



Impacts of leaf age on the spectral and physiochemical traits of trees in Amazonian forest canopies

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Abstract

This doctoral research presents the first comprehensive analysis of the morphological, biochemical and spectral leaf traits of canopy and emergent tropical trees during natural (*in situ*) leaf ageing. It adopts an interdisciplinary approach and combines multiple scales of analysis to generate insights into the effects of natural leaf ageing on our current understanding of tropical leaf trait variation, chemometric models used to spectrally predict leaf traits, and together with other leaf phenological processes, on remotely-sensed vegetation indices (VIs) commonly used to monitor canopy dynamics in tropical evergreen forests.

The first research paper of this thesis (**Chapter 4**) examines the effects of leaf age on morphological and biochemical leaf traits and demonstrates that leaf age is a significant driver of leaf trait variation in Amazonian canopy trees and that leaf age differences could potentially account for a significant fraction of what we currently understand as intra- and interspecific leaf trait variation. It also highlights that age-related trait variation within and between individual trees could play a significant role in shaping community composition and structure of tropical canopies.

The leaf traits examined in **Chapter 4**, among others, have been shown to directly influence the spectral reflectance behaviour of leaves. Therefore, **Chapter 5** investigates the effects of leaf age on leaf spectral properties within

and across a tropical canopy tree community. This study reveals that trees with diverse leaf properties age in a similar manner in terms of spectral properties. This is one of the most important findings of this thesis and lead to the development of a novel chemometric partial least square regression (PLSR) model to predict leaf age from hyperspectral data. This model extends the utility of current spectroscopic methods and introduces a simple and efficient approach for predicting and monitoring leaf age in lowland tropical forests with important implications for remote sensing. Additionally, this study is the first to provide evidence of age-related reflectance changes in leaves that have significant impacts on vegetation indices commonly used to monitor productivity and canopy dynamics in tropical evergreen forests.

Considering the findings of the previous two research chapters, **Chapter 6** investigates if chemometric PLSR models used to spectrally predict some of the important leaf traits for plant physiology and economy (leaf mass per area, LMA; water content, LWC; phosphorous, P; nitrogen, N; and carbon, C content) investigated in the previous two research chapters could be significantly biased by variation in leaf age. This is particularly relevant as the current standard protocol is to use only “fully expanded mature leaves” to calibrate these models. This study demonstrates that PLSR models developed using the current standard protocol display age/temporal sensitivity, which has important

implications for forest canopy communities with both synchronised and unsynchronised leaf phenology.

The final research chapter of this thesis, **Chapter 7**, demonstrates that the phenological age-related changes in leaf spectral properties reported in **Chapter 5** are also expressed at the canopy scale but influenced by both canopy leaf area (CLA) and the leaf phenological behaviour of individual trees. This study also reveals that the seasonality of greenness VIs such as NDVI and EVI2 are more strongly correlated to phenological changes in CLA than changes in leaf reflectance and proposes that NDWI (water content VI) which was found to be strongly correlated to age-related changes in leaf reflectance should complement greenness VIs in phenological studies. Furthermore, by combining leaf, canopy and community scale phenological observations, this study shows that complex and diverse leaf phenological behaviours exhibited by tropical canopy trees, at both the individual and community scale, challenge our current ability to remotely sense tropical canopy dynamics. Finally, this chapter highlights the need for more widespread phenological studies that examine the interaction, covariation, asynchrony and unique behaviours of tropical phenological processes at different scales. Such studies would enable the development of a significant mechanistic understanding of what creates and drives different phenological mosaics identified by remote sensing studies across tropical forests and in modelling their effects on water and carbon fluxes in tropical forest ecosystems.

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and
to the loving memory of Jan Bryant

**“It is the questions we can't answer that teach us the most. They teach us
how to think. If you give someone an answer, all they gain is a little fact.
But give them a question and they will look for their own answers.”**

— adapted from Patrick Rothfuss, *The Wise Man's Fear*

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Acronyms, Notations and Abbreviations

AIC	Akaike information criterion
All	Mixed-age PLSR model
ANCOVA	Analysis of covariance
ATP	Adenosine triphosphate molecule
Bare	Leafless canopy
BB	Broad bands
C	PLSR coefficients
C	Cellulose
Cal	Calibration
C _{area}	Leaf carbon content on an area basis (mg cm ⁻²)
CEH	Centre of ecology & hydrology
C _{mass}	Leaf carbon content on a mass basis (%)
CO ₂	Carbon dioxide
Com	Community
CVs	Coefficients of variation
D	Developmental PLSR models
δ ¹³ C	Delta 13-carbon (‰)
DBH	Diameter at breast height
EVI	Enhanced vegetation index
EVI2	Two-band enhanced vegetation index
fAPAR	Fraction of absorbed photosynthetically active radiation
GLOPNET	Global plant trait network
GP	Green peak (550nm)
IT	Individual tree
L	Lignin
LA	Leaf area (cm ⁻²)
LAI	Leaf area index
LES	Leaf economic spectrum

LM	Leaf mass (g)
LMA	Leaf mass per unit area (g m^{-2})
LT	Leaf thickness (mm)
LTD	Leaf tissue density (mg cm^{-3})
LWC	Leaf water content (%)
M	Mature leaves fully grown and structurally developed
MODIS	Moderate resolution imaging spectroradiometer
MRE	Measured resorption efficiency
N_{area}	Leaf nitrogen content on an area basis (mg cm^{-2})
NB	Narrow bands
NDVI	Normalised difference vegetation index
NDWI	Normalised difference water index
NIR	Near infrared spectral domain (700-1400nm)
nm	Nanometer
N_{mass}	Leaf nitrogen content on a mass basis (%)
O	Old leaves showing initial signs of senescence
P_{area}	Leaf phosphorus content on an area basis (mg cm^{-2})
PLSR	Partial least squares regression
P_{mass}	Leaf phosphorus content on a mass basis (%)
PRI	Photosynthetic reflectance index
RE	Red edge spectral domain (650-810nm)
RMSE	Root mean square error
RRE	Real resorption efficiency
S	Senescent PLSR models
S	Senescent leaves in the process of dying and abscising
S1	Senescent leaves in the process of dying and abscising (1 st month sample)
S2	Senescent leaves in the process of dying and abscising (2 nd month sample)
SMA	Standardised major axis
SuBD/ShE	Sun brevi-deciduous/Shade evergreen leaf phenological strategy

Su/ShAE	Sun/Shade asynchronous evergreen leaf phenological strategy
SWIR	Shortwave infrared spectral domain (1400-2500nm)
SWIR 1	Shortwave infrared spectral domain (1450–1940nm)
SWIR 2	Shortwave infrared spectral domain (1940–2450nm)
Tukey's HDS	Tukey-Kramer honestly significant difference test
Val	Validation
VIPs	Variables importance of prediction
VIs	Vegetation indices
VIS	Visible spectral domain (390-700nm)
W*	PLSR spectral weights
Wk	Week
Y/M	Developing leaves collected after 4 weeks of active leaf expansion
Y1 or D1	Developing leaves collected after 1 week of active leaf expansion
Y2 or D2	Developing leaves collected after 2 weeks of active leaf expansion
Y3 or D3	Developing leaves collected after 3 weeks of active leaf expansion

Thesis Overview

This thesis is presented according to the requirements for the University of Oxford's DPhil by submitted papers route. It is divided into **8 Chapters**. **Chapter 1** is the **Introduction**, which provides a scientific context and highlights gaps in the current knowledge that this DPhil research aims to address. **Chapter 2** presents the **Research Approach** that was adopted to fulfil the aims of this DPhil project. It provides an overview of the data collection design, the field campaigns, the research sites and a link to a video record of the fieldwork undertaken for this doctoral research. In **Chapter 3**, the **Literature Review** aims to provide background information for the topics that this thesis will cover. **Chapters 4 to 7**, present the four main research chapters (papers) produced by this doctoral thesis with the **Appendix** presenting a collaboration paper that is linked to **Chapter 5**. In **Chapter 8**, the **Discussion and Conclusions** summarise and analyse the main findings of this doctoral thesis. This chapter discusses the implications of these findings, the general relevance of the work and suggests future areas of research.



Chapter 1 Introduction

1.1 Research background

Tropical forests contain around 55% of the global carbon stores of all terrestrial ecosystems (Malhi *et al.*, 2002a). They account for approximately 50% of terrestrial photosynthesis (Field *et al.*, 1998) and strongly regulate regional and global climate (Nobre *et al.*, 1991; Betts *et al.*, 2007). These globally important roles are mediated through a simple structural unit: the leaf (Clark *et al.*, 2008). Primary productivity, and carbon and water exchange in forests, and between forests and the atmosphere, are controlled by the number of leaves, their photosynthetic capacity and their response to environmental drivers such as water, light and temperature (Borchert, 1994). This is why the study of the spatial variation and seasonality of tropical leaf phenology (timing of leaf flushing and abscission) and the extent to which climate factors drive it is a subject of much current interest.

The phenological dynamics of the Amazon forest may exert a strong influence on regional and global climate, hydrological and carbon cycles. This is due to the large areal extent of the region (5.4 million km²; Malhi *et al.*, 2008), the role it plays in regional meteorology (Nobre *et al.*, 1991; Aragao, 2012) and the magnitude of the carbon stocks stored there (Malhi *et al.*, 2002a). Research into its phenology has produced ambiguous results, however. Site-based plot and flux tower studies in some old-growth Amazon forests show depressed

photosynthetic activity in the dry season due to moisture stress (Malhi *et al.*, 1998) while studies at other sites show dry season increases in canopy photosynthesis (Saleska *et al.*, 2003). The effects of the Amazon dry season on forest productivity has also been investigated through field (Jipp *et al.*, 1998; Nepstad *et al.*, 2002; Doughty & Goulden, 2008; Phillips *et al.*, 2009; Brando *et al.*, 2010; Chave *et al.*, 2010; among others), modelling (Tian *et al.*, 1998; Williams *et al.*, 1998; Potter *et al.*, 1999; Asner *et al.*, 2000b; Asner & Martin, 2008b) and remote sensing studies (Huete *et al.*, 2006b; Myneni *et al.*, 2007; Saleska *et al.*, 2007). Some of these field and modeling studies have suggested decreased forest productivity in the dry season, with field studies reporting litterfall increases of 10-25% (Chave *et al.*, 2010), leaf area index (the one-sided green leaf area per unit ground area in broadleaf canopies; LAI) decreases of up to 25% during dry season (Myneni *et al.*, 2007) and a widespread decline in growth and increase in mortality among large trees during the severe drought of 2005 (Phillips *et al.*, 2009). On the other hand, some remote sensing papers describe a “green-up” of Amazon forests during the same 2005 drought event, suggesting that fresh young leaves cause a boost in tropical productivity under increased solar radiation (Huete *et al.*, 2006a; Saleska *et al.*, 2007). These contradictory findings are partially due to the heterogeneous availability of resources across the Amazon region, i.e. “green-up” effects during dry season reported by remote sensing studies focus on the less seasonal, more light limited (due to almost constant cloud cover) Amazonian evergreen rainforests while field

and model studies emphasise decreased productivity and leaf abscission due to drought stress in more seasonal forests. There is, therefore, a need to expand our knowledge on the primary productivity of these ecosystems, taking into account their spatial heterogeneity in order to improve our understanding of the phenological dynamics and biogeochemical function of Amazonian forests (Aragao *et al.*, 2009). More recent remote sensing studies such as Anderson *et al.* (2010; 2011; 2012), Bradley *et al.* (2011), Saatchi *et al.* (2013), Silva *et al.* (2013), Guan *et al.* (2015), Xu *et al.* (2015) and Wu *et al.* (2016a) are making headway in acknowledging and highlighting the effects that the high variability in climate (particularly rainfall regimes), dry season length and radiation seasonality has on creating different phenological mosaics identified across the Amazonian basin.

Satellite remote sensing can provide an important tool for studying phenological dynamics at scales beyond those possible with field studies. However, the interpretation of remotely sensed data must be done with caution as quality assessment of remotely sensed data/products has up to now primarily focused on the spatial resolution of methods used to generate monthly images with little effort placed on different sources of seasonal reflectance variability and the need for validation with on-the-ground observations. Remote sensing studies (see Huete *et al.*, 2006b; Myneni *et al.*, 2007; Saleska *et al.*, 2007) often interpret seasonal and interannual changes in the canopy of Amazonian forests as

changes in leaf area or flushes of new leaves with increased photosynthetic capacity. However, satellite remote sensing uses spectral reflectance data to measure canopy greenness which is a composite property of canopy chlorophyll content, canopy structure, and canopy leaf area. These three canopy elements vary intra-annually with leaf phenology, which can impact pixel level reflectance data. Furthermore, the interpretations put forward by Huete *et al.* (2006a), Myneni *et al.* (2007) and Saleska *et al.* (2007), offered little or no on-the-ground corroboration, nor have there been any field studies specifically designed to investigate the effects that leaf phenology can have on remotely sensed canopy dynamics. It is likely that changes in the physiochemical and reflectance properties of leaves with age together with other leaf phenological processes have as much influence as leaf area on the intra- and inter-annual changes identified by remote sensing studies. More recent remote sensing studies such as Brando *et al.* (2010), Bradley *et al.* (2011), Silva *et al.* (2013), Guan *et al.* (2015), Xu *et al.* (2015) and Wu *et al.* (2016a) have indeed suggested that the effect of age-related variation in the spectral reflectance properties of leaves together with seasonal changes in LAI may have a significant contribution towards explaining the “green-up” effect identified by earlier remote sensing studies. No study to date, however, has conducted *in situ* measurements of leaf and canopy reflectance as it varies over the leaf life-cycle nor explored how the complex and diverse leaf phenological behaviours displayed by tropical canopy trees can affect the vegetation indices we use to monitor the leaf phenological

dynamics of evergreen tropical forests. This study will specifically address these issues through detailed studies at two Amazonian field sites in French Guiana and Peru.

1.2 Research aims

The specific aims of this doctoral research are:

1. To investigate the role of leaf age as a fundamental driver of intraspecific leaf trait variation.
2. To assess how leaf physiochemical and spectral traits change with age by conducting field physiochemical measurements (i.e. leaf area; LA, mass; LM, thickness; LT, tissue density; LTD, mass per area; LMA, and water; LWC, phosphorus; P_{mass} , nitrogen; N_{mass} , carbon; C_{mass} and $\delta^{13}\text{C}$ content and spectro-radiometry.
 - 2a To identify the most significant changes in leaf reflectivity during different stages of the leaf life-cycle and determine how these changes are related to changes in physiochemical leaf traits (LMA, LWC, P_{mass} , N_{mass} and C_{mass}).
 - 2b To evaluate if leaf spectra can provide an efficient and accurate tool to predict leaf age.
3. To explore if (a) the relationship between spectra and leaf traits such as LMA, LWC, P_{mass} , N_{mass} and C_{mass} varies with age and (b) if leaf age affects the predictability of these traits from spectroscopic data.

4. To analyse how age-related phenological changes in leaf reflectance are expressed at the canopy level in trees with diverse leaf types and phenological behaviours and how this affects different spectral regions.
5. To investigate how age-related phenological changes in leaf reflectance and canopy leaf area (CLA) affect vegetation indices commonly used to measure vegetation greenness (the Normalised Difference Vegetation Index; NDVI and the Enhanced Vegetation Index; EVI) and vegetation water content (the Normalised Difference Water Index; NDWI).
6. To explore how the interaction, covariation and/or unique spectral behaviours of canopy phenological processes contribute to reported remotely sensed canopy dynamics in the tropics.

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Chapter 2 Research Approach

2.1 Field data collection

To achieve the aims of this DPhil project, a field data collection design involving leaf, tree and plot level data was implemented (**Figure 2.1.1**). This data collection involved extended field campaigns in two remote Amazonian primary evergreen tropical rainforest sites with moderate dry season in French Guiana and Peru.

These intensive and physically demanding field campaigns required the use of advanced tree-climbing techniques to reach the crowns of canopy and emergent trees 29-60m tall on a daily basis. Advanced arborist tree-climbing equipment and techniques were used to repeatedly access and move around the crowns of all sampled trees (8 in French Guiana and 12 in Peru) in order to (1) monitor their leaf life-cycle, (2) collect branches with leaf samples of different ages (e.g. developmental, mature, old and senescent leaves as explained in **Figure 4.2.4.1**) from different vertical canopy positions (upper, mid and lower canopy), and (3) find, establish and continuously access at least three different top-of-canopy positions for each sampled tree from which canopy-level multispectral reflectance measurements could be collected. The author of this thesis accompanied by a climbing assistant performed more than 800 individual tree climbs and together with a lab assistant processed >100,000 to collect the field data necessary to achieve the aims of this DPhil research project (see **Figure 2.1.1** and **Figure 2.3.1**).

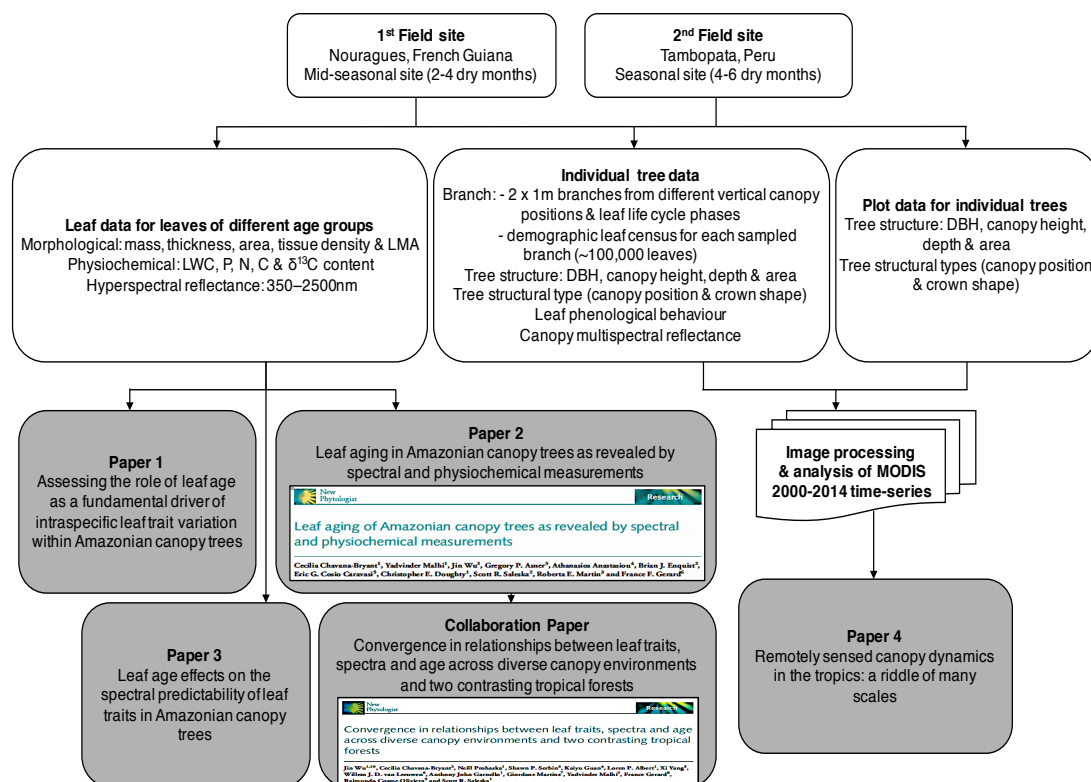


Figure 2.1.1 Diagrammatic representation of the research approach adopted for this DPhil project together with the four main research papers and one collaboration paper resulting from this approach.

2.2 Research field sites

As mentioned before, data collection was conducted at two primary evergreen tropical rainforest sites with moderate dry season in the Amazonian basin (**Figure 2.2.1** and **Figure 2.2.2**). The first field campaign was carried out in 2010 at the Grand Plateau (4°08'N, 52°7'W) in Nouragues field station, French Guiana. This site is located in primary tropical evergreen rainforest characteristic of the Guiana Shield region within the Nouragues National Reserve in North-eastern Amazonia. The Nouragues Grand Plateau is arranged as two adjoining 10ha plots that are subdivided into 1ha plots. The long-term tree database at this site

was established between 1992-1994 when trees over 30cm in diameter at breast height (DBH) were mapped, measured, and tagged. The soil at this site has been classified as oxisol. It presents a highly diverse vegetation with a closed canopy of 35 ± 5 m mean height. This is a moderately seasonal site with a 2-3 month-long dry season (months with <100mm rainfall), mean annual rainfall of 3,476 mm and a daily mean temperature that ranges between 20.3°C and 33.5°C. Long-term meteorological and litterfall monitoring have been carried out since 1987.

The second field campaign (and the focus of the four research chapters in this thesis) was conducted in and around two tropical evergreen rainforest sites located within the Tambopata National Reserve in the Madre de Dios region of Peru during 2011–2012. The two study plots TAM-06 (12°50'24"S, 69°17'59"W) and TAM-09 (12°49'48"S, 69°16'48"W) are part of the Global Ecosystems Monitoring (GEM) intensive sampling sites in the RAINFOR Amazon Forest Inventory Network (Malhi *et al.*, 2002b). Both plots are located within lowland primary tropical rainforest and lay between 215m and 220m above sea level on Holocene (TAM-06) and Pleistocene (TAM-09) terraces with Haplic alisol soils (Quesada *et al.*, 2010). They are seasonal sites with a four to six month-long dry season (months with <100 mm rainfall) between June and October, mean annual rainfall of 1900 mm (this may be lower than the long-term mean due to severe droughts in 2005 and 2010 (Lewis *et al.*, 2011) and mean annual air temperature

of 24.4°C. A detailed description of the geomorphology, climate and forest carbon cycle of this site can be found in Malhi *et al.* (2014). Vegetation at these study sites is highly diverse and presents a fairly open canopy with mean canopy heights of $27 \pm 8\text{m}$.

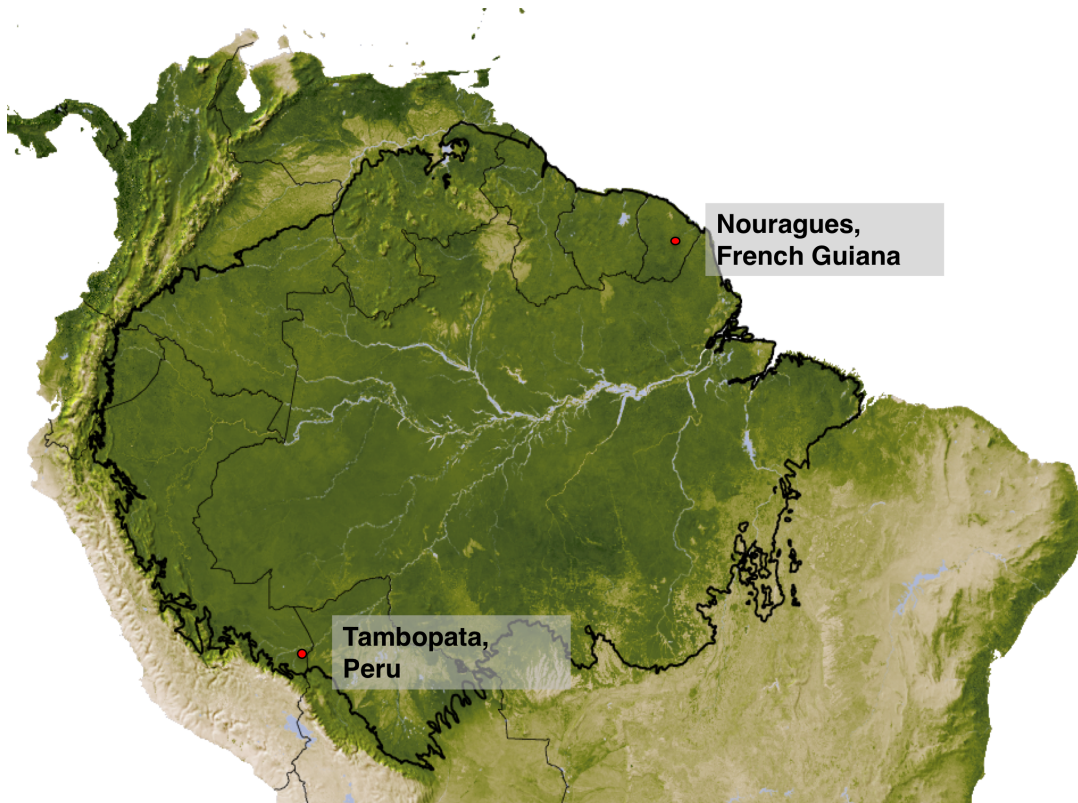


Figure 2.2.1 Map of the Amazon forest showing the location of the 2 long-term intensive sampling sites, Inselberg, French Guiana and Tambopata, Peru, where fieldwork for this DPhil project was conducted. Map by Robert Simmon (NASA GSFC) based on EVI data from Kimel Didan, Vegetation Index and Phenology Lab., The University of Arizona.

Moderately seasonal evergreen rainforests represent a larger fraction of the total Amazon extent than aseasonal rainforests. The research sites at French Guiana and Peru were selected to contrast the heterogeneity these Amazonian forests

experience in terms of dry season (**Figure 2.2.2** presents the dry season spatial distribution within the Amazonian basin).

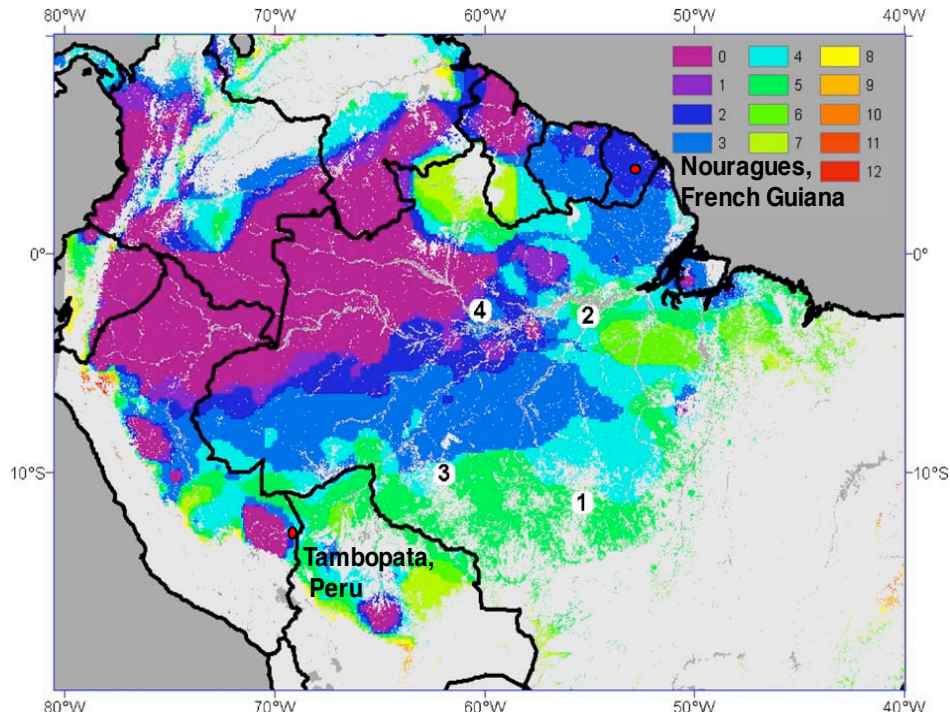


Figure 2.2.2 Spatial distribution of dry season (number of months with <100 mm rainfall) in tropical South America. Map from Xiao *et al.* (2006) based on monthly precipitation data from the South American Precipitation Dataset (Willmott & Webber, 1998). Although annual rainfall is high (>1500 mm/yr), there still exist distinct dry and wet seasons for a large proportion of the Amazon region (Keller *et al.*, 2004). The length of dry season varies substantially across the region, with most areas ranging from 0 to 6 months in a year (Malhi *et al.*, 2002b).

2.3 Tropical canopy leaf phenological dataset

This DPhil project collected (to my knowledge) the first phenological leaf and canopy dataset for tropical canopy trees. This unique phenological dataset, holding >8,000 leaf and canopy records from 20 sampled trees from different species (**Figure 2.3.1**), was used to investigate the morphological,

physiochemical, and spectral changes of tropical canopy trees associated with natural leaf ageing. The trees from which leaves were sampled represent a wide variety of leaf types (e.g. compound and simple leaves), leaf sizes, shapes, leaf thickness and waxiness within each site. This sampling made it possible to assess the influence of age on leaf morphological, physiochemical and spectral traits within individual trees and across local tropical canopy tree communities with diverse leaf properties.

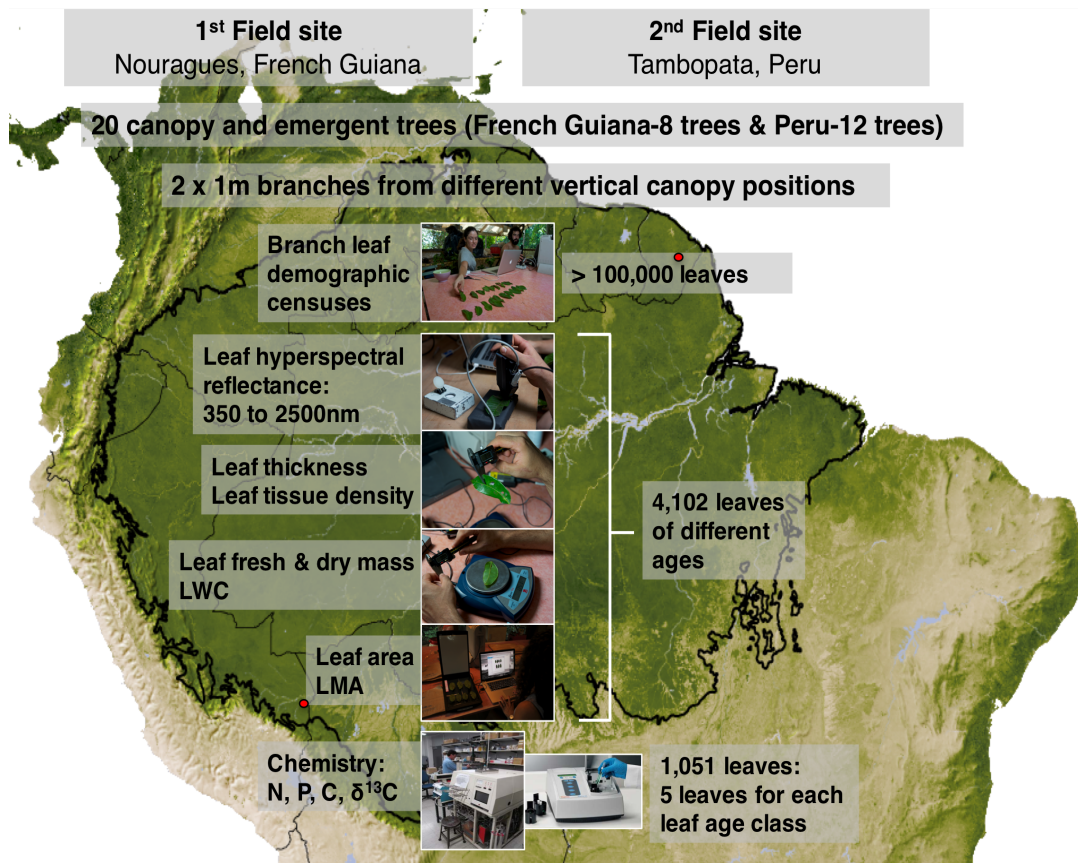


Figure 2.3.1 Overview of leaf level data collected in the field and the chemical analyses performed on some of these leaves.

The larger and more comprehensive phenological leaf dataset collected in Peru was used to produce the four research papers of this doctoral thesis with the last paper also including canopy level spectroscopy, plot level tree structural measurements and time-series of vegetation indices (VIs) from the Moderate Resolution Imaging Spectrometer (MODIS) (**Figure 2.1.1**). Additionally, the Peruvian dataset was used in a collaborative paper entitled “Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests” (see Appendix section).

Due to the large amount of field data collected for this doctoral research, the French Guiana leaf phenological dataset (containing both sun and shade leaves), the Peruvian dataset for shade leaves and life-cycle herbivory data for both sites remain to be explored in future research papers. These papers will be able to make comparisons in leaf phenology between two tropical forest sites with contrasting soil and forest properties, and for the first time, explore herbivory rates throughout the leaf life-cycle of tropical canopy trees. Also, due to the large number of leaves collected for each sampled leaf age (15 to 30 leaves) at both study sites, numerous leaf samples have been stored safely and are available for future studies on leaf venation and internal structure through the leaf life-cycle.

A video record of the field data collection undertaken for this doctoral thesis can be seen in <https://vimeo.com/208748496>.

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Chapter 3 Literature Review

Recent remote sensing studies have used a suite of terrestrial ecosystem variables such as vegetation indices (VI's), leaf area index (LAI), fraction of photosynthetically active radiation (FPAR), and gross primary production (GPP) from the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor to analyse Amazon forest metabolism and canopy dynamics (Huete *et al.*, 2006a; Huete *et al.*, 2006b; Myneni *et al.*, 2007; Saleska *et al.*, 2007). Much attention has been given to these studies, as they are the first to analyse seasonal and interannual dynamics at multiple scales and to offer a synoptic view of the entire Amazon region. Synoptic analyses could have never been achieved through ground sampling given the sheer size and diversity of the region and have only recently been made possible using the sampling system for land surface monitoring offered by MODIS and other satellites.

3.1 MODIS satellite sensor

The MODIS sensor was designed to provide improved monitoring of land, ocean, and atmosphere research. The design of the land-imaging component combines characteristics of the Advanced Very High Resolution Radiometer (AVHRR) and the Landsat Thematic Mapper, adding spectral bands in the middle and long-wave infrared (IR). The sensor has 36 spectral bands, seven of which are designed for the study of vegetation and land surfaces: blue band 1 (459-479nm), green band 2 (545-565nm), red band 3 (620-670nm), near infrared

bands 4 and 5 (NIR₁: 841-875nm; NIR₂: 1230-1250nm), and shortwave infrared bands 6 and 7 (SWIR₁: 1628-1652nm; SWIR₂: 2105-2155nm). Daily global imagery is provided at spatial resolutions of 250m (bands 1 and 2), 500m(bands 1 to 7) and 1km (bands 1-7) (Justice *et al.*, 1998).

MODIS is a particularly effective instrument for monitoring tropical canopies as mid- and long-wave IR spectral bands for improved atmospheric and cloud characterisation have been included to permit both the removal of atmospheric effects on surface observations (cloud contamination, sensitivity to seasonal variable atmosphere water vapour and aerosol conditions) and the provision of atmospheric measurements (Justice *et al.*, 1998). These improvements together with the multiscale sampling system and the frequency of data collection (twice daily) have made the compilation of stable monthly composites of MODIS multispectral data into well-calibrated time-series possible and realised the potential for a global systematic monitoring system for the land surface. The suite of MODIS land products are now used in terrestrial land cover, carbon, and water science, in quantifying terrestrial surface changes resulting from climatic forcing and human-induced and natural disturbances and more recently to analyse forest metabolism and dynamics (Huete *et al.*, 2006b). Other medium resolution satellite sensors such as MERIS (300m spatial resolution) and the ATSR series (1km spatial resolution) also provide freely available multispectral data time-series that are shorter and have a more limited spectral range (up to

1200nm) than those of MODIS. High resolution or hyperspectral satellite sensors such as Hyperion and SPOT are not able to provide the time-series necessary for the analysis of vegetation seasonal and interannual dynamics nor are their data freely available.

3.2 MODIS land products

The MODIS instrument onboard NASA's Earth Observation System Terra and Aqua satellites, measures a combination of basic spectral reflectance, albedo and land surface temperature (LST) variables. It began acquiring data with an improved radiometric and geometric rectification system and spatial resolution in 2000 (Justice *et al.*, 2002). These data are calibrated, geolocated, and processed for cloud filtering and atmospheric correction. Further data processing involves the application of a suite of algorithms to generate gridded rasters of higher order variables, such as vegetation indices (VI's), leaf area index (LAI), fraction of absorbed photosynthetically active radiation (fPAR) and gross primary production (GPP). These higher order variables are geophysical datasets called "products" and are generated with explicit pixel-level quality flags. Available MODIS products undergo updates as the latest algorithms are applied to the instrument's data. A collection numbering scheme is used to differentiate among different reprocessing runs, with the highest collection number reflecting the most recently improved or reliable version of the product (Anderson *et al.*, 2010).

MODIS products use spectral reflectance data to measure canopy greenness, a composite property of canopy structure, leaf area, and canopy chlorophyll (Asner, 1998) . This is done through spectral transformations of two or more bands designed to enhance the contribution of different vegetation properties and to convert reflectance data into quantitative biophysical variables (Huete *et al.*, 2002), which are then further processed into products that generate vegetation indices, vegetation index-based LAI estimates and/or fPAR.

3.3 What do land products measure?

Vegetation indices (VIs) are designed to optimise the measurement of different leaf biochemical constituents such as chlorophyll, water content and vegetation biophysical properties (*e.g.* LAI and fPAR). However, the vegetation signal in a remote measurement can be influenced by the atmosphere above and the soil below, thus, “enhanced” vegetation indices have been developed to partially correct for these effects and also compensate for the bidirectional geometry of measurement (Baret & Guyot, 1991). As a result, more than a dozen vegetation indices are reported in the literature and shown to correlate well with vegetation amount (Myneni *et al.*, 2007), fPAR (Asrar *et al.*, 1984), vegetation conductance and photosynthetic capacity (Sellers *et al.*, 1992), and seasonal atmospheric carbon dioxide variations (Tucker *et al.*, 1986). Details of the two most common vegetation indices, which are also the standard VIs offered by MODIS, can be found in **Table 3.3.1**. A third VI, the Normalised Difference Water Index (NDWI),

is included as water content is an important diagnostic feature in the study of canopy phenological dynamics. This is because leaf water is an important factor regulating canopy temperature and moisture stress, both of which are particularly acute in tropical forest canopies and have been observed to cause concerted leaf abscission (Nepstad *et al.*, 2002).

Table 3.3.1 Vegetation indices commonly used for detection of vegetation changes and extraction of canopy biophysical parameters.

Vegetation index	Description	Equation	Strengths, weaknesses and complementarities between VIs
Normalised difference vegetation index (NDVI)	This is the most widely used VI and is a normalised ratio of the near infrared (NIR) and red bands. The MODIS NDVI is referred to as the “continuity index” to the existing 20+ year NOAA-AVHRR derived NDVI time series which could be expanded by MODIS data to provide a longer term data record.	$NDVI = (P_{NIR} - P_{Red}) / (P_{NIR} + P_{Red})$ where, P_{NIR} and P_{Red} are the surface bidirectional reflectance factors for their respective MODIS bands.	The strength of the NDVI is in its ratioing concept, which reduces many forms of multiplicative noise (illumination differences, cloud shadows, atmospheric attenuation, and certain topographic variations) present in multiple bands. Its main disadvantage is the inherent nonlinearity of ratio-based indices and the influence of additive noise effects (Huete <i>et al.</i> , 2002). The NDVI also exhibits scaling problems and is known to saturate at LAI values of 3 to 4m ² /m ² , which is below the global mean LAI value of 4.7m ² /m ² in tropical forests (Asner <i>et al.</i> , 2003). The EVI overcomes these problems using atmospheric correction, cloud and cloud shadow removal, and standardisation of sun-surface-sensor geometries with bidirectional reflectance distribution function (BRDF) models. The EVI is also more responsive to canopy structural variations, including LAI, canopy type and architecture, and plant physiognomy, as it relies on NIR canopy reflectance which is less prone to saturate, particularly with moderate resolution pixels (Gao <i>et al.</i> , 2000). The two VIs complement each other as the NDVI is chlorophyll sensitive whereas the EVI is more responsive to canopy structural variations. Together they improve upon the detection of vegetation changes and extraction of canopy biophysical parameters. NDWI can be useful in canopy phenology studies as it could help clarify the effects that drought has on leaf abscission and flushing at different temporal and spatial scales. However, NDWI does not completely remove the background soil reflectance effects, similar to NDVI.
Enhanced vegetation index (EVI)	This is an ‘optimised’ index designed to enhance the vegetation signal with improved sensitivity in high biomass regions (such as the tropics) and improved vegetation monitoring through a de-coupling of the canopy background signal and a reduction in the atmosphere influences.	$EVI = [P_{NIR} - P_{Red} / P_{NIR} + (C_1 \times P_{Red} - C_2 \times P_{Blue}) + L] G$ where P_{NIR} = atmospherically-corrected (Raleigh and ozone absorption) surface reflectances; L = canopy background adjustment that addresses non-linear, differential NIR and red radiant transfer through a canopy; and C_1, C_2 = coefficients of aerosol resistance, which use the blue band to correct for aerosol influence on the red band. In the MODIS-EVI algorithm: $L=1$, $C_1=6$, $C_2=7.5$ and G (gain factor)=2.5	
Normalised difference water index (NDWI)	NDWI uses two near-IR bands (860 and 1240nm) to measure liquid water molecules in canopies. It uses these bands as absorption by vegetation liquid water near 0.86 μ m and 1.24 μ m is weak. Canopy scattering enhances the water absorption signal, as a result, NDWI is sensitive to changes in liquid water content of canopies. Also, atmospheric scattering effects in the 0.86-1.24 μ m region are weak so NDWI is less sensitive to atmospheric effects than NDVI (Gao, 1996).	$NDWI = [P(860nm) - P(1240nm)] / [P(860nm) + P(1240nm)]$ Where, P represents the radiance in reflectance units. Both the 860nm and the 1240nm bands are located in the high reflectance plateau of canopies. They sense similar depths through canopies.	

Remote estimates of leaf area index (LAI) and fraction of photosynthetically active radiation absorbed by vegetation (fPAR) enhance simple vegetation index products as they represent two important biophysically complementary ways of describing the Earth's vegetated surfaces (Fensholt *et al.*, 2004). LAI is defined as the one-sided green leaf area per unit ground area in broadleaf canopies and gives an estimate of the green leaf area of terrestrial vegetation (Myneni *et al.*, 1997). fPAR is a measure of the fraction of the incoming radiation in the photosynthetically active radiation spectral region (400-700nm) that is absorbed by leaves (Sellers *et al.*, 1992), thus, expresses the canopy's energy absorption capacity and is directly related to primary production (GPP). However, to understand what VI, LAI and fPAR measure it is necessary to understand the physical basis of spectral remote sensing and the relationship between vegetation reflectance spectra and leaf biochemical constituents.

The association between the biochemical and structural properties of leaves and the spectral quality of light they transmit, reflect and absorb has long been recognised (e.g. Knippling, E, 1970). Satellite remote sensors acquire brightness or reflectance data in separate regions of the electromagnetic spectrum. When incident solar radiation reaches the vegetation, it may be (1) absorbed by pigments (mainly chlorophylls a and b, carotenoids and anthocyanins), water and other leaf constituents, (2) specularly reflected at the surface of a leaf, and/or (3) diffused by the canopy. The probability that each event occurs is influenced by

the canopy configuration and its optical properties. Radiation not absorbed by the leaf emerges diffused on both sides of the leaf, thus the leaf albedo is the sum of leaf hemispherical reflectance and transmittance (Huete *et al.*, 2002).

Vegetation has a unique spectral signature that enables it to be distinguished from other types of land cover. A typical reflectance vegetation spectrum with highlighted common wavelength bands used in remote sensing studies of vegetation can be seen in **Figure 3.3.1**. Vegetation signatures can be identified by the high NIR reflectance but generally low reflectance in the visible part of the spectrum. Reflectance is low in the visible part of the spectrum due to light absorption by chlorophyll and other pigments for photosynthesis. Pigment light absorption is highest (thus, reflectance lowest) in both the blue and red regions of the spectrum with a drop at the green region which gives rise to the green colour of vegetation and the spectral “green peak” around 550nm. A distinct feature of vegetation spectra is the rapid increase in reflectance in the narrow wavelength region of 650-810nm, referred as the red edge (RE). This abrupt increase in reflectivity is a manifestation of the transition from the strong chlorophyll absorption region near 675nm to a region dominated by light scattering caused by the intercellular leaf structure in the near infrared (NIR) region (700-1400nm) (Horler *et al.*, 1983). There are no strong absorption features in the NIR region, reflectance remains high with the actual magnitude controlled by the cellular structure of the leaf, the nature of the complex cavities within the spongy

mesophyll and palisade tissues in the leaf and the internal reflection of infrared radiation within these cavities (Gausman, H.W. *et al.*, 1970). Water absorbs radiant energy throughout the short-wave infrared (SWIR) region (1400-2500nm), with strong absorption bands centred at 970, 1200, 1450, 1940 and 2500nm (Gao & Goetz, 1995).

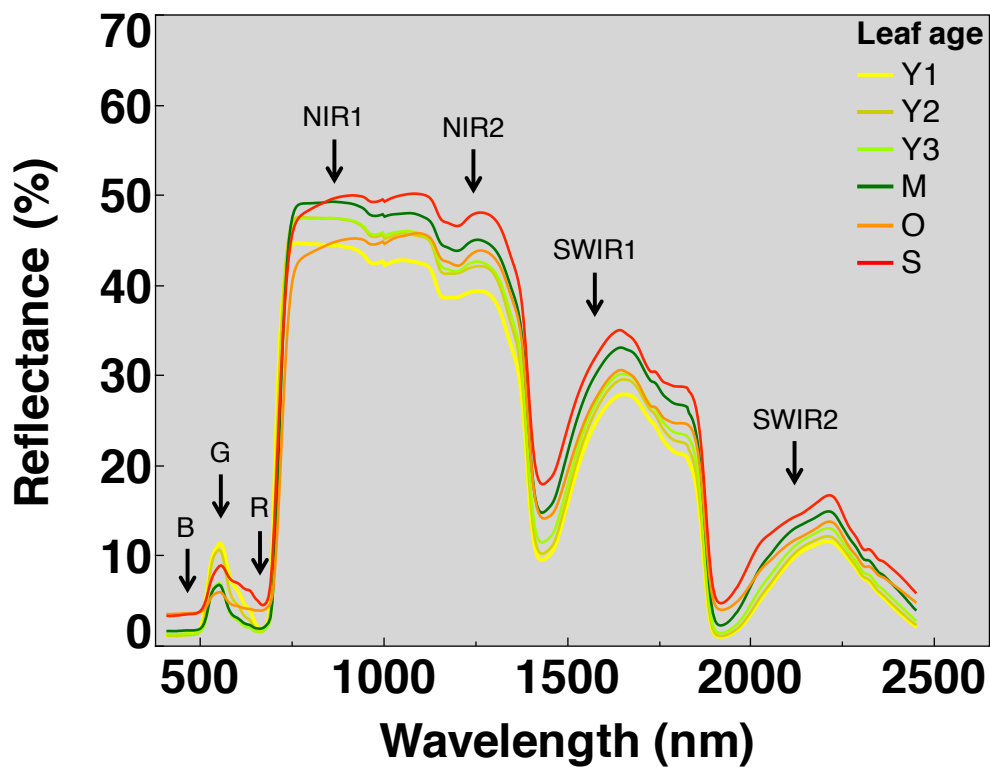


Figure 3.3.1 Example reflectance spectrum of leaves of different age groups for one sampled tree species in this study – *Bertholletia excelsa*. The labelled arrows indicate the common wavelength bands used in remote sensing studies of vegetation: Blue band= B; 430-480nm, Green band= G; 520-570nm (Green peak at 550-555nm), Red band= R; 630-700nm, Near infrared band 1= NIR₁; ~850nm, Near infrared band 2= NIR₂; ~1240nm, Short-wave infrared band 1= SWIR₁; ~1640nm and Short-wave infrared band 2= SWIR₂; ~2130nm.

Remote VI and LAI products capture the strong contrast between the chlorophyll absorption maximum near 670nm and the lack of absorption in the adjacent NIR region around 850nm through combinations of broadband Red and NIR reflectance. By dividing NIR/Red reflectance, remote VI and LAI products characterise the relationship between these two spectral regions and use this to evaluate vegetation chlorophyll content and cellular structure to determine vegetation type, condition and density.

3.4 Remote sensing studies analysing canopy dynamics in the Amazon

The remote sensing studies mentioned at the beginning of this review (Huete *et al.*, 2006a; Huete *et al.*, 2006b; Myneni *et al.*, 2007; Saleska *et al.*, 2007) used the MODIS EVI and LAI products to characterise seasonal and interannual changes in Amazonian vegetation. Huete *et al.* (2006a) used remote sensing imagery techniques to show how vegetation optical properties vary across the Amazon region. Their analysis of five years (2000-2005) of the MODIS EVI data along a climate-transect of variable rainfall and photosynthetically active radiation (PAR) regimes found a 25% increase in vegetation greenness during the dry season across most Amazon forests. Saleska *et al.* (2007) analysed the response of Amazon forests to the drought event of 2005 in order to find out if drought reduced whole-canopy photosynthesis. They found that intact forest canopy greenness (using a MODIS EVI anomaly distribution) significantly

increased during 2005 in the drought affected areas with 64% of EVI anomalies displaying a positive response to drought. Myneni *et al.* (2007) used five years (2000-2005) of MODIS LAI data to show how the seasonality in green leaf cover (LAI) varies across the Amazon region. They found that the MODIS-derived LAI increased by around 25% in most Amazon forests during the dry season. These three studies propose that the variations they detected are driven by seasonality in solar radiation rather than in rainfall and suggest that intact Amazon forests may be more resilient than many ecosystem models assume. This, in turn, assumes that canopy greenness and LAI in the dry season are a metric of sensitivity to long-term drought. However, while some field studies have found solar radiation may be a trigger for the flushing of new leaves during the dry season (Wright & van Schaik, 1994) in the less seasonal, more light limited (due to cloud cover) evergreen rainforests, it may also lead to concerted leaf abscission in more seasonal forests as found in studies by Chave *et al.* (2010), Doughty and Goulden (2008) and Nepstad *et al.* (2002). In their analysis of a large number of published and unpublished litterfall datasets covering 80 sites across the Amazon region Chave *et al.* (2010) found that litterfall seasonality was positively correlated spatially with rainfall seasonality. Furthermore, Huete *et al.* (2006a), Myneni *et al.* (2007) and Saleska *et al.* (2007) do not address other important sources of seasonal reflectance variability such as phenological changes in the spectral properties of leaves with age, the complex and diverse leaf phenological behaviours displayed by tropical canopy trees and the

interaction of these with other leaf phenological processes exhibited by tropical canopy communities and which can sharply impact pixel level reflectance data.

The effect that leaf age can have on remotely sensed canopy spectral reflectance is illustrated in **Figure 3.4.1**. It is likely that changes in the reflectance properties of leaves with age have as much influence as leaf area on the seasonal and interannual changes in the canopies of the Amazon forests identified by these early remote sensing studies. Indeed, more recent work from Asner and Martin (2008b), Doughty and Golden (2008), Anderson *et al.* (2010; 2011; 2012), Brando *et al.* (2010), Bradley *et al.* (2011) and Silva *et al.* (2013) have repeatedly suggested that the effect of age-related variation in the spectral reflectance properties of leaves and varying shadow patterns with seasonal changes in LAI may have a significant contribution towards explaining the “green-up” effect identified by the earlier remote sensing studies.

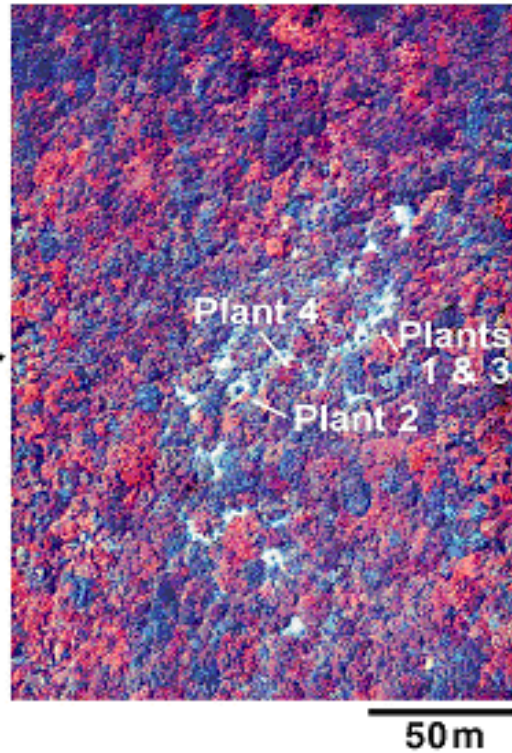


Figure 3.4.1 Colour infrared photograph of the INPA campina collected in September 1991 (image from Roberts *et al.*, 1998). Canopy infrared reflectance variation in this image is due to leaf age. Plants with new foliage are bright red, while plants with old foliage are dark red or blue tinted. Open sand is white. The locations of study plants are shown.

3.5 Tropical phenology and the effect of leaf ageing on spectral behaviour

The few leaf phenological studies that exist for tropical evergreen canopy communities, have previously reported a range of leaf phenological behaviours broadly described as patterns ranging from rapid shedding of short-lived, mesic leaves early in the dry season in deciduous species to irregular exchange in brevi-deciduous or constant exchange in evergreen species of long-lived, coriaceous leaves, and from complete intraspecific synchrony to complete

asynchrony (Vanschaik *et al.*, 1993; Wright & van Schaik, 1994; Reich, 1995; Reich *et al.*, 2004). The relative proportion of leaf cohorts in tropical trees is influenced by the time of season and recent climatic conditions. Seasonal water stress and light limitation have been identified as the two major phenological drivers in tropical forests (van Schaik *et al.*, 1993; Borchert, 1994; Wright & van Schaik, 1994; Reich, 1995; Borchert *et al.*, 2002; Singh & Kushwaha, 2005) with Amazonian forests growing under a range of seasonal and annual rainfall and solar radiation regimes. Thus, the role that seasonal water stress or light limitation have as phenological drivers will depend on their availability at different sites.

Anatomical, biochemical and physiological properties of leaves vary as a function of life strategy (evergreen vs. deciduous), physical environment, light quality, life span and leaf age (Reich *et al.*, 1999). Many changes in leaf properties are associated with changes in the spectral quality of light reflected, transmitted and absorbed by leaves (Gausman, H.W. *et al.*, 1970; Knipling, E, 1970; Woolley, 1971; Roberts *et al.*, 1998) and the manner in which chemical and anatomical changes associated with leaf ageing influence leaf spectra has also been recognised (Gausman, H.W. *et al.*, 1970; Sinclair *et al.*, 1971). The latter is clearly illustrated in **Figure 3.4.1** from Roberts *et al.* (1998). Spectral changes in leaves due to ageing happen mainly in both the visible and NIR portion of the spectrum. Changes in the visible portion of the spectrum characterise expanding

and maturing vegetation with absorption increasing to a peak as leaves achieve maturity and NIR absorption increasing as leaves senesce. In other words, reflectance of developing leaves in the visible bands decreases as they achieve maturity due to increased absorption driven by higher concentrations of pigments (chlorophyll *a* and *b*, carotenoids and anthocyanins), while in senescing leaves visible reflectance increases due to die-off of chlorophyll and NIR reflectance declines due to internal structural disintegration. Decreased NIR reflectance at the leaf scale may have a larger impact at the canopy scale by dampening NIR scattering within a canopy and thus decreasing canopy reflectance. This may provide another mechanism for producing changes in vegetation indices as a steady decrease in the ratio of NIR to red reflectance would imply a decrease in leaf area even if no change had occurred (Roberts *et al.*, 1998). However, to date, only one study, Roberts *et al.* (1998), has directly investigated the effect of leaf ageing on the leaf reflectance of tropical tree species. A better understanding of mechanisms that contribute to the spectral properties of different tropical forest communities will help in the classification and extraction of ecological information (including phenological dynamics) from remote sensing. Moreover, before the remote-sensing analysis of tropical canopy dynamics is used as a diagnostic tool to evaluate tropical forest resilience, a greater understanding of the effects of phenological age-related changes in leaf reflectance and its interaction with other complex and diverse canopy phenological processes such as leaf phenological behaviours of tropical trees and phenological asynchrony between trees affect

the remote sensing products commonly used to monitor the canopy dynamics of evergreen tropical canopy forests.

