portions of the EM spectrum (Rouse Jr *et al.* 1974):

[4] $$NDVI = \frac{(R_{NIR} - R_{RED})}{(R_{NIR} + R_{RED})}$$

Sims and Gammon's (2002) refinement, the modified normalized difference index (mND705), narrows the spectral range sampled and includes additional information from the blue part of the spectrum to increase the index's sensitivity to changes in leaf pigment levels.

[6] $$mND705 = \frac{(R_{750} - R_{705})}{(R_{750} + R_{705} - 2R_{445})}$$

le Maire *et al.*'s (2004) double difference (DD) index was developed to overcome the site- or dataset- specificity inherent in many SVIs, and was found by the authors to be more sensitive to pigment levels than many more complex approaches (ie. Neural networks, derivative-based analysis) of estimating these pigment contents.

[7] $$DD = (R_{749} - R_{720}) - (R_{701} - R_{672})$$

4.2.5 Classification Analysis

In order to evaluate the effect of changing successional stage on leaf-level spectral classification of tree species at these sites, a classification approach was used after the method described in Hesketh and Sánchez-Azofeifa (2012). A suite of variables were derived from the raw spectral data and used as inputs to a classification and cross referencing process to uncover the effect that varying stand age has on the accuracy of the classification process.

The raw spectra were transformed two ways. First, principle component analysis (PCA) was performed on the spectra so as to reduce the dimensionality and redundancy inerrant in hyperspectral data while retaining the variation contained in each band (Kalácska *et al.* 2007a;

Schowengerdt 1996). The first 4 principle components were retained, accounting for more than 97% of the variation in the original spectra.

Second, two spectral indices were included to stand in for important biophysical variables: pigment and water content. While physical measurements of theses leaf traits were collected, we chose spectral proxies for the classification analysis to address the potential for automated, database-style spectral classification as addressed by Asner and Martin (2008) Castro-Esau *et al.* (2006). The particular indices selected were chosen to allow direct comparison with the results described in Hesketh and Sánchez-Azofeifa (2012). Merzlyak *et al.*'s (1999) Plant Senescence Reflectance Index (PSRI) was included to provide an estimate of chlorophyll and carotenoid pigments. PSRI increases proportionate to the carotenoid/chlorophyll molar ratio, and stands as an indicator of the balance between these key pigments. Penuelas et al's (1993) Water Index (WI) was included to provide a spectral representation of leaf water content.

[8] $$PSRI = \frac{(R_{680} - R_{500})}{(R_{750})}$$

[9] $$WI = \frac{R_{900}}{R_{970}}$$

These six variables (PC1-PC4, PSRI, WI) were used as inputs to the nonparametric decision tree classifier See 5 (Rulequest Research, St Ives, NSW, Australia, 2008). See5 includes a cross-reference function to allow for the evaluation of the accuracy of a classification tree trained on a given successional stage when applied to data from a different stage (e.g. testing a classifier trained on early successional stage data on data from the late stage). The classification was run nine times at each of the two sites: three times with the training and testing datasets taken from the same stage (early, intermediate, and late), and a further six using all possible combinations of successional stages for the training and testing datasets. Classification accuracy was reported as a percentage (# individuals from the testing dataset classified correctly / total # individuals).

4.3 Results

4.3.1 Do leaf structural and chemical traits vary with successional stage?

We found that the potential for variability in leaf chemical and structural traits among early, intermediate, and late successional stages was site specific. At SNRP, 5 of the 7 traits measured showed significant variability with the exception of water content and phosphorous content (Table 4-4). At CCBR, with just over half the mean annual rainfall as measured at SNRP, none of the 7 traits showed significant variability according to successional stage.

Site # Species	CCBR 9	SRNP 14
Water	0.278*	0.13*
Thickness	0.267	**0.001**
SLA	0.312*	**0.005***
Chlorophyll	0.203*	**<0.001***
Carotenoids	0.127*	**0.001***
mND705	**0.031***	**0.001***
DD	**0.002***	**<0.001***
N	0.726	**0.05**
P	0.233	0.166*

Table 4-4. *p*-values from one-way analysis of variance among the three successional stages at each study sites. Boldface text indicates differences significant to the 0.05 level. Values with an * indicate data which did not satisfy ANOVA assumptions and Kruskal-Wallis results were substituted.

Considering the structural variables (leaf thickness, water content, and specific leaf area), we found significant differences among the three successional stages in leaf thickness and SLA at SRNP but not at CCBR (Figure 4-2, Table 4-4). Figure 4-2b shows a pattern of decreasing thickness with each later successional stage at SRNP (H= 20.003, *p*= 0.001), where no noticeable trend exists in the data at CCBR. Looking more closely at differences between the stages themselves, a pairwise comparison of leaf thickness among successional stages shows significant differences between early and intermediate (U= 1674, *p*= 0.001) and early and late stages (U: 1414, *p*= <0.001) at SRNP, while again, no significant difference was noted at CCBR (Table 4-5).

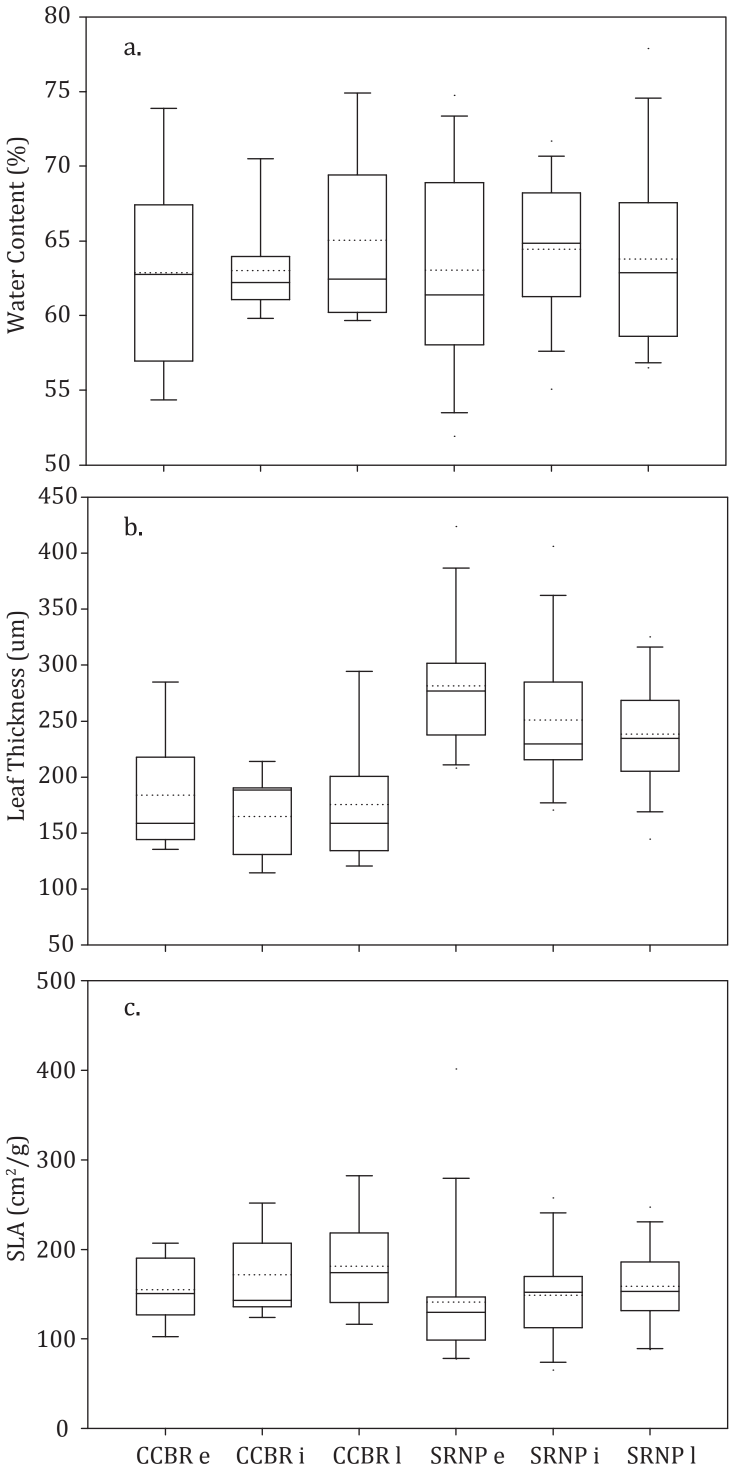

Figure 4-2. Structural Variables at the two study sites.
a. Leaf Thickness, b. Water Content, c. Specific Leaf Area.

a.	**Water**	**Thickness**	**SLA**	**Chl.**	**Car.**	**N**	**P**	**mND705**	**DD**
CCBR	0.142	0.423	0.112	0.317	0.089	0.604	0.243	**0.039**	**0.029**
SRNP	0.253	**<0.001**	**0.001**	**<0.001**	**<0.001**	**0.018**	0.444	**<0.001**	**<0.001**

b.	**Water**	**Thickness**	**SLA**	**Chl.**	**Car.**	**N**	**P**	**mND705**	**DD**
CCBR	1.000	0.176	0.468	**0.036**	**0.034**	0.5	0.655	0.819	**0.32**
SRNP	0.051	**0.001**	**0.038**	**<0.001**	**<0.001**	**0.005**	0.105	**<0.001**	**<0.001**

c.	**Water**	**Thickness**	**SLA**	**Chl.**	**Car.**	**N**	**P**	**mND705**	**DD**
CCBR	0.165	0.704	0.516	0.375	0.834	0.791	0.107	**0.02**	**0.001**
SRNP	0.253	0.348	0.341	0.072	**0.028**	0.266	0.266	0.496	0.696

Table 4-5. *p*-values from pairwise comparisons between successional stages at each study site. Boldface results indicate significance at 0.05. * indicates that t-test assumptions were not met, so Mann-Whitney was performed instead. Successional stage combinations: a. early-late, b. early-intermediate, c. intermediate-late.

Similar trends were noted among the SLA measurements. SRNP showed significant differences among the three stages (H= 10.591, *p*= 0.005), with the greatest variation found between the early-intermediate and early-late pairings (U= 1951.5, *p*= 0.038; U= 1667, *p*=0.001, respectively). Even though Figure 4-2c indicates an increasing trend in mean SLA with increasing succession at CCBR, these differences were found to be insignificant both overall and between the paired stages.

In spite of the indications of a rainfall-mediated trend in trait variation between the two sites, variation in leaf water content was found to be insignificant in all tests. Further, there were no general trends observable in the data in Figure 4-2a, other than an increase in the mean value between the intermediate and late stages at CCBR.

Variation in leaf chemistry (nitrogen and phosphorus levels) was generally found to be not significant between the stages, with the exception of N at SRNP (H=9.389, *p*= 0.009) (Figure 4-3). While the significant increase in nitrogen between both the early-intermediate and early-late successional stages (U=36, *p*=0.005; U=46, *p*= 0.018) result in a general increasing trend in the N:P ratio along the successional gradient (Figure 4-3c), this trend itself is not significant. Investigating this pattern between the drier CCBR site and the wetter SRNP, Figure 4-4 shows the linear relationship between nitrogen and phosphorous contents. Where CCBR shows very little variation in the slope of the regression line between successional stages, the slope of the line at SRNP increases by more than a factor of 6 between the early and intermediate stages, with a continued, but milder increase between intermediate and late.

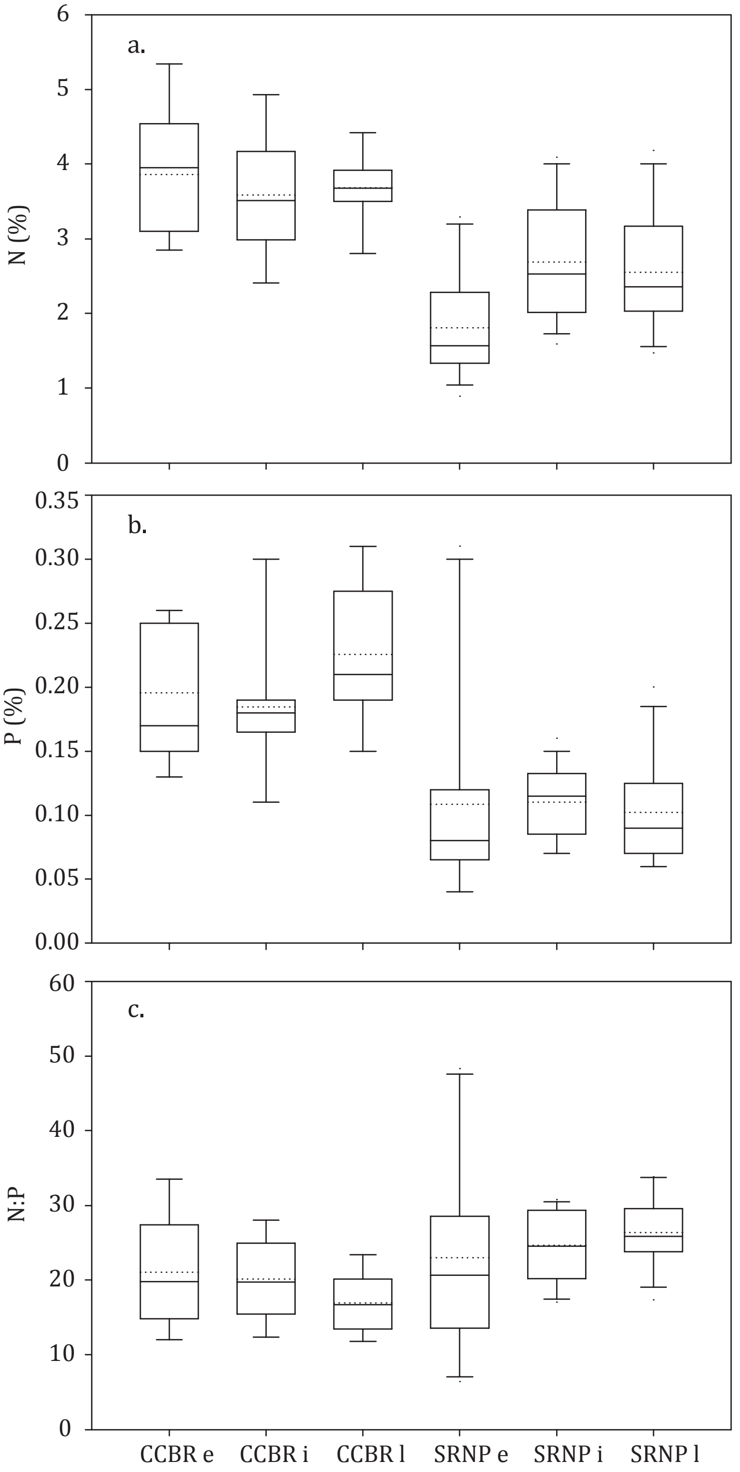

Figure 4-3. Chemical Variables at the two study sites. a. Nitrogen, b. Phosphorous, c. N : P Ratio.

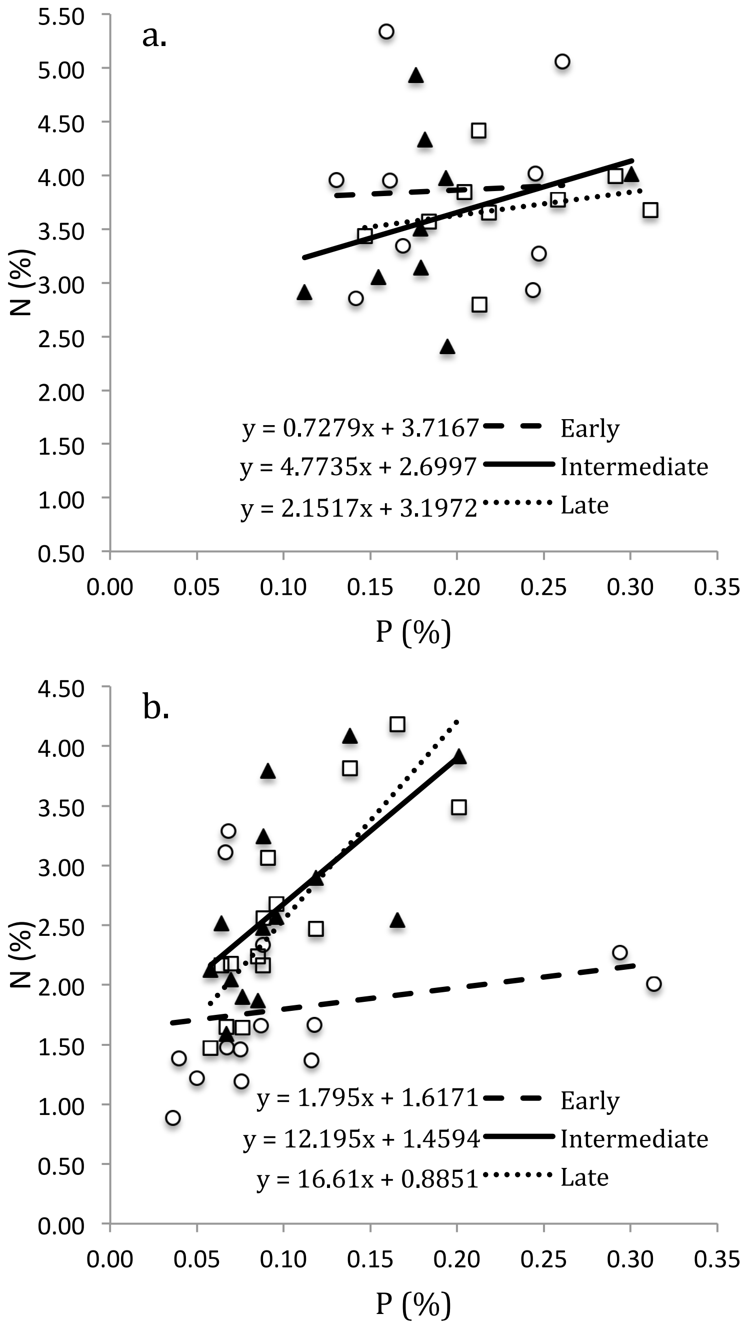

Figure 4-4. Changes in the relationship between Nitrogen and Phosphorous at the two study sites. Open circles are early stage species, solid triangles are intermediate, and open squares are late. a. CCBR, Mexico; b. SRNP, Costa Rica.