3.6 References

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Chapter 4 Statement: *In revision*

I begin the research chapters of this thesis by addressing the first aim of my research: “**to investigate the role of leaf age as a fundamental driver of intraspecific leaf trait variation**”. I use the leaf physiochemical trait data (i.e. leaf area (LA), mass (LM), thickness (LT), tissue density (LTD), mass per area (LMA), and water (LWC), phosphorus (P_{mass}), nitrogen (N_{mass}), carbon (C_{mass}) and $\delta^{13}\text{C}$ content) collected from leaves of different ages (from early expansion to maturity and senescence) to demonstrate that leaf age is a significant driver of trait variability within and across tropical canopy trees, therefore, measures of intra- and interspecific trait variation should account for leaf age in field collection protocols. We provide practical field and analytical recommendations to better account for leaf age. Trait analyses presented in this chapter also reveal that age-related trait variation within and between individual trees is an important temporal axis of variation in LES traits, and could therefore play a significant role in shaping community composition and structure of tropical canopies.

Author Contribution Statement

C.C-B., Y.M. and F.F.G. planned and designed the research. C.C-B. planned and conducted fieldwork and field data collection with logistical support from E.G.C. Data integration and quality assurance was done by C.C-B and A.A. Data analysis was performed by C.C-B. Chemical analyses were undertaken by B.J.E.

at the University of Arizona. C.C-B. wrote the manuscript with contributions from F.F.G., B.J.E., L.P.B., N.S.R., R.E.M., G.P.A. and Y.M.

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Chapter 4 Assessing the role of leaf age as a fundamental driver of intraspecific leaf trait variation within Amazonian canopy trees

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Abstract

- Intraspecific variation in functional traits reflects plasticity to microclimatic variation and influences ecosystem processes and remotely-sensed canopy dynamics. Leaf ageing, in turn, can be a fundamental driver of intraspecific variation in leaf traits. Yet, the extent to which leaf age affects leaf traits in tropical forest canopies is poorly understood.
- We measured physiochemical leaf traits from early expansion to maturity and senescence of 1099 leaves from 12 lowland Amazonian trees in southern Peru to investigate the variability within canopy trees and across a tropical canopy tree community due to natural leaf ageing.
- We demonstrate that leaf age is a significant driver of trait variability within and across trees, therefore, measures of intra- and interspecific trait variation should account for leaf age in field collection protocols. We also show the impact traditional leaf sampling and mass- and area-based nutrient estimates has on resorption studies in tropical trees. We provide practical field protocols and analytical recommendations to address these issues.
- We reveal that age-related trait variation within and between individual trees is an important axis of variation in LES traits, and could therefore play a significant role in shaping community composition and structure of tropical canopies.

Key Words

Tropical canopy trees; Leaf traits; Leaf mass per area (LMA); Leaf economic spectrum (LES); Age-related trait variation; Life-cycle functional strategies; Intraspecific variation; Community assembly

4.1 Introduction

Morphological and biochemical leaf traits play a critical role in regulating plant growth, energy and material cycling in terrestrial food webs, ecosystem net primary productivity and global biogeochemical cycling (Enquist *et al.*, 2007; Violle *et al.*, 2007). Furthermore, examination of the coordinated variation in the longevity, morphology, chemistry and metabolism of leaves, as measured by their mean trait values, has helped explain leaf economics, species strategies, community assembly and ecosystem structure and function (Grime, 1977; Westoby *et al.*, 2002; Diaz *et al.*, 2004; Wright *et al.*, 2004; Ackerly & Cornwell, 2007; Reich *et al.*, 2007; Savage *et al.*, 2007; Reich, 2014).

Trait-based ecology has generally considered species as the basic unit of variation and assumed that intraspecific trait variability is relatively small compared to interspecific variation (Reich *et al.*, 1999; Garnier, 2001; Cornelissen *et al.*, 2003; Wright *et al.*, 2004). It has, therefore, also been assumed that species can be reasonably characterised by their mean trait values. However, recent studies have shown that intraspecific leaf trait variation

can be greater than previously assumed (Albert *et al.*, 2010a; Albert *et al.*, 2010b; Messier *et al.*, 2010; Perez-Ramos *et al.*, 2012) with some studies revealing that intraspecific leaf trait variation can be large enough to influence rates of nutrient cycling (Lecerf & Chauvet, 2008) and play a fundamental role in species coexistence and plant community assembly (Lichstein *et al.*, 2007; Clark, 2010; Jung *et al.*, 2010; Albert *et al.*, 2011; Bolnick *et al.*, 2011; Long *et al.*, 2011; Paine *et al.*, 2011; Pruitt & Ferrari, 2011; Violle *et al.*, 2012).

Measuring intraspecific trait variability is important as it reflects a species' ability to respond to environmental variation through adaptation (or genetic variability) and/or acclimation (or phenotypic plasticity). Assessing the degree of intraspecific variation within and between individuals is also important for new remote sensing methodologies, such as imaging spectroscopy, attempting to differentiate species and quantify functional diversity in order to scale from traits to landscapes and ecosystems (Asner & Martin, 2008b; Asner & Martin, 2008a; Asner *et al.*, 2009; Asner *et al.*, 2011a; Asner *et al.*, 2014; Asner *et al.*, 2015). Here, we report on a source of intraspecific trait variability that, so far, has been largely neglected in the tropics (but see Kitajima *et al.*, 1997; Kitajima *et al.*, 2002) –the leaf trait variability generated by natural leaf ageing in a tropical forest evergreen canopy.

Many leaf-scale physiochemical, anatomical and morphological traits such as leaf pigmentation, nutrient content, intercellular structure, leaf mass per area (LMA),

leaf water content (LWC) have been shown to change with leaf age (Field & Mooney, 1983; Carter *et al.*, 1989; Miller *et al.*, 1991; Reich *et al.*, 1991; Kitajima *et al.*, 1997; Kitajima *et al.*, 2002; Escudero & Mediavilla, 2003; Ma *et al.*, 2011; Mediavilla *et al.*, 2011). Natural leaf ageing can therefore, be a fundamental driver of changes in leaf traits, and largely regulate ecosystem processes and remotely-sensed canopy dynamics. Leaf demography (i.e. leaf age distribution) has indeed been proposed as a major driver of seasonal productivity in carbon-rich tropical evergreen forests (Asner *et al.*, 2000b; Huete *et al.*, 2006b; Doughty & Goulden, 2008; Brando *et al.*, 2010; Anderson *et al.*, 2011; Bradley *et al.*, 2011; Samanta *et al.*, 2012) and it is possible that our measures of traits from tropical species may be overly influenced by variation in leaf age. However, the study of intraspecific leaf trait variability in tropical forests presents a methodological hurdle, i.e., most species in the tropics are rare (Hubbell, 1979; Gentry, 1988). This rarity translates into a high diversity of leaf traits as a result of high species diversity with low population abundance. This hurdle is most acute in tropical evergreen canopies, where only one individual can be found per hectare for most canopy tree species (Gentry, 1988; Ter Steege *et al.*, 2000). For example, the brazil nut *Bertholletia excelsa* (one of the relatively common Amazonian canopy tree species included in our study, see Table 1) has one or two canopy trees per hectare throughout the Tambopata National Reserve (Gentry, 1988), the location of our study. Consequently, our study focuses on individual canopy and

emergent trees to capture the variability due to natural leaf ageing within individual canopy trees and across a local canopy tree community.

We evaluate the influence of age on leaf trait variability within and across tropical canopy trees to assess the following questions: (1) is age-related trait variation a significant driver of trait variation, (2) are life-cycle trait trajectories different between trees (3) what are the implications of (1) and (2) for canopy nutrient budgets. To address these questions, we measured the morphological, nutrient, and isotopic changes of 1099 top-of-canopy sunlit leaves of different ages from 12 individual canopy trees (different species), in a lowland Amazonian tropical forest in southern Peru. These trees represented a wide variety of leaf types (e.g. compound and simple leaves), leaf sizes, shapes, leaf thickness and waxiness. This sampling allowed us to:

1. Quantify how much leaf trait variation is generated by leaf age in a tropical canopy tree community with diverse leaf properties.
2. Assess if variation in morphological and biochemical traits throughout the leaf life-cycle shows similar patterns within and across canopy trees.
3. Measure how leaf nutrient concentrations and budgets change during the leaf life-cycle.

4. Analyse how nutrient stocks moved in and out of leaves through their life-cycle, and the impact traditional leaf sampling and mass- and area-based nutrient estimates can have on resorption studies in tropical trees.

This paper presents a detailed assessment of the relationships of individual morphological and biochemical leaf traits with age, and of the combined LES trait trajectories and nutrient budgets across the leaf life-cycle of individual trees. It is complemented by a companion paper also in this special section, (Chavana-Bryant *et al.*, 2016) in which we examine the efficiency of hyperspectral leaf reflectance as an efficient and accurate tool to monitor leaf age by using a spectra-based partial least squares regression (PLSR) model to predict age. Chavana-Bryant *et al.* (2016) is focused on the possibility of remotely sensing leaf age, and therefore necessarily on the relationship between reflectance characteristics and leaf traits. In contrast, this paper examines in detail the nature and causes of variation of morphological and biochemical leaf properties throughout the leaf life-cycle.

4.2 Materials and Methods

4.2.1 Field site

Data were collected in and around the borders of two lowland tropical evergreen rainforest plots located within the Tambopata National Reserve in the Madre de Dios region of Peru in southwestern Amazonia (Chavana-Bryant *et al.*, 2016).

The two study sites are part of the RAINFOR Amazon Forest Inventory Network (2002b) and GEM intensive monitoring network, with RAINFOR codes TAM-06 (12°50'24"S, 69°17'59"W) and TAM-09 (12°49'48"S, 69°16'48"W). The sites are located between 215m and 220m above sea level within lowland closed-canopy primary tropical evergreen forest growing on Haplic alisol soils (Quesada et al., 2010). They are fairly seasonal sites with a four to five month-long dry season (number of months with <100 mm rainfall) between June and October, mean annual rainfall of 1900 mm (this may be lower than the long-term mean due to droughts in 2005 and 2010 (Lewis et al., 2011) and mean annual air temperature of 24.4°C. A detailed description of the geomorphology, climate and forest carbon cycle of this site is available in Malhi et al. (2014).

4.2.2 Tree sampling strategy

The tree sampling for this study focused on canopy and emergent trees (**Table 4.2.2.1**). Monitoring such trees in situ presents considerable challenges. Canopy walkways and towers, where they exist, do not provide sufficient access across a large number of crowns. Canopy cranes are ideal for such sampling, but there are only a few across the tropics. Hence, we resorted to repeated climbing and sampling of a number of large trees. The total number of trees sampled in this study was limited by the need to sample structurally sound trees large enough to withstand the repeated climbing required to monitor leaf life-cycle (we use this term to refer to all the leaf ages measured in this study, from early leaf expansion

to senescence) and sample leaves of different ages, and the difficulty involved in climbing large trees with ropes to minimise damage caused by repeated climbing. We therefore, chose to maximise variability within our leaf dataset by sampling 12 trees, each of a different species, representing a wide variety of leaf types (e.g. compound and simple leaves), leaf sizes and shapes, and leaf thickness and waxiness. This sampling allowed us to examine the variability due to natural leaf ageing of a range of leaf types, sizes, shapes, thickness and waxiness within individual trees and across a community of canopy trees with diverse leaf properties. Due of our lack of replication within species, we are not able to draw general conclusions about trait variation during the leaf life-cycle of particular species. We are however, able to explore relationships (1) between leaf traits and leaf age within individual trees along their leaf life-cycle, and, by examining across all 12 trees, (2) explore if there is evidence of general relationships or developmental pathways for leaf traits along the leaf life-cycle.

According to the “Crown Illumination Index” (Clark & Clark, 1992; Keeling & Phillips, 2007), eight of our sampled trees were canopy trees and four were emergent trees. Diameter at breast height (DBH) among sampled trees averaged 171 cm (range: 117-237 cm). The sampled trees averaged 32 m in height (range: 29-35 m), compared to a mean tree height of 27 ± 8 m ($\pm$ SE) for canopy trees $\geq$ 40cm DBH in local plots. Canopy depth, the difference between tree height and

height at the lowest branch of the canopy, among our sampled trees averaged 9.6 m (range: 6.3-15.1 m).

Trees were monitored for bud development, new leaf emergence, general leaf condition and signs of leaf senescence. During developmental leaf phases (see Y1, Y2, Y3 and Y/M in **Figure 4.2.4.1**), individual trees were sampled on a weekly basis for up to four consecutive weeks. During the mature leaf phase, individual trees were sampled for up to three months, while during the senescent leaf phase, individual trees were sampled for up to two months for old leaves and up to three months for senescent leaves (see “leaf age classification” section below).

Table 4.2.2.1 Species and structural information of trees sampled for leaf morphological and biochemical traits. Table also published in Chavana-Bryant *et al.* (2016).

Tree code	Species	Family	DBH (m)	Tree canopy position	Height (m)	Canopy depth (m)
1	<i>Licania brittoniana</i> Fritsch	Chrysobalanaceae	1.88	Canopy	29.50	10.50
3	<i>Simarouba amara</i> Aubl.	Simaroubaceae	1.62	Canopy	29.00	8.35
4	<i>Ruizodendron ovale</i> R.E. Fr. (Ruiz & Pav.)	Annonaceae	1.23	Canopy	32.62	10.07
5	<i>Aspidosperma parvifolium</i> A. DC.	Apocynaceae	1.57	Emergent	33.70	8.40
6	<i>Couratari macrosperma</i> A.C. Sm.	Lecythidaceae	1.43	Emergent	33.74	6.27
7	<i>Licania longistyla</i> (Hook. F.) Fritsch	Chrysobalanaceae	1.43	Canopy	30.30	8.70
8	<i>Bertholletia excelsa</i> Bonpl.	Lecythidaceae	2.30	Emergent	34.00	11.60
9	<i>Tachigali chrysaloidea</i> van der Werff	Caesalpiniaceae	2.10	Canopy	29.70	7.60
10	<i>Clarisia racemosa</i> Ruiz & Pav.	Moraceae	1.58	Canopy	33.75	9.25
11	<i>Eschweilera coriacea</i> (DC.) S.A. Mori	Lecythidaceae	1.88	Emergent	33.32	7.78
12	<i>Gautteria boliviana</i> H. Winkl.	Annonaceae	1.17	Canopy	31.90	11.34
13	<i>Pouteria franciscana</i> Baehni	Sapotaceae	2.37	Canopy	35.39	15.09

DBH, diameter at breast height

4.2.3 Branch sampling

Two top-of-canopy (fully exposed to direct sunlight) apical metre-long branches were collected each time trees were sampled. Sampled branches were cut, labelled, promptly lowered to the ground and recut under water to maintain hydraulic connectivity and minimise leaf desiccation during transport to the field lab, where leaf sampling and morphological (fresh leaf weight and area) measurements were carried out within two hours of the branches being cut. Demographic leaf counts (number of leaves from each leaf age group present) were also collected for all sampled branches.

4.2.4 Leaf age classification

We sampled the developmental, mature and senescent leaf stages (the detailed leaf age classification used in this study can be found in **Figure 4.2.4.1** and images illustrating the leaf life-cycle of some of our sampled trees in **Figure S 4.8.5**) for eight trees and also sampled some leaf ages from four other trees (see **Table S 4.8.1**). This tree sampling allowed us to build a well-balanced leaf phenological dataset in which developing leaves were sampled after 1, 2, 3 and 4 weeks of active expansion; mature leaves were sampled at different stages (newly matured, mid and end of maturity); and senescent leaves were also sampled at different stages (i.e. old leaves not yet abscising and senescent

leaves starting abscission through to advanced senescence when most leaves had been abscised from the tree crown).

Based on phenological observations of our trees and the pronounced dry season peaks in leaf flush and leaf fall at our site (Malhi *et al.*, 2014), we assigned a 13-month (57 weeks) leaf lifecycle for all trees, which allows for 7 weeks of active leaf expansion, 6.5 months of maturity, 2 months of old age and 3 months of senescence. This 13-month leaf lifecycle is similar to the mean leaf lifespan of 1.6 year found by Reich *et al.* (2004) for well-lit trees in an Amazonian *terra firme* mature forest canopy, although that study was based on a forest in a less seasonal climate and growing on poorer soils, where longer leaf lifespans can be expected.

Leaf developmental phase ending in maturity				
		Age codes	Age (weeks)	Leaf description
		Y1	1	Young1 – recently emerged leaf, collected once expansive growth has been active for ~ one week. Leaf very fragile and thin, small in size, has no rigidity (may fold when held up), its colour may be yellow or light yellow/green.
		Y2	2	Young2 – leaf still fragile and thin, hasn't achieved full size, has some rigidity (will not fold if held up), its colour is now definitely light green.
		Y3	3	Young3 – leaf not fragile anymore, but still thin or close to achieving full thickness and rigidity, has almost achieved full size or has achieved full size, its colour is now darker light green.
		M	7-35	Mature – fully-grown and structurally developed leaf that has achieved a fully darkened green colour. Leaves in this age class are in good condition and show no signs of epiphylls or senescence. Newly matured, mid and end of maturity leaves are included in this age class. Up to three months were sampled for individual trees.
Leaf senescent phase ending in abscission				
Leaf in best condition	Leaf in worst condition	Age codes	Age (weeks)	Leaf description
		O	36-44	Old – leaf near the end of its life cycle (~ 2 months before senescence and abscission); distinguished from previous age class by darker (shade leaf) and/or yellowing (sun leaf) pigment, and by initial signs of epiphylls and/or senescence. Up to two consecutive months were sampled for individual trees.
		S	45-57	Senescent – leaf in the process of dying and abscising; distinguished by loss of chlorophyll and/or onset of decomposition. Senescent leaves starting to abscise through to advanced senescence (most canopy leaves abscised) are included in this age class. Up to three consecutive months were sampled for individual trees.

Figure 4.2.4.1 Leaf life-cycle of *Bertholletia excelsa* illustrating the leaf age classification used in this study (adapted from Lowman & Box, 1983). The Young/Mature (Y/M) leaf age class is not included as it was sampled only for two trees for which Y3 leaves still displayed a light green colouration. When sampled, Y/M leaves were close to maturity, had achieved full size, full thickness and rigidity but their green colour was not yet fully darkened. Leaf age for developmental phase (one to four weeks) to maturity (seven weeks) was determined from witnessing leaf emergence. If leaf emergence was not observed, leaf age was determined based on leaf condition for mature and old leaves. For senescent leaves, an additional classification criteria was that extensive leaf abscission was observed within the canopy of the sampling tree; sampled leaves for this leaf age were those easily abscised with branch movement. Colour images (not to scale) show how leaves from 3 weeks old to senescent age (see leaves in best condition for both old and senescent leaves) can be easily mis-sampled (see also **Figure S 4.8.5**). Leaf age classification also published in Chavana-Bryant *et al.* (2016).

4.2.5 Leaf traits

We measured morphological traits such as leaf mass per area (LMA) and traits related to expansive (leaf area; LA and leaf thickness; LT) and structural (leaf mass; LM and leaf tissue density; LTD) leaf growth. For biochemical traits, we measured leaf water content (LWC), nitrogen, phosphorus and carbon content on both a mass (N_{mass} , P_{mass} and C_{mass}) and area (N_{area} , P_{area} and C_{area}) basis and stable isotope content ($\delta^{13}\text{C}$). A detailed description of the leaf traits and methods used in leaf trait measurements are provided in **Methods S 4.8.1**.

4.2.6 Statistical analyses

We used a subset of 184 tree species from the GLOPNET dataset (Wright et al., 2004) to put our P, N and LMA data into a pantropical context and to explore the leaf life-cycle resource use economy of our individual trees (GLOPNET biome codes = TROP RF and TROP FOR; growth form code = T). SMA slopes calculated on $\log_{10}$ -transformed variables were used to examine the scaling relationship between LA and LM.

To explore patterns in trait variation with age across individual trees, regression analysis was used a subset of eight trees with five or more sampled leaf age classes. We used the Akaike information criterion (AIC) and R^2 value to identify each best-fit regression model. Tukey-Kramer HSD multiple comparison post-hoc tests were used to assess differences among mean trait values from different leaf

age classes within each tree. Analysis of covariance (ANCOVA) was used to determine if trait-age relationships were significantly different between individual trees when appropriate. Trait-age relationships for individual trees were found to be significantly different (idiosyncratic) in all cases. Community trait-age relationships we report were, therefore, calculated based on mean responses for all 12 sampled trees.

As per advise in van Heerwaarden *et al.* (2003) and due to the large tree-specific age-dependent mass and area variations we observed among individual trees, we used total nutrient leaf stocks to analyse how nutrient stocks moved in and out of leaves through their life-cycle. We first calculated mean total nutrient (P and N) stocks for the youngest sampled leaves (Y1), mature (M) and senescent (S) leaves for the subset of eight trees with five or more sampled leaf age classes and then calculated nutrient stock change (%) between consecutive leaf ages (i.e. Y1 vs M and M vs S, the latter traditionally denominated “resorption efficiency”). We also report (1) changes in mean area and mass, and (2) measured resorption efficiency (MRE) on a mass basis as traditionally calculated in resorption studies for comparison purposes.

Statistical analyses were performed with JMP Pro version 10.0.2

(<http://www.jmp.com>) except for SMA regressions, which were performed using

the SMATR package in R (RStudio Version 0.98.1028). All statistical tests were considered to be significant at $P < 0.05$.

4.3 Results

4.3.1 Variation in leaf traits with age

We found large variation in morphological and physiochemical traits throughout the life-cycle of our sampled trees (**Table 4.3.1.1**). Among morphological traits, LM displayed the highest variation with a 3.3 to 11-fold difference across individual trees, followed by LA which varied 2.7 to 4.5-fold, LMA which varied 1.5 to 3.3-fold and LT and LTD which both varied 1.5 to 2.5-fold. Among physiochemical traits, P_{mass} had the highest variation of 1.8 to 5.8-fold across individual trees, followed by N_{area} and C_{area} which varied 1.6 to 5.6 and 1.5 to 4-fold, respectively. N_{mass} and P_{area} had lower variations of 1.4 to 2.9 and 1.6 to 2.9-fold, respectively, with C_{mass} displaying the least variation among chemical leaf traits of 1.1 to 1.5-fold. Finally, LWC varied 1.3 to 2-fold and $\delta^{13}\text{C}$ varied 0.9-fold. Furthermore, the age-related coordinated variation in LES traits P_{mass} , P_{area} , N_{mass} and N_{area} and LMA among our 12 sampled trees resulted in our dataset roughly covering the entire GLOPNET interspecific variation for pantropical trees (**Figure 4.3.1.1** and **Figure S 4.8.1**).

Table 4.3.1.1 Summary statistics for morphological and biochemical traits by leaf age class (Y/M leaf age not included as only two trees were sampled for this age class). M = mean, SD = standard deviation and Range = minimum and maximum values observed for each leaf age and N = number of samples. Data for LWC, LMA, P_{mass} , N_{mass} and C_{mass} also shown in Chavana-Bryant *et al.* (2016).

Leaf age class	Leaf morphological traits						Leaf biochemical traits						
		Area (cm ²)	Mass (g)	Thickness (mm)	Tissue density (mg cm ⁻³)	LMA (g m ⁻²)	LWC (%)	P _{mass} (%)	N _{mass} (%)	C _{mass} (%)	P _{area} (mg cm ⁻²)	N _{area} (mg cm ⁻²)	C _{area} (mg cm ⁻²)
Y1	M (SD)	52.00 (55.22)	0.35 (0.34)	0.19 (0.07)	379 (68)	72.8 (25.1)	71 (5)	0.23 (0.08)	2.66 (1.03)	50.22 (2.29)	0.016 (0.006)	0.19 (0.08)	3.73 (1.33)
	Range	13.14 – 240.23	0.07 – 1.52	0.13 – 0.40	229 – 543	34.6 – 135.5	60 – 81	0.11 – 0.40	0.85 – 4.19	46.74 – 55.13	0.007 – 0.036	0.05 – 0.33	1.65 – 7.05
	N	121	121	121	121	121	121	40	40	40	40	40	40
Y2	M (SD)	63.58 (56.34)	0.51 (0.44)	0.22 (0.05)	384 (71)	85.4 (34.9)	68 (6)	0.22 (0.06)	2.77 (0.82)	49.66 (2.16)	0.017 (0.004)	0.22 (0.07)	4.28 (1.79)
	Range	21.88 – 263.76	0.16 – 2.11	0.15 – 0.37	221 – 567	37.6 – 187.4	55 – 79	0.10 – 0.31	1.46 – 4.08	45.46 – 53.60	0.011 – 0.032	0.12 – 0.36	2.03 – 8.82
	N	110	110	110	110	110	110	35	35	35	35	35	35
Y3	M (SD)	65.17 (55.46)	0.62 (0.55)	0.24 (0.06)	409 (47)	96.9 (26.4)	63 (5)	0.18 (0.06)	2.55 (0.69)	49.12 (1.60)	0.016 (0.003)	0.24 (0.06)	4.74 (1.33)
	Range	22.37 – 264.12	0.19 – 2.36	0.15 – 0.39	315 – 526	47.4 – 166.1	51 – 73	0.08 – 0.33	1.45 – 3.69	45.28 – 52.87	0.012 – 0.023	0.14 – 0.35	2.49 – 7.57
	N	110	110	110	110	110	110	35	35	35	35	35	35
M	M (SD)	57.08 (42.80)	0.68 (0.48)	0.25 (0.04)	481 (50)	122.5 (26.3)	53 (5)	0.11 (0.03)	2.19 (0.54)	50.33 (3.95)	0.013 (0.003)	0.27 (0.07)	6.22 (1.61)
	Range	22.32 – 265.21	0.25 – 3.20	0.19 – 0.40	368 – 607	76.1 – 199.7	44 – 62	0.06 – 0.19	1.40 – 3.68	45.04 – 59.72	0.007 – 0.022	0.14 – 0.43	3.62 – 10.02
	N	230	2230	2230	230	230	230	65	65	65	65	65	65
O	M (SD)	57.70 (41.83)	0.82 (0.66)	0.27 (0.07)	528 (81)	138.7 (28.3)	48 (5)	0.11 (0.04)	2.14 (0.34)	51.73 (4.58)	0.014 (0.004)	0.29 (0.04)	7.29 (1.67)
	Range	20.33 – 208.14	0.20 – 3.20	0.15 – 0.43	384 – 792	90.4 – 202.7	38 – 60	0.05 – 0.18	1.42 – 2.93	38.92 – 59.47	0.008 – 0.021	0.23 – 0.43	4.07 – 10.71
	N	150	150	150	150	150	150	50	50	50	50	50	50
S	M (SD)	58.37 (35.39)	0.81 (0.57)	0.27 (0.07)	511 (78)	137.0 (32.2)	48 (5)	0.11 (0.04)	2.16 (0.47)	50.65 (4.51)	0.014 (0.005)	0.29 (0.08)	7.24 (1.82)
	Range	19.32 – 214.58	0.21 – 3.42	0.18 – 0.45	377 – 725	79.4 – 210.5	34 – 58	0.04 – 0.18	1.07 – 2.89	35.83 – 57.39	0.007 – 0.029	0.15 – 0.51	4.11 – 10.86
	N	321	321	321	321	321	321	80	80	80	80	80	80
ALL	M (SD)	58.30 (44.76)	0.68 (0.55)	0.25 (0.07)	467 (88)	116.45 (37.73)	56 (10)	0.15 (0.07)	2.37 (0.68)	50.34 (3.72)	0.015 (0.005)	0.26 (0.08)	5.84 (2.10)
	Range	13.14 – 265.21	0.07 – 3.42	0.13 – 0.45	221 – 792	34.60 – 210.5	34 – 81	0.04 – 0.40	0.85 – 4.19	35.83 – 59.72	0.007 – 0.036	0.05 – 0.51	1.65 – 10.86
	N	1072	1072	1072	1072	10572	1072	315	315	315	315	315	315

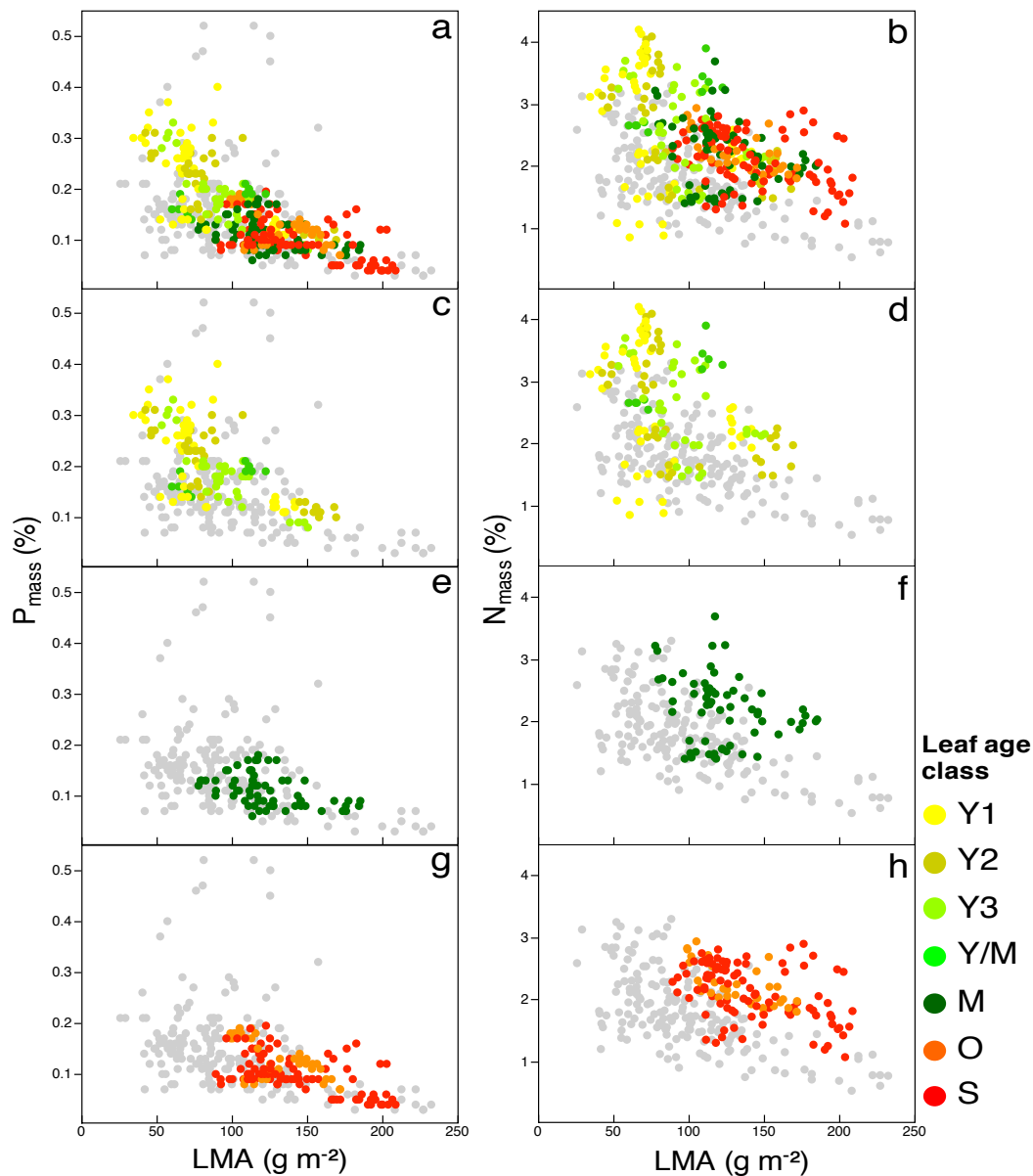


Figure 4.3.1.1 Variation in the combined leaf economic spectrum (LES) traits P_{mass} -LMA and N_{mass} -LMA among sun leaves of different ages from our 12 sampled trees (colour coded data points) versus the GLOPNET dataset of tree species (grey data points) (Wright et al., 2004). Individual GLOPNET data points represent mean traits from mature leaves of 184 pantropical tree species, while data points from our phenological dataset represent traits from 320 individual leaves of different ages from 12 lowland Amazonian tropical canopy and emergent trees. Graphs “a” and “b” present leaves of all ages versus the GLOPNET dataset, “c” and “d” show only developmental (Y1, Y2, Y3 and Y/M) leaves, “e” and “f” show only mature leaves and “g” and “h” show senescent leaves.

4.3.2 Age effects on physiochemical traits relationships within and across individual trees

Except for LA, all morphological traits displayed highly significant positive trait-age relationships within and across individual trees (**Figure 4.3.2.1**). LMA displayed the strongest relationship; leaf age explained 62-94% of the variation in LMA within individual trees and 40% across the tree community. LTD displayed the second strongest relationship; leaf age explained 17-90% of the variation in LTD within individual trees and 40% of the variation across the community. A divergent trait-age relationship was found for LA in *Bertholletia excelsa* (see **Figure 4.3.2.1a** and **Table 4.3.2.1**) as only leaves with significantly smaller LA ($p = <0.0001$) survived into old and senescent ages, which resulted in a negative relationship with age for this tree.

Table 4.3.2.1 Summary of trait-age relationships between morphological and physiochemical leaf traits (on both a mass and area basis) and leaf age (weeks). Only the subsample of eight trees with $\geq$ four leaf ages are included in regression analyses. The AIC method was used to define the best-fit regression model for trait-age relationships for each individual tree.

Species (code)	Leaf morphological traits						Leaf biochemical traits								
	N	Area (cm ²)	Mass (g)	Thickness (mm)	Tissue density (mg cm ⁻³)	LMA (g m ⁻²)	LWC (%)	N	δ ¹³ C (‰)	P _{mass} (%)	N _{mass} (%)	C _{mass} (%)	P _{area} (mg cm ⁻²)	N _{area} (mg cm ⁻²)	C _{area} (mg cm ⁻²)
<i>Licania brittoniana</i> (1)	91	- r ² =0.006	↑ r ² =0.23	↑ r ² =0.66	↑ r ² =0.35	↑ r ² =0.67	↓ r ² =0.68	25	↑ r ² =0.82	↓ r ² =0.72	↓ r ² =0.33	↑ r ² =0.22	↓ r ² =0.58	↑ r ² =0.38	↑ r ² =0.66
<i>Simarouba amara</i> (3)	150	↑ r ² =0.32	↑ r ² =0.55	↑ r ² =0.50	↑ r ² =0.26	↑ r ² =0.67	↓ r ² =0.90	50	↑ r ² =0.70	↓ r ² =0.93	↓ r ² =0.65	- r ² =0.002	↓ r ² =0.78	- r ² =0.005	↑ r ² =0.77
<i>Ruizodendron ovale</i> (4)	96	↑ r ² =0.48	↑ r ² =0.82	↑ r ² =0.76	↑ r ² =0.65	↑ r ² =0.83	↓ r ² =0.92	30	↑ r ² =0.56	↓ r ² =0.72	↓ r ² =0.65	↓ r ² =0.38	↓ r ² =0.42	↑ r ² =0.42	↑ r ² =0.87
<i>Couratari macrosperma</i> (6)	113	↑ r ² =0.11	↑ r ² =0.60	↑ r ² =0.73	↑ r ² =0.90	↑ r ² =0.90	↓ r ² =0.84	35	↑ r ² =0.92	↓ r ² =0.89	↓ r ² =0.71	↑ r ² =0.84	↑ r ² =0.19	↑ r ² =0.70	↑ r ² =0.91
<i>Bertholletia excelsa</i> (8)	125	↓ r ² =0.20	↑ r ² =0.25	↑ r ² =0.90	↑ r ² =0.62	↑ r ² =0.94	↓ r ² =0.87	40	↑ r ² =0.91	↓ r ² =0.76	↓ r ² =0.82	↑ r ² =0.19	- r ² =0.01	↑ r ² =0.17	↑ r ² =0.89
<i>Clarisia racemosa</i> (10)	68	↑ r ² =0.62	↑ r ² =0.80	↑ r ² =0.50	↑ r ² =0.64	↑ r ² =0.77	↓ r ² =0.83	20	↓ r ² =0.52	↓ r ² =0.79	↑ r ² =0.87	- r ² =0.07	↑ r ² =0.31	↑ r ² =0.85	↑ r ² =0.73
<i>Guatteria boliviana</i> (12)	82	↑ r ² =0.50	↑ r ² =0.70	↑ r ² =0.56	↑ r ² =0.60	↑ r ² =0.70	↓ r ² =0.85	25	↑ r ² =0.64	↓ r ² =0.76	↑ r ² =0.63	↓ r ² =0.85	- r ² =0.001	↑ r ² =0.90	↑ r ² =0.78
<i>Pouteria franciscana</i> (13)	12	↑ r ² =0.20	↑ r ² =0.73	↑ r ² =0.15	↑ r ² =0.60	↑ r ² =0.85	↓ r ² =0.94	35	↑ r ² =0.77	↓ r ² =0.93	↓ r ² =0.82	↑ r ² =0.66	↓ r ² =0.52	↑ r ² =0.17	↑ r ² =0.89
Community	1073	- r ² =0.009	↑ r ² =0.17	↑ r ² =0.16	↑ r ² =0.41	↑ r ² =0.43	↓ r ² =0.73	260	↑ r ² =0.22	↓ r ² =0.44	↓ r ² =0.13	↑ r ² =0.07	↓ r ² =0.05	↑ r ² =0.18	↑ r ² =0.42

Legend

- ↑ = positive relationship with $P < 0.0001$
- ↓ = negative relationship with $P < 0.0001$
- ↑ = positive relationship with $0.001 < P < 0.05$
- ↓ = negative relationship with $0.001 < P < 0.05$
- = non-significant relationship

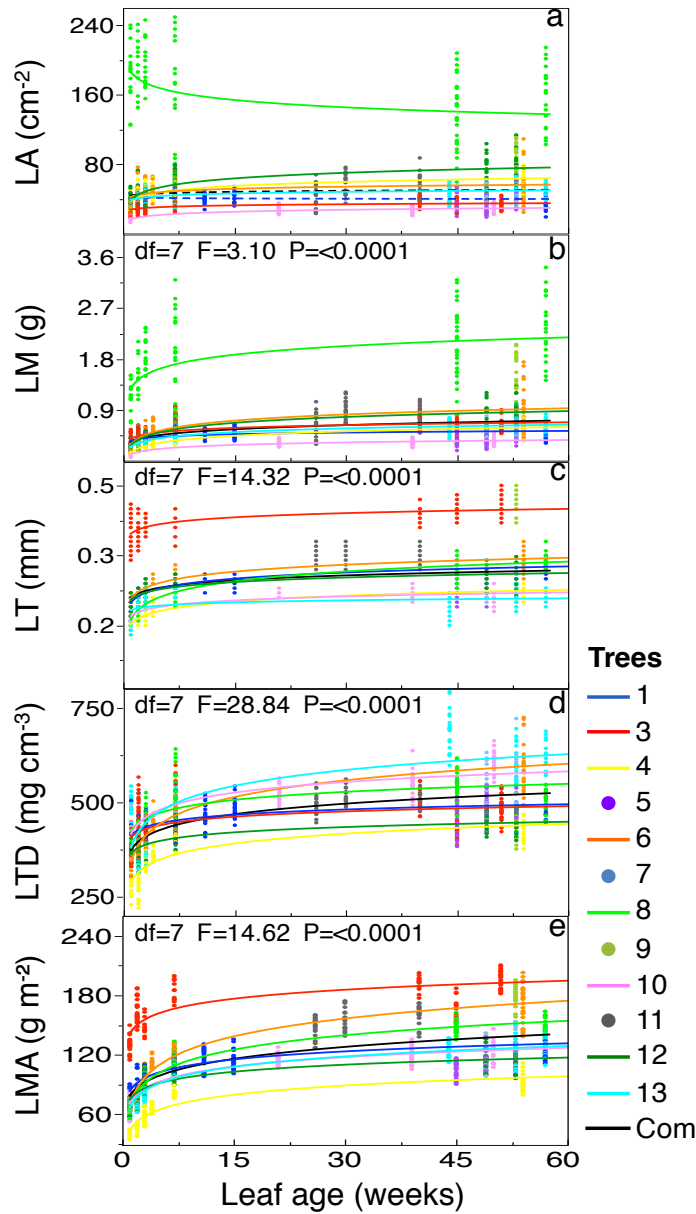


Figure 4.3.2.1 Relationships between morphological leaf traits and leaf age. Regressions were fitted as a function of log-transformed age with axes showing linear age values. Colour lines represent individual tree regressions (performed only for trees with four or more sampled leaf ages) and black lines represent community regressions (12 trees included; Com). Detailed regression results can be found in **Table 4.3.2.1**. Individual leaf trait values are used as data points. Dashed lines are non-significant regressions. All significant regression $P < 0.001$. When appropriate, summary of tests for homogeneity of slopes (ANCOVA) across trees are provided for trait-age relationships, df= degrees of freedom, F= test result and P value. LMA data also published in Chavana-Bryant *et al.* (2016).

We observed significantly higher mean LMA in old and/or senescent leaves compared to mature leaves across all individual trees ($p = <0.0001$ for most individual trees). This significant increase was the result of continued structural growth (i.e. increase in LM) throughout the life-cycle of individual trees while most leaf expansive growth (i.e. LA) was completed during the third week of active leaf expansion (isometric slopes = 1.10-1.96 and $R^2 = 0.65-0.91$ across individual trees; **Figure S 4.8.2**).

Diverse patterns emerged for physiochemical leaf trait-age relationships (**Figure 4.3.2.2** and **Table 4.3.2.1**). In general, physiochemical leaf traits relationships were stronger (more significant and with a greater R^2) across individual trees on a mass basis than on an area basis, except for leaf C_{mass} . LWC consistently displayed the strongest trait-age relationship among physiochemical and all traits analysed (**Table 4.3.2.1**), followed closely by leaf P_{mass} and C_{area} . Leaf age explained 68-93% of the variation in LWC, 60-93% of the variation in P_{mass} , and 65-91% of the variation in C_{area} across individual trees.

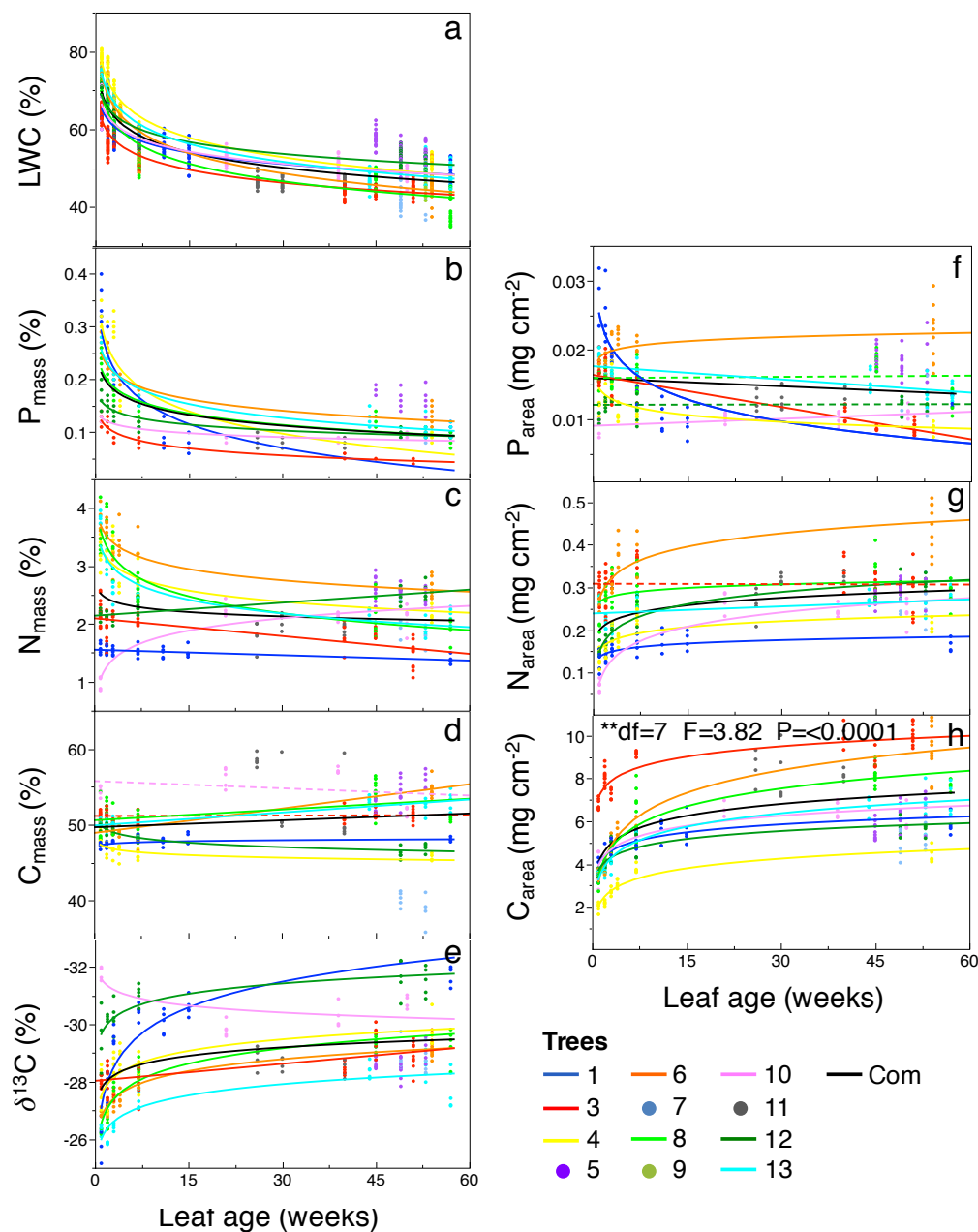


Figure 4.3.2.2 Relationships between physiochemical leaf traits and leaf age (weeks). Colour lines represent individual tree regressions (performed only for trees with four or more sampled leaf ages) and black lines represent community regressions (12 trees included; Com). Detailed regression results can be found in **Table 4.3.2.1**. Individual leaf trait values are used as data points. Dashed lines are non-significant regressions. All significant regression $P < 0.001$. When appropriate, summary of tests for homogeneity of slopes (ANCOVA) across trees are provided for trait-age relationships, df= degrees of freedom, F= test result and P value. LWC, P_{mass} , N_{mass} and C_{mass} data also published in Chavana-Bryant *et al.* (2016).

LWC decreased monotonically with age across all individual trees (**Figure 4.3.2.2a** and **Table 4.3.2.1**), however, regression slopes were found to be heterogeneous for this and all other trait-age relationships (**Figure 4.3.2.1** and **Figure 4.3.2.2**) analysed. Leaf $\delta^{13}\text{C}$ displayed tree-specific trait-age relationships, it increased both linearly and logarithmically with age within and across all trees, except for *C. racemosa* (**Figure 4.3.2.2e**).

P_{mass} showed monotonic decrease with age across all individual trees, while P_{area} displayed tree-specific relationships with age (**Figure 4.3.2.2b** and **f** and **Table 4.3.2.1**). P_{area} increased or decreased either linearly or logarithmically or remained constant with age. At the community level, trait-age relationships for P_{mass} and P_{area} were negative but had different significance ($R^2=0.44$ and $R^2=0.05$, respectively).

N_{mass} showed tree-specific relationships with age, it increased or decreased either linearly or logarithmically with age (**Figure 4.3.2.2c** and **g** and **Table 4.3.2.1**). N_{area} increased logarithmically (6 trees) or linearly (1 tree) or remained stable (1 tree) with age. Across the tree community, N_{mass} decreased logarithmically ($R^2=0.19$) and N_{area} increased logarithmically ($R^2=0.10$).

C_{mass} displayed diverse trait-age relationships, it increased linearly (4 trees), remained stable (2 trees) or decreased logarithmically (2 trees) with age. C_{area}

increased logarithmically with age across all individual trees and the community (**Figure 4.3.2.2d** and **h** and **Table 4.3.2.1**).

4.3.3 Coordinated trait variation with age for individual trees

We analysed the age-related coordinated variation in LES traits P_{mass} , P_{area} , N_{mass} , N_{area} and LMA for the subset of eight trees with four or more sampled leaf age classes in reference to the GLOPNET interspecific variation for pantropical trees (**Figure 4.3.3.1** and **Figure S 4.8.4**). We found the patterns in the combined leaf life-cycle trajectories of these key LES traits indicated that (1) individual trees appear to have different life-cycle functional strategies, (2) individual trees can cover a surprising amount of interspecific variation in LES traits through their leaf life-cycle, and (3) the different LES traits are unevenly variable both within individual trees and across the leaf life-cycles of these trees.

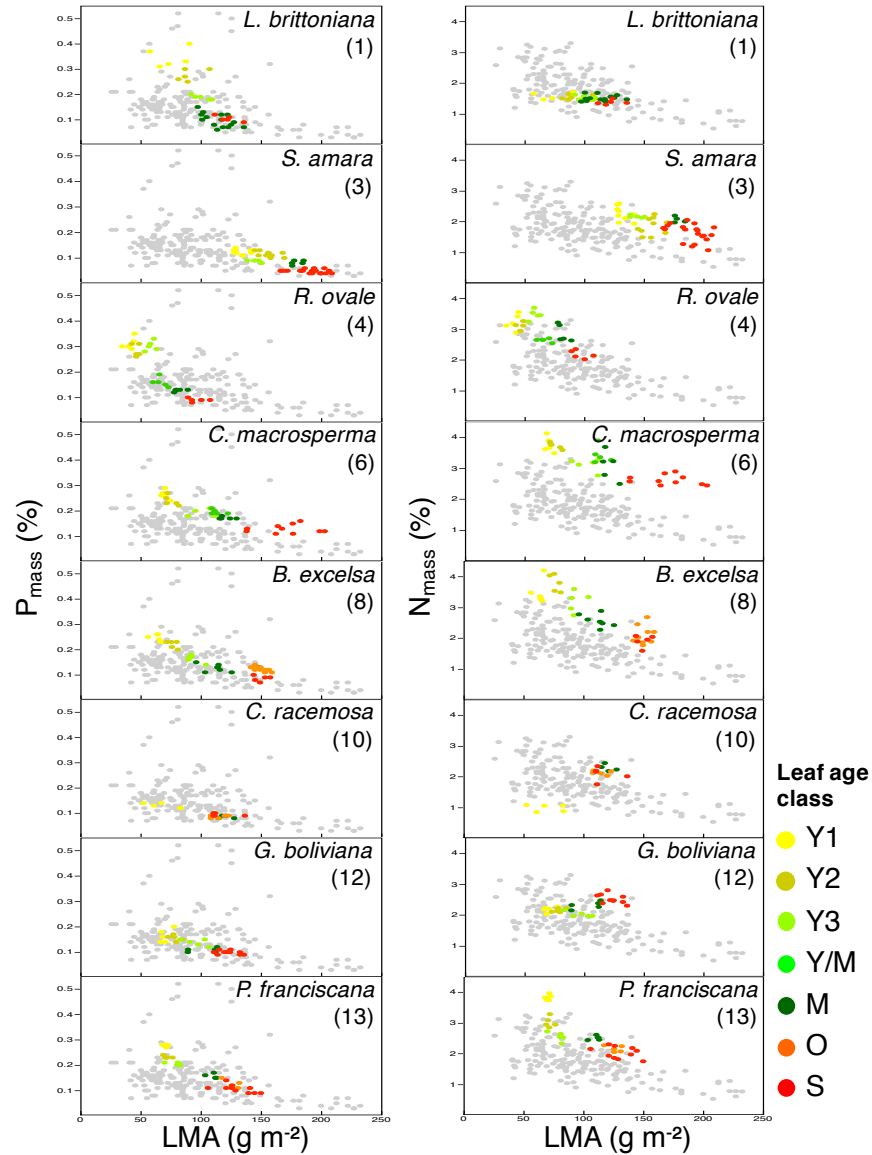


Figure 4.3.3.1 Combined life-cycle trajectories in leaf economic spectrum (LES) traits P_{mass} –LMA and N_{mass} –LMA from eight of our sampled trees (data points colour coded by leaf age) versus the GLOPNET dataset of tropical tree species (grey data points) (Wright *et al.*, 2004). Individual GLOPNET data points represent mean traits from mature leaves of 184 pantropical tree species, while data points from our phenological dataset represent traits from individual leaves of different ages from eight lowland Amazonian tropical canopy and emergent trees. Only trees (tree codes in brackets) with four or more sampled leaf ages are included in these graphs. Leaf age classification: Y1, Y2, Y3 and Y/M are developing leaves collected after 1, 2, 3 and 4 weeks of active leaf expansion, respectively; M are mature leaves; O are old leaves showing initial signs of senescence; S are senescent leaves in the process of dying and abscising.

4.3.4 Leaf life-cycle changes in morphological traits (LM and LA) and nutrient (P and N) stocks for individual trees

Mean morphological and nutrient stock changes (%) between early developing leaves (Y1) and mature leaves (M) followed expected patterns (increase with age) with dilution effects (decrease with age) clearly observed for trees 1, 8 and 13 for P and also for N in tree 8 (**Table 4.3.4.1a** and **Table 4.3.4.1b**). However, we observed tree-specific changes in both mean morphological traits and nutrient stocks between mature vs senescent leaves (S). Senescent leaves displayed larger mean mass and area than mature leaves in 6 and 5 individual trees, respectively. Mean nutrient stocks were smaller in senescent leaves of 4 individual trees while 4 trees displayed larger nutrient stocks in senescent leaves. We also found substantial tree-specific differences between our nutrient stock change calculations for mature vs senescent leaves from those calculated with traditional MRE (see columns P change vs P MRE and N change vs N MRE in **Table 4.3.4.1b**).

Table 4.3.4.1a Summary of mean total leaf nutrient (phosphorus and nitrogen; P and N) stocks, and mean leaf mass (LM) and area (LA) for early developing (Y1), mature (M) and senescent leaves. We also report mean morphological and nutrient content change (%) between leaf ages (Y1 vs M and M vs S) and measured resorption efficiency (MRE) on a mass basis between M and S leaves (as is most commonly used in traditional resorption studies), equations used in these calculations can be seen in **Figure S 4.8.2**.

Species (tree code)		Mean leaf P content (mg)			Mean leaf N content (mg)			Mean leaf mass (g)			Mean leaf area (cm ²)		
		Y1	M	S	Y1	M	S	Y1	M	S	Y1	M	S
<i>Licania brittoniana</i> (1)	M	0.90	0.50	0.38	4.14	7.65	5.15	268.30	495.30	405.80	35.69	43.75	32.64
	SE	0.15	0.04	0.05	0.71	0.42	0.83	20.77	11.11	28.00	1.75	0.81	1.98
	N	5	15	5	5	15	5	15	60	15	15	60	15
<i>Simarouba amara</i> (3)	M	0.43	0.57	0.26	7.79	14.11	7.79	313.30	662.00	621.30	24.46	35.88	32.11
	SE	0.03	0.06	0.01	0.76	1.25	0.43	16.03	40.18	19.47	1.12	2.02	1.04
	N	5	5	5	5	5	5	15	15	15	15	15	15
<i>Ruizodendron ovale</i> (4)	M	0.46	0.49	0.56	4.74	11.17	13.60	123.80	376.70	580.00	31.09	46.55	63.17
	SE	0.05	0.04	0.03	0.49	1.24	0.58	7.69	15.88	24.74	1.73	2.10	2.83
	N	5	5	5	5	5	5	15	15	20	15	15	20
<i>Couratari macrocarpa</i> (6)	M	0.59	1.24	1.24	8.35	22.18	24.85	202.00	623.50	932.80	29.22	51.89	53.82
	SE	0.02	0.10	0.19	0.32	2.16	3.20	8.00	30.15	78.74	1.02	2.20	3.85
	N	5	5	10	5	5	10	15	20	30	15	20	30
<i>Bertholletia excelsa</i> (8)	M	3.01	1.99	1.73	41.84	40.44	38.65	1147.30	1974.40	2341.50	186.19	202.12	157.92
	SE	0.40	0.33	0.10	4.59	6.39	2.48	65.08	173.25	127.43	8.78	15.42	7.59
	N	5	5	5	5	5	5	15	20	20	15	20	20
<i>Clarisia racemosa</i> (10)	M	0.15	0.31	0.36	1.14	8.14	7.88	116.30	319.50	373.30	16.44	27.66	30.19
	SE	0.02	0.02	0.03	0.23	0.82	0.65	12.38	11.66	13.74	1.12	0.87	0.92
	N	5	5	5	5	5	5	15	20	20	15	20	20
<i>Gautteria boliviana</i> (12)	M	0.46	0.84	0.98	6.07	18.13	25.21	282.50	726.60	874.00	40.00	71.36	77.65
	SE	0.09	0.05	0.07	0.90	1.00	2.07	21.68	22.14	45.19	2.68	1.54	3.36
	N	5	5	5	5	5	5	15	15	20	15	15	20
<i>Pouteria franciscana</i> (13)	M	0.68	0.60	0.74	9.48	9.70	15.32	248.00	478.70	616.70	35.31	43.69	48.05
	SE	0.03	0.04	0.08	0.55	0.58	1.14	5.45	27.17	28.60	0.85	2.45	1.91
	N	5	5	5	5	5	5	15	15	20	15	15	20

Table 4.3.4.1b Summary of mean morphological and nutrient content change (%) between leaf ages (Y1 vs M and M vs S) and measured resorption efficiency (MRE) on a mass basis between M and S leaves (as is most commonly used in traditional resorption studies), equations used in these calculations can be seen in **Figure S 4.8.2**.