4.3.2 Do leaf pigment content and the associated SVIs vary with successional stage?

Both the total chlorophyll and carotenoid levels were significantly different between successional stages at SRNP, but not at CCBR (H= 73.25, *p*= <0.001 and H= 42.26, *p*= 0.001) for chlorophyll and carotenoids, respectively. The increasing trend from early to late stages in both pigments at SRNP (Figure 4-5) resulted in significant differences between the not just early-intermediate and early-late pairs (t=-8.544, *p*= <0.001 and U= 617, *p*= <0.001 for chlorophyll, t= -5.216, *p*= <0.001 and U= 1004, p= <0.001 for carotenoids), but also between the paired intermediate and late stages (Chl: U= 2018, *p*= 0.072; Car: U= 1921, *p*= 0.028). This was the only example of significant difference in a physical (non-optical) trait between the intermediate and late stages. At CCBR, both pigments exhibited a non-significant decreasing trend across the three stages as opposed to the strong increases noted at SRNP. This decrease was significant only between the early and intermediate stages (t= 2.13, *p*=0.036 and t= 2.152, *p*= 0.034 for chlorophyll and carotenoid contents, respectively).

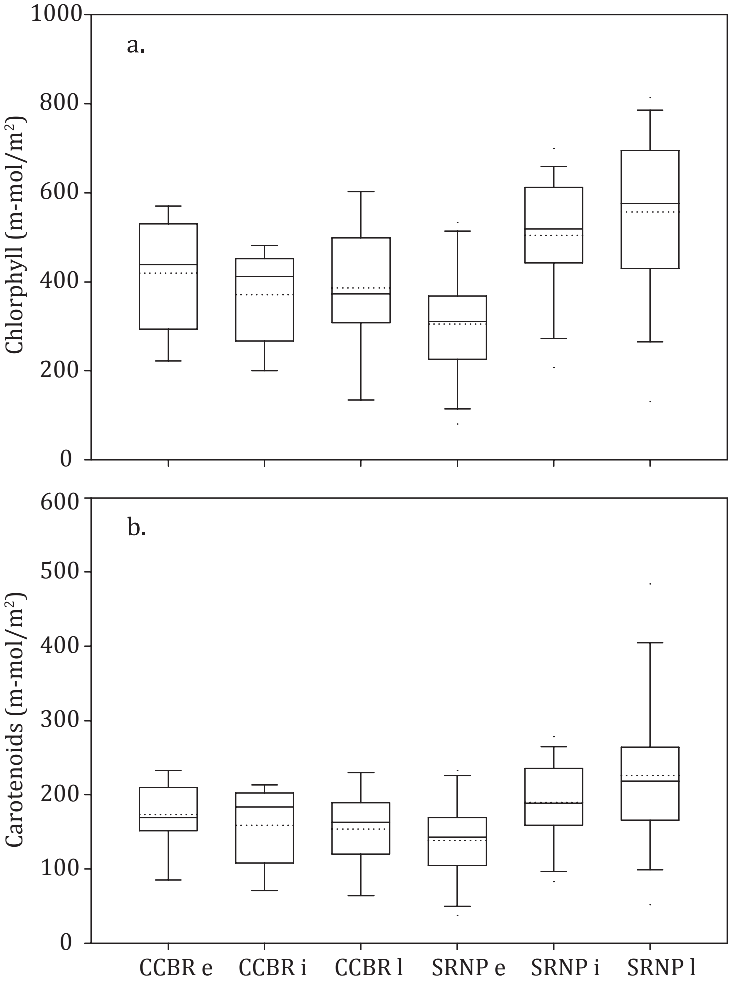

Figure 4-5. Pigment content at the two study sites. a. Total Chlorophyll, b. Carotenoids.

The SVI's included in the spectral analysis were designed to be sensitive to these pigment levels (le Maire *et al.* 2004; Sims and Gamon 2002), and should therefore follow similar trends. This is generally the case at SRNP, where strongly significant differences were noted across the three stages (F=118.41, p= 0.001 for mND705 and H= 102.51, p= <0.001 for DD). Both indices however show a slight decline from the intermediate to late stages, as compared to the increase noted in chlorophyll levels (Figures 4-6a and 4-5a). This is reflected in the insignificant difference between these stages, though significance is retained among the early-intermediate and early-late combinations (t= -12.626, t= 12.552 and U= 362, U= 345.5 for mND705 and DD, all with *p*= <0.001). We also found significant differences between the indices in the ANOVA results at CCBR, the only case in this analysis to do so (H = 6.955, *p*=0.031 and F= 6.459, *p*= 0.002 for mND705 and DD). This difference was reflected for mND705 and DD at both the early-late (t=-2.098, *p*= 0.039 and t= 2.223, *p*= 0.029) and intermediate-late stage (U= 723.5, *p*= 0.02 and t= 3.334, *p*= 0.001) pairings.

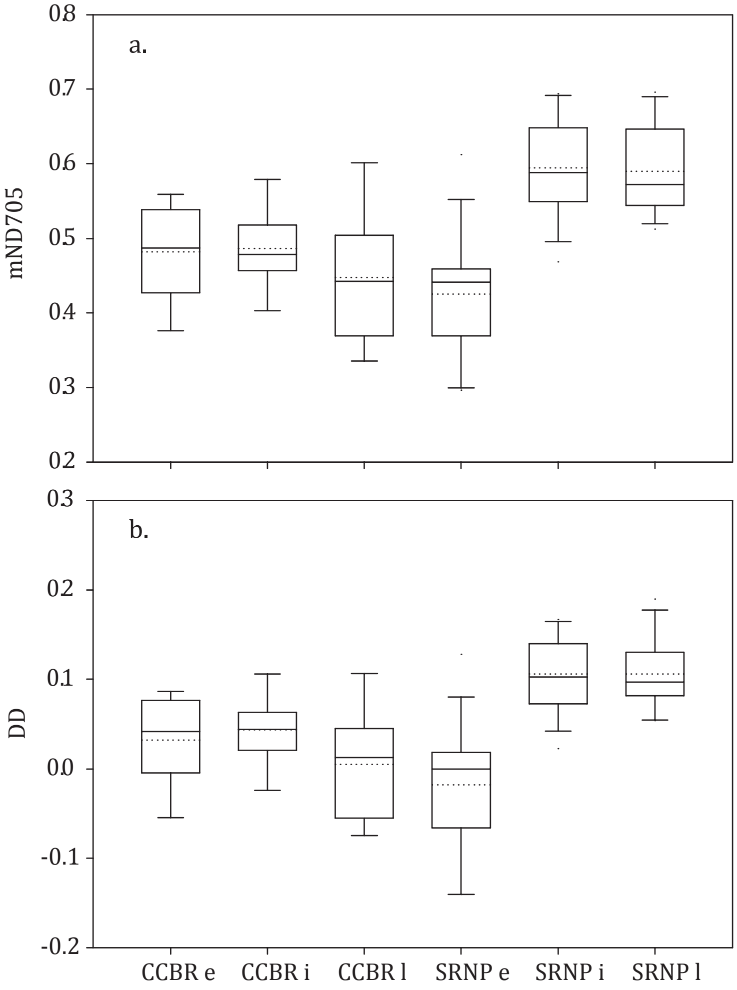

Figure 4-6. SVIs related to leaf pigments at the two study sites. a. mND705, b. DD.

4.3.3 Does the strength of correlations among leaf traits vary with successional stage?

Tables 4-6 and 4-7 address the correlation between the measured physical and optical leaf traits at changing levels of aggregation, with the three successional stages at each site grouped (Table 4-6) and for each successional stage at each site separately (Table 4-7). In general, the strength of correlations, and the number of strongly related variables increased with increasing specificity of the data set, in spite of the decreasing size of the sample.

	Water	**Thick**	**SLA**	**CHL**	**CAR**	**N**	**P**	**mND705**	**DD**
Water		0.37	**0.00**	0.75	0.85	0.36	0.18	0.17	**0.04**
Thick	**0.00**		**0.00**	**0.00**	0.87	**0.00**	**0.00**	**0.00**	0.11
SLA	**0.00**	**0.00**		0.09	0.94	0.19	0.16	0.21	0.17
CHL	0.73	0.83	0.36		**0.00**	**0.00**	**0.00**	0.06	0.99
CAR	0.68	0.99	0.77	**0.00**		**0.00**	**0.01**	0.98	0.32
N	**0.02**	0.07	0.51	**0.00**	0.15		**0.00**	**0.00**	0.55
P	**0.01**	**0.04**	0.61	**0.00**	0.13	**0.00**		**0.00**	0.55
mND705	0.17	0.16	**0.03**	0.49	0.98	0.47	0.77		**0.01**
DD	0.35	0.77	0.37	0.29	0.50	0.86	0.96	0.36	

SRNP

CCBR

Table 4-6. *p*-values from Pearson correlation between leaf traits from the combined successional stages at each of the CCBR and SRNP study sites. Boldface values indicate significance of $p \leq 0.05$.

a.	Water	Thick	SLA	CHL	CAR	mND705	DD	N	P
Water		0.24	**0.00**	0.09	0.15	**0.01**	**0.00**	0.46	0.36
Thick	**0.01**		**0.00**	**0.02**	0.86	**0.00**	**0.00**	0.21	0.73
SLA	**0.03**	**0.00**		0.93	0.49	0.18	0.30	0.93	0.65
CHL	**0.00**	0.19	**0.00**		**0.00**	**0.00**	**0.00**	0.89	0.80
CAR	0.12	0.17	**0.01**	**0.00**		**0.00**	**0.00**	0.31	0.62
mND705	**0.02**	**0.01**	0.46	**0.01**	0.56		**0.00**	**0.03**	0.69
DD	**0.00**	0.05	0.93	**0.00**	0.21	**0.00**		**0.05**	0.73
N	0.29	0.33	0.08	0.10	0.22	0.32	0.17		0.46
P	0.95	0.87	0.83	0.26	0.40	0.36	0.45	0.89	

SRNP

b.	Water	Thick	SLA	CHL	CAR	mND705	DD	N	P
Water		0.09	**0.00**	0.43	0.15	**0.04**	0.06	0.22	**0.03**
Thick	0.91		**0.00**	**0.04**	0.13	**0.00**	**0.00**	**0.04**	0.11
SLA	**0.00**	**0.00**		**0.01**	0.45	0.55	0.84	0.51	0.35
CHL	0.28	0.54	0.90		**0.00**	0.90	0.72	0.65	0.42
CAR	0.37	0.72	0.74	**0.00**		0.80	0.50	0.13	0.72
mND705	0.74	0.26	0.88	0.93	0.74		**0.00**	**0.04**	0.10
DD	0.64	0.44	0.77	0.49	0.29	**0.00**		**0.03**	0.08
N	0.23	0.31	0.12	0.35	0.26	**0.04**	0.07		**0.00**
P	0.55	0.39	0.54	0.48	0.38	0.10	0.89	0.38	

SRNP

c.	Water	Thick	SLA	CHL	CAR	mND705	DD	N	P
Water		0.26	**0.00**	0.57	0.50	0.97	0.74	0.08	0.22
Thick	0.06		0.06	0.19	**0.04**	0.15	0.13	**0.03**	**0.01**
SLA	**0.00**	**0.00**		0.88	0.51	0.84	0.93	0.07	**0.04**
CHL	0.30	0.12	0.36		**0.00**	**0.00**	**0.01**	0.45	0.83
CAR	0.20	0.08	0.14	**0.00**		0.54	0.98	0.78	0.35
mND705	0.47	0.29	0.45	**0.02**	0.14		**0.00**	0.35	0.62
DD	0.44	0.13	0.71	**0.01**	0.06	**0.00**		0.31	0.48
N	0.96	0.54	0.39	0.65	0.83	0.27	0.31		**0.00**
P	0.19	0.48	0.77	0.28	0.11	0.87	0.85	0.51	

SRNP

CCBR

Table 4-7. *p*-values from Pearson correlation between leaf traits by successional stage at each of the CCBR and SRNP study sites. Boldface values indicate significance of $p \leq 0.05$. a. Early Stage, b. Intermediate Stage, c. Late Stage.

Only a handful of traits are significantly related at all three successional stages (Table 4-7). Specific leaf area is well correlated with the other structural variables (r= 0.46 and -0.62; 0.52 and -0.38 for water content and leaf thickness at CCBR and SRNP, respectively, *p*= 0.00 in all cases), very much in keeping with the findings of Wright *et al.* (2004a). SLA did not, however, correlate with either pigment levels or foliar chemistry (with the exception of N at CCBR with r= 0.43, *p*= 0.03)), contrary to the findings of Asner *et al.* (2009). When considering the successional stages separately, the chemical variables did not correlate consistently with other traits across all three stages, though nitrogen showed a significant relationship with mND705 in the intermediate stage at CCBR (r= 0.68, *p*= 0.04), and phosphorus and nitrogen were related in the intermediate and late stages at SRNP (r= 0.72 and 0.84, respectively, *p*= 0.00. Significant relationships were noted between both SVIs and chlorophyll at both sites in the early and late successional stages (r= 0.37 and 0.47, 0.35 and .041 for mND705 and DD at CCBR early and late stages, respectively, *p*≤ 0.02. r= 0.35 and 0.34, 0.38 and .032 for mND705 and DD at SRNP early and late stages, respectively, *p*≤ 0.01), but not at either site in the intermediate stage.

4.3.4 Do leaf optical properties vary with successional stage?

Leaf spectral reflectance, shown in Figures 4-7 and 4-8, shows patterns of variability attributable to differences in moisture availability at each site. Figures 4-7d and 4-8d show the areas of greatest change in the spectrum between the early and late stages, as that was the pairing which showed the strongest and most significant differences between the biophysical traits. At SRNP, this difference tended to be maximized along the "red edge" (~720 nm). This is the region of the spectrum used in the calculation of the two chlorophyll-sensitive SVIs used here, which were found without exception to be significantly different (p= <0.001) in the same pairing of successional stages. The differences at CCBR were less dramatic, but were generally shifted further towards the near-infrared region of the spectrum, more indicative of changes in structural and water-controlled features between the early and late successional stages.

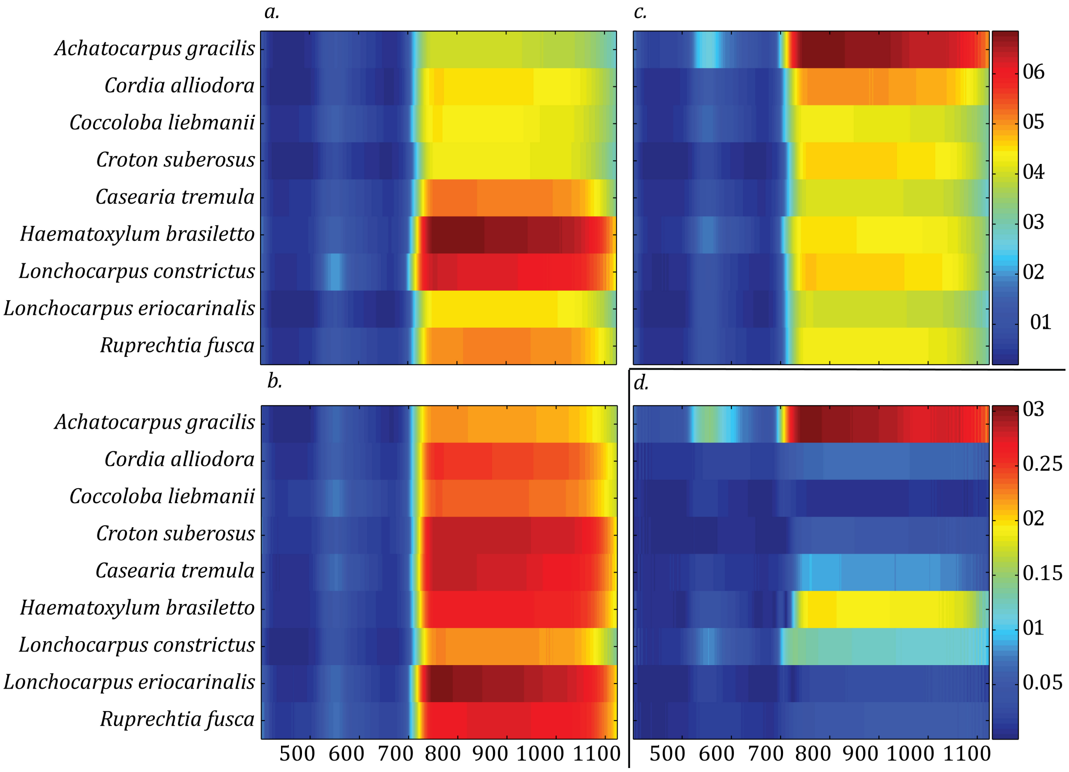

Figure 4-7. Spectral reflectance at: a. early, b. intermediate, c. late, and d. difference between e and l for CCBR. Panels a, b, and c share a common scale bar.