Species (tree code)		Mass change (%)	Area change (%)	P change (%)	N change (%)	Mass change (%)	Area change (%)	P change (%)	N change (%)	P MRE (%)	N MRE (%)
		Y1 - M	Y1 - M	Y1 - M	Y1 - M	M - S	M - S	M - S	M - S	M - S	M - S
<i>Licania brittoniana</i> (1)	M	84.61	22.59	-45.00	84.67	-18.07	-25.40	-23.21	-32.68	6.28	17.83
<i>Simarouba amara</i> (3)	M	111.30	46.71	30.86	81.07	-6.15	-10.50	-53.44	-44.80	50.39	41.19
<i>Ruizodendron ovale</i> (4)	M	204.28	49.71	7.30	135.54	53.97	35.71	13.69	21.78	26.16	20.91
<i>Couratari macrosperma</i> (6)	M	208.66	77.58	111.50	165.44	49.61	3.73	-0.47	12.05	33.48	25.10
<i>Bertholletia excelsa</i> (8)	M	72.09	8.56	-33.94	-3.36	18.59	-21.87	-12.68	-4.41	26.37	19.40
<i>Clarisia racemosa</i> (10)	M	174.72	68.24	109.29	615.38	16.84	9.15	15.86	-3.18	0.84	17.14
<i>Gautteria boliviana</i> (12)	M	157.20	78.41	82.25	198.65	20.29	8.82	16.27	39.06	3.34	-15.60
<i>Pouteria franciscana</i> (13)	M	93.02	23.75	-11.65	2.31	28.83	9.98	23.06	57.94	4.48	-22.60

4.4 Discussion

4.4.1 How much leaf trait variation is generated by leaf age in a tropical canopy tree community with diverse leaf properties?

Our leaf samples represent a range of leaf types (e.g. compound and simple leaves), leaf sizes, shapes, thickness and waxiness (Chavana-Bryant *et al.*, 2016). This, together with the variation in leaf traits we recorded throughout the natural (*in situ*) life-cycle of our 12 canopy and emergent tropical trees (**Table 4.3.1.1**) resulted in a dataset that covered large parts of the global leaf trait variability reported in large-scale/global studies such as those of Wright *et al.* (2004), Poorter *et al.* (2009) and Lloyd *et al.* (2010). For example, the age-related variation we found in P_{mass} , P_{area} and N_{mass} and N_{area} with reference to LMA among our 12 sampled trees roughly covered that of the interspecific variation found for 184 pantropical trees in the GLOPNET dataset (**Figure 4.3.1.1** and **Figure S 4.8.1**). Our dataset range of $\delta^{13}\text{C}$ values, -25.8 to -33.2, also matched those reported by Lloyd *et al.* (2010) for 204 rainforest trees growing across the Amazon basin. To put our dataset into a wider perspective, LMA (34.6 - 211g/m²), LWC (34 - 81%), P (0.04 - 0.40%), N (0.85 – 4.19%) and C (36 - 60%) exhibited ranges that cover 53, 89, 50, 67 and 100 %, respectively, of the values reported by Asner *et al.* (2011a) for 6136 humid tropical forest trees covering Australasia, Borneo, the Caribbean and Neotropics, Pacific Islands and Paleotropics. This high variability in leaf traits demonstrates that age can be a significant driver of several important leaf traits currently measured in functional

trait studies. It also raises the possibility that leaf age differences could potentially account for a significant fraction of what we currently understand as intra- and interspecific leaf trait variation.

This last implication becomes particularly relevant when we consider that single-sample field campaigns collect leaves encountered at the time of sampling.

Although the standard protocol is to collect “fully expanded mature sun leaves” (Cornelissen *et al.*, 2003), in practice, a range of leaves of different ages, from leaves close to the end of their full expansive development (see example images for Y3 and M leaves in **Figure S 4.8.5**) to senescent leaves (see “leaf in best condition” example images for Old and Senescent leaves in **Figure 4.2.4.1**) can and do end up being collected. We therefore propose, that leaf trait collection campaigns require additional protocols to better account for leaf age differences and offer the following practical recommendations: (1) only leaves found after the closest growth scar to the end of a branch should be collected (except for species with successive leaf production, this will ensure that leaves have more or less the same age) and (2) to more accurately identify the leaf age class of the sampled leaves, a printed version of **Figure 4.2.4.1** and **Figure S 4.8.5** could be used as field leaf age classification guides and (3) a ranking system used to classify the general leaf age stage of the canopy from which leaves are collected (i.e. 1- leaves from canopies starting their life-cycles if young developing leaves are present in the canopy, 2a- leaves from early to mid-mature canopies if only

fully-developed, healthy-looking leaves are present in the canopy or 2b- leaves from end of maturity to old canopies if fully-developed leaves show signs of ageing but not abscission in the canopy; and 3- leaves from canopies coming to the end of their life-cycle if leaves show signs of senescence, epiphylls and abscission in the canopy).

4.4.2 Leaf age as a significant driver of leaf trait variation within individual trees

Consistent with previous studies in both tropical forests (Kitajima *et al.*, 1997; Kitajima *et al.*, 2002) and non-tropical forests (Field & Mooney, 1983; Escudero & Mediavilla, 2003; Niinemets & Kull, 2003; Niinemets *et al.*, 2004b; Niinemets *et al.*, 2005; Mediavilla *et al.*, 2011), our data show strong age effects for leaf traits at the individual tree level, with these effects more diverse at the community level for chemical traits. Since all trees in this study were sampled in and around the borders of two well-defined, homogeneous sites, environmental conditions such as climate and general soil characteristics can largely be ruled out as driving factors for the observed variation in leaf morphology and biochemistry. Rather, the reported differences appear to reflect innate tree-specific characteristics due to leaf age.

Except for LA, in terms of morphology, our results demonstrate monotonic but idiosyncratic logarithmic increases across all individual trees, with the largest

change in these traits happening during leaf expansion. Much of the morphological trait variation within individual trees was explained by leaf age (**Table 4.3.2.1** and **Figure 4.3.2.1**), with the highest variation explained for LMA both within individual trees ($R^2=0.65-0.95$) and across the community ($R^2=0.43$).

We observed rapid expansive growth (i.e. increase in LA and LT) among the leaves of all our sampled trees. After two weeks of active leaf expansion all trees had achieved 80 to 100 % of the mean LA and 85 to 100% of the mean LT of mature leaves while LM increase (and its two associated leaf traits - LTD and LMA) took 3 to 4 weeks to achieve similar levels of development. These leaf expansion periods are similar to those found for four tropical trees (12, 18 and 25 days) by Cai *et al.* (2005) and (2 weeks) Ogawa *et al.* (1995). Furthermore, fast LA and LT accumulation rates in early developing leaves would be expected to be evolutionarily selected in tropical forests where herbivory pressures by grazing and phytophagous invertebrates are particularly high (Lowman, 1992; Metcalfe *et al.*, 2014).

Turgor changes due to high LWC drove the rapid leaf expansion we observed in early developing leaves (Hsiao *et al.*, 1998; Sobrado, 2008). Across individual trees, leaves at ≤ 1 week of active expansion had a mean LWC of 65-79 % and at 2 weeks of 57-76 % LWC. High LWC in early development was then followed by highly significant and strong patterns of decrease in LWC with age. This resulted

in LWC displaying the highest correlation with leaf age of all traits analysed, with age explaining most of the variation in LWC both within individual trees ($R^2=0.68-0.93$) and across the community ($R^2=0.73$). This high correlation between LWC and age could help us to better understand the effect of age on important leaf physiological variables such as stomatal conductance, transpiration, photosynthesis and respiration which have been found to be intimately related to LWC (Kramer & Boyer, 1995). We also found LWC to be highly correlated to LMA (**Figure S 4.8.3**) with leaves seemingly replacing water with mass as they aged but rarely decreasing below a 38 % LWC.

Continued leaf structural growth (increase in dry matter) was observed throughout the leaf life-cycle (**Figure 4.3.2.1 and Figure S 4.8.2**), and was achieved by remarkably high morphological plasticity displayed throughout the leaf life-cycle of individual trees. For example, in *B. excelsa*, a species with one of the largest leaf sizes among Amazonian canopy trees (mean mature LA = $202.12 \text{ cm}^2 \pm 33.7$), leaves with the largest LA were shed before they became old, leaving behind old and senescent leaves 22% smaller but with significantly higher mean LMA as result of higher mean LTD (11%) and LT (24%) compared to mature leaves. By contrast, in *S. amara*, a species with very thick, waxy and small compound leaflets (mean LT for mature leaflets = $0.45 \text{ mm} \pm 0.03$), old and senescent leaflets displayed significantly higher LMA due to significantly lower mean LT (12%) but significantly higher mean LTD (13%) than mature leaflets. Heavy herbivory pressures on early developing leaves could be the initial driver

of our observed positive LMA trend with age, which then continued to be selected for due to the timing in which new leaves mature in many tropical trees (dry season) and finally reinforced by the higher survival rates of leaves with the highest LMA. The different morphological strategies by which all our individual trees arrived at increased LMA with age appears to be an age-related/temporal mechanism that helps trees to maintain year-round canopies that can withstand the harsh growing conditions (tropical forests have the highest solar radiation and heat load per area due to high solar declination; Mulkey *et al.*, 1996) experienced at the top of the tropical canopy. Furthermore, as LMA is intimately connected to the resource-use economy of individual plants, these different morphological strategies may therefore reflect not only adaptive (to the harsh canopy environment) but also life-cycle competitive and/or coexistence functional strategies that can have important consequences for tropical tree community assembly and ecosystem structure.

To further explore the possibility of different life-cycle functional strategies for individual canopy trees, we examined the coordinated variation of the two other LES traits included in our study -nitrogen (N) and phosphorus (P)- with reference to LMA and mapped them against that of the GLOPNET variation for 184 pantropical trees (**Figure 4.3.3.1** and **Figure S 4.8.4**). We found the patterns in the combined life-cycle trajectories of these key LES traits to be rich in information, with 3 main findings to emphasise: (1) individual trees do appear to have different life-cycle functional strategies, (2) individual trees can cover a

surprising amount of the GLOPNET interspecific variation in LES traits through their life-cycle, and (3) the different LES traits are unevenly variable both within individual trees and across the life-cycles of these trees.

First, the different life-cycle LES traits' combined trajectories for individual trees confirm what we observed in individual trait-age relationships for P and N on both mass and area basis (**Figure 4.3.2.2** and **Table 4.3.2.1**). P_{mass} was the only trait that demonstrated monotonic decreases across all individual trees, while P_{area} , N_{mass} and N_{area} displayed tree-specific relationships with age. In other words, the increase in LMA with age exaggerated decreases in mass-based P content (due to dilution effects) across all trees but not always for N content. In contrast, the increase in LMA with age helped to maintain or increase P and N content across the leaf life-cycle of all trees on an area basis. Individual trees, therefore, displayed different combined ranges in P and N and LMA, which resulted in different life-cycle strategies in nutrient allocations in combination with the increase in LMA with age (**Figure 4.3.3.1** and **Figure S 4.8.4**).

It is also important to note that individual trees covered large parts of the GLOPNET interspecific trait variation in LMA, and P and N content on both a mass and area basis through their life-cycles (**Figure 4.3.3.1** and **Figure S 4.8.4**). Continuing with the same two trees used to exemplify different age-related morphological strategies –*S. amara* and *B. excelsa*–, we find that the life-cycle trait trajectories of these two trees not only cover large parts of the GLOPNET

ranges of interspecific variation in LMA, and P and N contents but they also showed difference in variation for each trait and occupied different parts of the GLOPNET interspecific ranges for these traits. All the above suggests that leaf age is an important axis of variation in LES traits with potentially important implications for tropical tree community assembly and ecosystem structure.

4.4.3 Implications of leaf life-cycle changes in morphological traits (LM and LA) to nutrient (P and N) stocks of individual tropical canopy trees

Although increased N & P stocks in the senescent leaves of four out of eight sampled trees may seem surprising, this was the result of increasing LMA with age modulating leaf nutrient stocks, which allowed for a higher level of diversity in leaf functioning and nutrient resorption physiology than previously reported for tropical canopy trees (**Table 4.3.4.1**). Similar results to those reported here have been reported previously in the literature. For example, increased LMA with age has been reported for sun leaves of seven tropical canopy trees by Kitajima *et al.* (2002), in evergreen trees presenting dry season phenology but growing in cold Mediterranean climates (Escudero & Mediavilla, 2003; Niinemets *et al.*, 2004b; Mediavilla *et al.*, 2011) and in a facultative drought-deciduous Californian chaparral shrub (Field & Mooney, 1983). Furthermore, two of the above studies also reported stable N_{area} due to LMA increase with age (i.e. decrease in N_{mass} was the result of dilution as LMA increased with age and no N resorption) in a

tropical canopy *Cecropia* tree (Kitajima *et al.*, 2002), and also increases in both P_{mass} and N_{mass} with age in four Mediterranean oaks (Niinemets *et al.*, 2004b). However, this is in stark contrast to the widely observed decrease in LMA during senescence in temperate deciduous trees (Wilson *et al.*, 2001).

We would also like to highlight important differences in the leaf sampling used in this study compared to traditional resorption studies: (1) contrary to the great majority of resorption studies which compare nutrient content from mature leaves from sunlit canopy areas with that of senescent leaves collected from litter traps which include both sun and shade leaves, advanced tree climbing techniques allowed us to ensure that only fully sunlit leaves were sampled for all leaf ages compared in this study; (2) sampled senescent leaves in this study were those easily detached from branches collected from senescent canopies already showing significant leaf abscission, therefore, our sampled senescent leaves had not undergone nutrient leaching as might be the case for senescent leaves collected from litter traps, and (3) our leaf phenological monitoring clearly showed that in all our sampled trees, senescent leaves were abscised in different conditions (see examples of collected senescent leaves in best and worst condition in **Figure 4.2.4.1** and also in **Figure S 4.8.5**), thus, our sampling reflected this and did not only include leaves fully devoid of green colouration as is the case in traditional resorption studies. These leaf sampling differences may have had a significant effect on the nutrient stock changes we report between

mature and senescent leaves in this study compared to those of traditional nutrient resorption studies.

We therefore suggest that resorption studies for tropical trees would benefit from leaf sampling that ensures that (1) the condition of collected senescent leaves is representative of the state in which they are abscised from trees, and (2) leaf sampling should try to ensure that senescent sun leaves are compared with mature sun leaves (and/or senescent shade vs mature shade leaves) although in practice this can be challenging for ground-based studies. Furthermore, considering the large variation in both mean mass (-18 to 54%) and area (-25 to 36%) changes between mature and senescent leaves we report across the individual trees in this study, we suggest that resorption studies for tropical trees should be based on total leaf nutrient stocks and not on mass- or area-based measurements. Due to all of the above, average declines in leaf mass previously reported in the literature (21% - van Heerwaarden *et al.*, 2003; 24.2% - Vergutz *et al.*, 2012) and leaf area (11% - van Heerwaarden *et al.*, 2003) are probably not applicable to tropical trees.

Finally, *S. amara* was the only tree that displayed resorption efficiencies similar to those reported in a traditional resorption study of 45 tree neotropical tree species (mean $40 \pm 13\%$ for N MRE and $70 \pm 13\%$ for P MRE based on a lignin-unit basis; Hättenschwiler *et al.*, 2008). *S. amara* displayed evergreen phenological

behaviour by replacing ~50 % of its canopy leaves (through a prolonged period of leaf abscission and flushing that lasted the entire dry season) while all other trees displayed brevi-deciduous phenology by fully exchanging their canopy leaves during the dry season (by initiating leaf flushing when most senescent leaves had been abscised or after 1-4 weeks of leaflessness), this could suggest that nutrient resorption might not be the optimal resource allocation strategy for all trees in a forest system that displays varied leaf phenological behaviours. The large differences in nutrient stocks we observed at the end of the leaf life-cycle of our sampled trees may therefore be another way in which leaf life-cycle nutrient stocks shape tropical tree community assembly and ecosystem structure.

4.5 Conclusions

This study demonstrates that leaf age is a significant driver of variation in leaf morphological and biochemical traits within and across Amazonian canopy trees. The age-related leaf trait variations we report here, have two important implications (1) trait variation due to leaf age within individuals can generate as much variation as interspecific variation and (2) leaf age differences could potentially account for a significant fraction of what we currently understand as intra- and interspecific leaf trait variation. We therefore propose that leaf trait collection campaigns require additional protocols to better account for leaf age and offer practical recommendations to achieve this. This study also demonstrates how leaf sampling and tree-specific differences in leaf mass and

area with age can have large effects on nutrient resorption estimates traditionally calculated on a mass- or area-basis. We provide sampling recommendations and suggest that resorption studies for tropical trees should be based on total leaf nutrient stocks.

The different life-cycle combined trajectories in leaf physiochemical functional strategies we observed among our sampled trees, which are intimately correlated to the resource-use economy and photosynthetic capacity of individual trees, suggests that there are more diverse solutions to the problem of life-cycle resource investment and return in tropical canopy leaves than we previously thought. Our findings highlight the need for more leaf trait life-cycle studies in tropical canopies, which are known to have high species diversity with low population densities and where these different life-cycle functional strategies could, therefore, play a vital role in community assembly and ecosystem structure.

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4.8 Supporting information

Supporting Information for manuscript: “Assessing the role of leaf age as a fundamental driver of intraspecific leaf trait variation within Amazonian canopy trees”.

Methods S 4.8.1

Leaf traits sampled

We focused on measuring several leaf traits that link to physiological and ecological functioning. We measured morphological traits such as leaf mass per area (LMA) and traits related to expansive (leaf area; LA and leaf thickness; LT) and structural (leaf mass; LM and leaf tissue density; LTD) leaf growth. LMA is the ratio of the dry mass of a leaf to its surface area, it is intimately connected to the resource use economy of the plant (Reich et al., 1997) as it can be understood as the leaf-level cost of light interception (Gutschick & Wiegand, 1988). LMA is, therefore, a key trait indicative of plant physiological processes ranging from light capture (Niinemets et al., 1999) to growth rates (Poorter et al., 2009) as well as plant life strategies (Westoby et al., 2002).

For biochemical traits, we measured leaf water content (LWC), nitrogen, phosphorus and carbon content on both a mass (N_{mass} , P_{mass} and C_{mass}) and area (N_{area} , P_{area} and C_{area}) basis and stable isotope content ($\delta^{13}\text{C}$). LWC is a useful indicator of plant water balance, since it expresses the relative amount of water

present on the plant tissues and it is intimately related to several leaf physiological variables, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration (Kramer & Boyer, 1995). Nitrogen is an integral component to the proteins of the photosynthetic machinery responsible for drawdown of carbon dioxide (CO₂) inside the leaf. Phosphorus, being a fundamental component of nucleic acids, lipid membranes and bioenergetic molecules such as ATP, is an essential nutrient in photosynthetic carbon assimilation and protein synthesis (Field, C. & Mooney, H. A., 1986). Thus, both N and P content have been directly linked to rates of photosynthetic activity in leaves (Raaimakers et al., 1995; Evans & Poorter, 2001; Reich et al., 2008, among others). Carbon is a major component in proteins and sugars (non-structural carbon) and cellulose and lignin (structural carbon), which in turn, are used to build the cell walls of leaf tissues (Kokaly et al., 2009). $\delta^{13}\text{C}$, describes the ratio of ¹³C to ¹²C within foliar tissues and indicates water use efficiency (Donovan & Ehleringer, 1994).

Leaf trait measurements

As previously described in Chavana-Bryant *et al.* (2016), we randomly selected a large number of replicates (15 to 30 leaves) for each leaf age class we sampled. Leaf petioles were removed and each individual leaf was then measured for thickness (LT), using a digital caliper, and fresh weight, using a high-precision balance with a 0.01g resolution (My Weigh, model: Durascale). Groups of

individually identified leaves were then colour scanned at 300 dpi resolution. ImageJ software (NIH, New York City, NY, USA) used to calculate individual leaf areas (LA). Scanned leaves were then individually placed in labeled paper bags and dried at 70°C for 72 hours before leaf dry weight (LM) was determined. LWC, LMA, leaf volume (LV) and leaf tissue density (LTD) were derived from these data as follows:

$$\% \text{ LWC} = \left(\frac{\text{fresh mass} - \text{dry mass}}{\text{fresh mass}} \right) \times 100 \quad (1)$$

$$\text{LMA} = \frac{\text{dry mass}}{\text{area}} \quad (2)$$

$$\text{LV} = \left(\frac{\text{thickness}}{10} \right) \times \text{area} \quad (3)$$

$$\text{LTD} = \frac{\text{dry mass}}{\text{LV}} \quad (4)$$

where: leaf fresh and dry mass are in grams (g), leaf area in square metres (m²) for LMA calculations and in square centimetres (cm²) for leaf volume calculations, leaf thickness in millimetres (mm) and leaf volume in cubic centimetres (cm³).

For *Simarouba amara*, a species with compound leaves, leaf trait values were measured on independent leaflets rather than the entire compound leaf as the leaflet green leaf portion is comparable to that measured in whole leaves for other species.

Nutrient content and isotopic composition for all leaves were measured

at the University of Arizona. For each tree, five dry leaves out of each leaf age class were randomly selected for chemical analysis. All dry leaf samples were then homogenised and ground to a fine powder. Total phosphorus (P) content per leaf was determined using persulfate oxidation followed by the acid molybdate technique (APHA, 1992). Phosphorus concentration was then measured colourimetrically with a spectrophotometer (Genesys20, Thermo Fisher Scientific, Madison, WI, USA). $\delta^{13}\text{C}$, phosphorus (P), nitrogen (N) and carbon (C) contents were measured by the Department of Geosciences Environmental Isotope Laboratory at the University of Arizona using a continuous-flow gas-ratio mass spectrometer (Finnigan Delta Plus XL, Thermo Fisher Scientific, Madison, WI, USA) coupled to an elemental analyser (Costech Analytical Technologies, Valencia, CA, USA). Samples of 1.0 mg ($\pm$ 0.1 mg) were combusted in the elemental analyser. Standardisation is based on acetanilide for elemental concentration, NBS-22 and USGS-24 for $\delta^{13}\text{C}$, and IAEA-N-1 and IAEA-N-2 for $\delta^{15}\text{N}$. Precision is at least $\pm$ 0.10 for $\delta^{13}\text{C}$ and $\pm$ 0.2 for $\delta^{15}\text{N}$ (1s), based on repeated internal standards.

Table S 4.8.1 Leaf sampling scheme by tree and leaf age class. Leaf age classification: Y1, Y2, Y3 and Y/M are developing leaves collected after 1, 2, 3 and 4 weeks of active leaf expansion, respectively; M are mature leaves; O are old leaves showing initial signs of senescence; S are senescent leaves in the process of dying and abscising. Table also published in Chavana-Bryant *et al.* (2016).

Tree species (codes)	Y1	Y2	Y3	Y/M	M	O	S	Total leaves by tree
<i>Licania brittoniana</i> (1)	15	15	20		60		15	125
<i>Simaruba amara</i> (3)	30	30	15		15	30	30	150
<i>Ruizodendron ovale</i> (4)	16	15	15	15	15		20	96
<i>Aspidosperma parvifolium</i> (5)						30	50	80
<i>Couratari macrosperma</i> (6)	15	20	15	15	20		29	114
<i>Licania l ongistyla</i> (7)							33	33
<i>Bertholletia excelsa</i> (8)	15	15	15		30	30	20	125
<i>Tachigali chrysaloïdes</i> (9)							20	20
<i>Clarisia racemosa</i> (10)	15				20	20	20	75
<i>Eschweilera coriacea</i> (11)					40	20		60
<i>Guatteria boliviana</i> (12)	15	15	15		15		40	100
<i>Pouteria Franciscana</i> (13)	15	15	15		15	20	41	121
Total leaves by age class	136	125	110	30	230	150	318	1099

Table S 4.8.2 Equations used to measure differences in nutrient content between leaves of different leaf age classes.

Description	Acronym	Equations	
Measured resorption efficiency	*MRE	$100 \times \left(1 - \frac{[X]_{senescent}}{[X]_{mature}} \right)$	where [X]= nutrient on a mass or area basis
Real resorption efficiency	*RRE	$100 \times \left(1 - \frac{X_{senescent}}{X_{mature}} \right)$	where X= total leaf nutrient stock
Percent change	% change	$100 \times \left(\frac{X_{senescent} - X_{mature}}{X_{mature}} \right)$	where X= total leaf nutrient stock

* Based on equations (2) and (3) from Heerwarden *et al.* (2003). It is important to note that in the MRE and RRE equations, there is an assumption that nutrient content is lower in senescent leaves compared to that of mature leaves, while our % change equation does not carry this assumption.

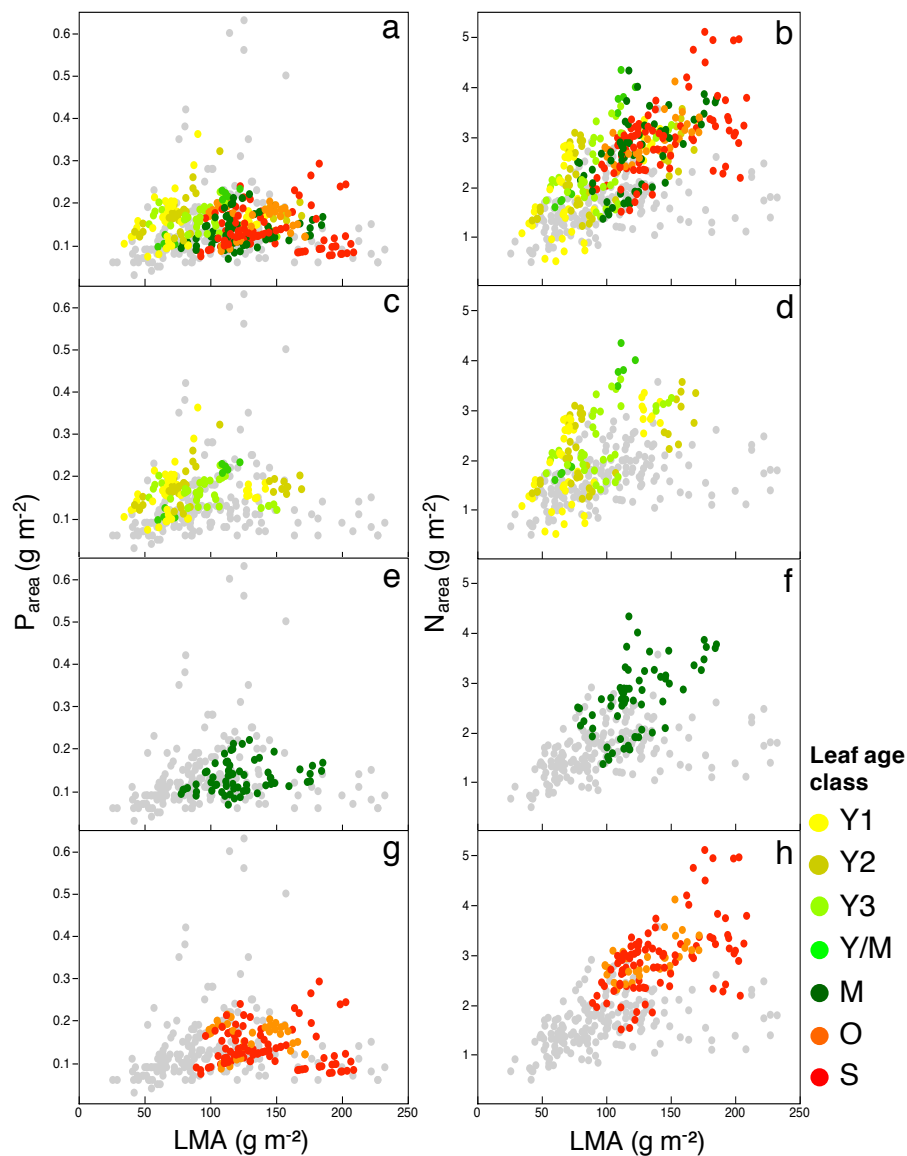
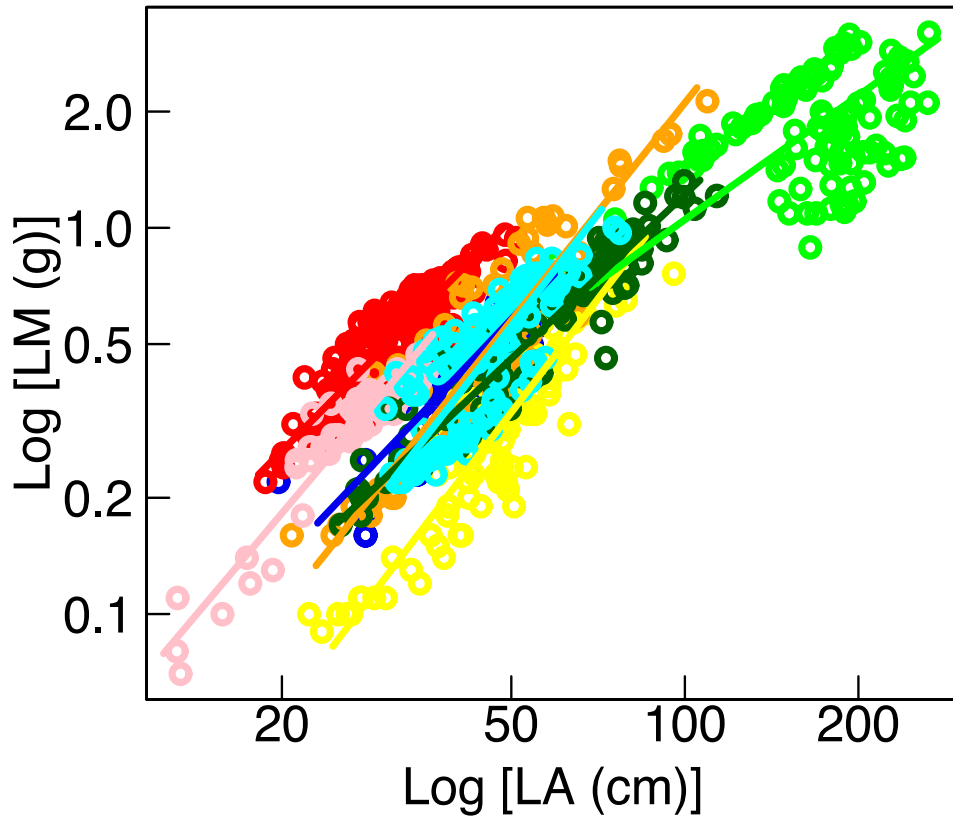


Figure S 4.8.1 Variation in the combined Leaf Economic Spectrum traits P_{area} –LMA and N_{area} –LMA among sun leaves of different ages from our 12 sampled trees (colour coded data points) versus the GLOPNET dataset for pantropical tree species (grey data points) (Wright *et al.*, 2004). Individual GLOPNET data points represent mean traits from mature leaves of 184 pantropical tree species, while data points from our phenological dataset represent traits from 320 individual leaves of different ages from 12 lowland Amazonian tropical canopy and emergent trees. Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in these graphs. Leaf age classification: Y1, Y2, Y3 and Y/M are developing leaves collected after 1, 2, 3 and 4 weeks of active leaf expansion, respectively; M are mature leaves; O are old leaves showing initial signs of senescence; S are senescent leaves in the process of dying and abscising.



Trees	Slope	R ²	N
<i>L. brittoniana</i> (1)	1.57 (1.41, 1.75)	0.65 (125)	
<i>S. amara</i> (3)	1.51 (1.40, 1.62)	0.80 (150)	
<i>R. ovale</i> (4)	1.96 (1.78, 2.14)	0.80 (96)	
<i>C. macrosperma</i> (6)	1.87 (1.71, 2.05)	0.77 (114)	
<i>B. excelsa</i> (8)	1.10 (0.92, 1.30)	0.18 (125)	
<i>C. racemosa</i> (10)	1.77 (1.63, 1.92)	0.89 (75)	
<i>G. boliviana</i> (12)	1.43 (1.33, 1.53)	0.91 (100)	
<i>P. franciscana</i> (13)	1.93 (1.73, 2.16)	0.65 (121)	

Figure S 4.8.2 Relationship between leaf mass (LM) and leaf area (LA) of individual leaves of different age classes from eight lowland Amazonian tropical canopy and emergent trees. Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in these SMA regression analyses. Individual leaf trait values are log-transformed and used as data points. Solid lines represent significant regressions (all P values= < 0.0001) for different trees.

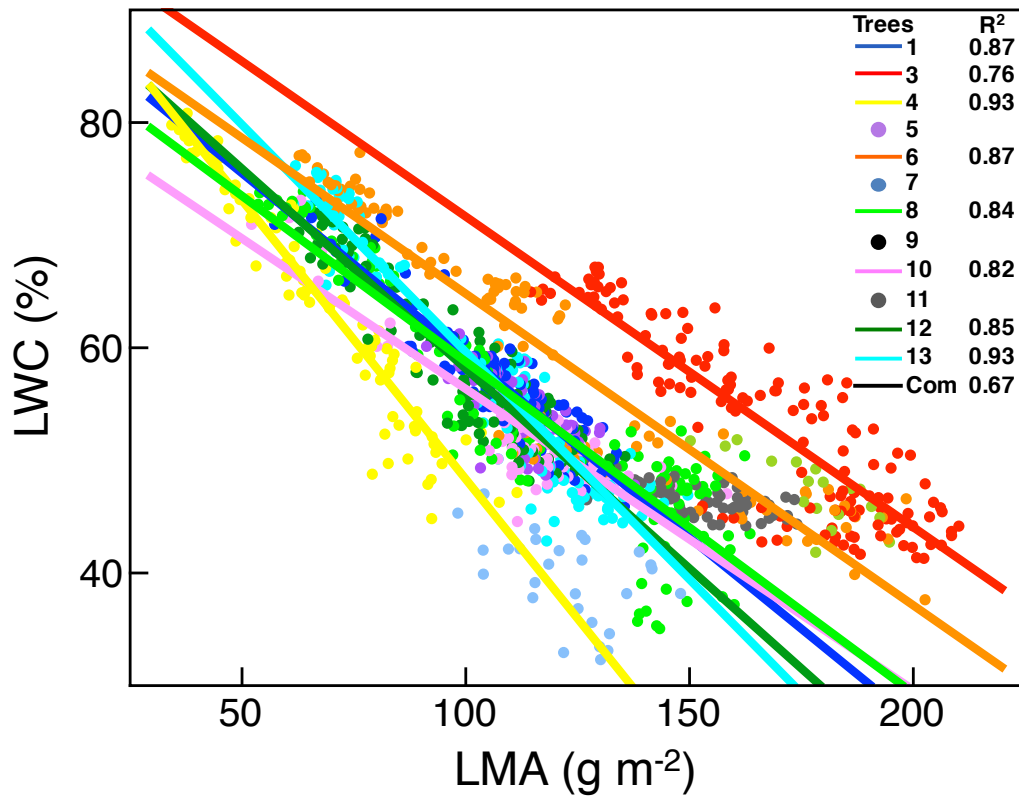


Figure S 4.8.3 Relationship between leaf water content (LWC) and leaf mass per area (LMA). Eight individual trees with four or more sampled leaf age classes are included in regression analyses (colour coded lines) with the community regression (black line) based on all 12 sampled trees. Individual leaf trait values are used as data points. All regressions are significant at P values = < 0.0001. Graph also published in Chavana-Bryant *et al.* (2016).

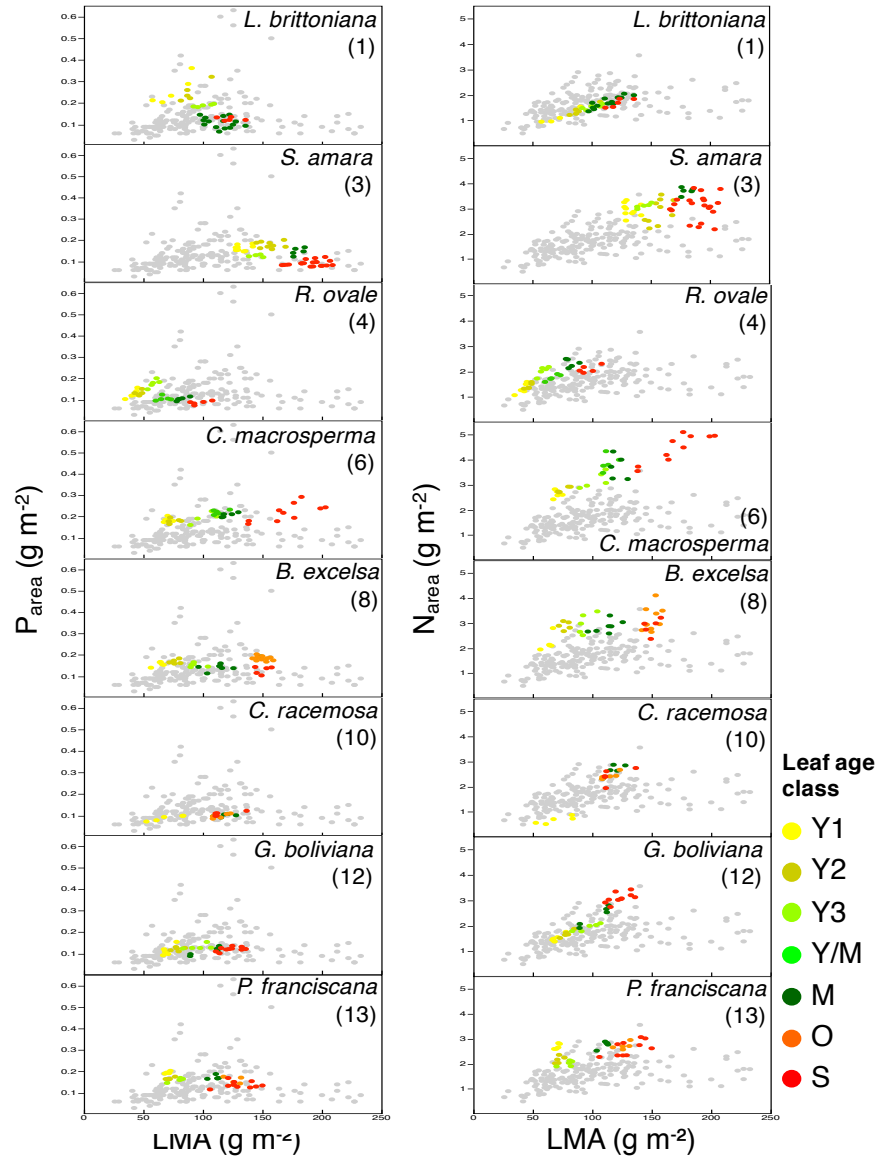


Figure S 4.8.4 Combined life-cycle trajectories in Leaf Economic Spectrum traits P_{area} – LMA and N_{area} – LMA from eight of our sampled trees (data points colour coded by leaf age) versus the GLOPNET dataset for pantropical tree species (grey data points) (Wright *et al.*, 2004). Individual GLOPNET data points represent mean traits from mature leaves of 184 pantropical tree species, while data points from our phenological dataset represent traits from individual leaves of different ages from eight lowland Amazonian tropical canopy and emergent trees (tree codes in brackets). Only trees (tree codes in brackets) with four or more sampled leaf ages are included in these graphs. Leaf age classification: Y1, Y2, Y3 and Y/M are developing leaves collected after 1, 2, 3 and 4 weeks of active leaf expansion, respectively; M are mature leaves; O are old leaves showing initial signs of senescence; S are senescent leaves in the process of dying and abscising.

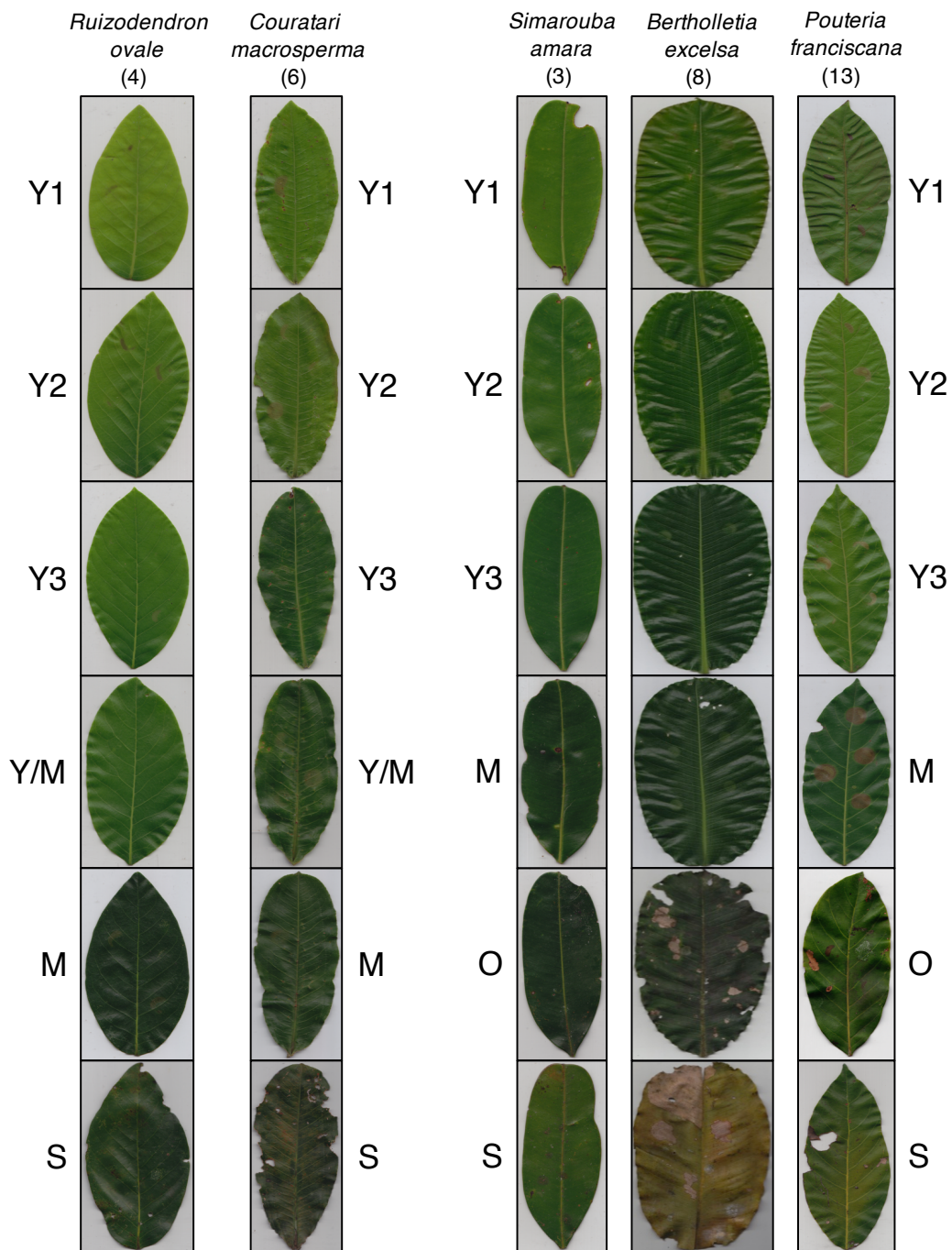


Figure S 4.8.5 Sampled leaves of different age classes, illustrating the leaf life-cycle of *Ruizodendron ovale*, *Couratari macrosperma*, *Simarouba amara* and *Pouteria franciscana*. (tree codes in brackets). Colour images are not to scale. Leaf age classification: Y1, Y2, Y3 and Y/M are developing leaves collected after 1, 2, 3 and 4 weeks of active leaf expansion, respectively; M are mature leaves; O are old leaves showing initial signs of senescence; S are senescent leaves in the process of dying and abscising.



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The leaf morphological and biochemical traits I examined in **Chapter 4**, have been shown to directly influence the spectral reflectance behaviour of leaves. Therefore, **Chapter 5** investigates the effects of leaf age on leaf spectral properties within and across individual canopy trees. This chapter addresses the spectral components of my second research aim: **“to assess how leaf spectral traits change with age, to (2.1) identify the most significant changes in leaf reflectivity during different stages of the leaf life-cycle and determine how these changes are related to changes in physiochemical leaf traits (LMA, LWC, P_{mass} , N_{mass} and C_{mass}); and (2.2) evaluate if leaf spectra can provide an efficient and accurate tool to predict leaf age”**. This study reveals how the underlying physiochemical age-related changes in the leaf traits of a community of Amazonian canopy trees are captured by hyperspectral leaf measurements, thus enabling leaf spectra to be used to predict leaf age. This novel approach extends the utility of spectroscopic methods to model leaf age. Spectral data is also used to calculate leaf-level vegetation indices (VIs), such as the normalised difference vegetation index (NDVI), the enhanced vegetation index (EVI), the photosynthetic reflectance index (PRI) and the normalised difference water index (NDWI), to for the first time, provide direct evidence that leaf age can have important impacts on these VIs commonly used to monitor canopy dynamics and productivity.

Author Contribution Statement

C.C-B., Y.M. and F.F.G. planned and designed the research. C.C-B. designed field data collection protocols and conducted fieldwork and field data collection with logistical support from E.G.C.C. Data integration and quality assurance were performed by C.C-B. and A.A. Data analysis was performed by C.C-B. with support from J.W. Chemical analyses were undertaken by B.J.E. at the University of Arizona. C.C-B. wrote the manuscript with contributions from F.F.G., Y.M., J.W., G.P.A., B.J.E., C.E.D., S.R.S. and R.E.M.

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Chapter 5 Leaf ageing of Amazonian canopy trees as revealed by spectral and physiochemical measurements

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Abstract

- Leaf ageing is a fundamental driver of changes in leaf traits, thereby, regulating ecosystem processes and remotely-sensed canopy dynamics.
- We explore leaf reflectance as a tool to monitor leaf age and develop a spectra-based partial least squares regression (PLSR) model to predict age using data from a phenological study of 1,099 leaves from 12 lowland Amazonian canopy trees in southern Peru.
- Results demonstrated monotonic decreases in leaf water- (LWC) and phosphorus content (P_{mass}) and increase in leaf mass per area (LMA) with age across trees; leaf nitrogen- (N_{mass}) and carbon content (C_{mass}) showed monotonic but tree-specific age responses. We observed large age-related variation in leaf spectra across trees. A spectra-based model was more accurate in predicting leaf age ($R^2 = 0.86$ and percent root mean square error %RMSE= 33) compared to trait-based models using single ($R^2 = 0.07$ to 0.73; %RMSE=7 to 38) and multiple predictors ($R^2 = 0.76$; %RMSE=28). Spectra and trait-based models established a physiochemical basis for the spectral age model. Vegetation indices (VIs) including the normalised difference vegetation index (NDVI), enhanced vegetation index 2 (EVI2), normalised difference water index (NDWI) and photosynthetic reflectance index (PRI) were all age-dependent.
- This study highlights the importance of leaf age as a mediator of leaf traits, provides evidence of age-related leaf reflectance changes that have

important impacts on VIs used to monitor canopy dynamics and productivity and proposes a new approach to predicting and monitoring leaf age with important implications for remote sensing.

Key Words

Canopy trees; leaf spectral properties; leaf age; leaf traits; leaf life-cycle; tropical forests; phenology; vegetation indices

5.1 Introduction

A central goal in ecosystem ecology is to link vegetation dynamics to spatial and temporal patterns in primary productivity and biogeochemical cycles. Recently, there has been much interest in understanding the inter- and intra-annual dynamics of tropical forest phenology using remote sensing methods (Asner *et al.*, 2000b; Saleska *et al.*, 2003; Asner *et al.*, 2004; Huete *et al.*, 2006a; Huete *et al.*, 2006b; Myneni *et al.*, 2007; Saleska *et al.*, 2007; Doughty & Goulden, 2008; Anderson *et al.*, 2010; Brando *et al.*, 2010; Samanta *et al.*, 2010; Anderson *et al.*, 2011; Bradley *et al.*, 2011; Anderson, 2012; Samanta *et al.*, 2012; Morton *et al.*, 2014) as they contain 55% of the global carbon stores (Pan *et al.*, 2011) and account for ~50% of terrestrial photosynthesis (Field *et al.*, 1998).

Satellite remote sensing offers an attractive tool for studying the canopy dynamics of tropical forests across large spatial scales. Remote sensing

products, such as the Moderate Resolution Imaging Spectroradiometer (MODIS) vegetation indices (VIs), use reflectance data from two or more spectral bands and ratio these data in different ways to provide estimates of canopy greenness – a composite property of both leaf- (leaf intercellular structure and biochemical composition) and canopy-level (canopy leaf area and structure) properties – and other vegetation biophysical properties such as leaf area index (LAI) and/or the fraction of absorbed photosynthetically active radiation (fAPAR) (Huete *et al.*, 2002).

Several remote sensing studies have used the MODIS LAI product (Myneni *et al.*, 2007) and the enhanced vegetation greenness index (EVI; an ‘optimised’ index designed to not saturate in high biomass regions such as the tropics; Huete *et al.*, 2006a) to investigate the inter-annual and seasonal canopy dynamics of the Amazonian basin. These studies report dry season increases in LAI and canopy greenness (a ‘green-up’ effect) across broad areas of Amazonian forests, attributing these increases to flushing of new leaves during the dry season. Although some studies have suggested that remotely detected seasonality in tropical forest canopy greenness might be attributed solely to sensor artifact (Galvão *et al.*, 2011; Morton *et al.*, 2014), other remote sensing studies (Doughty & Goulden, 2008; Brando *et al.*, 2010; Bradley *et al.*, 2011; Samanta *et al.*, 2012) propose that leaf demography, the effect of age-related variation in photosynthetic efficiency and the spectral reflectance properties of leaves may

significantly contribute to explaining this ‘green-up’ effect. If variation in leaf traits, photosynthetic efficiency and associated reflectances is large enough to influence remotely sensed patterns, these effects should be detectable in individual leaves as they develop and age. However, with the exception of Roberts *et al.* (1998), no study to date has conducted *in situ* measurements to investigate age effects on leaf reflectivity of canopy trees and on the VIs we use to monitor tropical canopy dynamics. Furthermore, an efficient and accurate approach for monitoring leaf age is still largely lacking.

We propose that hyperspectral leaf reflectance can provide an efficient and accurate tool with which to monitor leaf age and intraspecific variation in leaf traits, and thereby enhance our understanding of the mechanisms underlying remotely detected patterns. Our proposed methodology takes advantage of the fact that many leaf-scale biochemical and anatomical traits that change with leaf age, for example, leaf pigmentation, nutrient content, intercellular structure, leaf mass per unit area (LMA) and leaf water content (LWC) (Field & Mooney, 1983; Carter *et al.*, 1989; Miller *et al.*, 1991; Reich *et al.*, 1991; Kitajima *et al.*, 1997; Kitajima *et al.*, 2002; Escudero & Mediavilla, 2003; Mediavilla *et al.*, 2011), have been shown to directly influence the spectral reflectance behaviour of leaves (Gausman, H. W. *et al.*, 1970; Knipling, EB, 1970; Roberts *et al.*, 1998; Datt, 1999; Sims & Gamon, 2003). A few studies have also observed significant intra-specific variability in leaf spectral reflectance of trees as a result of differences in

leaf age (Roberts *et al.*, 1998; Datt, 1999; Lewis, 2002). This study set out to jointly investigate the spectral, morphological (LMA), and biochemical (leaf water (LWC), phosphorus (P_{mass}), nitrogen (N_{mass}) and carbon (C_{mass}) contents) changes associated with natural leaf ageing of 1099 leaves within and across 12 Amazonian canopy trees in order to address the following questions: (1a) How do leaf spectral properties vary with age and how are these variations related to leaf morphological and biochemical traits? (1b) Which spectral domains show most variation associated with leaf ageing? (2) Can leaf age be predicted from spectral properties, within and across individual trees, and what are the underlying mechanisms that mediate the leaf-level spectral age model? (3) What is the sensitivity of VIs to leaf-level age-dependent reflectivity and what are the implications when VIs are used to infer landscape greenness and productivity in tropical evergreen forests?

By answering these questions, we aimed to reveal how the underlying morphological and biochemical basis of leaf ageing in a selection of Amazonian canopy trees with diverse leaf properties is captured by hyperspectral leaf measurements, to offer a new spectral approach for determining leaf age and, using leaf-level VIs as proxies, to explore how leaf lifecycle effects can potentially influence the seasonal variation of remotely sensed canopy VIs.

Our paper represents the first attempt to study and predict tropical leaf age using hyperspectral approaches. This paper is complemented by two companion

papers. In Chavana-Bryant *et al.* (in review) we explore the role of leaf age as a fundamental driver of intraspecific trait variation within Amazonian canopy trees. In a follow-up paper, Wu *et al.* (2016b) test the generality of the spectral model we develop here, by application to an independent data set from a very different Amazonian forest site in Brazil with slow forest and canopy dynamics, and it is also tested for contrasting canopy (sun versus shade) light conditions.

5.2 Materials and Methods

5.2.1 Field site

Data were collected in and around two lowland tropical rainforest plots located within the Tambopata National Reserve in the Madre de Dios region of Peru in southwestern Amazonia. The two study sites are part of the RAINFOR Amazon Forest Inventory Network (Malhi *et al.*, 2002b) and GEM intensive monitoring network, with RAINFOR codes TAM-06 (12°50'24"S, 69°17'59"W) and TAM-09 (12°49'48"S, 69°16'48"W). The sites are located between 215 and 220 m above sea level within lowland closed-canopy primary tropical rain forest growing on Haplic alisol soils (Quesada *et al.*, 2010). They are fairly seasonal sites with a 4-6 month-long dry season (number of months with < 100mm rainfall) between June and October, mean annual rainfall of 1900mm (this may be lower than the long-term mean because of severe droughts in 2005 and (Lewis *et al.*, 2011) and a mean annual air temperature of 24.4°C. A detailed description of the

geomorphology, climate and forest carbon cycle of this site is given by Malhi *et al.* (2014).

Trees at our sites, as in other tropical forests, exhibit a range of phenological behaviours, from rapid shedding of short-lived, mesic leaves early in the dry season in deciduous species to irregular or constant leaf exchange of long-lived, coriaceous leaves in brevi-deciduous and evergreen species (Borchert *et al.*, 2002), and from complete intraspecific synchrony to complete asynchrony (Vanschaik *et al.*, 1993). Eleven out of 12 trees in this study displayed brevi-deciduous phenology (i.e. initiated leaf flushing when most senescent leaves had been abscised or after 1-4 weeks of leaflessness) and exchanged their leaves before or during the period of the dry season. One tree (*Simarouba amara*) displayed evergreen phenology and exchanged only ~50% of its canopy through a prolonged period of leaf abscission and flushing that lasted the entire dry season.

5.2.2 Tree sampling strategy

The tree sampling for this study focused on canopy and emergent trees (**Table 5.2.2.1**), as the chemical properties of top-of-canopy leaves drive the spectral characteristics of tropical forest canopies (Clark *et al.*, 2005; Asner & Martin, 2008a) as detected by remote sensing. According to the “Crown Illumination Index” (Clark & Clark, 1992; Keeling & Phillips, 2007), eight of our sampled trees

were classified as canopy trees and four were emergent trees. Diameter at breast height (DBH) among sampled trees averaged 171cm (range 117-237cm). The sampled trees averaged 32m in height (range 29-35m), compared with a mean ($\pm$ SE) tree height of 27 ± 8 m for canopy trees ≥ 40 cm DBH in local plots. Canopy depth, the difference between tree height and height at the lowest branch of the canopy, among our sampled trees averaged 9.6m (range 6.3-15.1m).

Table 5.2.2.1 Species and structural information of trees sampled for leaf spectral and physiochemical measurements.

Tree code	Species	Family	DBH (m)	Tree canopy position	Height (m)	Canopy depth (m)
1	<i>Licania brittoniana</i> Fritsch	Chrysobalanaceae	1.88	Canopy	29.50	10.50
3	<i>Simarouba amara</i> Aubl.	Simaroubaceae	1.62	Canopy	29.00	8.35
4	<i>Ruizodendron ovale</i> R.E. Fr. (Ruiz & Pav.)	Annonaceae	1.23	Canopy	32.62	10.07
5	<i>Aspidosperma parvifolium</i> A. DC.	Apocynaceae	1.57	Emergent	33.70	8.40
6	<i>Couratari macrosperma</i> A.C. Sm.	Lecythidaceae	1.43	Emergent	33.74	6.27
7	<i>Licania longistyla</i> (Hook. F.) Fritsch	Chrysobalanaceae	1.43	Canopy	30.30	8.70
8	<i>Bertholletia excelsa</i> Bonpl.	Lecythidaceae	2.30	Emergent	34.00	11.60
9	<i>Tachigali chrysaloidea</i> van der Werff	Caesalpinioidae	2.10	Canopy	29.70	7.60
10	<i>Clarisia racemosa</i> Ruiz & Pav.	Moraceae	1.58	Canopy	33.75	9.25
11	<i>Eschweilera coriacea</i> (DC.) S.A. Mori	Lecythidaceae	1.88	Emergent	33.32	7.78
12	<i>Gautteria boliviana</i> H. Winkl.	Annonaceae	1.17	Canopy	31.90	11.34
13	<i>Pouteria franciscana</i> Baehni	Sapotacea	2.37	Canopy	35.39	15.09

DBH, diameter at breast height

Monitoring canopy and emergent trees *in situ* presents considerable challenges. Canopy walkways and towers, where they exist, do not provide sufficient access across a large number of crowns. Canopy cranes are ideal for

such sampling, but there are only a few across the tropics. Hence, we resorted to repeated climbing and sampling of a number of large trees. The total number of trees sampled in this study was limited by the need to sample structurally sound trees large enough to withstand the repeated climbing required to monitor their leaf lifecycle and sample leaves of different ages, and the difficulty involved in climbing large trees with ropes to minimize damage caused by repeated climbing.

We therefore chose to maximize variability within our leaf data set by sampling 12 different tree species with a wide variety of leaf types (e.g. compound and simple leaves), sizes, shapes, thicknesses and waxiness. This sampling allowed us to examine the variability attributable to natural leaf ageing of a range of leaf types, sizes, shapes, thicknesses and waxiness within individual trees and across a canopy tree community with diverse leaf properties. As a consequence of our lack of replication within species, we are not able to draw general conclusions about the spectral variation during the leaf lifecycle of particular species. We are, however, able to explore relationships between leaf traits, spectra and leaf age within individual trees, and, by examining across all 12 trees, explore if there is evidence of general relationships or developmental pathways for leaf traits, spectra and leaf age across a canopy tree community with diverse leaf properties.

5.2.3 Leaf age classification

We sampled the developmental, mature and senescent leaf phases (the detailed leaf age classification used in this study can be found in **Figure 5.2.3.1**) for eight trees and also sampled some leaf ages from four other trees (see **Table S 5.8.1** and the 'Branch sampling' section in **Methods S 5.8.1**). This tree sampling allowed us to build a well-balanced leaf phenological data set in which developing leaves were sampled after 1, 2, 3 and 4 weeks of active expansion; mature leaves were sampled at different stages (newly matured, mid-maturity and end of maturity); and senescent leaves were also sampled at different stages (i.e. old leaves not yet abscising and senescent leaves starting abscission through to advanced senescence when most leaves had been abscised from the tree crown).

Based on phenological observations of our trees and the pronounced dry season peaks in leaf flush and leaf fall at our site (Malhi *et al.*, 2014), we assigned a 13-month (57 weeks) leaf lifecycle for all trees, which allows for 7 weeks of active leaf expansion, 6.5 months of maturity, 2 months of old age and 3 months of senescence. This 13-month leaf lifecycle is similar to the mean leaf lifespan of 1.6 year found by Reich *et al.* (2004) for well-lit trees in an Amazonian *terra firme* mature forest canopy, although that study was based on a forest in a less seasonal climate and growing on poorer soils, where longer leaf lifespans can be expected.

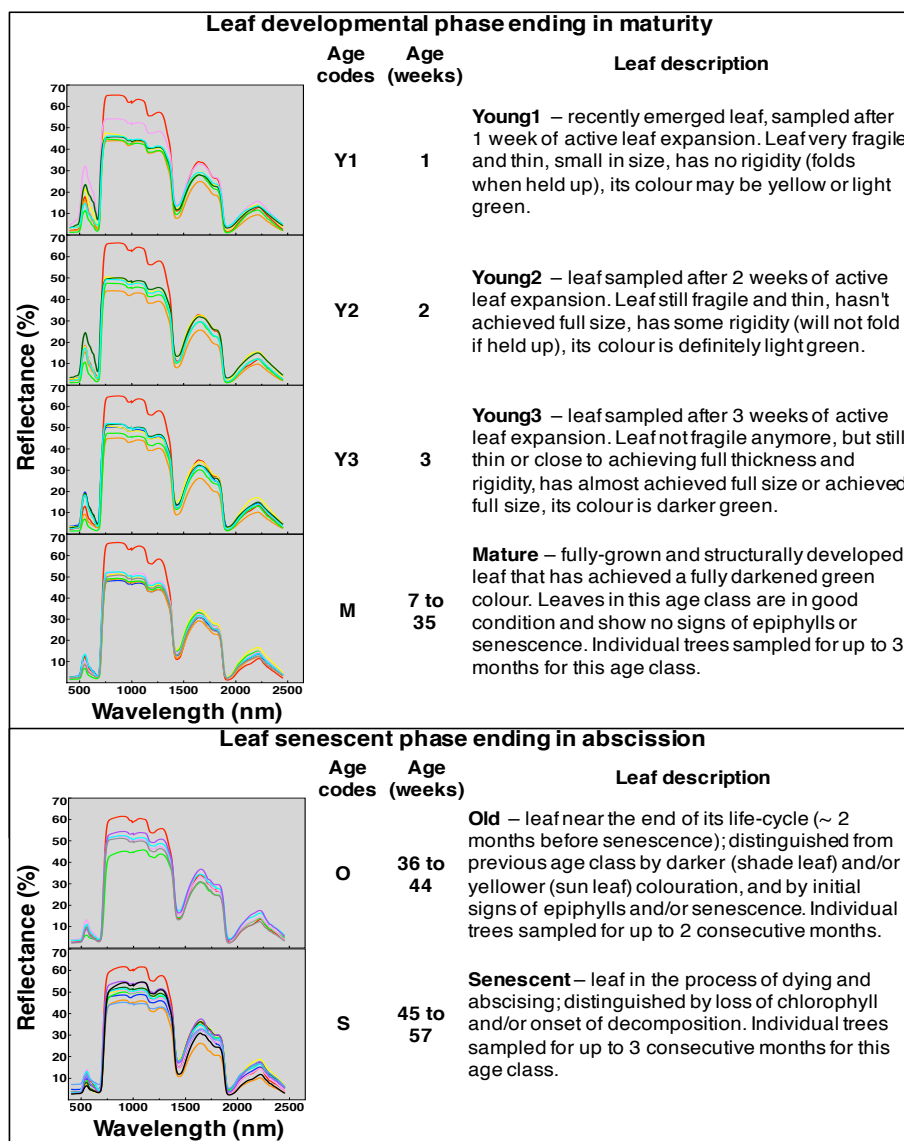


Figure 5.2.3.1 Leaf age classification used in this study (adapted from Lowman & Box, 1983). Graphs show mean reflectance (15–30 leaves) by age class for sun leaves of 12 Amazonian canopy and emergent trees. The young/mature (Y/M) leaf age class is not included as it was sampled only for two trees for which Y3 leaves still displayed a light green colouration. Y/M leaves are close to maturity and have achieved full size, full thickness and rigidity but their green colour is not yet fully darkened. Coloured lines represent individual trees: 1- dark blue; 3- red; 4- yellow; 5- purple; 6- orange; 7- light blue; 8- light green; 9- black; 10- pink; 11- grey; 12- dark green; 13- cyan. Leaf age for developmental phase to maturity was determined by witnessing leaf emergence and initiation of active leaf expansion. If leaf emergence was not observed, leaf age was determined based on leaf condition for mature and old leaves. For senescent leaves, an additional classification criterion was that extensive leaf abscission was observed within the canopy of the sampling tree; sampled leaves for this leaf age were those easily abscised with branch movement.

5.2.4 Morphological and biochemical leaf traits

We selected LMA, LWC, P_{mass} , N_{mass} and C_{mass} as the ensemble of leaf traits to measure (full methodological details are included in **Methods S 5.8.1**). Each of these leaf traits has important links to physiological and ecological functioning and a demonstrated contribution to the spectral signatures of vegetation (Curran, 1989; Asner *et al.*, 2009). A detailed exploration of these traits and their relationships to leaf age can be found in **Chapter 4**. We assessed leaf waxiness qualitatively according to leaf glossiness and categorised the eight main tree species into *Simarouba amara*, *Licania brittoniana* and *Clarisia racemosa* as having high leaf waxiness, and *Ruizodendron ovale*, *Couratari macrosperma*, *Bertholletia excelsa*, *Gautteria boliviana* and *Pouteria franciscana* as having low leaf waxiness.

5.2.5 Leaf spectroscopic measurements

We randomly selected 15–30 leaves from each sampled leaf age class for spectral analysis. Leaves were cut (at the base of the petiole) from their branch just before reflectance measurements were collected. Measurements were collected using a field spectrometer (FieldSpec Pro; Analytical Spectral Devices, Boulder, CO, USA) with a spectral range of 350–2500nm and sampling intervals of 1.4nm from 350 to 1000 and 2nm from 1000 to 2500nm. The spectrometer was fitted with a contact probe (High Intensity Contact Probe; Analytical Spectral Devices) which has its own calibrated light source designed to minimise

measurement errors associated with stray light. To ensure measurement quality, the spectrometer was optimized every 5 minutes, spectra for every leaf were white referenced to a calibration panel (Spectralon; Lasphere, Durham, NH, USA) and an average of 25 spectrum, dark current and white reference measurements were used to improve the signal-to-noise ratio of the spectra. Furthermore, leaves were placed on a custom-designed measurement block covered in a low-reflectivity (3%) material with a firm cushion fitted underneath to allow the contact probe to be pressed against the leaf without causing damage, while ensuring that no light escaped the measurement. Four reflectance measurements were collected and averaged for each leaf (dependent on leaf size). This protocol delivered high-quality calibrated spectra that did not require smoothing or other filters. The full-range leaf spectral data were trimmed at the far ends (<412 and $>2450\text{nm}$).

In addition to classifying leaves by their age and to account for potential effects of leaf coatings and natural leaf damage on spectra, we recorded the presence of epiphylls (which was always in very low abundance) and/or necrosis for each leaf surface we measured and used only reflectance measurements free of epiphylls and necrosis.

A short video documenting the fieldwork conducted for this study can be seen in <https://vimeo.com/208748496>.

5.2.6 Statistical analysis

We used regression analysis to examine the relationships of leaf traits with age (log-transformed) for each individual tree. Only trees with four or more sampled leaf age classes were used in these analyses with the Akaike information criterion (AIC) and R^2 values used to identify the best-fit regression models (**Figure 5.2.6.1**). For the same subset of trees, we calculated and compared mean reflectance (%; **Figure 5.2.6.2**) and reflectance variability (measured as coefficients of variation (CVs); bottom graph in **Figure 5.2.6.3** and **Figure S 5.8.1**) for the different age cohorts to investigate age effects on leaf reflectivity.

We used a partial least squares regression (PLSR) modelling approach (Geladi & Kowalski, 1986; Wold *et al.*, 2001) to predict the age of individual leaves from leaf spectral properties. To do this, we subsampled within the eight trees to predict leaf age within these individual trees, and combined leaves from all 12 trees and across ages to predict a mean leaf age for our canopy leaf community. We also used stepwise (forward) multiple regression to empirically model leaf age (log-transformed) across all trees using the five leaf traits (LMA, LWC, P_{mass} , N_{mass} and C_{mass}) and to assess the contribution of these traits to the leaf age prediction. The results of step-wise regressions were then compared to those of the spectral leaf age PLSR model results and used to explore the mechanisms behind the spectral leaf age model.

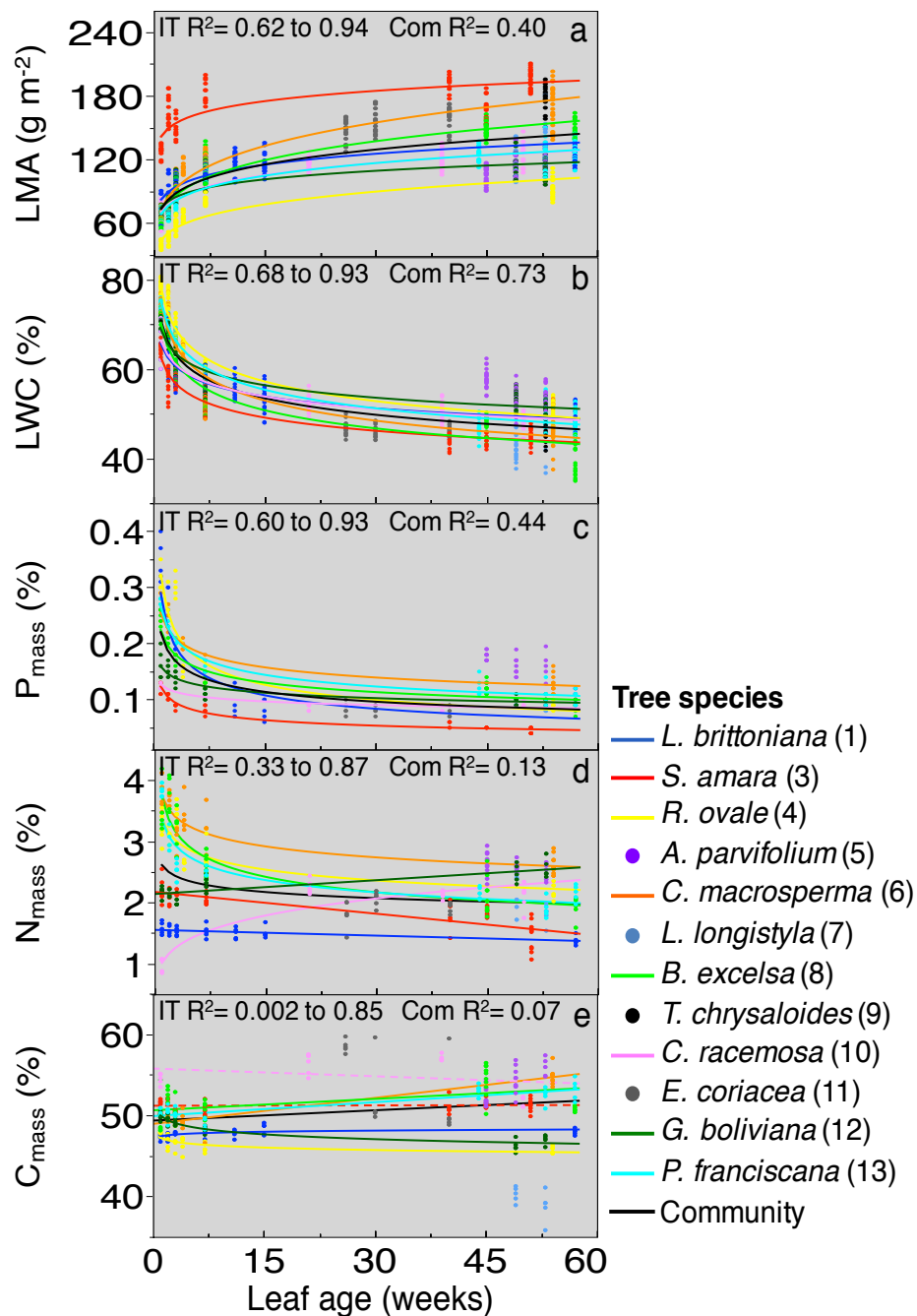


Figure 5.2.6.1 Relationships between leaf traits and leaf age (log-transformed). Coloured lines represent individual tree regressions (performed only for trees with four or more sampled leaf age classes; IT R²) and black lines represent community regressions (12 trees included; Com R²). Individual leaf trait values are used as data points. Dashed lines are nonsignificant regressions. All significant regressions were P < 0.001. LMA, leaf mass per unit area; LWC, leaf water content; P_{mass}, leaf phosphorus content; N_{mass}, leaf nitrogen content; C_{mass}, leaf carbon content.

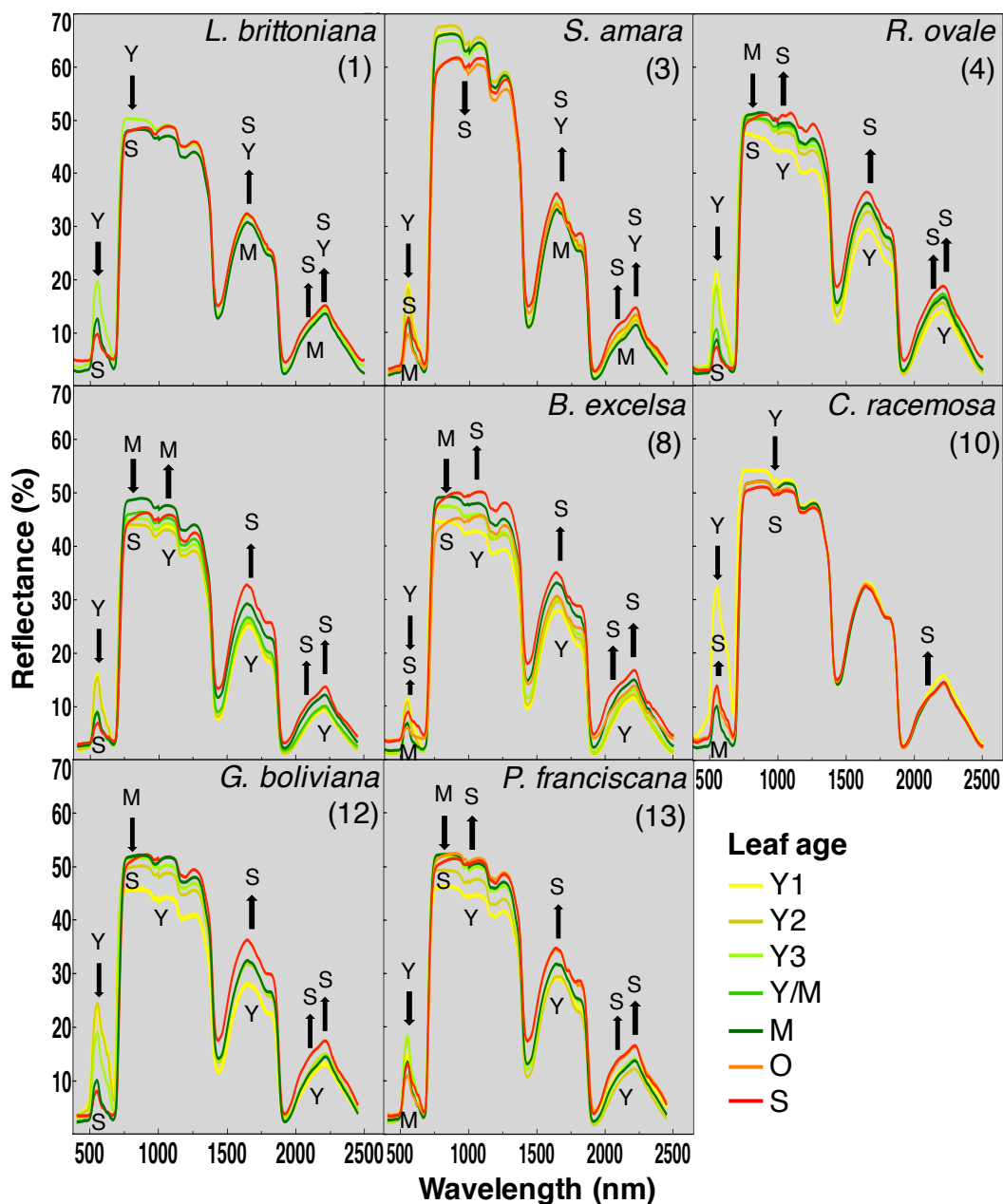


Figure 5.2.6.2 Mean reflectance (15–30 leaves) graphs by age class for sun leaves of eight Amazonian canopy and emergent trees. Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in this analysis. Leaf age classes: Y1, Y2, Y3 and young/mature (Y/M) are developing leaves collected after 1, 2, 3 and 4 wk of active leaf expansion, respectively; M, mature leaves; O, old leaves showing initial signs of senescence; S, senescent leaves in the process of dying and abscising. Standard error of mean reflectance for all leaf ages across trees is below 0.2 for most wavelengths and below 0.4 for all. Arrows indicate the direction of spectral change through different leaf ages.

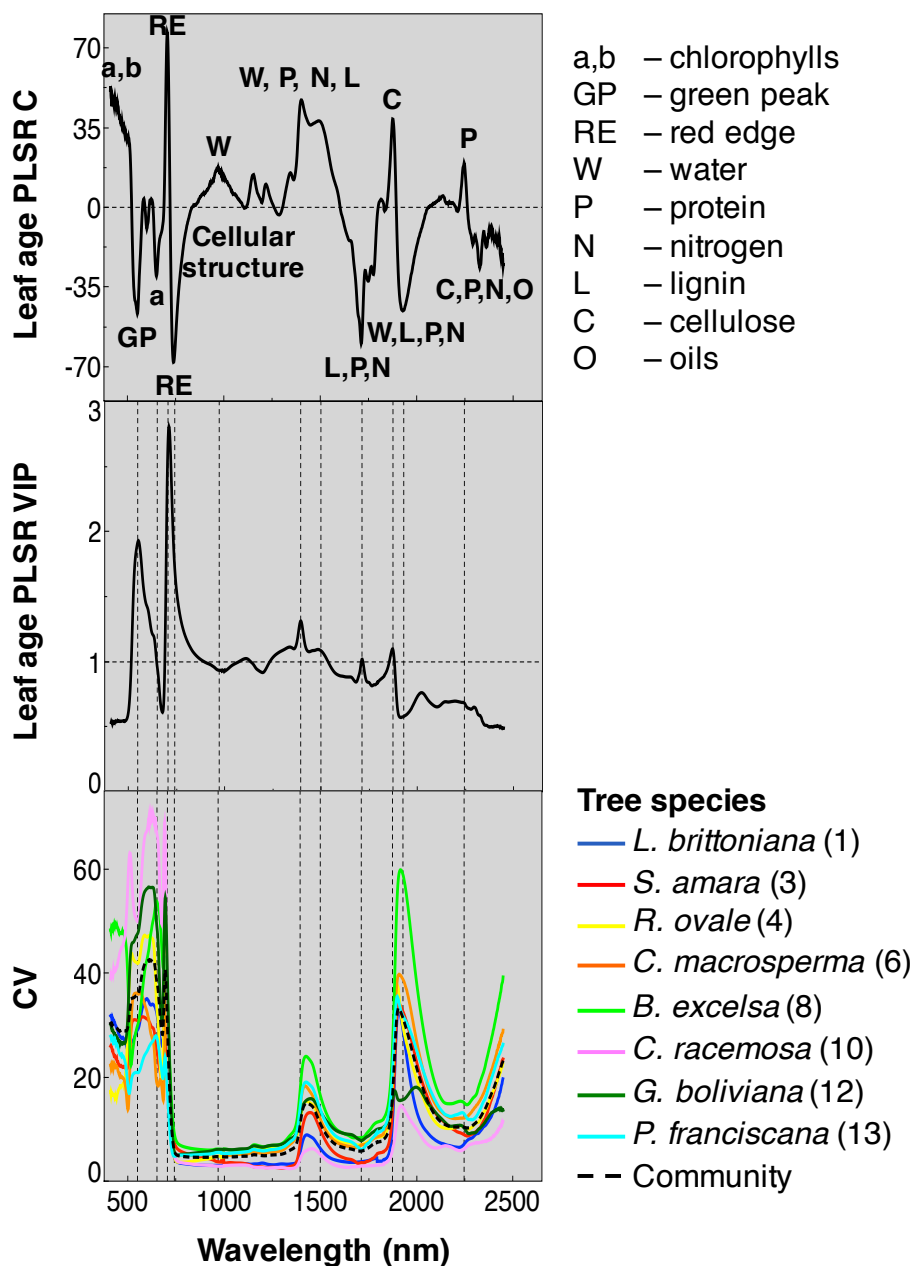


Figure 5.2.6.3 Spectral coefficients (C) and variable importance of prediction (VIP) for the partial least squares regression (PLSR) leaf age model, and spectral coefficients of variation (CV) plots. Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in CV plots, with lines representing individual trees (coloured lines) and the tree community (dashed black line). Dashed vertical lines in VIP and CV plots are peaks in the leaf age PLSR coefficients. The horizontal dashed line in the VIP plot represents the value at which VIPs are considered significant for the leaf age PLSR model. Leaf physiochemical absorption features corresponding to the coefficient peaks of the leaf age PLSR model are based on Knippling (1970), Curran (1989), Elvidge (1990) and Fourty *et al.* (1996).

PLSR reduces the large predictor matrix down to a relatively few, uncorrelated latent factors. As in Asner *et al.* (2011b; 2014; 2015) and Serbin *et al.* (2014), we performed a one-time randomised 70:30 split of our data into calibration and independent testing sets. We adopted a 1009 permutation 10-fold cross-validation (i.e. 1000 total permutations in which the calibration data set was further subdivided: 90% for calibration and 10% for validation) for each PLSR model. We evaluated the performance of PLSR models using two main metrics: R^2 and root mean square error (RMSE). We identified the optimal number of latent factors by selecting the model that minimised RMSE and maximised R^2 . The model performance was further characterised by using the independent testing set. We report the model performance for both the calibration (Cal) and independent testing (Val) sets and also provide the RMSE as a percentage of the sample mean (% RMSE) following Asner *et al.* (2011a; 2011b) (**Table 5.2.6.1**).

To examine the spectral link between leaf age and leaf traits, we regressed the leaf age spectral weights (w^* = PLSR spectral weights for each latent factor) for the first five PLSR latent factors against those of the leaf traits (and **Figure S 5.8.2**), and examined the variation explained by the PLSR latent factors for leaf age and traits (**Figure 5.2.6.5**). We also report the leaf age model PLSR spectral coefficients and variable importance of prediction (VIPs) (Fig. 4; Geladi & Kowalski, 1986; Wold *et al.*, 2001). PLSR analyses were performed using custom functions along with the MCS function from LibPLS (<http://www.libpls.net>) and the

PLSREGRESS function in Matlab (version R2014a; Mathworks Inc., Natick, MA, USA).

Table 5.2.6.1 Summary statistics and partial least squares regression (PLSR) modelling results for leaf age.

Tree	Leaf age (Weeks)									
	<i>N</i>	<i>M</i> (SD)	Range	<i>F</i>	<i>R</i> ²		RMSE		% RMSE	
					Cal	Val	Cal	Val	Cal	Val
T1	95	15 (17)	3-57	7	0.95	0.98	4.01	3.75	25	27
T3	120	20 (22)	1-51	7	0.93	0.91	5.70	6.00	22	25
T4	96	14 (21)	1-54	6	0.98	0.97	2.67	2.01	18	17
T6	114	16 (22)	1-54	8	0.97	0.94	4.12	3.96	23	27
T8	125	22 (23)	1-57	7	0.97	0.97	4.15	4.03	17	16
T10	75	33 (17)	1-57	8	0.96	0.96	3.43	3.15	11	10
T12	85	26 (25)	1-53	5	0.98	0.98	3.46	3.79	14	15
T13	121	28 (25)	1-57	8	0.97	0.94	4.19	4.75	20	22
All	1027	26 (23)	1-57	7	0.86	0.84	8.47	8.05	33	32

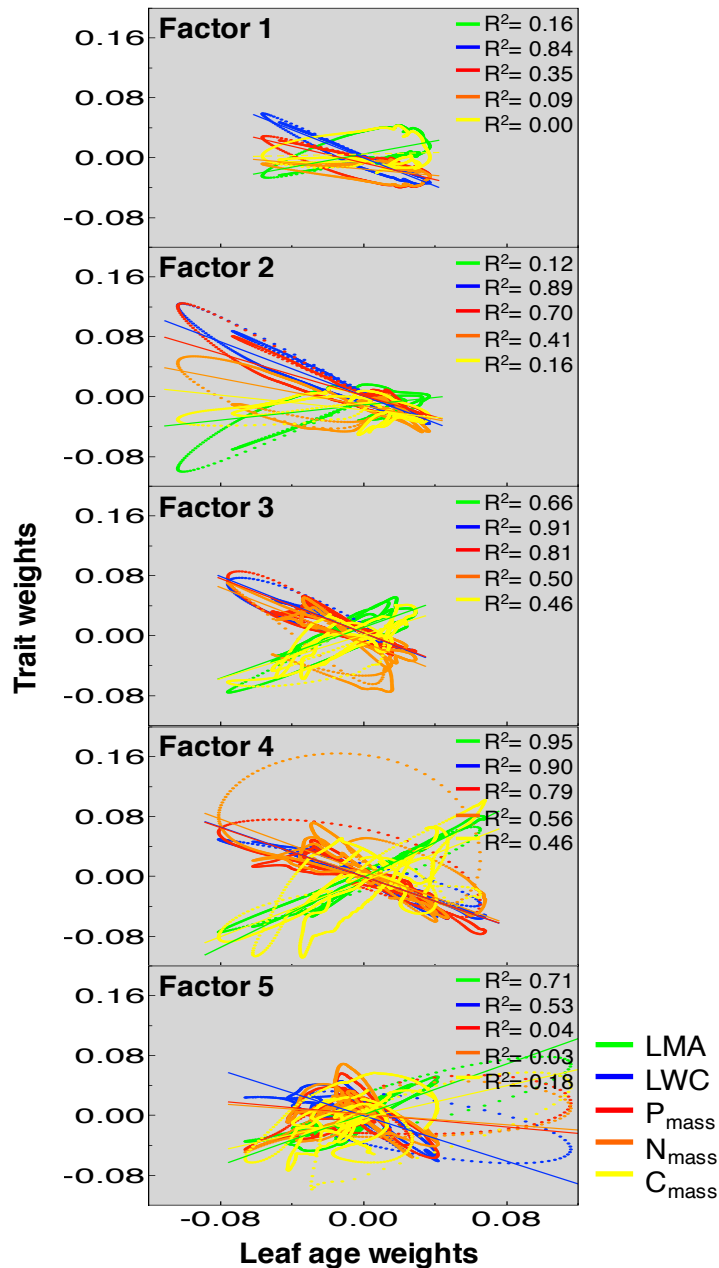


Figure 5.2.6.4 Relationships between leaf age spectral weights (w^*) and spectral weights for leaf mass per unit area (LMA), leaf water content (LWC), phosphorus content (P_{mass}), nitrogen content (N_{mass}) and carbon content (C_{mass}) (see also **Figure S 5.8.2**). Only spectral weights for the first five partial least squares regression (PLSR) latent factors (which cumulatively explain 83% of leaf age variation) are reported. Coloured data points and lines represent spectral weight values and regressions, respectively, between leaf age and the different leaf traits: LMA (green), LWC (blue), P_{mass} (red), N_{mass} (orange) and C_{mass} (yellow). Dashed regression lines are nonsignificant. All significant regressions were $P < 0.0001$.

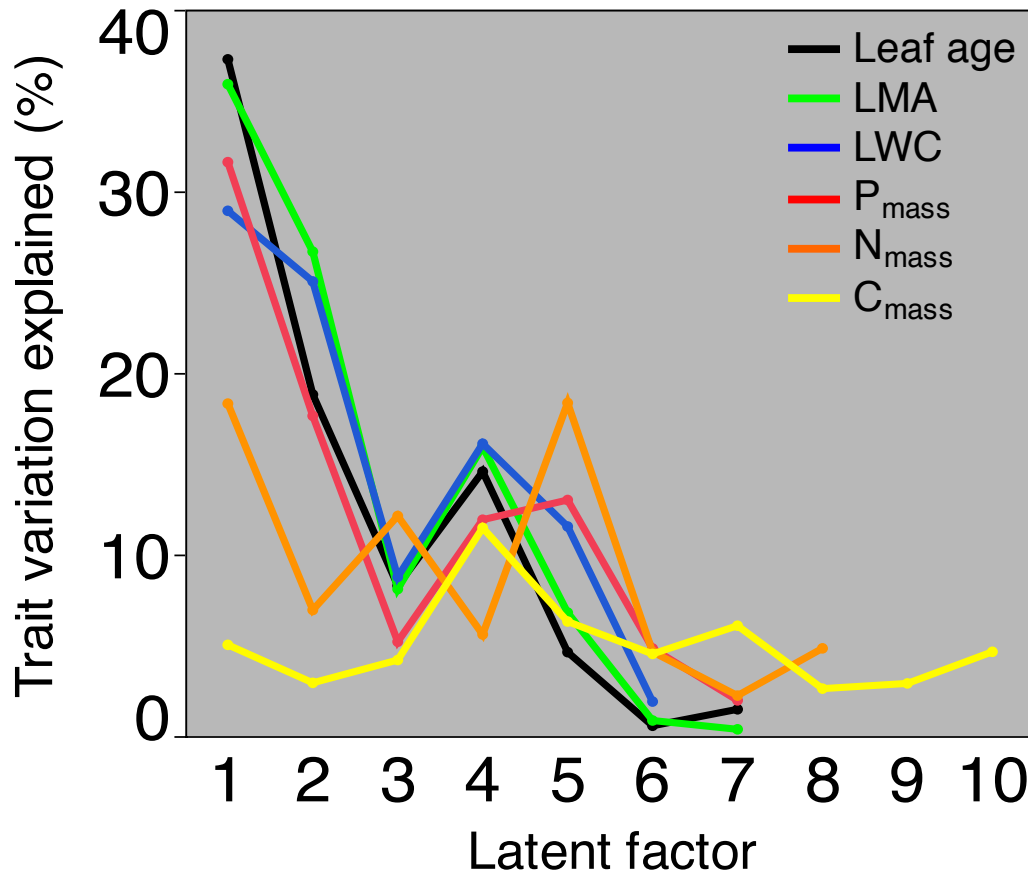


Figure 5.2.6.5 Per cent variation explained for leaf age, leaf mass per unit area (LMA), leaf water content (LWC), phosphorus content (P_{mass}), nitrogen content (N_{mass}) and carbon content (C_{mass}) by partial least squares regression (PLSR) latent factors.

Finally, we explored the effect that changes in leaf reflectivity attributable to age can have on VIs commonly used to measure vegetation greenness (normalised difference vegetation index (NDVI) and enhanced vegetation index (EVI)), light-use efficiency (photosynthetic reflectance index (PRI)) and water content (normalised difference water index (NDWI)). We used the two-band EVI (EVI2) as atmospheric corrections are not relevant to this study and derived variants of both NDVI and EVI2 using narrow bands (NB), broad bands (BB) and red edge bands (RE: spectral region between 680 and 780 nm produced by the

combination of strong absorption by leaf pigments in the red region and strong reflectance in the near infrared due to scattering in the leaf mesophyll and the absence of absorption by pigments; Woolley, 1971; Horler *et al.*, 1983). We calculated these VIs for each of our leaves using individual leaf spectra, the MODIS filter response functions (Xiong, 2015) and equations summarised in **Table S 5.8.2**. Mean values and confidence intervals were then calculated for each sampled leaf age class within individual trees and at the community level (i.e. across all 12 trees) (**Figure 5.2.6.6**). We report differences (measured in %change) in VIs attributable to age.

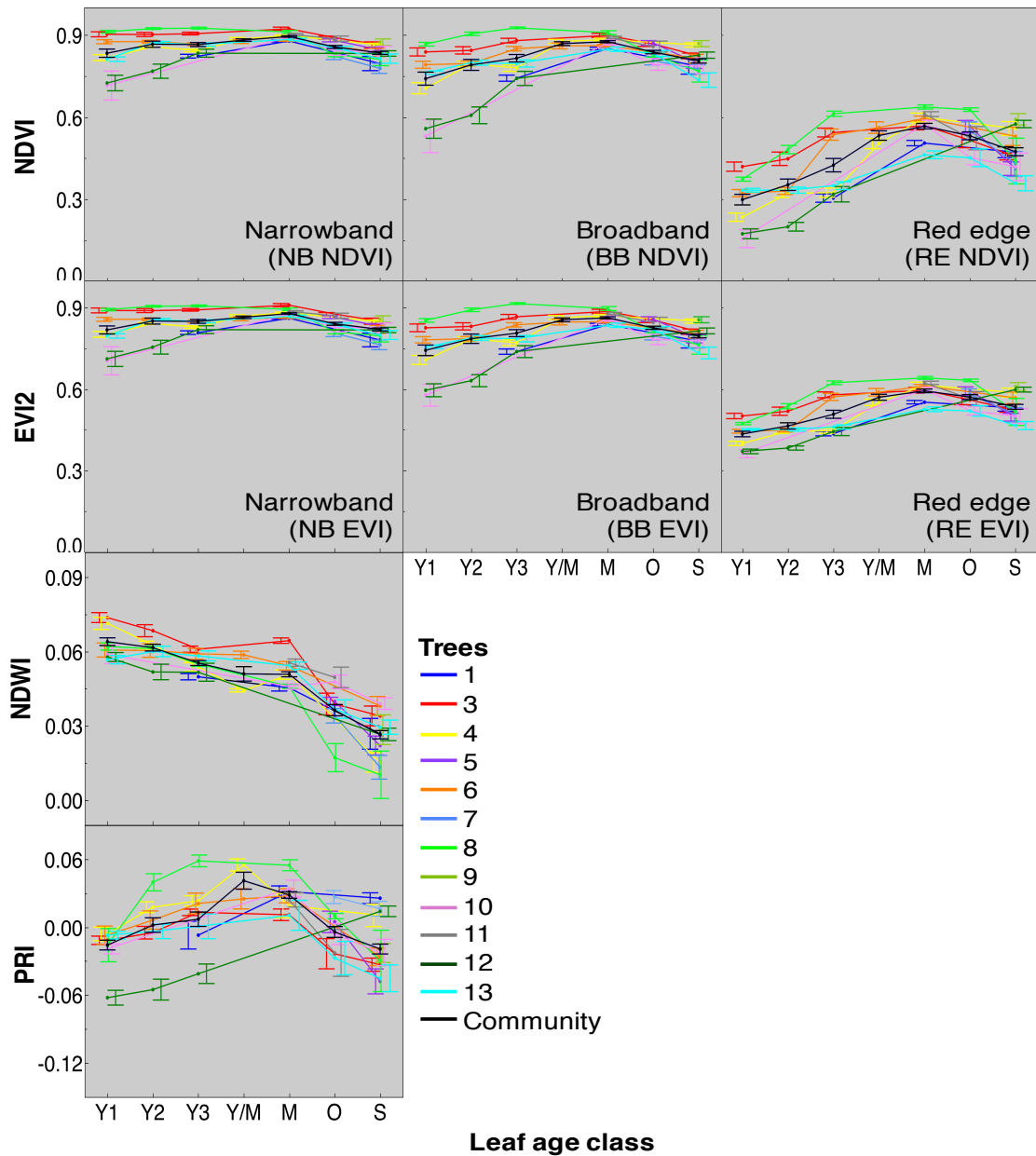


Figure 5.2.6.6 Variation in vegetation indices (VIs) commonly used to measure vegetation greenness (broadband normalised difference vegetation index (BB NDVI) and broadband enhanced vegetation index 2 (BB EVI2); narrowband (NB) NDVI and NB EVI2; and red edge (RE) NDVI and RE EVI2), water content (normalized difference water index (NDWI)) and light use efficiency (photosynthetic reflectance index (PRI)) attributable to leaf age. Mean values with 95% confidence intervals were calculated for each sampled leaf age class within individual trees (coloured lines) and across all trees (black lines). Leaf age classes: Y1, Y2, Y3 and young/mature (Y/M) are developing leaves collected after 1, 2, 3 and 4 wk of active leaf expansion, respectively; M, mature leaves; O, old leaves showing initial signs of senescence; S, senescent leaves in the process of dying and abscising.

5.3 Results

5.3.1 Age-dependent morphological and biochemical leaf properties

LWC, LMA and P_{mass} showed strong correlations with age: for LWC, $R^2 = 0.65$ – 0.93 in individual trees and $R^2 = 0.73$ across the community; for LMA, $R^2 = 0.62$ – 0.94 in individuals and $R^2 = 0.40$ across the community; for P_{mass}, $R^2 = 0.60$ – 0.93 in individuals and $R^2 = 0.44$ across the community; all P-values < 0.0001 (**Figure 5.2.6.1**; see also **Figure S 5.8.3**). N_{mass} displayed weaker age–trait relationships ($R^2 = 0.33$ – 0.87 in individuals and $R^2 = 0.13$ across the community; all P-values < 0.0001). C_{mass} showed both significant and nonsignificant age–trait relationships depending on individual trees. Age-related changes in leaf traits for our sampled trees have been extensively described and analysed in **Chapter 4**. In addition to varying age–trait relationships across different leaf traits, we found that relationships also varied across individual trees for both N_{mass} and C_{mass}. N_{mass} increased or decreased either logarithmically or linearly across trees, while C_{mass} either remained stable or increased linearly or decreased logarithmically with age. LWC, LMA and P_{mass} displayed similar age–trait relationships among individual trees, with LWC and P_{mass} displaying a logarithmic decrease and LMA a logarithmic increase.

Table 5.3.1.1 Summary statistics for leaf morphological and physiochemical traits by leaf age class (young/mature (Y/M) leaf age not included as only two trees were sampled for this age).

Leaf age class		Leaf traits				
		LMA (g m ⁻²)	LWC (%)	P _{mass} (%)	N _{mass} (%)	C _{mass} (%)
Y1	M (SD)	72.8 (25.1)	71 (5)	0.23 (0.08)	2.66 (1.03)	50.22 (2.29)
	Range	34.6 – 135.5	60 - 81	0.11 – 0.40	0.85 – 4.19	46.74 – 55.13
	N	121	121	40	40	40
Y2	M (SD)	85.4 (34.9)	68 (6)	0.22 (0.06)	2.77 (0.82)	49.66 (2.16)
	Range	37.6 – 187.4	55 - 79	0.10 – 0.31	1.46 – 4.08	45.46 – 53.60
	N	110	110	35	35	35
Y3	M (SD)	96.9 (26.4)	63 (5)	0.18 (0.06)	2.55 (0.69)	49.12 (1.60)
	Range	47.4 – 166.1	51 - 73	0.08 – 0.33	1.45 – 3.69	45.28 – 52.87
	N	110	110	35	35	35
M	M (SD)	122.5 (26.3)	53 (5)	0.11 (0.03)	2.19 (0.54)	50.33 (3.95)
	Range	76.1 – 199.7	44 - 62	0.06 – 0.19	1.40 – 3.68	45.04 – 59.72
	N	230	230	65	65	65
O	M (SD)	138.7 (28.3)	48 (5)	0.11 (0.04)	2.14 (0.34)	51.73 (4.58)
	Range	90.4 – 202.7	38 - 60	0.05 – 0.18	1.42 – 2.93	38.92 – 59.47
	N	150	150	50	50	50
S	M (SD)	137.0 (32.2)	48 (5)	0.11 (0.04)	2.16 (0.47)	50.65 (4.51)
	Range	79.4 – 210.5	34 - 58	0.04 – 0.18	1.07 – 2.89	35.83 – 57.39
	N	321	321	80	80	80
ALL	M (SD)	116.45 (37.73)	56 (10)	0.15 (0.07)	2.37 (0.68)	50.34 (3.72)
	Range	34.60 – 210.5	34 - 81	0.04 – 0.40	0.85 – 4.19	35.83 – 59.72
	N	1099	1099	315	315	315

5.3.2 Age-dependent spectral properties

We observed significant variation in both the amplitude and shape of leaf-level reflectivity as leaves aged, and also similar patterns in age-induced spectral changes in individual trees of various leaf types (**Figure 5.2.6.2**). The main

observed patterns of age related spectral changes were: visible (VIS; spectral domain: 390–700nm) reflectance decreased with leaf development until leaves reached maturity and then increased as leaves achieved old and/or senescent age status. Age-induced reflectivity changes in this spectral domain were especially pronounced at 550nm (green peak); NIR reflectance (spectral domain: 700-1400nm) increased as leaves expanded, significantly for low-wax leaves and only slightly for high-wax leaves. However, NIR reflectance collapsed in senescent leaves, with 3–10% decreases (across individual trees) in 700–850nm; shortwave-infrared (SWIR; spectral domain: 1450–2500nm) continuously increased as leaves aged in low-wax leaves, while high-wax leaves displayed less change, with structural carbon absorption features appearing at ~2000–2250nm during senescence; and red-edge (spectral domain: 650–810nm) shifted towards longer wavelengths as leaves expanded and reached maturity and towards shorter wavelengths during senescence. Differences in mean reflectance between mature leaves and all other leaf ages at individual tree level are reported in **Figure S 5.8.1**.

We also observed age effects in terms of reflectance variability (measured as CV) across individual trees (bottom graph in **Figure 5.2.6.3**). Reflectance variability was highest in the visible region (CV= 2–53%) and generally lowest in the NIR, except for the region between the red edge and 850nm where we observed CVs of up to 50%. Reflectance variability was also high in the SWIR 1

region (1450-1940nm; CV= 4–35%), with main water absorption features at c .1450 and 1940nm showing peaks in variability (CV= 4–18% and 7–40%, respectively). Furthermore, variability was higher in the SWIR 2 (1940–2450nm) than in the SWIR 1 region (CV= 4–35% versus 1–11%, respectively). We found no consistent leaf age effect on CVs across the whole spectrum (**Figure S 5.8.4**).

5.3.3 Spectra-based leaf age model

We treated leaf age as an important but spectrally unexplored leaf property, and applied PLSR analyses to model leaf age from leaf level hyperspectral measurements. We tested this spectra-based age model at both individual tree and community levels. Our results revealed that leaf spectra could accurately and precisely be used to predict leaf age within individual trees ($R^2 = 0.91–0.98$; %RMSE= 10–27 in individual trees), with leaf age spanning from 1 to 57 weeks (**Table 5.2.6.1**). Leaf-level spectra could also predict leaf age across all 12 trees ($R^2 = 0.86$; %RMSE= 33). Furthermore, the number of optimal latent factors for the spectral age models showed little variation for individual trees (from five to eight factors) and for the community-level model (seven factors). These results confirmed that leaf-level spectra could be used to classify leaves into young, mature and senescent leaf ages at both individual tree and community levels.

5.3.4 Trait-based leaf age model

Results from the ‘Age-dependent morphological and biochemical leaf properties’ subsection indicate that there may be correlations among traits, in particular between LMA and LWC (**Figure S 5.8.5**), which makes their relative importance in modelling leaf age difficult to establish. We therefore performed two separate step-wise (forward) regression analyses excluding one of these traits in each model. The best leaf age model was Model 1 (LMA excluded), which included LWC, N_{mass} and C_{mass} , respectively, and predicted the variation of leaf age across trees with an accuracy of $R^2 = 0.76$ and a precision of %RMSE= 32 (**Table 5.3.4.1**). Model 2 (LWC excluded) included P_{mass} , LMA and N_{mass} , respectively, and predicted the variation of leaf age across species with both a lower accuracy of $R^2 = 0.50$ and precision of %RMSE= 46 (**Table 5.3.4.1**).

These results indicated that LWC was the single most important leaf trait for modelling leaf age, with a contribution to the leaf age prediction of $R^2 = 0.73$, while P_{mass} and LMA, the next most important traits, had contributions of $R^2 = 0.45$ and $R^2 = 0.05$, respectively. Both N_{mass} and C_{mass} had negligible contributions (see **Table 5.3.4.1**). These results agreed with the analysis based on single leaf traits for the subset of eight trees for which we had multiple leaf age data (see the ‘Age-dependent morphological and biochemical leaf properties’ subsection and **Figure 5.2.6.1**), where we observed that LWC displayed the strongest trait–age relationship, followed by P_{mass} and LMA. Furthermore, due to negative correlation

between LMA and LWC (see **Figure S 5.8.5**), most of the contribution of LMA to leaf age prediction is included within the LWC contribution to the model.

Table 5.3.4.1 Results of step-wise (forward) regression modelling for leaf age (log-transformed) using leaf water content (LWC), phosphorus content (P_{mass}), nitrogen content (N_{mass}) and carbon content (C_{mass}) as predictor parameters for Model 1 and leaf mass per unit area (LMA), P_{mass} , N_{mass} and C_{mass} for Model 2.

Model 1 summary	Model step	Selected parameters	R^2	P
$R^2 = 0.76$	1	LWC	0.7339	< 0.0001
RMSE = 2.68	2	N_{mass}	0.7487	< 0.0001
%RMSE = 32	3	C_{mass}	0.7584	0.0003
AICc = 735.90				
Model 2 summary	Model step	Selected parameters	R^2	P
$R^2 = 0.50$	1	P_{mass}	0.4484	< 0.0001
RMSE = 3.81	2	LMA	0.5019	< 0.0001
%RMSE = 46	3	N_{mass}	0.5042	0.2269
AICc = 939.40				

5.3.5 Covariance in spectra-age and spectra-trait models: a mechanistic basis for the spectral age model

An analysis of the relationship between the PLSR model weights for leaf age and the five conventional leaf traits across the first five PLSR latent factors (**Figure 5.2.6.3** and **Figure S 5.8.2**) revealed that the strongest relationship was found

between leaf age and LWC weights across all five factors ($R^2 = 0.53\text{--}0.91$; all P-values <0.0001), followed by LMA ($R^2 = 0.12\text{--}0.95$; all P-values <0.0001) and P_{mass} ($R^2 = 0.04\text{--}0.81$; all P-values <0.0001). Factor weights for both N_{mass} ($R^2 = 0.03\text{--}0.56$; all P-values <0.0001) and C_{mass} ($R^2 = 0.16\text{--}0.46$; all P-values <0.0001 with one nonsignificant relationship) showed weaker relationships. Furthermore, the variation explained for leaf age and each of the leaf traits by these PLSR latent factors showed similar patterns as those of the regression analysis of the spectral weights (**Figure 5.2.6.5**). The patterns of variation explained by each latent factor suggest close spectral relationships between leaf age, LWC, LMA and P_{mass} , while the patterns of variation explained for both N_{mass} and C_{mass} indicated that they might provide different, but complementary, information to the overall spectral age relationship.

5.3.6 Vegetation indices change with leaf age

The results of VI calculations for leaves of different ages revealed that all leaf-level VIs analysed in this study were age dependent, including indices of water content (NDWI), greenness (broadband, narrowband, and red edge NDVI and EVI2) and light-use efficiency (PRI) (**Figure 5.2.6.6**). Across all trees, the NDWI displayed high sensitivity to leaf age with a continuous decrease in values with age, while the greenness VIs and PRI initially increased with leaf development (from youngest to mature cohorts), and then declined when leaves were at old and senescent stages. Among the greenness VIs, the red edge NDVI (RE NDVI)

and EVI2 (RE EVI2) were the most sensitive to leaf age, with the youngest leaves displaying mean decreases of 26–72% and 14–39%, respectively, and senescent leaves showing mean decreases of 7–31% and 4–20%, respectively, relative to mature leaves for individual trees. The broadband NDVI (BB NDVI) and EVI2 (BB EVI2) displayed mean decreases of 4–39% and 5–33% (for youngest leaves), respectively, and 3–15% (for senescent leaves) for individual trees. The narrow band NDVI (NB NDVI) and EVI2 (NB EVI2) displayed much smaller mean decreases of 1–20% (for the youngest leaves) and 4–11% (for senescent leaves). However, the most dramatic sensitivity to leaf age was observed for the PRI, which decreased on average 133–200% in the youngest leaves and 50–300% for senescent leaves for individual trees. At the community level (across all trees), NDWI values were on average 22% higher for the youngest and 43% lower for senescent leaves compared with mature leaves. RE NDVI and EVI2 were 47% and 33% lower for the youngest leaves, respectively, and 18% lower for senescent leaves. BB NDVI and EVI2 values were 16% and 22% lower for the youngest leaves and 9% and 11% lower for senescent leaves, respectively, while NB NDVI and EVI2 values were also lower for both the youngest and senescent leaves (7% and 11%, respectively). PRI displayed the lowest values for both the youngest (167%) and senescent leaves (200%). The different sensitivities we found in the greenness VIs (NDVI and EVI2) when derived using different spectral bands/regions (see **Table S 5.8.2**) demonstrate that, whereas the narrow band VIs capture too little of the spectral leaf age

signal, the broad band VIs miss the spectral domain that has a maximum correlation with leaf age – the RE (see top graph in **Figure 5.2.6.3**). The red edge VIs capture this spectral domain, and thus are the most sensitive to leaf age.

5.4 Discussion

To our knowledge, our study is the first to comprehensively analyse the morphological (LMA), biochemical (LWC, P_{mass} , N_{mass} and C_{mass}) and spectral leaf traits of canopy and emergent tropical tree species during natural (*in situ*) leaf ageing. Our leaf samples represent a range of leaf types (e.g. compound and simple leaves), sizes, shapes, thicknesses and waxiness. This, together with the variation of these leaf traits across the leaf lifecycle (see **Table 5.3.1.1**), resulted in a data set that covered large parts of the global leaf trait variability reported for mature leaves. To put our data set into perspective, LMA (34.6– 214.2 g m⁻²), LWC (34–81%), P (0.04–0.40%), N (0.85–4.19%) and C (36–60%) exhibited ranges that cover 53, 89, 50, 67 and 100%, respectively, of the values reported by Asner *et al.* (Asner *et al.*, 2011a; Asner *et al.*, 2011b) for 6136 humid tropical forest trees and also covered large parts of the ranges reported by others previously for global leaf trait variation (Wright *et al.*, 2004; Poorter *et al.*, 2009). The high variability found in this leaf phenological data set highlights the importance of considering leaf age in biochemical, physiological and spectral leaf trait studies, which could have wider implications across forests at the global scale.