4.3.5 Is spectral variation between successional stages sufficient to affect the accuracy of species level classification?

Spectral variability between successional stages had a strong negative influence on classification accuracy (Figure 4-9). The highest accuracy was found at SRNP, classifying within the intermediate stage (82.5%), with the lowest at CCBR, using a classifier trained in the early stage to classify data from the intermediate (7.4%). The various combinations are pictured in Figure 4-9 with the resulting classification accuracies listed in Table 4-8a. Without exception, classification accuracy was substantially higher when classifying within stages, rather than across stages, with an average decrease in accuracy by a factor of 4. (Table 4-8b).

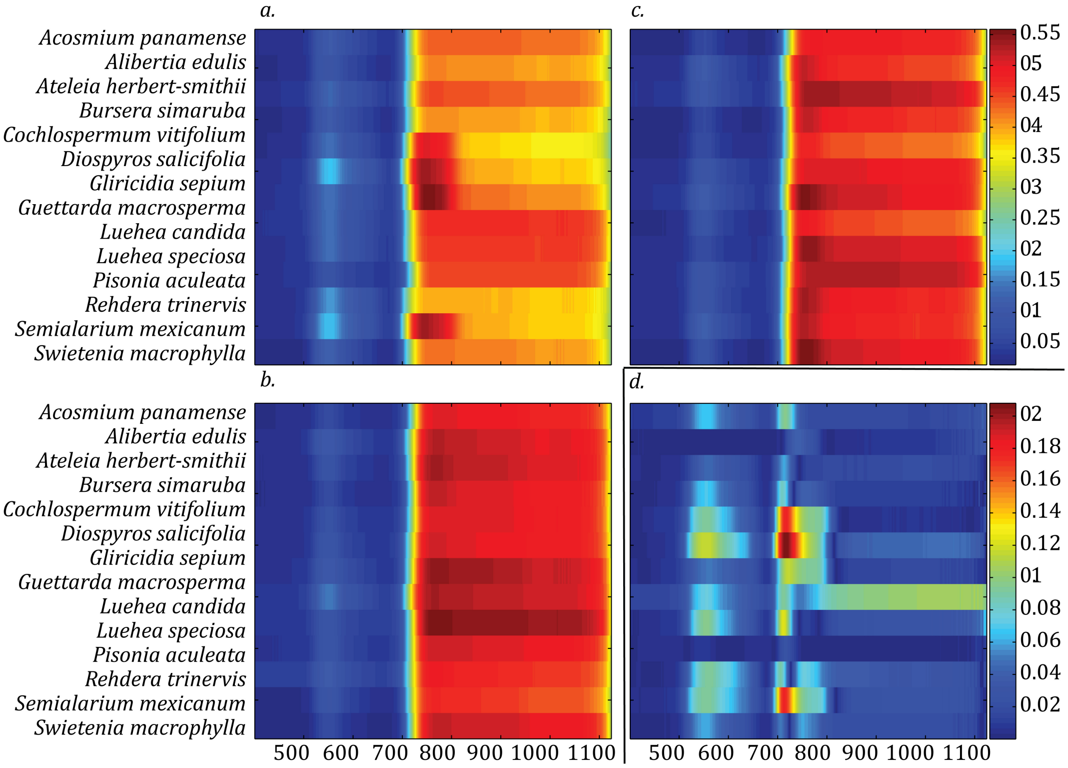

Figure 4-8. Spectral reflectance at: a. early, b. intermediate, c. late, and d. difference between e and l for SRNP. Panels a, b, and c share a common scale bar.

Examination of the between-stage classification results showed that a large portion of the between-stage accuracy was attributable to a limited number of species (*Achatocarpus gracilis* and *Croton suberosus* at CCBR and *Acosmium panamense*, *Gliricidia sepium*, *Luehea candida*, and *Rehdera trinervis* at SRNP). To test the effect of the presence of a small number of species which show consistent spectral properties across successional stages, these species were removed from the dataset and the See5 classification run a second time. The resulting within-stage accuracy was increased in the early stage at both sites, but decreased slightly in the intermediate and late stages (Table 4-8a). With only one exception however (where the classifier was trained on the early and tested on the intermediate stage at CCBR), accuracy was substantially decreased in all the between-stage classifications, indicating that the stability of classification results was largely the product of spectral consistency among a handful of species. Mean classification accuracy within successional stages was relatively constant at both sites following the removal of the above species (Table 4-8b). Mean between-stage accuracy, however, was decreased by approximately 17.2% at CCBR and 38.7% at SNRP, resulting an increase in the overall decrease in accuracy of 25.5% (Table 4-8b).

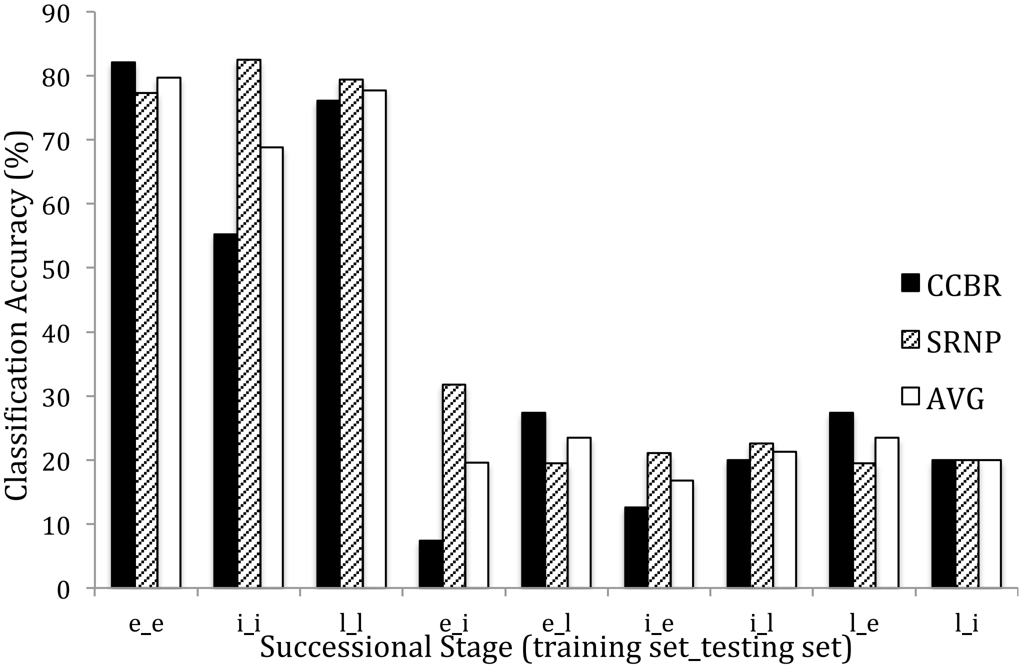

Figure 4-9. Accuracy of the See5 classification process for each training_testing stage combination. Accuracy levels for each site and stage combination in Table 4-8.

a Stages	CCBR all	CCBR adj.	SRNP all	SRNP adj.	Avg all	Avg adj.
e_e	82.09	92.31	77.32	82.35	80.91	87.33
i_i	55.22	53.85	82.47	79.41	76.45	66.63
l_l	76.12	71.15	79.38	77.94	82.39	74.55
e_i	7.41	9.52	31.79	13.33	23.35	11.43
e_l	27.41	20.95	19.49	15.56	20.35	18.25
i_e	12.59	12.38	21.03	14.81	17.59	13.60
i_l	20.00	11.43	22.56	11.85	19.74	11.64
l_e	27.41	23.81	19.49	17.04	18.41	20.42
l_i	20.00	16.19	20.00	18.52	18.06	17.35

b Accuracy	CCBR all	CCBR adj.	SRNP all	SRNP adj.	Avg all	Avg adj.
Within	71.14	72.44	79.73	79.90	79.92	76.17
Between	18.89	15.65	22.86	14.02	19.91	14.83
Factor	3.77	4.63	3.49	5.70	4.01	5.13

Table 4-8. a. See5 Classification accuracy: Expressed as a percentage of individuals classified correctly versus the number of individuals in the testing data set. The clear columns (labeled all) include all the common species as listed in Table 1 while the shaded columns (adj.) refer to classification accuracy following the removal of anomalously consistent species (2 from CCBR and 4 from SRNP). b. Mean classification accuracy within successional stages and between stages. Factor is the magnitude of the variation between within- and between-stage classification, expressed as the ratio of within- to between-stage accuracy.

Of the six inputs to the classifier, the two with a clear ecological interpretation are the SVIs PSRI and WI. When these variables were compared between the six removed species and those remaining (7 at CCBR and 10 at SRNP), significant differences were found in all stages except for PSRI at CCBR early and late (U=1305, *p*= 0.154 and U= 1422, *p*= 0.420) and WI at SRNP late (U= 3324, *p*= 0.72) and CCBR early (U=1221, *p*= 0.061). Mann-Whitney tests on the biophysical variables controlling these indices showed significantly higher water content values at all three stages in CCBR

(U= 10, 62, 34 for early, intermediate and late, *p*= <0.001 for all) and in the intermediate stage at SRNP (U=338, *p*= 0.036).

4.4. Discussion

The results presented here indicate that leaf structural and chemical traits, as well as leaf pigment content, have the potential to differ significantly between forest successional stages at a given site. This trait variability is influenced by site characteristics, so that the extent and direction of changes detected between successional stages are not the same at CCBR and SRNP. Additionally, the spectral reflectance of leaves was found to be variable between successional stages to the extent that automated species level classification was impossible when successional variability was not taken into account.

4.4.1 Variation in leaf traits in response to forest successional stage

Variability between successional stages among the plant traits evaluated here was generally significant in ANOVA analysis at SRNP, but only the SVIs showed significant differences between stages in the same tests at CCBR (Table 4-3). Traits exhibiting significant differences between successional stages tended to be those following a clear trend from early to late succession. Such a trend is noticeable at SRNP in SLA, pigments, SVIs and the N:P ratio (increasing) and leaf thickness (decreasing). The general lack of statistically significant differences between the three successional stages at CCBR noted in Table 4-3 may be attributable to the fact that this trend was not observed. For all structural and biochemical traits except for water content and SLA (Figure 4-2a and c), the mean value at the intermediate stage ran contrary to the general trajectory established by the early and late stages.

The between-stage variability also represents a deviation from the Leaf Economics Spectrum (LES) proposed by Wright et al (2004a). Where the LES describes functional trade-offs that apply globally across a broad range of species and climates, our results point to the presence of local-scale variability that doesn't fit this pattern. This deviation is reflected in a recent study by Funk and Cornwall (2013), who also report context-dependent variability that doesn't fit the pattern described by the LES. Both their results and ours underscore an important point: While relationships such as the LES are effective tools for understanding global-scale patterns in leaf traits, these patterns don't necessarily hold at the community level, where local-scale variation may produce different results.

The number of significantly related leaf traits remained unchanged at SRNP across the three successional stages, with 13 significant relationships found at each stage, though the related traits were inconsistent from stage to stage. Leaf traits at CCBR showed fewer correlations overall, though similar general patterns emerged. SLA was consistently related with leaf water content and leaf thickness, and chlorophyll and carotenoid pigments showed significant relationships at all stages. Contrasting with SRNP, the greatest number of correlations was found at the early successional stage (16), decreasing to 6 at each of the intermediate and late stages. There is little in the literature with which to compare variation of trait-correlations in response to changing forest succession, but this trend is consistent with Poorter *et al.* (2008), who note that relationships between traits are much stronger for younger, smaller trees than for more mature individuals.

4.4.2 Impact of site characteristics on trait variability

Variability in leaf traits at the site level was strongly divergent between CCBR and SRNP. The high degree of difference between stages found here is consistent with Asner *et al.* (2009), who studied variability in chemical and spectral properties of 162 species across a climate/precipitation gradient in Australia. They found that variation was greatest at lowland sites with high temperature and moderate precipitation. SRNP has a mean annual rainfall of 1500 mm, slightly below the range they describe, but is intensely variable, with values upwards of 2500 mm reported (Janzen 1993). Water limitation is undoubtedly a greater factor at CCBR, with mean annual precipitation of just over half the SRNP average.

Soil characteristics are also more favorable for growth at SRNP, with Cambisol-dominated topsoil and a relatively high organic carbon content (2.48% by weight). CCBR by contrast it dominated by sandy Regosols, with a much lower organic content (0.41%) (FAO *et al.* 2012). It may be that this water-stressed environment, common to all successional stages, restricts the development of distinct leaf traits associated with advancing successional stage.

Differences in the extent of successional variability leaf traits between the sites may be related to differences in the criteria used in establishing the permanent plots. The plots at CCBR were established based on time since abandonment, (Avila-Cabadilla *et al.* 2009), whereas those at SRNP were defined based on stand structural characteristics (Arroyo Mora *et al.* 2005; Kalácska *et al.* 2004a). The environmental constraints at CCBR, notably poorer soils and lower mean precipitation, may prevent the development of functionally distinct collections of leaf traits within the temporal boundaries applied to the selection of the plots. The plots at SRNP, selected based on stand characteristics rather than time since abandonment and less constrained resources, may have a greater likelihood of containing a more distinct successional profile of leaf traits.

4.4.3 Variability in leaf spectral reflectance

Variability in spectral reflectance between successional stages (Figures 4-7 and 4-8) is concentrated in different parts of the electromagnetic spectrum at each of the study sites. While within-stage variation between species is unremarkable, examination of the spectral regions that exhibit the greatest difference between the early and late stages (the most significantly different pairing for the majority of leaf traits) shows two distinct patterns. At CCBR, the drier of the two sites, spectral variation between the stages tended to be greatest in the region controlled primarily by leaf structural and moisture characteristics (Gates *et al.* 1965), whereas the region of greatest difference at SRNP is governed most strongly by leaf pigment content, particularly chlorophyll (Boyer *et al.* 1988; Gates *et al.* 1965).

This pattern of differences in key spectral regions points to a fundamental difference in plant function according to differences in site characteristics, and particularly in response to different precipitation regimes. The variation noted here seems to indicate physiological changes in leaf characteristics related to moisture stress at the relatively dry CCBR site, where leaf structure at SRNP appears to be more consistent between the stages. Instead, at SNRP where precipitation is less likely to be a limiting factor, variation is expressed instead as changes in the relative abundances of photosynthetic pigments between the stages. Castro-Esau *et al.* (2004), Sánchez-Azofeifa *et al.* (2009), and Hesketh and Sánchez-Azofeifa (2012) have all noted similar patterns of leaf properties in response to differences in precipitation regimes. It seems clear that at the site level, environmental constraints driven by micro-climate and soil type may influence the assemblage of traits most affected by changing successional stage. Accordingly, not just the extent of spectral variation but also the location of that variation within the spectrum vary as well.

4.4.4 Impact of forest succession on classification accuracy.

Within each site and successional stage, classification accuracy was relatively high, with a maximum value (before the removal of the most consistent species) of 82.5% at SRNP in the intermediate stage. The lowest accuracy, 55.2%, was found at the same stage in CCBR, though all other within-stage classifications had accuracies above 76.0%. This is slightly lower than previous findings by Clark *et al.* (2005), who report 92.0% accuracy of a set of 7 rainforest species collected in Costa Rica, but in the range of Castro-Esau *et al.* (2006) who found accuracies of 80.0% or higher at a collection of dry to wet tropical sites throughout Costa Rica, Panama, and Mexico. Our relatively low classification accuracy as compared with Clark *et al.* (2005) is likely a result of differences between the data sets and classifiers used. While we have intentionally limited the number of spectral inputs to our classification with an interest toward evaluating the plausibility of simple and efficient automated species discrimination, they include a greater number of spectral bands in their analysis (30 wavebands to our 6). This accords with similar species level classification by leaf chemistry where accuracy was found to increase with increasing dimensionality of the input dataset, rising from approximately 55% using 6 inputs to 90% with 20 (Greg Asner, 2010, personal correspondence).