Variation of leaf spectral characteristics with age and their relationship to leaf structure and biochemistry. Consistent with previous studies in both tropical forests (Kitajima *et al.*, 1997; Kitajima *et al.*, 2002) and nontropical forests (Field & Mooney, 1983; Escudero & Mediavilla, 2003; Niinemets & Kull, 2003; Niinemets *et al.*, 2004a; Niinemets *et al.*, 2005; Mediavilla *et al.*, 2011), our data show strong age effects for leaf traits within individual trees, with more diverse effects at the tree community level. Our results demonstrate that leaf traits such as LMA, LWC and P_{mass} display age-dependent monotonic increases (LMA) or decreases (LWC and P_{mass}) across individual trees, with the largest change in these traits happening during leaf expansion. However, interestingly, both N_{mass} and C_{mass} displayed tree specific relationships with age. We also show that these trait–age relationships mostly remained significant even when young leaves were not included in the analyses (see **Figure S 5.8.3**). These results suggest that reconstructing life histories of multiple physiochemical leaf properties could be possible and that a single calibration model may suffice for LWC, LMA and P_{mass} , while a general model for N_{mass} and C_{mass} could be more challenging. The sampling of traits through their leaf lifecycle for a much wider range of leaf types could allow us to better calibrate for these trait life histories. However, the variation within the same species remains to be explored, and we cannot infer relationships for particular species because of a lack of replication within species in our dataset. Furthermore, as in Kitajima *et al.* (Kitajima *et al.*, 1997; Kitajima *et al.*, 2002), our results suggest that structural limitations to photosynthesis as a

result of increased LMA (which has been associated with increases in total structural carbohydrates and lignin, leaf thickness and density; Niinemets *et al.*, 1999; Poorter & Bongers, 2006; Villar *et al.*, 2006) and biochemical limitations to photosynthetic capacity may be age-dependent. Thus, introducing leaf ageing as a major axis of leaf trait variation would facilitate predictions of seasonal variation in photosynthesis in tropical forests.

Leaf-level spectral properties also show strong age dependence, with the most age-sensitive spectral domains found to be: green peak (550nm), red edge (680–780nm), NIR (700–850nm), and around the main water absorption features (~1450 and 1940nm) across species. This is because the age-dependent changes in morphological and biochemical leaf traits we described earlier have a direct influence on the spectral properties of leaves (Carter *et al.*, 1989; Miller *et al.*, 1991; Roberts *et al.*, 1998; Datt, 1999; Sims & Gamon, 2002). Across all trees, reflectance in the visible spectrum (VIS; 412–700nm) which is dominated by pigment (chlorophylls, anthocyanins and carotenoids) light absorption (Knipling, EB, 1970; Elvidge, 1990) and controlled by the amount and status of these pigments (Billings & Morris, 1951), showed a concave response with age (**Figure 5.2.6.2**). Reflectance decreased continuously across this spectral domain as chlorophyll developed in young leaves, with the most marked changes observed at the green peak (550nm); following maturity, reflectance then increased throughout the VIS but with marked increases at ~570–630nm as

chlorophyll (the main photosynthetic pigment) broke down relatively rapidly compared with carotenoids during senescence (Biswall, 1995). In agreement with previous studies by Horler *et al.* (1983), Boochs *et al.* (1990) and Roberts *et al.* (1998), we observed red edge shifts attributable to leaf age. NIR reflectance (700–1400nm) showed differences in behaviour between high-wax and low-wax leaves. Low-wax leaves displayed a monotonic increase in NIR reflectance until maturity, while high-wax leaves displayed little change in this spectral domain until leaves achieved senescence. Observed NIR increases are associated with the development of the complex internal cellular structure in young leaves whereby photon scattering and reflectance increase as the number of air–cell wall–water interfaces within leaves increases (Gausman, H. W. *et al.*, 1970; Woolley, 1971). However, epicuticular waxes and/or thicker cuticles (which have been shown to enhance NIR reflectance by Reicosky & Hanover (1978) and Mulroy (1979), respectively) appear to mask the effect of intercellular development in young high-wax leaves. During senescence, however, both high-wax and low-wax leaves displayed a marked collapse in NIR reflectance between 700 and 850nm due to decreased photon scattering as a result of the breakdown of intercellular structure as leaves senesced. The shortwave infrared spectral domain (SWIR; 1400–2500nm: dominated by water absorption and biochemical features related to proteins and nitrogen, cellulose, lignin, and other carbon constituents; Woolley, 1971; Gao & Goetz, 1995; Fourty *et al.*, 1996; Kokaly *et al.*, 2009; Asner *et al.*, 2015) displayed similar patterns to the NIR. In low-wax

leaves, SWIR reflectance showed clear monotonic increase as LWC decreased with age, while waxes and/or cuticles also appeared to mask the decreasing LWC signal in high-wax leaves, which displayed little change until senesce.

The age effects in leaf reflectivity we have described could have potentially significant impacts for spectral species classification and many of the VIs commonly used to monitor vegetation productivity (e.g. PRI) and greenness (e.g. EVI and NDVI). In the following section, we offer an innovative hyperspectral methodology that can be used to predict leaf age and to disentangle drivers of intraspecific trait and spectral variation. We offer this method as a first step in separating the age signal from species spectral data used for classification and identifying seasonal patterns in VIs attributable to leaf age.

5.4.1 Spectral leaf age model: underlying mechanisms

The results of our PLSR modelling demonstrate the ability of spectroscopic data to characterise leaf age for individual trees ($R^2 = 0.91\text{--}0.98$; %RMSE = 10–27 across trees) and across a canopy tree community ($R^2 = 0.86$; %RMSE = 33) with a wide range of different leaf types (**Table 5.2.6.1**). We found that trees with leaves of different spectral properties aged in a similar manner (**Figure 5.2.6.2**) that could be captured by a single calibrated model. We observed higher model performance for individual trees than across the tree community. Model performance could be improved with more extensive sampling within two leaf types highlighted by our study: a gradient of leaf waxiness as this trait appears to interfere with the age spectral signal in the NIR and SWIR in high-wax leaves, and a gradient of LWC/LMA given the strong correlation between these two traits and their coupling with leaf structure and age. Our results reinforce and, more importantly, extend the utility of spectroscopic methods to model leaf age.

Unlike using leaf traits individually (**Figure 5.2.6.1**) or as multiple predictors in a step-wise regression model (**Table 5.3.4.1**) to predict leaf age, our PLSR approach captures the simultaneous contribution of many important absorption properties of leaves (**Figure 5.2.6.3**) across the leaf spectrum, yielding a much more robust model. The spectral domains of greatest variance (calculated as CVs) among our sampled trees and thus potentially containing the most information related to variation in leaf age were captured by both the PLSR

coefficients (C) and the variable importance of prediction (VIP) (**Figure 5.2.6.3**). Furthermore, the highly significant individual trait– age relationships, the step-wise regression and the PLSR analyses demonstrate that LWC is the primary trait related to leaf age and that the residual differences between predicted leaf age and LWC might be explained by complementary information (in descending order of importance) from LMA, P_{mass} , N_{mass} and C_{mass} .

The strong spectral relationships between leaf age and LWC are evidenced in the high leaf age PLSR coefficient peaks we observed at the water absorption features (970, 1450 and 1940nm). Spectral relationships between leaf age and LMA are related to the changing internal leaf cellular structure with age and expressed in PLSR coefficient peaks in the NIR (with particularly high values for 700–850nm, a spectral region found to have a positive relationship with leaf thickness by Knapp & Carter (1998), and to be a function of the percentage of intercellular air space in the mesophyll and the ratio of mesophyll surface area to leaf area by Slaton *et al.* (2001), Aasamaa *et al.* (2005) and Castro & Sanchez-Azofeifa (2008)). The relationship between leaf age and P_{mass} and N_{mass} is related to the build-up during development, functioning during maturity and collapse during senescence of the leaf photosynthetic apparatus, these relationships are reflected in PLSR coefficients peaks at the VIS (particularly the green peak at 550nm), RE (680–750nm) and SWIR (1510, 1690, 1940 and 2240nm). The spectral leaf age and C_{mass} relationship is reflected in PLSR

coefficient peaks at 1420–1450, 1490, 1690, 1820, 1940 and 2340nm and related to structural carbohydrates: polyphenolics and cellulose. Similar patterns of age-related variation in leaf reflectivity (**Figure 5.2.6.2** and **Figure 5.2.6.3**) to those we discuss in subsection ‘Variation of leaf spectral characteristics with age and their relationship to leaf structure and biochemistry’ have also been reported for nontropical species (Dillen *et al.*, 2012; Yang *et al.*, 2014). Therefore, variations of our spectral age model could potentially apply to leaves from other tropical and nontropical forests. Furthermore, the use of hyperspectral remote sensing to predict leaf age could be a powerful indirect method for estimating leaf traits that are a function of leaf age. Calibration with a wider range of leaf types/species will, of course, be required, but there is a tantalizing possibility that calibration will need to be carried out just once to establish the minima and maxima for groups of leaf types/species within gradients of waxiness and LWC/LMA.

5.4.2 Leaf-level age-dependent vegetation indices: impacts on ecosystem processes and satellite remote sensing

All leaf-level VIs we analysed, which measure different aspects of leaf morphological and biochemical properties, showed age dependence at both the individual tree and community levels (**Figure 5.2.6.6**). Red edge-derived greenness VIs were most sensitive to the leaf age signal, supporting the PLSR spectral-age model high coefficients and VIPs found for this spectral domain

(**Figure 5.2.6.3**). It could be an interesting exercise to derive pseudo-RE NDVI and EVI using MODIS bands 14 and 15 and explore their seasonal variation compared with those of the traditional MODIS NDVI and EVI.

Age-dependent VIs in this study were derived from top-of-canopy sun leaves, which drive the spectral characteristics of the upper tropical canopy (Clark *et al.*, 2005; Asner & Martin, 2008a). This suggests that the age-dependent leaf reflectance of top-of-canopy leaves can significantly influence remote sensed vegetation indices. Indeed, the decreases in community-level VI values (PRI= 200%; NDWI= 43%; RE NDVI and RE EVI2 = 18% for both VIs) we observed in senescent leaves relative to newly matured dry season leaves are well above or similar to the 25% dry season increase in the MODIS EVI reported by Huete *et al.* (2006a) across seasonally dry Amazon forests. Furthermore, field-based studies (Doughty & Goulden, 2008; Brando *et al.*, 2010; Chave *et al.*, 2010; Malhi *et al.*, 2014) have shown that many trees in tropical evergreen forests exchange their leaves in and around the dry season. Canopy LAI, however, does not necessarily change with season, as most trees lose and flush leaves before and during the dry season asynchronously within and across species. Canopy-level VIs will, therefore, be influenced by both leaf quantity and age distribution. Consequently, in the dry season, canopy VIs would reflect the mixture of senescent (i.e. leaves with lower VIs from trees that have not yet exchanged their leaves), young (i.e. new developing leaves with rapidly increasing VIs) and

mature leaves (i.e. newly matured leaves with higher Vis attained during the dry season) present. After the dry season, most leaves in the canopy would probably be mature, however, this does not imply a stable canopy VI. As mature leaves age and evolve towards senescence, their morphological, biochemical, and thus spectral properties gradually change towards lower VIs before the dry season. Our findings therefore support previous studies by Huete *et al.* (2006a), Doughty & Goulden (2008) and Brando *et al.* (2010) that propose that seasonality observed in MODIS EVI and NDVI may be attributable to flushes of new leaf growth in the dry season.

Increasing epiphyll cover with leaf age (Roberts *et al.*, 1998; Toomey *et al.*, 2009) has been suggested to potentially affect remotely sensed VIs. However, from the rare epiphyll cover we encountered in our field observations from this site and another two sites in French Guiana and Brazil (C. Chavana-Bryant, pers. comm.), it seems that epiphyll effects on satellite-based VIs may be negligible.

5.5 Conclusions

This study demonstrates the significant contribution of leaf age to variation in leaf morphological, biochemical and spectral traits within individual Amazonian canopy trees. The age-related leaf trait variations we report here have two important implications: trait variation attributable to leaf age within individual trees can generate as much variation as intra- and interspecific variation; and leaf age

differences could potentially account for a significant fraction of what we currently understand as intra- and interspecific leaf trait variation. However, leaf age has historically been difficult to measure. This work proposes a simple and efficient approach for predicting and monitoring leaf age in lowland tropical forests using hyperspectral data.

Our proposed spectral age model will need to be further tested at other sites and with wider data sets and canopy-level modelling. However, the results of the testing of our spectral age model at a different Amazonian forest site (Wu *et al.*, 2016b) provides support for the generality of our spectral age model for sun leaves of tropical forests.

Finally, this study is the first to directly investigate the effect of leaf age on commonly used VIs such as NDVI, EVI2, NDWI and PRI. We demonstrate that, at the leaf-level, these VIs display age sensitivity, with NDWI and PRI displaying high sensitivity. This observed sensitivity, and its timing, suggest that seasonal variations in remotely sensed VIs previously reported for Amazonian tropical forests may be strongly influenced by leaf age effects, although seasonal variation in canopy three-dimensional structure may also be important. Our findings suggest that tropical canopy spectral signatures may have relatively tractable and quantifiable variation with leaf age, opening up the possibility of tracking their leaf demography through remote sensing.

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5.8 Supporting information

Supporting Information for manuscript: “Leaf aging of Amazonian canopy trees as revealed by spectral and physiochemical measurements”.

Methods S 5.8.1

Branch sampling

Sampled trees were monitored frequently for bud development, new leaf emergence, general leaf condition and signs of leaf senescence. During developmental leaf phases, individual trees were sampled on a weekly basis for up to four consecutive weeks. During the mature leaf phase, individual trees were sampled for up to three consecutive months, while during the senescent leaf phase, individual trees were sampled for up to two months for old leaves and up to three months for senescent leaves.

Two top-of-canopy (fully exposed to direct sunlight) apical metre-long branches were collected each time trees were sampled. Sampled branches were cut, labelled, promptly lowered to the ground and recut under water to maintain hydraulic connectivity and minimise leaf desiccation during transport to the field lab, where leaf sampling and morphological (fresh leaf weight and area) measurements were carried out within two hours of the branches being cut. Demographic leaf counts were also collected for all sampled branches.

Leaf traits

We measured an important morphological trait – leaf mass per area (LMA). LMA is the ratio of the dry mass of a leaf to its surface area, it is intimately connected to the resource use economy of the plant (Reich et al., 1997) as it can be understood as the leaf-level cost of light interception (Gutschick & Wiegel, 1988). LMA is, therefore, a key trait indicative of plant physiological processes ranging from light capture (Niinemets et al., 1999) to growth rates (Poorter et al., 2009) as well as plant life strategies (Westoby et al., 2002).

In terms of biochemical traits, we measured leaf water- (LWC), nitrogen- (N_{mass}), phosphorous- (P_{mass}) and carbon content (C_{mass}). LWC is a useful indicator of plant water balance, since it expresses the relative amount of water present on the plant tissues and it is intimately related to several leaf physiological variables, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration (Kramer & Boyer, 1995). Nitrogen is an integral component to the proteins of the photosynthetic machinery responsible for drawdown of carbon dioxide (CO_2) inside the leaf while phosphorus, being a fundamental component of nucleic acids, lipid membranes and bioenergetic molecules such as ATP, is an essential nutrient in photosynthetic carbon assimilation and protein synthesis (Field, C. & Mooney, H. A., 1986). Thus, both N_{mass} and P_{mass} have been directly linked to rates of photosynthetic activity in leaves (Raaimakers et al., 1995; Evans & Poorter, 2001; Reich et al., 2008, among others). Carbon is a major

component in cellulose and lignin, which are used to build the cell walls of leaf tissues (Kokaly et al., 2009).

Leaf trait measurements

We randomly selected a large number of replicates (15 to 30 leaves) for each leaf age class we sampled. Leaf petioles were removed and leaves then measured for thickness (LT) using a digital caliper and fresh weight using a high-precision balance with a 0.01g resolution (My Weigh, model: Durascale). Groups of individually identified leaves were then colour scanned at 300 dpi resolution. ImageJ software (NIH, New York City, NY, USA) was used to calculate individual leaf area (LA). Scanned leaves were then individually placed in labeled paper bags and dried at 70°C for 72 hours before dry weight (LM) was determined for each leaf. LWC, LMA, leaf volume (LV) and leaf tissue density (LTD) were derived from these data as follows:

$$\% \text{ LWC} = \left(\frac{\text{fresh mass} - \text{dry mass}}{\text{fresh mass}} \right) \times 100$$

$$\text{LMA} = \frac{\text{dry mass}}{\text{area}}$$

$$\text{LV} = \left(\frac{\text{thickness}}{10} \right) \times \text{area}$$

$$\text{LTD} = \frac{\text{dry mass}}{\text{volume}}$$

where: leaf fresh and dry mass are in grams (g), leaf area in square metres (m²) for LMA calculations and in square centimetres (cm²) for leaf volume

calculations, leaf thickness in millimetres (mm) and leaf volume in cubic centimetres (cm³).

For *Simarouba amara*, a species with compound leaves, leaf trait values were measured based on independent leaflets rather than the entire compound leaf as the leaflet green leaf portion is comparable to that measured in whole leaves for other species.

Nutrient content for all leaves were measured in the lab at the University of Arizona. For each tree, five dry leaves out of each leaf age class were randomly selected for chemical analysis. All dry leaf samples were then homogenised and ground to a fine powder. Total phosphorous (P) content per leaf was determined using persulfate oxidation followed by the acid molybdate technique (APHA, 1992). Phosphorus concentration was then measured colourimetrically with a spectrophotometer (Genesys20, Thermo Fisher Scientific, Madison, WI, USA). Phosphorous (P), nitrogen (N) and carbon (C) contents were measured by the Department of Geosciences Environmental Isotope Laboratory at the University of Arizona using a continuous-flow gas-ratio mass spectrometer (Finnigan Delta Plus XL, Thermo Fisher Scientific, Madison, WI, USA) coupled to an elemental analyser (Costech Analytical Technologies, Valencia, CA, USA). Samples of 1.0 mg (+/- 0.1 mg) were combusted in the elemental analyser.

Table S 5.8.1 Leaf sampling scheme by tree, canopy position and leaf age class. Leaf age classes: Y1, Y2, Y3 and young/mature (Y/M) are developing leaves collected after 1, 2, 3 and 4 wk of active leaf expansion, respectively; M, mature leaves; O, old leaves showing initial signs of senescence; S, senescent leaves in the process of dying and abscising.

Tree codes	Canopy position	Y1	Y2	Y3	Y/M	M	O	S	Total leaves by tree
1	SU	15	15	20		60		15	125
3	SU	30	30	15		15	30	30	150
4	SU	16	15	15	15	15		20	96
5	SU						30	50	80
6	SU	15	20	15	15	20		29	114
7	SU							33	33
8	SU	15	15	15		30	30	20	125
9	SU							20	20
10	SU	15				20	20	20	75
11	SU					40	20		60
12	SU	15	15	15		15		40	100
13	SU	15	15	15		15	20	41	121
Total leaves by age class		136	125	110	30	230	150	318	1099

Table S 5.8.2 List of vegetation indices (VIs) and corresponding equations used in this study.

Vegetation index	Acronym	Equations	
Narrow band normalised difference vegetation index	NB NDVI	$\frac{\rho_{800} - \rho_{680}}{\rho_{800} + \rho_{680}}$	where ρ_{800} = measured percent reflectance at 800nm and ρ_{680} = measured percent reflectance at 680nm
Narrow band enhanced vegetation index 2	NB EVI2	$2.5 \times \frac{\rho_{800} - \rho_{680}}{\rho_{800} + 2.4 \times \rho_{680} + 1}$	
Broad band normalised difference vegetation index	BB NDVI	$\frac{NIR - RED}{NIR + RED}$	where NIR= measured reflectance of MODIS NIR broadband (819 to 898 nm) using the MODIS filter response functions and RED= measured reflectance of MODIS RED broadband (614 to 682nm) using the MODIS filter response functions.
Broad band enhanced vegetation index 2	BB EVI2	$2.5 \times \frac{NIR - RED}{NIR + 2.4 \times RED + 1}$	
Red edge normalised difference vegetation index	RE NDVI	$\frac{\rho_{750} - \rho_{705}}{\rho_{750} + \rho_{705}}$	where ρ_{750} = measured percent reflectance at 750nm and ρ_{705} = measured percent reflectance at 705nm
Red edge enhanced vegetation index 2	RE EVI2	$2.5 \times \frac{\rho_{750} - \rho_{705}}{\rho_{750} + 2.4 \times \rho_{705} + 1}$	
Normalised difference water index	NDWI	$\frac{\rho_{857} - \rho_{1240}}{\rho_{857} + \rho_{1240}}$	where ρ_{857} = measured percent reflectance at 857nm and ρ_{1240} = measured percent reflectance at 1240nm
Photosynthetic reflectance index	PRI	$\frac{\rho_{531} - \rho_{570}}{\rho_{531} + \rho_{570}}$	where ρ_{531} = measured percent reflectance at 531nm and ρ_{570} = measured percent reflectance at 570nm

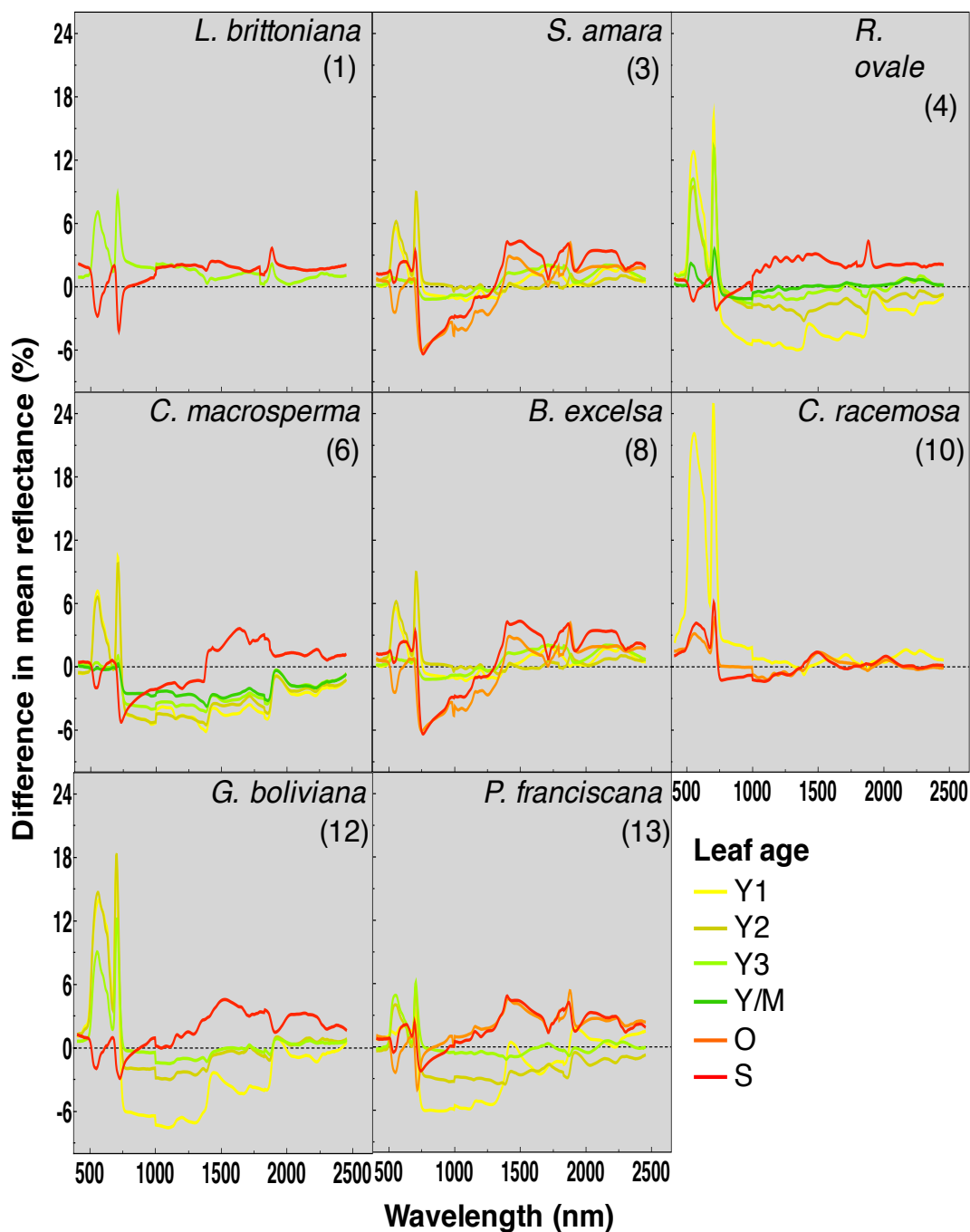


Figure S 5.8.1 Difference in mean reflectance between mature leaves and other leaf age classes for sun leaves of eight Amazonian canopy and emergent trees. Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in this figure. Leaf age classes: Y1, Y2, Y3 and young/mature (Y/M) are developing leaves collected after 1, 2, 3 and 4 wk of active leaf expansion, respectively; M, mature leaves; O, old leaves showing initial signs of senescence; S, senescent leaves in the process of dying and abscising.

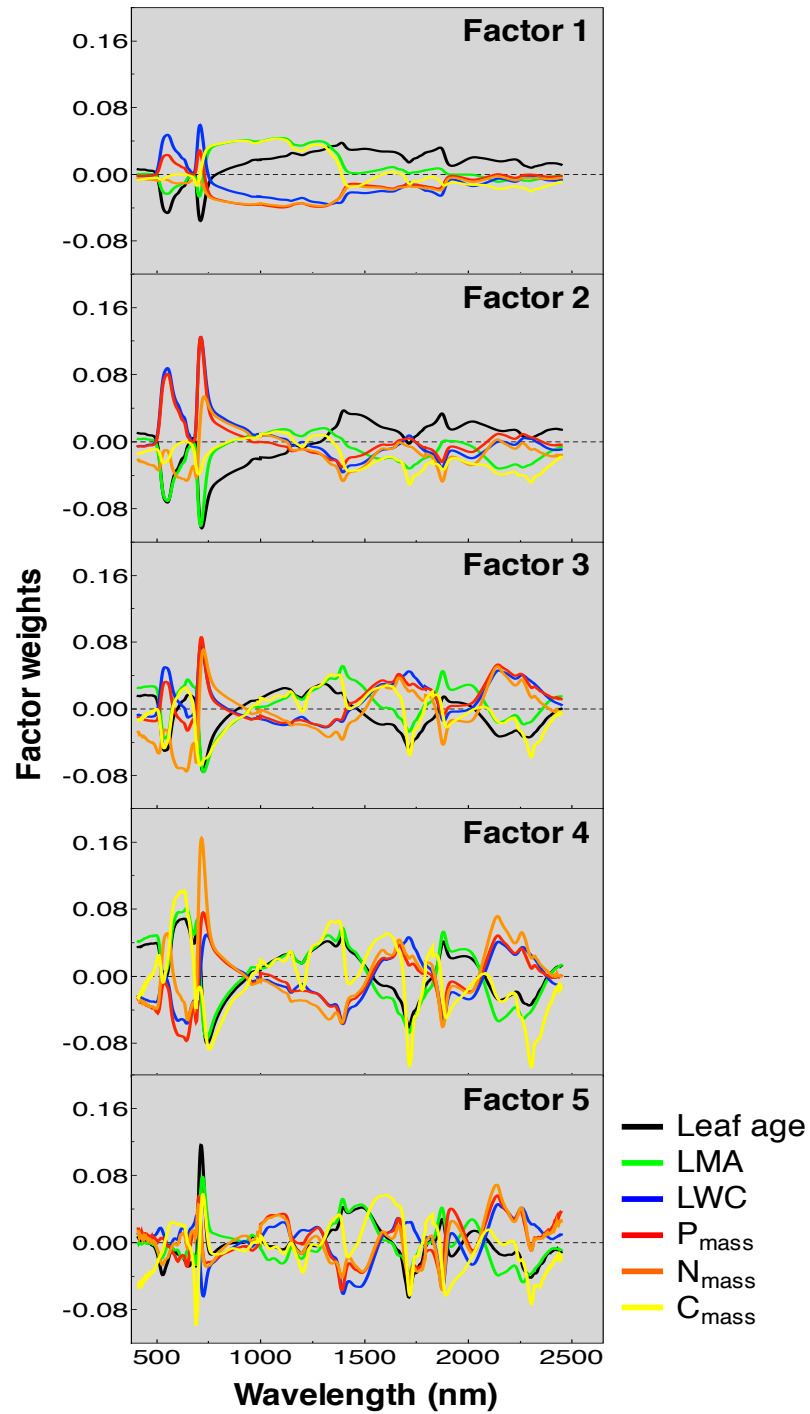


Figure S 5.8.2 Spectral weights (w^*) for leaf age, leaf mass per area (LMA), leaf water content (LWC), phosphorous content (P_{mass}), nitrogen content (N_{mass}) and carbon content (C_{mass}). Only spectral weights for the first five PLSR latent factors (which cumulatively explain 83% of leaf age variation) are reported. Colour lines represent spectral weights for: leaf age (black), LMA (green), LWC (blue), P_{mass} (red), N_{mass} (orange) and C_{mass} (yellow).

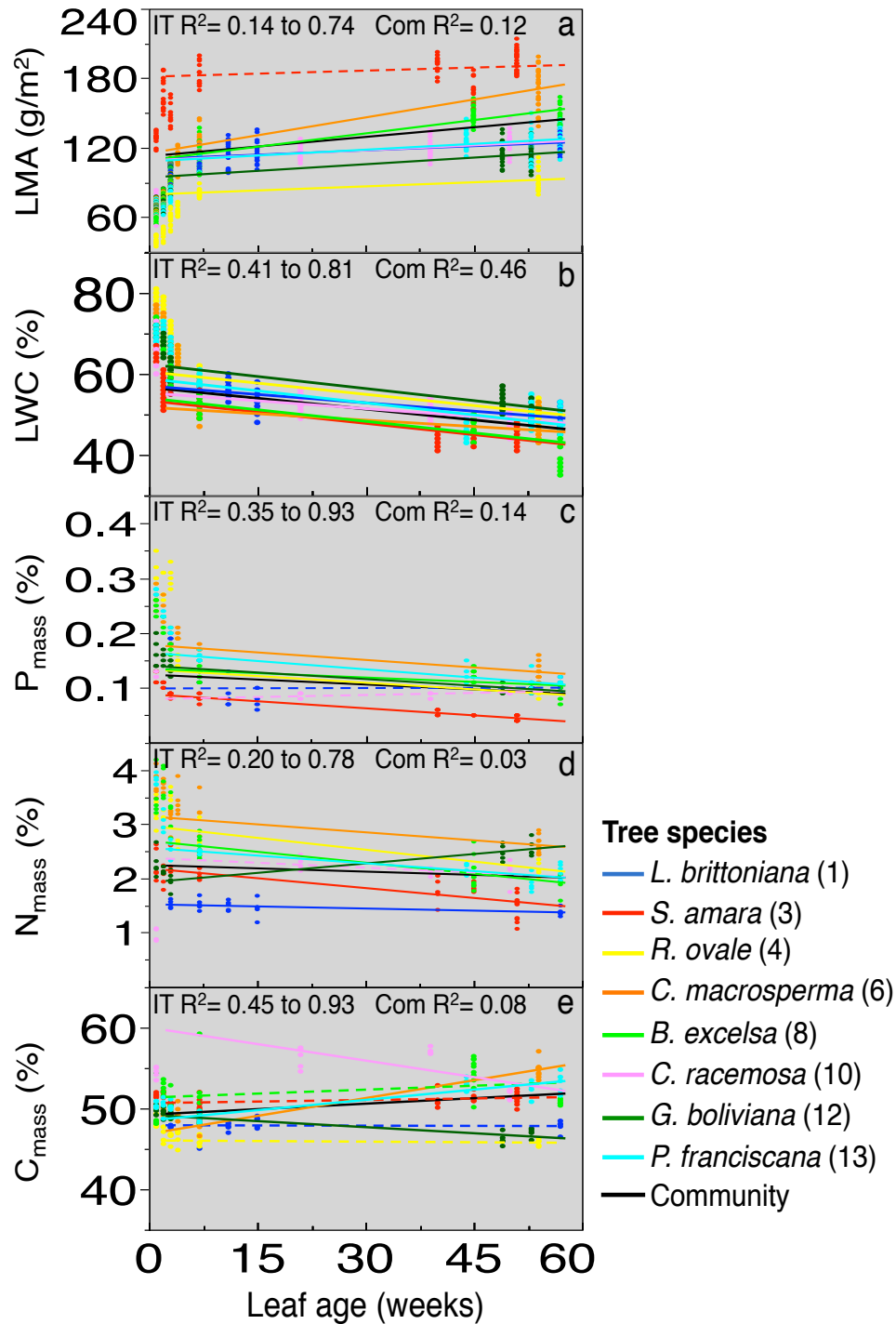


Figure S 5.8.3 Relationships between leaf traits and leaf age (not including developing leaves). Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in these regression analyses. Individual leaf trait values are used as data points. Dashed lines are non-significant regressions. IT R^2 are for individual trees and Com R^2 are for all trees. All significant regressions were $P < 0.05$.

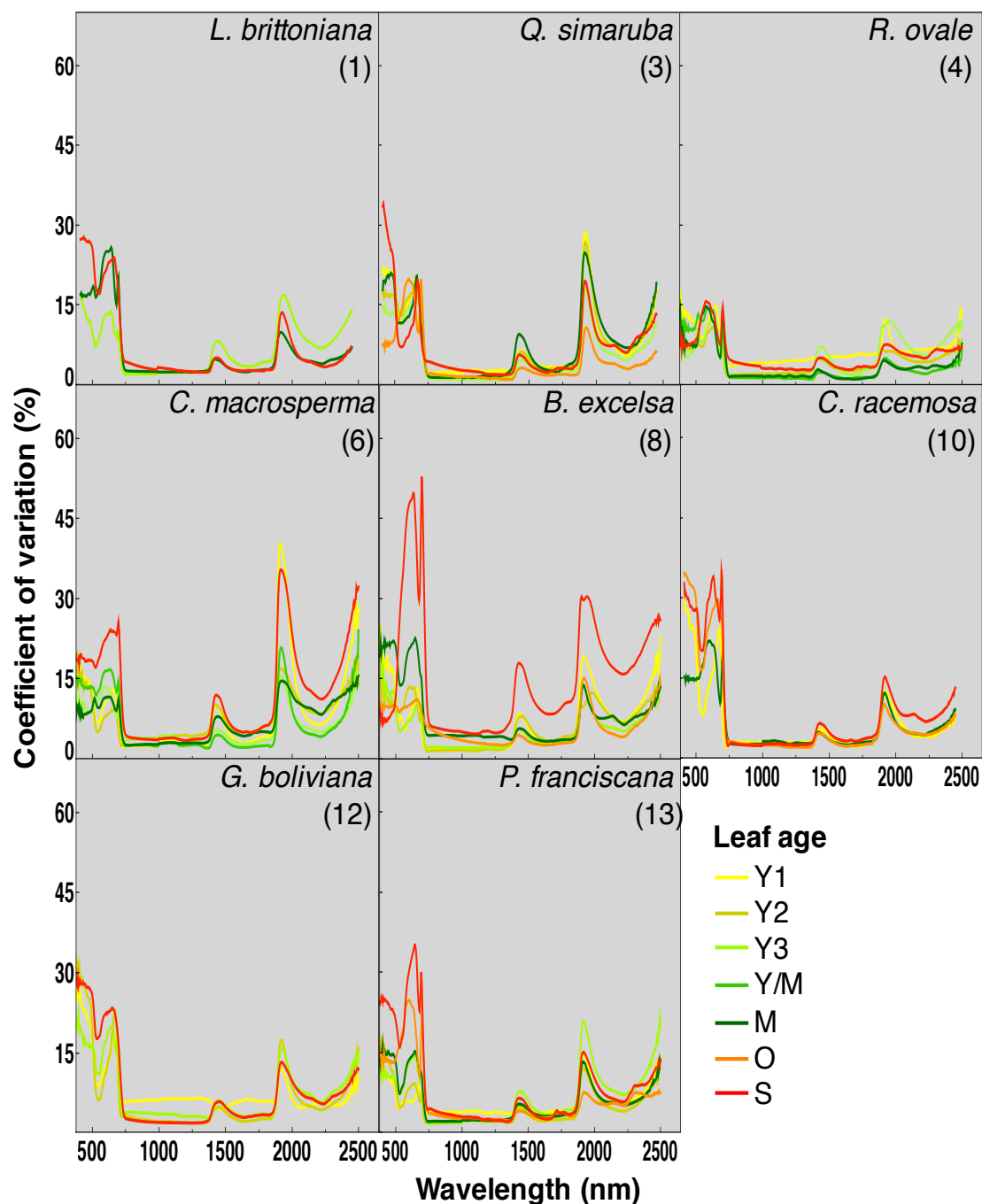


Figure S 5.8.4 Spectral coefficients of reflectance variation (CV) by leaf age class for sun leaves of eight Amazonian canopy and emergent trees. Only trees (tree codes in brackets) with four or more sampled leaf age classes are included in this figure. Leaf age classes: Y1, Y2, Y3 and young/mature (Y/M) are developing leaves collected after 1, 2, 3 and 4 wk of active leaf expansion, respectively; M, mature leaves; O, old leaves showing initial signs of senescence; S, senescent leaves in the process of dying and abscising.

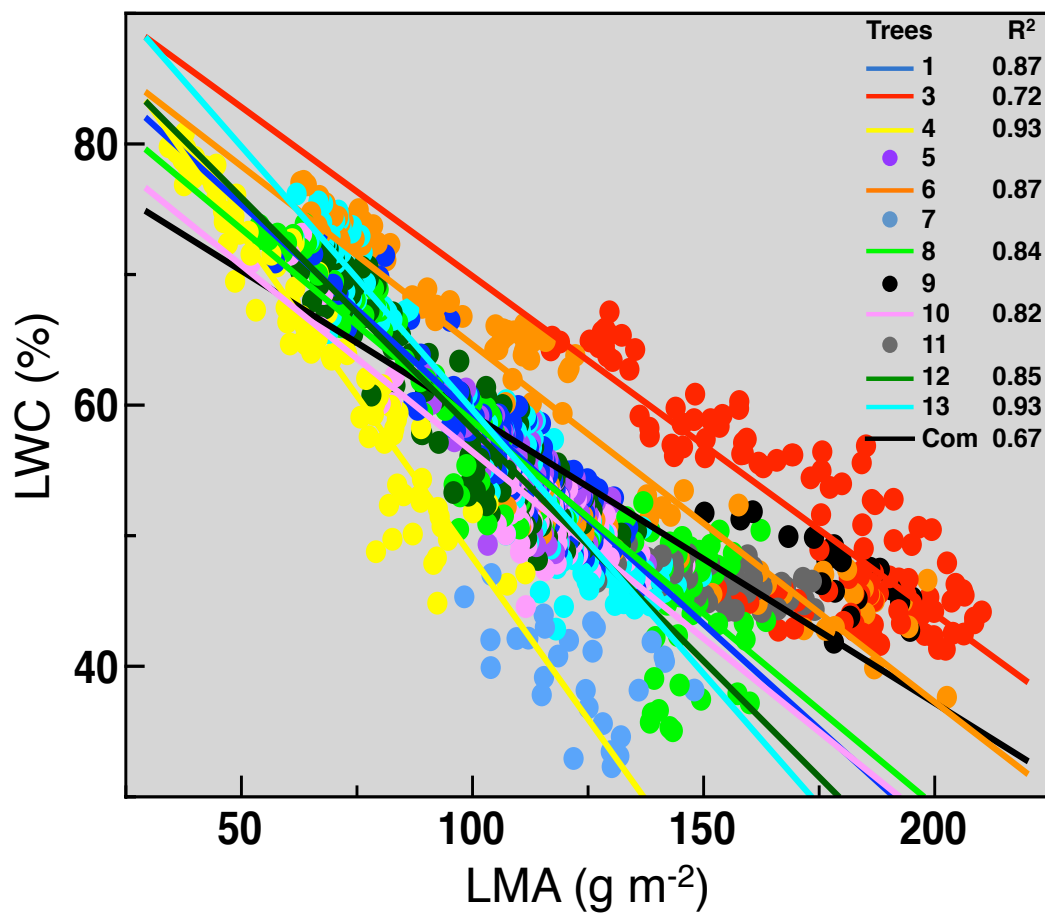


Figure S 5.8.5 Relationship between leaf water content (LWC) and leaf mass per area (LMA). Individual leaf trait values are used as data points. Only trees with four or more sampled leaf age classes are included in regression analyses. Solid lines represent significant regressions (all $P < 0.0001$) for individual trees (colour lines) and across all 12 trees (black line; Com).



Chapter 6 Statement: *Submitted for publication*

The first two research chapters of this thesis demonstrated that morphological, biochemical and spectral properties change significantly through time and/or seasonally as leaves age. Therefore, **Chapter 6** investigates if chemometric partial least square regression (PLSR) models used to predict leaf traits from leaf and canopy spectroscopic measurements could be significantly biased by variation in leaf age. This chapter addresses the third aim of my research: “**to explore if (a) the relationship between spectra and leaf traits such as LMA, LWC, P_{mass} , N_{mass} and C_{mass} varies with age and (b) if leaf age affects the predictability of these traits from spectroscopic data**”. Physiochemical (LMA, LWC, P_{mass} , N_{mass} and C_{mass}) and hyperspectral leaf data were used to develop PLSR models to spectrally predict these five leaf traits important to plant physiology and economy. PLSR models for leaves of different leaf age groups – developmental (D), mature (M), senescent (S) and mixed-age (All)- were developed for each leaf trait. Models for all traits, except LMA, displayed age/temporal sensitivity. Models for all five traits displayed age/temporal sensitivity when models developed using leaves from one leaf age groups were used to predict the traits of leaves of other leaf age groups. These results indicate that the standard practice of using only “fully expanded mature leaves” to calibrate models used for remote sensing estimates of tree canopy traits may introduce differential error that can bias results depending on the phenological state of canopy leaves under study.

Author Contribution Statement

C.C-B., Y.M. and F.F.G. planned and designed the research. C.C-B. designed field data collection protocols and conducted fieldwork and field data collection with logistical support from E.G.C.C. Data integration and quality assurance were performed by C.C-B. and A.A. Data analysis was performed by C.C-B. with support from A.A. Chemical analyses were undertaken by B.J.E. at the University of Arizona. C.C-B. wrote the manuscript with contributions from Y.M., F.F.G and B.J.E.

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Chapter 6 Leaf age effects on the spectral predictability of leaf traits in Amazonian canopy trees

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Abstract

Recent work has shown that leaf traits and spectral properties change through time and/or seasonally as leaves age. Current field and hyperspectral methods used to estimate canopy leaf traits could, therefore, be significantly biased by variation in leaf age. To explore the magnitude of this effect, we used a phenological dataset comprised of leaves of different leaf age groups – developmental (D), mature (M), senescent (S) and mixed-age (All) from eight different canopy and emergent tropical trees in southern Peru. We developed partial least squares regression (PLSR) models to spectrally predict five leaf traits important to plant physiology and economy - leaf mass per unit area (LMA) and water (LWC), phosphorous (P_{mass}), nitrogen (N_{mass}) and carbon content (C_{mass}). We observed trait-specific age effects in models of four of the traits we analysed, with LMA models the only ones to display no age effects. Age effects were also reflected in spectral coefficients and variable importance in projection (VIPs) differences observed between models developed from different leaf age groups. Models for all five leaf traits also displayed age/temporal sensitivity when we used models developed using leaves from one leaf age group to predict the traits of leaves of other leaf age groups. Importantly, the mature (M) models displayed the worst overall performance when predicting the traits of leaves from other leaf age groups. These results indicate that the standard practice of using ‘fully expanded mature leaves’ to calibrate remotely sensed estimates of tree canopy traits introduces differential error that can bias results depending on the

phenological stage of canopy leaves. To achieve temporally stable models, spectranomic studies would benefit from calibrating models with leaves of different leaf age groups as they present themselves through the growing season. We discuss the implications of this for surveys of canopies with synchronised and unsynchronised leaf phenology.

Highlights

- Leaf traits and hyperspectral properties change as leaves age
- Sample bootstrapping tested the impact of leaf age on PLSR model trait estimates
- Trait-specific age effects on model performance, coefficients and VIP were observed
- Trait estimates of models calibrated with mature leaves showed the worst performance
- PLSR model precision was more widely affected by leaf age than accuracy

Key Words

Amazon; Tropical evergreen forests; Leaf chemistry; Chemometrics; PLSR; Leaf phenology; Leaf traits; Leaf age effects; Imaging spectroscopy

6.1 Introduction

Morphological and biochemical properties of leaves are important indicators of plant physiology and play a critical role in regulating plant growth and energy and material cycling in terrestrial food webs (Wright *et al.*, 2004; Messier *et al.*, 2010; Mercado *et al.*, 2011; Niinemets *et al.*, 2015). The ability to characterise the variation of key functional leaf traits in space and time, is therefore, central to improving our understanding of ecosystem function, seasonal productivity and global biogeochemical cycling (Reich *et al.*, 1997; Chavana-Bryant *et al.*, in review; Enquist *et al.*, 2007; Violle *et al.*, 2007)

In the last few decades, remote sensing – specifically high fidelity imaging spectroscopy – has played an increasingly important role in the study of the diversity and functional ecology of traits of leaves, canopies and ecosystems (Curran, 1989; Blackburn & Milton, 1995; Fourty *et al.*, 1996; Asner, 2001; Townsend *et al.*, 2008; Asner *et al.*, 2009; Kokaly *et al.*, 2009; Ustin *et al.*, 2009; Asner *et al.*, 2011a; Serbin *et al.*, 2014). Imaging spectroscopy uses high fidelity measurements of reflected light in narrow sampling intervals (typically $\leq 10\text{-}15$ nm) over the entire reflected solar spectrum (i.e. 400-2500 nm) together with chemometric partial least square regression (PLSR) techniques to link leaf and canopy spectroscopic measurements with their traits. From boreal to tropical environments, an extensive body of work has used PLSR techniques to estimate vegetation traits associated with light capture and growth (i.e. pigments, nitrogen,

phosphorus, LMA, LWC and soluble carbon) and structure and defense (i.e. lignin, cellulose and total carbon) (Richardson & Reeves, 2005; Asner *et al.*, 2009; Asner *et al.*, 2011a; Féret *et al.*, 2011; Ramoelo *et al.*, 2011; Axelsson *et al.*, 2013; Qi *et al.*, 2014; Serbin *et al.*, 2014; Asner *et al.*, 2015; Singh *et al.*, 2015). A major goal of these chemometric PLSR techniques, whether used at the leaf level or embedded in a canopy model for upscaling purposes, is to develop generic models to predict leaf traits across space and time. However, the standard protocol in these studies has been to use “fully expanded mature leaves” (Cornelissen *et al.*, 2003) and to apply the resulting models across time/seasons. The implicit assumption in this methodology –is that measures of leaf traits in ‘mature’ leaves are minimally influenced by differences in leaf age. However, besides a recent study by Yang *et al.* (2016) which assessed the potential of using leaf spectroscopy to predict leaf traits across their life cycle at two temperate deciduous forests, no study to date has addressed the potential temporal/seasonal element of PLSR models in evergreen tropical forests.

Leaf traits change through time and/or seasonally as leaves age. Previous studies in both tropical (Kitajima *et al.*, 1997; Kitajima *et al.*, 2002; Chavana-Bryant *et al.*, 2016; Chavana-Bryant *et al.*, in review; Wu *et al.*, 2016b) and non-tropical forests (Field & Mooney, 1983; Escudero & Mediavilla, 2003; Niinemets & Kull, 2003; Niinemets *et al.*, 2004b; Niinemets *et al.*, 2005; Mediavilla *et al.*, 2011) have shown strong age effects on leaf traits of individual trees and that many of

these age effects can have direct influence on the spectral reflectance behaviour of leaves (Gausman, H. W. *et al.*, 1970; Knipling, EB, 1970; Roberts *et al.*, 1998; Datt, 1999; Sims & Gamon, 2003). Furthermore, a recent study by Chavana-Bryant *et al.* (2016) demonstrated that age-related spectral changes in leaves are distinct enough to enable the prediction of leaf age across trees of different species with diverse leaf properties from hyperspectral leaf reflectance measurements. It is, therefore, very likely that current hyperspectral methods used to estimate canopy leaf traits are affected by leaf age.

In this study, we assess the implicit assumption that leaf aging – even in mature fully expanded leaves – has little effect on predictions of canopy traits. We quantify the magnitude of the effect of leaf aging on canopy trait predictions and explore its implications for vegetation systems with synchronised (e.g. temperate and deciduous tropical forests) and unsynchronised leaf phenology (e.g. evergreen tropical forests). Significantly, in vegetation systems with synchronised leaf phenology there is a time of year when only mature leaves are present in the canopy, while in unsynchronised systems such as tropical evergreen forests, different proportions of leaves of different ages will be present throughout the annual cycle due to a range of species-specific leaf phenological behaviours, and subtle seasonal trends due to intra- and interspecific phenological asynchrony (Borchert, 1983; van Schaik *et al.*, 1993; Reich, 1995; Borchert *et al.*, 2002). In other words, potential age effects on PLSR models for synchronised vegetation

systems could be observed at the beginning and ending of the growth season while in unsynchronised systems they could be observed throughout the annual cycle.

To test the implicit assumption that leaf aging has little effect on predictions of canopy traits, we collected a phenological leaf dataset comprised of leaves of different leaf age groups –developmental (D), mature (M), senescent (S) and mixed-age (All)- from eight different canopy and emergent tropical trees.

Chemometric PLSR models were developed using data subsets comprised of the different leaf age groups to answer the following questions: Does the robustness of PLSR models vary with leaf age? Does leaf age affect PLSR model performance (precision and accuracy) differently for different leaf traits? How well do PLSR models, developed using leaves from a single leaf age group, predict the traits of leaves of other leaf age groups? And what are the implications for best practice when assessing leaf traits using spectranomic approaches?

6.2 Materials and Methods

6.2.1 Field site

Data were collected in and around the borders of two lowland tropical rainforest plots located within the Tambopata National Reserve in the Madre de Dios region of Peru in southwestern Amazonia. The two study sites are part of the RAINFOR

Amazon Forest Inventory Network (Malhi *et al.*, 2002b) and GEM intensive monitoring network, with RAINFOR codes TAM-06 (12°50'24"S, 69°17'59"W) and TAM-09 (12°49'48"S, 69°16'48"W). The sites are located between 215m and 220m above sea level within lowland closed-canopy primary tropical rain forest growing on Haplic alisol soils (Quesada *et al.*, 2010). They are fairly seasonal sites with a four to five month-long dry season (number of months with <100mm rainfall) between June and October, mean annual rainfall over the period 2005-2012 of 1900mm (this may be lower than the long-term mean because of droughts in 2005 and 2010 (Lewis *et al.*, 2011) and mean annual air temperature of 24.4°C. A detailed description of the geomorphology, climate and forest carbon cycle of this site is given by Malhi *et al.* (2014).

6.2.2 Tree sampling

As top-of-canopy leaves are expected to be the main contributors to the spectral response of forest canopies (Clark *et al.*, 2005; Asner & Martin, 2008a), the tree sampling for this study focused on canopy and emergent trees (**Table 6.2.2.1**). A total of eight trees were sampled. According to the “Crown Illumination Index” (Clark & Clark, 1992; Keeling & Phillips, 2007), six of these were canopy trees and two were emergent trees. Diameter at breast height (DBH) among sampled trees averaged 152cm (range: 117-237cm). The sampled trees averaged 32m in height (range: 29-35m), compared to a mean tree height of $27 \pm 8\text{m}$ ($\pm$ SE) for canopy trees $\geq 40\text{cm}$ DBH in local plots. Among the sampled trees, canopy

depth, the difference between tree height and height at the lowest branch of the canopy, averaged 10.3 m (range: 6.3-15.1m).

Table 6.2.2.1 Species and structural information of trees sampled for leaf physiochemical and spectral traits.

Tree code	Species	Family	DBH (m)	Tree canopy position	Height (m)	Canopy depth (m)
1	<i>Licania brittoniana</i> Fritsch	Chrysobalanaceae	1.88	Canopy	29.50	10.50
3	<i>Simarouba amara</i> Aubl.	Simaroubaceae	1.62	Canopy	29.00	8.35
4	<i>Ruizodendron ovale</i> R.E. Fr. (Ruiz & Pav.)	Annonaceae	1.23	Canopy	32.62	10.07
5	<i>Aspidosperma parvifolium</i> A. DC.	Apocynaceae	1.57	Emergent	33.70	8.40
6	<i>Couratari macrosperma</i> A.C. Sm.	Lecythidaceae	1.43	Emergent	33.74	6.27
7	<i>Licania longistyla</i> (Hook. F.) Fritsch	Chrysobalanaceae	1.43	Canopy	30.30	8.70
8	<i>Bertholletia excelsa</i> Bonpl.	Lecythidaceae	2.30	Emergent	34.00	11.60
9	<i>Tachigali chrysalioides</i> van der Werff	Caesalpinioideae	2.10	Canopy	29.70	7.60
10	<i>Clarisia racemosa</i> Ruiz & Pav.	Moraceae	1.58	Canopy	33.75	9.25
11	<i>Eschweilera coriacea</i> (DC.) S.A. Mori	Lecythidaceae	1.88	Emergent	33.32	7.78
12	<i>Gautteria boliviana</i> H. Winkl.	Annonaceae	1.17	Canopy	31.90	11.34
13	<i>Pouteria franciscana</i> Baehni	Sapotacea	2.37	Canopy	35.39	15.09

DBH, diameter at breast height

Monitoring canopy and emergent tropical trees *in situ* presents considerable challenges. Canopy walkways and towers, where they exist, do not provide sufficient access across a large number of crowns. Canopy cranes are ideal for such sampling, but there are only a few across the tropics. Hence, we resorted to repeated climbing and sampling of a number of large trees. The total number of trees sampled in this study was limited by the need to sample structurally sound

trees large enough to withstand the repeated climbing required to monitor leaf life-cycle and sample leaves of different ages, and the difficulty involved in climbing large trees with ropes to minimise damage caused by repeated climbing. We therefore, chose to maximise variability within our leaf dataset by sampling 8 trees, each a different species, with a wide variety of leaf types (e.g. compound and simple leaves), leaf sizes and shapes, and leaf thickness and waxiness. This sampling allowed us to examine the trait and spectral variability due to natural leaf aging in a tropical canopy tree community with diverse leaf properties.

Trees were monitored frequently for bud development, new leaf emergence and development, general leaf condition and signs of leaf senescence. We sampled the developmental, mature and senescent leaf life-cycle phases for all eight trees. During leaf development, individual trees were sampled on a weekly basis from early leaf development (after $\leq$ one week of active leaf expansion) until most leaf area expansion had been achieved (up to four consecutive weeks after active leaf expansion). During the mature leaf phase, individual trees were sampled monthly at different stages (i.e. newly matured, mid-maturity and end of maturity) for up to three months. Similarly, during the senescent leaf phase, individual trees were sampled at different stages (i.e. old leaves not yet abscising and senescent leaves starting abscission through to advanced senescence when most leaves had been abscised from the tree crown) for up to two months for old leaves and up to three months for senescent leaves. Example images and a

detailed description of the leaf classification used in this study can be found in **Figure 6.2.2.1.**

Two top-of-canopy (fully exposed to direct sunlight) apical metre-long branches were collected each time trees were sampled. Sampled branches were cut, labelled, promptly lowered to the ground and recut under water to maintain hydraulic connectivity and minimise leaf desiccation during transport to the field lab, where leaf spectral and morphological (fresh leaf weight and area) measurements were carried out within two hours of the branches being cut. Demographic leaf counts were also collected for all sampled branches.

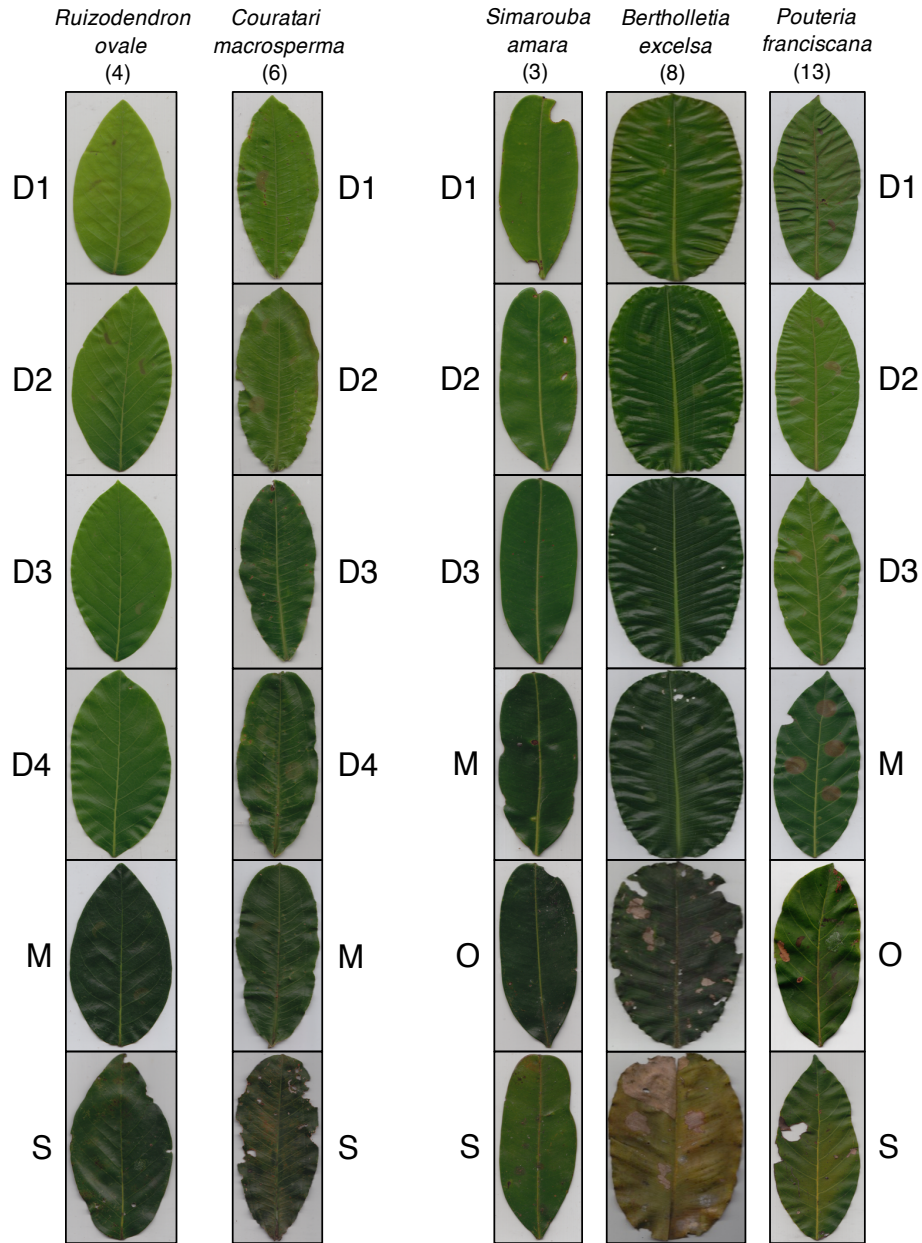


Figure 6.2.2.1 Sampled leaves of different age classes, illustrating the leaf life-cycle of some of our sampled trees: *Ruizodendron ovale*, *Couratari macrosperma*, *Simarouba amara*, *Bertholletia excelsa* and *Pouteria franciscana* (tree codes in brackets). Leaf age classification: D1, D2, D3 and D4 are developing leaves in the process of gaining area, thickness and green colouration and collected after 1, 2, 3 and 4 weeks of active leaf expansion, respectively; M are fully-grown and structurally developed mature leaves that have achieved a fully darkened green colour; O are old leaves showing initial signs of epiphylls and/or senescence and near the end of their life cycle (~2 months before senescence and abscission) and; S are senescent leaves displaying loss of chlorophyll and/or onset of decomposition and in the process of dying and actively abscising.

6.2.3 Leaf spectroscopic and trait measurements

We randomly selected 15 to 30 leaves from each sampled leaf age class for spectral analysis. Leaves were cut (at base of petiole) from their branch just before reflectance measurements were collected. Measurements were collected using a field spectrometer (Analytical Spectral Devices FieldSpec Pro, Boulder, Colorado, USA) with a spectral range of 350-2500 nm and sampling intervals of 1.4 nm from 350-1000 nm and 2 nm from 1000-2500 nm. The spectrometer was fitted with a contact probe (Analytical Spectral Devices High Intensity Contact Probe, Boulder, Colorado, USA) which has its own calibrated light source designed to minimise measurement errors associated with stray light. To ensure measurement quality, the spectrometer was optimised every 5 minutes, spectra for every leaf was white referenced to a calibration panel (Spectralon, Lasphere, Durham, New Hampshire, USA) and an average of 25 spectrums, dark current and white reference measurements were used to improve the signal-to-noise ratio of the spectra. Furthermore, leaves were placed on a custom-designed measurement block covered in a low reflective (3%) material with a firm cushion fitted underneath to allow for the contact probe to be pressed against the leaf without causing damage, while ensuring that no light escaped the measurement. Four reflectance measurements were collected and averaged for each leaf (dependent on leaf size). This protocol delivered high-quality calibrated spectra that did not require smoothing or other filters. The full-range leaf spectral data were trimmed at the far ends (<412nm and >2450nm).

In addition to classifying leaves by their age, we recorded the presence of epiphylls (which was always in very low abundance) and/or necrosis for each leaf surface we measured. To account for potential effects of leaf coatings and natural leaf damage on spectra, we only used reflectance measurements free of epiphylls and necrosis.

We chose LMA, LWC, P_{mass} , N_{mass} and C_{mass} as the ensemble of leaf traits to measure. Each of these traits is important to leaf physiological and ecological functioning (Field, C. & Mooney, H. A. , 1986; Gutschick & Wiegell, 1988; Donovan & Ehleringer, 1994; Kramer & Boyer, 1995; Raaimakers *et al.*, 1995; Evans & Poorter, 2001; Westoby *et al.*, 2002; Reich *et al.*, 2008; Kokaly *et al.*, 2009; Poorter *et al.*, 2009) and have demonstrated contributions to the spectral signatures of vegetation (Curran, 1989; Asner *et al.*, 2009). Variations in leaf morphology, measured as leaf mass per unit area (LMA), correspond to the fundamental tradeoff in leaf construction costs vs light-intercepting surface area and are driven by a range of environmental controls (Niinemets, 2007; Poorter *et al.*, 2009). Leaf water content (LWC) is a useful indicator of plant water balance, since it expresses the relative amount of water present in plant tissues and it is intimately related to several leaf physiological variables, such as leaf turgor, growth, stomatal conductance, transpiration, photosynthesis and respiration (Kramer & Boyer, 1995). Nitrogen is an integral component to the proteins of the photosynthetic machinery responsible for drawdown of carbon dioxide (CO_2)

inside the leaf while phosphorus, being a fundamental component of nucleic acids, lipid membranes and bioenergetic molecules such as ATP, is an essential nutrient in photosynthetic carbon assimilation and protein synthesis (Field, C. & Mooney, H. A., 1986). Thus, both N and P content have been directly linked to rates of photosynthetic activity in leaves (Raaimakers *et al.*, 1995; Evans & Poorter, 2001; Reich *et al.*, 2008, among others). Carbon content in leaves has two functionally different components - non-structural and structural carbon. Non-structural carbon (i.e. sugars, starch and pectin) is produced and mainly stored in leaves and constitutes the mobile energy store of plants (Evans, 1989; Chapin *et al.*, 1993.), while structural carbon (i.e. lignin and cellulose) is generated to support strength and longevity, and to decrease palatability to herbivores (Melillo *et al.*, 1982).

All sampled leaves were measured for LMA and LWC while P_{mass} , N_{mass} and C_{mass} was measured for 5 randomly selected leaves out of each sampled age class. For LMA and LWC calculations, leaf petioles were removed and each sampled leaf was then measured for fresh mass, using a high-precision balance with a 0.01g resolution (My Weigh, model: Durascale). Groups of individually identified leaves were then colour scanned at 300 dpi resolution. ImageJ software (NIH, New York City, NY, USA) used to calculate individual leaf areas. The scanned leaves were then individually placed in labeled paper bags and

dried at 70°C for 72 hours before leaf dry mass was determined. LWC and LMA were derived from these data as follows:

$$\% \text{ LWC} = \left(\frac{\text{fresh mass} - \text{dry mass}}{\text{fresh mass}} \right) \times 100$$

$$\text{LMA} = \frac{\text{dry mass}}{\text{area}}$$

where leaf fresh and dry mass are in grams (g) and leaf area in square metres (m²).

For *Simarouba amara*, a species with compound leaves, leaf trait values were measured on independent leaflets rather than the entire compound leaf as the leaflet green leaf portion is comparable to that measured in whole leaves for other species.

Leaf nutrient content was measured at the Department of Geosciences Environmental Isotope Laboratory at the University of Arizona. For each tree, five dry leaves out of each leaf age class were randomly selected for chemical analysis. All dry leaf samples were then homogenised and ground to a fine powder. Total phosphorus (P) content per leaf was determined using persulfate oxidation followed by the acid molybdate technique (APHA, 1992). Phosphorus concentration was then measured colourimetrically with a spectrophotometer (Genesys20, Thermo Fisher Scientific, Madison, WI, USA). Phosphorous (P),

nitrogen (N) and carbon (C) contents were measured a continuous-flow gas-ratio mass spectrometer (Finnigan Delta Plus XL, Thermo Fisher Scientific, Madison, WI, USA) coupled to an elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA). Samples of 1.0 mg (+/- 0.1mg) were combusted in the elemental analyser. Precision is at least ± 0.10 based on repeated internal standards.

6.2.4 Partial least square regression (PLSR) models: latent variables and model performance

We used chemometric PLSR models to link leaf level reflectance spectra and trait measurements. PLSR is a standard statistical approach utilised in chemometric analyses and is designed to handle high predictor collinearity and situations where the number of predictor variables (i.e. 2039 wavelengths) is much higher than the number of observations. PLSR reduces the large predictor matrix down to a relatively few, uncorrelated latent variables. As in Asner *et al.* (2011b; 2014; 2015) and Serbin *et al.* (2014), we performed a one-time randomised 70/30 split of our data into calibration and independent testing sets. We adopted a 100 $\times$ permutation 10-fold cross validation i.e. 1000 total permutations in which the calibration data set was further subdivided: 90% for calibration and 10% for validation) for each PLSR model. We evaluated the performance of PLSR models using two main metrics: R^2 (i.e. precision) and root mean square error (RMSE, i.e. accuracy) (Lin & Torbeck, 1998; Hodgson &

Bresnahan, 2004; Walther & Moore, 2005; Asner *et al.*, 2011a). We identified the optimal number of latent factors for each leaf trait-leaf age model permutation by minimising RMSE and maximising R^2 . The number of optimal factors varied between 4-6 for LMA models of different leaf age groups and between 6-8 factors for all other traits, we therefore standardised to 6 latent factors for all LMA models and to 8 factors for all other leaf trait models. We report the model performances for the independent testing sets and also provide the RMSE as a percentage of the sample mean (% RMSE) following Asner *et al.* (2011b).

PLSR analyses were performed using custom functions along with the MCS function from LibPLS (<http://www.libpls.net>) and the PLSREGRESS function in Matlab (Version R2014a, Mathworks Inc., Natick, MA, USA).

6.2.5 Experimental design

We used bootstrapping to produce 10 subsets of 60 leaf samples using leaves from each of four different leaf age groups (i.e. developmental (D), mature (M), senescent (S) and mixed-age (All)). These subsets were then used to develop PLSR models for each of the five leaf traits under analysis. A flow-diagram detailing how we developed different sets of 10 PLSR models per leaf age group-trait combination can be seen in **Figure 6.2.5.1**.

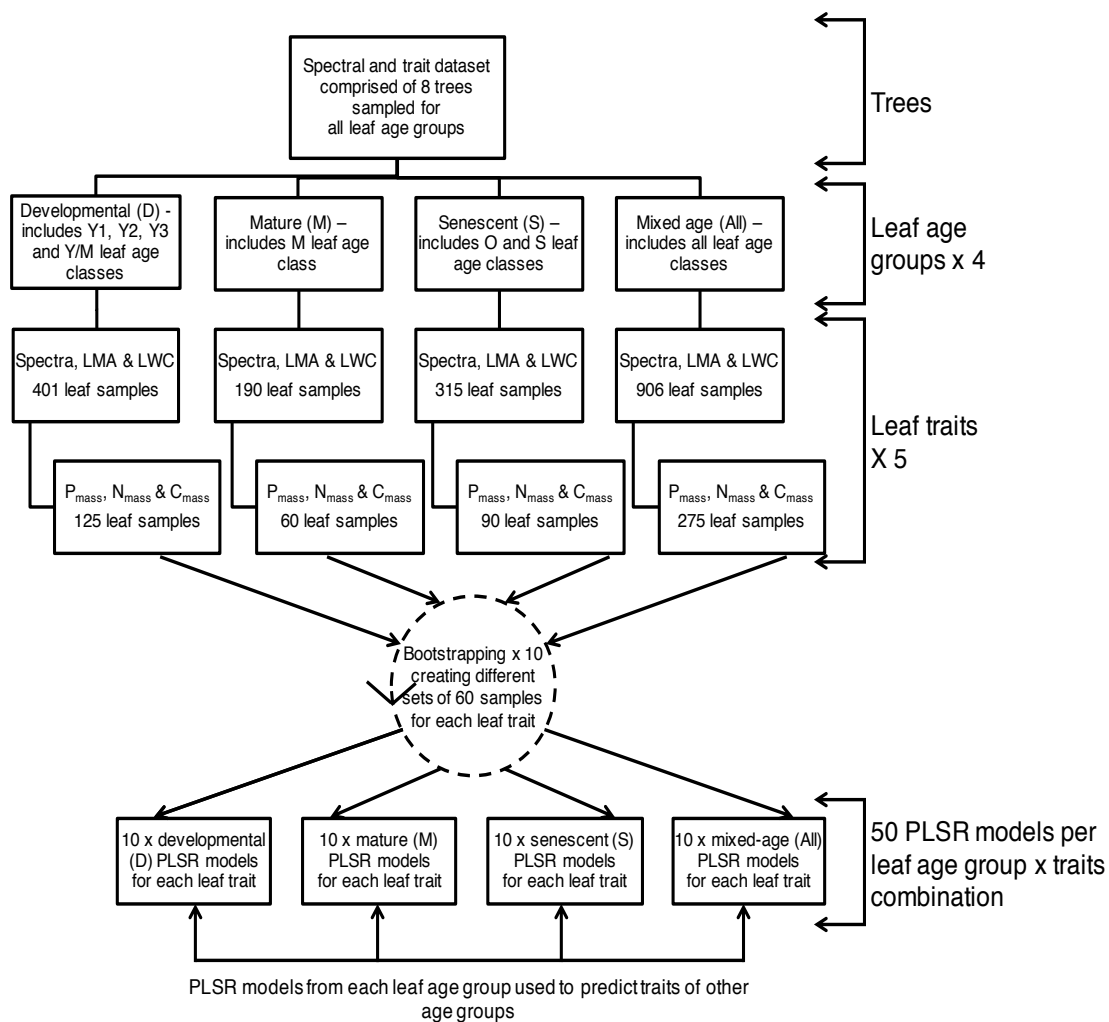


Figure 6.2.5.1 Data subsets used to create different partial least squares (PLSR) models to evaluate the impact of leaf age on these chemometric models. PLSR models “All” were created using an equal number of samples from each of the three different leaf age groups.

We report the trait summary statistics for the four different leaf age group data subsets used to develop these models, the mean results (R^2 and RMSE) of the 10 PLSR models for each leaf age group-trait combination and the standardised number of PLSR latent variables used to predict leaf traits across all leaf age

groups. To evaluate the robustness of the PLSR models across leaf age groups, we used the 10 models from each group to predict the traits of leaves from the other three age groups. We report the R^2 , RMSE and RMSE as a percentage of the sample mean; %RMSE, mean spectral coefficients and their correlations between leaf age groups, and the mean relative importance of each wavelength in predicting leaf traits (VIPs) of these 10 PLSR model predictions for each leaf age group-trait combination. Additionally, results of sensitivity analyses show stable R^2 and RMSE values beyond leaf sample sizes of 60 (**Figure S 6.8.1**). Model accuracy (RMSE) was less sensitivity to sample size than model precision (R^2).

Finally, we explored the leaf traits and spectral range and coefficients of variation (CV) of the different leaf age groups to further elucidate the impact of leaf age on PLSR models. Tukey's HSD tests were used to compare the leaf trait means for the different leaf age groups.

6.3 Results

We examined the performance of PLSR models built using leaves from different leaf age groups (developmental (D), mature (M), senescent (S) and mixed-age (All)) when predicting (1) individual leaf traits within leaf age groups and (2) individual leaf traits across different leaf age groups.

We found that within all four leaf age groups, individual traits were predicted in the same descending order of precision: LMA>LWC>P_{mass}>N_{mass}>C_{mass}. However, there were trait-specific differences in the performance of PLSR models for different leaf age groups (**Table 6.3.1**). Among all traits, LMA was predicted with the highest precision and was the only trait to display consistently high prediction precision and accuracy across all leaf age groups ($R^2 = 0.94-0.96$ and %RMSE= 5-8; **Table 6.3.1**). LWC was also predicted with high precision and accuracy, however, precision dropped by 11-12% for the senescent leaf age group compared to the other three leaf age groups (**Table 6.3.1**). Chemical traits were predicted with lower precision than LMA and LWC, but not always the lowest accuracy (**Table 6.3.1**). For example, C_{mass} showed the lowest prediction precision (R^2) of 0.58-0.70 but the highest accuracy of 2-5 %RMSE, while prediction precision was higher but accuracy lower for both P_{mass} ($R^2 = 0.84-0.92$ and %RMSE= 9-13) and N_{mass} ($R^2 = 0.76-0.87$ and %RMSE= 9-14) across leaf age groups. Furthermore, differences in prediction precision (R^2) between leaf age groups were higher among the chemical traits. The developmental leaf age group (D) displayed the highest prediction precision for both P_{mass} and N_{mass} with predictions 3-8% and 3-11% less precise, respectively, for other leaf age groups. Prediction precision for C_{mass} was highest for the senescent leaf age group (S) and lowest in the mixed-age leaf age group (All $R^2 = 12\%$ lower compared to S).

Table 6.3.1 Leaf trait summary statistics and mean PLSR modelling results by leaf age group. PLSR modelling results include the standardised number of PLSR latent factors used to predict leaf traits across all leaf age groups and the standardised number of samples used to build these models with the total number of leaf samples for each leaf age group in brackets.

Leaf trait		Leaf age groups			
		D (8 trees)	M (8 trees)	S (8 trees)	All (8 trees)
LMA (g/m ²)	M (SD)	90.69 (33.52)	123.83 (26.03)	141.45 (31.78)	116.61 (38.39)
	Range	34.60 – 187.39	76.07 – 199.67	79.42 – 210.49	34.60 – 210.49
	N	60 (401)	60 (190)	60 (315)	60 (906)
	R ²	0.96	0.94	0.96	0.94
	RMSE	5.89	6.09	6.52	9.15
	% RMSE	6	5	5	8
	Factors	6	6	6	6
LWC (%)	M (SD)	67 (6)	53 (4)	47 (4)	57 (10)
	Range	51 - 81	44 - 62	35 - 57	35 - 81
	N	60 (401)	60 (190)	60 (315)	60 (906)
	R ²	0.91	0.91	0.80	0.92
	RMSE	1.95	1.40	1.72	2.62
	% RMSE	3	3	4	5
	Factors	8	8	8	8
P _{mass} (%)	M (SD)	0.19 (0.07)	0.11 (0.03)	0.09 (0.03)	0.15 (0.07)
	Range	0.08 – 0.35	0.06 – 0.18	0.04 – 0.16	0.04 – 0.40
	N	60 (125)	60	60 (90)	60 (275)
	R ²	0.92	0.85	0.87	0.89
	RMSE	0.02	0.02	0.01	0.02
	% RMSE	10	12	9	13
	Factors	8	8	8	8
N _{mass} (%)	M (SD)	2.73 (0.84)	2.19 (0.55)	2.05 (0.41)	2.37 (0.72)
	Range	0.85 – 4.19	1.40 – 3.68	1.07 – 2.89	0.85 – 4.19
	N	60 (125)	60	60 (90)	60 (275)
	R ²	0.87	0.84	0.76	0.76
	RMSE	0.28	0.23	0.21	0.36
	% RMSE	10	9	10	14
	Factors	8	8	8	8
C _{mass} (%)	M (SD)	49.87 (2.07)	50.68 (4.18)	51.59 (3.24)	50.28 (2.91)
	Range	44.85 – 55.13	45.04 – 60.14	45.27 – 57.71	44.85 – 60.14
	N	60 (125)	60	60 (90)	60 (275)
	R ²	0.68	0.66	0.70	0.58
	RMSE	1.08	2.57	1.91	1.89
	% RMSE	2	5	3	4
	Factors	8	8	8	8

When we analysed the performance of PLSR models built using one leaf age group to predict the traits of the other three leaf age groups, we found that the mixed-age model (All) displayed the best performance (**Figure 6.3.1a-t**). When predicting leaf traits for the leaves of the mixed-age group (All; **Figure 6.3.1d, h, l, p and t**) the developmental model (D) showed the second-best performance in terms of both precision and accuracy, however, prediction precision still dropped by 10, 10 and 26% when the D-model was used to predict the chemical leaf traits (P_{mass} , N_{mass} and C_{mass} , respectively). We also found that models displayed trait-specific age sensitivity. LMA was the only leaf trait that was predicted with relatively high precision and accuracy ($R^2 = 0.78-0.96$ and $\%RMSE = 5-18$ across leaf age groups) regardless of the age model used (**Figure 6.3.1a-d**) while C_{mass} models displayed large decreases in prediction precision ($R^2 = 36-53\%$) with some age models unable to predict C_{mass} for leaves of other leaf age groups (**Figure 6.3.1q-t**). For developmental leaves, PLSR models built using mature leaves (M) displayed the worst performance for LMA, LWC, N_{mass} with both M- and S-models unable to predict C_{mass} for this leaf age group (**Figure 6.3.1a, e, i, m and q**). For mature leaves, D-models showed the worst performance for LMA, LWC and N_{mass} with S-models unable to predict C_{mass} for this leaf age group (**Figure 6.3.1b, f, j, n and r**). For senescent leaves, D-models displayed the worst performance for LMA and LWC with M-models showing the worst performance for P_{mass} and unable to predict N_{mass} and C_{mass} for this leaf age group (**Figure 6.3.1c, g, k, o and s**).

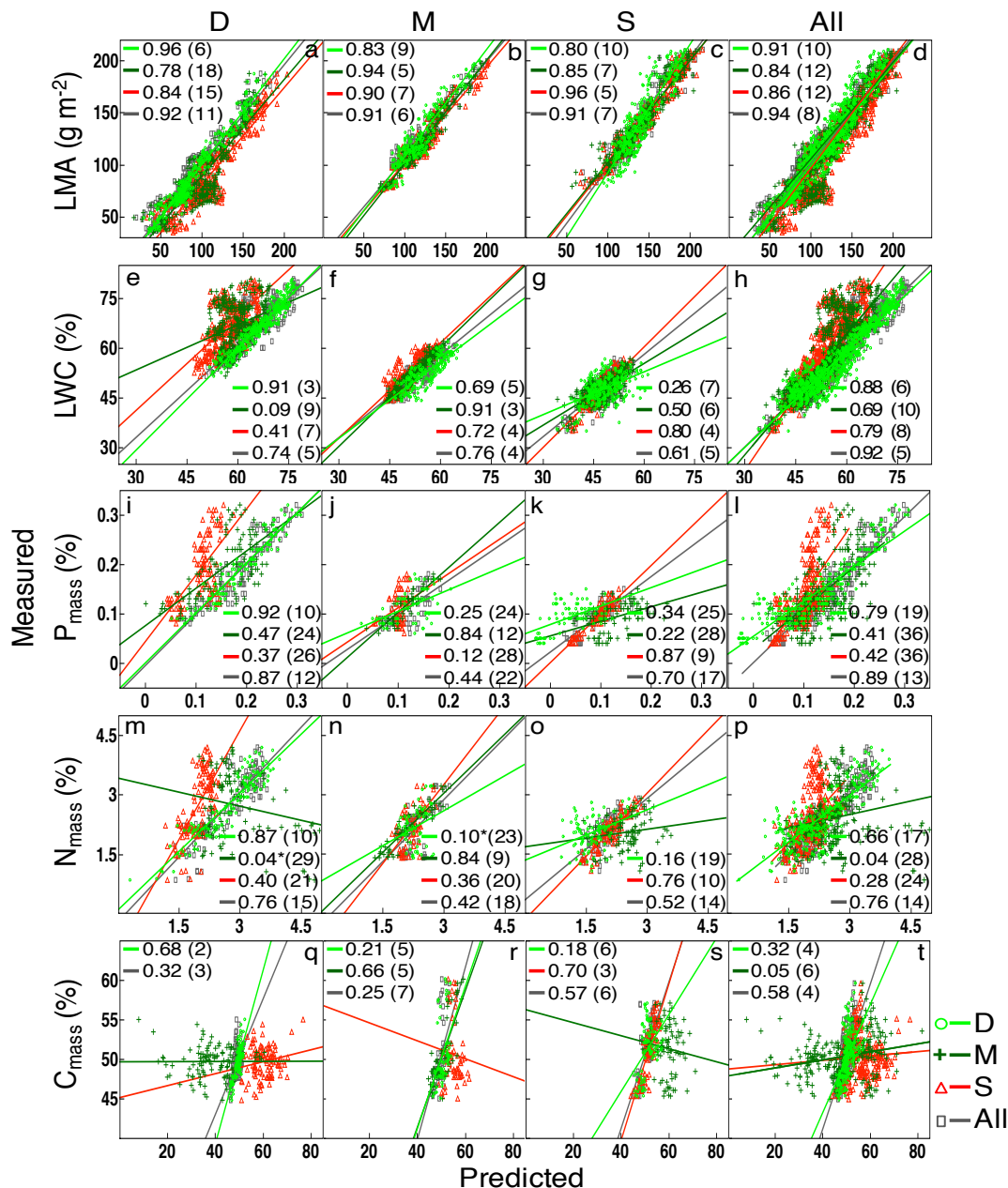


Figure 6.3.1 Partial least squares regression (PLSR) results for measured vs spectrally predicted leaf traits. Leaf traits (LMA, LWC, P_{mass}, N_{mass} and C_{mass}) were spectrally predicted for the four different leaf age groups (columns left to right: D= developmental, M= mature, S= senescent and All= mixed-age) using PLSR models calibrated with leaves of the different leaf age groups. For leaves of each leaf age group, we report R² and (%RMSE) for the four different PLSR leaf age models: Developmental model= light green, Mature model= dark green, Senescent model= red and Mixed-age model= dark grey. Non-significant regressions are not reported, all significant regression *P* values= <0.0001, except for * were *P* value= 0.03.

We found differences in spectral PLSR coefficients (**Figure 6.3.2**) and VIPs (**Figure S 6.3.2**) due to age. Only correlations between coefficients for LMA models were found to be consistently high across leaf age groups (**Figure 6.3.2**) with correlations also high between developmental (D) and mixed-age (All) leaf age groups for four out of five traits (LMA, LWC, P_{mass} and N_{mass}).

Summary trait statistics (trait mean, standard deviation and range) for each leaf age group are provided in **Table 6.2.5.1** and shown in **Figure 6.3.3**. The developmental (D) leaf age group displayed the largest trait ranges for LMA, LWC, P_{mass} and N_{mass} , covering most of the ranges in these traits shown by the mature and senescent leaf age groups. However, mean LMA significantly increased between consecutive leaf age groups ($p = <0.0001$ for both D-M and M-S leaf age group comparisons), while mean LWC and P_{mass} significantly decreased ($p = <0.0001$ for both D-M and M-S leaf age group comparisons). Mean N_{mass} continuously decreased between leaf age groups but only significantly between D and M leaf age groups ($p = <0.001$). In contrast, all leaf age groups showed similar trait range and means for C_{mass} .

Spectral data displayed similar patterns to those shown by the trait data. Reflectance range and coefficients of variation (CVs) across most of the spectrum (350-2450nm; **Figure 6.3.4**) were highest for the D leaf age group and lowest for the M age group.

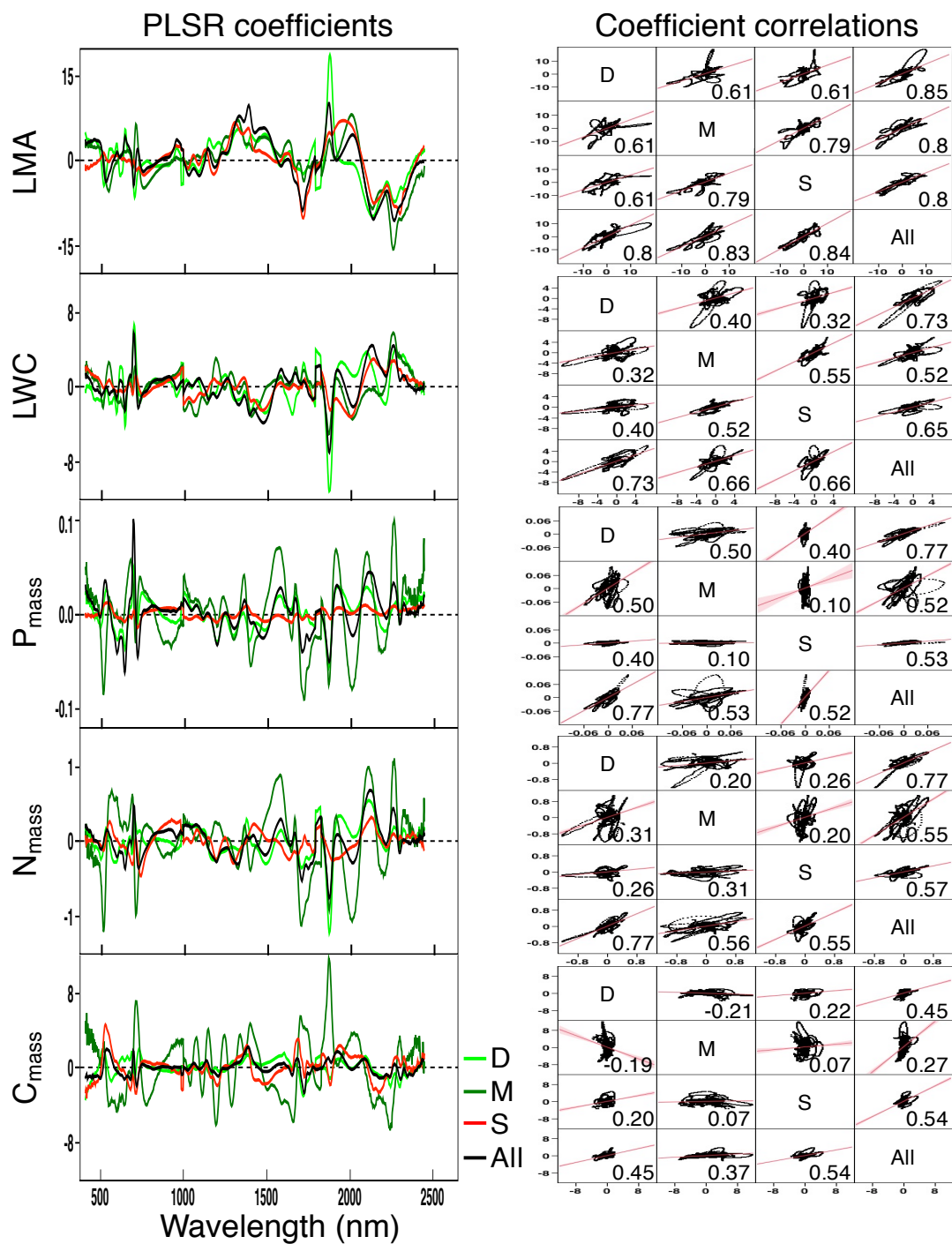


Figure 6.3.2 Partial least squares regression (PLSR) coefficients by leaf age group (left column) and their correlation (right column). Leaf traits: leaf mass per unit area (LMA), leaf water content (LWC), phosphorous (P_{mass}), nitrogen (N_{mass}) and carbon (C_{mass}) content. Leaf age groups: D= developmental (light green), M= mature (dark green), S= senescent (red) and All= mixed-age (black).

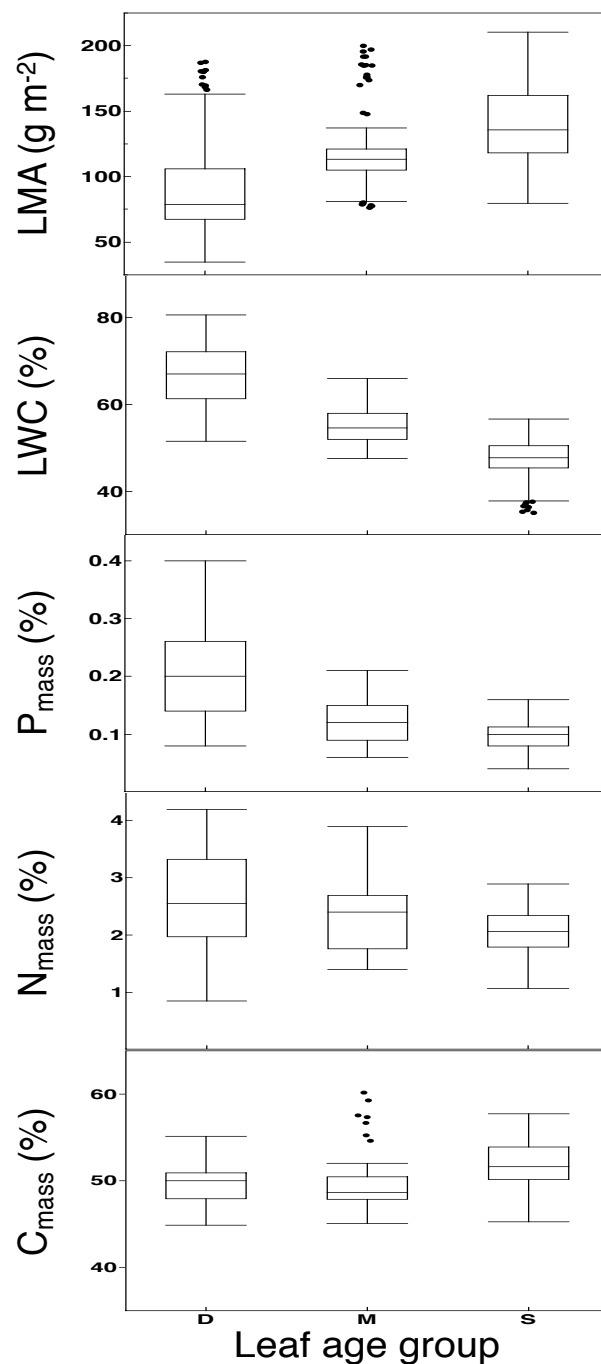


Figure 6.3.3 Trait summary statistics by leaf age group for sun leaves of eight Amazonian emergent and canopy trees. Leaf age groups: D= developmental, includes leaves after one to four weeks of active expansion; M= mature, includes newly matured, mid and end of maturity leaves; and S= senescent, includes old leaves showing initial signs of senescence and senescent leaves starting to abscise through to advanced senescence (most leaves abscised).

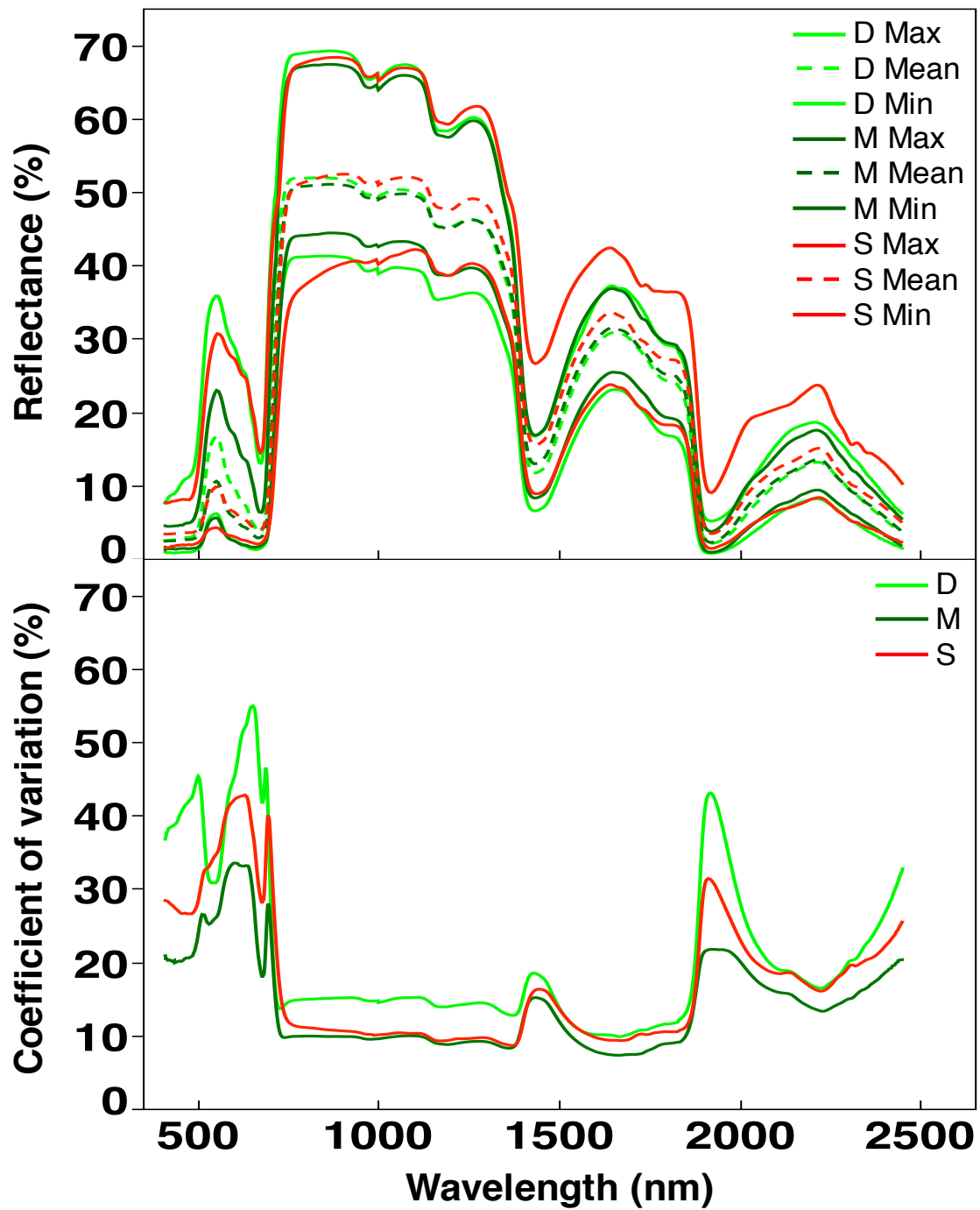


Figure 6.3.4 Spectral summary statistics by leaf age group for sun leaves of eight Amazonian emergent and canopy trees. Leaf age groups: D= developmental, includes leaves after one to four weeks of active expansion; M= mature, includes newly matured, mid and end of maturity leaves; and S= senescent, includes old leaves showing initial signs of senescence and senescent leaves starting to abscise through to advanced senescence (most canopy leaves abscised).

6.4 Discussion

6.4.1 Does the robustness of spectral PLSR models vary with leaf age and does leaf age affect model performance when predicting different leaf traits?

We found that within each leaf age group, the five individual leaf traits we analysed were predicted in the same descending order of precision:

$LMA > LWC > P_{mass} > N_{mass} > C_{mass}$. These results are comparable with previous studies using PLSR approaches (Richardson & Reeves, 2005; Asner *et al.*, 2011a; Asner *et al.*, 2011b; Serbin *et al.*, 2014), and more importantly, indicate that leaf trait-spectra relationships are stable within the different leaf life-cycle stages (i.e. developmental, mature and senescent). However, we did observe important trait-specific age effects on the performance of these PLSR models.

In terms of PLSR models for individual traits, LMA models were the only ones to display no age effects. LMA was not only predicted with the highest precision among all traits but also consistently across all leaf age groups ($R^2 = 0.94-0.96$ and %RMSE = 5-8; **Table 6.3.1**). Although P_{mass} models displayed lower prediction precisions than LMA across leaf age groups ($R^2 = 0.85-0.92$; **Table 6.3.1**), these were also relatively consistent across age groups (2-7 and 1-4% differences in R^2 and %RMSE, respectively between age groups). However, age effects were identified for LWC, N_{mass} and C_{mass} . LWC models displayed a significant decrease in precision of 11-12 % for the senescent leaf age group (S;

Table 6.3.1). This decrease in model precision may be due to a diminished magnitude of the optical signal for LWC in senescent leaves as a result of their significantly lower mean LWC (20 and 6% lower than mean LWC in developing and mature leaves, respectively, **Table 6.3.1** and **Figure 6.3.1**) together with mixed trait-age effects in the NIR (700-1400nm) and SWIR (1400-2500nm) spectral regions. These spectral regions are directly correlated to LWC but also indirectly to LMA in the NIR and both directly and indirectly to biochemical features such as nitrogen, phosphorus, proteins and non-structural (sugars and starch) and structural (cellulose and lignin) carbon constituents in the SWIR region. N_{mass} models also showed decreased prediction precisions of 8-11% (decreases in R^2 compared to M and D age groups, respectively) for both senescent (S) and mixed-age (All) leaf age groups while C_{mass} models displayed decreased precisions of 8-12% for the mixed-age model. This was most likely the result of tree-specific relationships with age displayed by both N_{mass} and C_{mass} as previously reported in **Chapters 4 and 5** (see also **Figure S 6.8.3**). In contrast, the model accuracy (measured as RMSE and %RMSE) is relatively stable across leaf age groups for all traits (%RMSE changes 2-3% for LMA; 1-2% for LWC; 1-4% for P_{mass} ; 1-5% for N_{mass} and 1-3% for C_{mass}).

Although the leaf traits included in this study have been previously analysed using PLSR approaches, our study is unique as it is the first to investigate the effects of leaf age on the PLSR models used to spectrally predict these traits.

Furthermore, the ability to predict these traits with high precision throughout the leaf life-cycle is important as these leaf traits are of central importance to plant physiology and economy and to ecosystem function and seasonal variation in productivity. Our findings suggest that spectrally predicting LWC and N_{mass} for senescent leaves and developing PLSR models that can predict N_{mass} and C_{mass} throughout the leaf life-cycle without significant loss in prediction precision may be more challenging.

6.4.2 What is the performance of chemometric PLSR models developed using leaves from one leaf age group when predicting the traits of leaves of other leaf age groups?

Chemometric approaches such as PLSR are known to be subject to instability when models developed with one dataset are used to predict chemicals from spectra in another dataset (Martens, 2001; Boulesteix & Strimmer, 2006). We therefore used models developed from datasets of different leaf age groups to test the robustness of these PLSR models across time (i.e. predict traits of leaves at different times of their life-cycle). Our results demonstrate that chemometric PLSR models for all five leaf traits we analysed displayed leaf age/temporal sensitivity. LMA was the trait that displayed the least age/temporal sensitivity. Although LMA could be predicted by models developed from the different leaf age group datasets, significant loss in prediction precision (and accuracy in the case of developmental leaves) was observed in most cases (4-18%) for leaves

from single leaf age groups when models from different single leaf age groups were used. None of the other four leaf traits analysed (LWC , P_{mass} , N_{mass} and C_{mass}) could be predicted by models developed from a different leaf age group dataset without significant loss of prediction precision, and in most cases, also accuracy. Furthermore, models of different leaf age groups were sometimes unable to predict N_{mass} and C_{mass} (**Figure 6.3.1**). Except for P_{mass} for the developmental leaf age group, even models developed using mixed age leaves (All) displayed significant decreases in precision (11-42%) and accuracy (8-10%) when predicting the traits of leaves from single age groups. These results were reflected in the spectral coefficients' differences we observed between models for individual leaf traits developed from different leaf age groups (**Figure 6.3.2**).

When we analysed the correlations between spectral coefficients of the different leaf age group models within individual traits, we observed that only the spectral coefficients of LMA models displayed consistently strong correlations between different leaf age groups (i.e. single leaf age group models can predict LMA for other leaf age groups), while the coefficients for models of different leaf age groups within the other four leaf traits displayed correlations of varying strength (i.e. single leaf age group models for these traits display age sensitivity and cannot consistently predict the leaf traits of other age groups) (**Figure 6.3.1** and **Figure 6.3.2**). Although previous chemometric studies using PLSR approaches have reported differences in spectral coefficients and VIPs for different leaf traits,

our study is the first to report differences in both of these metrics within leaf traits due to leaf age (**Figure 6.3.2** and **Figure S 6.8.2**).

As it is standard practice to develop chemometric PLSR models using only “fully expanded mature leaves” (Cornelissen *et al.*, 2003), it is important to highlight two of our main findings (1) that the mature (M) models displayed the worst overall performance when predicting the traits of leaves from other leaf age groups (**Figure 6.3.1**) and (2) current models developed using only mature leaves are likely to display leaf age/temporal sensitivity. The uncertainty introduced is a reduction in precision for all traits, and a directional bias mainly for P_{mass} , N_{mass} and C_{mass} . For example, with the exception of LMA models, in vegetation systems with synchronised leaf phenology such as temperate and deciduous tropical forests, prediction precision would be significantly decreased if PLSR models were used to predict the leaf traits of leaves collected at different times in the growing season (i.e. early or late in the growing season). Therefore, to achieve temporally stable PLSR models, it would be important to calibrate these models with leaves of different leaf age groups (i.e. collected at different times of the growing season).

Our results also demonstrate that except for C_{mass} , traits of leaves of mixed age groups (All) could be predicted with only small loss in precision (3-10%) and accuracy (0-6%) by developmental (D) models. The developmental (D) leaf age

group covered most of the trait and spectral range of both the mature (M) and senescent (S) age groups (**Figure 6.3.3** and **Figure 6.3.4**), therefore, except for C_{mass} , D models performed well when predicting the traits of leaves of mixed age (All) groups (**Figure 6.3.1**). This highlights the importance of covering a sufficient range of values when calibrating chemometric models. More importantly, this finding can have important implications for evergreen tropical forests where most field and aerial spectranomic campaigns are conducted during the dry season, a time when field-based studies (Doughty & Goulden, 2008; Brando *et al.*, 2010; Chave *et al.*, 2010; Malhi *et al.*, 2014 among many others; Chavana-Bryant *et al.*, 2016) have shown that many trees in these forests exchange their leaves through a wide variety of phenological behaviours. These behaviours include dry season shedding of short lived, mesic leaves in deciduous species, brief loss of leaves (typically 1-4 weeks) prior to flowering or before a flush of new leaves in brevi-deciduous species and irregular or constant leaf exchange of long-lived, coriaceous leaves in evergreen species. Furthermore, intra- and interspecific phenological asynchrony (Borchert, 1983; van Schaik *et al.*, 1993; Reich, 1995; Borchert *et al.*, 2002) result in different proportions of leaves of different ages being present in tropical evergreen canopies throughout the annual cycle. Therefore, current chemometric PLSR models calibrated with only mature leaves are likely to experience different degrees of sensitivity to leaf age throughout the annual cycle, which could explain some of the model residuals in published studies (Asner *et al.*, 2009; Asner *et al.*, 2011a; Féret *et al.*, 2011; Axelsson *et al.*,

2013; Qi *et al.*, 2014; Asner *et al.*, 2015). Leaf age sensitivity would be more pronounced in spectranomic studies where PLSR models calibrated with only mature leaves are used to predict traits at canopy and landscape scales where leaves of different leaf ages are present. Spectranomic studies in evergreen tropical forests may therefore benefit from moving away from standard practice (sampling only fully expanded mature leaves) and instead sample any leaves encountered in the field at the time of year of interest. The following protocols from Chavana-Bryant *et al.* (in review) can be used to keep track of leaf age differences during field collection campaigns: (1) only leaves found after the closest growth scar to the end of a branch should be collected (except for species with successive leaf production, this will ensure that leaves have more or less the same age) and (2) to more accurately identify the leaf age class of the sampled leaves, a printed version of **Figure 6.2.2.1** could be used as field leaf age classification guides and (3) a ranking system used to classify the general leaf age stage of the canopy from which leaves are collected (i.e. 1- leaves from canopies starting their life-cycles if young developing leaves are present in the canopy, 2a- leaves from early to mid- mature canopies if only fully-developed, healthy-looking leaves are present in the canopy or 2b- leaves from end of maturity to old canopies if fully-developed leaves show signs of aging but not abscission in the canopy; and 3- leaves from canopies coming to the end of their life-cycle if leaves show signs of senescence, epiphylls and abscission in the canopy).

Finally, developing temporally stable chemometric models (i.e. models that are stable across the growing season) for some leaf traits may be more challenging than for others. For example, C_{mass} may be a particularly challenging trait as not only does this trait display tree-specific age relationships but total C_{mass} remains more or less stable throughout the leaf life-cycle while the ratios of the two different carbon constituents (non-structural and structural carbohydrates) in leaves changes with leaf age. It is therefore possible, that the loss in prediction precision we observed in C_{mass} models for leaves of mixed age (All) is the result of the anti-correlation of spectral features between non-structural (sugars and starch) and structural (lignin and cellulose) carbohydrates previously identified in Asner *et al.* (2015).

6.5 Conclusions

We have demonstrated the importance of leaf age in the development of chemometric models used to estimate leaf traits from hyperspectral radiometry. Leaf age affects both the precision and accuracy of trait estimates. This effect varies with trait and precision is more widely affected than accuracy. To achieve temporally stable models, spectranomic studies would benefit from moving away from the standard practice of focusing on mature leaves and instead calibrate models with leaves of different leaf age groups. In regions where leaf phenology is strongly synchronised (e.g. temperate and deciduous tropical forests) this would involve collecting leaves at different times of the growing season, while in

regions with unsynchronised leaf phenology (e.g. evergreen tropical forests) it would involve randomly sampling leaves encountered in the field at the time of year of interest or across the year.

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6.8 Supporting information

Supporting Information for manuscript: “Leaf age effects on the spectral predictability of leaf traits in Amazonian canopy trees”.

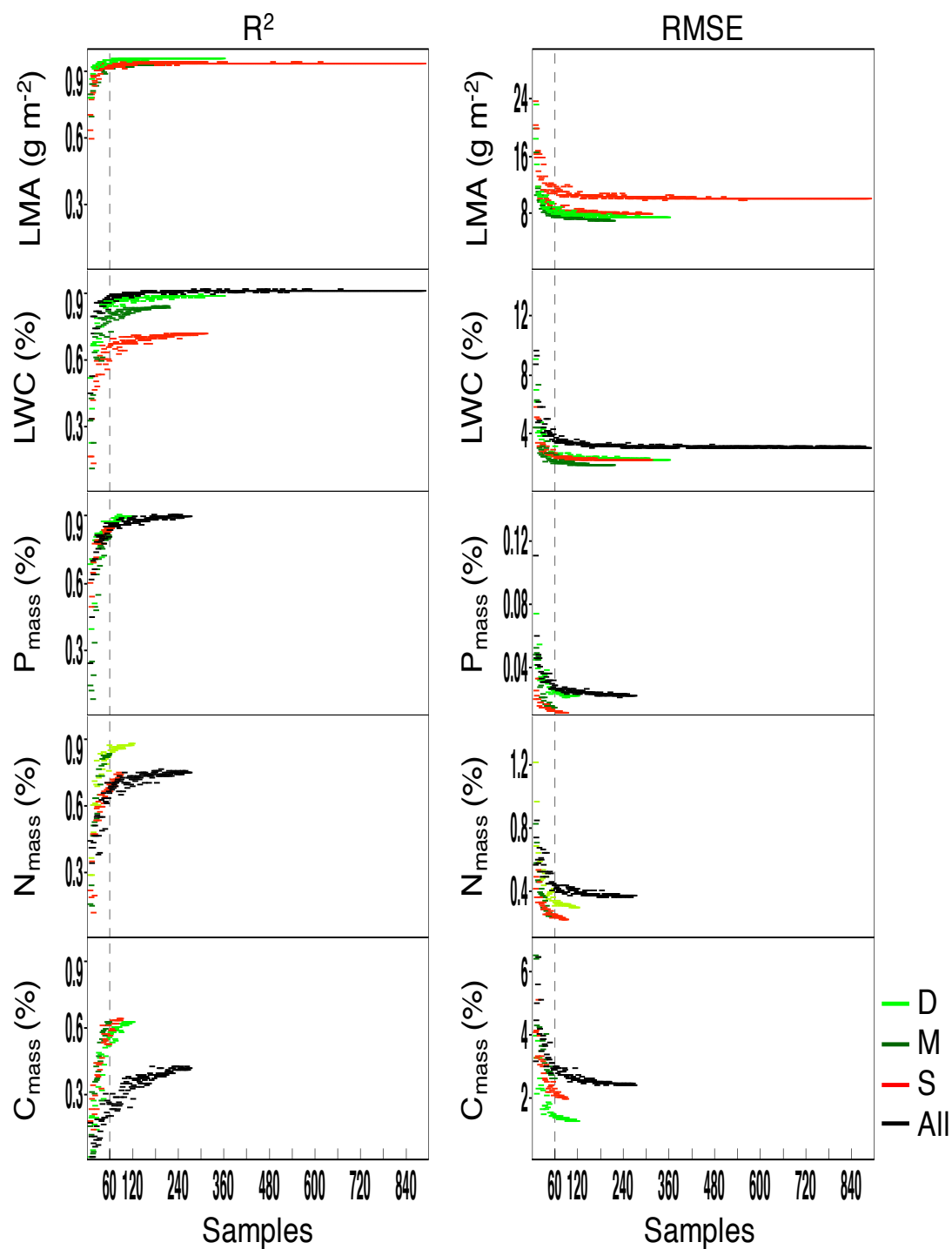


Figure S 6.8.1 Partial least square regression (PLSR) model sensitivity to sample size for measured leaf traits by age group (D= developmental, M= mature, S= senescent and All= mixed-age). PLSR models were run using six latent factors for leaf mass per area (LMA), and eight latent factors for all other traits.

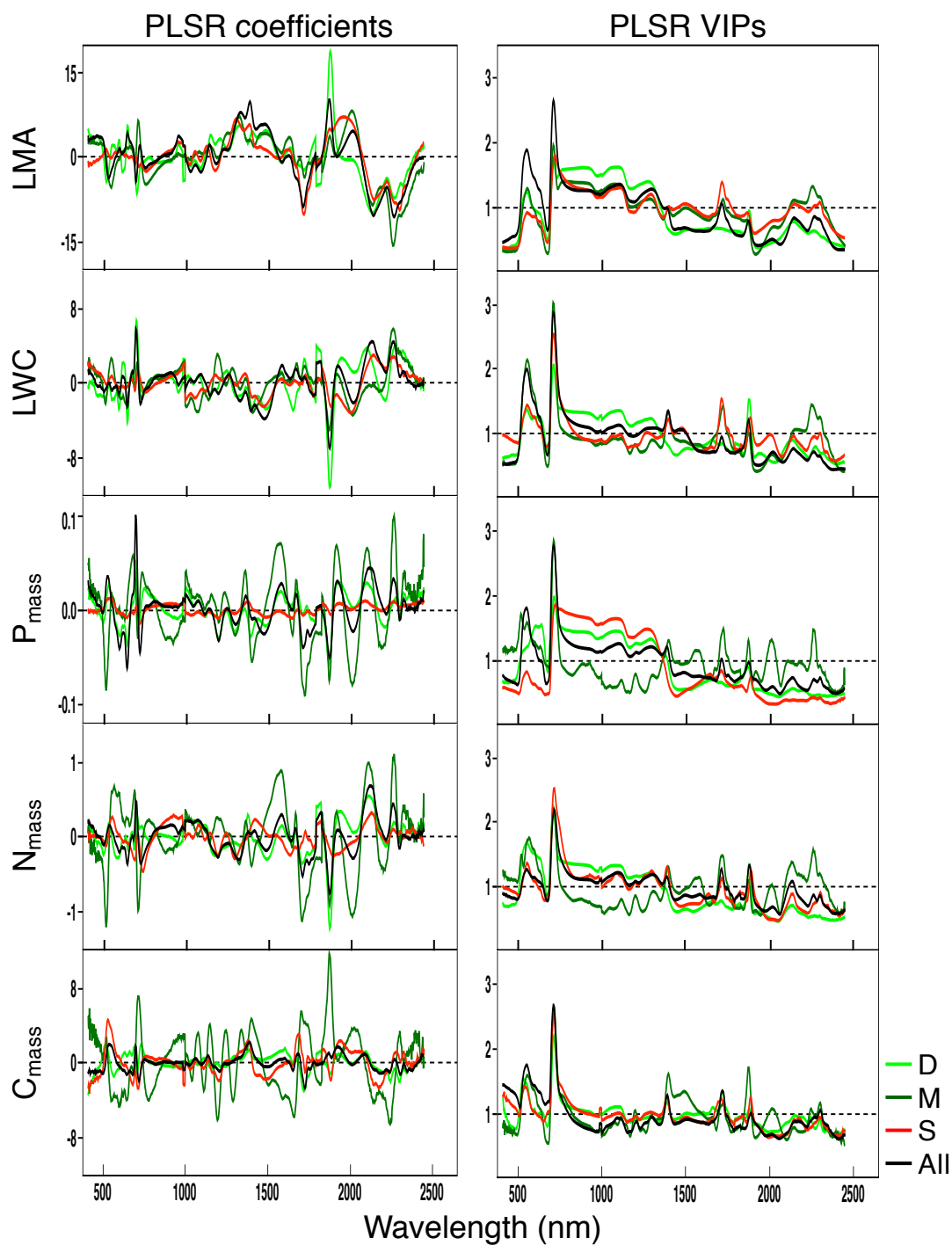


Figure S 6.8.2 Partial least squares regression (PLSR) coefficients and variable importance in projection (VIPs) for leaf traits: leaf mass per area (LMA), leaf water content (LWC), phosphorous (P_{mass}), nitrogen (N_{mass}) and carbon (C_{mass}) content by leaf age group. Leaf age groups: D= developmental, M= mature, S= senescent and All= mixed-age.