While a negative relationship between sample size and classification accuracy was expected to manifest as increased accuracy following the removal of the six anomalously consistent species, this was only found to be true in the early successional stage. Castro-Esau *et al.* (2006) project a linear decrease from 85.0% with 20 species to 69.0% with 100 species. However, in spite of the modest sample size resulting from a focus on species common to all three successional stages, our average within-stage accuracy was typically lower than both their projections and the results of Hesketh and Sánchez-Azofeifa (2012), achieved with the same classifier used here on 30 species in the Panamanian dry forest (Table 4-7b).

The decrease in accuracy from within-stage to between-stage classification, was consistent and unambiguous, with a typical reduction to approximately 25% of the within-stage accuracy where all common species were included, and 20% where the six anomalously consistent species were removed from the classification. Apart from their high between-stage classification accuracy, the species removed (*Achatocarpus gracilis* and *Croton suberosus* at CCBR and *Acosmium panamense, Gliricidia sepium, Luehea candida*, and *Rehdera trinervis* at SRNP) exhibit little in common with each other. They do not cluster taxonomically, though the *Acosmum* and *Gliricidia* species share the family *Fabaceae*. As discussed above, it is likely that water availability and retention at the leaf level plays an important roll in determining leaf spectral characteristics, and this is evident in the classification results. This conclusion supports the findings of Castro-Esau *et al.* (2004), who found that lianas and trees at two sites in Panama could be accurately discriminated between using spectral reflectance at a dry forest site, but not at a rainforest site. Clearly, the role of microclimate in general and precipitation as well as soil moisture in particular must be considered in any classification undertaken in the seasonally dry tropics.

4.4.5 Applications and directions for further study

While investigation into leaf biophysical and spectral variation of the type described here is important from the perspective of better understanding the plant physiology of tropical dry forests and contributing to the dialogue surrounding the development of spectral taxonomic relationships (Asner and Martin 2008; Ustin and Gamon 2010), this variation is of particular importance in assessing the potential for reliable species-level discrimination of plant species or functional types using airborne remote sensing. One of the goals of remote sensing of forests is the use of these data for automated retrieval of biodiversity and species information (Asner and Martin 2008). The requirement for species discrimination is that interspecies variability in leaf properties is greater than intra-species variability (Castro-Esau *et al.* 2006). It follows then that an understanding of the scope and variation of plant traits across any number of environmental (ie. temperature, precipitation, altitude) and temporal (seasonal, phenological, successional) gradients is integral to the development of techniques that can be applied with confidence over a variety of environments.

Recent promising results, such as those of Clark *et al.* (2005) and Asner and Martin (2008) point to the potential for monitoring biodiversity via high spatial and spectral resolution imaging, but both ignore the temporal dimension, which has been demonstrated to be an important contributor to leaf-level spectral variability (Hesketh and Sánchez-Azofeifa 2012). Further, the majority of studies into leaf trait variability and classification in the tropics have taken place in rainforest environments, while seasonally dry forests are largely overlooked. This is important, as numerous studies have demonstrated that these environments may yield entirely distinct patterns of trait relationships from more humid forests (Sánchez-Azofeifa *et al.* 2009). Castro-Esau *et al.* (2004) showed that discrimination between trees and lianas at two sites in Panama was influenced largely by the precipitation regime at the site level. They found that spectral characteristics expressed during the dry season at a seasonal site allowed for accurate discrimination between functional groups, whereas differences were less clear at a rainforest site.

Sánchez-Azofeifa *et al.* (2009) found a distinct pattern of leaf traits among liana species in dry versus rainforest environments. Drought adaptation by lianas, noted by Schitzer (2005) and Andrade et al (2005), are expressed as higher spectral reflectance and transmittance and lower absorbance, resulting in better heat management and a lower risk of water stress. These traits are most pronounced in dry environments where the advantage they impart relative to host trees is maximized. While our study focus solely on tree leaves, in light of the impact of lianas on canopy spectral response (Sánchez-Azofeifa and Castro-Esau 2006) and the tendency for lianas species to show distinct patterns in leaf biophysical and optical characteristics, particularly in drier environments (Sánchez-Azofeifa *et al.* 2009), investigation into the effect of successional stage on liana leaf traits would be an important contribution to the overall understanding of leaf spectral and biophysical variation in the tropics. Liana species are an increasingly important component of tropical canopies in the Americas (Schnitzer *et al.* 2011) and an understanding of their unique leaf traits as well as variability in liana loading of host trees is an important element in interpreting tropical canopies.

If the classification procedures described here are to be adapted to the canopy scale for use with airborne spectroscopy, variation in forest structural and compositional characteristics between successional stages becomes an important consideration. Species diversity tends to increase and stem density tends to decrease asymptotically with time since abandonment (Powers *et al.* 2009). Kalácska *et al.* (2005) detail the variation in leaf area index (LAI) plant area index (PAI) and canopy openness between successional stages at three tropical sites, including CCBR and SRNP. Decreasing canopy openness and increasing vertical complexity moving from early to late successional stages control the importance of the herbaceous understory at the forest floor in contributing to canopy-level spectral reflectance. That is, where the spectral properties of understory vegetation are an important contributor to overall stand-level reflectance in early stage forests, this contribution decreases as the forest matures and the canopy becomes more closed. While these differences allowed for discrimination between successional stages based on a linear relationship between LAI/PAI and vegetation indices calculated from Landsat 7 ETM+ imagery, this variation, coupled with the potential for inter-species spectral and biophysical variability in addition to changing species composition between successional stages (e.g. Kalácska *et al.* 2004a) suggests a very complex set of variables. As a result, the site-to-site portability of relationships between optical and structural stand characteristics may be impacted.

4.5 Conclusions

We demonstrate here the potential for significant variation in leaf biophysical and spectral characteristics between early, intermediate, and late stages of succession. Site characteristics and microclimate have a clear impact on the magnitude of the difference between successional stages, with the more productive Costa Rican site exhibiting a clearer distinction between leaf traits among the three successional stages. The trend of asymptotic increase in complexity following a disturbance described by Powers *et al.* (2009) is echoed by a pronounced change in leaf properties between the early and intermediate stages, with a more modest change between the intermediate and late. While this variation doesn't occur to the same extent in both of the environments studied, in both cases it was sufficient to inhibit accurate species level classification based on leaf spectral reflectance. Classification accuracies of approximately 80% were eroded by a factor of 4 to 5 when applied without consideration for successional stage.

This study represents an alternative approach to a trend in the ecophysiological literature for studies combining large assemblages of species for evaluation against a variety of environmental conditions, but overlook local-scale variability and the influence of microclimate (e.g. Asner and Martin 2008; Asner *et al.* 2009; Martin and Asner 2009). This local variation, even within species, is measurable and complex, influenced by site characteristics, temperature and precipitation regimes, and patterns of land-use history. A continued exploration of these characteristics, expanded to include other structural groups such as liana species and the herbaceous elements of the forest understory will provide a valuable addition to larger, species-intensive analysis.

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Chapter 5: Effect of season and successional stage on leaf properties in a tropical dry forest

5.1 Introduction

Dry forests in the neotropics, characterized by a naturally fragmented distribution (Pennington *et al.* 2009), have been further fragmented by deforestation related to commercial logging, agriculture, pasture, and human-induced fires (Calvo-Alvarado *et al.* 2009; Colon and Lugo 2006; Corlett 1995; Rudel *et al.* 2002; Silver *et al.* 2000). These forests have been heavily exploited since early colonial times, with climatic and edaphic conditions conducive to development and cultivation (Ewel 1999; Quesada *et al.* 2009; Tosi and Voertman 1964). As a result, they are among the world's most threatened ecosystems (Gentry 1982; Janzen 1988; Murphy and Lugo 1986a), with more than 40% deforestation in dry forests regions reported in Latin America (Olson 2000). In Mexico, which accounts for 38% of the total dry forest coverage in the neotropics (Portillo-Quintero and Sánchez-Azofeifa 2010), nearly 60% of original dry forests have been converted to other uses, and much of what remains exists as fragments in marginalized areas, such as on steep slopes (Trejo and Dirzo 2000). Miles *et al.* (2006) estimate that while 97% of tropical dry forests globally are under threat by some combination of climate change, fragmentation, fire, and conversion for agriculture, only 28.5% enjoys any kind of protected status. Even these forests may be threatened, as fragmentation of surrounding forests increases (DeFries *et al.* 2005; Sánchez-Azofeifa *et al.* 2009).

This pattern of disturbance has lead to the recognition of secondary forests, or those in various stages of regeneration, as the tropical forests of the future (Corlett 1994; Joseph Wright and Muller-Landau 2006; Wright 2005; Wright and Muller-Landau 2006). Because secondary forests are more efficient carbon sinks than mature forests (Brown 1993; Dixon *et al.* 1994; Fearnside and Guimaraes 1996; Uhl *et al.* 1988), an understanding of the distribution of these forests is an important element of global carbon models (Kimes *et al.* 1999). A need for estimates of the extent and distribution of secondary forests in the tropic has led to a call for techniques to monitor this forests using satellite remote sensing (Arroyo-Mora *et al.* 2005; Castillo *et al.* 2012; Foody *et al.* 1996; Steininger 1996).

Longstanding interpretation of the successional characteristics of tropical dry forests predict a more rapid recovery following disturbance than would be expected in wet forests due to a supposed simplicity in their structure and composition and a dominance of clonal reproduction through coppicing (Ewel 1977; Murphy and Lugo 1986b; Segura *et al.* 2003). In fact, high levels of phylogenetic (Gillespie 2005) and genetic (Quesada *et al.* 2004) diversity indicate a number of sexual systems as the primary mode of forest reproduction (Quesada *et al.* 2009). This supports the idea of a system of wind and animal dispersed seeding, as suggested by Janzen (Janzen 1988), which may actually be slower and more susceptible to disturbance due to the seasonality of the growing season and the reliance on granivory and zoophily (Bawa 1990; Cascante *et al.* 2002; Fuchs *et al.* 2003; Quesada *et al.* 2001). Castillo *et al.* (2011) confirm this using LiDAR data to map dry forest canopy heights, finding a pattern of dome-shaped canopies which reflects vertebrate-dispersed seeding.

The successional pathways initiated by secondary forest regeneration give rise to a temporal gradient of successional stages, which influence the structure and composition of forested areas (Kalácska *et al.* 2007b; Kalácska *et al.* 2004; Kalácska *et al.* 2005). Mapping studies have indicated that at the pixel scale, the reflectance characteristics of secondary forests "merged" with those of mature stands after anywhere from 2 to 14 years, depending on the spatial and spectral resolutions of the sensors used (Fearnside 1982; Steininger 1996). In the dry forests, the pronounced seasonality introduces a second temporal gradient. This wet/dry seasonality results in a very dynamic canopy which has a pronounced impact on the discrimination of forest types (Portillo-Quintero and Sánchez-Azofeifa 2010), functional groups (Castro-Esau *et al.* 2004; Kalácska *et al.* 2007a) and species (Hesketh and Sánchez-Azofeifa 2012) at scales ranging from the leaf to the stand.

Succession in secondary forests can be measured using a number of different methods, including palynology (analysis of pollen in the fossil record), stand reconstruction,and the long-term monitoring of permanent forest plots, but the chronosequence method has become one of the most commonly used hazdon *et al.* 2007). Chronosequences, or space-for-time substitutions, use multiple plots of different ages, or times-since-abandonment, to stand for different forest successional stages (Kalácska *et al.* 2005; Lehmkuhl *et al.* 2003), allowing for the simultaneous study of different successional stages. Criticism of the method is based on the assumptions that 1. biotic and abiotic components of all sites follow the same history; and 2. that the variability found between sites in a chronosequence is in fact the result of changing successional stage rather than other environmental or anthropogenic factors (Johnson and Miyanishi 2008). This assumption is difficult to prove, though the topographic conditions, land use histories, and substrates underlying chronosequence plots are standardized to limit the potential for variable successional pathways (Quesada *et al.* 2009). While the common alternative, the tracking of permanent plots, may be ideal in principle, the high financial and human investment necessary to establish long-term studies limits the number of sites. This, coupled with the high environmental and species variability in tropical environments makes if difficult to rely on these studies to fully represent the variety of potential successional pathways (Guariguata and Ostertag 2001). Further, while chronosequence analysis has predicted trends in such characteristics as basal area (Chazdon *et al.* 2007; Pascarella *et al.* 2000) more effectively than species richness (Pascarella *et al.* 2000; Sheil 2001) and species density (Chazdon *et al.* 2007; Johnson and Miyanishi 2008), it is the only way to address the potential variation in leaf traits in response to changing successional stage. No other method allows for data collection in a narrow temporal window, standardizing for both intra- and inter-annual variation in environmental conditions at the site level.

Because an assessment of the variability in leaf properties is an important element of remote monitoring, various studies have investigated this variation along environmental (Asner and Martin 2008; Asner *et al.* 2009; Martin *et al.* 2007) and functional (Castro-Esau *et al.* 2004; Sánchez-Azofeifa *et al.* 2009) gradients. Few studies, however, have been directed towards the temporal properties of these highly variable forests. Better understanding the variability in leaf biophysical and optical properties in response to forest succession is a critical step in establishing methods for classifying and monitoring secondary forests.

As such, the objective of this study is to investigate the impact of seasonality and successional stages on leaf traits and spectral reflectance at a very dry tropical forest in Mexico. First we examine the variability among a suite of leaf traits along a successional gradient during the wet and dry seasons, addressing differences between seasons as well as between pairings of successional stages. Second, we explore the effect of seasonality on leaf spectral properties. Finally, we address the effect of variation in leaf spectral reflectance due to changing season and successional stage on the potential for accurate species classification.

5.2 Methods

5.2.1 Study Site

Data for the analysis were collected at the Chamela-Cuixmala Biosphere Reserve (CCBR) in the Province of Jalisco, Mexico (19°22’ – 19°35’N, 104°56” – 105°03’W). The site falls toward the dry end of the rainfall spectrum for tropical dry forest as defined by Sánchez-Azofeifa *et al.* (2005), with mean annual precipitation of 741 mm (±256 mm) occurring during the short wet season of June through October (http://www.ibiologia.unam.mx/ebchamela/). An average temperature during 1978 – 2009 of 25.2° C (±1.3° C)(Kalácska *et al.* 2004; Maza-Villalobos *et al.* 2011) is relatively cool among tropical dry forests, as defined above.

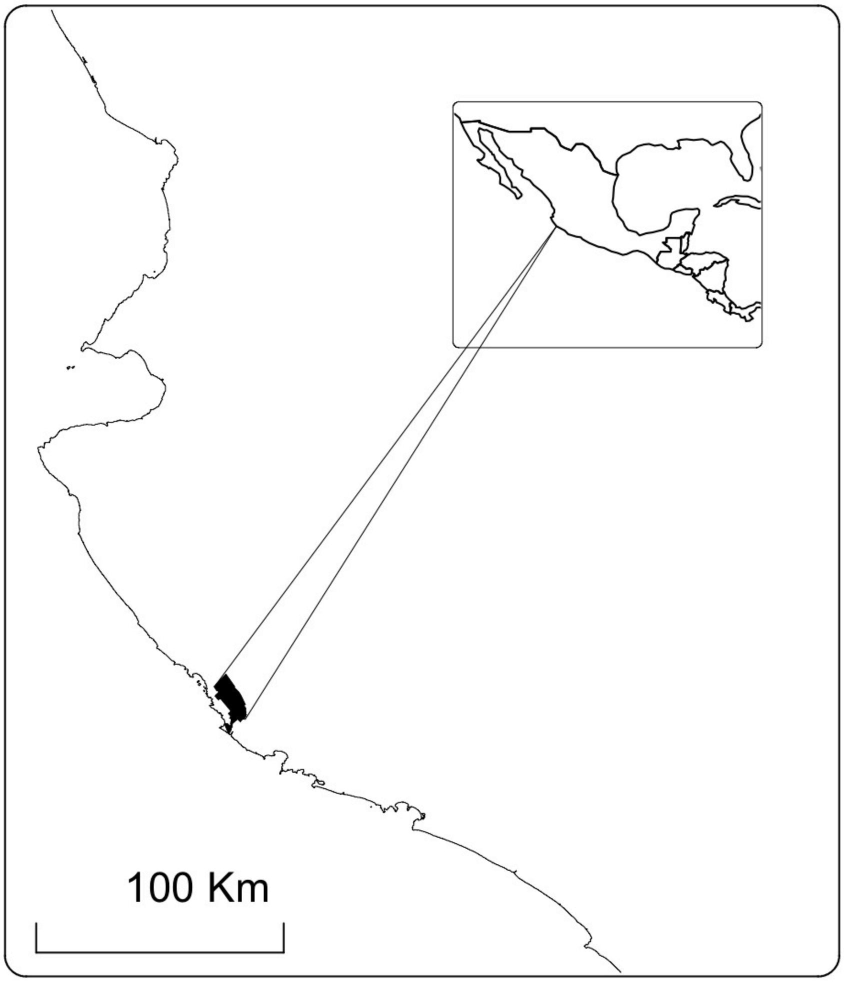

Figure 5-1. Map showing the location of the Chamela Cuixmala Biosphere Reserve (CCBR), in Jalisco, Mexico.