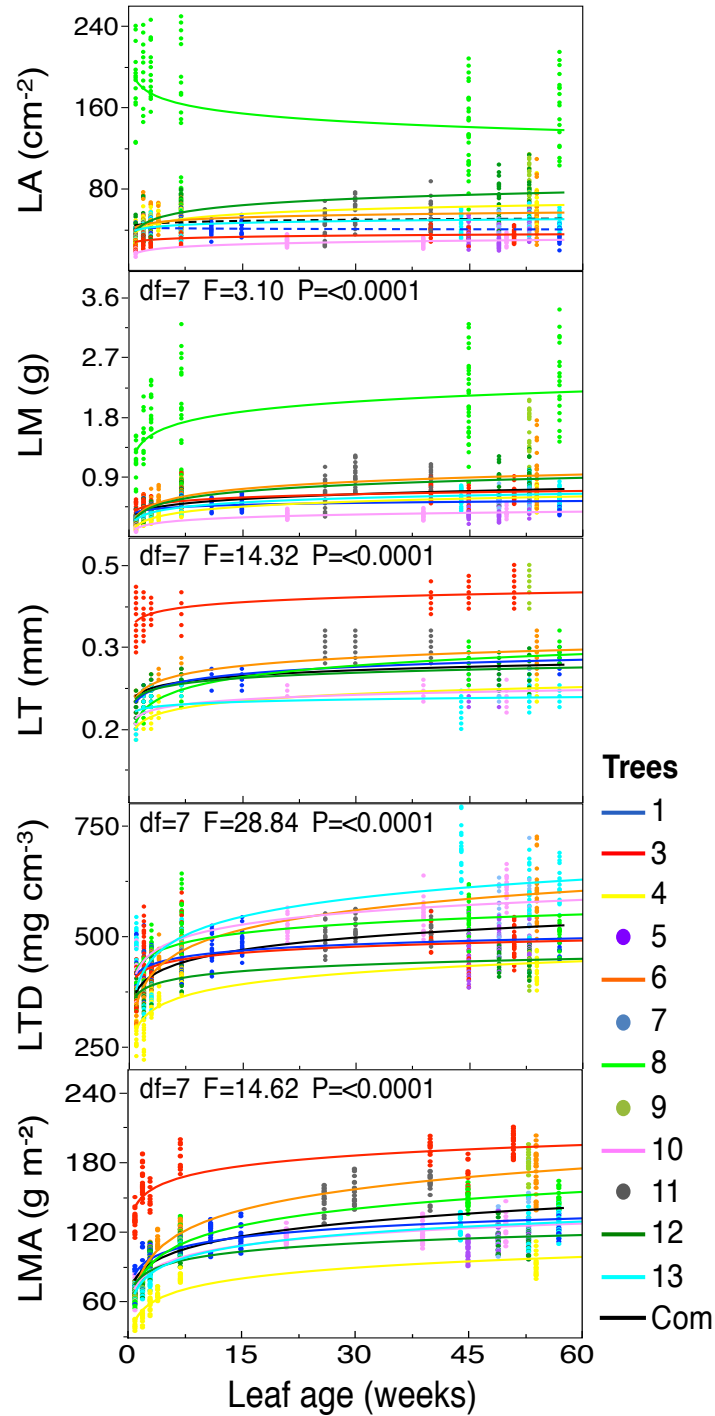


Figure S 6.8.3 Relationships between leaf traits and leaf age (log-transformed). Coloured lines represent individual tree regressions (performed only for trees with four or more sampled leaf age classes; $IT R^2$) and black lines represent community regressions (12 trees included; $Com R^2$). Individual leaf trait values are used as data points. Dashed lines are nonsignificant regressions. All significant regressions were $P < 0.001$. LMA, leaf mass per unit area; LWC, leaf water content; P_{mass} , leaf phosphorus content; N_{mass} , leaf nitrogen content; C_{mass} , leaf carbon content.



Chapter 7 Statement: *soon to be submitted for publication*

Chapter 7 investigates if the phenological age-related changes in leaf spectral properties reported in **Chapter 5** are also expressed at the canopy scale and in canopy vegetation indices (VIs) such as NDVI, EVI2 and NDWI. This chapter also adopts a broader perspective to investigate the different mechanisms that contribute to the seasonal spectral properties of tropical forest communities as captured by remote sensing vegetation indices. This final research chapter addresses the fourth to sixth aims of my research: “**(4) to analyse how age-related phenological changes in leaf reflectance are expressed at the canopy level in trees with diverse leaf types and phenological behaviours, and how this affects different spectral regions, (5) to investigate how phenological age-related changes in leaf reflectance and canopy leaf area (CLA) affect greenness vegetation indices commonly used to measure vegetation greenness (the Normalised Difference Vegetation Index; NDVI and the Enhanced Vegetation Index; EVI) and vegetation water content (the Normalised Difference Water Index; NDWI), and (6) to explore how the interaction, covariation and/or unique spectral behaviours of other complex and diverse canopy phenological processes contribute to reported remotely sensed canopy dynamics in the tropics**”. This study reveals that at the canopy scale, the expression of age-related phenological changes in leaf reflectance depends on both canopy leaf area (CLA) and the leaf phenological

behaviour of individual trees. It also demonstrates that at the community scale, the complex interaction, covariation, asynchrony and unique behaviours of tropical phenological processes create complex seasonal dynamics that are difficult to capture using a single remote sensing monitoring tool. Finally, the findings in this chapter highlight the need for more widespread multiscale phenological studies if a significant mechanistic understanding of what creates and drives different phenological mosaics identified across Amazonian forests is to be achieved.

Author Contribution Statement

C.C-B., Y.M. and F.F.G. planned and designed the research. C.C-B. designed field data collection protocols and conducted fieldwork and field data collection with logistical support from E.G.C.C. Data integration and quality assurance were performed by C.C-B. and A.A. Data analysis was performed by C.C-B, with the exception of the MODIS Fourier analyses which were performed by C.T.G. Chemical analyses were undertaken by B.J.E. at the University of Arizona. C.C-B. wrote the manuscript with contributions from Y.M. and F.F.G.

Chapter 7 Remotely sensed seasonal canopy dynamics

in the tropics: a riddle of many scales

Authors

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Abstract

- Leaf demography has been proposed as a major driver of seasonal productivity in carbon-rich tropical evergreen forests yet tropical leaf phenology is poorly understood.
- At the individual tree canopy scale, we found that (1) age-related phenological changes in leaf reflectance are expressed at the canopy scale but influenced by both canopy leaf area (CLA) and the leaf phenological behaviour of individual trees; (2) the seasonality of greenness VIs such as NDVI and EVI2 are more strongly correlated to phenological changes in CLA than changes in leaf reflectance; and (3) NDWI (water content VI) which was found to be strongly correlated to age-related changes in leaf reflectance could complement greenness VIs in phenological studies.
- At the community scale, more vertically complex tropical evergreen canopy communities display more complex leaf phenological behaviours that are more difficult to monitor. Furthermore, other community scale phenological sources of reflectance and structural canopy variation such as (1) leaf phenological asynchrony within and between trees; (2) short periods of leaflessness in brevi-deciduous trees that are difficult to detect; and (3) lack of seasonality displayed by particular evergreen leaf phenological behaviours can produce dampening effects on the seasonal amplitudes of annual cycles observed by remote sensing VIs.

- By combining leaf, canopy and community scale phenological observations we show that complex and diverse leaf phenological behaviours exhibited by tropical canopy trees challenge our current ability to remotely sense tropical canopy dynamics. This highlights the need for more widespread phenological studies that examine the interaction, covariation, asynchrony and unique behaviours of tropical phenological processes at different scales to achieve a significant mechanistic understanding of what creates and drives different phenological mosaics identified across the Amazonian forests and in modelling their effects on water and carbon fluxes across the Amazon.

Key Words

Tropical phenology; canopy reflectance; NDVI; EVI; phenological behaviours; evergreen tropical forests; tropical canopy trees; asynchrony

7.1 Introduction

Tropical forests contain around 13% of the global carbon stores of all terrestrial ecosystems (Malhi *et al.*, 2002a). They account for approximately 50% of terrestrial photosynthesis (Field *et al.*, 1998) and strongly regulate regional and global climate (Nobre *et al.*, 1991; Betts *et al.*, 2007). These globally important roles are mediated through a simple structural unit: the leaf (Clark *et al.*, 2008). Primary productivity, and carbon and water exchange in forests, and between forests and the atmosphere, are controlled by the number of leaves, their photosynthetic capacity and their response to environmental drivers such as water, light and temperature (Borchert, 1994). This is why the study of the spatial variation and seasonality of tropical leaf phenology (the timing of life-cycle events such as leaf flushing, developmental phases and leaf abscission) and the extent to which climate factors drive it is a subject of much current interest.

Although studying the phenological dynamics of tropical forests is important as they may exert a strong influence on regional and global climate, hydrological and carbon cycles, conducting field phenological studies in tropical forests which are tens of meters tall, rich in species and have high leaf area index (LAI; $>4\text{m}^2/\text{m}^2$) is very challenging. Only a small number of field-based leaf phenological studies in tropical forests exist, for example, there are only a few studies relevant to the ecology of evergreen tropical forest canopies in Amazonia, the forest type under study here (Frankie *et al.*, 1974; van Schaik *et al.*, 1993;

Wright & van Schaik, 1994; Reich, 1995; Reich *et al.*, 2004). Satellite remote sensing can provide an important tool for studying phenological dynamics at scales beyond those possible with field studies. This is because satellite remote sensing provides the only means of viewing large portions of the Earth's surface at regular intervals and gather a large amount of information relevant to the dynamics and function of ecosystems.

Several remote sensing studies have used the LAI and vegetation indices products (VIs) from the Advanced Very High Resolution Radiometer (AVHRR) and Moderate Resolution Imaging Spectrometer (MODIS) to investigate different aspects of Amazonian leaf phenology. These remote sensing products use reflectance data from two or more spectral bands and ratio this data in different ways to provide estimates of canopy greenness -a composite property of both leaf (leaf intercellular structure and biochemical composition) and canopy (canopy leaf area and structure) properties- and other vegetation biophysical properties such as leaf area index (LAI) and/or the fraction of absorbed photosynthetically active radiation (fAPAR) (Huete *et al.*, 2002). For example, Asner *et al.* (2000a) used the AVHRR Normalised Difference Vegetation Index (NDVI) to investigate El Niño effects on Amazonian phenology and productivity, Silva *et al.* (2013) used this same product to examine the large-scale heterogeneity of Amazonian phenology. Several other studies using the MODIS Enhanced Vegetation Index (EVI; an optimised index designed to be more

responsive to canopy structural variations and to not saturate at high LAI values such as those found in tropical forests (Huete *et al.*, 2002)) identified a “greening-up” in Amazonian forests during the dry season, and studied its relationship to seasonal radiation, precipitation and the flushing of new leaves in the Amazon region (Xiao *et al.*, 2006; Anderson *et al.*, 2007; Myneni *et al.*, 2007; Saleska *et al.*, 2007; Doughty & Goulden, 2008; Brando *et al.*, 2010; Anderson *et al.*, 2011; Bradley *et al.*, 2011; Anderson, 2012).

The remote sensing studies mentioned above, used the seasonal amplitude of annual cycles in time-series of NDVI and EVI to investigate the temporal signal of leaf phenology of forest canopy communities included within different satellite image pixels. However, both experimental and modelling studies have established that the reflectance properties of individual leaves are insufficient to describe the remotely sensed response, as canopies are assemblages of green (many separate leaves that vary in number, size, orientation, shape and clumping patterns) and non-green plant parts (bark, twigs, flowers etc.) and shadow, with soil or leaf litter reflectance less important in densely vegetated tropical canopies (Gausman, H. W. *et al.*, 1970; Myneni *et al.*, 1989; Jacquemoud, 1993; Baret *et al.*, 1994; Fourty *et al.*, 1996; Jacquemoud *et al.*, 1996; Asner, 1998; Blackburn, 1998; Datt, 1999; Sims & Gamon, 2002; Huete *et al.*, 2006b; Huang *et al.*, 2007; Asner & Martin, 2008b; Chavana-Bryant *et al.*, 2016). In this study, we examine processes related to leaf phenology at the leaf, individual tree crown, plot and

satellite pixel scale, in a Peruvian evergreen Amazonian tropical forest that exhibits moderate seasonality. We start by analysing how age-related changes in leaf reflectance are expressed at the canopy scale in trees with diverse leaf types and phenological behaviours and how this affects different spectral regions controlled by leaf pigment content (i.e. visible spectral domain: 350-700nm; VIS), internal structure (i.e. near infrared spectral domain: 700-1400nm; NIR), water content (i.e. short infrared spectral domain: 1400-2500nm; SWIR and NIR), and biochemicals (i.e. SWIR: 1400-2500nm). We then examine how age-related changes in leaf reflectance and canopy leaf area (CLA) affect canopy vegetation indices: (i) the Normalised Difference Vegetation Index (NDVI) and (ii) Enhanced Vegetation Index (EVI) which are commonly used to measure vegetation greenness, and (iii) the Normalised Difference Water Index (NDWI) used to measure vegetation water content. Finally, we consider how other complex and diverse canopy phenological processes such as leaf phenological behaviours and between tree phenological asynchrony, contribute to our reported remotely sensed canopy dynamics. To date, no studies have explored the relative importance that each of these scale-dependent phenological processes can have on remotely sensed NDVI and EVI time-series. Yet, our ability to better understand and interpret these remote sensed VI products used to monitor tropical leaf phenology hinges on our ability to resolve the interaction of these processes, and their potential covariance or unique behaviours.

7.2 Materials and methods

7.2.1 Field site

Data were collected in and around the borders of two lowland tropical rainforest plots located within the Tambopata National Reserve in the Madre de Dios region of Peru in southwestern Amazonia. The two study sites are part of the RAINFOR Amazon Forest Inventory Network and GEM intensive monitoring network, with RAINFOR codes TAM-06 (12°50'24"S, 69°17'59"W) and TAM-09 (12°49'48"S, 69°16'48"W). The sites are located between 215m and 220m above sea level within lowland closed-canopy primary tropical rain forest growing on Haplic alisol soils (Malhi *et al.*, 2002b). They are fairly seasonal sites with a four to five month-long dry season (number of months with <100 mm rainfall) between June and October, mean annual rainfall of 1900 mm (this may be lower than the long-term mean due to droughts in 2005 and 2010 (Quesada *et al.*, 2010)) and mean annual air temperature of 24.4°C. A detailed description of the geomorphology, climate and forest carbon cycle of this site is given by Malhi *et al.* (Lewis *et al.*, 2011).

7.2.2 Tree, branch and leaf sampling

Detailed descriptions of the tree, branch and leaf sampling used in this study can be found in Chavana-Bryant *et al.* (2016). Data collection was achieved through repeated climbing and sampling of 12 canopy and emergent trees (eight canopy and four emergent trees), the main contributors to the spectral response of forest

canopies (**Table 7.2.2.1**). To maximise variability within our leaf dataset we selected trees from different species, these different trees species represent a wide variety of leaf types (e.g. compound and simple leaves), leaf sizes and shapes, and leaf thickness and waxiness. This sampling allowed us to examine the spectral variability due to natural leaf aging in a tropical canopy tree community with diverse leaf properties.

Trees were monitored for bud development, new leaf emergence and development, general leaf condition and signs of leaf senescence. We sampled the developmental, mature and senescent leaf life-cycle phases for eight trees and also sampled some leaf age groups for four other trees. A detailed description of the leaf age classification used in this study can be found in **Figure 7.2.2.1**.

Table 7.2.2.1 Species, tree structural type and phenological information of sampled trees for leaf and canopy reflectance. Detailed descriptions of leaf phenological behaviours can be found in **Table 7.3.5.1**.

Tree code	Species	Family	DBH (m)	Tree canopy Position (Shape)	Height (m)	Canopy depth (m)	Leaf phenological behaviour	Flowering
1	<i>Licania brittoniana</i> Fritsch	Chrysobalanaceae	1.88	Canopy (vertically arranged)	29.50	10.50	Sun brevi-deciduous/Shade evergreen (SuBD/ShE)	Sep-Oct
3	<i>Simarouba amara</i> Aubl.	Simaroubaceae	1.62	Canopy (spherical)	29.00	8.35	Evergreen (~50% leaves exchanged during the dry season)	Flowers started to emerge mid-Aug with full flowering during Sep & Oct & falling during Nov
4	<i>Ruizodendron ovale</i> R.E. Fr. (Ruiz & Pav.)	Annonaceae	1.23	Canopy (vertically arranged)	32.62	10.07	Brevi-deciduous	
5	<i>Aspidosperma parvifolium</i> A.DC.	Apocynaceae	1.57	Emergent (umbrella-like)	33.70	8.40	Brevi-deciduous	
6	<i>Couratari macrosperma</i> A.C. Sm.	Lecythidaceae	1.43	Emergent (spherical)	33.74	6.27	Brevi-deciduous	
7	<i>Licania longistyla</i> (Hook.F.) Fritsch	Chrysobalanaceae	1.43	Canopy (umbrella-like)	30.30	8.70	Brevi-deciduous	
8	<i>Bertholletia excelsa</i> Bonpl.	Lecythidaceae	2.30	Emergent (umbrella-like)	34.00	11.60	Brevi-deciduous	
9	<i>Tachigali chrysalioides</i> van der Werff	Caesalpinioideae	2.10	Canopy (umbrella-like)	29.70	7.60	Brevi-deciduous	
10	<i>Clarisia racemosa</i> Ruiz & Pav.	Moraceae	1.58	Canopy (umbrella-like)	33.75	9.25	Brevi-deciduous	
11	<i>Eschweilera coriacea</i> (DC.) S.A. Mori	Lecythidaceae	1.88	Emergent (umbrella-like)	33.32	7.78	Brevi-deciduous	Flowers started to emerge at the end of Sep with full flowering during Oct & Nov
12	<i>Gautteria boliviana</i> H. Winkl.	Annonaceae	1.17	Canopy (spherical)	31.90	11.34	Evergreen (senescent leaves actively abscising and few new leaves emerging during the dry season with majority of leaf abscission and emergence at beginning of wet season)	Aug-Nov
13	<i>Pouteria franciscana</i> Baehni	Sapotaceae	2.37	Canopy (vertically arranged)	35.39	15.09	Sun/Shade asynchronous evergreen (Su/ShAE)	

DBH, diameter at breast height

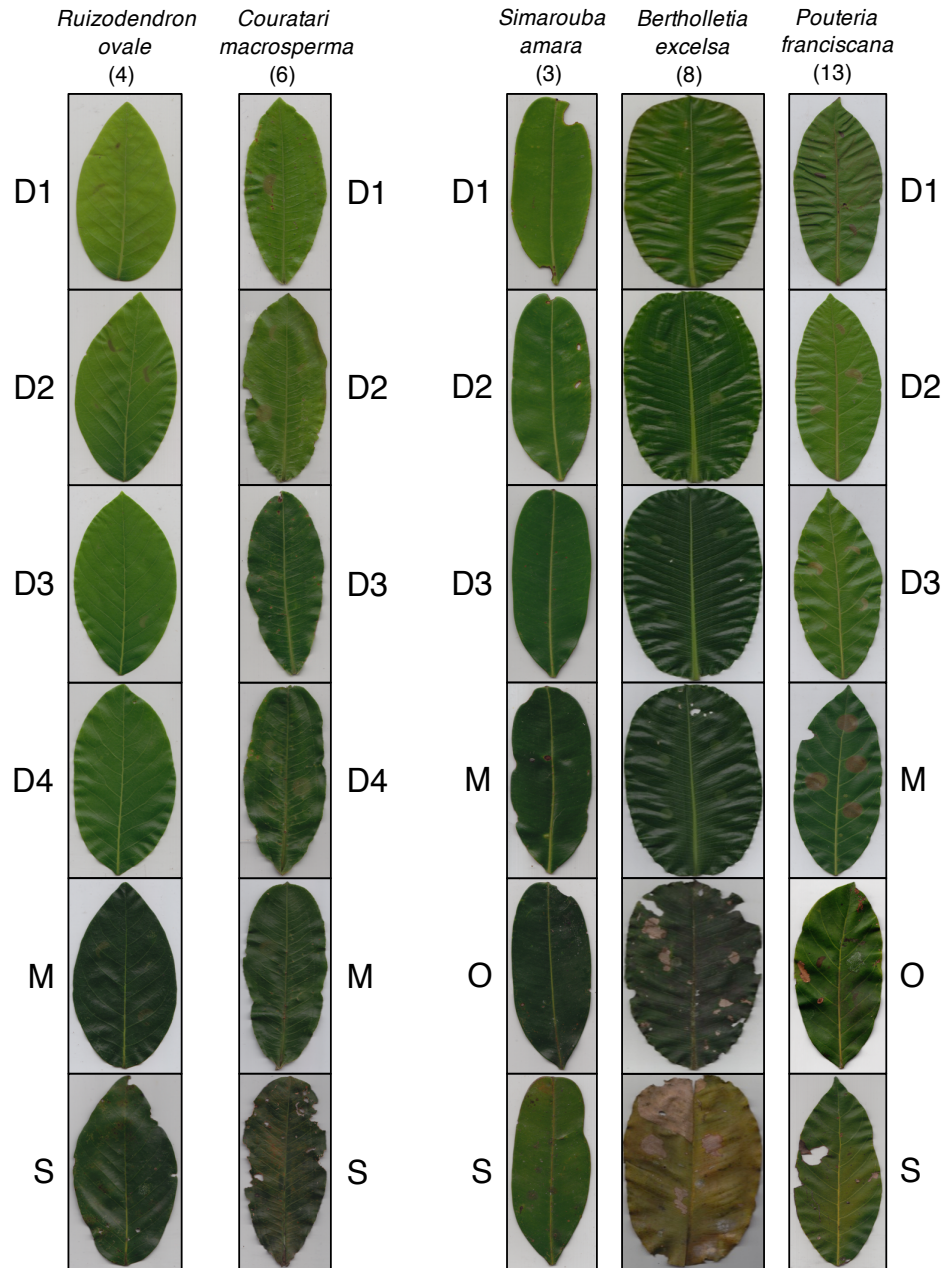


Figure 7.2.2.1 Sampled leaves of different age classes, illustrating the leaf life-cycle of some of our sampled trees: *Ruizodendron ovale*, *Couratari macrosperma*, *Simarouba amara*, *Bertholletia excelsa* and *Pouteria franciscana* (tree codes in brackets). Leaf age classification: D1, D2, D3 and D4 are developing leaves in the process of gaining area, thickness and green colouration and collected after 1, 2, 3 and 4 weeks of active leaf expansion, respectively; M are fully-grown and structurally developed mature leaves that have achieved a fully darkened green colour; O are old leaves showing initial signs of epiphylls and/or senescence and near the end of their life cycle (~2 months before senescence and abscission) and; S are senescent leaves displaying loss of chlorophyll and/or onset of decomposition and in the process of dying and actively abscising.

During leaf development, individual trees were sampled on a weekly basis from early leaf development (one week after active leaf expansion) until full leaf area expansion had been achieved (up to four consecutive weeks after active leaf expansion). During the mature leaf phase, trees were sampled monthly at different stages (i.e. newly matured, mid-maturity and end of maturity) for up to three months. Similarly, during the senescent leaf phase, individual trees were sampled at different stages (i.e. old leaves not yet abscising and senescent leaves starting abscission through to advanced senescence when most leaves had been abscised from the tree crown) for up to two months for both old and senescent leaves.

7.2.3 Demographic leaf counts and canopy leaf area

Each time a tree was sampled, two top-of-canopy (fully exposed to direct sunlight) apical metre-long branches were collected. Sampled branches were recut under water and a small bag full of water attached to the base of each branch to maintain hydraulic connectivity and minimise leaf desiccation during transport to the field lab. Leaf spectral and morphological (fresh leaf weight and area) measurements were carried out within two hours of the branches being cut. Demographic leaf counts (i.e. number of leaves from each leaf age group present) were collected for all sampled branches. We calculated leaf area for each branch we sampled using the demographic leaf data and the mean leaf area for each leaf age class present as follows:

$$LA = (N_{la} * A_{la}) + (N_{lb} * A_{lb}) + \dots \quad (1)$$

where N_{la} = number of leaves present from leaf age group “a” and A_{la} = mean leaf area for that leaf age group. The mean leaf area (LA) of the two branches was used to represent canopy leaf area (CLA).

7.2.4 Leaf and canopy spectroscopic measurements

We collected reflectance measurements at both the leaf and canopy level. For leaf level measurements, 15 to 30 leaves were randomly selected from each sampled leaf age group. In the field lab, leaves were cut (at base of petiole) from their branch just before reflectance measurements were collected.

Measurements were collected using an ASD field spectrometer (Analytical Spectral Devices FieldSpec Pro, Boulder, Colorado, USA) using a contact probe. Four reflectance measurements were collected and averaged for each leaf (dependent on leaf size). This protocol delivered high-quality calibrated spectra that did not require smoothing or other filters. The full-range leaf spectral data were trimmed at the far ends (<412nm and >2450nm). Full protocol details can be found in Chavana-Bryant *et al.* (2015). To account for potential effects of leaf coatings and natural leaf damage on spectra, we only used reflectance measurements free of epiphylls and necrosis.

Nadir 1m² top-of-canopy reflectance measurements were collected from 3 to 4 different canopy positions (**Figure 7.2.4.2a** and **Figure 7.2.4.2b**). Reflectance

measurements were collected using a multispectral radiometer (MSR32R, Cropscan Inc., MN, USA) fitted with 32 sensors, to provide the reflectance for 16 bands (16 upward and 16 downward facing sensors to measure incoming and reflected radiation, respectively). The 16 bands were strategically selected to collect information on canopy pigment content (VIS; visible bands: 470-700nm), red edge (RE; bands: 650-810nm), water content (bands: 970 and 1200nm), and simulate MODIS bands 1-6 (detailed band information is provided in **Figure 7.2.4.1** and **Table S 7.8.1**. Measurements were collected under clear sky conditions with a solar zenith angle close to 40°.

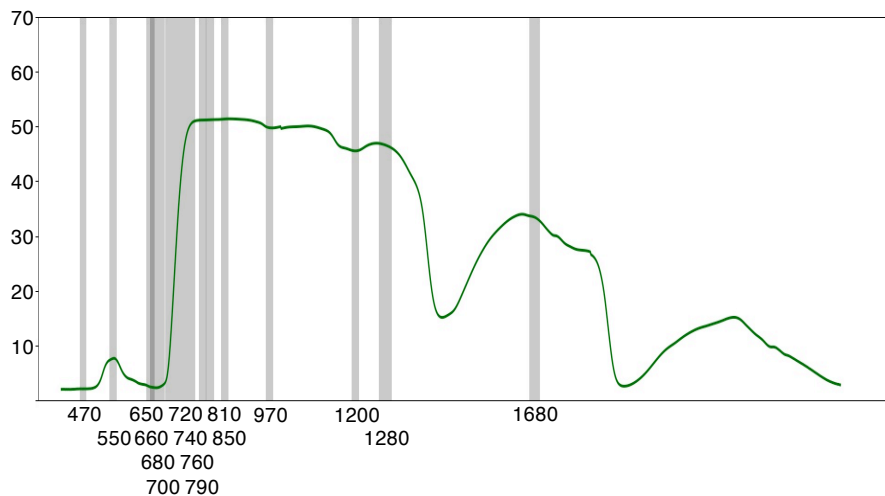


Figure 7.2.4.1 Multispectral radiometer band information. The centre wavelength (CWL) for each band is displayed with grey areas showing the full width at half maximum (FWHM) for each band. Full band information is provided in **Table S 7.8.1**. The hyperspectral mean reflectance (green line) of *Bertholletia excelsa* mature leaves is provided as reference.



Figure 7.2.4.2a Top-of-canopy reflectance measurements were laboriously collected using advanced arborist tree climbing techniques. Measurements from 3-4 different sampling positions for each sampled tree required (1) clear sky conditions, (2) collection within 30 minutes to minimise the impact of changes in shadows and (3) a solar zenith angle close to 40° to avoid shading the canopy target with the spectrometer. Meeting these optimal sampling conditions frequently required multiple sampling attempts.



Figure 7.2.4.2b op-of-canopy reflectance measurements were laboriously collected using advanced arborist tree climbing techniques. Measurements from 3-4 different sampling positions for each sampled tree required (1) clear sky conditions, (2) collection within 30 minutes to minimise the impact of changes in shadows and (3) a solar zenith angle close to 40° to avoid shading the canopy target with the spectrometer. Meeting these optimal sampling conditions frequently required multiple sampling attempts.

We collected and averaged 15 reflectance measurements for each canopy position, averaged measurements were then averaged by individual tree. The same canopy positions within each sampled tree were resampled in order to

capture canopy reflectance at different phases of the leaf life-cycle (i.e. developmental, mature and senescent phases). Due to uneven emergence of new leaves within tree canopies, the developmental leaf phase was sampled once leaves had emerged at all sampling positions for each individual tree. Developmental canopy reflectance measurements are therefore, the result of a mix of leaves of different developmental age groups (i.e., D1, D2 and D3; see **Figure 7.2.2.1**). Developmental canopy life-cycle phase (or canopy age group) was assigned according to the most dominant developmental leaf age group present in the canopy at the time of sampling.

7.2.5 Analyses of leaf- and canopy-level reflectance and vegetation indices (VIs), and canopy leaf area (CLA)

We compared mean reflectance of the different leaf age groups sampled at both the leaf and canopy level within individual trees. We evaluated absolute values and relative difference values (RD):

$$RD = \left[\frac{(\rho_c - \rho_l)}{\rho_l} \right] * 100 \quad (2)$$

where ρ_c = canopy reflectance and ρ_l = leaf reflectance.

We explored the effect that age-related changes in leaf reflectance can have on three commonly used VIs at both the leaf and canopy level: the normalised difference vegetation index (NDVI) and the enhanced vegetation index (EVI) are VIs used to measure vegetation greenness, while the normalised difference

water index (NDWI) is used to measure vegetation water content. We used the two-band EVI (EVI2) as atmospheric corrections are not relevant to this study. We calculated these VIs for each leaf and canopy spectral sample, using the MODIS filter response functions (Xiong, 2015) and the equations summarised in **Table S 7.8.2**. For ease of comparison, all three VIs were standardised to a common 0-1 scale. Mean VI values and standard error (SE) were calculated for each sampled leaf and canopy age group within individual trees and at the community level (i.e. across all 12 trees). We report differences (measured in % change) in VIs between different leaf age groups. We analysed the relationships of canopy VIs to age-dependent changes in both leaf reflectance (i.e., leaf VIs) and canopy leaf area (CLA). We also performed independent samples Student's t-tests to compare the means of leaf versus canopy VIs for the mature age groups.

7.2.6 Leaf phenological behaviours and plot-level structural tree data

We monitored the leaf phenological behaviours and the timing of these behaviours for the twelve trees included in this study during the 2011 dry season. We also collected plot level structural data (diameter at breast height; DBH, total height and canopy depth) for all trees and palms with a DBH ≥ 10 cm in plot TAM-06. Trees and palms were classified by their canopy position (i.e., emergent, canopy or sub-canopy) with trees also classified by their canopy type

(shape) and possible leaf phenological behaviours according to their tree crown structural type (canopy position + crown shape).

7.2.7 MODIS vegetation indices (VIs) time-series analyses

NDVI and EVI 13-year time-series (Apr 2000-Jun 2014) were derived from the 16-day 500m MODIS VI products (EVI and NDVI). These input time-series were acquired from LPDAAC and are referred to as MOD13A1. We used the Fourier Transform (FT) method to test for the presence of regular annual cycles in the NDVI and EVI time-series for both our plots TAM-06 and TAM-09 following the approach described in Bradley *et al.* (2016). The time-series underwent a standard linear de-trending before the FT was applied to allow for the analysis of a 'stationary' time-series (Weedon, 2003). We derived the power spectra for the 13-year time-series to establish the presence and significance of the spectral peak that coincides with the frequency matching of the annual cycle. To be significant, this spectral peak had to exceed the 95% confidence level defined by the lag-1 autoregressive spectral background model AR1 or 'red noise'. The power spectra of this spectral peak can then be used as a measure of the amplitude of the annual cycle. For a 13-year 16-day time-series, this method requires a time-series of 100 or more good quality observations (out of a total of 320 observations), with at least one observation per month. The time-series of the MODIS pixel containing TAM-06 did not fulfill this requirement, so the time-series of a nearby pixel was used instead (12°49'59" S, 69°18'53" W).

7.3 Results

7.3.1 Leaf age phenological effects on canopy reflectance

Canopy reflectance displayed similar age effects as those displayed by leaf reflectance (age effects on the physiochemical and spectral leaf properties of our sampled trees have been described in detail in Chavana-Bryant *et al.*, 2016) but mostly showed lower reflectance values throughout the spectral range (**Figure 7.3.1.1**). The main observed patterns of age-related spectral changes in canopy versus leaf reflectance were, except for the canopies of *Licania brittoniana* and *Simarouba amara* (trees 1 and 3, respectively): in the visible spectrum (VIS; multispectral bands 470-700nm), both leaf and canopy reflectance showed concave responses with age (decreasing until maturity and increasing in old/senescent leaves). The strong age-induced changes observed in leaves at band 550nm (green peak) were not observed in canopy reflectance. As previously reported for leaf reflectance, canopy near infrared reflectance (NIR; multispectral bands 700-1280nm) displayed strong age effects with canopies comprised of waxy leaves (trees 1, 3 and 10) displaying less pronounced effects. However, canopy NIR reflectance for *S. amara* (tree 3) displayed contrasting patterns with age from those shown by its leaf reflectance. Significantly, the NIR reflectance collapse observed in senescent leaves between 700-850nm (3-10% mean decrease compared to mature leaves across individual trees) was even more dramatic in canopy reflectance, which displayed mean decreases of 7-28% across trees. Canopy shortwave-infrared reflectance (SWIR; multispectral band

1680nm) generally increased with age but showed no obvious differences in canopies composed of high wax leaves as did leaf SWIR reflectance. Significant increases (across VIS bands: 28-109%) and decreases (across NIR and SWIR bands: 30-46%) in reflectance were also observed when we sampled the bare canopy of one of our trees (*Ruizodendron ovale*; tree 4).

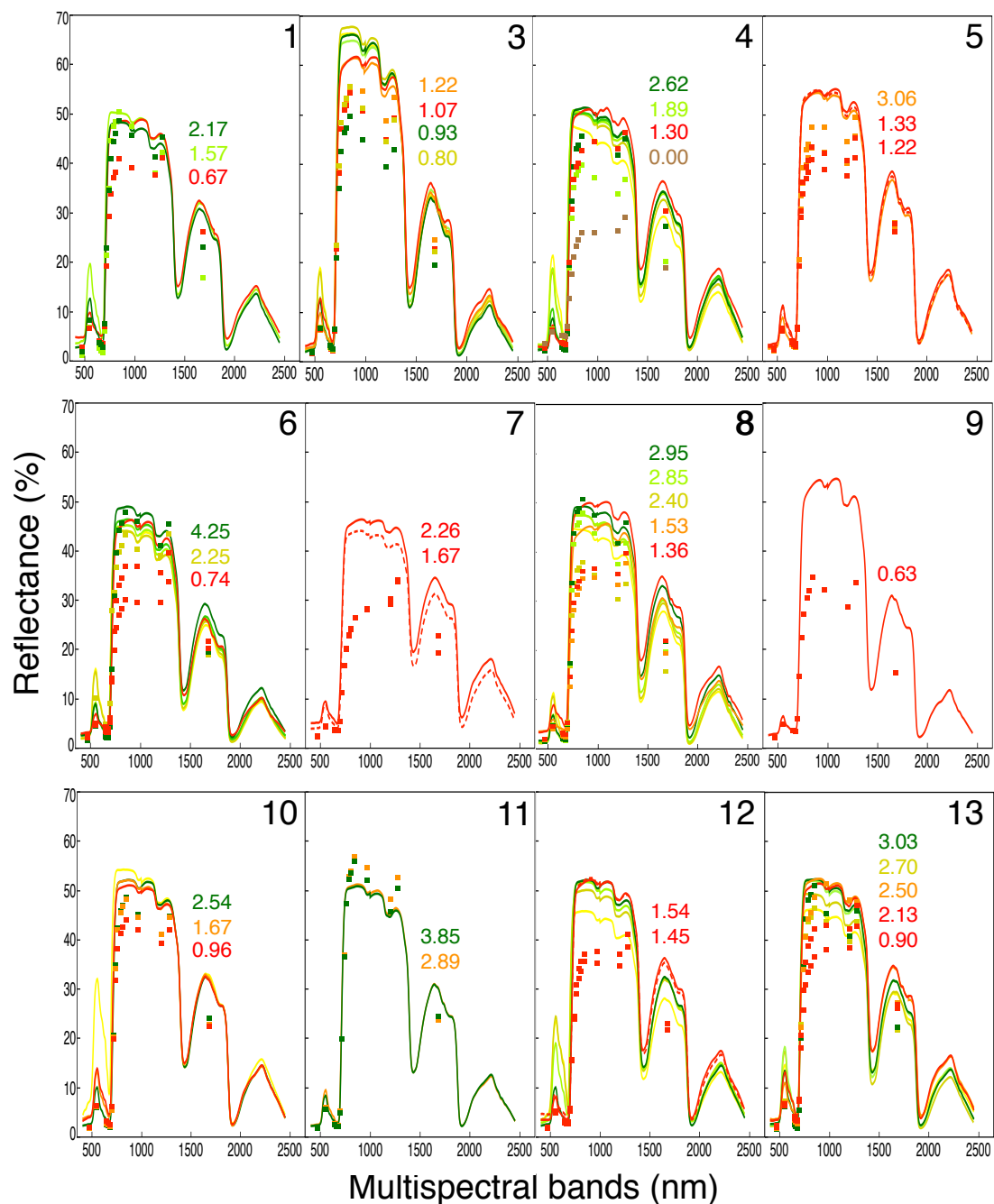


Figure 7.3.1.1 Hyperspectral mean leaf reflectance (solid and dashed lines) and multispectral (16 bands) mean canopy reflectance (squares) for each sampled leaf age group within individual trees (1-13). The estimated canopy leaf area (colour-coded by leaf age group and arranged in descending order) for each canopy measurement is also shown. Leaf age groups: Developmental leaves collected after one (light yellow), two (dark yellow) and three (light green) weeks of active leaf expansion; Mature leaves (dark green); Old leaves (orange); and Senescent leaves (red).

7.3.2 Sensitivity of canopy reflectance to leaf reflectance

We found that the canopy reflectance of different age groups (i.e. developmental, mature and senescent), showed the strongest sensitivity to leaf reflectance (i.e. smallest differences) in the NIR bands 760-1280nm, followed by the SWIR band 1680nm, with the weakest sensitivity (i.e. largest differences) displayed in the VIS and early RE bands 470-740nm (**Figure 7.3.2.1**). Furthermore, mature canopies displayed the strongest overall sensitivities of 2-15% across the NIR bands 760-1280nm, ~20-30% in the SWIR band 1680nm and ~10-45% across the VIS & early RE bands 470-740nm; followed by senescent canopies showing sensitivities of ~10-40%, ~20-35% and 25-55%, respectively; while developmental canopies exhibited the weakest overall sensitivities of ~10-30%, ~20-45% and ~35-65%, respectively.

Variability in canopy sensitivity to leaf reflectance also displayed both wavelength and age-dependent patterns (**Figure 7.3.2.1**). In terms of wavelength, VIS generally displayed the least variability with bands 470 and 680 showing the smallest variability and band 550 (green peak) the largest variability. RE bands 700 and 720 displayed the largest variability within NIR and SWIR bands. Finally, senescent canopies generally displayed larger variability across the whole spectral range.

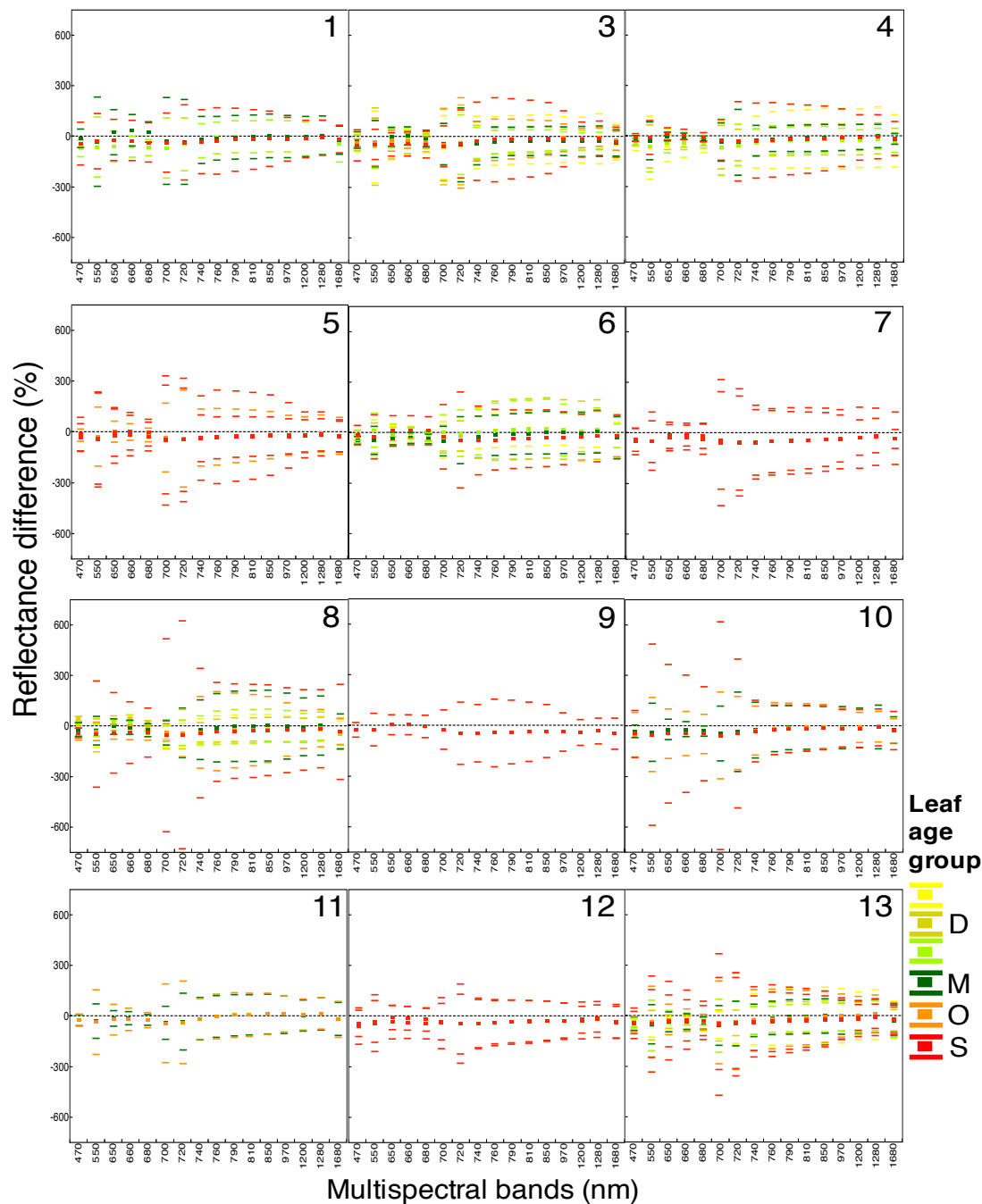


Figure 7.3.2.1 Sensitivity of canopy reflectance to leaf reflectance (measured as the difference between mean canopy and mean leaf reflectance (squares) relative to the mean leaf reflectance $\pm$ SD (lines)) for each leaf age group sampled within individual trees (1-13). Radiometer band filters were used to calculate the reflectance of the 16 radiometer bands from the hyperspectral leaf reflectance. Leaf age groups: D= developmental leaves collected after one (light yellow), two (dark yellow) and three (light green) weeks of active leaf expansion; M= mature leaves (dark green); O= old leaves (orange); and S= senescent leaves (red).

7.3.3 Leaf age phenological effects on canopy leaf area

Strong age effects were also observed on canopy leaf area (CLA) across all our sampled trees (**Figure 7.3.1.1**). With the exception of *S. amara* (tree 3), mature canopies displayed the highest CLA across trees, followed by developing canopies with senescent canopies displaying the lowest CLA. In **Figure 7.3.3.1** we show the leaf turnover in terms of the observed canopy leaf area and canopy leaf life-cycle phase for each sampled during the 2011 dry season.

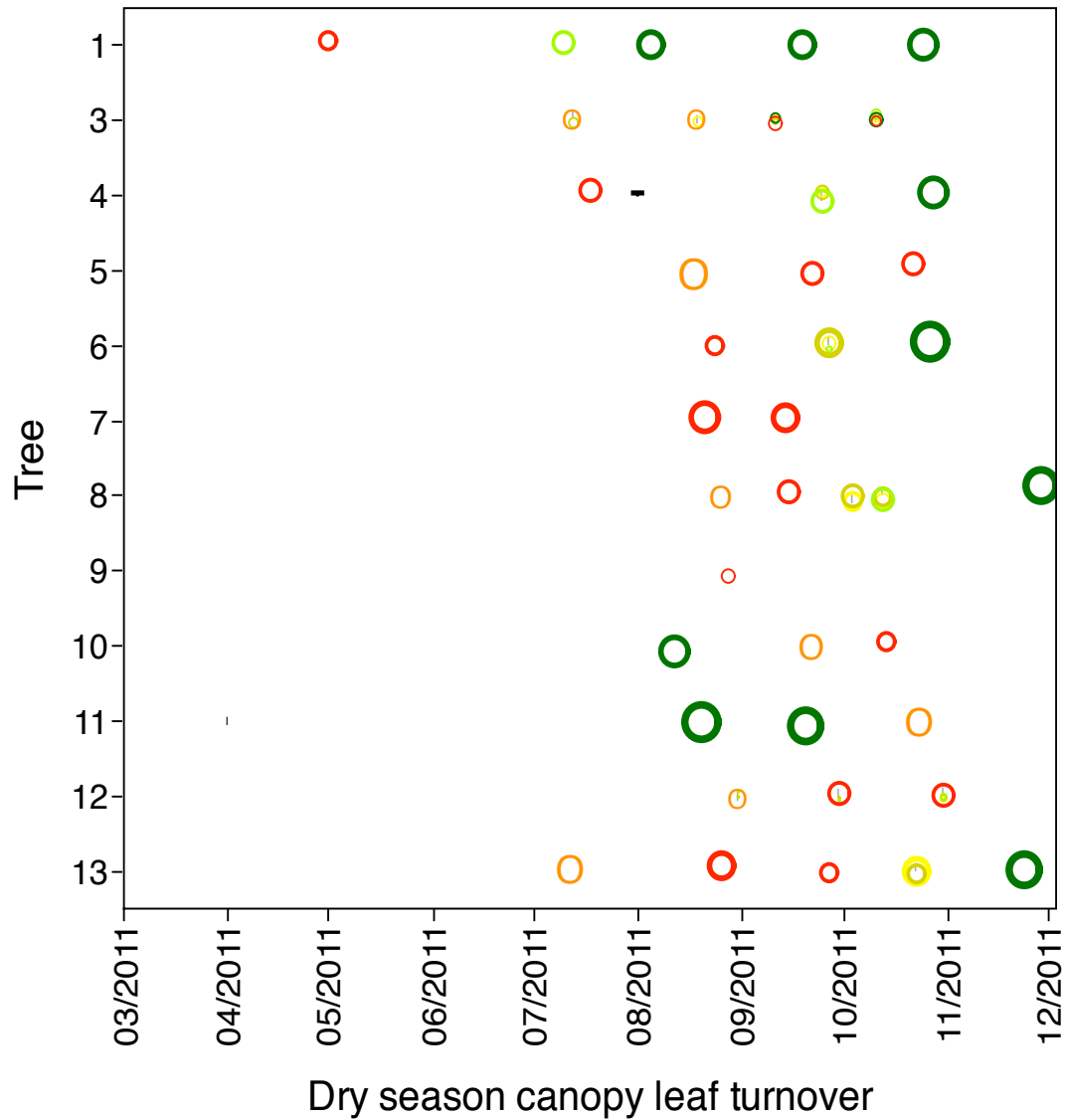


Figure 7.3.3.1 2011 dry season canopy leaf turnover for each of our sampled trees. The different canopy leaf life-cycle phases per tree are represented by colour coded circles. Canopy life-cycle phases: immature phase when canopies are composed of newly emerged immature leaves (I), D= developmental phase when canopy leaves undergoing early (light yellow circles), mid (dark yellow circles) or advanced (light green circles) development; M= mature phase when canopy leaves are mature (dark green circles); O= old phase when canopy leaves are near the end of their life-cycle (two to three months before senescence and abscission) and showing initial signs of epiphyll cover and/or senescence (orange circles); S= senescent phase when canopy leaves are in the process of dying and abscising and can be distinguished by loss of chlorophyll or onset of decomposition (red circles); and canopy leaflessness phase when canopies are leafless for an entire month (-). The size of circles representing different canopy leaf life-cycle phases is proportional to the estimated canopy leaf area (CLA) for that phase.

7.3.4 Leaf age phenological effects on canopy vegetation indices (VIs)

As previously reported for leaf VIs in Chavana-Bryant *et al.* (2016), the greenness (NDVI and EVI2) and water content (NDWI) VIs analysed here also revealed age sensitivity at the canopy level (**Figure 7.3.4.1**). With the exception of *S. amara* (tree 3), individual trees displayed continuous decrease in NDWI as their canopies aged, while both NDVI and EVI2 increased as new leaves developed, reached maximum values at maturity and then declined as the leaves in their canopies reached old and senescent stages. Interestingly, the range of values for both leaf and canopy EVI2 were 0.01-0.05 greater than those of NDVI. Across individual trees, the NDVI and EVI2 of developing and senescent canopies displayed, relative to mature canopies, mean decreases of 3-15% and 4-19%, and 12-56% and 14-61%, respectively. Across individual trees, NDWI was on average 12-43% higher in developmental canopies and 10-68% lower in senescent canopies.

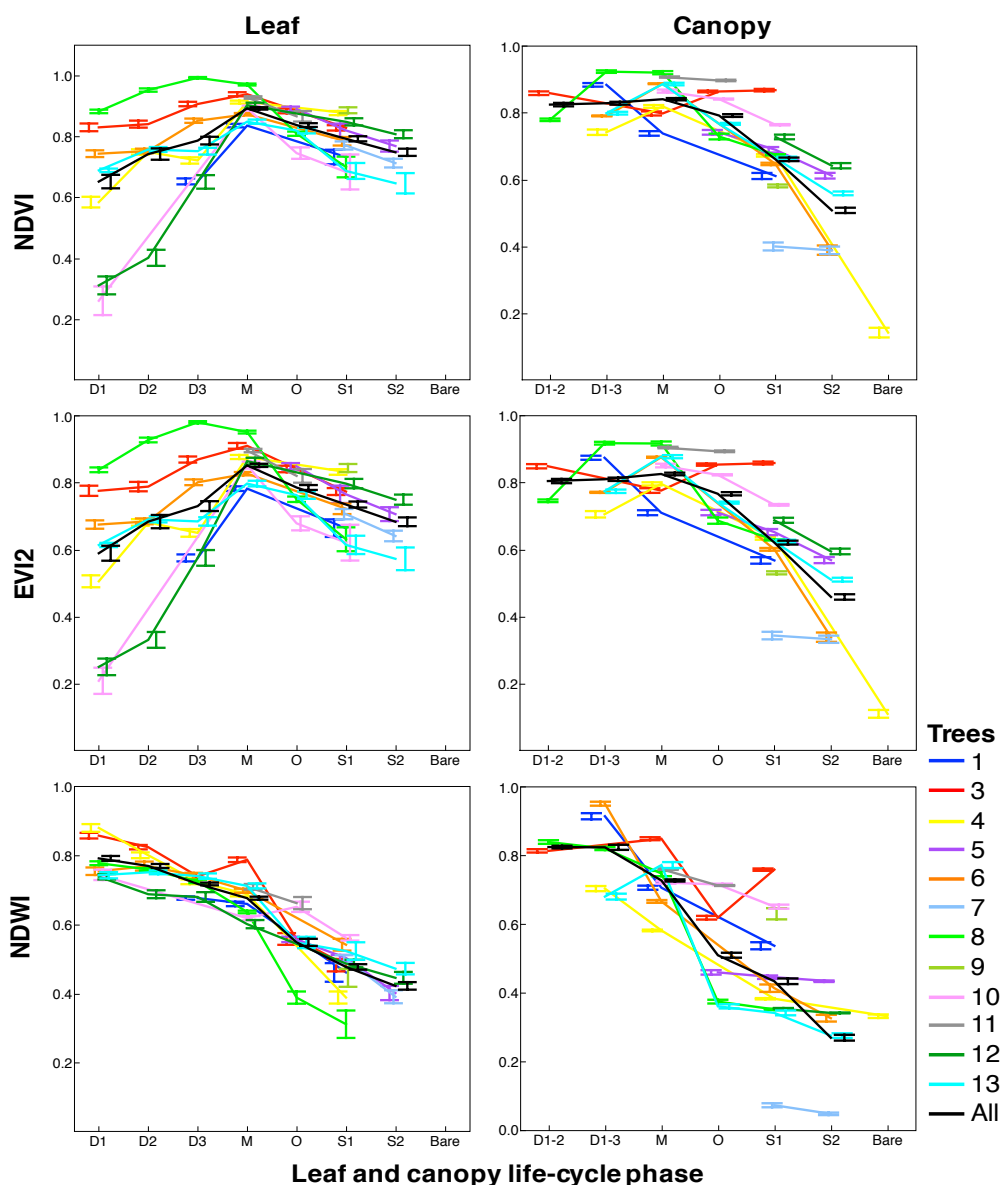


Figure 7.3.4.1 Variation in leaf and canopy level vegetation indices (VIs) attributable to leaf age or canopy life-cycle phase. VIs: normalised difference vegetation index (NDVI); enhanced vegetation index 2 (EVI2); and normalised difference water index (NDWI). Mean values with standard error (SE) were calculated for each sampled leaf age group or canopy life-cycle phase within individual trees (coloured lines) and across all trees (black lines). Leaf age groups: D1, D2 and D3= developing leaves collected after 1, 2 and 3 weeks of active leaf expansion, respectively; M= mature leaves; O= old leaves; and S1 & S2= senescent leaves. Due to uneven emergence of new leaves within tree canopies, the developmental canopy life-cycle phase was assigned according to the most dominant developmental leaf age group present in the canopy at the time of sampling. We also sampled the “bare” canopy life-cycle phase for one tree which, among our sampled trees, remained leafless for the longest period of time (one month).