Successional stages at CCBR have been defined as chronosequences on the basis of time since abandonment following a land-use pattern of cultivation followed by pasture (Avila-Cabadilla *et al.* 2009). At the time of data collection, plots located in the early stage had been abandoned 3 – 5 years, those in the intermediate stage for 8 – 12 years, and those listed as mature for a minimum of 50 years. A more detailed account of the land use history and site characteristics is provided in the previous chapter as well as in Avila-Cabadilla *et al.* (2009) and Alvarez-Añorve *et al.* (2012).

5.2.2 Data Collection

Leaf samples were collected and analyzed during 2009 and 2010. The 2009 collection was carried out in September at the height of the wet season (WET). Data from 2010 collection was collected in late in October during the senescent period and is referred to here as dry season data (DRY), though the trees had not yet begun to drop their leaves. The list of species analyzed is detailed in Table 5-1. Protocols for site selection, leaf collection and transportation were the same as those described in chapter 4.

The leaf traits included in the study are listed in Table 5-2. Structural traits (water content, leaf thickness, and specific leaf area (SLA)) and leaf level spectral reflectance were collected within 6 hours of leaf harvest, during which these properties are relatively stable (Foley *et al.* 2006). Leaf spectral reflectance (400 – 1100 nm) was measured at this time using a PP Systems Unispec SC spectrometer (PP Systems, Amesbury, MA). Two vegetation indices were calculated: the mND705 (Sims and Gamon 2002) and the double difference (DD) (le Maire *et al.* 2004). These two indices have been shown to be more reliably applied across a variety of site conditions and species assemblages than many of the alternative indices (le Maire *et al.* 2004).

a.	**Family**	**Genus species**
	Achatocarpaceae	*Achatocarpus gracilis*
	Flacourtiaceae	*Casearia tremula**
	Boraginaceae	*Cordia alliodora**
	Polygonaceae	*Coccoloba liebmanii*
	Euphorbiaceae	*Croton suberosus*
	Leguminosae	*Haematoxylum brasiletto**
	Leguminosae	*Lonchocarpus constrictus**
	Leguminosae	*Lonchocarpus eriocarinalis**
	Polygonaceae	*Ruprechtia fusca**

b.	**Family**	**Genus species**
	Leguminosae	*Caesalpinia caladenia*
	Flacourtiaceae	*Casearia tremula**
	Boraginaceae	*Cordia alliodora**
	Euphorbiaceae	*Croton pseudoniveus*
	Leguminosae	*Haematoxylum brasiletto**
	Leguminosae	*Lonchocarpus constrictus**
	Leguminosae	*Lonchocarpus eriocarinalis**
	Polygonaceae	*Ruprechtia fusca**

Table 5-1. Species included in the investigation. a. Wet season; b. Dry season. Species marked with an asterisk are common to both seasons.

Leaf pigment content (chlorophyll a, b, total chlorophyll, and carotenoids) was estimated by a process of Dimethyl Sulfoxide (DMSO) digestion and absorption spectroscopy performed on frozen leaf cores transported back from the field site (Hiscox and Israelstam 1979; Richardson *et al.* 2002). Foliar chemistry (nitrogen and phosphorous) were estimated from dry leaf material using a combustion element analyzer (N) and colorimetric analysis (P) (Richards 1993). Chapter 4 outlines more exhaustive description of the instruments and laboratory procedures followed in the estimation of each trait.

5.2.3 Statistical Analysis

Summary statistics were calculated for each of the measured variables (Table 5-2). In order to measure variation between successional stages, one-way ANOVA analysis was carried out on the wet and dry season data for each season. Wherever the ANOVA assumptions were not met, the non-parametric Kruskal-Wallis H-test was substituted.

Pairwise analysis of each trait was carried out in two ways: First, differences between paired successional stages were analyzed to determine the locations along the successional gradient where each trait showed greatest variability. The pairings used were early and late (E/L) successional stages, early and intermediate (E/I), and intermediate and late (I/L), and the tests were carried out separately for each season. Second, differences between wet and dry seasons were evaluated separately for each trait at each successional stage. The pairwise analyses use Student's t-test where possible, and the non-parametric Mann-Whitney U-test where the assumptions for parametric statistics were not met. Variability in the interrelatedness of the leaf traits across the successional gradient was evaluated using correlation analysis on both the combined successional stages for each season, and for each stage separately.

a.	Stage	Mean	SD
Water Content (%)	e	62.87	6.18
	i	62.62	5.17
	l	64.99	7.22
Thickness (um)	e	181.75	49.56
	i	164.40	37.64
	l	175.42	53.00
SLA (cm2/g)	e	157.89	35.90
	i	172.05	56.33
	l	181.20	64.54
Chl (m-mol/m^2)	e	419.63	132.43
	i	363.71	113.09
	l	386.03	150.32
Car (m-mol/m^2)	e	173.19	47.17
	i	150.94	49.79
	l	154.02	56.78
mND705	e	0.48	0.06
	i	0.48	0.06
	l	0.45	0.09
DD	e	0.03	0.05
	i	0.04	0.05
	l	0.00	0.06
N %	e	3.86	0.83
	i	3.59	0.75
	l	3.68	0.41
P %	e	0.20	0.05
	i	0.19	0.05
	l	0.23	0.05
N:P	e	21.04	6.85
	i	20.11	4.99
	l	16.94	3.69

b.	Stage	Mean	SD
Water Content (%)	e	53.82	4.70
	i	56.26	5.51
	l	50.19	7.12
Thickness (um)	e	228.65	92.31
	i	187.08	86.91
	l	205.61	78.02
SLA (cm2/g)	e	104.44	55.58
	i	122.59	67.28
	l	157.97	50.58
Chl (m-mol/m^2)	e	399.14	173.48
	i	385.15	171.25
	l	358.35	170.20
Car (m-mol/m^2)	e	130.70	34.78
	i	153.11	64.60
	l	134.33	63.30
mND705	e	0.53	0.06
	i	0.48	0.08
	l	0.51	0.06
DD	e	0.08	0.05
	i	0.04	0.07
	l	0.07	0.06
N %	e	2.50	0.30
	i	2.25	0.37
	l	2.41	0.31
P %	e	0.16	0.03
	i	0.20	0.05
	l	0.23	0.09
N:P	e	16.69	3.39
	i	12.41	4.40
	l	12.02	4.85

Table 5-2. Leaf traits collected during the a. Wet season, and b. Dry season.

5.2.4 Classification Analysis.

The effect of spectral variability between seasons and successional stages on the potential for accurate classification of tree species was estimated through the application of a non-parametric decision tree classifier

(See5, Rulequest Research, St Ives, NSW, Australia, 2008). As inputs to the classifier, a set of 6 variables was calculated from the raw spectral data. Inputs 1 – 4 were the first four principal components of the spectra from each species, used here to reduce the high correlation and redundancy exhibited by hyperspectral data (Kalácska *et al.* 2007a; Schowengerdt 1996). To link the spectral inputs to biophysical properties, Merzlyak *et al.*'s (1999) plant senescence reflectance index (PSRI) was used to estimate the chlorophyll and carotenoid balance of each species, and Peñulas *et al.*'s (1993) water index (WI) was included as a spectral surrogate for measured leaf water content.

The effect of variability along seasonal and successional gradients was evaluated by applying the classifier in two ways. First, each season was treated individually, and training/testing combinations were established between the successional stages whereby a classifier trained on data from one successional stage was then tested for accuracy when used to classify data from another stage. All possible combinations of within- and between-stage classification were tested and the accuracy reported as the percentage of species in the testing set classified correctly in each pairing. Second, the effect of seasonality within each successional stage was explored by comparing the accuracy of classifiers trained and tested within a single season with those trained on wet season data, then applied to data from the dry season and vice versa. This seasonally cross-referenced approach was repeated for each successional stage. To normalize the datasets between the seasons, we limited the classification to species common to both in both. As a result, this second round of classifications included only the species marked with an asterisk in Table 5-1.

5.3 Results

Differences in leaf traits were noted throughout the analysis, between both seasons and successional stages. One-way ANOVA analysis (Table 5-3) uncovered a greater magnitude of difference at the trait level between successional stages during the dry season than during the wet. Water content (H= 17.65, p= 0.001), SLA (H=14.30, p= 0.001), Carotenoids (H= 7.18, p= 0.028), and both vegetation indices (mND705: H= 8.45, p= 0.015; DD: H= 9.47, p= 0.003) all show significant differences among the three successional stages during the dry season. During the wet, only the chlorophyll index mND705 shows significant variation (H= 9.96, p= 0.031).

Season **# Species**	**Wet** 9	**Dry** 8
Water Content	0.278*	**0.001***
Thickness	0.312	0.073*
SLA	0.312*	**0.001***
Chlorophyll	0.203*	0.609*
Carotenoids	0.127*	**0.028***
mND705	**0.031***	**0.015***
DD	0.219*	**0.003***
N	0.730	0.361
P	0.222	0.099

Table 5-3. Results of one-way ANOVA analysis of differences between the three successional stages (early intermediate, and late) during each season. Significant results are in bold (*p*= <0.05). Results marked with an * indicate substitution of Kruskal-Wallis test where ANOVA assumptions were unmet.

5.3.1 Structural Traits

Among the three structural traits considered, water content, leaf thickness, and SLA (Figure 5-2), we found the greatest difference between seasons in water content, which showed significant differences between seasons at all successional stages (U= 0.00 and *p*= <0.001 at all stages) (Table 5-4). Differences in water content were also significant between all three pairings of successional stages, with *p* values ranging from 0.023 (U= 564.5) between E/L, to <0.001 (U=381.5) between the I/L (Table 5-5).

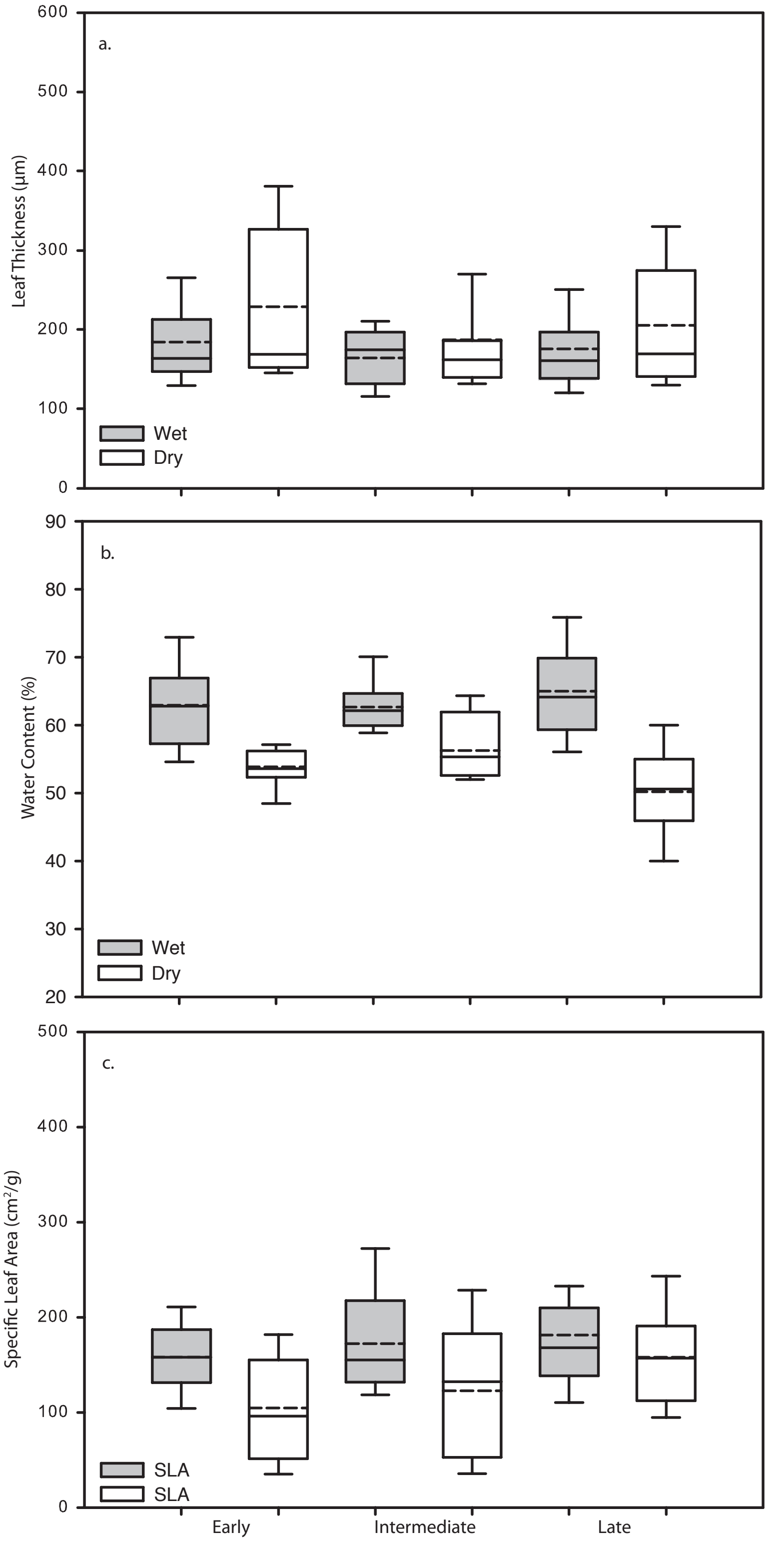

Figure 5-2. Structural Variables: a. Leaf thickness, b. Water content, c. Specific Leaf Area

a.	**Water**	**Thick**	**SLA**	**Chl**	**Car**	**mND705**	**DD**	**N**	**P**	**N:P**
Wet	0.142	0.423	0.112	0.317	0.089	**0.039**	**0.029**	0.604	0.243	0.793
Dry	**0.023**	0.194	**<0.001**	0.301	0.817	0.186	0.243	0.583	**0.038**	0.061

b.	**Water**	**Thick**	**SLA**	**Chl**	**Car**	**mND705**	**DD**	**N**	**P**	**N:P**
Wet	1.000	0.176	0.468	**0.036**	**0.034**	0.819	0.32	0.5	0.655	0.161
Dry	**0.010**	**0.023**	0.231	0.725	0.076	**0.003**	**0.002**	0.196	0.091	0.055

c.	**Water**	**Thick**	**SLA**	**Chl**	**Car**	**mND705**	**DD**	**N**	**P**	**N:P**
Wet	0.165	0.704	0.516	0.375	0.834	**0.02**	**0.001**	0.791	0.107	0.155
Dry	**<0.001**	0.353	**0.027**	0.329	0.088	0.437	0.26	0.445	0.442	0.876

Table 5-4. *p*-values from pairwise comparisons by successional stage. a. early / late pairing, b. early / intermediate pairing, and c. intermediate / late pairing. Results in bold indicate significant differences (*p*= <0.05). Results marked with an * indicate substitution of Mann-Whitney test where t-test assumptions were unmet.

Stage	**Water**	**Thick**	**SLA**	**CHL**	**CAR**	**mND705**	**DD**	**N**	**P**	**N:P**
E	**< 0.001**	0.051	**< 0.001**	**0.04**	**< 0.001**	**0.002**	**< 0.001**	**0.001**	0.075	0.229
I	**< 0.001**	0.802	**0.006**	0.755	0.809	0.801	0.721	**0.002**	0.265	**0.008**
L	**< 0.001**	0.168	0.140	0.313	0.061	**< 0.001**	**< 0.001**	**< 0.001**	0.885	**0.049**

Table 5-5. *p*-values from pairwise comparisons of wet and dry season results at each successional stage. Significantly-different (*p*= <0.05) pairings are highlighted.

SLA varied according to a similar pattern, with significant seasonal differences in the E/I successional stages (U= 407, *p*= <0.001 and U= 558, *p*= 0.006, respectively). Again, variation between paired successional stages was restricted to the dry season. There was an increasing trend from early to late stage, with significant differences noted between the E/L (U= 397, *p*= <0.001) and I/L (U= 569, *p*= 0.027) stages.

All three traits were strongly correlated during the wet season, both with the successional stages grouped and separated (Tables 5-6 and 5-7). No pattern emerged in the correlation of these structural traits during the dry season though leaf thickness and water were significantly related in the intermediate stage (r= 0.48, p= <0.001, and SLA was related to both water content (r= 0.63) and thickness (r= -0.71)in the late (p= <0.001 for both).

	Water	**Thick**	**SLA**	**CHL**	**CAR**	**N**	**P**	**mND705**	**DD**
Water		0.08	0.15	0.07	0.72	0.90	0.22	0.65	0.71
Thick	**0.00**		0.32	**0.00**	**0.00**	0.57	0.60	0.25	0.23
SLA	**0.00**	**0.00**		0.70	**0.02**	0.63	0.62	0.77	0.73
CHL	0.73	0.83	0.36		**0.00**	0.72	0.23	**0.03**	**0.04**
CAR	0.67	0.99	0.77	**0.00**		0.73	0.70	0.77	0.70
N	0.17	0.16	**0.03**	0.49	0.98		0.24	0.18	0.18
P	0.35	0.77	0.37	0.29	0.50	0.36		0.35	0.51
mND705	**0.02**	0.07	0.51	**0.00**	0.15	0.47	0.86		**0.00**
DD	**0.01**	**0.04**	0.61	**0.00**	0.13	0.77	0.96	**0.00**	

DRY

WET

Table 5-6. *p*-values from Pearson correlation between leaf traits with all three successional stages summed. Significantly-related (*p*= <0.05) pairings are highlighted.

a.	Water	Thick	SLA	CHL	CAR	mND705	DD	N	P
Water		0.08	0.15	0.07	0.72	0.65	0.71	0.10	0.72
Thick	**0.01**		0.32	**0.00**	**0.00**	0.25	0.23	0.44	0.23
SLA	**0.03**	**0.00**		0.70	**0.02**	0.77	0.73	0.35	0.32
CHL	**0.00**	0.19	**0.00**		**0.00**	**0.03**	**0.04**	0.14	0.65
CAR	0.12	0.17	**0.01**	**0.00**		0.73	0.70	0.91	0.53
mND705	**0.02**	**0.01**	0.46	**0.01**	0.56		**0.00**	0.60	0.72
DD	**0.00**	0.05	0.93	**0.00**	0.21	**0.00**		0.68	0.69
N	0.29	0.33	0.08	0.10	0.22	0.32	0.17		0.96
P	0.95	0.87	0.83	0.24	0.40	0.36	0.45	0.89	

DRY

b.	Water	Thick	SLA	CHL	CAR	mND705	DD	N	P
Water		**0.00**	0.81	0.28	0.31	0.61	0.56	0.85	0.38
Thick	0.91		0.82	**0.01**	0.07	0.19	0.09	0.38	0.16
SLA	**0.00**	**0.00**		0.06	**0.01**	**0.00**	**0.00**	0.50	0.55
CHL	0.28	0.54	0.90		**0.00**	**0.01**	**0.01**	0.31	0.78
CAR	0.37	0.72	0.74	**0.00**		**0.01**	**0.01**	0.30	0.72
mND705	0.74	0.26	0.88	0.93	0.74		**0.00**	0.90	0.63
DD	0.64	0.44	0.77	0.49	0.29	**0.00**		0.81	0.62
N	0.23	0.31	0.12	0.35	0.26	**0.04**	0.07		0.48
P	0.55	0.39	0.54	0.48	0.38	1.00	0.89	0.38	

DRY

c.	Water	Thick	SLA	CHL	CAR	mND705	DD	N	P
Water		0.54	**0.00**	0.54	0.54	**0.02**	**0.01**	0.41	0.52
Thick	0.06		**0.00**	0.05	**0.02**	0.22	0.23	0.98	0.64
SLA	**0.00**	**0.00**		0.34	0.40	0.17	0.10	0.30	0.57
CHL	0.30	0.12	0.36		**0.00**	0.27	0.58	0.41	0.59
CAR	0.20	0.08	0.14	**0.00**		0.64	0.30	0.98	0.82
mND705	0.47	0.29	0.45	**0.02**	0.14		**0.00**	0.09	0.64
DD	0.44	0.13	0.71	**0.01**	0.06	**0.00**		0.06	0.88
N	0.96	0.54	0.39	0.65	0.83	0.27	0.31		0.44
P	0.19	0.48	0.77	0.28	0.11	0.87	0.85	0.51	

DRY

WET

Table 5-7. *p*-values from Pearson correlation between leaf traits for each site at each successional stage. a. early, b. intermediate, c. late. Significantly-related ($p = <0.05$) pairings are highlighted.

5.3.2 Chemical Traits

Foliar nitrogen levels were significantly lower during the dry season at all successional stages (U= 2, *p*= <0.001; U= 4, *p*= 0.002, and U= 1, *p*= <0.001 at early, intermediate, and late) (Figure 5-3, Table 5-4) though differences between stages were non significant for either season (Table 5-5). Phosphorous was strongly, but not significantly different between wet and dry seasons only for the early successional stage (U= 17, *p*= 0.075). Between successional stages, phosphorous showed significant variability between the E/l stages (U= 12, *p*= 0.038) and strong but non-significant variability between the E/I (t = -1.81, *p*= 0.091).

Nitrogen concentration followed a similar pattern of decrease between the early and intermediate stages, then an increase to an intermediate value in the late. Phosphorous exhibited a general pattern of increase with increasing successions, though that increase was concentrated between the E/I stages during the dry season (Figure 5-3a and b). Patterns in the resulting N:P ratio show no consistency in their pattern between seasons (Figure 5-3c). Differences in the ratio between successional stages were strong but not significant between the early and both intermediate and late stages (t= 2.038, *p*= 0.061 and t= 2.089, *p*= 0.055). N:P varied significantly between seasons in both the intermediate and late stage (U= 8, *p*= 0.006 and U= 15, *p*= 0.049).

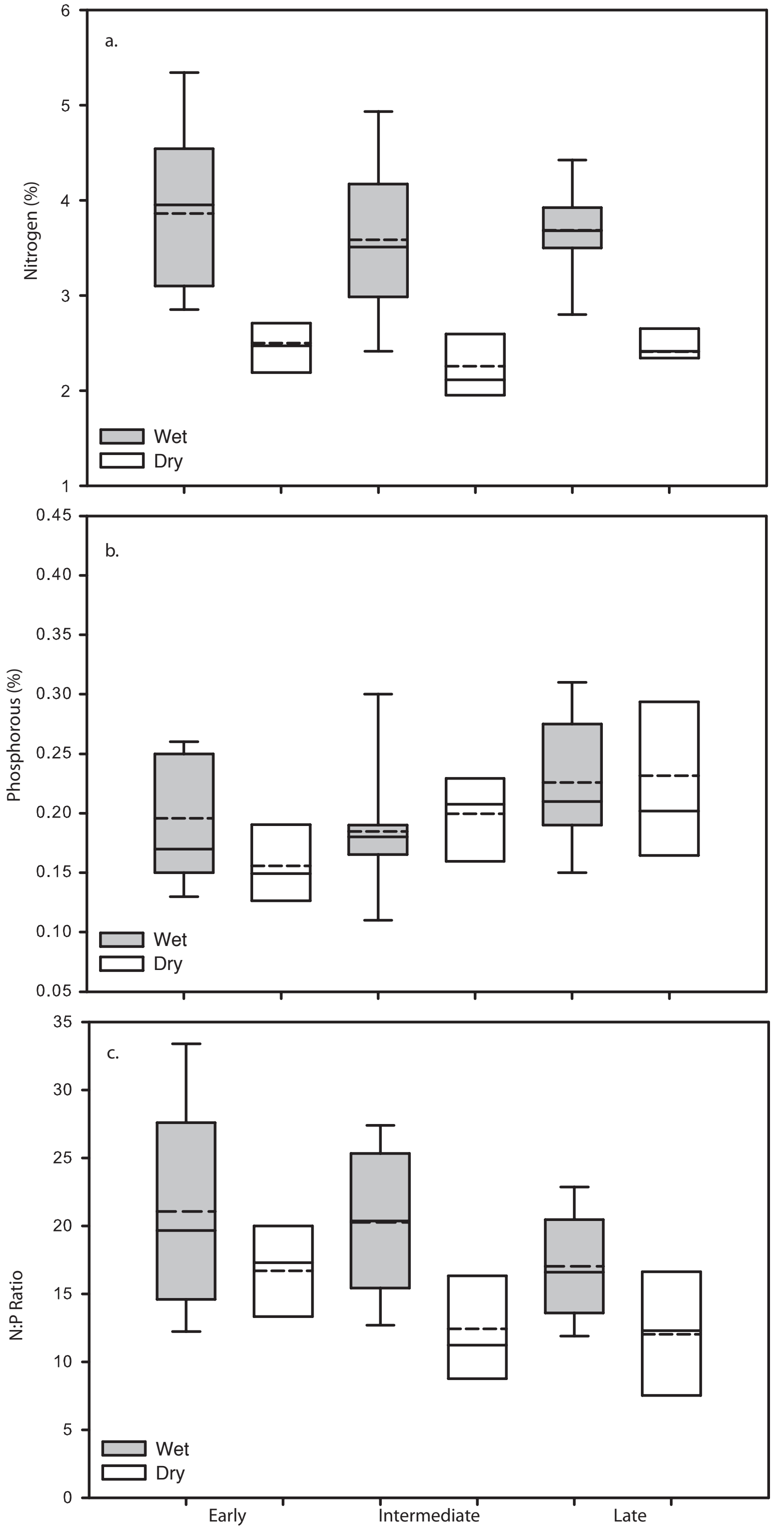

Figure 5-3. Foliar Chemicals. a. Nitrogen, b. Phosphorous, c. N:P ratio

Correlations between nitrogen and phosphorous were weak, both with the successional stages grouped and separated. No relationships were noted among leaf chemical levels and any of the other traits measured.

5.3.3 Leaf Pigments and SVIs

Of the leaf pigments, the only significant difference between the seasons was in carotenoid levels in the early successional stage (U= 326, p= <0.001), though differences in that pigment in the late stages were nearly significant as well (U= 687, p= 0.061) (Figure 5-4, Table 5-4). Carotenoids were significantly different in the wet season between the E/I pair (t= 2.152, p= 0.034) and non significantly different in the dry between the E/I and I/L pairs (U= 450, p= 0.076 and U= 622, p= 0.088). The only significant variation in chlorophyll content was found between the E/I stages during the wet season (t= 2.130, p= 0.036) (Table 5-5).

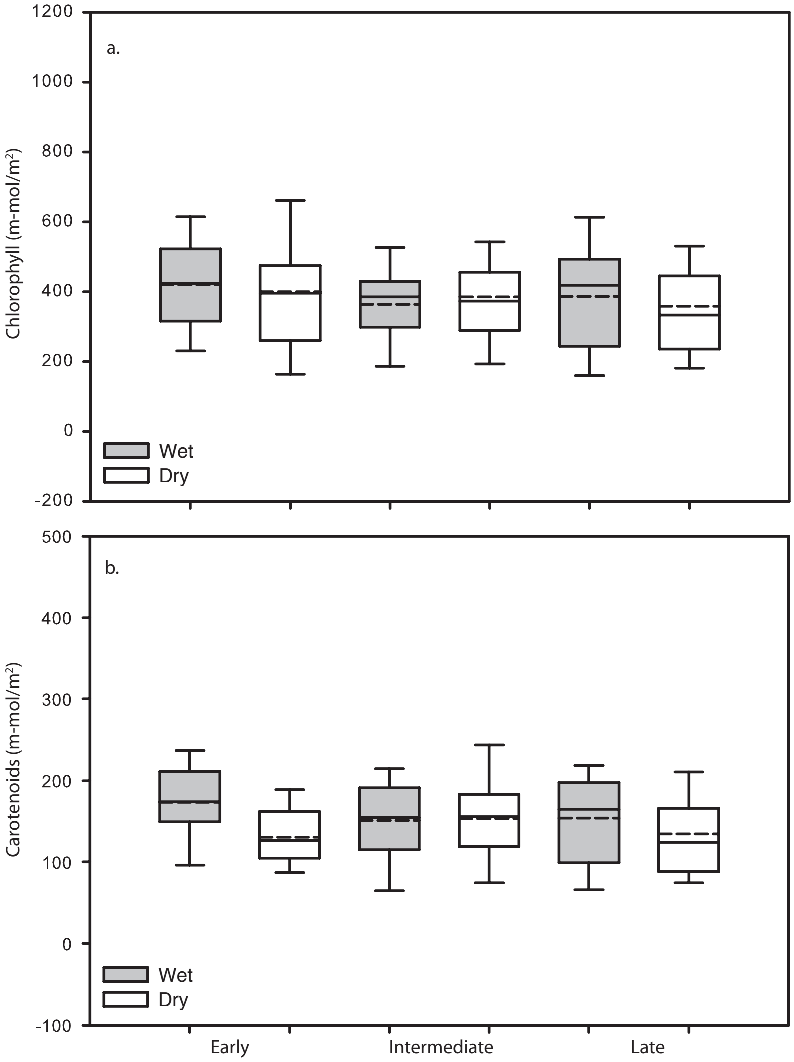

Figure 5-4. Leaf Pigments. a. Chlorophyll, b. Carotenoids

The two pigments were significantly related to each other both at the level of the season, with all successional stages grouped, and at each individual stage(r= 0.97 or higher at all sites and stages, *p*= <0.001 for all) (Tables 5-6 and 5-7). The spectral vegetation index mND705 was correlated with chlorophyll in the early and late stages during the wet season (r= 0.37 and 0.34; *p*= 0.01 and 0.02, respectively) and the early and intermediate stages during the dry (r= 0.35 and 0.39; *p*= 0.03 and 0.01, respectively), as well as carotenoids during the intermediate stage during the dry season (r= 0.41, *p*= 0.01) (Figure 5-5). DD was correlated to a similar extent in all cases. Additionally, mND705 was related during the wet season to water content and leaf thickness in the early stage (r = -0.36 and -0.37; *p*= 0.02 and 0.01, respectively) and nitrogen in the intermediate stage (r = 0.68, *p*= 0.04). During the dry season it was related to SLA during the intermediate stage (r= 0.42, *p*= 0.00) (Table 5-7).

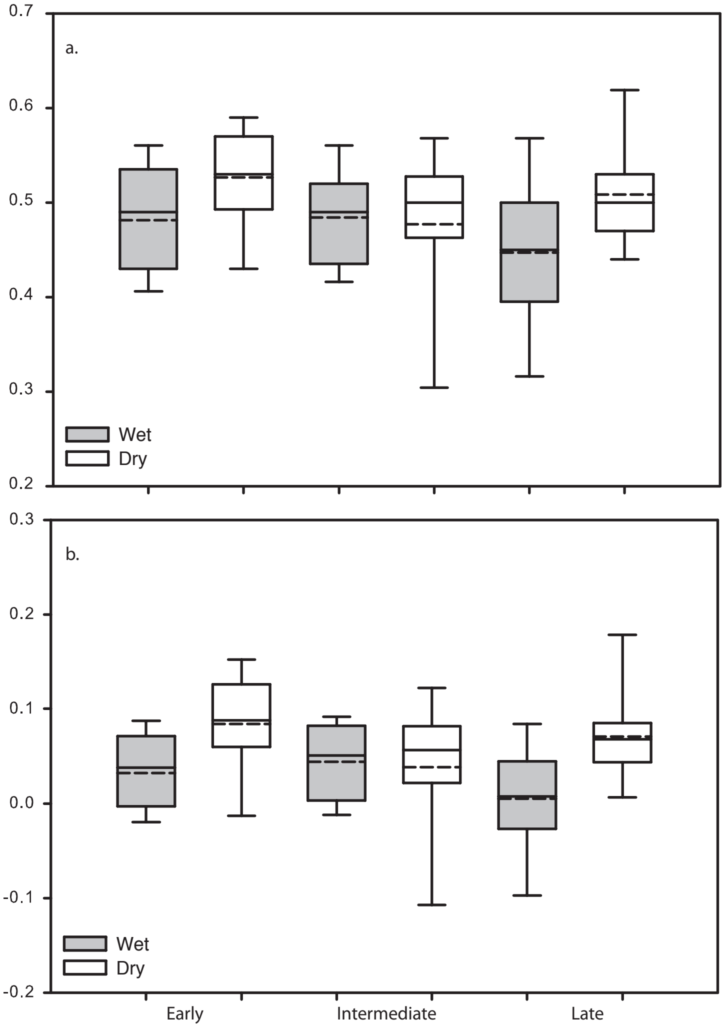

Figure 5-5. Spectral Vegetation Indices. a, mND705, b. DD

The indices exaggerate the variability in pigment content, and show significant variation between seasons in the early and late successional stages (mND705: U= 546.5, *p*= 0.002 and U= 495.5, *p*= <0.001. DD: U= 409, *p*= <0.001 and U= 362, *p*= <0.001) (Table 5-4). Between successional stages, both indices were variable between the E/L and I/L stages in the wet season, and between the E/I stages in the dry season.

5.3.4 Spectral Variability

Variation in leaf spectra, averaged across the six species that underwent spectral classification, was expressed differently during the wet and dry seasons. Figure 5-6a shows the absolute difference in mean reflectance values between paired successional stages (early/intermediate, early/late, and intermediate/late). Spectral variation was concentrated in the range of 750 to 1050 nm, the spectral region most indicative of leaf moisture and structural properties (Gates *et al.* 1965). The same differences between the stages, when calculated for the dry season, show an increased prominence of the spectral regions centered at 550 and 700 nm, regions sensitive to plant pigment concentrations (Boyer *et al.* 1988). Additionally, the relative magnitude of the variability between the successional pairs was reversed, with the greatest difference found between the intermediate/late pair and the least between the early/intermediate pair during the wet season and the inverse during the dry. The early/late successional pairing was intermediary during both seasons.