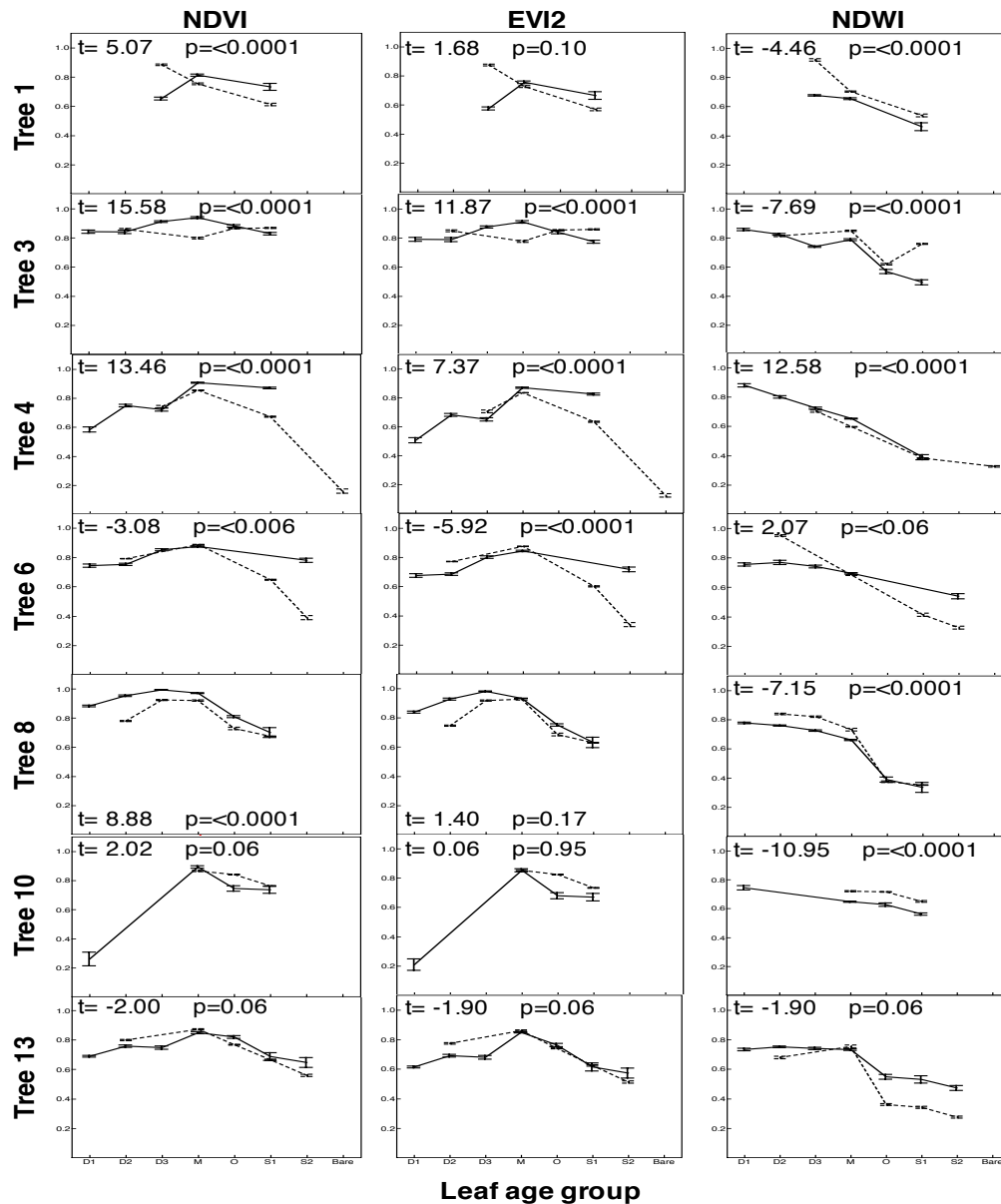


Figure 7.3.4.2 Variation in leaf (solid lines) and canopy (dotted lines) level NDVI, EVI2 and NDWI attributable to leaf age. Mean values with standard error (SE) were calculated for each sampled leaf age class within individual trees. Summary results for independent-samples t-tests between means of leaf and canopy VIs for the mature life-cycle phase are provided, t = test result and P value. Leaf age groups: D1, D2 and D3 are developing leaves collected after 1, 2 and 3 weeks of active leaf expansion, respectively; M= mature leaves; O= old leaves; and S1 & S2= senescent leaves. Due to uneven emergence of new leaves within tree canopies, the developmental canopy life-cycle phase was assigned according to the most dominant developmental leaf age group present in the canopy at the time of sampling. We also sampled the “bare” canopy life-cycle phase for one tree which,

among our sampled trees, remained leafless for the longest period of time (one month).

When we examined leaf and canopy VIs of individual trees, for which we sampled multiple leaf age groups, we found that except for *S. amara* (tree 3), leaf and canopy VIs were closest in value at the mature life-cycle phase (**Figure 7.3.4.2**). This was particularly true for EVI2, for which t-tests showed no significant differences in the means between leaf and canopy VIs for the mature leaf age group in four out of seven trees, and for which differences were observed to be smallest between means for the other leaf ages groups.

Finally, when we analysed the relationships between age-related changes in canopy VIs vs age-related changes in leaf VIs and in canopy leaf area (**Figure 7.3.4.3**), we found that both canopy greenness VIs were more strongly correlated to changes in canopy leaf area (NDVI and EVI2 $R^2 = 0.45$) than changes in leaf VIs (NDVI $R^2 = 0.24$ and EVI2 $R^2 = 0.28$) while changes in canopy NDWI were correlated only to changes in leaf NDWI ($R^2 = 0.45$).

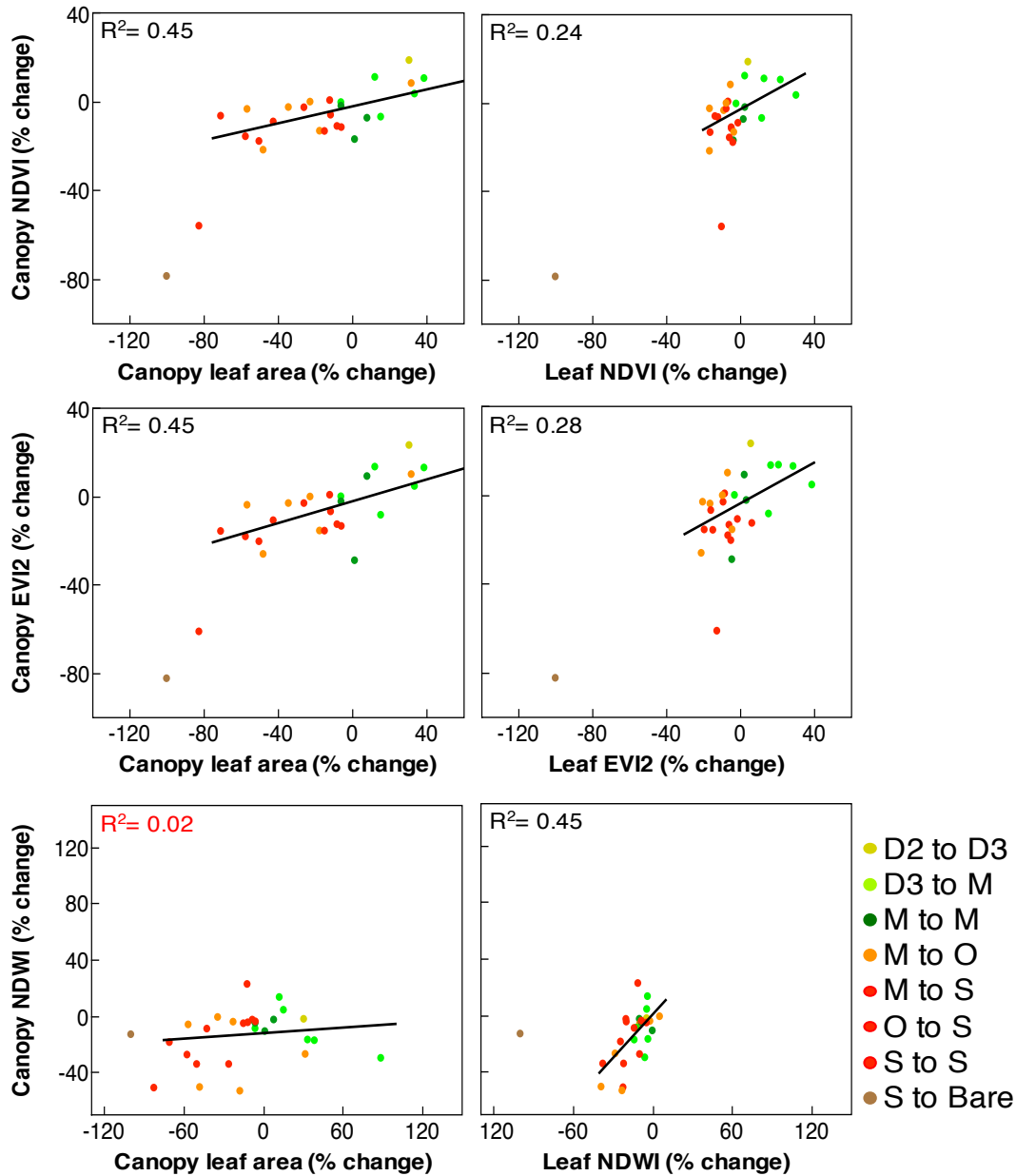


Figure 7.3.4.3 Relationships between age-dependent changes in canopy vegetation indices (VIs) and age-dependent changes in leaf reflectivity (VIs) and canopy leaf area (CLA). R^2 values in black are significant at the <0.005 level and R^2 in red is nonsignificant. Leaf age groups: D2 and D3 are developing leaves after 2 and 3 weeks of active leaf expansion, respectively; M= mature leaves; O= old leaves; and S1 & S2= senescent leaves. Due to uneven emergence of new leaves within tree canopies, the developmental canopy life-cycle phase was assigned according to the most dominant developmental leaf age group present in the canopy at the time of sampling. We also sampled the “bare” canopy life-cycle phase for one tree which, among our sampled trees, remained leafless for the longest period of time (one month).

7.3.5 Leaf phenological behaviours

In **Table 7.2.2.1** we report the leaf phenological behaviours of the twelve trees in this study, which are described in detail in **Table 7.3.5.1**. Two of these behaviours, sun/shade asynchronous evergreen (Su/ShAE) and sun brevideciduous/shade evergreen (SuBD/ShE), have not, to our knowledge, been previously reported in the literature, and are in fact, combinations of other well-known behaviours which are made possible by crown shapes that are vertically complex (i.e. crowns that are vertically arranged and are comprised of both sun and shade leaves). These leaf phenological behaviours were also observed in 4 other trees with vertically complex crowns we monitored at a site in French Guiana (unpublished data). In **Table 7.3.5.2**, by combining the canopy position and crown shape data we collected for every tree (DBH >10cm) within plot TAM-06 with the leaf phenological behaviours we report, we present the possible leaf phenological behaviours for each tree structural type found within this plot.

Table 7.3.5.1 Leaf phenological behaviours derived from our field monitoring of 12 trees with varied tree crown structural types.

Leaf phenological strategies	Description
Brevi-deciduous	• Canopy composed of only sun leaves or both sun and shade leaves • Senescent leaves abscised simultaneously • New leaves emerge simultaneously or sequentially • Tree remains bare for a brief period of time (1 to 4 weeks)
Evergreen	• Canopy composed of only sun leaves • ~ 50% of the leaf area is abscised and replaced with new leaves during the dry season and results in a canopy composed of two leaf growth seasons of varying proportions during the dry season. • Canopy never bare • Or • Canopy composed of only sun leaves or both sun and shade leaves • Senescent leaves start abscising and few new leaves emerging during the dry season with the majority of leaf abscision and emergence during the beginning of the wet season. This results in a canopy composed of two leaf growth seasons with senescent leaves dominating during the dry season and mature and new leaves dominating during the beginning of the wet season. • Canopy never bare
Sun/Shade asynchronous evergreen (Su/ShAE)	• Canopy composed of both sun and shade leaves • Senescent sun leaves are abscised simultaneously and new leaves flushed soon after • Shade leaves are retained and become exposed to sun for a brief period • New sun leaves expand • Shade leaves are abscised once sun leaves are fully expanded and new shade leaves are flushed soon after • Canopy never bare
Sun brevi-deciduous/Shade evergreen (SuBD/ShE)	• Canopy composed of both sun and shade leaves • Senescent sun leaves are abscised simultaneously and new leaves flushed soon after • Shade leaves are retained with leaves from up to three different growth seasons becoming exposed to sun • New sun leaves expand • Shade leaves from the oldest growth season start abscising once new sun leaves are fully expanded and new shade leaves are flushed soon after. Shade leaves from oldest growth season completely abscised by end of dry season • Canopy never bare

Table 7.3.5.2 Structural summary statistics for trees and palms (with $\geq 10\text{cm}$ DBH) when classified according to their canopy positions and crown structural type (canopy position + crown shape). We also present the possible leaf phenological behaviours trees could exhibit based on their crown structural type.

N	Canopy position (crown shape)	Leaf type	DBH (cm)		Height (m)		Canopy depth (m)		Possible leaf phenological behaviours linked to tree crown structural type				
			Mean (SD)	Range (Min - Max)	Mean (SD)	Range (Min - Max)	Mean (SD)	Range (Min - Max)	*Deciduous	Brevi-deciduous	Evergreen	Sun/Shade asynchronous evergreen (SSAE)	Sun brevi-deciduous/Shade evergreen (SBD/SE)
27	Emergent tree (umbrella-like)	sun	392.3 (160.7)	171 - 910	33.3 (3.4)	28.4 - 39.8	8.3 (1.3)	5.8 - 10.9	✓	✓	✓		
12	Emergent tree (spherical)	sun	412.1 (190.7)	217 - 829	35.6 (4.9)	29.3 - 46.2	11.2 (1.1)	9.5 - 13.3	✓	✓	✓		
22	Canopy tree (umbrella-like)	sun	254.4 (80.9)	156 - 465	26 (2.3)	21.6 - 29.7	7.3 (1.5)	4.3 - 9.5	✓	✓	✓		
20	Canopy tree (vertically arranged)	sun & shade	260.5 (87.8)	130 - 507	28.4 (2.9)	22.8 - 35.7	10.8 (2.3)	9 - 18.6	✓	✓	✓	✓	✓
178	Sub-canopy tree	shade	153.3 (49)	99 - 371	12.7 (3.4)	4.3 - 20.6	4.5 (1.7)	0.4 - 9.4	✓	✓	✓		
49	Palm canopy		221.2 (56.1)	118 - 326	25.2 (2.9)	19 - 31.6	4.9 (2)	1.7 - 9.7			✓		
69	Palm Sub-canopy		189.6 (55.9)	103 - 317	13.3 (4.4)	4.5 - 19.8	2.9 (1.2)	0.7 - 7.8			✓		

* The deciduous leaf phenological behaviour is included in this table as it can be present in tropical evergreen forests although the brevi-deciduous behaviour is more common.

7.3.6 MODIS VI time-series analyses

The MODIS NDVI time-series for both of our sites display little change, however the Fourier transform results still identify a 95% significant annual cycle, but with very low amplitude values (TAM06= 0.010 and TAM09= 0.013; **Figure 7.3.6.1a** and **b**). The EVI time-series shows a stronger annual cycle (99% significant), with slightly larger amplitude values (TAM06= 0.030 and TAM09= 0.038; **Figure**

7.3.6.1a and **c**) and peaks centred around the end of October to the end of February (TAM06= 0.030 and TAM09= 0.038; **Figure 7.3.6.1a** and **c**).

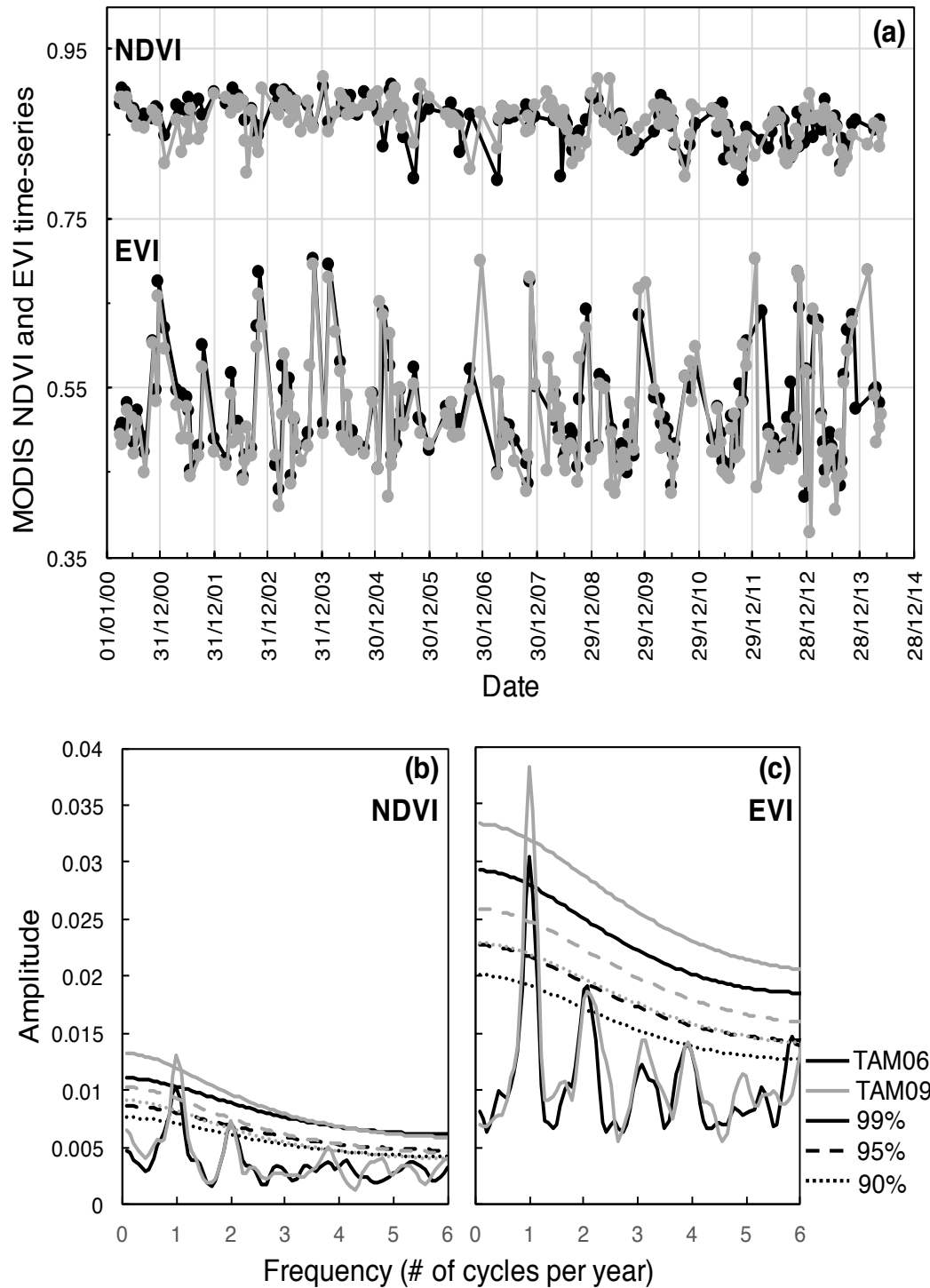


Figure 7.3.6.1 MODIS NDVI and EVI time-series for our two study plots TAM-06 and TAM-09 (a) and their Fourier transform power spectrum plots (b) and (c).

7.4 Discussion

7.4.1 Leaf age phenological effects on canopy reflectance

Age-related changes in the physiochemical and spectral leaf properties of leaves were extensively reported and discussed in Chavana-Bryant *et al.* (2016). Here, we focus on canopy scale phenological processes and start by examining how the age effects on leaf spectral properties of our sampled trees are represented at the canopy scale.

Although our canopy multispectral reflectance data covered a narrower spectral range (detailed information for the 16 multispectral bands used in this study can be found in **Figure 7.2.4.1** and **Table S 7.8.1**) than that of the full-range hyperspectral leaf reflectance data (350-2500nm), we found similar leaf age effects on canopy reflectance, albeit with lower canopy reflectance observed throughout the spectral range. We also found that the sensitivity of canopy reflectance to the reflectance properties of its leaf constituents displayed both age- and wavelength-dependency (**Figure 7.3.1.1** and **Figure 7.3.2.1**).

The lower reflectance throughout the spectral range that we observed at canopy scale is the result of higher photon absorption due to multiple downward scattering within crowns, i.e. into shade (Ollinger, 2011). In terms of phenological leaf age effects, differences between leaf and canopy reflectance were smallest in mature canopies and in the near infrared spectral region (NIR). Mature

canopies have the highest canopy leaf area (CLA) (**Figure 7.3.1.1** and **Figure 7.3.2.1**), which, combined with more planophile leaf angles (personal observations), increases the contribution of leaves to the canopy reflectance and reduces the impact of below canopy shade. In the NIR, higher CLA further enhances the contribution of leaves to canopy reflectance through increased intracellular upward scatter. Mature canopies therefore, present an enhanced leaf spectral signal for remote sensing while this is not the case for developing nor senescent canopies. Differences between leaf and canopy reflectance are larger for both developmental and senescent canopies. In developmental canopies this is the result of the heterogeneous emergence and development among leaves present in the canopy (i.e. heterogeneous development of leaf photosynthetic apparatus and structure), lower CLA and more erectophile leaf angles (personal observations), and in senescent canopies due to a heterogeneous breakdown among senescent canopy leaves (i.e. heterogeneous breakdown of the photosynthetic apparatus and leaf structure) and a rapidly decreasing CLA due to leaf abscission.

Previous remote sensing and modelling studies have identified the enhanced expression of leaf-level spectral information under high LAI conditions while variation in LAI and leaf angle distribution (LAD) have been recognised as the main canopy factors affecting the expression of leaf-level spectral information at the canopy scale (Baret *et al.*, 1994; Asner & Martin, 2008b; Ollinger, 2011). In a

modelling study, Asner (1998) reported that in low canopy LAI scenarios (in our study, low CLA is found in developing and senescent canopies), leaf spectral variability (in our study, leaf spectral variability is the result of leaf ageing) played a small role in driving canopy reflectance while at high canopy LAI (i.e. mature canopies in our study), the effects of leaf spectral variability were more pronounced. Here, by examining the phenological effects of leaf spectral variability and CLA, we provide empirical results that support these findings: we found that leaf-level spectral information was more weakly represented (i.e. larger differences between leaf and canopy reflectance) at canopy scale in developing and senescent canopies. We observed larger differences between leaf and canopy reflectance of 15-20% in visible reflectance (VIS), 8-25% in NIR and 5-15% in short infrared reflectance (SWIR) in developmental and senescent canopies compared to mature canopies.

In terms of spectral regions, across canopies of different leaf life-cycle phases (i.e. developmental, mature and senescent) leaf-level spectral information was most strongly represented (i.e. smallest differences between leaf and canopy reflectance) at canopy scale in the NIR and most weakly (i.e. largest differences) in the VIS. Furthermore, the NIR reflectance collapse reported for senescent leaves between 700-850 nm (3-10% mean decrease compared to mature leaves across individual trees) in Chavana-Bryant *et al.* (2016) was even more dramatic in the canopy signal, with senescent canopies displaying mean decreases of 7-

28% in this spectral region across trees. Finally, the largest NIR reflectance collapse in senescent canopies was observed in band 850nm which is the NIR band used in many VIs. This is a significant finding as it has been reported (Ollinger *et al.*, 2008) that vegetation indices involving VIS and NIR (such as NDVI and EVI) are often driven to a greater extent by variation in NIR reflectance than in the VIS. As Ollinger *et al.* (2008) note, this is not that surprising if we consider that multilayered canopies are very effective at absorbing visible light, which causes reflectance in this region to be very low and have little variation while the NIR is the spectral region in which leaves are most optically transparent and is therefore dominated by structural properties at the leaf, stem and canopy scales which are often correlated to tree physiological functioning. All the above introduces the question of how the phenological age-related changes in leaf and canopy reflectance and in CLA we have described so far are expressed in canopy scale vegetation indices. We will explore this topic in the next section.

7.4.2 Leaf age phenological effects on canopy vegetation indices (VIs)

As reported in the previous section for canopy reflectance, canopy greenness (NDVI and EVI2) and water content (NDWI) VIs also revealed sensitivity to leaf age/life-cycle phase (**Figure 7.3.4.1**). Both NDVI and EVI2 showed maximum values in mature canopies with mean decreases (relative to mature canopies) across individual trees of 3-15% and 4-19% in developing canopies, and 12-56% and 14-61% in senescent canopies, respectively. Across individual trees, NDWI

decreased continuously as canopy leaves aged with values 12-43% higher on average in developmental canopies and 10-68% lower in senescent canopies. When we examined leaf and canopy VIs of individual trees for which we sampled multiple canopy life-cycle phases, we found that with the exception of one tree (3), leaf and canopy VIs displayed no difference in mean value or were closest in value at the mature life-cycle phase (**Figure 7.3.4.2**), matching the patterns observed between leaf and canopy spectral reflectance.

Figure 7.3.4.3 shows the relative contribution of leaf age, expressed through the leaf VIs, and canopy leaf area (CLA) to canopy VIs. Both canopy NDVI and EVI2 (i.e. VIs associated to vegetation greenness) showed changes that were more strongly correlated to changes in CLA (NDVI and EVI2 $R^2 = 0.45$) than to changes in leaf VIs (NDVI $R^2 = 0.24$ and EVI2 $R^2 = 0.28$). In contrast, the changes in canopy NDWI (i.e. VI associated to vegetation water content) were correlated only to changes in leaf NDWI ($R^2 = 0.28$). In these analyses, it is also important to note that these relationships break down when CLA in senescent canopies becomes very low and in bare canopies, as exemplified by the outliers displayed in the graphs but which were not included in these correlation analyses **Figure 7.3.4.2**. Significantly, a phenological study of the seasonal variation in spectral reflectance of deciduous tree canopies by Blackburn & Milton (1995) found that NDVI was closely related to percent canopy cover (%canopy cover is related to LAI as %canopy cover $\sim$ canopy gap fraction and canopy gap fraction is used to

calculate LAI). Furthermore, their study reports that despite a large increase in the number of dead and senescent leaves in the canopy which one would associate with changes in the rates of physiological processes, NDVI remained high until the phase of rapid leaf abscission. Our results also show that both canopy greenness VIs we analysed –NDVI and EVI2- are more closely correlated to CLA than to leaf age (a proxy for physiological processes) but still, the correlations with leaf age are highly significant. In contrast, the water content VI - NDWI- was only correlated to leaf age. This is not surprising if we consider that in Chavana-Bryant *et al.* (2016) we found that leaf water content (LWC) was highly correlated to leaf age ($R^2 = 0.68-0.93$ across individual trees and $R^2 = 0.73$ at community tree level) and that LWC is a direct (at specific water absorption spectral features) and indirect (across the spectrum through its covariation with many physiological processes) driver of leaf reflectance.

So far, we have not discussed the contrasting patterns we identified between the leaf and canopy reflectance and leaf and canopy VIs for one of our sampled trees, *Simarouba amara* (tree 3). This omission is intentional as these contrasting patterns are linked to the evergreen phenological leaf behaviour displayed by this tree. In the next section, we will discuss (1) the leaf phenological behaviours we encountered among our sampled trees, (2) how these behaviours are linked to tree canopy structural types and (3) how the tree structural types found within

one of our study plots (TAM-06) can provide information about the possible distribution of these leaf phenological behaviours within this plot.

7.4.3 Leaf phenological behaviours in tropical trees are both diverse and complex within and across tree crowns

In **Table 7.2.2.1** we introduce the phenological behaviours observed among our sampled trees with **Table 7.3.5.1** providing detailed descriptions of these behaviours. Eight out of our twelve sampled trees displayed the brevi-deciduous leaf phenological behaviour and exchanged their leaves before or during the period of the dry season. Leaf emergence for these trees started when most senescent leaves had been abscised or after a short period of leaflessness that lasted ~10 days to 1 month across individual trees. Two of our sampled trees displayed divergent forms of the evergreen leaf phenological behaviour and two trees displayed combinations/variations of the brevi-deciduous and evergreen leaf phenological behaviours, which to our knowledge have not been previously reported in the literature.

Of the two trees displaying evergreen leaf phenology, *Gautteria boliviana* (tree 12), slowly started to exchange its senescent leaves with new leaves nearing the end of the dry season with the bulk of leaf exchange happening at the beginning of the wet season from November to December. Although *G. boliviana* simultaneously exchanged its senescent for new leaves and did not display a

period of leaflessness, its canopy still showed a largely synchronised leaf age distribution at any one time, i.e. periods when one leaf age group largely dominated its canopy. For example, old and then senescent largely leaves dominated during the dry season, developing leaves dominated at the beginning of the wet season with only mature leaves present during the rest of the wet season. *Simarouba amara* (tree 3) displayed a different type of evergreen leaf phenology, it exchanged ~50 of its canopy for new leaves during the dry season. Its canopy was therefore, always comprised of leaves from two different growth seasons with all leaf life-cycle phases observed at any one time for this tree representing a canopy in constant flux. The developmental phase for *S. amara* is characterised by a canopy containing ~30% developmental leaves and ~70% old leaves from the previous growth season; the mature phase by a canopy containing ~50% of mature leaves; the old phase by a canopy comprised of old leaves from two growing seasons; and the senescent phase by a canopy in which significant abscission of the leaves from the oldest growth season has occurred and new leaves are emerging.

As already mentioned, the last two of our sampled trees displayed combinations/variations of canopy brevi-deciduous and evergreen leaf phenological behaviours. The canopies of both trees were characterised by more vertically complex crown structures made up of both sun and shade leaves with these two leaf types displaying different/asynchronous leaf phenological

behaviours. In *Licania brittoniana* (tree 1), we observed a sun brevi-deciduous/shade evergreen (SuBD/ShE) leaf phenological behaviour. The senescent sun leaves in this tree displayed brevi-deciduous leaf phenology, which were abscised simultaneously and promptly replaced with new leaves. Shade leaves displayed evergreen phenology and were retained for up to three growth seasons. As sun leaves made up most *L. brittoniana*'s canopy, this tree displayed a marked decline in CLA after senescent sun leaves were abscised but was never totally leafless. In *Pouteria franciscana* (tree 13), we observed asynchronous brevi-deciduous leaf phenology between its sun and shade canopy leaves (Su/ShAE), i.e., sun leaves were abscised and promptly replaced by new leaves while shade leaves were retained and exposed to full sun for the brief period (~3-4 weeks) it took for sun leaves to achieve an advanced state of leaf expansion. Shade leaves were then abscised and promptly replaced with new leaves. This within-canopy asynchronous leaf phenology resulted in *P. franciscana* displaying a decline in CLA during senescence but was by no means crown leafless as most its canopy was made up of shade leaves.

The diverse phenological behaviours we observed across our sampled trees draw attention to the complexity of leaf phenology that can be found both within individual trees and across tropical canopy tree communities with diverse tree species and crown structural types. Based on our observations, we present in **Table 7.3.5.2** the possible leaf phenological behaviours for trees within plot TAM-

06 according to their tree crown structural type. As can be clearly seen in this table, the more vertically complex a tropical canopy tree community is the more diverse and complex its leaf phenological behaviours could be. This highlights the difficulties that both field and remote sensing studies encounter when attempting to monitor the complex seasonal leaf phenological dynamics of tropical canopy tree communities.

7.4.4 Remotely sensed seasonal canopy dynamics in the tropics:

interaction, covariation, asynchrony and unique behaviours

The results of the Fourier transform analyses of the NDVI and EVI 13-year time-series for both of our study plots (TAM-06 and TAM-09) identified significant but subtle annual cycles for both VIs with EVI showing a stronger annual cycle (**Figure 7.3.6.1**). The NDVI time-series displayed little change while the EVI time-series showed clear peaks centred around the end of the dry season to the beginning of the wet season. The stronger annual cycle observed for EVI is not surprising as NDVI is known to saturate at high LAI values such as those found in tropical forests (Huete *et al.*, 2002).

We will focus on the stronger annual cycle identified for EVI to explore how the interaction, covariation and/or unique behaviours of the different leaf phenological canopy processes we have described in previous sections, and others we will introduce in this section contribute to the observed small but seasonal peaks

centred around the end of the dry season-beginning of wet season (end of October to the end of February) and to the noisy patterns of lower EVI values during the rest of the year. Starting with the observed EVI peaks, our findings in section 7.4.1 suggest that this is the time of year tropical canopies present a maximised leaf spectral signal for remote sensing as this is when the most synchronised leaf phenological phase and the highest LAI is displayed at the community/landscape scale in tropical canopies. It has been well documented that dry season is the time when concerted albeit asynchronous leaf turn-over is observed among tropical trees, therefore, by the end of the dry season/beginning of the wet season most tropical trees have exchanged their senescent leaves for new leaves. This means that, at this time of year, tropical canopies present their most synchronised leaf phenological phase with most trees displaying newly matured healthy leaves and the highest amount of leaves at the community level.

Figure 7.3.3.1 exemplifies this pattern, at a much smaller scale, for our sampled trees. The opposite effect can be observed during the dry season when the remote sensing leaf spectral signal is weakened by the highly asynchronous timing of leaf turn-over displayed between tropical trees at the community scale. The dry season in tropical canopy communities, therefore, presents the least synchronised leaf phenological phase, with canopy communities simultaneously displaying heterogeneous mosaics containing new leaves in different stages of development, newly matured leaves and senescent leaves in different stages of structural breakdown together with rapidly fluctuating CLA.

The few leaf phenological studies that exist for tropical evergreen canopy communities, have previously reported a range of leaf phenological behaviours broadly described as patterns ranging from rapid shedding of short-lived, mesic leaves early in the dry season in deciduous species to irregular exchange in brevi-deciduous or constant exchange in evergreen species of long-lived, coriaceous leaves, and from complete intraspecific synchrony to complete asynchrony (Vanschaik *et al.*, 1993; Wright & van Schaik, 1994; Reich, 1995; Reich *et al.*, 2004). The diverse and complex leaf phenological behaviours that we report here (see section 7.4.3) can also add extra sources of variation of reflectance and canopy structure that produce a dampening effect on the amplitudes of annual cycles observed by remote sensing VIs. Similarly, the within-crown asynchronous leaf phenological behaviours that our sampled trees with vertically complex crowns displayed, and which results in these trees avoiding leaflessness, can also produce a dampening effect on the amplitudes of remotely sensed annual cycles. Furthermore, the 16-day compositing approach used to create the MODIS 16-day VIs is likely to result in the VIs failing to reveal the short periods of leaflessness ($\leq$ one month) that we observed across our brevi-deciduous sampled trees, and thus, may not be suitable for sensing the full leaf phenological canopy dynamics of tropical canopy tree communities. Also worthy of note is the unique behaviour in the canopy reflectance and VIs for the different life-cycle phases (i.e. developmental, maturity and senescence) in one of our sampled trees - *Simarouba amara* (tree 3)- which was the result of the

particular type of evergreen leaf phenological behaviour displayed by this tree. As described in the previous section, the canopy of *S. amara* was always comprised of leaves from two different growth seasons with the leaf life-cycle phases observed at any one time for this tree representing a canopy in flux. This resulted in *S. amara*'s canopy reflectance, CLA and both greenness VI's displaying their lowest values at the end of the dry season/start of the wet season when most other trees displayed mature canopies with the highest values in canopy reflectance, NDVI, EVI and CLA. Although this particular evergreen leaf phenological behaviour was observed in only one of our sampled trees, this is not an uncommon behaviour in tropical evergreen canopy communities. A phenological study at a lowland rainforest in Costa Rica with mean annual rainfall of 4000mm and one "long" dry season from late January-April and a second, shorter dry season during August-October by Frankie *et al.* (1974) reported that 28% of the 93 "overstorey" (canopy and sub-canopy trees >15m tall) trees they studied displayed similar evergreen leaf phenology as displayed by *S. amara*. The general lack of seasonality displayed by this type of leaf phenological behaviour (see **Figure 7.3.4.2**) could therefore, have a significant dampening effect on the remotely sensed seasonal dynamics of tropical canopy communities in which this leaf phenological behaviour is common.

7.5 Conclusions

The complex and diverse leaf phenological behaviours exhibited by tropical canopy trees challenge our current ability to remotely sense tropical canopy dynamics. We have demonstrated that, at the individual tree canopy scale, the effect of age-related phenological changes in leaf reflectance depends on both canopy leaf area (CLA) and the leaf phenological behaviour of individual trees. We have also shown that the seasonality of greenness VIs such as NDVI and EVI2 are more strongly correlated to phenological changes in CLA than changes in leaf reflectance while NDWI (water content VI) is strongly correlated to age-related changes in leaf reflectance and should complement greenness VIs in phenological studies. At the community scale, leaf phenological behaviours of individual trees are affected by their crown structural type with more vertically complex tropical evergreen canopy communities displaying more complex leaf phenological behaviours that are more difficult to monitor. Furthermore, other community scale phenological sources of reflectance and structural canopy variation such as (1) leaf phenological asynchrony within and between trees, (2) short periods of leaflessness in brevi-deciduous trees that are difficult to detect and (3) lack of seasonality displayed by the evergreen leaf phenological behaviour, can produce dampening effects on the seasonal amplitudes of annual cycles observed by remote sensing VIs. Finally, recent studies such as Bradley *et al.* (2011), Anderson (2012), Guan *et al.* (2015), Xu *et al.* (2015) and Wu *et al.* (2016a), that are starting to identify different phenological mosaics across

Amazonian forests, highlight the need for more widespread phenological studies such as the one we present here. These are necessary if we are to achieve a significant mechanistic understanding of what creates and drives these phenological mosaics and in modelling their effects on water and carbon fluxes across tropical forests.

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7.8 Supporting information

Supporting Information for manuscript: “Remotely sensed seasonal canopy

dynamics in the tropics: a riddle of many scales”.

Table S 7.8.1 Multispectral radiometer band information.

Centre wavelength (CWL)	Bandwidth (BW)	Range			FWHM Cut on	FWHM Cut off
		Cut on	Cut off	FWHM		
470	30	455	485	8.9	461	479
550	30	535	565	9.6	540	560
650	30	635	665	10.9	639	661
660	30	645	675	10.9	649	671
680	30	665	695	9.1	671	689
700	40	685	725	9.7	690	710
720	40	700	740	10.4	710	730
740	40	720	760	10.4	730	750
760	30	745	775	10	750	770
790	30	775	805	9.8	780	800
810	30	795	825	10.7	799	821
850	70	815	885	10.1	840	860
970	30	955	985	10.2	960	980
1200	50	1170	1220	9.9	1190	1210
1240	50	1215	1265	16.7	1223	1257
1680	40	1660	1700	14	1666	1694

Table S 7.8.2 List of vegetation indices used in this study and their corresponding equations.

	Vegetation index	Acronym		Equations
Leaf level	Normalised difference vegetation index	NDVI	$\frac{NIR - RED}{NIR + RED}$	where NIR= measured percent reflectance of MODIS NIR broadband (819 to 898 nm) using the MODIS filter response functions and RED= measured percent reflectance of MODIS RED broadband (614 to 682 nm) using the MODIS filter response functions.
	Enhanced vegetation index 2	EVI2	$2.5 \times \frac{NIR - RED}{NIR + 2.4 \times RED + 1}$	
	Normalised difference water index	NDWI	$\frac{\rho_{857} - \rho_{1240}}{\rho_{857} + \rho_{1240}}$	
Canopy level	Normalised difference vegetation index	NDVI	$\frac{NIR - RED}{NIR + RED}$	where NIR= measured percent reflectance of Cropscan NIR band 850 and RED= measured percent reflectance of Cropscan bands 650 + 660 / 2.
	Enhanced vegetation index 2	EVI2	$2.5 \times \frac{NIR - RED}{NIR + 2.4 \times RED + 1}$	
	Normalised difference water index	NDWI	$\frac{NIR - SWIR}{NIR + SWIR}$	



Chapter 8 Thesis Discussion

8.1 Main insights and implications

The overall aim of this doctoral thesis was to explore and highlight the importance of a fundamental source of variability that has been largely neglected in both trait and remote sensing communities –the variability generated by natural leaf ageing and other phenological processes in tropical forest evergreen canopies. The study of these temporal/seasonal sources of variability in tropical canopy communities which are tens of metres tall and rich in species with low population abundances poses many practical challenges. However, as the findings in this doctoral thesis have demonstrated, this temporal/seasonal variation is embedded but not directly addressed within our current understanding of many important physiological processes at leaf, canopy and community scales and also within the field and analytical protocols we use to collect and analyse these processes.

8.1.1 Leaf age as a fundamental driver of intraspecific leaf trait variation in Amazonian canopy trees

In the first research chapter of this thesis, I examined the variability in physiochemical leaf traits generated by natural leaf ageing within canopy trees and across a tropical evergreen canopy tree community with diverse leaf properties. This study revealed high age-related variability in leaf traits within individual trees and at the community scale. For example, the variation in leaf

traits found among the leaves of the 12 sampled trees in this study covered large parts of the leaf trait variability reported in large-scale/pantropical studies such as those of Wright *et al.* (2004), Poorter *et al.* (2009), Lloyd *et al.* (2010) and Asner *et al.* (2011a). In terms of individual trees, I analysed the coordinated variation of three leaf economic spectrum (LES) traits –nitrogen (N) and phosphorous (P) with reference to LMA- and mapped them against that of the variation in traits for 184 pantropical trees in the Wright *et al.* (2004) dataset. This analysis showed that individual trees can cover a surprising amount of the interspecific trait variation displayed by the pantropical tree dataset, while at the same time, occupying different parts of the trait space covered by this dataset. These findings have three important implications (1) trait variation due to leaf age within individuals can generate as much variation as interspecific variation, (2) leaf age differences could potentially account for a significant fraction of what we currently understand as intra- and interspecific leaf trait variation and (3) different life-cycle combined trajectories in LES traits, which are intimately correlated to the resource-use economy and photosynthetic capacity of individual trees, could play a vital role in community assembly and ecosystem structure in tropical canopy communities with high species diversity with low population densities. These implications become particularly relevant when we consider that single-sample field trait campaigns collect leaves encountered at the time of sampling. Although the standard protocol is to collect “fully expanded mature sun leaves” (Cornelissen *et al.*, 2003), in practice, a range of leaves of different ages, from

leaves close to the end of their full expansive development (see example images for Y3 and M leaves in **Figure 5.2.3.1** and **Figure S 4.8.5**) to senescent leaves (see “leaf in best condition” example images for Old and Senescent leaves in **Figure 5.2.3.1**) can and do end up being collected. I therefore propose that leaf trait collection campaigns require additional protocols to better account for leaf age differences and offer practical field protocols to help address these issues.

Finally, the advanced tree climbing techniques used during my field data collection and the range of leaf types and close monitoring of the diverse and complex leaf phenological behaviours displayed by sampled trees allowed me to identify important leaf sampling and analytical considerations for leaf nutrient resorption studies. In summary, the findings in this research chapter suggest that diverse and complex leaf phenological behaviours may offer more diverse solutions to the problem of life-cycle resource allocation, including nutrient resorption physiology, in tropical evergreen canopy communities.

8.1.2 Leaf aging of Amazonian canopy trees as revealed by spectral and physiochemical measurements

In this study, I introduce the use of a chemometric partial least squares regression (PLSR) model that extends the utility of current spectroscopic methods to deliver a novel, efficient and accurate tool for predicting and monitoring leaf age in lowland tropical forests. This spectral age model takes

advantage of the fact that many of the leaf-scale biochemical and anatomical traits that change with age (some of which I analysed in my first research chapter) have been shown to directly influence the spectral reflectance behaviour of leaves, and as this study reveals, trees with diverse leaf properties age in a similar manner in terms of spectral properties. A physiochemical basis for the spectral age model is presented and identifies leaf water content (LWC) as the primary physiochemical and spectral trait related to leaf age. It is important to highlight that as can be seen in the collaboration paper included in the Appendix section of this thesis, the generality of this spectral model has been successfully tested by application to an independent data set from a different Amazonian forest site in Brazil with slow forest and canopy dynamics and also for contrasting canopy (sun versus shade) light conditions and validated.

This study is also the first to directly investigate and demonstrate that at the leaf level, commonly used vegetation indices (VIs) such as NDVI, EVI2, NDWI and PRI display age sensitivity. This observed sensitivity, and its timing, suggest that seasonal variations in remotely sensed VIs previously reported for Amazonian tropical forests may be strongly influenced by leaf age effects, although seasonal variation in canopy three-dimensional structure may also be important. Leaf age effects on canopy scale remotely sensed VIs is further investigated in the last chapter of this thesis but the findings in this chapter do strongly suggest that tropical canopy spectral signatures may have relatively tractable and quantifiable

variation with leaf age, opening the possibility of tracking their leaf demography through remote sensing.

8.1.3 Leaf age on the spectral predictability of leaf traits in Amazonian canopy trees

The findings of the previous two research chapters in this thesis demonstrate that both physiochemical and spectral leaf traits are affected by leaf age. This raised the question of whether the chemometric PLSR models used to spectrally predict some of the important leaf traits for plant physiology and economy (leaf mass per area, LMA; water content, LWC; phosphorus, P; nitrogen, N and C content) investigated in these previous chapters could be significantly biased by variation in leaf age. This question is particularly relevant as the current standard protocol is to use only “fully expanded mature leaves” to calibrate these models. To explore the magnitude of this effect, I used a subset of my phenological dataset comprised of leaves of different leaf age groups –developmental (D), mature (M), senescent (S) and mixed-age (All) from eight different sampled canopy trees. I developed PLSR models to spectrally predict leaf mass per unit area (LMA) and water (LWC), phosphorous (P_{mass}), nitrogen (N_{mass}) and carbon content (C_{mass}). The results of this analysis show trait-specific age effects in models of four of the five traits I analysed, with LMA models the only ones to display no age effects. This study also demonstrates that PLSR models for all five leaf traits displayed age/temporal sensitivity when we used models developed using leaves from one

leaf age group to predict the traits of leaves of other leaf age groups. Importantly, the mature (M) models displayed the worst overall performance when predicting the traits of leaves from other leaf age groups. These results are significant as they indicate that the standard practice of using ‘fully expanded mature leaves’ to calibrate remotely sensed estimates of tree canopy traits introduces differential error that can bias results depending on the phenological stage of canopy leaves. I therefore propose that to achieve temporally stable PLSR models, hyperspectral remote sensing studies would benefit from calibrating models with leaves of different leaf age groups as they present themselves through the growing season. This would involve leaf sampling at different times (early, mid and end) of the growing season in vegetation systems with synchronised leaf phenology (e.g. temperate and deciduous forests) and randomly sampling leaves of all ages encountered in the field during the dry season or at a particular time of year of interest in systems with unsynchronised leaf phenology such as evergreen tropical forests.

8.1.4 Remotely sensed seasonal canopy dynamics in the tropics: a riddle of many scales

In the final research chapter of this thesis, I adopted a broader perspective to examine the different leaf phenological processes that contribute to the seasonal spectral properties of a tropical forest community in Tambopata, Peru as captured by remote sensing vegetation indices. This study is particularly relevant

as leaf demography has been proposed as a major driver of seasonal productivity in carbon-rich tropical evergreen forests yet tropical leaf phenology is poorly understood. At the individual tree canopy scale, this study shows that (1) age-related phenological changes in leaf reflectance are expressed at the canopy scale but are affected by both canopy leaf area (CLA) and the leaf phenological behaviour of individual trees; (2) the seasonality of greenness VIs such as NDVI and EVI2 are more strongly correlated to phenological changes in CLA than changes in leaf reflectance; and (3) NDWI (water content VI) which was found to be strongly correlated to age-related changes in leaf reflectance should complement greenness VIs in phenological studies. At the community scale, this study revealed that more vertically complex tropical evergreen canopy communities display more complex leaf phenological behaviours that are more difficult to monitor. Furthermore, other community scale phenological sources of reflectance and structural canopy variation such as (1) leaf phenological asynchrony within and between trees; (2) short periods of leaflessness in brevi-deciduous trees that are difficult to detect; and (3) lack of seasonality displayed by the evergreen leaf phenological behaviour can produce dampening effects on the seasonal amplitudes of annual cycles observed by remote sensing VIs. By combining leaf, canopy and community scale phenological observations this study demonstrates that complex and diverse leaf phenological behaviours exhibited by tropical canopy trees challenge our current ability to remotely sense tropical canopy dynamics.

8.2 Concluding remarks and future research needs

The body of work of this doctoral thesis helps to establish the fundamental co-variation among leaf traits, spectra and age in tropical tree canopies. It proposes that studies that seek insights into trait and spectral variation and mechanisms that regulate resource investment and return in leaves should expand their field and analytical methodologies to include various leaf phenological stages and their effects on key physiological traits. This thesis provides practical field and analytical protocols to address some of these issues. Furthermore, the spectral age model introduced in **Chapter 5** and successfully tested at a different Amazonian forest site in Brazil and with a completely different set of tree species (Wu *et al.*, 2016b) present a strong case for the future use of hyperspectral remotely sensed observations to monitor and map leaf demography across tree crowns and whole landscapes, and to study the temporal dynamics of tropical forest canopies.

With respect to the detection and interpretation of leaf phenology in tropical evergreen forests, **Chapter 7** presents a benchmark study that highlights the need for more widespread phenological studies that examine the interaction, covariation, asynchrony and unique behaviours of tropical phenological processes at different scales. Global forest inventory and intensive monitoring networks around the tropics such as RAINFOR and GEM, many of which already monitor LAI and leaf litter on a bi-monthly or monthly basis, would benefit hugely

from implementing additional leaf phenological protocols that catalogue the vertical structure of canopy trees, the phenological synchrony/asynchrony between these trees and identify key phenological behaviours present in their sites. Implementing these protocols at sites located within different phenological mosaics identified across the Amazon Basin and other tropical evergreen forests with different canopy tree composition and structure (e.g. dipterocarp forests of SE Asia) should be prioritised. These additional phenological protocols would help establish a monitoring network that would provide ground validation for remote sensing studies and build a significant mechanistic understanding of what creates and drives different phenological mosaics identified across the tropics in studies by Bradley *et al.* (2011) and Xu *et al.* (2015). This, in turn, could help incorporate these phenological mosaics and seasonal dynamics into large-scale vegetation models.

As the introduction to this thesis has shown, tropical leaf phenology has been a subject of much debate in recent remote sensing studies. These studies have reported higher values in vegetation indices (both NDVI and EVI) at the end of the dry season (Huete *et al.*, 2006a; Xiao *et al.*, 2006; Brando *et al.*, 2010), which have been interpreted or attributed to increases in leaf area index (LAI) (Myneni *et al.*, 2007) or the changes in leaf spectral properties from the exchange of old leaves for new ones during the dry season (Xiao *et al.*, 2006; Doughty & Goulden, 2008; Brando *et al.*, 2010). Although both of these interpretations have

some supporting evidence, each has also its shortcomings. For example, attributing remotely sensed (RS) seasonal dynamics in VIs solely to changes in spectral leaf properties due to the exchange of old for new leaves ignores field studies that clearly show seasonal dynamics in leaf area and litterfall (Asner *et al.*, 2004; Malhado *et al.*, 2009; Chave *et al.*, 2010). On the other hand, attributing RS dynamics only to LAI changes ignores the differences in leaf physiochemical (which are related to leaf photosynthetic capacity) and spectral properties between old and new leaves, which have been clearly established for tropical canopy leaves for the first time in this thesis.

I propose that the way to reconcile these divergent views is to bring together the different scales at which the diverse and complex tropical leaf phenological processes work. As I have shown in **Chapter 5**, leaf level VIs that measure vegetation greenness (NDVI and EVI), water content (NDWI) and light-use efficiency (PRI) are affected by age-related changes in leaf spectral properties. However, when leaf age phenological changes are considered at the individual tree canopy scale, these leaf level age-related spectral changes go hand in hand with changes in canopy leaf area (CLA). As **Figure 7.3.4.3** in **Chapter 7** makes clear, both changes in leaf spectral properties and CLA are phenological processes that happen simultaneously and should not be considered in isolation. This figure also shows that canopy NDWI can be a more representative proxy for the contribution of age-related spectral changes to RS seasonal canopy

dynamics. Furthermore, when the scale of observation of leaf phenological processes is broadened, it becomes clear that, the phenological asynchrony between trees, short periods of leaflessness in brevi-deciduous trees and diverse and complex evergreen phenological behaviours during the dry season challenge the ability of RS VIs to capture the canopy dynamics during this time of year, which is the season of maximum dynamism in leaf phenology. However, the higher values reported in VIs and ecosystem photosynthesis (Huete *et al.*, 2006a; Myneni *et al.*, 2007; Saleska *et al.*, 2007; Brando *et al.*, 2010; Bradley *et al.*, 2011; Silva *et al.*, 2013; Guan *et al.*, 2015; Xu *et al.*, 2015; Wu *et al.*, 2016a) at the end of dry season/beginning of the wet season are the result of a combination of (1) maximum CLA displayed by individual trees with newly matured canopies (see **Figure 7.3.1.1**), (2) maximum LAI displayed at the community/landscape scale as most trees have exchanged their old leaves for new ones during the dry season, and (3) the maximised spectral and photosynthetic properties of newly matured leaves. The relative contribution of changes in leaf area versus changes in spectral properties of leaves to RS VIs remains to be unraveled but perhaps the use of the spectral leaf age model introduced in **Chapter 5** with data from new generation hyperspectral remote sensing instruments could prove a useful tool in this endeavour.

In conclusion, this thesis represents a novel exploration of the seasonal variation in biochemical, morphological and spectral properties of a tropical forest canopy,

a difficult-to-access “frontier” of the biosphere that is rarely studied in detail. This thesis has shown that leaf age and canopy phenology weave together to generate the seasonal variation of canopy spectral signatures of “apparently” evergreen forest canopies, it has also shown that there is much promise in inferring canopy demography from new technologies such as hyperspectral remote sensing, which in turn, has the potential of revolutionising our understanding of the biosphere in coming years.

8.3 References

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Appendix – Collaboration paper

Wu J, Chavana-Bryant C, Prohaska N, Serbin SP, Guan K, Albert LP, Yang X, van Leeuwen WJD, Garnello AJ, Martins G, et al. 2016b. Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests. *New Phytologist* **214**: 1033-1048.

Wu *et al.* (2017) is a follow-up paper to Chavana-Bryant *et al.* (2017), which is presented in this thesis in **Chapter 5**. It tests the generality of the spectral age model developed in Chavana-Bryant *et al.* (2017) by application to an independent data set from a very different Amazonian forest site in Brazil with slow forest and canopy dynamics, and it is also tests for contrasting canopy (sun versus shade) light conditions.

Author contribution statement

J.W., S.R.S., **C.C-B.**, L.P.A. and S.P.S. planned and designed the research. J.W., S.R.S., N.P., L.P.A., **C.C-B.**, R.C.O. and G.M. designed, performed and/or supported Brazil field data collection. Data integration and quality assurance at the Brazil site were performed by J.W. J.W. and S.P.S. designed the MATLAB version of PLSR analysis with input from **C.C-B.** A.J.G. helped process leaf traits data at the Brazil site. Data analysis was performed by J.W. J.W. wrote the manuscript in close collaboration with S.R.S. and **C.C-B.**, with contributions from S.P.S., F.G., Y.M., K.G., X.Y., L.P.A. and W.J.D.vL.