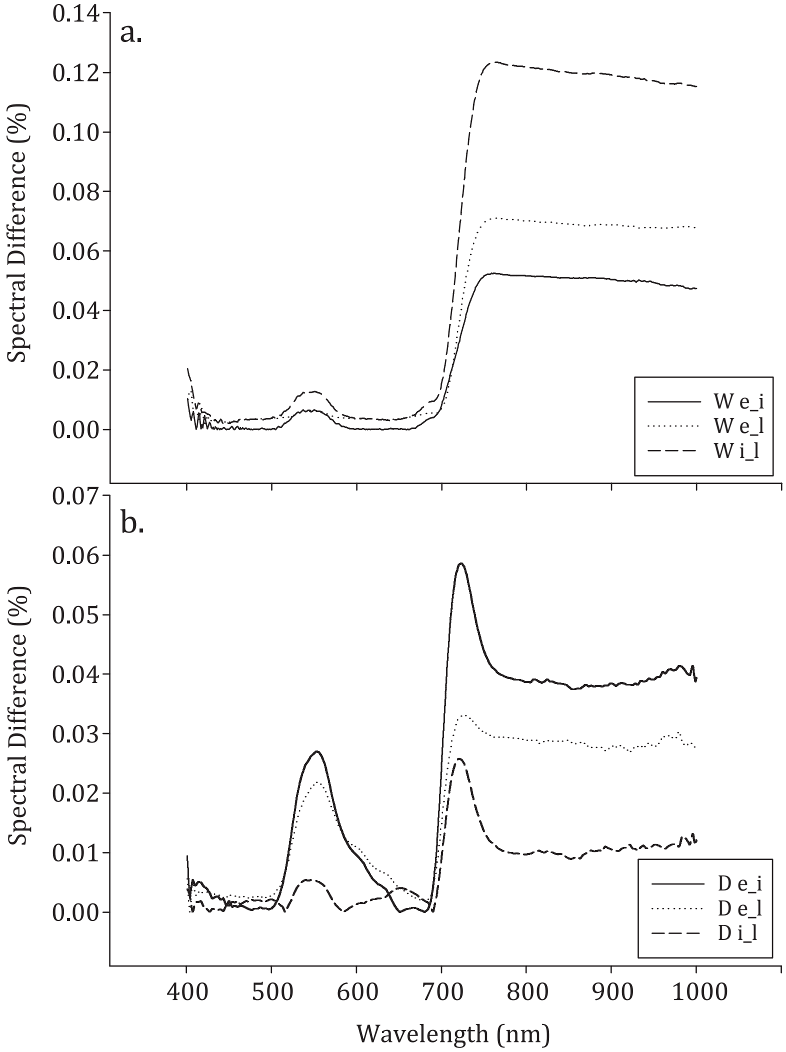

Figure 5-6. Absolute difference in reflectance between successional stages during the: a. Wet season, and b. Dry season. Legend codes indicate the successional stages between which the spectral difference was calculated. e_i: difference between early and intermediate stages, e_l: difference between early and late stages, and i_l: difference between intermediate and late stages.

Figure 5-7 shows a pattern of increasing spectral difference between seasons with advancing successional stages. In the early stage, spectral differences are almost entirely concentrated in wavelengths longer than 750 nm. While the magnitude of spectral difference in this region increases with successional stage, so does the difference at the "green peak" region around 550 nm.

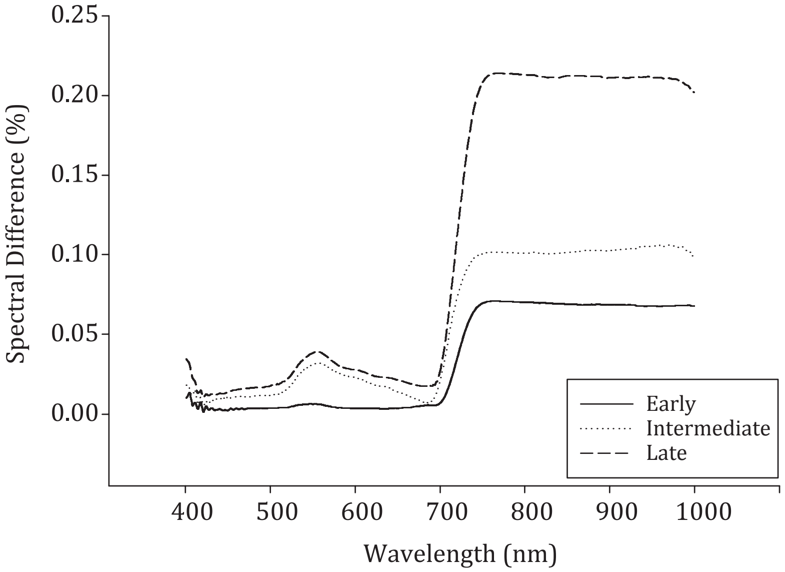

Figure 5-7. Absolute difference between average spectral reflectance in the wet versus the dry season at each successional stage.

The impact of variability in leaf spectral properties is shown in Figures 5-8 and 5-9, which detail the effect of changing successional stage and season on the accuracy of an automated non-parametric classifier. Considering the effect of succession on spectral classification, in all but one case (the intermediate stage during the wet season) accuracy was a minimum of 75% when testing the classifier on data collected in the same successional stage for which it was trained (Table 5-8a). This accuracy erodes substantially when the classifier is tested on data from a stage different from the training data. In the most extreme case, when a classifier trained during the wet season in the early stage is then applied to intermediate stage data from the same season the accuracy drops by 75% (from 82% to 7%). This trend is maintained during each season for all combinations of successional stages, with an average reduction in classification accuracy by a factor of 4.4.

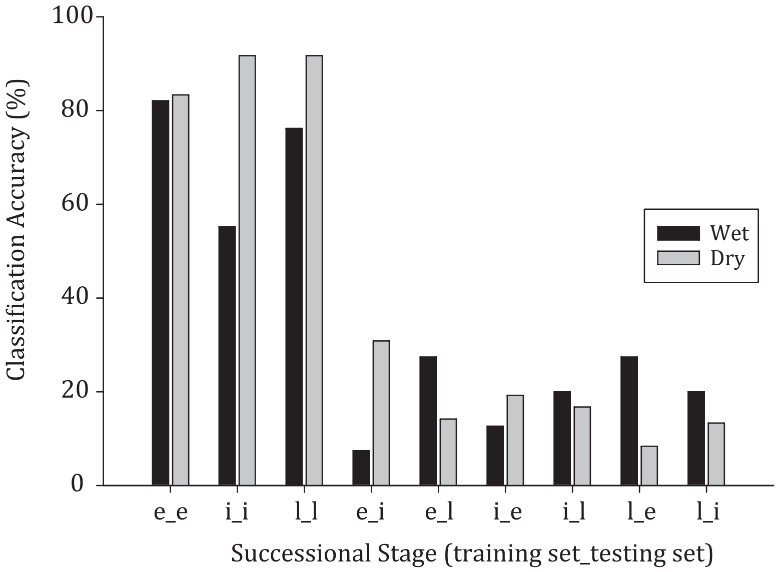

Figure 5-8. See5 classification accuracy: Effect of successional stage by season. X-axis labels indicate the seasons from which the training and testing data were taken from. d: dry season, w: wet season.

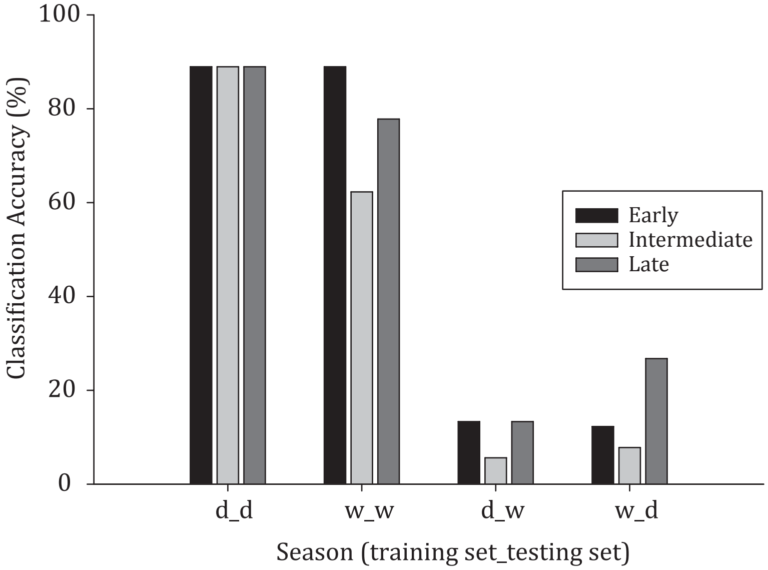

Figure 5-9. See5 classification accuracy: Effect of season by successional stage. X-axis labels indicate the successional stages from which the training and testing data were taken from. e: early, i: intermediate, l: late.

The importance of the temporal domain is reinforced when comparing the impact of changing season on classification results at each successional stage. Accuracy when classifiers are trained and tested on the same season/stage is consistently over 75% (again, the intermediate stage during the wet season is an exception). When these same classifiers are then tested on data from the opposite season, accuracy is a fraction of the within-season results (Table 5-8b). This reduction is variable across successional stages, with the greatest decrease noted in the intermediate stage where accuracy is reduced by a factor of 11.33. Even in the late stage, where classification is least impacted by seasonal variation, the erosion in accuracy is more than 75%.

a.	Wet	Dry	Mean
e_e	82.09	83.33	82.71
i_i	55.22	91.67	73.45
l_l	76.12	91.67	83.9
e_i	7.41	30.83	19.12
e_l	27.41	14.17	20.79
i_e	12.59	19.17	15.88
i_l	20.00	16.67	18.34
l_e	27.41	8.33	17.87
l_i	20.00	13.33	16.67
Within	71.14	88.89	80.02
Between	18.18	17.86	18.02
Factor	3.91	4.98	4.44

b.	Early	Intermediate	Late
d_d	88.89	88.89	88.89
w_w	88.89	62.22	77.78
d_w	13.33	5.56	13.33
w_d	12.22	7.78	26.67
Within	88.89	75.56	83.33
Between	12.78	6.67	20.00
Factor	6.96	11.33	4.17

Table 5-8. See5 classification accuracy, expressed as a percentage of individuals classified correctly versus the number of individuals in the testing data set. a. Cross-validated comparing successional stages. b. Cross-validated comparing wet and dry seasons within each successional stage. Factor is the magnitude of the variation between the within- and between-stage classification, expressed as the ration of within- to between-stage accuracy.

5.4 Discussion

Our results suggest a potential for significant differences between leaf structural traits, pigment levels, and chemical concentrations during the wet and dry seasons in a tropical dry forest, but that these differences vary with changing successional stage (Tables 5-4 and 5-5). Further, these differences translate into variability in spectral reflectance of a magnitude that negatively impacts the potential for accurate classification of even a modestly sized data set based on species-level spectral characteristics.

5.4.1 Differences in leaf traits between seasons

Unsurprisingly, water content was the leaf trait that varied most strongly between seasons, with a significance of p= < 0.001 regardless of successional stage. Evidence from previous studies that precipitation regimes are a strong determinant of leaf traits at the site level (Sánchez-Azofeifa *et al.* 2009) supports the notion that this variability in leaf water content is a driver to the patterns of seasonal variation described here. Variation among traits identified as contributors to the leaf economic spectrum (LES) (Reich *et al.* 1997; Wright *et al.* 2004) show responses to seasonality, as photosynthetic capacity declines with leaf senescence. The LES asserts a pattern of covariance between foliar nitrogen, SLA, and leaf lifespan correlated to changes in maximum photosynthetic capacity (A_{max}) that is essentially consistent across biomes and climate at a global scale.

In our site-level analysis, nitrogen in particular shows a strong decrease from wet to dry season at all successional stages (Figure 5-3a, Table 5-4). In keeping with the LES, we also observed corresponding seasonal decreases in SLA in both the early and intermediate stages (the late stage was non-significant, but relatively strong, with $p = 0.14$). Basing our expectations on the LES, a similar decrease in chlorophyll content should have been associated with decreased dry-season productivity, but significant differences in pigment concentration were only noted in the early successional stage. The SVIs mND705 and DD that correlated significantly with chlorophyll levels in the majority of stage/season combinations (Table 5-6) did, however, present differences between the seasons in the late stage.

A_{max} was not measured as part of the data collection for this study, and as a result we cannot speak directly to the consistency of the relationship between the traits comprising the LES. We can, however, point to important differences in leaf traits between seasons within sites. Given that these traits have a well-established impact on productivity (Falster *et al.* 2011; Poorter and Bongers 2006), and that mapping and monitoring efforts using remotely sensed data often exploit variability in the photosynthetically-active region of the electromagnetic spectrum, it follows that these seasonal differences can impact estimation of the extent and distribution of these seasonal forests.

5.4.2 The importance of forest succession in seasonal variability in leaf traits

While trait-level variation in leaf properties according to changing forest successional stage was not as pronounced as that attributable to seasonal variation, we noted consistent patterns of increase or decrease along the successional gradient (Fig 5-2). Interestingly, in 5 of the 9 traits considered during the wet season the trajectory defined by the early and late stages was not conformed to by the data from the intermediate stage. During the dry season 6 of 9 traits followed this trend. This tendency for the intermediate successional stage to deviate from a trajectory established by the early and late stages suggests that plots established according to time since abandonment may not reflect the structural and physiological changes attributable to forest succession.

This calls into question the assumptions underlying the chronosequence approach to evaluating successional pathways, namely that species at an intermediate successional stage, according to the time since abandonment, are indeed following a linear trajectory which will lead to the same climax state as the late stage plots. While we're unaware of any previous research directly relating variability in leaf traits to forest successional stage in tropical dry forests, Loebeck *et al.* (2013) noted different trajectories of trait variation according to stand age in wet versus dry tropical forests in Mexico. Where the measured leaf traits in the dry season indicated a shift from conservative to acquisitive strategies with increasing stand age, likely driven by an need for heightened drought tolerance in the harsher and more open early successional stage, in the wet forest, the reverse trend was noted, suggesting light, rather than water, limitation was the driving force. The fact that our data tend away from a linear trajectory from early to late stage may indicate either differences in the environmental conditions or the successional pathways at the intermediate stage sites, or it may be that a more detailed chronosequence (ie. with more age classes represented) would serve to smooth out the general trends indicated by the early and late stage measurements.

Land use history typically explains the creation of successional pathways in secondary forests in the dry tropics (Castillo *et al.* 2005). That said, the progression from pioneer to climax stages of forest development is then moderated by site characteristics (e.g. species composition topography, soil type, latitude) and microclimate (Corlett 1994; Denslow 1996; Janzen 1983, 1988, 2000). Arroyo-Mora *et al.* (2005) demonstrated an approach using high-resolution satellite imagery (Landsat ETM+ and IKONOS) to discriminate between early, intermediate, and late successional stages in Costa Rica. Rather than time since abandonment, they define successional stages via forest structural characteristics and species assemblages (Kalácska *et al.* 2004). Though the analysis focuses on the relationship between spectral reflectance and woody area index and forest structure at the canopy scale, their results suggest far less overlap between successional stages than is evident in the spectral indices included here (Figure 5-5). The influence of the criteria for the selection of successional stages is also reflected in an earlier study, described in chapter 4, which found greater variation attributable to succession during the wet season at the same Costa Rican sites detailed by Arroyo-Morro *et al.* (2005) than in the Mexican sites used here, defined based on time since abandonment.

5.4.3 Spectral variability

Variation in leaf spectra between seasons was concentrated above 750 nm, the spectral region most indicative of leaf moisture and structural properties (Gates *et al.* 1965) (Figure 5-7). This is consistent with previous findings that spectral variation in dry environments (CCBR has mean annual rainfall positioned towards the low end of the spectrum ascribed to tropical dry forests (Sánchez-Azofeifa *et al.* 2005)) is concentrated in wavelength regions most influenced by water stress, as opposed to the region dominated by leaf pigment properties (500 – 750 nm) as found in more humid environments (Hesketh and Sánchez-Azofeifa 2012). The magnitude of this variation changed between successional stages, however, with seasonal near infrared variation in the late stage almost 4 times the values noted in the early. This reflects the inverse trends in measured water content during each season: while mean leaf water content raised from 62.9% in the early stage to 65.0% in the late during the dry season, water dropped during the dry season from 53.8% in the early to 50.2% in the late stage (Table 5-2). Likewise, the more modest increase in reflectance at the "green peak" of 550 nm is related to increases in variability in leaf chlorophyll with increasing succession.

Within-season variability in leaf reflectance showed marked differences between the wet and dry datasets (Figure 5-6). During the wet season, differences between paired successional stages characterized by peaks in water-dominated wavelengths with a shape similar to the wet/dry season differences in Figure 5-7. During the dry season, however, the differences attributable to water features were minimized, and marked increases in spectral difference between successional stages were found both centered at 550 nm and 725 nm. These spectral regions, controlled largely by leaf pigment content are used in the calculation of spectral indices to estimate chlorophyll content and general vegetation health and abundance (le Maire *et al.* 2004; Sims and Gamon 2002). This increase in variability in these regions corroborates the results of Castro-Esau *et al.* (2004), who reported an ability to discriminate between tree and liana species during the dry season at a dry forest sites in Panama, while finding the same distinction difficult or impossible during the rainy season or at a rainforest site. Extrapolating to coarser spatial scales, Kalácska *et al.* (2007b) stress the importance of considering seasonality, evaluating the utility of EO-1 Hyperion data to map forest structural and floristic diversity using dry season imagery. Portillo and Sánchez-Azofiefa (2010) also found that dry season imagery from MODIS was more effective in mapping the extent of tropical dry forests than imagery from the wet season. While these canopy scale analyses also take into account seasonal variability in stem density, vertical structure, and species composition (Kalácska *et al.* 2004) which is revealed during the dry season, we suggest that the differences noted here in leaf spectral response play a role in these findings as well.

5.4.4 Variability in classification accuracy

Classification within seasons was accomplished with high accuracy in all cases, and particularly during the dry season. Here results are consistent with those of Clark *et al.* (2005), who report overall classification accuracy of 92.0% using a set of 7 species at the scale of individual tree crowns at La Selva Biological Station in Costa Rica. Accuracy was lower in almost all cases during the wet season due to the extent and locations of spectral variability discussed in the previous section. Minimum within-season classification accuracy was obtained in the intermediate successional stage during the wet season, and is attributable to a decrease in spectral variability in the 550 nm region controlled by leaf pigment content.

Testing the results across seasons provided reductions in classification accuracies similar to that of Hesketh and Sánchez-Azofeifa (2012), who found a reduction in average classification accuracy of a set of 8 tree species at a dry forest site from 90.2% within seasons to 16.6% between seasons, a decrease by a factor of 5.45. The notable deviation from this (Table 5-8b) is the difference in classification accuracy between seasons in the intermediate successional stage, but this is attributable to the compounding of the low wet season classification accuracy in this stage with the cross-seasonal classification. Restructuring the classification to control for seasonality resulted in a more modest reduction in accuracy when cross-classifying, this time combining successional stages (Table 5-8a).

5.5 Conclusions

We demonstrate here that leaf structural and chemical traits as well as leaf spectral reflectance vary substantially along two temporal gradients in a tropical dry forest: seasonal and successional. First, variation according to wet/dry seasonal cycles is significant, particularly in traits related to water content and photosynthesis. Along the same seasonal gradient, spectral reflectance differed enough that automated species-level classification was impossible when data from the two seasons were combined. This seasonal variation compounded variation between forest successional stages among biophysical traits, and the decreased moisture availability during the dry season produced markedly different patterns in spectral variation between stages than was noted during the wet season.

There has been a trend toward studies of leaf traits searching for patterns at scales which attempt to unify processes across broad ranges of species and biomes (Asner and Martin 2008; Reich *et al.* 1997; Wright *et al.* 2004). These analyses describe general trends but tend to mask the importance of local variation. While an assemblage of traits may array well along a gradient spanning many biomes, variability at the site and species level according to gradients in age, phenology and microclimate are important. Mobilizing broad patterns in leaf traits in concert with remote sensing analysis for the monitoring of forest characteristics is an attractive proposition, but our results suggest that any detailed analysis must also account for local-scale variation and an appreciation of the temporal dynamics of the environment under investigation, including seasonal variation in micro-climatological conditions and soil moisture.

While this poses challenges for species classification, leaf trait and spectral variation driven by successional changes indicates a potential for the refinement of forest inventories to include information on ecological succession. Studies have shown the advantages of data from particular phenophases for the remote estimation of LAI (Kalácska *et al.* 2005), structural group discernment (Castro-Esau *et al.* 2004), and land cover classification (Portillo and Sánchez-Azofeifa 2010). While we've demonstrated here a negative impact of spectral variability on the accuracy of a relatively simple classifier, this same variability may prove a useful data source for the differentiation of secondary forests and successional classes.

In the context of initiatives such as REDD+, remote sensing is currently used to monitor deforestation, but assessing forest degradation and stages of regeneration are more complicated (De Sy *et al.* 2012). A better understanding of the variability in leaf biophysical properties and the resulting spectral characteristics is the first step towards methods for remote monitoring of forest successional stage, which may be related to stand biomass and incorporated into carbon budgets that can in turn inform conservation and resource management efforts. The results presented here indicate potential for leaf trait variability to direct discrimination of forest age classes, but also point to the need for further investigation into the temporal dynamics of leaf properties, particularly in highly seasonal environments.

5.6 References

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Chapter 6: Conclusions and Implications

6.1 Introduction

An essential element for the utility of remote sensing observations in the monitoring of forest environments is an understanding of the consistency of relationships between biophysical variables and their spectral properties across various geographic and temporal gradients. The effectiveness of automated biodiversity assessment as proposed by Asner and Martin (2008) as well as the scalability of the relationships described in the worldwide leaf economics spectrum (LES) (Reich *et al.* 1997; Wright *et al.* 2004) rely on this assumption of consistency among leaf properties and the relationships between them. That this assumption holds at spatial scales of the continental and greater, as well as within certain biomes such as the Amazon rainforest is likely, but its limits have been insufficiently studied in other regions. In particular, tropical dry forests, characterized by high species diversity and intense wet-dry seasonality, represent a potential obstacle to the universal application of these concepts.

To date, the majority of studies addressing the variability in physical and spectral leaf traits in the tropics have focused on non-temporal drivers to trait variability, with attention paid to variables such as site characteristics (Castro-Esau *et al.* 2006), genetics (Martin *et al.* 2007), climate (Asner *et al.*

2009), and rainfall (Townsend *et al.* 2007). While remote sensing analyses identifying the extent and location of forest types (Miles *et al.* 2006; Portillo-Quintero and Sánchez-Azofeifa 2010), evaluating forest productivity (Gamon *et al.* 2005; Kalácska *et al.* 2005; Káacska *et al.* 2005), and mapping forest structure (Alves and Skole 1996; Kimes *et al.* 1999; Kimes *et al.* 1998) have all noted the influence of phenology, direct investigation of temporal variation at the leaf scale has been underexplored.

The work represented in this thesis addresses the issue of temporal variability in leaf biophysical and spectral properties, which, while not unique to tropical dry forest environments, are particularly important in the context of remote detection and monitoring of these ecosystems at all spatial scales. Chapter 2 contextualizes this issue via a review of the body of literature on remote sensing in the dry tropics. Chapter 3 tackles the highly seasonal nature of tropical dry forests, and the resulting impact on the potential for spectral classification of tree and liana species. Chapters 4 and 5 address the effects of the conversion of forests from primary to secondary, and the variability in leaf structural, chemical, and spectral traits imposed by the process of forest succession. As a whole, the thesis contributes to the understanding temporal variability in leaf properties, and the effect this has on the application of remote sensing tools to the monitoring of dry forest characteristics.

6.2 Conclusions and Contributions

Remote sensing analysis has become a critical tool in the assessment of the nature and distribution of forests. Study into forest structure, productivity, and biodiversity using remote sensing techniques has been particularly valuable in assessing difficult to reach areas and evaluating spatial and temporal patterns that may be difficult or impossible to survey using traditional ecological methods. Chapter 2, "*Review of remote sensing of tropical dry forests*", stands as the only synoptic review of the state of remote sensing research focused purely on tropical dry forest environments. Categorized broadly under the subheadings of tropical dry forest classification and mapping, assessment of forest health and productivity, species and structural group discrimination, and the differentiation of structural types and successional stages, the included literature demonstrates the progress made towards the establishment of linkages between fry forest ecology and remote sensed measurements, particularly during the past decade. These linkages form the basis of remote sensing analysis, where spectral information stands as a proxy for direct measurement of biophysical variables. The challenges to establishing these linkages in the dry tropics identified in the literature, while dependent on the scale and focus of the analysis, are related to the temporal characteristics of the biome.

Alongside the demonstration of the importance of research into dry forests, the review's most significant contribution is the consistent evidence of the importance of the dynamic characteristics of dry forests. In particular, forest phenology is shown to have a profound impact on the discrimination of dry forest boundaries (Portillo-Quintero and Sánchez-Azofeifa 2010), the modeling of forest structural characteristics (Kálacska *et al.* 2005), and the separation of species (Hesketh and Sánchez-Azofeifa 2012) and structural groups (Castro-Esau *et al.* 2006; Castro-Esau *et al.* 2004).

High temporal variability, coupled with the high biodiversity found in tropical environments, lends a unique set of challenges to remote sensing analysis of tropical dry forests. These conclusions not only comprise a contextual framework for the following chapters, but suggest the overall need for the incorporation of a dynamic or temporal dimension into investigation of dry forest characteristics and function. Looking forward, the review suggests gaps in our knowledge of the importance of tropical dry forest dynamics that may be addressed by the targeting of future research and the application of emergent technology. Widespread study of dry forest temporal characteristics is hobbled at the leaf level by the expense and time associated with rigorous fieldwork, and at the canopy scale by the trade-offs between spatial resolution and return time of currently-available satellite data, as well as a relative lack of hyperspectral data. Advances in wireless sensor network (WSN) technology have the potential to fill in these temporal gaps, with the ability to monitor variability local-scale environmental variables such as humidity, temperature, photosynthetically active radiation, and leaf temperature in near real-time (Sánchez-Azofeifa *et al.* 2011). When coupled with more traditional spectral and biophysical surveys of forest characteristics, data from WSNs may provide insight into the environmental drivers to observed variability in physical and optical properties.

As the availability and quality of hyperspectral data at spatial scales from the leaf to the canopy increases, the potential for accurate species level discrimination of trees and other elements of the forest canopy is closer to being realized. While research to date has demonstrated the possibility of accurate discrimination between a limited number of species based on spectral reflectance, the prospect of efficient automated biodiversity monitoring suggested by Asner and Martin (2008) is an optimistic indication of the future. In Chapter 3, *"The effect of seasonal spectral variation on species classification in the Panamanian tropical forest",* we test one of the prerequisites for this type of analysis, namely that the spectral properties of an assemblage of species are sufficiently consistent through time that a database of spectral signatures can be created and used to guide an automated classification approach. In dry forests, where intense wet/dry seasonality and a strong phenological profile are the norm, this variability presents a significant obstacle to accurate species discrimination.

Our results, which consider 43 species of trees and lianas collected during both wet and dry seasons at two forest sites suggest that a non-parametric classification approach had little difficulty distinguishing among species at a given site, provided all the data was collected during the same season. Classification accuracy in this scenario was consistent with Clark *et al.* (2005), but dropped by more than a factor of 10 when the classifier was applied to data from another season. We demonstrate here that the influence of seasonality on leaf spectral properties is pronounced and unambiguous. While the prospect of implementing an efficient automated method of biodiversity assessment as proposed by Asner and Martin (2008) is attractive, this study shows clearly that in seasonal environments such as tropical dry forests, variability in spectral reflectance between the seasons precludes accurate classification which does not take into account the temporal domain.

The consistency of spectral signatures across phenophases is a requirement for database-type automated classification, and the spectral variability indicated by this study casts serious doubt on the prospect of the implementation of such a process. Through a direct focus on temporal changes in leaf spectral properties and the incorporation of innovative techniques for the quantification of spectral variation, this study is an important contribution to the understanding of the spectro-temporal dynamics of tropical dry forests. Our results support those of Castro-Esau *et* *al.* (2006) and indicate that an understanding of the role of phenologically-induced spectral variation must be an integral component of any classification exercise, certainly in seasonally dry forests, but potentially in forests with less dramatic annual cycles.

Whether or not secondary forests are indeed the tropical forests of the future (Wright 2005; Wright and Muller-Landau 2006), there is no question that intensive human development and conversion have altered the distributions and character of much of what has historically been primary tropical dry forest (Castillo *et al.* 2005). Chapter 4, *"Successional variation in spectral and biophysical leaf traits in two Latin American tropical dry forests"* addresses the impact of changes in forest successional stage, on both biophysical and spectral leaf properties measured during the wet season at dry forests in Costa Rica and Mexico.

Statistical analysis of a suite of traits including leaf structural, chemical, and spectral properties showed a pattern of significant differences among early, intermediate, and late successional stages at the Costa Rican site, while the level of difference found at the Mexican site was insignificant among all but the spectral measurements. Differences in the scale of variation between the two study sites may be attributable to the criteria used in defining the successional stages. Where the Mexican sites were established using a chronosequence based on time since abandonment, the sites in Costa Rica were defined based on forest structural characteristics. Though the Mexican sites were selected to control for topographic and edaphic variations that would influence the rate of succession, our results echo Arroyo-Mora *et al.* (2005), suggesting that the more empirical criteria used at the Costa Rican field sites results in more pronounced variability between the successional stages. Looking ahead, this consideration for the criteria by which these stages are identified may be an important element in establishing consistent definitions of successional stages and, accordingly, an understanding of trait variation that is more relatable from site to site.

Classification analysis was carried out to address the impact that this biophysical and spectral variation may have on efforts to monitor biodiversity via remote sensing. Species-level spectral classification using a non-parametric classifier showed a reduction in accuracy by a factor of between 3.7 and 5.7 when species were classified using a classifier trained on the same species but from a different successional stage. An essential contribution of the study was the consistency of the species composition of the sample at each successional stage, effectively controlling for variation imposed by taxonomy and isolating the temporal trend. Additionally, while this study reinforces the importance of temporal dynamics, this time related to forest successional stage, in controlling spectral variation, we also show that the biophysical and chemical traits which control spectral reflectance may be measurably affected by the same successional dynamics.

Combining the temporal scales addressed in the previous chapters, Chapter 5, *"Effect of season and successional stage on leaf properties in a tropical dry forest"*, looks at the interplay between the seasonality inherent in dry forest environment and the levels of succession created by secondary forest regeneration in controlling both biophysical and spectral leaf properties. Using a similar set of analyses as those described in Chapter 4, we found difference in the successional variability between the wet and dry seasons at a dry forest site in Mexico. Significant variation was found among leaf structural, pigment, and spectral properties during the dry season, while only spectral response varied during the wet. As found in chapter 4, the level of variation between the early and intermediate successional stages tended to be greater than between the intermediate and late.

Classification analysis showed an average reduction in classification accuracy attributable to variation due to changing successional stage by a factor of 3.9 during the wet season and 5.0 during the dry. When controlling for successional stage and testing the effect of season, accuracy was reduced by a factor of 4.2 in the late successional stage, 7.0 in the early, and 11.3 in the intermediate. Analysis of leaf spectral reflectance by season showed a shift in the region of greatest variability from wavelengths controlled by photosynthetic pigment levels during the wet season to those controlled primarily by leaf water content during the dry.

Overall, the study shows that significant physiological variability, attributable to both seasonality and changes in forest successional stage, translates into variability in leaf spectral reflectance to the extent that spectral classification of species is negatively impacted. As with chapters 3 and 4, the strict control of the species assemblage sampled at each successional stage was an essential element of the experimental design. While this consistency allows in our case for the focus to be placed on variability associated with successional and environmental variables, it will be important in future to better assess how this variability changes in nature and magnitude at the level of individual species. This relationship between species and the magnitude of variation may be of particular importance in less species-rich temperate forests where each species represents a greater proportion of the overall forest cover.

6.3 Overall Significance

In recent years, study into the relationships between leaf traits has focused on continental- to global-scale, cross biome comparisons that seek to unify covariance of leaf properties, such as the LES (Reich *et al.* 1997; Wright *et al.* 2004) and the concept of Spectranomics put forth by Asner and Martin (2008). Both these concepts show that useful and statistically significant trends may be found through the consideration of huge datasets combining thousands of species from across a wide variety of locations and biomes.

However, while these concepts are valuable at the scales at which they were developed, they fail to highlight the potential for significant variability of leaf biophysical and spectral properties at smaller scales (Funk and Cornwell 2013) and are not supported by work carried out at more local scales.

The risk of overlooking the role played by trait variation induced by phenology, microclimate, or site characteristics in the development of classification or monitoring procedures is that highly accurate spectral classification of the type reported by Clark *et al.* (2005) and Asner and Martin (2008) are not repeatable in any environment where this variability is pronounced. These issues would likely be compounded when scaling to the crown and stand scales by intra-species variation as described by Zhang *et al.* (2006).

The studies presented here contribute unambiguous evidence of trait variability in dry forests at the site level, not just between sites but also in response to the temporal dynamics at a given site. This temporal variability, associated with the intense seasonality characteristic of the dry forest biome as well as with changing successional stages resulting from the regeneration pathways of secondary forests, directly impacts not just the universal applicability of the above relationships, but also any strategies for the remote mapping and monitoring of this important class of tropical forests.

The development of remote sensing and GIS tools for tropical forest monitoring is ongoing and a great deal of work remains to refine and validate these techniques. By focusing of the temporal characteristics of seasonally dry tropical forests, the studies presented here address a major gap in our understanding of the extent of physiological variability of leaf traits and the resulting implications for remote monitoring. The most significant contribution of this thesis, then, is the reminder that in spite of the attractiveness of unified theories of trait relationships and automated, database-style biodiversity assessment, the importance of local scale ecological and temporal variation must not be underestimated.

6.4 References

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